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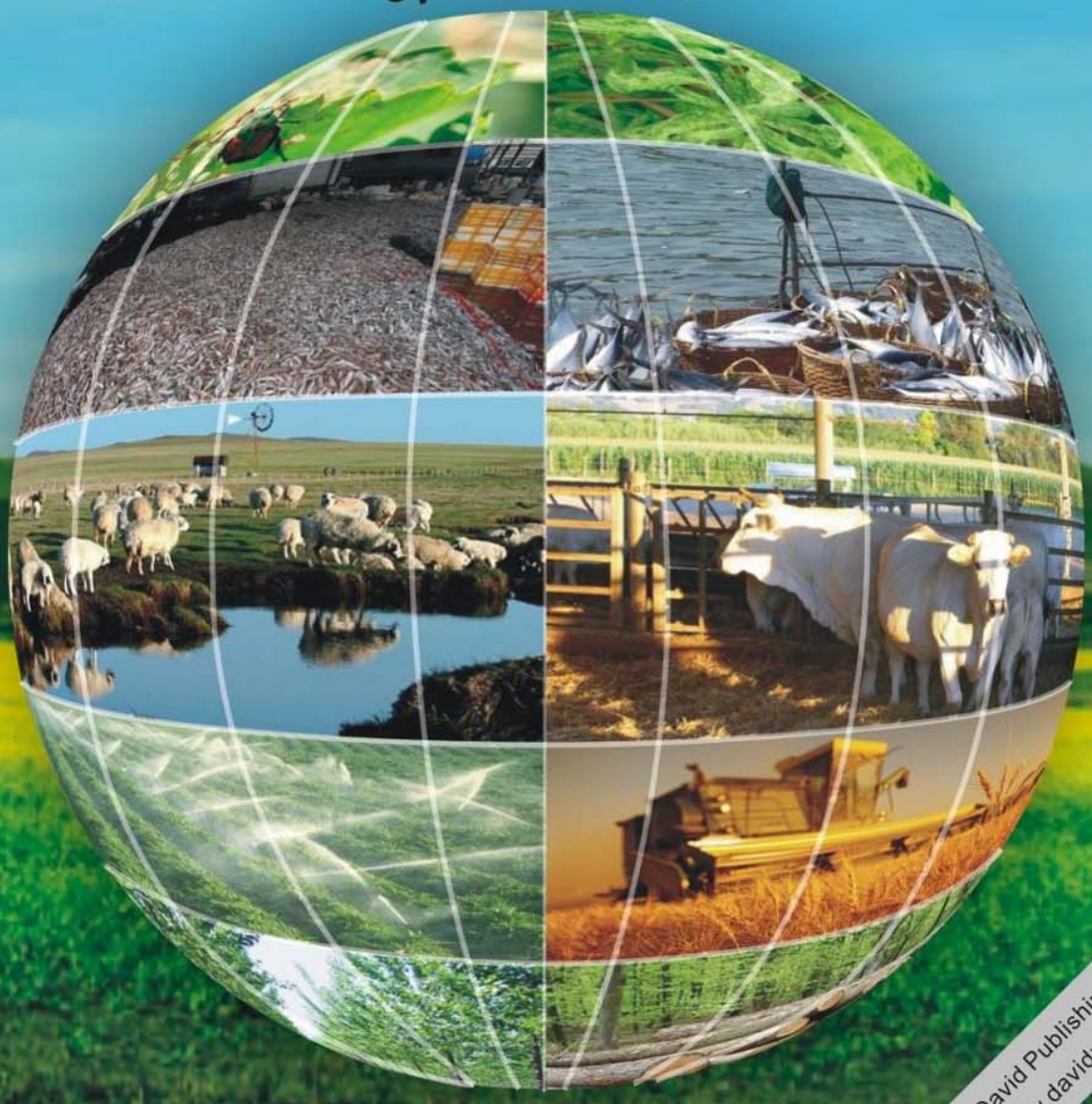

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Development of RAPD-Derived STS Markers for Genetic Diversity Assessment in Melon (*Cucumis melo* L.)

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Abstract: Random amplified polymorphic DNA (RAPD) has been used widely in diversity studies, including population structure and phylogenetics at all taxonomic levels. However, there is a problem in stability and repeatability of RAPD in some cases. Therefore, conversion of RAPD markers into new type of PCR-based marker to overcome low levels of repeatability of RAPD marker is needed. The aim of this study was to develop sequence-tagged site (STS) markers by designing specific primers based on RAPD marker sequences to provide the potential markers for analyzing genetic diversity of melon germplasm. Eight RAPD-STS markers were successfully converted from RAPD markers and have two polymorphism types; A20 and B99 showed different sizes of fragment; A22, A31, A57, B15, B71 and C00 showed presence/absence polymorphism in melon germsplasm. The applicability of new RAPD-STS markers has been demonstrated by comparing genotype analysis of 41 melon accessions using RAPD and RAPD-STS markers. Both of RAPD markers and RAPD-STS markers divided them into two major clusters. However, the RAPD-STS markers were more polymorphic than RAPD markers (polymorphic index content (PIC) values were 0.346 and 0.274, respectively). Mantel's test showed significant correlation (r = 0.896, P < 0.01) between RAPD-STS dendrogram and RAPD dendrogram. Furthermore, RAPD-STS markers could give more information in population structure and identify admixture individuals by using STRUCTURE software. Eight RAPD-STS markers developed in this study are useful for genetic diversity analysis and population studies in melon.

Key words: Sequence-tagged site (STS), RAPD, *Cucumis melo* L., genetic diversity, marker.

1. Introduction

Melon (*Cucumis melo* L.) is considered as one of the most morphologically diverse species among the major cucurbit crops, even among vegetables. Pitrat [1] has classified 15 botanical groups of melon, including five botanical groups in *Cucumis melo* ssp. *agrestis* (*acidulus*, *conomon*, *momordica*, *makuwa*, *chinensis*) and 10 botanical groups in *Cucumis melo* ssp. *melo* (*chate*, *flexuosus*, *tibish*, *adana*, *ameri*, *cantalupensis*, *chandalak*, *reticulatus*, *inodorus*, *dudaim*). However, recent findings of the African and Asian melons not included in this taxonomy caused confusion [2, 3]. Application of molecular markers to assess genetic diversity is essential to refine morphological-based classification.

Abundant of molecular markers are applicable to reveal the variation within melon accessions, including restriction fragment length polymorphisms (RFLP) [4], random amplified polymorphic DNA (RAPD) [5, 6], simple sequence repeats (SSR) [7] and single nucleotide polymorphisms (SNP) [8]. PCR-based markers, especially RAPD and SSR, have received much attention; however, RAPD markers are dominant markers and incapable to detect heterozygote, and also difficult to reproduce RAPD profile between different laboratories due to their lack

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of specificity. In contrast, SSR often require high resolution and laboratory intensive techniques. Therefore, genetic diversity analysis requires a new type of marker targeted to specific sites and needs no manipulation of amplified products for polymorphism detection.

In broad sense, STS markers are generated by a pair of primers (18-21 nucleotides), which are designed based on known DNA sequences. In narrow sense, STS markers are produced based on the conversion from RAPD/AFLP/RFLP markers which are referred as another name sequence characterized amplified region (SCAR). STS marker was firstly introduced by Olson et al. [9] as a DNA landmark in physical map of human, which occurred only once in a genome or chromosome. STS marker is now used as a valuable genetic marker to distinguish individuals with many advantages, including high specificity and reproducibility, low time-consuming and easiness to conduct [10-14].

The goal of this study was to develop RAPD-STS markers by designing specific primer sets from RAPD sequences to provide potential markers for genetic diversity analysis in melon.

2. Materials and Methods

2.1 DNA Materials

A total of 24 melon accessions were selected based on RAPD profile from previous studies for screening polymorphism of RAPD-STS markers [5, 6]. The polymorphic RAPD-STS markers were used to genotype 41 melon accessions [6].

Total DNA from melon leaves was extracted by cetyl trimethyl ammonium bromide (CTAB) method as described by Murray and Thompson [15] with minor modifications.

2.2 Cloning and Sequencing of RAPD-Generated DNA Fragments

RAPD reactions were performed and analyzed following the method as described by Nhi et al. [6]. The bright and highly reproducible products of 16 RAPD markers were excised from agarose gel and purified by QIAquick PCR purification kit (QIAGEN, USA). The RAPD fragments were cloned into pCR2.1-TOPO of the TOPO TA cloning kit (Invitrogen, USA) followed by the manufacturer's instructions and sequenced by using an ABI PRISMR 3730 DNA Analyzer (Applied Biosystems, USA).

2.3 RAPD-STS Primer Design

To design the primer set specific to each RAPD marker sequence, the additional 5' and 3' flanking sequences were obtained from the melon genome database by BLAST using the marker sequence as a query[1]. Based on the genomic sequence, including RAPD marker sequence and 5' and 3' flanking sequences obtained, primers consisting of 18-21 nucleotides were designed by Primer3[2]. Whole or part of the original RAPD primer sequence was included in the new primers.

2.4 RAPD-STS Analysis

The 10 μL PCR mixture for RAPD-STS analysis comprised of 50 ng of genomic DNA, 1 μL PCR buffer (Sigma, USA: 10 mM Tris-HCl, pH 8.3, 50 mM KCl), $MgCl_2$, 0.1 mM dNTP, 0.25 μM of each primer and 0.25 U Taq polymerase (Sigma, USA). Amplification reactions were performed using an iCycler (Bio-Rad, USA). The PCR cycle started with an initial denaturing step at 95 °C for 3 min, followed by 35 cycles of 1 min at 95 °C, 1 min for annealing and 2 min at 72 °C. The final step was at 72 °C for 5 min. The annealing temperature and $MgCl_2$ concentration were adjusted depending on RAPD-STS primer sets.

The PCR products were separated on 1.5% agarose gels in TBE buffer, stained with ethidium bromide, and visualized under ultraviolet light.

[1] http://melonomics.net/.
[2] http://bioinfo.ut.ee/primer3-0.4.0/primer3/.

2.5 Data Analysis

The RAPD marker band and RAPD-STS marker band were scored as 1 for present and 0 for absent. From these data, the polymorphic index content (PIC) was calculated according to Anderson et al. [16]. Genetic similarity (GS) among accessions was calculated as described by Apostol et al. [17] and their genetic distance (GD) was calculated with Eq. (1):

$$GD = 1\text{-}GS \quad (1)$$

A dendrogram was constructed by the PHYLIP program using the unweighted pair group method with arithmetic mean (UPGMA) method. Mantel's test of XLSTAT software was used to find out the correlation between dendrograms constructed by RAPD-STS markers and RAPD markers, respectively. Population structure and identification of admixed individuals was performed using STRUTURE 2.2 software.

3. Results and Discussions

3.1 Development of RAPD-STS Markers

A total of 18 polymorphic bands of RAPD markers were cloned and successfully sequenced. Basically, based on the internal sequence within the cloned RAPD markers, RAPD-STS markers were designed either by adding 10 to 14 bases to 3' end of the original primer sequence [11, 14, 18] or by settling inner side of the cloned RAPD fragments without regard to the sequence of the RAPD primers [19, 20]. Upstream and downstream of sequences flanking to the RAPD marker sequence were obtained using BLAST tool. RAPD-STS primers were then designed (Table 1).

3.2 Detection Efficiency and Sensitivity of RAPD-STS Markers

Three out of 18 RADP-STS markers (A23, B32 and B68) failed to amplify. Fifteen primers successfully produced a single band of expected sizes from melon genome, except for A22. Marker A22-RAPD-STS amplified a 350-bp fragment, which was much smaller than those amplified by A22-RAPD (1,520 bp-fragment). The sequence of 350 bp-fragment was 99.99% identical to the downstream sequence of 1,520 bp-fragment.

Reference accessions, including "plus accessions" (can be amplified according to RAPD profile) and "minus accessions" (cannot be amplified according to RAPD profile), were used for selecting the polymorphic marker among 15 RAPD-STS markers. Two different types of polymorphism were observed. A20 and B99 showed different sizes of fragment, while A22, A31, A57, B15, B71and C00 showed presence or absence of band (Fig. 1). For the latter six markers, positive control primer sets shown in Table 2 were combined in the PCR reaction and multiplex PCR was performed, in order to confirm the absence of marker band. The other seven markers showed no polymorphism.

In this study, the loss of polymorphism was observed in 10 out of 18 RAPD-STS markers. The difficulty in polymorphism reproduction was also observed by Horejsi et al. [11], Mammadov et al. [13] and Paran et al. [14]. Paran et al. [14] explained that the lack of specificity in RAPD primer can lead to mismatch at the primer sites and produce the artifactual bands (false positives) corresponding to rearranged fragments. Therefore, the sequence data of RAPD marker fragment did not provide information on these mismatches explains why RAPD-STS markers lost the polymorphism when converted from RAPD markers.

3.3 Validation of RAPD-STS Markers

To confirm the accuracy and stability of new RAPD-STS markers (A20, A22, A31, A57, B15, B71, B99 and C00), 41 melon accessions were genotyped and compared with RAPD profile [6]. All markers produced clear and reproducible polymorphic fragments in the 41 accessions, except for C00. C00 did not show polymorphism among 41 melon accessions so that it was eliminated during establishment of phylogenetic tree.

Table 1 Sequence of 18 RAPD-STS primer sets and their expected size.

RAPD-STS marker	Primer sequence	Expected size (bp)
A20	ATAGATCACCTAGCGGGACCA ACGAACTCAAACCGGGACCA	800
A22	TTTTCCAAGAACGGGAAGG GGTGAAGAAGCCAAACTACCA	350
A23	AGTGGTGGTATACCTGT CAGGAGTAAGCCGAATC	1,200
A26	GTTAATGGAGCTGCGTATTCA TGAGGATTCATTAGGCAAAGC	1,400
A31	GGCAGCAGTGGTGGCATC GGAACAAATAAGTGTGTGGTATC	800
A41	AATAGACCCTTGTACGGTAT GATGAGGAGTTGGTACTGTAT	930
A57	GCTAATAAGCAATTGGCGAAC GCCAAAGATCGATTGTCGAA	800
B15	CCTTGGCATCGGTATGTA CTTGGCATCGGCACCTTT	600
B32	TTTTTACGTACGTGGATACCAA TCATCTAAAATTAATGGATCGT	700
B68	CACACTCGTCATATACA CACACTCGTCATGTTT	1,078
B71	GGACCTCCATCGATAA GGACCTCCATCGTATCC	1,220
B84-600	CTTATGGATCCGCTTGGTATG CTTATGGATCCGACGAAGAG	600
B84-700	CTTATGGATCCGTTTGGTAA CTTATGGATCCGACGAAGAG	700
B86	TCGAGCGAACGTAATGAAGA TACTGTTATCCCGAGCGAATG	1,370
B96-750	AAAGACTGCTACCAAAACGAA TGGTCGTGTGAATACTATGGA	750
B96-850	GGCTACAAAGGTGAAAACTATGG TGAGATTATGAATCCTATGTATG	850
B99	TTCTGCTCGAAACTCTAGG TTCTGCTCGAAATACAAGAAA	1,550
C00	GAGTTGTATGCGGAGTTGGA GTTGTATGCGATAAAGTCAACA	1,350

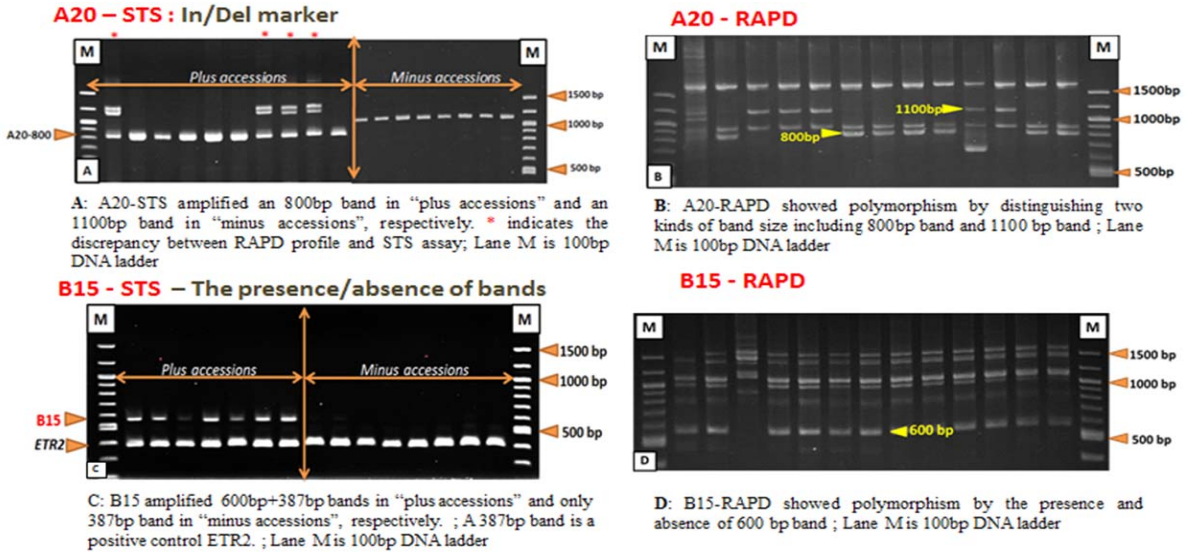

Fig. 1 The comparison of two RAPD-STS markers with the corresponding RAPD markers.

Table 2 PCR conditions of eight sets of RAPD-STS primers and the PIC values of RAPD-STS and RAPD polymorphism in 41 melon accessions.

RAPD-STS markers	Annealing temperature (°C)	Concentration of $MgCl_2$ (mM)	Expected size (bp)	Type of polymorphism	Positive control primers[a]	Amount of primers[b]	PIC[c]	
							RAPD-STS	RAPD
A20	62	2.5	800 1,100	InDel	Not used		0.450 0.433	0.485 0.433
A22	62	2.5	350	Presence/absence	ACO1-MS3	2:1	0.485	0.476
A31	64	2.5	800	Presence/absence	ACO1-MS3	2:1	0.414	0.314
A57	62	2.5	800	Presence/absence	ETR-MS2	1:1	0.450	0.000
B15	62	2.5	600	Presence/absence	ETR-MS2	2:1	0.136	0.136
B71	60	2.5	1,220	Presence/absence	B86-1370	2:1	0.214	0.214
B99	64	2.5	1,400 1,550	InDel	Not used		0.393 0.136	0.136 -
C00	62.5	2.0	1,350	Presence/absence	ETR-MS2	2:1	0.000	0.000

[a] Sequence of positive control primers:

ACO1-MSF3CCACCTCCTCTCTTTCCCACATA ACO1-MSR3 CTCCTTAAACCTCTCTTCCATAC

ETR-MSF2ATCGATTGTTGAAGCAACTTT ETR-MSR2GAGACCCAGAAAGGCGTTTAG

B86-1370-F2 TCGAGCGAACGTAATGAAGA B86-1370-RTACTGTTATCCCGAGCGAATG

[b] amount of RAPD-STS primers (amount of positive control primers).

-: means the marker band was not scored by Nhi et al. [6].

The average PIC value of all the RAPD-STS markers was 0.346, with a maximum of 0.485 for A22 and a minimum of 0.136 for B99 and B15 (Table 2). Most of PIC values obtained from RAPD-STS markers were higher than those obtained from RAPD markers. Thus, RAPD-STS markers were more polymorphic than RAPD markers.

3.4 Genetic Diversity Analysis of Melon Germplasm

A total of 41 melon accessions were used to assess their genetic diversity. Of these, 27 accessions were collected from Vietnam. These accessions mainly belong to var. *conomon* and var. *makuwa*, and have a large phenotypic diversity and a low genetic diversity [6]. Despite of the low genetic diversity of 41 melon accessions, both of RAPD markers and RAPD-STS markers still divided them into two major clusters (Fig. 2). Cluster I consisted of 28 accessions, which were dominated by andromonoecious type, except for VN139, whereas the remaining accessions were included in cluster II, which was further divided into two subclusters. Subcluster IIa included 10 monoecious accessions, while subcluster IIb was composed of three andromonoecious accessions of European and American origin. Furthermore, the correlation between RAPD-STS based dendrogram and RAPD based dendrogram was 0.896 by Mantel's test.

Based on the membership coefficients, the accessions which had the coefficients over 70 % were assigned to the corresponding subgroups, while others categorized as the admixture (Fig. 3). With RAPD-STS markers, a monoecious accessions VN139 is considered as admixture form with inferred value from cluster I and cluster II is 69% and 31%, respectively. The admixture is likely the result of hybridization between two groups. As one more remarkable case, VN13 was regarded as admixture with inferred value from cluster II quite high (34.8%) by RAPD analysis, while this value was only 3.4% by RAPD-STS analysis. In other words, the genetic architecture of divergent accessions can be estimated by assessing the STRUTURE of the population using RAPD-STS markers.

In short, the results derived from analysis of genetic diversity and population structure strongly supported that eight RAPD-STS markers can be utilized as efficient molecular markers to analyze diversity of melon.

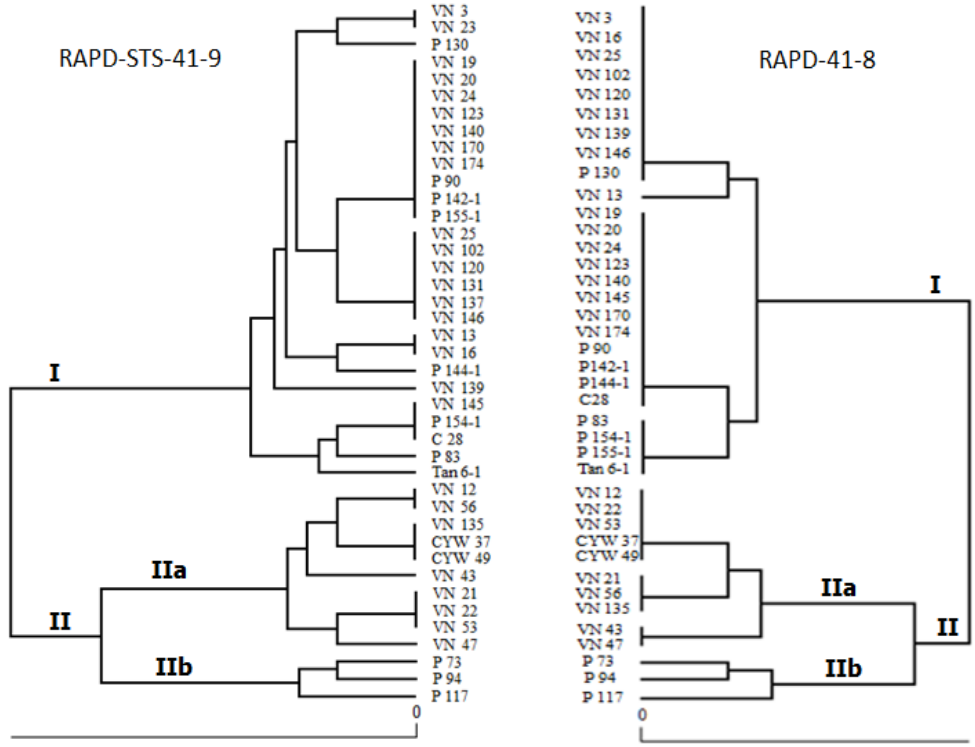

Fig. 2 Comparison of phylogenetic trees constructed by RAPD-STS analysis (RAPD-STS-41-9) and RAPD analysis (RAPD-41-8).

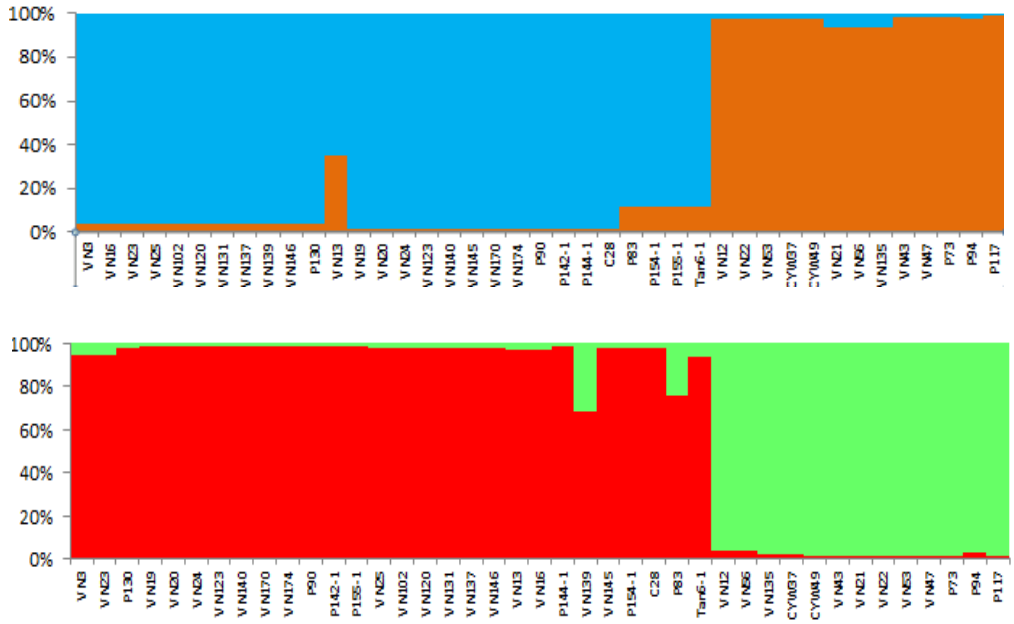

Fig. 3 Bar plots for individual melon accessions by Structure 2.2 using the admixture model based on eight RAPD markers (upper) and nine RAPD-STS markers (lower).

4. Conclusions

In general, this study contributed advantages of STS markers compared with RAPD markers. With the use of primer sets designed using the external sequences of the cloned RAPD fragments, it can be identified the position of RAPD-STS markers in the melon genome, which is meaningful not only for diversity analysis but also for mapping projects. Conversion of RAPD markers into RAPD-STS markers was not easy task. Especially, the polymorphism loss tended to occur at high frequency. However, the success of conversion of eight RAPD-STS markers (A20, A22, A31, A57, B15, B71, B99 and C00) will be useful for genetic diversity analysis as well as population mapping. Two RAPD-STS markers A20 and B99 showed polymorphism with different sizes of fragment; while the remaining markers (A22, A31, A57, B15, B71 and C00) showed presence/absence polymorphism. These eight markers could amplify various sizes of marker fragment ranging from 350 bp to 1,350 bp, indicating that they are valuable for diversity analysis. Indeed, validation of these eight markers using 41 melon accessions was initial evidence for the applicability of them. In order to widely apply the set of these RAPD-STS markers, it is suggested that these eight markers need to continue being validated on various melon populations. If they are stable and reliable, eight markers should be used as efficient molecular markers to analyze diversity of melon and also *Cucumis* sp..

References

[1] Pitrat, M. 2008. "Melon (*Cucumis melo* L.)." In *Handbook of Crop Breeding,* edited by Prohens, J., and Nuez, F. Vol. 1. New York: Springer, 283-315.

[2] Renner, S. S., Schaefer, H., and Kocyan, A. 2007. "Phylogenetics of *Cucumis* (Cucurbitaceae): Cucumber (*C. sativus*) Belongs in an Asian/Australian Clade Far from Melon (*C. melo*)." *BMC Evol. Biol.* 7: 58.

[3] Sebastian, P., Schaefer, H., Telford, I. R. H., and Renner, S. S. 2010. "Cucumber (*Cucumis sativus*) and Melon (*C. melo*) Have Numerous Wild Relatives in Asia and Australia, and the Sister Species of Melon Is from Australia." *Proceedings of the National Academy of Sciences* 107 (32): 14269-73.

[4] Neuhausen, S. L. 1992. "Evaluation of Restriction Fragment Length Polymorphism in *Cucumis melo*." *Theor. Appl. Genet.* 83 (3): 379-84.

[5] Tanaka, K., Nishitani, A., Akashi, Y., Sakata, Y., Nishida, H., Yoshino, H., and Kato, K. 2007. "Molecular Characterization of South and East Asian Melon, *Cucumis melo* L., and the Origin of Group Conomon var. *makuwa* and var. *Conomon* Revealed by RAPD Analysis." *Euphytica* 153 (1): 233-47.

[6] Nhi, P. T. P., Akashi, Y., Hang, T. T. M., Tanaka, K., Aierken, Y., Yamamoto, T., Nishida, H., Long, C. L., and Kato, K. 2010. "Genetic Diversity in Vietnamese Melon Landraces Revealed by the Analyses of Morphological Traits and Nuclear and Cytoplasmic Molecular Markers". *Breed. Sci.* 60: 255-66.

[7] Aierken, Y., Akashi, Y., Nhi, P. T. P., Halidan, Y., Tanaka, K., Long, B., Nishida, H., Long, C. L., Wu, M. Z., and Kat, K. 2011. "Molecular Analysis of the Genetic Diversity of Chinese Hami Melon and Its Relationship to the Melon Germplasm from Central and South Asia." *Japan. Soc. Hort. Sci.* 80 (1): 52-65.

[8] Blanca, J., Esteras, C., Ziarsolo, P., Pérez, D., Fernández-Pedrosa, V., Collado, C., Pablos, R. R., Ballester, A., Roig, C., Cañizares, J., and Picó, B. 2012. "Transcriptome Sequencing for SNP Discovery across *Cucumis melo.*" *BMC Genomics* 13: 280.

[9] Olson, M., Hood, L., Cantor, C., and Botstein, D. 1989. "A Common Language for Physical Mapping of the Human Genome." *Science* 245: 1434-5.

[10] Heikrujam, M., Sharma, K., Kumar, J., and Agrawal, V. 2014. "Generation and Validation of Unique Male Sex-Specific Sequence Tagged Sites (STS) Marker from Diverse Genotypes of DIOECIOUS Jojoba-Simmondsia CHINENSIS (Link) Schneider." *Euphytica* 199 (3): 363-72.

[11] Horejsi, T., Box, J. M., and Staub, J. E. 1999. "Efficiency of Randomly Amplified Polymorphic DNA to Sequence Characterized Amplified Region Marker Conversion and Their Comparative Polymerase Chain Reaction Sensitivity in Cucumber." *J. Amer. Soc. Hort. Sci.* 124 (2): 128-35.

[12] Kanno, A., Kubota, S., and Ishino, K. 2014. "Conversion of a Male-Specific RAPD Marker into an STS Marker in *Asparagus officinalis* L.." *Euphytica* 197 (1): 39-46.

[13] Mammadov, A. C., Li, X. M., and Wang, R. C. 2010. "Development of STS and CAPS Markers Specific to Genomes in the Tribe Triticeae." *Proc. of ANAS (Biological Sciences)* 65 (5-6): 122-31.

[14] Paran, I., and Michelmore, R. W. 1993. "Development of

Reliable PCR-Based Markers Linked to Downy Mildew Resistance Genes in Lettuce." *Theor. Appl. Genet.* 85 (8): 985-93.

[15] Murray, M. G., and Thompson, W. F. 1980. "Rapid Isolation of High Molecular Weight Plant DNA." *Nucleic Acids Res.* 8 (19): 4321-6.

[16] Anderson, J. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D., and Sorrells, M. E. 1993. "Optimizing Parental Selection for Genetic Linkage Maps." *Genome* 36 (1): 181-6.

[17] Apostol, B. L., Black, W. C., Miller, B. R., Reiter, P., and Beaty, B. J. 1993. "Estimation of the Number of Full Sibling Families at an Oviposition Site Using RAPD-PCR Markers: Applications to the Mosquito *Aedes aegypti*." *Theor. Appl. Genet.* 86 (8): 991-1000.

[18] Kiran, U., Khan, S., Mirza, K. J., Ram, R., and Abdin, M. Z. 2010. "SCAR Markers: A Potential Tool for Authentication of Herbal Drugs." *Fitoterapia* 81 (8): 969-76.

[19] Mekuria, G. T., Sedgley, M., and Collins, G. 2002. "Development of a Sequence-Tagged Site for the RAPD Marker Linked to Leaf Spot Resistance in Olive." *J. Amer. Soc. Hort. Sci.* 127 (4): 673-6.

[20] Monna, L., Miyao, A., Inoue, T., Fukuoka, S., Yamazaki, M., Zhong, H. S., Sasaki, T., and Minibe, Y. 1994. "Determination of RAPD Markers in Rice and Their Conversion into Sequence-Tagged Sites (STSs) and STS-Specific Primers." *DNA Res.* 1 (3): 139-48.

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Pig Breeds (GF24) Introducing to Central Vietnam and Reproductive Performance

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Abstract: The aim of this study was to characterize the reproductive performance of new pig breed introduced to Central Vietnam. The data were collected on farm by field studies with 30 individuals, during a period of one year to record and register productive and reproductive indicators. The GF24 female breeding pigs imported from US for Vietnam through Green feed Asia were intended for reproductive performance. Results obtained for first coming heat, first mating, first litter farrowing age, pregnancy time and dry sow were as follows: 166.2 ± 2.94 days, 200.44 ± 2.14 days, 133.79 ± 2.29 days, 317.75 ± 2.19 days, 117.31 ± 0.31 days and 8.27 ± 0.86 days, respectively. The average number of piglets born alive and stillbirths per litter were 11.58 ± 0.52 and 0.65 ± 0.27 respectively; both traits were affected by the parity of the sows. The individual weight at birth of piglets was 1.38 ± 0.12 kg and the number of weaned piglets was 9.58 ± 0.53. The feeding in the farms was characterized by using commercial feed (80%) and agricultural remainder (20%) as balanced feed. As a conclusion, this breed offers a potential progress in livestock for rural and industrial areas and is a contribution to the development of pig husbandry in Vietnam.

Key words: GF24 pig breed, reproductive performance, adaptation, Central Vietnam.

1. Introduction

Pig production systems, including backyard, free grazing, semi-intensive and intensive, are together developed in Vietnam, and the systems in the coming years will increase the number of pigs and improve productivity and meat quality by researching and putting the formula feed to new hybrid that suits the natural conditions of Vietnam. Rural and peri-urban pig farming is a form of production characterized by a low scale of activity, basically for subsistence [1]. New producers who are dedicated to this kind of industrial pig husbandry under family usually raise reproductive sows [2, 3]. The genetic quality will be improved much by exotic breeds as Pietrain, Duroc, Landrace and Yorkshire, combination breed as PIC and now inducing of GF24 for adaptability to a new environment to enable them to produce meat and reproductive performance with a minimum input. GF24 is a product of collaboration between two lines' harmonization of pig key PIC: 1050 line × 1040 line. The 1050 line has superior characteristics of fertility with about 28 weaned/sows/year, while 1040 line is superior in weight gain and meat quality. About physical appearance, pigs GF24 have average appearance, long blow, white body, back flat, erect, small head, short muzzle, strong legs, small bones and large thigh muscles grow vertically. GF24 is a new-generation line of pigs, which carries the increased appetite gene and should grow and develop very fast with low feed consumption, average gained of 1 kg of meat/1.1 kg bran. GF24 pigs carry the gene for increasing litter size and milk production, have a high resistant to diseases and also carry genes resistant to *E. coli*. Consistent with climate and farming conditions in Vietnam, backyard husbandry systems must be considered as a peculiar productive stratum within the context of national pig farming. They are unlikely to disappear and therefore cannot be ignored,

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and so they must be studied in greater depth to know better the levels of productivity and the limitations to achieving higher rates of production. In intensive pig farms, it is common practice to routinely evaluate the system in the pursuit of greater productivity. By the way, there is a lack of information concerning levels of productivity in region and country [4]. Yet, this type of pig husbandry has many owners. Performance in a pig enterprise should be guided by specific target levels, with the aim of obtaining higher profits through an adequate business control. When pig farmer of small farm scale want to have a wide vision of the farm and regional productivity, it is essential to apply methods to illustrate this through the productive indicator analysis [1, 5, 6]. To achieve this, the producer needs a record, containing all information necessary to realize such an evaluation. Householders of pig farms in small scale generally do not have records about quantification of animal production; moreover, they rarely have access to technical support to guide them in this task. The study aimed in investigation of a new pig breed to Central Vietnam and introduction of the breed to communities to increase reproductive performance and a higher income for small scale pig farms.

2. Materials and Methods

2.1 Animals and Feeding

The observations were made from January to July 2015, and the production data in a small industrial farm in Quang Binh, imported 30 gilts of GF24 pig breed from Green-Feed Company, origin from USA, were recorded and analyzed. The type of pig that prevailed in the study was the "exotic". The "improved" breeds in the farms were not included in the study. The 30 breeding stocks and gilts were allocated into five groups, each one with six gilts on eight months of age. Housing system is built in a closed model, microclimate in the barn is regulated by a system of indoor units installed in cages and fan assembly sequence at the end of the range cage to maintain proper temperatures in 22-26 °C, suitable wind speeds from 1.0-1.5 m/s. Booths of sows reviews rectangular cage floor paved with cement plates, the floor pens have drainage systems stool, urine, toilet stalls. This system of waste resulting from the barn to the final biogas system at the waste was taken to the reservoir.

Sows are allocated into five groups, each one with six gilts, and arranged for easy coordination week care and management. Nutrients and feeding regime composed by 80% of commercial compound feed and 20% of agricultural wastes: commercial feed, 15% and 16.5% CP, 3,100 and 3,200 kcal digestible energy (DE), 5.5% ash and 14% moisture, and agricultural wastes as sweet potato leaves, rice bran for GF07 and GF08, respectively, are used (Table 1).

2.2 Measurements and Methods

The following parameters were registered: number of piglets born alive, number of stillbirths, individual and litter weight at birth and at weaning, number of weaned piglets, pre-weaning mortality, weaning age, percentage of stillborn piglets, feed conversion, and age and weight at market. The methodology used to evaluate and characterize the "runt" pigs was described by Refs. [4, 6]. The next reproductive parameters were registered as following: interval from

Table 1 Dietary composition and feeding regime.

Diets	Moisture (%)	CP (%)	ME (kcal/kg)	CF (%)	Ca (%)	P (%)	Lysine (%)
GF 07	14.0	15.0	3,100	10.0	0.9-1.5	0.6-1.2	0.8
GF 08	14.0	16.5	3,200	6.0	0.9-1.5	0.6-1.2	0.9-1.5
Feeding regime	Gilt		Pregnancy		Suckling		Dry
	Restricted (GF07)		Restricted (GF08)		Restricted and *ad-libitum* (GF08)		Restricted (GF07)

CP: crude protein; ME, Metaboliable Energy; CF: crude fat; GF07: GreenFeed 07 line; GF08: GreenFeed 08 line.

weaning to first service, interval from weaning to effective service, lactation period, fertility percentage, abortion percentage, productive life span, productive cycle, and interval between farrowings.

The statistical analysis of the data consists of central trend measurements. An analysis of variance was performed to evaluate the effect of parity on the number of piglets born alive and dead. The model included parity and error with farms as replicates. For lactating sow, at the 23th day the weaning is conducted, on the 20th days the caloric needs half demand amount of 3 kg/sow, and on weaned day sows not eat, but are injected able vitamins A, D, E of 7 mL/sow. And sows were moved through distribution and the waiting for mating. Sows were checked and waited for insemination of sow and is conducted for coming litter. Each sow is mated two times and transferred to pregnancy crate and pens for care [1], coordinating complete to record by sow cards for herd management. After finishing coordinate, sows are kept quiet, restricted movement, kept in place coordination within 1-2 days, and then sorted sows are weekly distributed for ease of management and care. Data was processed on an Excel spreadsheet. Using some statistical parameters to characterize the degree of concentration as the mean, mean error and confidence level at $P < 0.05$.

3. Results

3.1 Reproductive Indicators of GF24 sows

The results from the reproductive criteria of sows are shown in Table 2. The variables of sows GF24 and assessing fertility indicated that first mating early and body weight with 133.75 ± 2.19 kg at age of 200.44 ± 2.14, reduced feed consumption for whole periods in each cycle, thereby improving economic efficiency for farmers or investors. Age of first estrus of GF24 is 166.20 days.

3.2 Estrus and Insemination

Age of first insemination of study subjects of GF24 is 200.44 days. It is lower than the set target of the GreenFeed Company is 209 days. This difference is due to the techniques applied in animal husbandry and ignores for first two estrus. The duration of standing estrus is often shorter in gilts compared to sows, but the effect of sow on estrus and ovulation timing is largely due to differences in weaning-to-estrus interval between parity groups. A sow's weaning-to-estrus interval is inversely related to the duration of estrus and onset of estrus-to-ovulation interval; sows who have short weaning-to-estrus intervals tend to have a long duration of estrus and a long onset of estrus-to-ovulation interval and vice versa.

Multiparous sows were expected to have a shorter duration of estrus and onset of estrus-to-ovulation interval. However, about the weaning-to-estrus interval, Koketsu et al. [7] reported a shorter duration of estrus cycle for litter 1 and 2, compared to litter 3 of sows (55 h vs. 62 h; $P < 0.001$). Table 2 also showed sows after weaning and litter one baby tended shorter duration of estrus, and had a shorter onset of estrus-to-ovulation interval than that of sows after litter four birth piglets (52.3 h vs. 54.6 h, and 38.7 h vs. 42.8 h; $P < 0.01$, respectively). Collectively, these data suggest that parity does have some affect on duration

Table 2 Reproductive indicators of GF24 sows (n = 30).

Variables	Mean	Standard error	Probability
First coming heat age (days)	166.20	2.94	0.060
First mating age (days)	200.44	2.14	0.043
First body weight (kg/head)	133.79	2.29	0.047
First litter age (days)	317.75	2.19	0.044
Pregnancy period (days)	117.31	0.51	0.006
Dry sow (days)	8.27	0.86	0.010

of estrus and onset of estrus-to-ovulation interval that is not related to weaning-to-estrus interval. Differences in weaning-to-estrus interval, duration of estrus and onset of estrus-to-ovulation intervals between primiparous and multifarious sows may be large enough to have important implications for the artificial insemination protocols of parity-specific herds. The volume of first insemination is also a factor affecting the productivity and quality of cubs. The study groups finding was 133.79 kg/head, while the GreenFeed Company's qualified set was 130-140kg/head. First calving age have a close relationship with the age of first sexual activity, age of first breeding and insemination success rate for the first time. The first calving age is determined by age of first insemination and the insemination at early age and high breeding success rate that increases duration of use sows, however time for first reproductive cycle longer (317.75 days). The successfully mated time of weaning is a indicator affecting calving interval, and reproductive performance of sows. The successful mating after weaning depends largely on the condition of stud and the body condition of sow before breeding , because too fat or too skinny will lead to slow up on pregnancy results. Time of successful insemination after weaning depends on care, sanitation and pens of sows, especially in a period of several days after birth, because it affects the recovery of the reproductive organs of sows. In addition, time of insemination after successful weaning depends on mating techniques to timely coordinate appropriate increase in pregnancy rates. To shorten the time of successful drying sows and decreasing the culling rate for sows in pig farms, farmers should have concentrated the time of feeding and care after prolonged weaning, and sows often get infections reproductive organs. Previous scientific results show that time after successful mating of sows weaning GF24 is 8.27 days.

3.3 Gestation and Farrowing

Gestation is characteristic for the species. But besides determined by genetic factors, pregnancy is also affected by care regime. Study of pregnancy period is 117.31 days. These results are suitable with gestation period of sow, as 110-118 days average [8, 9].

3.4 Reproductive Performance

To assess the fertility of sows GF24, keep track of details on the sow herself, while also monitor reproductive performance on the cubs of the study subjects. The research results are presented in Table 3. The average number of piglets born alive and stillbirths per litter were 11.58 and 0.65, respectively. Both traits were affected by the parity of the sows. The individual weight at birth was 1.38 kg, the number of weaned piglets 9.58, and age and weight at marketing were 210 days and up to 86 kg, respectively.

4. Discussion

The numbers of newborns, i.e., the total of all the offspring, include number of born alive, litter size and stillbirths. This criterion can assess whether the egg is fertilized, the technical level and stud foster care for

Table 3 Reproductive performance variables of GF24 sows (n = 30).

Variables	Mean	Standard errors	Probability
Average number of piglets born alive (number)	11.58	0.52	0.01
Average number of stillbirths (number)	0.65	0.27	0.01
Weight at birth average (kg/head)	1.38	0.12	0.00
Weight at weaning average (kg/head)	6.59	0.19	0.00
Average number of weaning piglets (number)	9.58	0.53	0.01
Lactation period (days)	23.27	0.36	0.01

pregnant sows. The number of piglets born more or less depends on whether the zygote is formed and capacity of feeding pregnant sows [1, 10]. The number of newborns/drive is the target of indirect selection to improve the number of piglets born alive per litter. In this study, the numbers of newborn are 11.58 piglets per litter. It is relatively low compared with the GreenFeed Company's standards given as 13 piglets. This difference may depend on technical conditions and ranches.

Number of piglets alive after 24 h is an indicator to assess the viability of the fetus, breeding capability of fetal pig and technical custody of pregnant sows of breeding farms. Research results in Table 3 shows the number of piglets alive after 24 h was 11.58 piglets per litter; it was lower than the company's standard of 12.45 piglets per litter, lower Landrace breed of 11.65 piglets per litter [11, 12] and higher than crossbred F1 (between Yorkshire and local Mong Cai breed) of 11.01 piglets per litter [5]. The number of breeding pigs selected to study was 10.24 piglets per sow, and the company's standard is 12 piglets per sow. Birth weight per litter related to the born alive and nutritional care regime affect the ability of piglet weight gain during the maternal period and post-weaning period.

Birth weight on target groups GF24 is 1.38 kg/piglet, and criteria of the company are 1.3-1.4 kg/head as average weaning weight to help assess the strength of the growth of the piglets in under mother period and the maternal ability of sows. Weaning weight depends on the uniformity of the piglets at birth, survival rate, uniformity at weaning, weaning weight, number of weaned piglets per litter and technical nursing care. Weaning weight reached of 6.59 kg/head, which is still lower than other exotic breeds in Vietnam [5, 6].

The number of weaned pigs in experiments on GF24 is 9.58 piglets/litter. Number of weaned pigs is as important indicators to assess present capabilities of the selected animal conservation. Piglets are sensitive to environmental impacts. To minimize the loss of piglets, his mother must have the appropriate technical measures, particularly preventive hygiene and stay warm for piglets. The mortality rate at birth is a lower than other breed. In addition, mortality and birth born type depend on specific regulations of each ranch. For this research, the specified volume of neonatal piglets alive after 24 h was selected from the householder's feedback of 0.9/piglet or more, not deformed piglets. Piglet mortality rate during follow-mother in this study was 0.65%. Percentage of piglets' death during follow-mother said the number of newborn piglets alive at weaning. This indicator significantly affects economic efficiency in livestock breeding sows through the weaning amount sold [1, 8, 10]. Percentage of piglets' death during follow-mother depends greatly on the care process of the selected piglets reared in suckling period. The piglets also depend on sows ability to care for new born piglets and the design sow crates for sow just enough to stand up and lie down so happens status sow piglets crushed the piglets to death [9, 11]. In addition, the male piglets at this stage must be castrated after 10 days of age, and in the following days given creeffeed for piglets to restrict pathogens [9, 13-15]. Adjust the appropriate temperature for the sow and piglets in the demand for higher heat for piglet sow a lot [16]. When the temperature drops very low, piglet diarrhea are prone to die, and the surviving piglets were treated, but also affected very long during the growth and development of pig later. These factors affect the rate of maternal death, time to weaning (days) affects approximately suckling interval and the suckling interval must impact on two factors that shorten the weaning date and time successfully inseminated after weaning. Shortening the number of weaning days contributes to reducing the gap between the two parities, thereby increasing the parity of the sow in a year while reducing the cost of breeding and increasing economic efficiency. Weaning time for this study of GF24 is 23.27 days.

5. Conclusions

There were obvious adapted abilities of the new GF24 pig breed introduced to Central Vietnam, and reproductive performance in the local feeding combination with commercial feed under husbandry conditions in the areas were higher than native and crossbreds.

Reproductive aspects were adapted in small scale pig farms in Central Vietnam under condition of semi-intensive pig production systems. On the positive side, the use of recycled wastes is an important contribution to sustainable resource utilization, as well as being a low cost system for the producer and a higher benefit.

References

[1] Duyet, H. N., Nguyen, Q. L., Cargill, C., and Nguyen, N. P. 2010. "Effect of Using Creep Boxes for Piglets on the Reproductive Performance of Mong Cai Sows Kept in Smallholder Farms in Thuy Phuong Commune, Thua Thien Hue Province." In *Proceedings of the 14th Animal Science Congress of the Asian-Australasian Association of Animal Production Societies (AAAP),* 535.

[2] Eisen, J. J., Kanis, E., and Kemp, B. 2000. "Sow Factors Affecting Voluntary Feed Intake during Lactation." *Livest. Prod. Sci.* 64 (2-3): 147-65.

[3] Elbers, A. R., Vernooy, J. C., Van Den Broek, J., and Verheijden, J. H. 1996. "Risk of Recurrence of Repeat Breeding in Sows with a Repeat Breeding in the First Parity." *J. Anim. Sci.* 74 (10): 2327-30.

[4] Vesseur, P. C., Kemp, B., and Den Hartog, L. A. 1994. "Factors Affecting the Weaning-to-Estrus Interval in the Sow." *J. Anim. Physiol. and Anim. Nutr.* 72: 225-33.

[5] Linh, Q. N., Tu, L. C., and Koops, W. J. 1999. "The Use of Pig Large White, Mong Cai and Their Crossbreeds in Different Farming Systems in Central Vietnam." M.Sc. thesis, Wageningen Agricultural University, The Netherlands.

[6] Linh, Q. N., Hoang, N. D., Nguyen, N. P., Cargill, C., and Trott, D. 2010. "Influence of Different Breeds, Feeding and Housing Systems on Sow Reproductive Performance in Central Vietnam." In *Proceedings of the 13th Animal Science Congress of the Asian-Australasian Association of Animal Production Societies (AAAP),* 212.

[7] Koketsu, Y. 1999. "Assessment of Sows Mating Efficacy during the Low Productive Period after Early Weaning: A Field Study." *Therio.* 51 (8): 1525-32.

[8] Jindal, R., Cosgrove, J., Aherne, F. X., and Foxcroft, G. R. 1996. "Effect of Nutrition on Embryonal Mortality in Gilts: Association with Progesterone." *J. Anim. Sci.* 74 (3): 620-4.

[9] Pope, W. F. 1994. "Embryonic Mortality in Swine." In *Embryonic Mortality in Domestic Species,* edited by Zavy, M. T., and Geisert, R. D. Boca Raton, FL: CRC Press, 53-77.

[10] Tsuma, V. T., Einarsson, S., Madej, A., Kindahl, H., and Lundeheim, N. 1996. "Effect of Food Deprivation during Early Pregnancy on Endocrine Changes in Primiparous Sows." *Anim. Reprod. Sci.* 41 (3-4): 267-78.

[11] Jindal, R., Cosgrove, J. R., and Foxcroft, G. R. 1997. "Progesterone Mediates Nutritionally Induced Effects on Embryonic Survival in Gilts." *J. Anim. Sci.* 75 (4): 1063-70.

[12] Kirkwood, R. N., Baidoo, S. K., and Aherne, F. X. 1990. "The Influence of Feeding Level during Lactation and Gestation on the Endocrine Status and Reproductive Performance of Second Parity Sows." *Can. J. Anim. Sci.* 70: 1119-26.

[13] Le Cozler, Y., Dagorn, J., Dourmad, J. Y., Johansen, S., and Aumaître, A. 1997. "Effect of Weaning-to-Conception Interval and Lactation Length on Subsequent Litter Size in Sows." *Livest. Prod. Sci.* 51: 1-11.

[14] Tantasuparuk, W., Lundeheim, N., Dalin, A. M., Kunavongkrit, A., and Einarsson, S. 2000. "Reproductive Performance of Purebred Landrace and Yorkshire Sows in Thailand with Special Reference to Seasonal Influence and Parity Number." *Therio.* 54 (3): 481-96.

[15] Tantasuparuk, W., Dalin, A. M., Lundeheim, N., Kunavongkrit, A., and Einarsson, S. 2001. "Body Weight Loss during Lactation and Its Influence on Weaning-to-Service Interval and Ovulation Rate in Landrace and Yorkshire Sows in the Tropical Environment of Thailand." *Anim. Reprod. Sci.* 65 (3-4): 273-81.

[16] Koketsu, Y., and Dial, G. D. 1998. "Interactions between the Associations of Parity, Lactation Length and Weaning-to-Conception Interval with Subsequent Litter Size in Swine Herds Using Early Weaning." *Prev. Vet. Med.* 37: 113-20.

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Determination of Ileal Amino Acid Digestibilities of Some By-Products for Chickens

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Abstract: The aim of this study was to determine the standardized ileal digestibility of amino acids in six by-product ingredients for broiler, i.e., copra meal, rapeseed meal, feather meal, hydrolyzed poultry meal, meat and bone meal, and shrimp head meal. Luong Phuong chickens at 35 days of age were used in the trials. Dietary protein in all assay diets was supplied solely by the test ingredient. In the case of protein-rich ingredients, dextrose was added to assay diets to obtain 20% of crude protein. Standardized ileal amino acid digestibilities were calculated by correcting the apparent ileal digestibility coefficients by basal endogenous amino acid losses. Results of study showed that the variation in ileal digestibility coefficients of amino acids was low in rapeseed meal and high in copra meal. Among animal protein meals, the lowest variation of digestibility among amino acids was observed in shrimp head meal. The high standardized ileal digestibilities of Arg, His, Leu + Ile, Thr, Trp, Val, and Phe were observed in hydrolyzed poultry meal and shrimp head meal. Meanwhile, the standardized ileal digestibility values of Lys, Thr, and Trp in feather meal were very low. Meat and bone meal and feather meal was the two least digestible amino acid ingredients.

Key words: Amino acid, by-product, chicken, standardized ileal digestibility.

1. Introduction

It is recognized that not all the nutrients in feed ingredients are available for production purposes, and a portion of nutrients is excreted undigested or not utilized [1]. Therefore, maximizing the efficiency of nutrient utilization, especially protein and amino acid, is very important. Knowledge of amino acid digestibility coefficients in feed ingredients and the requirement of digestible amino acids for a defined production target enables the formulation of diets more close to chicken's requirements [2]. Formulating diets based on digestible amino acids allows increasing the diversity and inclusion levels of non-traditional ingredients, despite the fact that they may contain less than optimal natural amino acid profiles and are poorly digested [1]. Such formulations have significant role in developing countries, where highly digestible conventional ingredients are not available [1]. Many study results indicated the beneficial effects of using ileal digestible amino acids in broiler diet formulations to increase the inclusion levels of poorly digestible ingredients, such as cotton meal, canola meal, meat and bone meal [3-7]. Furthermore, diet formulations based on digestible amino acid improve the precision of formulation, offer economic benefits, ensure more predictable bird performance and reduce nitrogen output from poultry operations [1, 2, 8].

However, a question often posed by commercial nutritionists is which digestible amino acid system is the most appropriate for use in poultry diet formulation [9]. Apparent digestibility measures the digestibility of amino acids of both dietary and endogenous origins [10]. While, standardized digestibility includes a correction for endogenous amino acid secretions [11]. The choice of the

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appropriate system of digestible amino acids may depend on the diet formulation method [9]. If diets are being formulated to least-cost using linear programming, then apparent ileal digestibility values are the most appropriate, as they take into account the endogenous cost of digestion. On the other hand, in case of formulating diets with computer simulation models, then standardized digestibility values will be relevant if the model corrects for the endogenous cost of digestion [9]. Notwithstanding, it should be appreciated that both digestible amino systems are better than the total amino acid system, and all systems have specific applications and shortcomings [9]. This study was carried out with the purpose of determining standardized ileal digestibility of amino acids in some by-product ingredients (copra meal, rapeseed meal, feather meal, hydrolyzed poultry meal, meat and bone meal, and shrimp head meal) for broiler.

2. Materials and Methods

2.1 Animals and Diets

The study was performed with Luong Phuong chickens at the Poultry Research Room and Central Lab, Faculty of Animal Husbandry and Veterinary Medicine, Hue University of Agriculture and Forestry from October 2013 to January 2014. A completely randomized experimental design in a single factor experiment was applied. A total of 180 35-day-old Luong Phuong chickens (completely matured digestive system) with uniform body weight 515 g/chick were assigned to six treatments for evaluation of apparent ileal amino acid digestibility in six test ingredients - copra meal, rapeseed meal, feather meal, hydrolyzed poultry meal, meat and bone meal, and shrimp head meal. Each treatment was replicated five times with 30 chicks per treatment. Every two chicks (one male and one female) were housed in a cage.

Dietary protein in all assay diets was supplied solely by the test ingredient [12, 13]. In the case of protein-rich ingredients, dextrose was added to assay diets to obtain 20% of crude protein [12]. Paper powder (3.0%) was added as a source of fiber in diets containing animal protein meals [12]. Celite (Celite® 545RVS, Nacalai Tesque, Japan) was added at 1.5% to all diets as a source of acid insoluble ash (AIA), which was used as an indigestible marker in the calculation of digestibility coefficients [14]. The nutritional value of test ingredients and the composition of experimental diets are presented in Tables 1 and 2.

2.2 Experimental Procedure

The experiment was implemented for seven days following the method of Bryden and Li [12]. Diets were provided *ad libitum* and water was available at all times [12]. At the 8th day of experiment, all chickens were euthanized. Ileal digesta was collected as described by Bryden and Li [12]. The ileum was defined as the portion of the small intestine extending from Meckel diverticulum to a point of 4 cm proximal to the ileo-caecal junction. The contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. Ileal digesta of six chickens within a replication were pooled and frozen at -20 °C immediately after collection.

2.3. Chemical Analysis and Calculations

Digesta samples were dried at 60 °C in forced-ventilation oven for 8 h. Feed and dried digesta samples were ground to pass through a 0.5 mm sieve and stored in airtight container at 4 °C for chemical analyses. All proximate composition of samples was analyzed following AOAC procedures [15] at Central Lab, Faculty of Animal Husbandry and Veterinary Medicine, Hue College of Agriculture and Forestry, Hue University. Dry matter (DM) content of samples was determined by oven drying at 130 °C for 3 h as AOAC official method 930.15 [15]. Nitrogen (N) content was determined by using Kjeltec 8200 system (Foss, Sweden) following AOAC official method

Table 1 Nutritive value of test ingredients (as fed basis).

Nutritive		Copra meal	Rapeseed meal	Feather meal	Hydrolyzed poultry meal	Meat and bone meal	Shrimp head meal
Total amino acid (%)	Arg	2.27	2.07	5.15	4.21	3.39	2.43
	His	0.34	1.06	0.75	1.14	0.89	5.88
	Ile + Leu	1.92	3.48	10.01	6.73	4.53	4.41
	Lys	0.50	1.97	2.02	0.58	2.31	1.88
	Met	0.30	0.65	0.49	1.18	0.68	0.53
	Phe	0.80	1.77	3.63	2.52	1.55	1.71
	Thr	0.61	1.44	3.50	2.46	1.57	1.26
	Trp	0.16	0.41	0.58	0.41	0.31	0.20
	Val	1.09	1.65	5.34	2.98	2.30	1.91
	Ala	0.73	1.56	3.85	4.00	3.03	2.00
	Asp	1.49	2.39	4.57	5.79	3.39	2.45
	Cys	0.20	0.81	4.09	0.58	0.48	1.63
	Glu	3.56	6.12	8.20	6.11	6.27	4.27
	Gly	0.78	1.83	7.08	5.62	6.83	1.66
	Pro	0.60	2.05	9.51	4.14	4.19	1.10
	Ser	0.80	1.37	7.94	3.40	1.57	0.90
	Tyr	0.56	0.94	1.96	1.83	1.05	1.36
Proximate composition (%)	DM	93.16	88.34	94.15	90.93	96.00	87.67
	CP	19.59	36.08	85.74	67.41	51.47	46.18
	EE	13.70	2.51	4.48	13.56	2.33	8.25
	CF	13.25	12.09	0.28	0.45	2.33	10.12
	Ash	7.02	6.97	3.64	6.88	3.91	14.56

Table 2 Composition and nutrient content of experimental diets.

Ingredient	Ratio (%)					
Copra meal	94.00	-	-	-	-	-
Rapeseed meal	-	55.50	-	-	-	-
Feather meal	-	-	23.30	-	-	-
Hydrolyzed poultry meal	-	-	-	30.00	-	-
Meat and bone meal	-	-	-	-	38.86	-
Shrimp head meal	-	-	-	-	-	45.53
Dextrose	-	38.05	65.55	58.85	49.99	43.32
Soybean oil	2.00	2.00	6.00	6.00	6.00	6.00
Paper powder	-	-	3.00	3.00	3.00	3.00
Vitamin premix*	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix**	0.25	0.25	0.25	0.25	0.25	0.25
$CaCO_3$	1.45	-	-	-	-	-
DCP	0.40	2.30	-	-	-	-
NaCl	0.20	0.20	0.20	0.20	0.20	0.20
Celite	1.50	1.50	1.50	1.50	1.50	1.50
Total	100	100	100	100	100	100
Nutrient content, as-fed basis						
Protein (%)	18.41	20.02	19.98	20.22	20.00	21.02
Metabolizable energy (kcal/kg)	2,223	995	1,127	1,383	1,135	1,259

* Bio-pharmachemie (Bio-ADE + B-complex premix), 1 kg contains: vitamin A, 3,100,000 UI; vitamin D3, 1,100,000 UI; vitamin E, 300 UI; vitamin B1, 320 mg; vitamin B2, 140 mg; niacinamide, 1,000 mg; vitamin B6, 600 mg; vitamin B12, 1,200 μg; vitamin C, 1,000 mg; acid folic,130 mg.

** Bio-pharmachemie (Bio-chicken minerals), 1 kg contains: Mn, 10,800 mg, Fe, 2,160 mg; Zn, 7,200 mg; Cu, 1,260 mg; iodine, 144 mg; Co, 21.6 mg; Se, 14.4 mg; acid folic, 40 mg; biotin, 4,800 μg; choline chloride, 20,000 mg.

984.13 [15]. Crude protein contents of the samples were calculated as N × 6.25. Ether extract, crude fiber and total ash contents of the samples were determined following AOAC official methods 920.39, 978.10, and 942.05 [15], respectively. Amino acids in digesta and feed were analyzed using LC/MS/MS system with EZ:faast™ amino acids analysis kit (Phenomenex) at Hai Dang Chromatography Scientific Services Joint Stock Company (Ho Chi Minh city, Vietnam). AIA content was assayed following the method of Vogtmann et al. [16].

Standardized ileal amino acid digestibilities were calculated by correcting the apparent ileal digestibility coefficients by basal endogenous amino acid losses [17], as shown in Eq. (1) [14]:

SID (%) = AID (%) + [basal endogenous amino acid losses (g/kg DM intake)/amino acid content of the raw material (g/kg DM) × 100] (1)

where, AID (%): apparent ileal digestibility coefficient; SID (%): standardized ileal digestibility coefficient; DM: dry matter.

In the authors' previous study, basal endogenous amino acid losses had been determined based on the concentration of amino acids in ileal digesta collected from chickens fed protein free diet, AIA contents in diet and the ileal digesta [17].

2.4 Data analysis

The mean value was calculated from five replicate values. The data were analyzed using Microsoft excel 2007.

3. Results and Discussion

The crude protein content and amino acid composition of test ingredients (Table 1) are within the ranges reported in Ref. [18]. The apparent and standardized ileal digestibilities of amino acids of six test ingredients are presented in Tables 3 and 4. The apparent ileal digetibilities of protein or amino acids were lower than the standardized ileal digetibilities. The variations between apparent and standardized ileal digetibilitie values were low in protein-rich ingredients, and vice versa.

There are no remarkable differences in the ileal digestibility coefficients of amino acids of rapeseed meal. This finding was similar to the previous study conducted by Ravindran et al. [13]. Regarding to copra meal, high variation of the standardized ileal

Table 3 Apparent ileal amino acid digestibility of test ingredients.

Amino acid	Apparent ileal digestibility (%)					
	Copra meal	Rapeseed meal	Feather meal	Hydrolyzed poultry meal	Meat and bone meal	Shrimp head meal
Arg	83.54	82.12	66.23	86.45	72.65	80.14
His	66.92	79.72	53.69	79.36	68.75	75.76
Ileu + Leu	68.00	73.79	68.44	79.89	72.14	77.90
Lys	63.44	71.4	51.55	79.09	64.21	74.01
Met	70.97	72.88	54.69	74.19	67.79	78.19
Phe	67.58	73.52	59.17	79.14	64.65	75.85
Thr	61.09	65.53	50.82	79.49	60.81	73.87
Trp	69.23	75.19	46.43	76.01	53.67	74.61
Val	73.31	71.45	64.33	75.66	68.88	76.99
Ala	66.62	73.85	75.96	73.91	68.99	71.99
Asp	59.33	70.08	66.70	67.74	62.89	77.26
Cys	58.85	72.94	45.30	67.88	48.02	75.85
Glu	62.94	82.47	65.77	76.23	72.68	76.48
Gly	60.87	71.2	79.26	73.07	70.80	72.87
Pro	55.84	71.01	71.04	80.74	72.88	72.93
Ser	55.04	69.29	74.77	69.08	64.94	72.15
Tyr	66.27	73.55	57.44	72.64	69.82	74.23

Table 4 Standardized ileal amino acid digestibility of test ingredients.

Amino acid	Standardized ileal digestibility (%)					
	Copra meal	Rapeseed meal	Feather meal	Hydrolyzed poultry meal	Meat and bone meal	Shrimp head meal
Arg	84.80	84.33	68.49	88.52	74.75	82.43
His	71.36	82.00	61.90	83.39	72.99	76.27
Ileu + Leu	71.70	77.06	71.32	83.11	76.04	81.04
Lys	67.49	73.04	55.63	82.84	66.39	76.11
Met	74.58	75.53	63.51	76.97	71.73	82.15
Phe	71.18	76.13	62.40	82.63	69.27	79.14
Thr	69.38	71.18	56.73	85.81	68.84	81.72
Trp	75.74	79.23	53.61	83.72	61.95	84.43
Val	77.23	75.59	67.59	80.03	73.50	81.34
Ala	70.77	76.96	79.16	76.22	71.48	74.92
Asp	63.00	73.76	71.58	70.63	66.92	81.62
Cys	70.42	77.44	47.57	79.93	59.91	78.58
Glu	64.99	84.39	69.40	79.88	75.58	79.82
Gly	67.05	75.44	82.04	75.69	72.56	78.56
Pro	62.36	74.09	72.72	84.59	75.22	79.92
Ser	60.37	74.32	76.97	72.93	71.71	81.45
Tyr	71.43	78.44	64.38	77.43	76.64	78.33

amino acid digestibilities was observed, ranging from 60.37%-84.80%. The difference in quality of rapeseed meal and copra meal may be explained due to the difference in concentrations of limiting amino acids and bulking properties.

The variation of digestibility between amino acids in shrimp head meal was lower than that in other animal protein ingredients. Standardized ileal digestibility of amino acids ranged from 74.92% to 84.43% in shrimp head meal. Meanwhile, the standardized ileal digestibility values of amino acid in feather meal, meat and bone meal ranged from 47.57% to 82.04% and from 59.91% to 76.64%, respectively. Among the essential amino acids, the standardized ileal digestibility values of Lys, Thr, and Trp in feather meal were very low. On the other hand, the standardized ileal digestibilities of Arg, His, Leu + Ile, Thr, Trp, Val, and Phe were very high in hydrolyzed poultry meal and shrimp head meal (Table 4). The low standardized ileal digestibility of amino acids was observed in feather meal, meat and bone meal. The variation in quality of meat and bone meal is likely to be caused by the correlation variability between muscle protein and collagen content in raw materials, or by processing conditions of the meals [7, 19, 20]. In poor-quality meat and bone meal, 50%-65% of total protein may be collagen [1]. Collagen is the major protein in bone, connective tissue, cartilage and tendon. Eastoe and Long [21] found that collagen is severely deficient in most indispensable amino acids and poorly digested because of the low level of collagenase in digestive tract.

4. Conclusions

The variation in ileal digestibility coefficients of amino acids was low in rapeseed meal and high in copra meal. Among animal protein meals, the lowest variation of digestibility between amino acids in shrimp head meal was observed. The high standardized ileal digestibilities of Arg, His, Leu + Ile, Thr, Trp, Val, and Phe was observed in hydrolyzed poultry meal and shrimp head meal. Meanwhile, the standardized ileal digestibility values of Lys, Thr, and Trp in feather meal were very low. Meat and bone meal and feather meal was the two least digestible amino acid ingredients.

References

[1] Ravindran, V. 2011. *Poultry Feed Availability and Nutrition in Developing Countries—Advances in Poultry Nutrition.* Rome, Italy: FAO.

[2] Hoehler, D., Lemme, A., Ravindran, V., Bryden, W. L., and Rostagno, H. S. 2006. "Feed Formulation in Broiler Chickens Based on Standardized Ileal Amino Acid Digestibility." In *Proceedings of VIII International Symposium on Aquatic Nutrition*.

[3] Fernandez, S. R., Zhang, Y., and Parsons, C. M. 1995. "Dietary Formulation with Cottonseed Meal on a Total Versus a Digestible Amino Acid Basis." *Poultry Science* 74 (7): 1168-79.

[4] Ravindran, V., and Bryden, W. L. 1999. "Evaluation of Meat and Bone Meal in Broiler Starter Diets Formulated on the Basis of Total or Digestible Amino Acids." *Pro. Aust. Poult. Sci. Sym.* 11: 169.

[5] Ravindran, V., and Bryden, W. L. 1999. "Evaluation of Broiler Diets Containing Graded Levels of Cottonseed Meal and Formulated on the Basis of Total or Digestible Amino Acids." *Pro. Aust. Poult. Sci. Sym.* 11: 168.

[6] Ravindran, V., Hew, L. I., and Bryden, W. L. 1998. "Broiler Feed Formulations with Canola Meal Based on Total or Digestible Amino Acids." *Pro. Aust. Poult. Sci. Sym.* 10: 209.

[7] Wang, X., and Parsons, C. M. 1998. "Effect of Raw Material Source, Processing System and Processing Temperatures on Amino Acid Digestibility of Meat and Bone Meals." *Poultry Science* 77: 834-41.

[8] Rostagno, H. S., Pupa, J. M. R., and Pac, M. 1995. "Diet Formulation for Broiler Based on Total Versus Digestible Amino Acid." *The Journal of Applied Poultry Research* 4 (3): 293-9.

[9] Bryden, W. L., and Li, X. 2010. "Amino Acid Digestibility and Poultry Feed Formulation: Expression, Limitations and Application." *Revista Brasileira de Zootecnia* 39: 279S-87S.

[10] Ravindran, V., and Bryden, W. L. 1999. "Amino Acid Availability in Poultry—*In Vitro* and *in Vivo* Measurements." *Australian Journal of Agricultural Research* 50 (5): 889-908.

[11] AFZ, Eurolysine Ajinomoto, Nutrition Aventis Animal, INRA and ITCF. 2000. "AmiPig—Standardised Ileal Digestibility of Amino Acids in Feedstuffs for Pigs." Accessed November, 2015. http://www.feedbase.com/amipig.php?Lang=E.

[12] Bryden, W. L., and Li, X. 2004. *Utilisation of Digestible Amino Acids by Broilers.* Australian: Rural Industries Research and Development Corporation (RIRDC).

[13] Ravindran, V., Hew, L. I., Ravindran, G., and Bryden, W. L. 2005. "Apparent Ileal Digestibility of Amino Acids in Feed Ingredients for Broiler Chickens." *Animal Science* 81 (1): 85-97.

[14] Garcia, A. R., Batal, A. B., and Dale, N. M. 2006. "A Comparison of Methods to Determine Amino Acid Digestibility of Feed Ingredients for Chickens." *Poultry Science* 86 (1): 94-101.

[15] Association of Official Analytical Chemists (AOAC). 1990. *Official Methods of Analysis,* 15 ed.. Arlington,Virginia, USA: AOAC.

[16] Vogtmann, H., Pfirter, H. P., and Prabucki, A. L. 1975. "A New Method of Determining Metabolisability of Energy and Digestibility of Fatty Acids in Broiler Diets." *British Poultry Science* 16 (5): 531-4.

[17] Thong, H. T, Chau, H. L. Q, An, N. X., and Cuong, V. C. 2014. "Determination of Basal Endogenous Protein and Amino Acids in Luong Phuong Chickens." *Journal of Animal Science and Technology* 12: 14-9.

[18] NRC. 2012. *Nutrition Requirements of Swine.* Washington, DC: National Academy Press.

[19] Aikison, J., and Capenter, K. J. 1970. "Nutritive Value of Meat Meals II: Influence of Raw Materials and Processing on Meat Meal Quality." *Journal of the Science of Food and Agriculture* 21 (7): 366-72.

[20] Skurray, G. R., and Herbert, L. S. 1974. "Batch Dry Rendering: Influence of Raw Materials and Processing Conditions." *Journal of the Science of Food and Agriculture* 25 (9): 1071-9.

[21] Eastoe, J. E., and Long, J. E. 1960. "The Amino Acid Composition of Processed Bones and Meat Meal." *Journal of the Science of Food and Agriculture* 11 (2): 87-92.

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Preliminary Studies on the Toxic Effects of Degradation Products of Oxytetracycline and Chlortetracycline on Rats

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Abstract: The paper aimed to study the toxic effects of the degradation products of oxytetracycline and chlortetracycline, such as, α-apo-oxytetracycline (α-apo-OTC), β-apo-oxytetracycline (β-apo-OTC), anhydro-chlortetracycline (ACTC) and 4epi-anhydro-chlortetracycline (EACTC) on rats. Male rats received oral doses of 10 mg/kg body weight/day of either α-apo-OTC, β-apo-OTC, ACTC or EACTC for 90 d. At the end of 90 d treatment, the body/organ weight, white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT) count, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were measured. The results revealed that the rats treated with α-apo-OTC or ACTC for 90 d did not affect body, organ weights or certain blood- and serum-factors, however, those treated with β-apo-OTC or EACTC exhibited significant decreases in body weights, RBC counts and HGB concentrations, as well as significant increases in BUN concentrations, SGOT and SGPT activities. The results of this study suggest that the toxic effects of β-apo-OTC or EACTC treatment could damage liver and kidney tissues of rats, as well as lead to the degeneration and necrosis in the hepatocytes.

Key words: Antibiotic, oxytetracycline and chlortetracycline degradation products, toxic effects, rats.

1. Introduction

Oxytetracycline and chlortetracycline are broad-spectrum antibiotics and are members of the tetracycline family. Their therapeutic use has been of particular interest for food producing animals because of their broad-spectrum activity and low cost for preventing and controlling diseases, and also used as feed additives for the promotion of weight gain. However, their over and improper use can result in the presence of their residues in edible tissues of animals, which can be toxic and dangerous for human health and potentially cause allergic reactions. Liver plays a central role in detoxification and excretion of many endogenous and exogenous compounds. Hence, any injury to it or impairment of its function has grave implication for the health of the affected person. The mechanisms involved in liver injury are complex and interactive, and can be artificially separated as chemical and immune injuries. The biochemical mechanisms concern various chemicals that are detoxified in the liver via cytochrome P-450 and conjugation. Toxic metabolites may alter plasma membrane, mitochondria, intracellular ion homeostasis, or degratative enzyme activity. Immune mechanisms involve cell cooperation, and are mediated by cytokines, nitric oxide and complement. Pathologic apoptosis is potentially an important mechanism of acute liver injury (hypoxia/reoxygenation, liver congestion, acetaminophen poisoning, posttransplant acute liver rejection, severe sepsis, viral hepatitis and alcoholic liver disease) [1]. In an earlier report, the administration of therapeutic doses of

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tetracycline to male rats induced oxidative stress by decreasing the antioxidant system, increased lipid peroxidation and concomitantly impaired testicular function, spermatogenesis and epididymal sperm physiology and morphology [2]. In another report, tetracycline caused hyperglycemia and injury to the pancreas, and caused significant oxidative stress to liver and pancreas by reducing the levels of antioxidant enzymes and glutathione (GSH) and elevating the levels of lipid peroxide formed. In addition, this drug induced an hepatotoxicity caused by fatty infiltration of the liver and damage to liver parenchymal cells [3]. Usually, meat is cooked before consumption, and therefore oxytetracycline, chlortetracycline as well as any degradation products formed could be subjected to consumption, which may be a threat to human health. Tetracycline degradation products have been found in foods after treatment with different thermal processing conditions [4-6]. Degraded tetracycline preparations have been associated with Fanconi-type syndrome—a reversible renal dysfunction [7]. Benitz and Diermeier [8] reported that anhydro-4-epi-tetracycline was the only degradation product which caused abnormal urinary functions similar to the Fanconi-type syndrome. Severe nephrotic changes were found in the kidneys of rats after being given very large oral doses (1,000 mg/kg/d), and in dogs after intravenous doses of 10 mg/kg and 20 mg/kg on successive days. So far, few reports are available for toxicity of degradation products of tetracycline, chlortetracycline and oxytetracycline on sludge and soil bacteria [9], as well as phytoplanktons [10], still no reports are available in rats. Therefore, the objective of this study was to determine the toxicity of oxytetracycline and chlortetracycline degradation products when they were orally administered to rats.

2. Materials and Methods

2.1 Chemicals

The anhydro-chlortetracycline (ACTC), 4-epianhydro-chlortetracycline (EACTC), α-apo-oxytetracycline (α-apo-OTC) and β-apo-oxytetracycline (β-apo-OTC) were purchased from Acros Organics (Geel, Belgium).

2.2 Animals

A total of 50 healthy rats (4-week-old male Sprague-Dawley) were individually housed in stainless steel cages lined with wood shavings and fitted with wire mesh tops. The rats were acclimated to ambient temperature (23 ± 2 °C), humidity and natural light/dark cycle. All rats were fed a standard rat chow, had free access to drinking water and were kept in the facilities for at least one week prior to use. After confirming their normal health status at the end of the acclimation period, 50 rats were randomly allocated to five groups, each consisting of 10 rats. Then they were then given the control or experimental diets for 90 d. The rats in group I served as control. The rats in groups II, III, IV and V received orally and daily 10 mg/kg body weight of α-apo-OTC, β-apo-OTC, ACTC or EACTC, respectively.

2.3 Clinical Observations

The behavior and appearance of all rats, including coat condition, skin, eyes and excretions, were monitored every 2 d throughout the study. Body, organ weights and macroscopic observation of liver were conducted on all surviving rats on the 90th days (the end of the trial).

2.4 Biochemical Analysis

Hematology and serum chemistry were conducted on all surviving rats on the 90th days (the end of the trial). Rats were fasted for 12 h before collecting blood samples. Whole blood from the inner canthus was collected with and without anticoagulant, and analyzed for white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count (PLT), urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT) and

serum glutamic pyruvic transaminase (SGPT).

2.5 Statistical Analysis

The data was present as mean value and standard deviation (SD). A one-way analysis of variance (ANOVA) was applied to evaluate the homogeneity variance of all of groups. ANOVA was used to compare the experimental groups. The response variable values of the treatment groups were compared to the control group using *t*-test. Differences between values were considered statistically significant at a $P < 0.05$. All analysis were carried out using the SPSS statistics software version 20.

3. Results and Discussion

3.1 Body and Organ Weights

The body weights, organ weights and their percentages are presented in Table 1. No significant differences in body weight were observed between control rats and treated rats with α-apo-OTC or ACTC. In contrast, the body weights of those rats treated with β-apo-OTC or EACTC showed significant changes compared with rats in the control group ($P < 0.05$). The groups treated with β-apo-OTC or EACTC showed significant increase in both absolute and relative weights of the liver and kidney as compared with the control group, whereas, no significant changes were observed in the weights of liver and kidney in the group treated with α-apo-OTC or ACTC, as compared with control group (Table 1).

Absolute heart weight was significantly decreased, whereas, the significantly increased changes were observed in the relative weight of lung in the group treated with β-apo-OTC, as compared with control group. Absolute and relative heart/testis weights in the groups treated with α-apo-OTC or ACTC were slightly decreased, but no significant difference was detected compared with the control group.

3.2 Hematology

Hematological analysis from the groups treated with β-apo-OTC or EACTC were significantly different from those obtained from the control group (Table 2). However, no differences were observed between rats in the control group and those in the groups treated with α-apo-OTC or ACTC. In the groups treated with β-apo-OTC or EACTC, there was significant increased ($P < 0.001$) in both WBC and PLT, as compared with the control animals. Although the WBC and PLT of rats in the groups treated with α-apo-OTC or ACTC were also slightly increased, no significant differences were detected compared with controls. RCB, HGB and HCT in the groups treated with β-apo-OTC, EACTC were significantly decreased

Table 1 Body weight, organs weight and percentage of organs weight per body weight in male rats treated with antibiotics as indicated in mean ± SD (n = 10).

Parameter	Control	α-apo-OTC	β-apo-OTC	ACTC	EACTC
Body weight (g)	359.02 ± 18.98	354.33 ± 20.26	341.57 ± 14.34*	348.92 ± 21.27	338.97 ± 20.36*
Liver weight (g)	13.43 ± 1.59	14.16 ± 1.84	14.97 ± 1.09*	14.34 ± 1.06	15.84 ± 2.23*
Liver weight (%)	3.76 ± 0.58	4.02 ± 0.61	4.39 ± 0.39*	4.12 ± 0.39	4.71 ± 0.82**
Kidney weight (g)	1.86 ± 0.14	1.93 ± 0.15	2.08 ± 0.15**	2.00 ± 0.17	2.13 ± 0.11***
Kidney weight (%)	0.52 ± 0.03	0.55 ± 0.06	0.61 ± 0.05***	0.57 ± 0.04	0.63 ± 0.04***
Lungs weight (g)	1.32 ± 0.12	1.35 ± 0.07	1.34 ± 0.10	1.39 ± 0.09	1.36 ± 0.12
Lungs weight (%)	0.37 ± 0.04	0.38 ± 0.04	0.39 ± 0.03*	0.40 ± 0.04	0.40 ± 0.04
Heart weight (g)	0.96 ± 0.08	0.92 ± 0.11	0.88 ± 0.09*	0.88 ± 0.10	0.87 ± 0.12
Heart weight (%)	0.27 ± 0.03	0.26 ± 0.03	0.26 ± 0.03	0.25 ± 0.03	0.26 ± 0.03
Testis weight (g)	2.32 ± 0.18	2.29 ± 0.15	2.16 ± 0.18	2.21 ± 0.18	2.11 ± 0.18*
Testis weight (%)	0.65 ± 0.06	0.65 ± 0.05	0.63 ± 0.04	0.64 ± 0.06	0.62 ± 0.08

Rats were treated orally and daily for 90 d by 10 mg/kg body weight of α-apo-OTC, β-apo-OTC, ACTC or EACTC.
*, ** and *** mean significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively, when compared with the control group.

Table 2 Hematological analyses of male rats after 90 d feeding (mean ± SD) (n = 10).

Parameter	Control	α-apo-OTC	β-apo-OTC	ACTC	EACTC
WBC ($\times 10^9$/L)	5.08 ± 0.29	5.50 ± 0.74	6.46 ± 0.67***	5.21 ± 0.60	6.71 ± 0.91***
RBC (10^{12}/L)	8.03 ± 0.76	8.43 ± 0.50	6.93 ± 0.65**	7.87 ± 0.70	6.30 ± 0.60***
HGB (g/dL)	13.61 ± 1.11	12.61 ± 1.27	10.96 ± 1.12***	13.62 ± 1.22	11.22 ± 1.46**
HCT (%)	48.54 ± 6.74	48.75 ± 3.19	41.68 ± 3.52*	44.10 ± 3.40	40.08 ± 4.38**
PLT ($\times 10^9$/L)	451.31 ± 47.60	489.41 ± 50.56	542.28 ± 41.60***	479.63 ± 45.87	586.84 ± 37.97***

Rats were treated orally and daily by 10 mg/kg body weight of α-apo-OTC, β-apo-OTC, ACTC or EACTC.
WBC: white blood cell count, RBC: red blood cell count, HGB: hemoglobin, HCT: hematocrit, PLT: platelet count.
*, ** and *** mean significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively, when compared with the control group.

Table 3 Serum enzyme activities and urea nitrogen in male rats after 90 d of feeding (mean ± SD) (n = 10).

Parameter	Control	α-apo-OTC	β-apo-OTC	ACTC	EACTC
SGOT (U/L)	50.22 ± 4.25	54.01 ± 3.84	74.38 ± 13.37***	52.15 ± 5.72	65.12 ± 10.07***
SGPT (U/L)	43.29 ± 6.12	45.02 ± 5.33	58.82 ± 9.48***	46.54 ± 6.29	61.78 ± 5.85***
BUN (mg/dL)	51.54 ± 8.81	46.86 ± 4.09	64.24 ± 9.49**	55.00 ± 5.65	69.84 ± 8.50***

Rats were treated orally and daily by 10 mg/kg body weight of α-apo-OTC, β-apo-OTC, ACTC or EACTC.
BUN: urea nitrogen, SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase.
*, ** and *** mean significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively, when compared with the control group.

when compared with that of the control group, whereas no significant changes occurred in the rats treated with β-apo-OTC or EACTC compared with control group.

3.3 Serum Chemistry and Macroscopic Changes

The effects of the tetracycline degradation products on various serum constituents are given in Table 3. Significant differences in some constituents were observed between rats in the control group and those in the groups treated with β-apo-OTC or EACTC. However, changes in the serum constituents of the groups treated with α-apo-OTC or ACTC did not show any significant differences compared with the controls. Both SGOT and SGPT in the groups treated with β-apo-OTC or EACTC were significantly increased in compared with the controls ($p<0.001$). Although the SGOT and SGPT of rats in the groups treated with α-apo-OTC or ACTC were also slightly increased, but not significant compared with controls. The administration of β-apo-OTC significantly increased BUN concentration ($P < 0.01$) compared to the control group, whereas the BUN in the group treated with EACTC was significantly increased ($P <$ 0.001) in comparison with that of the control group.

At the 90th days, degeneration and necrosis were observed in the hepatocytes of rats in the groups treated with either β-apo-OTC or EACTC (Fig. 1). While no necrosis was observed in the livers of rats in the groups treated with α-apo-OTC or ACTC, other morphological changes occurred.

The present study indicated that when a dose of either β-apo-OTC or EACTC (10 mg/kg body weight) orally administered for 90 d, the body weight of rats significantly decreased, and concomitantly there were significant increases in both the absolute and relative weights of the liver and kidney as compared with the control. This did not result from malnutrition, since they had a normal daily food intake. These findings demonstrate that both β-apo-OTC and EACTC have toxic effects of on rats. Further, both the β-apo-OTC and EACTC treated rats showed a decrease in the RBC count and HGB concentration in their blood, whereas the WBC count and the PLT count were both significantly increased compared with the control group.

In the present study, serum SGOT, SGPT and BUN activities were used as markers of liver and kidney

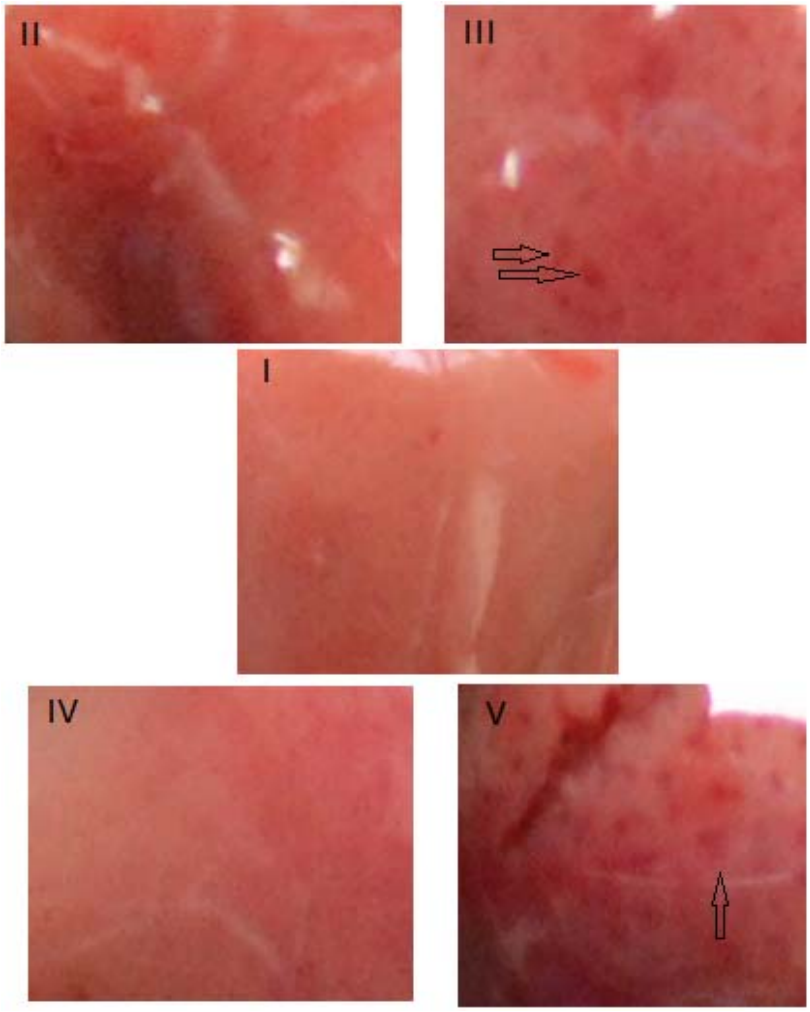

Fig. 1 Representative images of livers from rats.
I: control rat showing normal morphology, II: rats treated with α-apo-OTC, III: rat treated with β-apo-OTC, IV: rat treated with ACTC, V: rat treated with EACTC.

damage. The animals treated with either β-apo-OTC or EACTC showed a significant increased in serum SGOT, SGPT and BUN. These data demonstrates that both β-apo-OTC and EACTC were toxic and appear to damage liver and kidney rats. The tetracycline degradation product, anhydro-4-epi-tetracycline was the only product which caused abnormal urinary findings of a type similar to the Fanconi-type syndrome. Severe nephrotic changes were found in the kidneys of rats and dogs treated with anhydro-4-epi-tetracycline [7]. Although several of the degradation products, including anhydrotetracycline (ATC) and ACTC, have been shown to be potent antibiotics on tetracycline resistant bacterial strains, and on the other hand, the products of chlortetracycline have adverse effects on fresh-water phytoplankton [8, 9], yet no studies have been conducted on the toxicity of the degradation products of oxytetracycline and chlortetracycline in animals.

4. Conclusions

The present study was designed to determine the toxicity of oxytetracycline and chlortetracycline degradation products upon oral administration to rats.

The results demonstrated that the rats treated with α-apo-OTC or ACTC for 90 d did not affect body, organ weights or certain blood- and serum-factors. The β-apo-OTC and EACTC, on the other hand, exhibited significantly toxic effects on male rats, significantly decreased body weights and RBC counts, lowered HGB concentrations, and increased WBC and PLT counts. Concomitantly, there were increases in serum SGOT, SGPT and BUN. In summary, the toxic effects of β-apo-OTC and EACTC in rats appeared to damage the liver and kidney, 'which ultimately led to necrosis of the hepatocytes. Further studies are suggested to explore the mechanisms of liver and kidney toxicity by β-apo-OTC and EACTC.

References

[1] Losser, M. R., and Payen, D. 1996. "Mechanisms of Liver Damage." *Semin. Liver Dis*. 16 (4): 357-67.

[2] Farombi, E. O., Ugwuezunmba, M. C., Ezenwadu, T. T, Oyeyemi, M. O., and Ekor, M. 2008. "Tetracycline-Induced Reproductive Toxicity in Male Rats: Effects of Vitamin C and N-Acetylcysteine." *Experimental and Toxicologic Pathology* 60 (1): 77-85.

[3] Asha, K. K., Sankar, T. V., and Nair, P. G. 2007. "Effect of Tetracycline on Pancreas and Liver Function of Adult Male Albino Rats." *J. Pharm Pharmacol.* 59 (9): 1241-8.

[4] Kuhne, M., Hamscher, G., Korner, U., Schedl, D., and Wenzel, S. 2001. "Formation of Anhydrotetracycline during a High-Temperature Treatment of Animal-Derived Feed Contaminated with Tetracycline." *Food Chem*. 75 (4): 423-9.

[5] Gratacós-Cubarsí, M., Fernandez-Garcia, A., Picouet, P., Valero-Pamplona, A., Garcia-Regueiro, J. A., and Castellari, M. 2007. "Formation of Tetracycline Degradation Products in Chicken and Pig Meat under Different Thermal Processing Conditions." *J. Agric. Food Chem*. 55 (11): 4610-6.

[6] Fedeniuk, R. W., Shand, P. J., and McCurdy, A. R. 1997. "Effect of Thermal Processing and Additives on the Kinetics of Oxytetracycline Degradation in Pork Muscle." *J. Agric. Food Chem*. 45 (6): 2252-7.

[7] Frimpter, G. W., Timpanelli, A. E., Eisenmenger, W. J., Stein, H. S., and Ehrlich, L. I. 1963. "Reversible 'Fanconi Syndrome' Caused by Degraded Tetracycline." *JAMA*. 184 (2): 111-3.

[8] Benitz, K. F., and Diermeier, H. F. 1964. "Renal Toxicity of Tetracycline Degradation Products." *Proc. Soc. Exp. Biol. Med.* 115 (4): 930-5.

[9] Halling-Sorensen, B., Sengelov, G., and Tjornelund, J. 2002. "Toxicity of Tetracyclines and Tetracycline Degradation Products to Environmentally Relevant Bacteria, Including Selected Tetracycline-Resistant Bacteria." *Arch. Environ. Contam. Toxicol.* 42 (3): 263-71.

[10] Guo, R. X., and Chen, J. Q. 2012. "Phytoplankton Toxicity of the Antibiotic Chlortetracycline and Its UV Light Degradation Products." *Chemosphere* 87 (11): 1254-9.

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Agronomic Characteristics of Induced Pepper Germplasm in 2015 at Thua Thien Hue

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Abstract: The agronomical characteristics of 14 induced pepper accessions from Korea were evaluated in spring-summer in 2015 at Thua Thien Hue in order to select pepper lines having high yield and good adaptation under local conditions for introducing new cultivar to enhance plant productivity and quality. Most of the accessions showed growth duration suitable to cropping pattern in Thua Thien Hue. Large variation in agronomical characteristics, such as plant height, number of leaves and branches, stem and flower color and fruit shape were observed among these induced pepper accessions. Plant height of all induced pepper accessions was higher than control check, ranging from 49.2 cm to 105.0 cm. YT2-1 had the highest fruit set rate (80.38%). All pepper accessions had higher yield compared with the control check (1.42 tons/ha), ranging from 3.71 tons/ha (YT4-1) to 28.34 tons/ha (YT4-3). YT1-2 had good characteristics and good quality. YT1-1 showed high resistance to insects and diseases. These accessions are potentially useful for breeding programme.

Key words: Germplasm, pepper, *Capsicum annumm*, Thừa Thiên Huế.

1. Introduction

Hot pepper (C*apsium annum* L.) belonging to family Solanaceae is an important cash crop used widely as spice trade in the world. Peppers (dry and green) were cultivated in 2011 about 3.8 million ha, out of which 3.3 million ha in developing and less developed countries of Asia (2.5 million ha) and Africa (0.8 m ha) [1]. Spice pepper is produced in great amount in the world [2]. Pepper contains more vitamins C than any other vegetable crop [3] and is also source of minerals (Ca, Fe, Na, P, S), amino acids (thiamin, axit oxalic, riboflavin...), proteins and lipids [4]. Recently, pepper becomes commercial products because of industrial process as chilli food (powder, pepper sauce…) and is considered as medicinal plant used in traditional medicine for several stimulating, antiseptic, digestive, disinfectant and anti-inflammatory functions [5].

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Hot pepper is a high economic traditional spicy vegetable and is cultivated popularly through Vietnam. Since 1995, some provinces have gotten high income due to that large area of pepper produce provides raw material for food factories and companies for consuming and exporting [6, 7]. Being one of mainly provinces for growing pepper, Thua Thien Hue has taken advantages of large sandy soil area to develop this spice crop. Thus, promoting pepper production has been a good condition to efficiently utilize land by intercropping or rotated cropping and also be a chance to utilize labour resource in Thua Thien Hue. However, local pepper varieties, such as Chia Voi and Sung Bo, which have low yield and infected disease, are popularly used by farmers in here. In addition, local farmers used to keep the seeds for next sowing seasons by themselves. This made seed contaminated and low yield. To develop pepper becoming commercial crop in this province, the selection of variety is the most important step. Thus, the present

study aimed to evaluate adaptability of induced hot pepper germplasm to local conditions and to select good lines based on their morphological and agronomical characters to provide good pepper line for breeding study and cropping in Thua Thien Hue.

2. Materials and Methods

2.1 Materials

Fourteen pepper lines collected from National Institute of Horticultural and Herbal Science (NIHHS) in Korea and one local variety (Chia Voi) as control were used in this study (Table 1).

2.2 Methods

2.2.1 Experimental Design, Time and Place

This experiment was designed in complete randomized design (CRD) without replication. Each plot had double rows with 16 plants. Spacing of 40 and 60 cm between plants and rows was used, respectively. Thirty days after sowing, seedlings were transplanted to open field. The cultivated techniques based on documents of national technical regulations on testing for value of cultivation and use of hot pepper and sweet pepper varieties of Ministry of Agriculture and Rural Development (QCVN 01-64: 2011/BNNPTNT) [8]. The experiment was conducted from January to May 2015 at greenhouse of Agronomy Faculty, Hue University of Agriculture and Forestry.

Table 1 List of peper accessions used.

No.	Name of accessions	Place of collection
1	YT1	NIHHS
2	YT2	NIHHS
3	YT3	NIHHS
4	YT4	NIHHS
5	KR1-1	NIHHS
6	KR1-2	NIHHS
7	KR1-3	NIHHS
8	KR1-4	NIHHS
9	YT1-1	NIHHS
10	YT1-2	NIHHS
11	YT2-1	NIHHS
12	YT4-1	NIHHS
13	YT4-2	NIHHS
14	YT4-3	NIHHS
15	Chia Voi (control)	Thua Thien Hue

2.2.2 Agronomy Characteristic Collection

Characteristics include morphological, plant structure, ability of growth, flowering, fruiting, setting seeds, fruit qualities, levels of pest and insects infected and productivity of pepper germplasm. Fruit qualities were found by some characteristics as fruit length, fruit width, wall thickness, fruit color, Brix, dry matter content and pungency. Quantitative and qualitative parameters observed, weighed or measured were used. Data was collected from five growth plants for each line. Predicted yield was formed by mean of fruit weight, number fruits per plant and the density of 40,000 individuals per ha. Actual yield (ton/ha) was calculated by total of fruits weight per hectare. The rate of fruits infected or harmed by some diseases or insects, such as anthracnose or fruitworm, were calculated as Eq. (1):

The rate of fruits infected or harmed (%) = (number of infected or harmed fruits)/ (total of fruit observed) (1)

The rate of cutworm and beet armyworm was recorded by number of individuals/m^2.

2.2.3 Analysis Method

Data was analyzed by Excel 2007, and evaluation of stability and adaptability was considered by standard deviation value (SD).

2.3 Weather Conditions during This Experiment

Table 2 showed the effect of weather conditions on the ability of the growth and the development of peper. Seedlings were affected by low temperature (19.5 °C) mostly in rainy days in January. In February, high number of rainy days, high humidity and low temperature impacted on tilling and transplanting. Temperature increasing and the most rainfall appearing in March influenced the periods of flowering and fruiting. The mean temperature and humidity reaching 25.9 °C and 87% in April, respectively,

Table 2 Some meteorological conditions recorded in spring-summer season 2015.

Month	Temperature (°C)			Rainfall (mm)		Humidity (%)		Evaporation (mm)	Total sunny hour (h)	Number of stormy day (d)	Number of drought day (d)
	T_{mean}	T_{max}	T_{min}	NRD	R_{mean}	H_{mean}	H_{min}				
January	19.5	29.1	13.3	12	70.8	89	56	47.4	119	0	0
February	21.8	33.5	14.5	11	64.2	90	61	44.5	135	2	0
March	25.1	35.8	18.6	6	180.1	88	48	64.3	167	1	2
April	25.9	39.0	16.1	10	151.7	87	44	67.6	198	3	3
May (1st-10th day)	29.1	37.0	23.5	3	10.8	79	52	-	85	-	-

Source: Center for Hydrometeorology Forecast of Thua Thien Hue Province, 2015.
NRD: number of rainy days; H: humidity.

Table 3 Growth and development periods of pepper germplasm (days).

Accession	Time from transplant to...			
	Branching	Flowering	1st harvesting	2nd harvesting
YT1	20	31	81	104
YT2	21	31	81	104
YT3	21	31	81	104
YT4	21	31	81	104
YT1-1	21	31	81	104
YT1-2	21	31	81	104
YT2-1	23	31	81	104
YT4-1	23	36	89	104
YT4-2	23	39	89	104
YT4-3	21	39	89	104
KR1-1	19	31	96	104
KR1-2	19	31	81	104
KR1-3	19	31	81	104
KR1-4	21	31	89	104
Control	23	41	96	104

were favourable for growth and development of pepper. However, the most of number of rainy days (10 days) occurred in May taking advantage for the development of anthracnose. In May, temperature at 29.1 °C, humidity at 79% and suitable rainfall promoted fruit ripen and harvest.

3. Results and Discussion

3.1 Time of Growth and Development

Time of growth and development is decided by genetic characters and conditions, such as water, temperature, humidity, light and cultivated techniques. The identification of growth time helps to control crops to usefully suit land and climate conditions of each production area. Time to finish periods of the growth and development is showed in Table 3. Time from transplant to appear the first branch ranged from 19 d to 23 d. KR1-1, KR1-2 and KR1-3 accessions showed the earliest time of branching (19 d), followed by YT1 (20 d). Whereas, YT2-1, YT4-1 and YT4-2 accessions had the latest time of branching with 23 d and the remaining accessions started branching at 21 d.

Control check had the latest time of flowering by 41 d. Almost flowering time of pepper lines was 31 d and earlier than control, except YT4-1 (36 d), YT4-2 and YT4-3 (39 d).

Most of accessions gave fruits at 81 d after transplanting to open field. YT4-1, YT4-2, YT4-3 and KR1-4 accessions fruited at 89 d. Whereas, fruiting of KR1-1 and control accessions was at 96 d. Although

KR1-1 had flowering time earlier, but fruited the latest. Follow observing, there were nine lines riped at same time (81 days after transplanting). KR1-1 and control check had latest time of harvesting (Table 3).

3.2 Vegetative Characteristics

Table 4 recorded the results of vegetative characteristics on pepper germplasm. Plant canopy width is ability for occupation spacing of plant and potentiality of branch distribution. This is meant to be suitable for disposition density of each variety. YT1-2 had the widest canopy diameter (91.6 cm), followed by YT2, YT1 and YT4-3 with 87.6 cm, 81.6 cm and 80.6 cm, respectively. The narrowest was observed in control (30.8 cm). The remaining accession had plant canopy width ranging from 50 cm to 80 cm.

The plant height was different among pepper accessions. YT1 had the highest plant (105 cm) and control accession had the lowest plant height (37 cm). The plant height of remaining accessions ranged from 49 cm to 90 cm. Number of leaves per stem was recorded approximately 12 leaves in YT4, YT1-1 and YT1-2. YT3, YT4-1 and YT4-2 lines had number of leaves equal with the control. The lowest number of leaves was observed in KR1-3 and KR1-4 (9.2 leaves).

Total of branches per plant ranged from 21 to 86. YT1-2 had the highest number of branch (86.2 branches), next one was YT4-3 (82.8 branches) and YT1-1 (82.6 branches). Control accession had the lowest number of branches (21.6 branches).

3.3 Flowering and Fruiting Ability

Characters of flowering and fruiting of pepper germplasm were presented in Table 5. The highest number of flowers belonged to YT4-3 (193.2 flowers), followed by YT1-1, KR1-2 and YT1 with 155.2, 140.8 and 133.6, respectively. Percentage of efficient flower ranged from 28.82% (control) to 80.38% (YT2-1). Number of efficient fruits varied widely from 9.2 fruits to 116 fruits. YT1 and control check had the lowest number of fruit (approximately 9 fruits), whereas YT4-3 reached 116 fruits and YT1-1 line got 91.2 fruits per plant. Number of fruits of remaining accessions ranged from 39.1 to 68.2. Percentage of fruiting was low at almost accessions ranging from 20% to 60%. The highest rate was found in YT1-2 line 68.20%), next was YT4-3 (60.25%) and YT1-1 (58.76%). Whereas, YT1 only had 6.88% percentage of fruiting.

3.4 Morphological Traits

Morphological characters of flower, fruit and seed are the main features of genotype distinguishing varieties' difference. Morphological traits were detected in a wide variation presented in Table 6.

Table 4 Vegetative characteristics of pepper germplasm.

Accession	Plant height (cm)	Plant canopy width (cm)	Number of leaves/stem	Total of branches
YT1	105.0 ± 5.0	81.6 ± 5.7	10.6 ± 0.4	70.8 ± 8.9
YT2	90.0 ± 3.6	87.6 ± 6.9	10.2 ± 0.2	41.4 ± 4.5
YT3	61.6 ± 6.8	65.6 ± 1.9	11.8 ± 0.7	41.8 ± 9.2
YT4	66.6 ± 4.2	69.2 ± 5.2	12.0 ± 1.4	52.4 ± 5.5
YT1-1	71.2 ± 8.8	77.4 ± 8.8	12.6 ± 1.3	82.6 ± 8.2
YT1-2	91.8 ± 13.7	91.6 ± 17.3	12.0 ± 1.3	86.2 ± 11.9
YT2-1	58.6 ± 4.2	66.6 ± 5.2	10.6 ± 0.7	38.6 ± 3.7
YT4-1	49.2 ± 5.4	65.2 ± 5.8	11.8 ± 1.1	41.2 ± 4.9
YT4-2	65.4 ± 3.3	65.2 ± 5.8	11.8 ± 1.1	66.0 ± 21.2
YT4-3	87.8 ± 13.5	80.6 ± 8.1	11.6 ± 1.8	82.8 ± 8.6
KR1-1	56.6 ± 2.9	59.2 ± 4.8	11.2 ± 0.5	48.4 ± 7.1
KR1-2	54.8 ± 5.8	54.2 ± 5.4	10.4 ± 0.5	46.8 ± 11.9
KR1-3	57.2 ± 3.6	59.8 ± 6.9	9.2 ± 0.4	35.6 ± 5.0
KR1-4	55.6 ± 5.0	59.8 ± 6.9	9.2 ± 0.4	46.0 ± 9.2
Control	37.0 ± 11.5	30.8 ± 8.9	11.8 ± 0.5	21.6 ± 9.2

Table 5 Characteristics of flowering and fruiting ability of pepper germplasm.

Accession	Number of flowers per plant	Percentage of efficient flowers (%)	Number of efficient fruits per plant	Percentage of fruiting (%)
YT1	133.6 ± 6.06	54.69	9.2	6.88
YT2	127.0 ± 38.63	69.76	54.1	42.59
YT3	92.6 ± 68.08	66.95	39.1	42,22
YT4	105.4 ± 23.45	76.66	51.8	49.15
YT1-1	155.2 ± 90.96	80.28	91.2	58.76
YT1-2	100.0 ± 29.98	67.60	68.2	68.20
YT2-1	104.0 ± 30.00	80.38	55.2	53.08
YT4-1	79.4 ± 37.96	45.59	17.2	21.66
YT4-2	101.6 ± 64.08	71.65	49.8	49.02
YT4-3	193.2 ± 33.17	77.74	116.4	60.25
KR1-1	114.8 ± 50.43	65.68	41.2	35.89
KR1-2	140.8 ± 114.30	67.90	53.8	38.21
KR1-3	84.0 ± 14.27	66.43	41.0	48.81
KR1-4	115.4 ± 33.65	72.27	42.6	36.92
Control	34.0 ± 14.44	28.82	9.4	27.65

Table 6 Morphological characteristics of pepper germplasm.

Accession	Pedicle position at anthesis	Pedicle position at fruiting	Colour				
			Stem	Leaf	Node	Unripe fruit	Ripe fruit
YT1	Pendant	Pendant	Dark green	Dark green	Dark violet	Dark green	Dark red
YT2	Pendant	Pendant	Light green	Green	Violet	Light green	Bright red
YT3	Pendant	Pendant	Green	Dark green	Light violet	Green	Bright red
YT4	Pendant	Pendant	Green	Dark green	Violet	Light green	Scarlet
YT1-1	Pendant	Pendant	Light green	Light green	Light violet	Green	Scarlet
YT1-2	Pendant	Pendant	Green	Dark green	Light violet	Green	Scarlet
YT2-1	Pendant	Pendant	Light green	Green	Dark violet	Light green	Bright red
YT4-1	Pendant	Pendant	Green	Dark green	Violet	Light green	Bright red
YT4-2	Pendant	Pendant	Light green	Green	Light violet	Light green	Bright red
YT4-3	Intermediate	Erect	Green	Dark green	Violet	Green	Bright red
KR1-1	Pendant	Pendant	Green	Green	Light violet	Light green	Dark red
KR1-2	Pendant	Pendant	Green	Green	Violet	Light green	Scarlet
KR1-3	Pendant	Pendant	Green	Green	Violet	Green	Dark red
KR1-4	Pendant	Pendant	Green	Green	Dark violet	Green	Scarlet
Control	Pendant	Pendant	Light green	Light green	Green	Light green	Orange

Pedicle position at anthesis and fruiting are concluded as pendant, intermediate or erect. All accessions had pendant flower and fruit, except YT4-3, which had intermediate flower habit and erected fruit. The colour of stem included dark green (YT1), light green (YT2, YT1-1, YT2-1, YT4-2, control) and green (the remained lines). YT1, YT3, YT4, YT1-2, YT4-1, and YT4-3 had dark green leaf, whereas light green leaves were observed in YT1-1 and Control check and green colour was found in remained lines. Dark green stem and leaves indicated earning high sunlight ability and strong photosynthesis.

The colour of node varied from light violet to dark violet. YT1, YT2-1 and KR1-4 lines had dark violet node, but the lighter one was observed in YT3, YT1-1, YT1-2, YT4-2 and KR1-1. Nodes of all remained lines were violet, except the control check which had green stem.

In the immature stage, colour of fruit was diversed from light green to dark green. Only YT1 line had dark green fruit. Green fruits were found in YT3, YT1-1, YT1-2, YT4-3, KR1-3 and KR1-4. The

remained accessions had light green fruits. In the mature stage, fruit colour changed from bright red, scarlet to dark red. Only fruits of the control check had orange color. Fruits which had bright red were observed in YT2, YT3, YT2-1, YT4-1, YT4-2 and YT4-3. Fruits of YT4, YT1-1, YT1-2, KR1-2 and KR1-4 had scarlet and the remained accessions had dark red, such as YT1, KR1-1 and KR1-3. Scarlet and bright red are the main colour of mature fruit because they suit demands of consumers, industrial process and exporting.

The results indicated that YT1-2, YT4-3 and YT1-1 showed morphological traits better than the other lines.

3.5 Qualitative Characters of Fruit

According to Bosland [9, 10], chilli are usually classified based on fruit characteristic, including pungency, color, shape, flavor, size and use. Fruit's qualitative characteristics of pepper germplasm were shown in Table 7. Fruit length ranged from 6.53 cm to 11.28 cm. The control check obtained the longest fruit (11.28 cm), next ones were YT4-2 (10.97 cm), KR1- 3 (10.86 cm) and YT4 (10.32 cm). The remained accessions had almost equal fruit length from 7.47 cm to 9.8 cm. Fruit width was the lowest in YT4-3 (1.38 cm) and the highest in YT4 (2.3 cm). Fruit width was greater than 2.0 cm in YT1, YT2-1, YT4-2, KR1-3 and control accession. Large fruit is more suitable for processing pepper [11].

Fruit wall thickness was different among accessions. Fruit of accessions had thick wall, such as YT1 (0.21 cm), YT4 (0.21 cm), KR1-3 (0.21 cm) and YT4-2 (0.2 cm). The remained accessions had fruit wall thickness from 0.15 cm to 0.19 cm. Brix is indicator to express content of solube solid. KR1-1 had the highest Brix (10.7), followed by YT1-2 (10). YT4-2 and control check had the lowest Brix at 4.1 and 5.8, respectively.

Dry matter content calculated by weight of 100 g of fresh fruit is one of qualitative demands for exporting pepper powder. The higher dry matter content is, the higher amount of dry powder get. KR1-3 had the highest dry matter content at 16.88%, followed by YT1-2 at 15.22%. The remained accessions had dry matter content over 10% except for YT4-2, KR1-1 and control check.

Pungent fruit is characterized for hot pepper. Pungency of YT1-1, YT1-2 and YT4-3 was higher that the control check. Flavor is meaning specific taste of fruit and takes appetite for customers. Flavor assessment had good results in almost accessions, except for YT2, YT3, YT2-1, YT4-1, YT4-2 and KR1-2.

Table 7 Qualitative characteristics of pepper germplasm.

Accession	Fruit length (cm)	Fruit width (cm)	Fruit wall thickness (cm)	Brix	Dry matter content (%)	Flavor	Fruit pungency	Dry fruit color
YT1	8.79 ± 1.42	2.16 ± 0.26	0.21 ± 0.04	8.4	13.10	Strong	Low	Dark red
YT2	8.48 ± 2.37	1.82 ± 0.22	0.18 ± 0.04	8.0	13.91	Light	Low	Bright red
YT3	7.94 ± 2.34	1.70 ± 0.23	0.18 ± 0.04	7.5	14.13	Light	Low	Bright red
YT4	10.32 ± 1.98	2.30 ± 0.28	0.21 ± 0.04	6.7	12.61	Strong	Low	Scarlet
YT1-1	9.51 ± 2.17	1.50 ± 0.38	0.18 ± 0.06	8.1	13.58	Strong	Medium	Scarlet
YT1-2	8.88 ± 0.98	1.47 ± 1.50	0.19 ± 0.05	10.0	15.22	Strong	High	Scarlet
YT2-1	8.90 ± 2.09	2.00 ± 0.36	0.19 ± 0.05	6.9	14.41	Light	Low	Bright red
YT4-1	6.53 ± 1.55	1.68 ± 0.26	0.15 ± 0.05	8.5	13.33	Light	Low	Bright red
YT4-2	10.97 ± 2.04	2.05 ± 0.34	0.20 ± 0.06	5.8	9.57	Light	Low	Bright red
YT4-3	8.36 ± 1.38	1.38 ± 0.25	0.14 ± 0.04	9.1	13.25	Strong	High	Bright red
KR1-1	7.97 ± 2.64	1.89 ± 0.38	0.17 ± 0.03	10.7	9.89	Strong	Low	Dark red
KR1-2	7.47 ± 2.35	1.75 ± 0.26	0.15 ± 0.05	9.0	12.50	Light	Low	Scarlet
KR1-3	10.86 ± 1.92	2.18 ± 0.14	0.21 ± 0.04	9.5	16.88	Strong	Low	Dark red
KR1-4	9.80 ± 1.69	1.96 ± 0.37	0.15 ± 0.04	9.0	14.29	Strong	Low	Scarlet
Chìa Vôi	11.28 ± 2.68	2.06 ± 0.23	0.16 ± 0.03	4.1	9.83	Strong	Low	Orange

3.6 Yield and Yield Components

By same weather condition and taking care techniques, productivity value shows preeminent variation. Yield depends on main factors, such as number of fruits per plant and fruit weight. The results of yield and yield components were recorded in Table 8.

Number of commercial fruits ranged from 7.6 to 110.4 fruits. YT4-3 had the highest number of fruit (110.4 fruits), followed by YT1-1 (77.2 fruits) and YT1-2 (66.8 fruits). Some accessions had low number of fruits, such control check, YT1 and YT4-1. The remained accessions' number of fruit ranged from 34.8 to 49.6. Followed observation, the reason caused low fruit number of few accessions was damaged by fruitworm.

Fruit weight depends on fruit size and fruit wall thickness. The biggest fruits were observed in KR1-4 (16.6 g) and KR1-3 (16.5 g). YT4, YT2-1, YT2 and control had almost equal fruit weight (ranging from 12.5 g to 13.1 g).

Fruit weight and number of fruits decide the yield of pepper. All of pepper lines gave predicted and actual yields higher than the control check. YT4-3 gave the highest yield (28.31 tons/ha) and YT4-1 gave the lowest yield (3.71 tons/ha).

3.7 Situation of Insects and Disease Affected on Pepper Germplasm

Insects and diseases are reasons which caused yield and fruit quality decreases. Level of infected insects and diseases depends on resistant ability of variety or line. During experiment, many objects harmed peppers, such as cutworm, diamondback moth, fruitworm, bedbug, spider, etc., affected yield and fruit quality. Effects of worm and diseases on pepper germplasm were presented in Table 9.

3.7.1 Cutworm (*Ggrotis ipsilon* Rott)

Cutworm damaged pepper germplasm during experiment. Density of cutworm was observed the highest in YT3 line (4.78 individuals/m^2) and the lowest in YT4-1, YT4-2, KR1-1, KR1-2 and KR1-3 (none damage). Percentage of damaged plant was the highest in YT4 (60%) and ranged from 20%-40% in remaining accessions.

3.7.2 Beet Armyworm (*Spodoptera exigua*)

The highest density of this worm was recorded in YT4-2 line (7.83 individuals/m^2) and its lowest was 0.43 individuals/m^2 in YT1-1. Percentage of harmed plant was the highest at 80% in YT1, YT2, YT1-2, YT4-1 and YT4-2. The remained accessions were damaged from 20%-60%.

Table 8 Yield and yield components of pepper germplasm.

Accession	Number of efficient fruits per plant	Number of commercial fruits per plant	Mean fruit weigh (g)	Predict yield (ton/ha)	Actual yield (ton/ha)
YT1	9.2	8.6	15.5	5.33	5.49
YT2	54.0	49.6	12.5	24.80	17.99
YT3	39.0	34.8	10.0	13.92	13.26
YT4	51.8	46.0	13.1	24.01	17.33
YT1-1	91.2	77.2	9.0	27.79	19.70
YT1-2	68.2	66.8	10.4	27.79	20.17
YT2-1	55.2	47.0	12.9	24.25	23.01
YT4-1	17.2	13.0	8.0	4.16	3.71
YT4-2	49.8	40.0	10.1	16.16	15.25
YT4-3	116.4	110.4	8.9	39.30	28.31
KR1-1	41.2	40.6	9.6	15.59	14.24
KR1-2	53.8	48.4	9.9	19.17	15.91
KR1-3	41.0	36.0	16.5	23.76	20.17
KR1-4	42.6	39.4	16.6	26.16	16.29
Control	9.4	7.6	13.1	3.98	1.42

Table 9 Effect of worms and diseases on pepper germplasm.

Accesion	Worm					Disease	
	Cutworm		Beet armyworm		Fruitworm	Soft rot	Anthracnose
	Number of individual/m^2	Percentage of harmed plant (%)	Number of individual/m^2	Rate of harmed plant (%)	Rate of harmed fruit (%)	Rate of harmed fruit (%)	Rate of harmed fruit (%)
YT1	0.43	20	3.48	80	13.04	0.00	6.52
YT2	1.74	20	6.09	80	11.85	0.37	0.37
YT3	4.78	40	2.61	60	10.26	3.08	1.54
YT4	2.61	60	2.17	60	7.72	0.39	0.00
YT1-1	0.87	20	0.43	20	2.41	0.66	0.44
YT1-2	0.43	20	2.61	80	1.17	0.88	0.29
YT2-1	0.43	20	0.87	40	11.96	13.04	1.81
YT4-1	0.00	0	3.04	80	10.47	1.16	3.49
YT4-2	0.00	0	7.83	80	2.41	1.20	1.61
YT4-3	1.30	40	3.91	60	2.75	1.03	0.86
KR1-1	0.00	0	2.61	40	2.91	1.46	0.00
KR1-2	0.00	0	1.74	40	2.60	0.37	0.00
KR1-3	0.00	0	2.17	60	3.41	0.00	0.98
KR1-4	1.74	20	1.30	40	3.29	1.41	1.41
Control	0.87	20	0.87	40	8.51	6.38	10.64

3.7.3 Fruitworm (*Helicoverpa armigera* Hiibner)

YT1 line was highly damaged at 13.04% by fruitworm, next ones were YT2, YT2-1, YT4-1 at 11.85%, 11.96%, 10.47%, respectively. The remained accessions were damaged by low percentage from 1.17% to 8.51%.

3.7.4 Soft Rot (*Erwinia carotovora*)

The damage by soft rot was found the highest in YT2-1 (13.04%), followed by control check (6.38%) and YT3 (3.08%). YT1 and KR1-3 were not damaged by this disease.

3.7.5 Anthracnose (*Colletotrichum capsici*)

Control accession had the highest rate of infection of anthracnose at 10.64%, next ones were at YT1 (6.52%) and YT4-1 (3.49%) lines. YT4, KR1-1 and KR1-2 were not infected by this disease. The remained accessions had damaged rate ranging from 0.29% to 1.81%.

4. Conclusions

As a result, the induced pepper germplasms were harvested earlier, and had higher yield and better quality compared with the control. The induced pepper accessions are diversed in morphological and agronomical features, as plant height, number of leaves or branches, colour of leaf, flower and fruit. YT2-1 had the highest percentage of effective flowers (80.38%), YT1-2 gave the highest percentage of fruiting (68.2%), whereas YT4-3 reached the highest yield (28.31 tons/ha). These pepper lines should be used for breeding of high yield varieties. YT1-1 was resistant to insects and diseases and should be used in breeding program for insect and disease resistance. YT1-2 showed good adaptability and fruit quality compared with other accessions under Thua Thien Hue weather conditions. The induced pepper lines which gave good characteristics should be designed in next experiments in different seasons and ecological areas to assess their potentiality to complete crop structure.

References

[1] Food and Agriculture Organization of the United Nations (FAO). 2013. "FAOSTAT." Accessed November, 2015. www.fao.org.

[2] Joshi, S., Thakur, P. C., Verma, T. S., and Werma, H. C. 1993. "Selection of Spice Paprika Breeding Lines." *Capsicum and Eggplant Newsletter* 12: 50-2.

[3] Dexiang, C. 1994. *Hot Pepper Varietal Trial*. The 12th Regional Training Report in Vegetable Production and

Research, ARC-AVRDC.

[4] Cannon, P. F., Bridge, P. D., and Monte, E. 2000. "Linking the Past, Present and Future of *Colletotrichum* Systematics." In *Colletotrichum: Host Specificity, Pathology and Host-Pathogen Interaction,* edited by Prusky, D., Freeman, S., and Dickman, M. B. St. Paul, Minnesota: APS Press, 1-20.

[5] Agradi, E., Regondi, S., and Rotti, G. 2005. *Aspects of Pharmaceutical Botany: Learn about Medicinal Plants.* Vol. 4. Italy: MediService, 154-6. (in Italian)

[6] Mai, P. A., Tran, V. L., and Tran K. T. 1995. *Vegetables*. Ha Noi: Agriculture Publish House, 183-9. (in Vietnamese)

[7] Anh, M. T. P. 1999. *Vegetable Cultivation.* Ha Noi: Farmer Publish House, 5-11. (in Vietnamese)

[8] Ministry of Agriculture and Rural Development (MARD). 2011. *Circular No. 48/2011/TT-BNNPTNT of the Ministry of Agriculture and Rural Development on National Technical Regulation on Crop Trial.* (in Vietnamese)

[9] Bosland, P. W. 1992. "Chiles: A Diverse Crop." *Hort. Technol.* 2 (1): 6-10.

[10] Bosland, P. W. 1994. "Chillies History, Cultivation and Uses." In *Species, Herbs and Edible Fungi.* New York, USA: Elsevier Publication, 347-66.

[11] Akinci, S., and Akinci, I. E. 2004. "Evaluation of Red Pepper for Spice (*Capsicum annuum* L.) Germplasm Resource of Kahramanmaras Region (Turkey)." *Journal of Biological Sciences* 7 (5): 703-10.

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Evaluation of Introduced Pepper Accessions for Agronomic Characteristics in Summer-Autumn Season 2014 at Thua Thien Hue

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Abstract: Five lines of introduced pepper obtained from National Institute of Horticultural and Herbal Science, Korea, namely YT1, YT2, YT3, YT4, KR1, and two local varieties, namely Chia Voi and Moi, were used in this study to evaluate bio-agronomy characteristics and yield components in summer-autumn season 2014 at Thua Thien Hue, Vietnam. The experiment was laid out in a random complete block design (RCBD) with three replications. The results showed that all of the introduced pepper accessions can grow under Thua Thien Hue condition. The introduced accessions as KR1, YT2 and YT4 showed good adaptability. These pepper lines had high yield and good fruit qualities, like Chia Voi. YT4 gave high number of leaves and plant height. Accession KR1 had the highest number of fruits per plant and yield (1397.8 kg/ha). Accession YT2 had high fruit setting rate and the biggest fruit (8.08 g). YT2 and YT4 had high yield, which were equivalent to Chia Voi. These accessions need to be conducted another trials in different areas and seasons to confirm their growth ability and can be used in crop structure at Thua Hien Hue province and breeding programs.

Key words: Pepper, *Capsicum* sp., solanaceae, Thừa Thiên Huế.

1. Introduction

Pepper (*Capsicum* sp.) belongs to the genus *Capsicum*, members of the Solanaceae family, is the second most importance vegetable crop after tomato. It originated from Peru and Bolivia [1], and is one of popular spicy vegetable throughout the world. Pepper fruit is considered as rich nutrients source, in 100 g of fresh pepper containing 3.7% protein, 0.6% lipid, 4.8% carbohydrate, 0.5% organic acid, 0.17 mg carotin, 103 mg vitamin C, 292 mg vitamin A and some mineral elements [2, 3]. In recent times, pepper becomes a valuable commodity, because it is not only fresh food in meal but also makes a lot of products and raw materials for food processing industry. It can be used in alcohol type to resist hoarseness, treat emerods, flatulence and dysentery [3]. Cultivation of pepper is high potential in Vietnam, because it is easy to plant, not choosy soil and suitable for many ecological areas. Pepper can harvest many times and is simple for treatment or process (dry, powder, pepper sauce). In addition, pepper powder is the first of exporting spice-vegetable commodities [4]. Within advantage characteristics, pepper overcomes market's risks, maintains stable prices and ensures producer's benefits. Pepper have been cultivated in Vietnam long time ago, but mainly in centre of Vietnam and suburban areas or densely populated areas of Southern and Northern parts.

In Thua Thien Hue, pepper is the most popular spicy vegetable and almost indispensable in every meals. Strengthening pepper's production is to utilize land efficiently, improve soil by alternate crops, intercropping and overlapping crops, and increase efficient economic. However, in Thua Thien Hue,

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pepper production area is decreased due to local variety having low level of resistance to disease, insect and high pollen contamination due to hot and humid weather. These caused local variety degraded and contaminated. To enhance value of pepper productions, selection of high yield and good qualities varieties are needed. The objective of this study was to evaluate induced pepper lines on growth ability, fruit qualities and yield to select promised lines for introducing into crop system and breeding programs in Thua Thien Hue.

2. Materials and Methods

2.1 Materials

A total of seven pepper accessions, including five introduced accessions—YT1, YT2, YT3, YT4, KR1 from National Institute of Horticultural and Herbal Science (NIHHS), Korea, and two local accessions—Chia Voi and Moi were used in this study (Table 1).

2.2 Methods

The experiment was performed during summer-autumn season from May to October 2014 in a greenhouse of Hue University of Agricultural and Forestry. The experiment was laid out in random complete block design (RCBD) with three replications. Each plot contained eight plants for with spacing of 80 cm between rows and 40 cm between plants. Density was 30,000 plants/ha.

The cultivated process based on documents of the national technical regulations on testing for value of cultivation and use of hot pepper and sweet pepper varieties of Ministry of Agriculture and Rural Development (QCVN01-64:2011/BNNPTNT) [5]. In nursery stage, seeds of each accession were sown in plastic trays comprising of 40% subsurface, 40% coconut fibre and 20% manure. Seedlings with 4-5 true leaves were transplanted to open field experiment. Total of fertilizer applied for experiment field included 15 tons manure/ha, 110 kg N/ha, 80 kg P_2O_5/ha and 140 kg K_2O/ha. The basal fertilizing contained 100% manure, 100% superphosphate, 1/3 amount potassium and 1/3 amount urea of total fertilizer were applied before transplanting. Fertilizers were supplied once a month after transplanting four weeks with potassium and urea. Watering to keep field humidity at 70%-75% was carried out regularly. Weeding and field hygiene were performed [6].

The experiment was evaluated for plant structure, morphological traits, growth traits, fruit's quantitative and qualitative characteristics, yield components and yield of seven pepper accessions. The evaluation was processed on 21 characteristics during the study. Data was collected from five plants per plot and analyzed by analysis of variance (one-way ANOVA) by Statistix 9.0 trial version. Differences between mean values were compared using Duncan's test at $P < 0.05$ [7].

3. Results and Discussion

3.1 Horticultural Characters

3.1.1 Plant Structure and Morphological Traits

Plant structure and morphological traits are depending on genetics of variety to help distinguish differences among pepper varieties. Peeraullee and Rangoo-Sanmukhiya [8] emphasized that morphological characterization is an important target. Plant materials collected for molecular analysis is based on difference of phenotype. There are three types of plant structure of pepper, such intermediate, erect and spread. According to Chaux and Foury [9], the plant habit is determined by the bifurcation of the main stem. Stem which is observed as intermediate (compact) type, divides many primary branches forming angle from 60° to 90°. So, this plant habit is considered stable and prevents falling down from strong wind. Erect plant habit is easy to fall, because plant height is high and primary branches make angle less than 45°. According to result from Table 2, almost accessions had intermediate habit, except for Moi which was erect. Stem of indeterminate habit type has one or two flowers developed per node, and shortened internodes never develop. This result is in the in agreement with

study of Zhani et al. [10].

The number of flower per node affects fruit number and actual yield. Only YT1 had one or two flowers per node, the remained pepper lines had one flower. This type is the typical flowering of the *Capsicum* species as one flower per axil located at each node and intersected by two branches [11]. Position of flower and fruit are related with specific of variety. Flower and fruit of Chia Voi was pendant, Moi was erect. Accessions YT1, YT2, YT3, YT4 and KR1 had pendant fruit. Normally, pendant fruit is bigger and longer than those which were erect.

Pepper flower is hermaphroditic and self-pollinated. However, high cross pollination ratio still occurs, because stigma is higher than anther in some accessions. Stigma of YT1, YT3 and Moi was higher than anther. Stigma of the remained accessions was the same position with anther.

Stem, leaf and node of pepper accessions were differed in color. Light green, green and dark green were observed in stem color, green and dark green were in leaf and light or dark violet were in node. Color of stem, leaf and node of accession Moi was same with those in YT2; YT4 and Chia Voi had the same color of stem and leaf. Both YT3 and KR1 had green stem and light violet node, but different leaf color (Table 2).

These results showed that accession YT4 had almost characteristics like Chia Voi. YT1 accession had a lot morphological traits alike Moi.

3.1.2 Growth Traits

Growth traits indicate adaptability of weather condition of the accessions. The results were recorded in Table 3. Number of leaves per plant not only depends on variety, but also expresses growth ability. Numbers of leave of the accessions were observed significantly different. It ranged from 8.16 to 13.11 leaves. Moi had the highest of leaves (13.11 leaves), whereas YT2 was the lowest (8.16 leaves). Accession YT4 had the highest number of leaves among introduced accessions, followed by YT1 (8.84 leaves).

Plant height is one of specificity of variety and related to plant structure. The results in Table 3 showed that Moi had erect plant structure and the highest plant height (99.72 cm) and significantly different from the other accessions obtained as intermediate structure. The highest plant of introduced accessions was found in KR1 (69.45 cm), follow by YT4 (62.21 cm) and YT2 (51.06 cm). YT3 had the lowest plant height (40.92 cm) and next was Chia Voi

Table 1 List of pepper accessions used.

Number	Accession name	Place of collection
Introduced accessions		
1	YT1	NIHHS
2	YT2	NIHHS
3	YT3	NIHHS
4	YT4	NIHHS
5	KR1	NIHHS
Local accessions		
6	Chia Voi	Thua Thien Hue
7	Moi	Thua Thien Hue

Table 2 Plant structure and morphological traits of pepper accessions.

Target accession	Plant habit	Characteristics of flower			Fruit position	Color			
		Number of flower per node	Position	Position of stigma compares with anther		Flower	Stem	Leaf	Node
YT1	IM	1 or 2	IM	Higher	P	W	DG	DG	DV
YT2	IM	1	IM	Same	P	W	DG	DG	LV
YT3	IM	1	IM	Higher	P	W	G	DG	LV
YT4	IM	1	IM	Same	P	W	LG	G	-
KR1	IM	1	IM	Same	P	W	G	G	LV
Chia Voi	IM	1	P	Same	P	W	LG	G	-
Moi	E	1	E	Higher	E	W	DG	DG	LV

-: means no observe data. IM = intermediate, E = erect, P = pendant, W = white, G = green, LG = light green, DG = dark green, DV = dark violet, LV = light violet.

Table 3 Growth traits of pepper accessions used in the study.

Accession	Number of leaves per plant	Plant height (cm)	Number of branches
YT1	8.84^{de}	47.32^{bc}	22.12^{a}
YT2	8.16^{e}	51.06^{bc}	32.11^{a}
YT3	8.64^{de}	40.92^{c}	19.31^{a}
YT4	9.54^{d}	62.21^{bc}	31.37^{a}
KR1	8.67^{de}	69.45^{b}	33.89^{a}
Chia Voi	11.50^{c}	43.72^{c}	23.33^{a}
Moi	13.11^{b}	99.72^{a}	33.67 ^{a}
$LSD_{0.05}$	0.92	22.26	19.44

$^{a-e}$ Mean different letters in a column indicate significantly difference (Duncan's test, $P < 0.05$).

(43.72 cm). The plant height of pepper in this study is similar results of Occhiuto et al. [12], Misra et al. [13] and Prasath et al. [14].

In pepper, more branches will give more flowers, because flower appears at where branch sprouts. Total of branches were different between accessions, ranging from 19.31 to 33.89, but there were no significant difference. The highest number of branches was found in KR1 (33.89 branches) and the lowest was YT3 (19.31 branches). Accession Moi, YT2 and YT4 obtained over 30 branches per plant. The result is in agreement with result of Misra et al. [13] that total of branches of pepper, which included primary, secondary and tertiary branches, ranged from 18 branches to 28 branches. Accessions YT4, KR1 and YT2 showed good adaptability under Thua Thien Hue weather conditions.

3.2 Fruit Quality Traits

Fruit traits are essential for pepper fruits used in the industry or cooking, either in natural or as paprika, or paste or dehydrated pepper or in conserves [15]. Characterization of fruit, such as shape, size, color, pungency and flavor, is used to classify chili varieties [16, 17]. The quality parameters including fruit length, diameter, wall thickness and fruit color are presented in Table 4. Fruit size was recorded by length and diameter. Fruit length ranged from 3.82 cm to 9.09 cm. Chia Voi had the longest fruit (9.09 cm), whereas Moi had the shortest one (3.82 cm). KR1 and YT2 had long fruit with 8.29 cm and 7.34 cm, respectively. Fruit diameter of Moi was the smallest and significant difference from other accessions. Fruit diameter of YT2 was found to be the widest (2.03 cm), next was Chia Voi (1.99 cm) and KR1 (1.89 cm). All of accessions gave ratio of length and diameter greater than 1. The fruit quality traits in this study is in agreement with those in studies of Misra et al. [13], Patel et al. [18], Akinci, S. and Akinci, I. E. [19]. Winch [20] found difference in the thickness of the pericarp related to the ability of the genotype in the partition of assimilation. Fruit wall thickness is one of genetic characteristics and rangs from 0.86 mm to 2.24 mm. YT4, KR1 and Chia Voi had the thickest fruit wall (from 2.22 mm to 2.24 mm) and Moi had the thinnest wall (0.86 mm), which were significantly different from other accessions. Montesano et al. [21] reported that their *Capsicum annuum* germplasm was obtained with fruit length 4.00-16.67 cm, fruit width 2.7-4.92 cm and fruit wall thickness 2.00-4.00 mm [12], while Occhiuto et al. [12] reported that their three clusters of pepper germplasm were found average fruit length in 4.34, 6.91 and 13.95 cm, average width in 6.21, 3.64 and 1.71 cm and average wall thickness in 3.6, 0.9 and 1.9 mm, respectively. The results from Table 4 showed that Chia Voi had light green fruits and Moi had dark green fruits at immature stage, whereas the other accessions had green fruits. Mature pepper fruit is produced for culinary applications and the color is an important determinant of quality of fresh and processed product. Dark red is better for powder processing. Fruit of almost accessions had dark red color at mature stage, except for KR1 and Chia Voi. The typical red color of fruit at mature stage is related

Table 4 Fruit characteristics of pepper accessions used in the study.

Accession	Fruit length (cm)	Fruit diameter (cm)	Fruit wall thickness (mm)	Fruit color	
				Immature	Mature
YT1	5.93^{d}	1.65^{a}	1.99^{ab}	G	DR
YT2	7.34^{bc}	2.03^{a}	2.12^{ab}	G	DR
YT3	6.38^{cd}	1.65^{a}	1.85^{b}	G	DR
YT4	6.26^{cd}	1.74 ^{a}	2.24^{a}	G	DR
KR1	8.29^{ab}	1.89^{a}	2.24^{a}	G	R
Chia Voi	9.09^{a}	1.99^{a}	2.22^{a}	LG	R
Moi	3.82^{e}	0.77^{b}	0.86^{c}	DG	DR
$LSD_{0.05}$	1.33	0.53	0.37		

$^{a-e}$ Mean different letters in each columns indicate significantly difference (Duncan's test, $P < 0.05$).
R = red, DR = dark red, LG = light green, DR = dark green.

Table 5 Yield and yield components.

Accession	Percentage of fruit setting (%)	Fruit weight (g)	Number of fruits per plant	Predicted yield (kg/ha)	Actual yield (kg/ha)
YT1	4.83^{c}	5.94 ^{b}	3.17^{c}	583.44^{bc}	386.45^{bc}
YT2	21.10^{ab}	8.08^{a}	12.18^{ab}	2,966.00^{a}	892.30^{ab}
YT3	12.83^{bc}	6.03^{b}	3.31^{c}	602.91^{bc}	490.450^{bc}
YT4	14.81^{abc}	7.00^{ab}	5.63^{bc}	1,210.10^{bc}	958.80^{ab}
KR1	24.56^{a}	7.25^{ab}	15.78^{a}	3,433.90^{a}	1,397.80^{a}
Chia Voi	22.72^{ab}	7.40^{ab}	7.69^{bc}	1,559.90^{b}	979.85^{ab}
Moi	6.74^{c}	1.35^{c}	4.50^{c}	96.77^{d}	79.85^{c}
$LSD_{0.05}$	10.89	1.46	6.61	1,316.50	685.300

$^{a-d}$ Mean different letters in each columns indicate significantly difference(Duncan's test, $P < 0.05$).

to transformation of chloroplasts into chromoplasts [22].

According to these results, KR1, YT2 and Chia Voi had big fruit and YT4 had the thickest fruit wall.

3.3 Yield and Yield Components

The results in Table 5 showed that percentage of fruit setting ranged from 4.83% to 24.56%. KR1 had the highest rate 24.56%, whereas YT1 had lowest rate 4.83%, followed by Moi 6.74%. These accessions were significant difference from other accessions. The largest fruit was observed in YT2 (8.08 g), followed by Chia Voi (7.40 g) and KR1 (7.25 g), whereas Moi contained the smallest fruit (1.35 g). The difference was highly significant among accessions. The result was similar to fruit weight (from 2.17 g to 19.84 g) of Prasath et al. [14] and average fruit weight was 8.37 g. The results showed fruit weight was increased due to increase of its width. Silva et al. [23] also reported that the accessions which showed greater fruit average also presented larger fruit diameter average. In addition, fruit wall thickness and fruit weight were positive correlated, which was reported by Lannes et al. [24]. Somashekhar and Salimath [25] and Schuelter et al. [26] described that the fruit weight was related inversely to fruit number per plant. Yield is one of important traits reflecting results and influenced by applied techniques during planting and caring process. Chili pepper may yield up to 18 tons/ha and sweet pepper up to 30 tons/ha in open fields [27]. There were significant differences among accessions in all of yield components and yield. Predicted yield ranged from 96.77 kg/ha to 3,433.90 kg/ha. KR1 had the highest predicted yield (3,433.90 kg/ha), next was YT2 (2,966 kg/ha), whereas Moi had the lowest predicted yield (96.77 kg/ha). The difference was highly significant among accessions.

Accession KR1 was obtained with the highest

actual yield (1,397.80 kg/ha), next was Chia Voi (979.85 kg/ha) and YT4 (958.80 kg/ha). Moi had the lowest yield (79.85 kg/ha). However, there was no significant difference among these accessions.

4. Conclusions

All of accessions can grow under Thua Thien Hue condition. Almost accessions had intermediate plant structure, which is easy to take care and avoid strong wind or storm. The anther was observed to be lower than stigma in YT1, YT3 and Moi, which caused high pollen contaminated ability. Fruits of control Moi were erect and small. KR1 had higher yield in comparison with control Chia Voi. Yield of YT2 and YT4 was equivalent to local variety Chia Voi. Besides, Chia Voi and Moi (local varieties), KR1, YT2, and YT4 showed good adaptability under Thua Thien Hue conditions based on horticultural traits. These lines were obtained with high yield and good quality. Therefore, these lines should be introduced to crop system and breeding programs in Thua Thien Hue.

References

[1] Splittstoesser, W. E. 1984. "Growing Individual Vegetables: Peppers." In *Vegetable Growing Handbook.* Connecticut, USA: AVI Publishing Company, Inc., 251-2.

[2] Ha, T. V., and Tran, B. Q. 2004. *Preserving Fresh Vegetables and Sell Products*. Hanoi: Agriculture Publisher. (in Vietnamese)

[3] Mai, A. T. P. 1999. *Techniques on Planting of Important Vegetable.* Hanoi: Agriculture Publication, 15-30 (in Vietnamese).

[4] Tran, T. K., and Nguyen, H. C. 2005. *Techniques on Safe, Clean Vegetables and Processing, Exporting.* Thanh Hoa: Thanh Hoa Publisher. (in Vietnamese)

[5] Ministry of Agriculture and Rural Development. 2011. *Circular No. QCVN 01-64:2011/BNNPTNT of National Technical Regulations on Testing for Value of Cultivation and Use of Hot Pepper and Sweet Pepper Varieties.* (in Vietnamese)

[6] Ministry of Agriculture and Rural Development (MARD). 2011. *Circular No. 48/2011/TT-BNNPTNT of National Technical Regulation on Testing Crop Varieties.* (in Vietnamese)

[7] Duncan, E. B. 1995. "*Multiple Range Test and Multiple F-Tests*." *Biometrics* 11 (1): 1-42.

[8] Peeraullee, N., and Ranghoo-Sanmukhiya, V. M. 2013. "Assessment of Genetic Diversity in Local Chilli (*Capsicum annuum*) Varieties in Mauritius." *Int. J. Agric. Biol*. 15 (5): 891-6.

[9] Chaux, C. I., and Foury, C. I. 1994. "Peppers." In *Vegetables Fruit—Vegetable Productions*. Paris: Lavoisier.

[10] Zhani, K., Hamdi, W., Sedraoui, S., Fendri, R., Lajimi, O., and Hannachi, C. 2015. "A Comparative Study of Morphological Characterization of Tunisian Accessions of Chili Pepper (*Capsicum frutescens* L.)." *International Research Journal of Engineering and Technology* 2 (4): 87-94.

[11] Erard, P., Bellamy, A., Berry, D., Buffière, A., Capy, A., Dumolin, J., Guillon, A., Hutin, C., and Izard, D. 2002. *Pepper*. Paris: CTIFL Publisher. (in France)

[12] Occhiuto, P. N., Peralta, I. E., Asprelli, P. D., and Galmarini, C. R. 2014. "Characterization of *Capsicum* Germplasm Collected in Northwestern Argentina Based on Morphological and Quality Traits." *Agriscientia* 31 (2): 63-73.

[13] Misra, S., Lal, R. K., Darokar, M. P., and Khanuja, S. P. S. 2011. "Genetic Variability in Germplasm Accessions of *Capsicum annuum* L.." *American Journal of Plant Sciences* 2: 629-35.

[14] Prasath, D., Ponnuswami, V., and Muralidharan, V. 2007. "Evaluation of Chilli (*Capsicum spp.*) Germplasm for Extractable Colour and Pungency." *Indian J. Genet*. 67 (1): 97-8.

[15] Pinto, C. M. F., Caliman, F. R. B., Moreira, G. R., Mattos, R. N., Rocha, P. R. R., Venzon, M., and Paula, J. T. J. 2007. "Pimenta (*Capsicum* spp.)." In *101 Cultures: Agricultural Techniques Book,* edited by Paula, J. T. J., and Venzon, M. Belo Horizonte: EPAMIG, 625-32.

[16] Bosland, P. W. 1992. "Chiles: A Diverse Crop." *Hort. Technol*. 2 (1): 6-10.

[17] Bosland, P. W. 1994. "Chillies: History, Cultivation and Uses." In *Species, Herbs and Edible Fungi,* edited by Charambous, G. New York: Elsevier Publication, 347-66.

[18] Patel, J. A., Shukla, M. R., Doshi, K. M., Patel, B. R., and Patel, S. A. 1998. "Combining Ability Analysis for Green Fruit Yield and Yield Components in Chilli (*Capsicum annuum* L.)." *Capsicum and Eggplant Newsletter* 17: 34-7.

[19] Akinci, S., and Akinci, I. E. 2004. "Evaluation of Red pepper for Spice (*Capsicum annuum* L.) Germplasm Resource of Kahramanmaras Region (Turkey)." *Pakistan Journal of Biological Science* 7 (5): 703-10.

[20] Winch, T. 2006. *Growing Food: A Guide to Food Production.* Hereford, UK: Springer.

[21] Montesano, V., Negro, D., Bitonte, D., Montemurro, F.,

De Lisi, A., and Sarli, G. 2014. "Characterization of a *Capsicum annuum* L. of a Germplasm Collection." *International Journal of Agri. Science* 4 (12): 499-508.

[22] Hornero-Méndez, D., and Minguez-Mosquera, M. I. 2000. "Xanthophyll Esterification Accompanying Carotenoid Overaccumulation in Chromoplast of *Capsicum annuum* Ripening Fruits Is a Constitutive Process and Useful for Ripeness Index." *J. Agric. Food Chem*. 48 (5): 1617-22.

[23] Silva, A. R., Nascimento, M., Cecon, P. R., Sapucay, M. J. L. C., Rêgo, E. R., and Barbosa, L. A. 2013. "Path Analysis in Multicollinearity for Fruit Traits of Pepper." *IDESIA (Chile)* 31 (2): 55-60.

[24] Lannes, S. D., Finger, F. L., Schuelter, A. R., and Casali, V. W. D. 2007. "Growth and Quality of Brazilian Accessions of *Capsicum chinense* Fruits." *Scientia Horticulturae* 112 (3): 266-270.

[25] Somashekhar, S. A. P., and Salimath, P. M. 2008. "Estimation of Gene Effects for Fruit Yield and Its Components in Chilli (*Capsicum annuum* L.)." *Karnataka Journal Agricultural Sciences* 21: 181-3.

[26] Schuelter, A. R., Pereira, G. M., Amaral, A. T., and Casali, V. W. D. 2010. "Genetic Control of Agronomically Important Traits of Pepper Fruit Analyzed by Hayman's Partial Diallel Cross Scheme." *Genetics Molecular Research* 9 (1): 113-7.

[27] Grubben, G. J. H., and El-Tahir, I. M. 2004. "*Capsicum annuum* L.." In *Plant Resources of Tropical Africa,* edited by Grubben, G. J. H., and Denton, O. A. Wageningen, Netherlands: Backhuys Publishers, 154-63.

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Identification of Rice Blast Resistance Genes in South Central Coast of Vietnam Using Monogenic Lines under Field Condition and Pathogenicity Assays

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Abstract: In the present study, the authors studied the interaction between 26 monogenic differentials carrying 26 blast resistance genes with 15 isolates of *Pyricularia oryzae* under field conditions and pathogenicity assays. The result in field conditions showed that area under disease progress curves (AUDPC) of monogenic lines was found to range from 0 to 142.3. Lijiangxintuanheigu (LTH) was susceptible in all of regions. The monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz, Pi1*, *Pi7(t)*, *Pik-m*, *Pi4(t)*, *Pish*, *Pi9(t)* and *Pita* were highly resistant to blast in all of regions. In plastic house net conditions, the percentage of virulent reactions of monogenic lines to 15 isolates was found to range from 23.1% to 84.6%. LTH was susceptible to all 15 isolates. All 26 monogenic lines were resistant to at least 3 isolates of *P. oryzae*, and the frequency of resistant reactions of the monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* were 80.0%, 93.3%, 93.3%, 80.0%, 73.3%, 86.7%, 73.3%, 80.0%, 80.0%, 80.0% and 80.0%, respectively. These findings suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* may be important *R* genes for preventing blast disease in South Central Vietnam. Based on these data, a useful strategy for managing rice blast disease by stacking pyramiding blast *R* genes against pathogenic *P. oryzae* isolates at hotspot in South Central Coast of Vietnam was proposed.

Key words: Rice, *P. oryzae*, blast rice, monogenic lines, resistance genes.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops for more than half of the world's population [1]. Rice blast, caused by *Pyricularia oryzae* Cav. (*P. oryzae*), is the most devastating rice disease that occurs all over the world [2]. The yield loss caused by blast vary widely depends on agro-systems and can reach several million tons, 10% to 30% of crops [3]. Under certain environmental conditions and cultural practices, 100% loss was observed [4]. Cultural practices, fungicides and genetic resistance are interchangeably used among different rice production areas worldwide to prevent blast disease [5-8]. Extensive and uncontrolled use of fungicides poses a significant concern for human health and environmental safety. Hence, utilization of multiple *R* genes with over lapped resistance spectra is one of the most powerful strategies for manage blast disease [9-11]. To date, 96 major blast *R* genes and quantitative trait locus (QTLs) have been identified and mapped using DNA markers [12]; 18 of these

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genes have been cloned and used for crop protection [13]. However, the management of rice blast caused by *P. oryzae* through the use of disease resistant cultivars has become difficult because of the highly variable nature of the pathogen as well as favorable environmental conditions for infection during the crop season. The virulence of rice-blast pathogen isolates in a population tends to shift from avirulence to virulence, rather than virulence to avirulence, on a given host [14]. Such variability has made it difficult to identify strategies for selecting or breeding rice cultivars that show durable resistance to blast populations in a given area. Most of the blast-resistant cultivars of rice succumb to the disease within 2-3 years of their introduction into a disease-prone area [15, 16]. Therefore, determining the effectiveness of blast *R* genes in a certain area is important in assisting the development of rice breeding programs, which generate rice with long lasting effective genetic resistance [17].

The monogenic lines have been important assets to identify blast *R* genes [18]. The first international rice blast differential system was developed in the 1960 to determine physiological races of *P. oryzae* [19]. Makill and Boman [20] developed a set of near-isogenic lines (NILs) derived from CO39 with 14 *R* genes, including *Pish*, *Pib*, *Piz-5*, *Pz-t*, *Pi5(t)*, *Piks*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7(t)*, *Pita* and *Pita-2*. In China, a set of NILs was developed with five blast *R* genes *Pib*, *Pik*, *Pik-m*, *Pik-p* and *Pita-2*, using a susceptible japonica-type genetic background, Lijiangxintuanheigu (LTH) [21]. A set of monogenic lines, containing 24 major *R* genes *Pia*, *Pii*, *Pik*, *Pik-s*, *Pik-m*, *Pik-h*, *Pik-p*, *Piz*, *Piz-5*, *Piz-t*, *Pita*, *Pita-2*, *Pib*, *Pit*, *Pish*, *Pi1*, *Pi3*, *Pi5(t)*, *Pi7*, *Pi9*, *Pi11*, *Pi12(t)*, *Pi19* and *Pi20(t)* in the blast-susceptible recurrent japonica variety LTH, was developed by the International Rice Research Institute (IRRI) [22]. This differential set is excellent materials that have been used to identify blast *R* genes worldwide [12, 23].

In Vietnam, rice blast disease is one of the most destructive diseases limiting rice production, because the fungal pathogen can infect and cause lesions on almost all organs of the plant [24]. In 2012, the total area of infection rice blast disease was 366,412 ha, of which 11,400 ha are severe infections, 8 ha are full lost, and reduction in milling yield is about 10%-25% [25]. The South Central Vietnam is within the "hotspot" areas of Vietnamese rice. Blast disease has been found in more than 2,054 ha in South Central Vietnam [25]. Although the major blast genes *Pita*, *Pib* and *Pik-m* are widely used, the effectiveness of these *R* genes at Central Vietnam has not been determined yet.

The present study was conducted to evaluate disease reactions of selected field blast isolates on 26 monogenic differentials, and provide useful information in formulating strategies for improving blast resistance.

2. Materials and Methods

2.1 Plant Materials

A set of blast differential varieties, consisting of 26 monogenic lines with 26 *R* genes (*Pia*, *Pii*, *Pik-s*, *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi2(t)*, *Piz-t*, *Pi4(t)*, *Pib*, *Pit*, *Pish*, *Pi1*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9*, *Pi12(t)*, *Pi19*, *Pik-m*, *Pi20*, *Pita2*, *Pita*, *Pi11(t)* and *Piz5* developed under IRRI was used. The recurrent parent was the blast-susceptible variety LTH, which was used as a susceptible control.

2.2 Field Experiment

2.2.1 Experimental Design

The experiment was conducted in farmer's field at the hotspot in Vietnam South Central Coast, including, Quang Nam (QN), Quang Ngai (QNG) and Binh Dinh (BD) province (Fig. 1). The chosen locations have a long history of strong blast disease presence and are mainly at poor alluvial soil.

The trial was laid down in a randomized complete block design. Each genotype was planted in two rows of 5 m long as an experimental unit to evaluate qualitative

Fig. 1 Different location of field trials at South Central Vietnam.

resistance to blast; distance between rows was 20 cm.

The fertilizers were applied at the rate of 100 kg, 50 kg and 50 kg per ha of N, P_2O_5 and K_2O, respectively. All P_2O_5 were used as basal at the time of sowing. The 30 kg N and 25 kg K_2O was uniformly broadcasted at 12 days after sowing (DAS), 40 kg N was used at 20 DAS and the rest on 45 DAS.

2.2.2 Disease Assessment

Ten plants were randomly (diagonally) selected from each plot (4 m × 2 m) and tagged. Disease scoring started from the 15th day after seeding and periodic survey was made one time every 7 days. The disease severity was calculated as disease severity (%), calculated as Eq. (1):

Disease severity (%) = (sum of all numerical rating)/(total number of leaves or plants observed) × maximum rating × 100 (1)

Disease assessment was scored based on the reaction of the differential varieties/cultivars. The lesions were scored as follows:

0 = no lesion observed;

1 = small brown specks off pinpoint size or large brown specks without sporulation center;

3 = small, roundish to slightly elongated necrotic sporulation spots, about 1-2 mm in diameter with a distinct brown margin or yellow halo;

5 = narrow or slightly elliptical lesions, 1-2 mm in breadth, more than 3 mm long with a brown margin;

7 = broad spindle-shaped lesion with yellow, brown, or purple margin;

9 = rapidly, coalescing small, whitish, grayish, or bluish lesions without distinct margins.

Symptom scores were attributed as average symptom observed in each experimental unit [26].

Area under disease progress curves (AUDPC) was calculated based on the Eq. (2) used by Das et al. [27]:

$$\text{AUDPC} = \sum_{i=1-(n-1)} [(Y_{i+1} + Y_i / 2) \times (t_{i+1} - t_i)] \quad (2)$$

where Y_i is percentage of disease at the ith observation, t_i is time at the ith observation, and n is total number of observation. AUDPC was calculated for each rice cultivar.

2.3 Pathogenicity Assays

2.3.1 Collection, Isolation and Culture Maintenance

A total of 200 samples of leave and panicle neck having blast rice symptom were collected from experimental fields of rice production areas of three provinces at South Central Vietnam (QN, QNG and BD) from 2014 to 2015. Single lesions were placed on glass rods in Petri dishes with wet filter paper and

incubated at 25 °C until sporulation. With a pointed capillary tube, spores will be picked from a single lesion under dissecting microscope and spread onto water agar in Petri dishes. A single spore was transferred on potato dextrose agar slant under a dissecting microscope and incubated from 8 d to 10 d at 26 °C. For maintenance of the isolates obtained from single spore culture, the colony was grown in sterilized filter paper, which was put on potato dextrose agar and incubated for 10 d. After that, only the paper pieces were transferred onto empty Petri plate for drying at 37 °C for 3 d; after drying, they were stored in small bottle with silica gel in a freezer at -10 °C [28]. List of blast isolates is presented in the Table 1.

2.3.2 Test Cultivars

Seedlings of 26 monogenic lines resistance to *P. oryzae* and one susceptible variety LTH were grown in the plastic house net in either plastic pots (25 × 25 × 30 cm) containing 4 kg of soil fertilized with N-P-K (20-20-10 g pot). Ten seeds of each line/cultivar were planted in a plastic pot. Plants were grown plastic house net at 25-30 °C for four weeks, until they reached the four leaf stage. The experiment was conducted in a randomized complete block design with three replications. Four-week-old plants (4-leaf stage) were inoculated with spore suspensions as described in the study of Berruyer et al. [29].

Table 1 ***P. oryzae*** **strains from center of Vietnam used in this study.**

Isolate	Origin	Cultivar
QN49	Quang Nam	BC15
QN95	Quang Nam	Xi23
QN125	Quang Nam	OM4900
QN186	Quang Nam	SV181
QN239	Quang Nam	KD18
QNG63	Quang Ngai	Thom 1
QNG109	Quang Ngai	KD18
QNG158	Quang Ngai	Q5
QNG193	Quang Ngai	BC15
QNG249	Quang Ngai	Thom 1
BD38	Binh Dinh	BC15
BD92	Binh Dinh	Q5
BD128	Binh Dinh	KD28
BD183	Binh Dinh	BC15
BD258	Binh Dinh	KD28

2.3.3 Inoculation and Disease Assessment

Five strains of *P. oryzae* selected in each province were used as representative monoconidial isolates for the present study. Four-week-old plants at the 4-5 leaf stage in each pot were inoculated by spraying 20 mL aqueous spore suspension (1×10^5 spores/mL) onto the leaves. Disease reactions were scored at the 7th days after inoculation on a scale from 0 to 5, in which 0 = no evidence of infection; 1 = brown specks smaller than 0.5-1 mm in diameter; 3 = roundish to elliptical lesions about 1-3 mm in diameter with gray centers and brown margins; 4 = typical spindle-shaped blast lesion, 3 mm or longer with little or no coalescence of lesions; 5 = same as 4, but half of one or more leaves killed by coalescence of lesions. Plants rated 0-3 were considered resistant, and those rated 4-5 were considered susceptible [30, 20]. For blast screening, the scores of five plants for each line were averaged and an actual infection means scales were used in analysis.

3. Results

3.1 Field Experiments

Table 2 showed AUDPC of leaf disease severity in the different monogenic line and at the different regions of the study. AUDPC figures varied among the different cultivars and were different for the three regions.

With respect to AUDPC of leaf disease severity, LTH (susceptible control) showed the highest percentage of spots, ranged from 203.8% to 352.6%, of which the highest is at BD province and the lowest at QN province. LTH was followed by the lines containing *R* genes: *Pia*, *Pii*, *Pik-s*, *Piz-t*, *Pib*, *Pit*, *Pi12(t)*, *Pi11(t)*, *Pi3*, *Piz5* and *Pi20(t)* which showed high disease severity in all of regions, with values ranging between 24.3 % and 142.3%. However, in each location, a single blast *R* gene can be effective in preventing blast disease, for example, *Pib* and *Piz-t* in

Table 2 AUDPC for monogenic lines resistance to *P. oryzae* in the field of Central Vietnam during the spring season of 2014.

Rice line	Gene name	AUDPC (%)		
		Quang Nam	Quang Ngai	Binh Dinh
IRBLa-A	*Pia*	104.6	142.3	105.3
IRBLi-F5	*Pii*	98.3	91.3	95.2
IRBLks-F5	*Pik-s*	93.2	132.7	92.7
IRBLk-ka	*Pik*	0.0	0.0	0.0
IRBLkp-K60	*Pik-p*	0.0	0.0	0.0
IRBLkh-K3	*Pik-h*	0.0	0.0	0.0
IRBLz-Fu	*Piz*	0.0	0.0	0.0
IRBLz5-CA	*Pi2(t)*	78.1	72.4	0.0
IRBLzt-T	*Piz-t*	104.2	94.2	0.0
IRBLta-K1	*Pi4(t)*	28.4	15.8	19.5
IRBLb-B	*Pib*	77.3	82.5	0.0
IRBLt-K59	*Pit*	95.4	92.5	60.4
IRBLsh-S	*Pish*	19.4	21.6	18.5
IRBL1-CL	*Pi1*	0.0	0.0	0.0
IRBL3-CP4	*Pi3*	64.2	68.3	94.6
IRBL5-M	*Pi5(t)*	28.4	82.4	0.0
IRBL7-M	*Pi7(t)*	0.0	0.0	0.0
IRBL9-W	*Pi9(t)*	29.3	27.3	32.4
IRBL12-M	*Pi12(t)*	95.2	87.2	24.3
IRBL19-A	*Pi19*	62.4	68.3	31.5
IRBLkm-Ts	*Pik-m*	0.0	0.0	0.0
IRBL20-IR24	*Pi20(t)*	97.3	132.8	57.3
IRBLta2-Pi	*Pita2*	74.3	72.5	0.0
IRBLta-CP1	*Pita*	22.4	19.3	0.0
IRBL11-Zh	*Pi11(t)*	123.7	27.6	48.5
IRBLz5-CA	*Piz5*	101.7	123.8	112.9
LTH		203.8	310.4	352.6

BD region. The cultivars that showed medium susceptibility were *Pi2(t)*, *Pi19*, *Pita2* and *Pi5(t)* with values ranging from 31.5% - 82.4%. Among them, blast *R* genes are still effective in preventing blast disease in some regions, such as *Pi2(t)*, *Pi5(t)* and *Pita2* in BD province (Table 2). *Pi4(t)*, *Pish*, *Pi9(t)* and *Pita* showed significantly resistance to *P. oryzae*. The highly resistant level to leaf blast in both two season and at all regions was observed in the monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7(t)* and *Pik-m* which with value of zero. This result suggested that effective genes for blast resistance in rice production areas in South Central Vietnam included *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7(t)*, *Pik-m*, *Pi4(t)*, *Pish*, *Pi9(t)* and *Pita.*

3.2 Pathogenicity Assays

3.2.1 Evaluation of Useful Blast *R* Genes

All monogenic lines were resistant to at least one isolate, indicating that the *R* genes they carry will have some impact in preventing blast disease. However, the recurrent parent LTH was susceptible to all tested isolated. Among 26 monogenic lines, monogenic lines carrying *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* showed strong resistance, with the percentages of resistant reactions were 80.0%, 93.3%, 93.3%, 80.0%, 73.3% 86.7%, 73.3%, 80.0%, 80.0%, 80.0% and 80.0%, respectively (Fig. 2). These results suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* are highly effective against blast disease in South Central Vietnam. Furthermore,

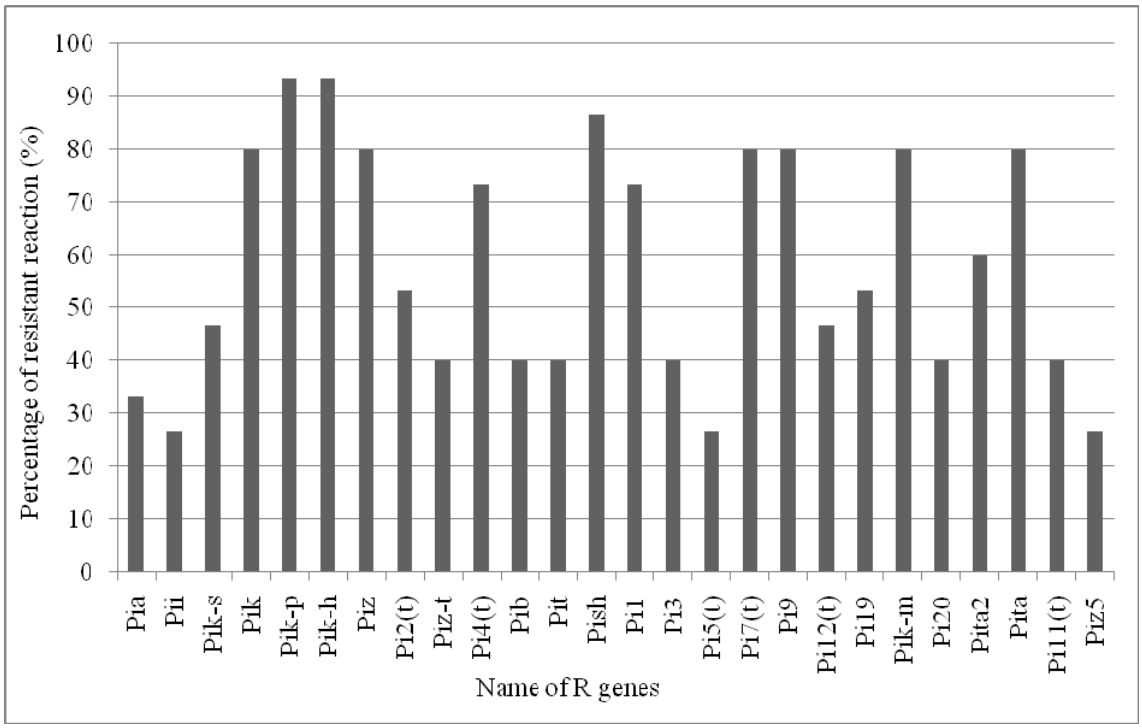

Fig. 2 Percentage of the resistant reaction of 26 monogenic lines containing an *R* gene to isolates of *P. oryzae*.

in each location, a single blast *R* gene can be effective in preventing blast disease. *Pik-h* in QN province; *Pik-p* and *Pish* in QNG province and *Pik*, *Pik-p*, *Piz*, *Pk-h*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m*, *Pita2* and *Pita* in BD province may be adequate to prevent blast disease (Table 3). This result is similar to the result of experiments in the field.

3.2.2 Virulence of *P. oryzae* Isolates

All isolates were virulent to one or more monogenic lines. The frequency of virulence was found to be from 23.1% to 84.6%. The frequency of virulence for isolate BD92 and BD258 was the lowest, which was 23.1%, and the greatest was QN186 which was 84.6% (Fig. 3). Overall, one or two isolates in each region were very virulent on the tested monogenic lines with *R* genes. Notably, the pathogenicity of isolates from QN province was higher than those of the isolates in the other province (Table 3). The most virulent isolates (QN186) also were found in the QN province.

3.2.3 A Strategy for Pyramiding Blast *R* Genes

In the present study, monogenic differentials carrying 26 major blast *R* genes (*Pia*, *Pii*, *Pik-s*, *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi2(t)*, *Piz-t*, *Pi4(t)*, *Pib*, *Pit*, *Pish*, *Pi1*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9*, *Pi12(t)*, *Pi19*, *Pik-m*, *Pi20*, *Pita2*, *Pita*, *Pi11(t)* and *Piz5*) were used to test the efficacy of resistance to 15 isolates from three provinces of South Central Vietnam. As a result, it can be better understand the resistance mediated by a specific *R* gene. Such as, the monogenic variety carrying the *Pi4(t)* gene was demonstrated to prevent infections by *P. oryzae* isolates in the BD rice growing regions, but not in the QN and QNG region. In addition, it was able to identify the effective major *R* gene(s) for different rice growing regions. Based on disease reactions, it is proposed that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pi1*, *Pi7(t)*, *Pi9*, *Pish*, *Pik-m* and *Pita* can be used as *R* gene donors for improved blast resistance in South Central Vietnam (Fig. 2). Futhemore, *Pik-p* and *Pik-h* appears to be resistant to most isolates and can be used as these blast *R* genes to aid in breeding rice variety resistance of blast disease in South Central Vietnam. A combination of *Pik-p* or *Pik-h* with one of

Table 3 Disease reaction of 26 rice monogenic lines with 15 isolates of *P. oryzae*.

R genes	QN 49	QN 95	QN 125	QN 186	QN 239	QNG 63	QNG 109	QNG 158	QNG 193	QNG 249	BD 38	BD 92	BD 128	BD 183	BD 258	Amount of incompatible isolates	Percent of Incompatible isolates (%)
Pia	R	S	S	R	S	S	R	S	S	S	S	S	R	S	R	5	33.3
Pii	S	S	S	S	S	S	S	R	S	R	S	R	S	S	R	4	26.7
Pik-s	S	R	S	S	R	S	R	S	R	S	R	S	R	R	S	7	46.7
Pik	S	R	R	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
Pik-p	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	14	93.3
Pik-h	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	14	93.3
Piz	R	R	R	S	S	R	R	R	R	S	R	R	R	R	R	12	80.0
Pi2(t)	S	R	S	S	R	R	S	S	S	R	R	S	R	R	S	8	53.3
Piz-t	S	R	S	S	R	R	S	S	R	S	S	R	S	S	R	6	40.0
Pi4(t)	S	S	R	R	R	R	S	R	R	S	R	R	R	R	R	11	73.3
Pib	R	S	S	S	S	S	R	S	S	S	R	R	R	R	S	6	40.0
Pit	S	S	R	S	S	R	S	S	S	R	S	R	S	R	R	6	40.0
Pish	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	13	86.7
Pi1	R	S	R	S	S	R	R	R	R	S	R	R	R	R	R	11	73.3
Pi3	S	S	S	S	S	R	R	S	S	S	R	R	R	R	S	6	40.0
Pi5(t)	S	S	R	R	S	S	R	S	S	S	S	S	R	S	S	4	26.7
Pi7(t)	R	S	R	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
Pi9	R	R	R	S	S	R	R	R	S	R	R	R	R	R	R	12	80.0
Pi12(t)	S	S	R	S	R	R	S	S	R	S	S	R	R	S	R	7	46.7
Pi19	S	R	R	S	S	S	R	S	R	R	S	R	S	R	R	8	53.3
Pik-m	R	R	S	S	R	R	R	R	R	S	R	R	R	R	R	12	80.0
Pi20	S	R	S	S	R	S	S	S	R	R	R	S	S	R	S	6	40.0
Pita2	S	S	R	S	R	R	S	S	R	S	R	R	R	R	R	9	60.0
Pita	R	R	R	S	R	R	S	R	R	S	R	R	R	R	R	12	80.0
Pi11(t)	S	S	R	S	R	S	R	S	R	S	S	R	S	S	R	6	40.0
Piz5	S	S	S	S	R	S	R	S	S	S	S	S	R	S	R	4	26.7
Number of virulence isolates	16	12	10	22	10	9	9	14	9	19	9	6	8	7	6		
Percent of virulence isolates (%)	61.5	46.2	38.5	84.6	38.5	34.6	34.6	53.8	34.6	73.1	34.6	23.1	30.8	26.9	23.1		

R = resistant; S = susceptible.

the genes (*Pik*, *Piz*, *Pi4(t)*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita*) can also be effective in preventing blast infections in South Central Vietnam (Table 3).

4. Discussion

In Vietnam, rice blast disease is managed by the use of resistance genes in combination with seed treatments with systemic fungicides and fungicidal foliar sprays. The use of host resistance has proven to be the most effective and economical method for controlling rice blast [31, 11]. It is necessary to know that host resistance, pathogenicity characteristics, including degree of pathogenicity, sporulation and spectrum of pathogenicity, and environmental conditions are the critical factors determining the degree of plant disease [2]. The key step of controlling disease is to know interaction between host resistance and the pathogen. Evaluation of rice germplasm and breeding lines with the selected isolates have been realized worldwide.

The different progenies from various crosses showed the different level of blast disease reaction. Such differential defense responses result from the interaction of dominant and resistant (*R*) gene of host

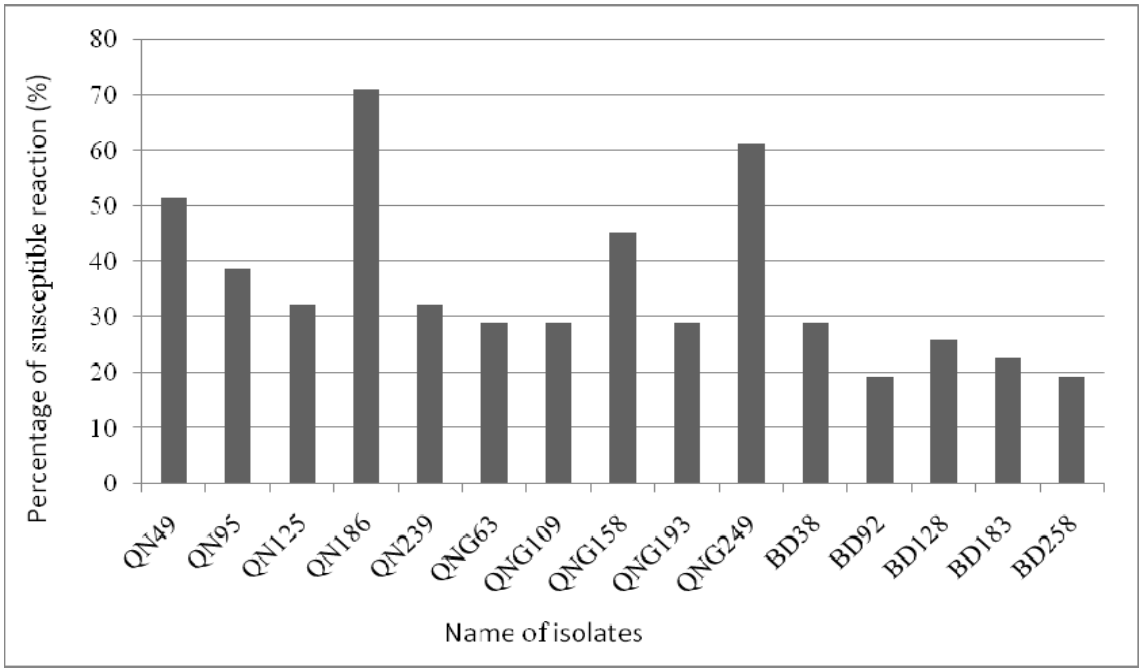

Fig. 3 Virulence frequency of the 15 *P. oryzae* isolates.

and avirulence (*AVR*) genes of pathogen as proposed gene-for-gene hypothesis [32]. In rice, the interaction between host plant and pathogen *P. oryzae* is well documented as gene-for-gene system [6]. The resistance and susceptible interaction on rice conferred by single amino acid substitution in *Pi-ta* leucine rich domain (LRD) or in the *AVR-Pi-ta* protease motif, result in loss of resistant in plant. Thus, the different genetic of rice lines used in this study showed different interaction host resistance and pathogen blast rice in South Central Vietnam.

In this study, the differential system consisting of 26 differential *R* genes and a universal susceptible LTH were used for rapid identification of the resistance spectra of *R* genes in field condition and greenhouse conditions in South Central Vietnam. Inoculation and evaluation were repeated three times, and the disease reaction of each variety was based on a standard of IRRI [26] and the method of Bastiaans [30]. The authors' results suggest that *Pik*, *Pik-p, Pik-h*, *Piz*, *Pi4(t)*, *Pi1*, *Pi7(t)*, *Pish*, *Pi9*, *Pik-m* and *Pita* are the most effective blast *R* genes in South Central Vietnam.

In the present study, 15 isolates were selected to represent the genetic diversity of rice blast populations at South Central Vietnam. Therefore, these results should provide a systematic way to map *R* genes in breeding parents and assist in the development of appropriate breeding strategies for achieving effective management of the blast disease. To our knowledge, this was the first identification of blast *R* genes for rice breeding in a specific rice growing area in South Central Vietnam.

5. Conclusions

Among 26 monogenic lines carrying 26 *R* genes evaluated on farmers field condition, seven monogenic lines containing genes *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi1*, *Pi7 (t)* and *Pik-m* are highly resistant, four lines (*Pi4(t)*, *Pish*, *Pi9(t) and Pita*) are moderately resistant, four lines are moderately susceptible and 11 lines were susceptible. Similarly, on greenhouse condition, 10 monogenic lines carrying genes *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* were high resistant with the percentages of resistant reactions were 80.0%, 93.3%, 93.3%, 80.0%,

73.3% 86.7%, 73.3%, 80.0%, 80.0%, 80.0% and 80.0%, respectively. These findings suggest that *Pik*, *Pik-p*, *Pik-h*, *Piz*, *Pi4(t)*, *Pish*, *Pi1*, *Pi7(t)*, *Pi9*, *Pik-m* and *Pita* may be useful for preventing blast disease in South Central Vietnam. A concerted effort should be made to incorporate these effective genes into adapted backgrounds and to develop gene combinations that can provide broad-based durable resistance to blast rice.

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References

[1] Khush, G. S. 2005. "What It Will Take to Feed 5.0 Billion Rice Consumers in 2030." *Plant Molecular Biology* 59 (1): 1-6.

[2] Ou, S. H. 1980. "Pathogen Variability and Host Resistance in Rice Blast Disease." *Annu. Rev. Phytopathol.* 18: 167-87.

[3] Valent, B. 1990. "Rice Blast as a Model System for Plant Pathology." *Phytopathology* 80 (1): 33-6.

[4] Cruz-Vazquez, C., Ramos-Parra, M., Itela-Mendoza, I., Garcia-Vazquez, Z., and Quintero-Martinez, M. T. 2007. "Relationships between Stable Fly Infestation with Some Physical Facility Characteristics and Sanitation Practices in Several Dairy Farms in the State of Aguascalientes, Mexico." *Vet. Parasitol.* 149 (3-4): 246-50.

[5] Webster, R. K., and Gunnell, P. S. 1992. *Compendium of Rice Diseases.* St. Paul, MN: American Phytopathological Society.

[6] Silue, D., Notteghem, D. J., and Tharreau, D. 1992. "Evidence for the Gene for Gene Relationship in the *Oryza sativa-Magnaporthe grisea* Pathosystem." *Phytopath*. 82 (5): 577-80.

[7] Long, D. H., Lee, F. N., and TeBeest, D. O. 2000. "Effect of Nitrogen Fertilization on Disease Progress of Rice Blast on Susceptible and Resistant Cultivars." *Plant Disease* 84 (4): 403-9.

[8] TeBeest, D. O., Guerber, C., and Ditmore, M. 2007. "Rice Blast." The Plant Health Instructor. The American Phytopathological Society. Accessed March, 2007. http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/RiceBlast.aspx.

[9] Ou, S. H. 1995. *Rice Diseases,* 2nd ed.. Kew, England: Commonwealth Mycological Institute, 380.

[10] Bonman, J. M., Khush, G. S., and Nelson, R. J. 1992. "Breeding Rice for Resistance to Pests." *Ann. Rev. Phytopathology* 30: 507-28.

[11] Sharma, T. R., Rai, A. K., Gupta, S. K., Vijayan, J., Devanna, B. N., and Ray, S. 2012. "Rice Blast Management through Host-Plant Resistance: Retrospect and Prospects." *Agric. Res*. 1 (1): 37-52.

[12] Koide, Y., Telebanco-Yanoria, M. J., Peña, F. D., Fukuta, Y., and Kobayashi, N. 2011. "Characterization of Rice Blast Isolates by the Differential System and Their Application for Mapping Resistance Gene, *Pi19(t)*." *J. Phytopathol*. 159 (2): 85-93.

[13] RoyChowdhury, M., Jia, Y., Jackson, A., Jia, M. H., Fjellstrom, R., and Cartwright, R. D. 2012. "Analysis of Rice Blast Resistance Gene *Pi-z* in Rice Germplasm Using Pathogenictiy Assays and DNA Markers." *Euphytica* 184 (1): 35-46.

[14] Lau, G. W., and Ellingboe, A. H. 1993. "Genetic Analysis of Mutations to Increased Virulence in *Magnaporthe grisea*." *Phytopathology* 83 (10): 1093-6.

[15] Kiyosawa, S. 1982. "Genetics and Epidemiological Modeling of Breakdown of Plant Disease Resistance." *Annual Review of Phytopathology* 20: 93-117.

[16] Bonman, J. M., and Mackill, D. J. 1988. "Durable Resistance to Rice Blast Disease." *Oryza* 25: 103-10.

[17] Chen, Q. H., Wang, Y. C., and Zheng, X. B. 2006. "Genetic Diversity of *Magnaporthe grisea* in China as Revealed by DNA Fingerprint Heliotypes and Pathotypes." *Phytopathology* 154 (6): 361-9.

[18] Jia, Y., and Moldenhauer, K. 2010. "Development of Monogenic and Digenic Rice Lines for Blast Resistance Genes *Pi-ta*, *Pi-kh*/*Pi-ks*." *J. Plant Regist*. 4 (2): 163-6.

[19] Atkins, J. G., Robert, A. L., Adair, C. R., Goto, K., Kozaka, T., Yanagida, R., Yamada, M., and Matsumoto, S. 1967. "An International Set of Rice varieties for Differentiating Races of *Pyricularia oryzae.*" *Phytopathology* 57: 297-301.

[20] Mackill, D. J., and Bonman, J. M. 1992. "Inheritance of Blast Resistance in Near-Isogenic Lines of Rice." *Phytopathology* 82 (7): 746-9.

[21] Ling, Z. Z., Mew, T. W., Wang, J. L., Lei, C. L., and Huang, N. 2001. "Development of Chinese Near Isogenic Lines of Rice and Their Differentiating Ability of Pathogenic Races of Blast Fungus." *Chin. Agric. Sci.* 1: 50-6.

[22] Telebanco-Yanoria, M. J., Ohsawa, R., Senoo, S., Kobayashi, N., and Fukuta, Y. 2008. "Diversity Analysis

[23] for Resistance of Rice (*Oryza sativa* L.) to Blast Disease (*Magnaporthe grisea* (Hebert) Barr.) Using Differential Isolates from the Philippines." *Plant Breed.* 127 (4): 355-63.

[24] Wang, J. C., Jia, Y., Wen, J. W., Liu, W. P., Liu, X. M., Li, L., Zhang, J. H., Jiang, Z. Y., Guo, X. L., and Ren, J. P. 2013. "Identification of Rice Blast Resistance Genes Using International Monogenic Differentials." *Crop Prot.* 45: 109-16.

[25] Le, M. T., Tsutomu, A., and Tohru, T. 2010. "Population Dynamics and Pathogenic Races of Rice Blast Fungus, *Magnaporthe oryzae* in the Mekong Delta in Vietnam." *J. Gen. Plant Pathol*. 76 (3): 177-82.

[26] Bureau of Statistics, Ministry of Agriculture and Rural Development (MARD). 2013. *Statistical Yearbook.* Vietnam: MARD.

[27] International Rice Research Institute (IRRI). 1996. *Standard Evaluation System for Rice.* Genetic Resources Centre, Manila, Philippines.

[28] Das, M. K., Rajaram, S., Mundt, C. C., and Kronstad W. E. 1992. "Inheritance of Slow Rusting Resistance to Leaf Rust in Wheat." *Crop Sci.* 32 (6): 1452-6.

[29] Filippi, M. C., and Prabhu, A. S. 2001. "Phenotypic Virulence Analysis of Isolates from Brazilian Upland Rice Cultivars." *Pesqu Agropecu Bras* 36 (1): 27-35.

[30] Berruyer, R., Adreit, H., Milazzo, J., Gaillard, S., Berger, A., Dioh, W., Lebrun, M. H., and Tharreau, D. 2003. "Identification and Fine Mapping of *Pi33*, the Rice Resistance Gene Corresponding to the *Magnaporthe grisea* Avirulence Gene *ACE1*." *Theor. Appl. Genet*. 107 (6): 1139-47.

[31] Bastiaans, L. 1991. "Ratio between Virtual and Visual Lesion Size as a Measure to Describe Reduction in Leaf Photosynthesis of Rice Due to Leaf Blast." *Phytopathology* 81 (6): 611-5.

[32] Bonman, J. M. 1992. "Durable Resistance to Rice Blast Disease: Environmental Influences." *Euphytica* 63 (1): 115-23.

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Evaluation on Agronomical Characteristics of F1 Hybrid Tomato Lines in Spring-Summer Season 2015 in Thua Thien Hue

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Abstract: The four F1 hybrid tomato lines (CT2011, CW2011, TS2011, and CLN2011) and two commercial F1 hybrid tomato cultivars used as control (TN52 and TN561) were evaluated for yield and yield components. The efforts were made to minimize reliance on the import of hybrid seed. The experiment was laid out in randomized complete block design (RCBD) with three replications in spring-summer season 2015. Data was recorded on five plants for each replication. The results indicated that all of F1 tomato hybrid lines grew well under Thua Thien Hue condition. CT2011 had the highest plant height, followed by TS2011 and CLN2011. CT2011 is a cherry type and obtained the highest percentage of fruit setting (82.87%) and the highest of Brix (4.97). This line also had high yield (70.91 tons/ha). TN52 had the highest yield (80.69 tons/ha) among the processing tomato lines, and TN561 had the biggest fruit (90.1 g). All of F1 hybrid tomato lines had high resistant level to late blight and bacterial wilt diseases.

Key words: Hybrid, bacterial wilt, late blight, *Solanum lycopersicum*, Thua Thien Hue.

1. Introduction

Tomato is one of important *Solanum* crops occupying 14% of world vegetable production in 2010 [1]. It is popular fruit vegetable and gives high fresh consumption in many countries, including Vietnam, because of its high nutrition and good adaptability under different agro-climatic conditions. Rick [2] indicated that in the US, tomatoes are ranked the first among fruits and vegetables by abundant sources of vitamins and minerals. In addition, tomatoes are rich of lycopene, a phytochemical that protects cells from oxidants [3]. Tomato is one of the most expected vegetables, not only rich nutrients but also high economic efficiency allows be cultivating and investing commonly in the central provinces of Vietnam, including Thua Thien Hue.

Due to high demand of tomatoes during entire year, enhancing tomato production and productivity are needed. Moreover, consumers are concerning about food safety due to pesticide residues on products. Thus, to restrict abuse of pesticides, the disease resistance cultivars are needed. Thus, many breeding programs aim to select F1 lines having good characteristics as well as resistant ability, high yield and good quality. Choudhary et al. [4] emphasized extensive utilization of heterosis to increase tomato production. In addition, Yordanov [5] indicated that the heterosis manifestation in tomato is in the form of the faster growth and development and increased productivity. However, farmers are used to use old cultivars or import new hybrid tomatoes for sowing next season, which caused qualitative decreasing,

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degeneration and high cost. In this study, the authors evaluated four F1 hybrid tomato lines, which were made at National Institute of Horticultural and Herbal Science (NIHHS), Korea, for their agronomical characteristics under Thua Thien Hue condition.

2. Materials and Methods

2.1 Materials

The four F1 hybrid tomato lines, namely CT2011, CW2011, TS2011 and CLN2011, which were made at NIHHS, Korea in 2011, and two commercial F1 tomato cultivars as control, namely TN52 and TN561, which are commonly used in Thua Thien Hue, were used in this study (Table 1).

2.2 Methods

The experiment was conducted during spring-summer season from January to May in 2015 at Agronomy Faculty of Hue university of Agriculture and Forestry. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each plot was 1.2 m^2 containing six plants for each replication. Tomato population was obtained of 50,000 plants/ha. The distance between row to row was 50 cm and the distance between plant to plant was 40 cm. Seeds were sown in a plastic tray. When the seedlings grew to reach 5-6 true leaves, they were ready to transplant into experimental field. Taking care methods were applied regularly and uniformly to keep plant health. According to the guidelines of the national technical regulations QCVN01-63:2011/BNNPTNT of the Ministry of Agriculture and Rural Development, the relevant field techniques for this study were applied.

Table 1 List of tomato lines used.

No.	Name of hybrid	Place of collection
01	CT2011	NIHHS
02	CW2011	NIHHS
03	TS2011	NIHHS
04	CLN2011	NIHHS
05	TN52	Thua Thien Hue
06	TN561	Thua Thien Hue

The data parameters were saved on quantitative and qualitative characteristics in open field. Data included day to finish growth stages (day); vegetative characteristic, such as growth type, plant height, number of nodes and height from foot to the first flower, length and width of leaf (cm); morphological traits on leaf and fruit, as shape, color or firmness, flower number per bunch, percentage of flowering, fruit size (length, diameter, wall thickness…); yield components and pest situation. The data of five plants per experimental plot were recorded regularly. The Statistic 10.0 trial version was used to analyze analysis of variance (one-way ANOVA) and compare differences between means values by Duncan's test at $P < 0.05$ [6].

3. Results and Discussion

3.1 Morphological and Vegetative Characteristic of Plant

Depending on the ability of growth and branching, growth types of tomatoes expressed different as indeterminate, determinate or semi-determinate. Growth habit of the F1 hybrids tomato lines was indeterminate or semi-determinate. CT2011, TS2011 and CLN2011 had indeterminate growth, and the others had semi-determinate (Table 2). Leaf shape included two types; potato leaf was found in CT2011, TN52 and TN561 hybrids, and regular leaf was found in the remains. Leaf color of tomato lines changed from green to dark green. Leaves of CT2011 and CW2011 had dark green color, TS2011 and CLN2011 had grey green color, and TN52 and TN561 had green color.

Significant difference in plant height was observed among F1 tomato hybrid lines. The highest plant height was obtained in CT2011 (277.13 cm), whereas the shortest plant height was observed in CW2011 (134.7 cm). The plant height of the remains ranged from 149.80 cm to 263.73 cm. Singh et al. [7] had evaluated of tomato hybrid (*Lycopersicon esculentum* Mill.) in Allahabad agro-climatic conditions during

Table 2 Vegetative traits of F1 hybrid tomato lines.

No.	Hybrids	Growth type	Leaf shape	Leaf color	Plant height (cm)	No of nodes from foot to 1st flower (node)	Height from foot to 1st flower (cm)	Leaf length (cm)	Leaf width (cm)
01	CT2011	I	PL	DG	277.13[a]	11.80[a]	52.47[b]	41.80[ab]	27.96[a]
02	CW2011	S-D	RL	DG	134.70[c]	11.13[ab]	64.67[a]	44.84[a]	31.60[a]
03	TS2011	I	RL	GG	263.73[a]	10.00[bc]	57.40[b]	43.28[ab]	28.41[a]
04	CLN2011	I	RL	GG	216.37[ab]	10.65[ab]	58.70[ab]	43.80[a]	29.51[a]
05	TN52	S-D	PL	Gr	149.80[bc]	9.60[bc]	45.73[c]	40.28[ab]	33.27[a]
06	TN561	S-D	PL	Gr	171.00[bc]	8.47[c]	39.80[c]	37.27[b]	30.65[a]
$LSD_{0.05}$					80.36	1.70	6.25	6.42	6.33

I = indeterminate, S-D = semi-determinate, PL = potato leaf, RL = regular leaf, DG = dark green, GG = grey green, Gr = green.
[a, b, c] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

Table 3 Flower and fruit characteristics of F1 tomato hybrid lines.

No.	Hybrid	Inflorescence type	No. of flowers per bunch (flower)	Percentage of fruiting (%)	No. of seeds
01	CT2011	Compound dichasium	8.61[a]	82.87[a]	78.87[abc]
02	CW2011	Compound dichasium	7.67[ab]	41.57[b]	82.63[ab]
03	TS2011	Compound dichasium	7.40[bc]	38.27[b]	30.37[d]
04	CLN2011	Compound dichasium	7.12[bc]	45.98[b]	51.13[cd]
05	TN52	Compound dichasium	7.31[bc]	41.07[b]	89.17[a]
06	TN561	Compound dichasium	6.45[c]	46.80[b]	55.27[bcd]
$LSD_{0.05}$			1.09	13.88	30.23

[a, b, c] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

the winter (rabi) season of 2011, and found that the best on the basic of plant height was 83.67 cm.

Number of nodes to first flower was significantly different between CT2011 and TN561 and expressed narrow variation. CT2011 had the highest number of nodes (11.80 nodes), whereas TN561 had the lowest number of node (8.47 nodes). There was significant difference in height from foot to first flower among F1 hybrid tomato lines. CW2011 had the longest (64.67 cm), and the shortest was observed in TN561 (39.80 cm). Leaves of CW2011 were the longest (44.84 cm), and the shortest one was control TN561 (37.27 cm). There was no significant difference about leaf width among F1 tomato hybrids lines. It ranged from 27.96 cm to 33.27 cm (Table 2).

3.2 Flowering Ability

Inflorescence type of all tomato lines was compound dichasium (Table 3). Significant differences were detected in number of flowers per bunch among F1 tomato hybrid lines. It ranged from 6.45 to 8.61 flowers per bunch. The highest number of flowers was found in CT2011 (8.61 flowers) and significantly different from the others, except CW2011 (7.67 flowers). The number of flowers of control TN561 was observed to be the lowest (6.45 flowers), followed by CLN2011, TN52 and TS2011 hybrids with 7.12, 7.31 and 7.40 flowers, respectively. The highest fruiting rate was observed in CT2011 (82.87%) and obviously different significance from the remains. Percentage of fruiting of TN561 was 46.80%, which was similar to CLN2011 (45.98%). The lowest percentage of fruiting was observed in TS2011 (38.27%). The analyzed data showed large variation on number of seeds. TN52 revealed the highest number of seeds per fruit (89.17 seeds), next were CW2011 (82.63 seeds) and CT2011 (78.87 seeds). Number of seeds per fruit of the remains ranged from 30.37 seeds to 55.27 seeds.

3.3 Fruit Morphological and Qualitative Characteristics

The morphological characteristics were described by one time observation and recorded in Table 4. Almost fruits of the F1 hybrid tomato lines had medium firmness, excepted for CT2011, which had soft fruits. Color of fruit shoulder changed from light green to dark green among F1 hybrids tomato lines. Fruits of CW2011, TS2011 and TN52 had light green shoulder before ripening. Dark green was observed in TN561. Fruits of CT2011 and CLN2011 had green shoulder. At maturity stage, all of F1 hybrid tomato lines revealed red fruit color. However, fruit flesh color was different between lines. Red color was observed in CT2011 and CLN2011, whereas orange-yellow was in CW2011, TS2011 and TN52. Fruit of TN561 had pink fruit flesh. The fruit shape is dependent on genetic and may distinguish between varieties. Recorded data described four type of fruit bottom shape, such as flat, point, pointless and depress. TN52 and TN561 had depressed fruit bottom, whereas CT2011 and CLN2011 had flat fruit bottom. Fruit bottom was pointed in CW2011 and pointed less in TS2011. Wetness of flesh and firmness of fruit are related. Only CT2011 obtained soft fruit, because it had wet flesh. Therewith, fruits of TS2011 and TN52 had wet flesh. Mild dry was found in the remains. Tasty of fruits of CT2011 was mild sour, and sour in TS2011, CLN2011 and TN52. Tasteless was found in CW2011 and TN561.

Fruit quality was described by parameters, including diameter, length, pericarp thickness, number of locules and Brix, and presented in Table 5. There was significant difference in fruit diameter among F1 tomato hybrid lines. TS2011 had the widest fruit (5.53 cm), followed by CLN2011 (5.47 cm), TN52 (5.44 cm) and TN561 (5.19 cm). The smallest fruit width was observed in CT2011 (2.84 cm). While Singh et al. [7] had reported that the tomato hybrid (*Lycopersicon esculentum* Mill.) line that well adapted in Allahabad agro-climatic conditions during the winter (rabi) season of 2011 had fruit diameter 6.47-6.65 cm (polar and radial), Farooq et al. [8] had reported that fruit diameter of tomato hybrid line ranged from 5.49 cm to 7.89 cm. Thus, significant differences in fruit diameter were observed in this study and previous studies [8, 9].

Table 4 Fruit morphological traits.

No.	Hybrid	Firmness	Color of fruit shoulder	Fruit color at maturity	Fruit flesh color	Shape of bottom fruit	Wetness (flesh)	Tasty
01	CT2011	Soft	Green	Red	Red	Flat	Wet	Mild sour
02	CW2011	Medium	Light green	Red	Orange-yellow	Point	Mild dry	Tasteless
03	TS2011	Medium	Light green	Red	Orange-yellow	Point less	Wet	Sour
04	CLN2011	Medium	Green	Red	Red	Flat	Mild dry	Sour
05	TN52	Medium	Light green	Red	Orange-yellow	Depress	Wet	Sour
06	TN561	Medium	Dark green	Red	Pink	Depress	Mild dry	Tasteless

Table 5 Fruit qualitative characteristics.

No.	Hybrid	Fruit diameter (cm)	Fruit length (cm)	Fruit firmness (mm)	No. of locules (locular)	Brix
01	CT2011	2.84^{c}	2.57^{c}	3.00^{c}	2.40^{d}	4.97^{a}
02	CW2011	5.09^{b}	4.89^{b}	4.73^{b}	4.43^{a}	2.95^{d}
03	TS2011	5.53^{a}	5.27^{a}	6.02^{a}	3.90^{abc}	3.35^{bc}
04	CLN2011	5.47^{ab}	5.11^{ab}	6.12^{a}	4.43^{a}	3.18^{cd}
05	TN52	5.44^{ab}	5.22^{a}	5.99^{a}	3.60^{bc}	3.45^{bc}
06	TN561	5.19^{ab}	5.06^{ab}	6.25^{a}	3.23^{c}	3.51^{b}
$LSD_{0.05}$		0.41	0.27	0.71	0.81	0.27

[a, b, c] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

Fruit length had wide variation among tomato lines ranged from 2.57 cm to 5.27 cm. TS2011 produced the longest fruit length (5.27 cm) and CT2011 had the shortest fruit length (2.57 cm). Fruit length of CLN2011, TN52 and TN561 lines were alike by 5.11, 5.22 and 5.06 cm, respectively.

Fruit firmness varies significantly among the hybrids. Fruit firmness of CT2011 was the lowest (3.00 mm), next was CW2011 (4.73 mm) and they are significantly different from the others. The fruit firmness of the remains ranged from 5.99 mm to 6.25 mm. The fruit firmness in this study was higher than those in study of Farooq et al. [8], similar to fruit firmness of Chaudhry et al. [10] and correlated with the studies of Kanno and Kanno [11] and Hall [12] who also observed significant differences in fruit firmness in different cultivars of tomato.

A significant difference was observed among tomato hybrids for number of locules. Number of locules showed wide variation from 2.40 locules to 4.43 locules. CW2011 and CLN2011 had the highest number of locules (4.43), whereas CT2011 had the lowest number of locules (2.40). The result in this study is similar with those in study of Farooq et al. [8] and correlated with the study of Kartofel [13] who obtained varied number of locules in different tomato cultivars.

There was significant difference in Brix among F1 tomato hybrid lines. The results indicated the highest Brix was found in CT2011 (4.97) and significantly different from the others. The lowest Brix was observed in CW2011 (2.95).

3.4 Yield Components and Yield

Yield and yield components of pepper were influenced by conditions of growth and cultivation, and potential of cultivar. Fruit weight and number of fruits per plant are relevant components to use as selection criteria for improving pepper yield. Fruits of almost F1 hybrid tomato lines were obviously different (Table 6). Fruits of CT201 were the smallest (16.47 g), which is belonged to cherry tomato. The remains ranged from 74.41 g to 90.1 g, which are belonged to processing tomato. TN561 had the biggest fruits (90.10 g), next was TN52 (87.64 g) and TS2011 (82.96 g). Chaudhry et al. [14] evaluated 10 tomato hybrids under plastic tunnel. The maximum fruit weight of 167.11 g was observed in Carmello. Chaudhry et al. [15] evaluated six indeterminate tomato hybrids under plastic tunnel, and Carmello gave the highest fruit weight of 163.33 g. Farooq et al. [8] had evaluated 17 tomato hybrids under plastic tunnel and got the result that the maximum individual fruit weight of 170.63 g was recorded in NTT-05-08. Singh et al. [7] had reported that fruit weight of the tomato hybrid (*Lycopersicon esculentum* Mill.) line which was well adapted in Allahabad agro-climatic conditions during the winter (rabi) season of 2011 was 72.27 g. The fruit weight of tomato hybrid line in this study is similar with previous studies.

There was significant difference in number of fruit per plant between CT2011 and the others. Only cherry type tomato hybrid CT2011 produced the highest number of fruits per plant (82.87 fruits), whereas the

Table 6 Yield and yield components.

No.	Hybrid	Fruit weigh (g)	No. of fruit per plant	Yield (ton/ha)
01	CT2011	16.47^{e}	82.87^{a}	70.91^{ab}
02	CW2011	74.41^{d}	41.57^{b}	39.03^{bc}
03	TS2011	82.96^{c}	38.27^{b}	38.39^{bc}
04	CLN2011	75.09^{d}	45.97^{b}	22.67^{c}
05	TN52	87.64^{b}	41.07^{b}	80.69^{a}
06	TN561	90.10^{a}	46.80^{b}	40.95^{bc}
$LSD_{0.05}$		2.40	13.88	36.92

[a-e] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

Table 7 Level of damage by diseases and insect.

No.	Hybrids	Percentage of wilted plant by bacteria wilt disease (%)	Index of late blight disease (%)	Percentage of harmed fruit by fruitworm (%)
01	CT2011	20.00[a]	25.33[b]	48.00[a]
02	CW2011	6.67[ab]	26.67[b]	6.67[b]
03	TS2011	0.00[b]	18.67[b]	9.33[b]
04	CLN2011	6.67[ab]	29.33[b]	20.67[b]
05	TN52	20.00[a]	78.67[a]	18.67[b]
06	TN561	20.00[a]	72.00[a]	56.67[a]
$LSD_{0.05}$		18.75	16.09	22.91

[a, b] Different letters in each columns indicate significantly different means (Duncan's test, $P < 0.05$).

remains had number of fruit per plant ranged from 38.27 to 46.80. Chaudhry et al. [16] evaluated 12 indeterminate tomato hybrids and reported that cherry hybrid gave significantly higher number of fruits per plan. While Singh et al. [7] had reported that the tomato hybrid (*Lycopersicon esculentum* Mill.) line that well adapted in Allahabad agro-climatic conditions during the winter (rabi) season of 2011 had 22 of fruits per plant, Farooq et al. [8] reported that fruit number of tomato hybrid ranged from 11.75 to 30.26. Thus, fruit number in this study is in agreement with previous studies.

TN52 had the highest yield (80.69 tons/ha), followed by CT2011 (70.91 tons/ha). Yield of the remains ranged from 22.67 tons/ha to 40.95 tons/ha, and were statistically at par with each other. CLN2011 produced the lowest actual yield (22.67 tons/ha), followed by TS2011 (38.39 tons/ha). Thus, among processing tomato type, control hybrid variety TN52 have the highest yield (80.69 tons/ha), followed by control TN561 (40.95 tons/ha), but there is no significant difference with the rest of hybrid tomato lines. Farooq et al. [8] have evaluated 17 tomato hybrids which had yield ranging from 34.75 tons/ha to 71.85 tons/ha. It is thus concluded that CT2011 had high yield, good quality and should be grown under local condition for cherry tomato.

3.5 Pest Situation

All of four new F1 hybrid tomato lines had high resistant level to bacteria wilt and late blight disease. Bacterial wilt caused by *Ralstonia solanacearum* is one of the most damaging soilborne diseases of many important crops worldwide. The results in Table 7 showed that almost F1 hybrid tomato lines were affected slightly by bacteria wilt disease, except for TS2011. CT2011, TN52 and TN561 had 20% of infected plants, whereas CW2011 and CLN2011 had 6.67% of infected plants. The winter-spring season is considered as appropriate environmental conditions (temperature, humidity, sun…) for late blight (*Phytopthora infestan*) development. Late blight disease was observed in all of F1 tomato hybrid lines. Index of late blight disease ranged from 18.67% to 78.67%. TN52 and TN561 had the highest susceptible level to late blight, up to 78.67% and 72.00%, respectively. The remains were infected at low level as 29.33% in CLN2011, 26.67% in CW2011 and 25.33% in CT2011. Fruitworm (*Helicoverpa zea*) damages tomato at fruiting development stages. Percentage of harmed fruit by fruitworm ranged from 6.67% to 56.67% among F1 tomato hybrid lines. Of these, TN561 had the highest damaged level (56.67%), followed by CT2011 (48%).

4. Conclusions

All F1 tomato hybrid lines grew well under Thua Thien Hue conditions. CT2011, TS2011 and TN561 lines were considered as displaying good growth and development ability. Fruits of TS2011, CLN2011 and TN52 lines were suit for transporting and process. CT2011 is a cherry tomato, which had small fruit and high Brix, and good for fresh food and decoration. TS2011 had higher predicted yield than TN52 and

TN561. CT2011 produced higher actual yield than TN561. Almost new F1 hybrid tomato lines had high resistant level to bacteria wilt and late blight diseases. The F1 tomato hybrid lines gave good characteristics and should be designed in next experiments in different seasons and ecological areas to assess their potentiality to complete crop structure.

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References

[1] Food and Agriculture Organization of the United Nations (FAO). 2010. *Plant Genetic Resource for Food and Agriculture.* Rome: FAO.

[2] Rick, C. M. 1980. "Tomato." In *Hybridization of Crop Plants,* edited by Fehr, W. R., and Hadley, H. H. Madison, USA: Am. Soc. Agron./Crop Sci. Soc. Am., 669-80.

[3] Giovannucci, E. 1999. "Tomatoes, Tomato Based Products, Lycopene and Cancer; Review of the Epidemiologic Literature." *J. Natl. Cancer Inst.* 91 (4): 317-31.

[4] Choudhary, B., Punia, R. S., and Sangha, H. S. 1965. "Manifestation of Hybrid Vigour in F1 and Its Correlation in F2 Generation of Tomato (*Lycopersicon esculentum* Mill.)." *Indian J. Hort.* 22: 52-9.

[5] Yordanov, M. 1983. "Heterosis in Tomato." In *Monograph on Theoretical* and *Applied Genetics 6,* edited by Frankel, R. Berlin: Springer-Verlag, 189-219.

[6] Duncan, D. B. 1995. "Multiple Range Test and Multiple *F*-Tests." *Biometrics* 11 (1): 1-42.

[7] Singh, A., Singh, A. C., and Prasad, V. M. 2013. "Evaluation of Tomato Hybrid (*Lycopersicon esculentum* Mill.) in Allahabad Agro Climatic Conditions." *Trends in Biosciences* 6 (6): 744-6.

[8] Farooq, M., Ullah, H., Nawab, N. N., and Qureshi K. M. 2013. "Evaluation of Indigenous Tomato Hybrids under Plastic Tunnel." *Pakistan J. Agric. Res.* 26 (2): 97-103.

[9] Chaudhry, M. F., Khokhar, M. A., Khokhar, K. M., Jeelani, G., and Mahmood, T. 2006. "Off Season Production and Correlation Studies of Tomato Hybrids under Plastic Tunnel." *Sarhad J. Agric.* 22 (2): 237-9.

[10] Chaudhry, M. F.,. Khokhar, K. M, Hussain, S. I., Mahmood, T., and Iqbal, S. M. 1999. "Comparative Performance of Some Local and Exotic Tomato Cultivars during Spring and Autumn Seasons." *Pakistan J. Arid Agric.* 2 (2): 7-10.

[11] Kanno, T., and Kamimura, S. 1985. "Studies on Fruit Firmness Structural Factors and Quality in Tomato." *Bull. Veg. and Ornamental Crop Station B. (Morioka)* 5: 27-41.

[12] Hall, C. B. 1987. "Firmness of Tomato Fruit Tissues According to Cultivars and Ripeness." *J. Amer. Soc. Hort. Sci.* 112 (4): 663-6.

[13] Kartofel, 1993. "New Tomato Hybrids." *Hort. Absts.* 63 (2): 1224.

[14] Chaudhry, M. F., Khokhar, K. M., Rahman, H., and Bhatti, M. H. 1998. "Comparative Studies on Early Production of Some Tomato Hybrids under Plastic Tunnel during Spring Season at Islamabad." *Sarhad J. Agric.* 14 (3): 203-5.

[15] Chaudhry, M. F., Khokhar, K. M., Ashraf, M., and Mahmood, T. 1995. "Performance of Six Tomato Hybrids under Plastic Tunnel during Winter Cultivars 1995." *Sarhad J. Agric.* 11 (3): 325-9.

[16] Chaudhry, M. F., Jeelani, G., Riaz, S., and Bhatti, M. H. 2003. "Yield Potential of Some Indeterminate Hybrids and an Open-Pollinated Variety of Tomato during Winter Season under Plastic Tunnel at Islamabad." *Pakistan J. Arid Agric.* 6 (1): 5-7.

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Evaluation of Promissing Sponge Gourd (*Luffa cylindrical*) Accessions in Summer-Autumn Season 2014 in Thua Thien Hue

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Abstract: Sponge gourd varieties having aroma after cooking have been degenerated because of cross pollination. Collection and evaluation of sponge gourd germplasm are needed for conservation and breeding of high quality varieties. The objective of the study was to identify varieties having high yield, good quality and aroma under local conditions. Ten promising sponge gourd accessions, including A2, A6, A7, A13, A16, A17, B29, B30, HN and QN were evaluated for growth, morphological traits, fruit quality and yield. The experiment was carried out in Hue University of Agricultural and Forestry from June to October in 2014. The results showed that all promising accessions grew well. Different morphological traits were observed among promising lines. Yield of accessions A7, A13 and A17 were higher than the others. Only fruits of accession B29 had aroma after cooking. These lines can be used in sponge gourd breeding programs.

Key words: Sponge gourd, *Luffa cylindrical*, Thua Thien Hue, aroma.

1. Introduction

Sponge gourd (*Luffa cylindrical*) is member of Cucurbitaceae family, *Luffa* genus. It is used as a vegetable either prepared like squash or eaten raw like cucumber [1]. In many developing country, old fruit is produced for wide applications in bathing and washing by fibrous vascular system, such as utensil cleaning sponges, bath sponge and adsorbent for heavy metal in waste water. Sponge gourd is a cross-pollinated crop and has 26 chromosomes ($2n$ = 26) [2]. Sponge gourd was first grown commercially in Japan in the early 1890s [3]. Sponge gourd is known as important medicine plant, especially in China. Fruits are used in the traditional Chinese medicine as an anthelmintic, stomachic and antipyretic phytomedicinal drug. Saponins from the leaves and fruits possess effect on anoxia and fatigue and immunological activity [4]. Additionally, the Luffin, a ribosome-inactivating protein isolated from *Luffa* seed, has been shown to be effective against growth of parasites, protozoa, insects, fungi and HIV [5]. *Luffa* seed has been shown to be effective against growth of parasites, protozoa, insects, fungi and HIV [6]. Nowadays, there are many researches who have mentioned application capacity of *Luffa* fibrous system and chemical compounds extracted from fruit, seed and leaf. Thus, sponge gourd is known not only in vegetable but also in industrial and science researching materials.

Sponge gourd is a tropical and sub-tropical plant which requires warm temperature. It is widely and easily cultivated in Vietnam. However, nowadays, Sponge gourd's growing area is limited in Vietnam; in addition, famers keep the seed of local varieties to continue next sowing season. In this case, crossing pollinating cause degraded and adulterated, thus

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leading to decreased quality of local variety, such as high yield, aroma and sticky. It's necessary to select and conserve good sponge gourd lines having high yield, good quality, and disease resistance to increase production efficiency. In the previous study, the sponge gourd germplasm consisting of 49 accessions was evaluated in Ha Noi [7]; however, selected promising accessions are needed to confirm their growth ability and fruit quality under local conditions. Therefore, the purpose of this study was to evaluate the potential of promising *Luffa cylindrical* accessions on yield and fruit quality under local conditions and to select good lines for breeding programs and introducing to crop system in Thua Thien Hue.

2. Materials and Methods

2.1 Materials

A total of 10 accessions, including eight accessions obtained from Plant Genetic Resource of Vietnam (A2, A6, A7, A13, A16, A17, B29 and B30) and two local aroma sponge gourd accessions HN (collected from Ha Noi) and QN (collected from Quy Nhon), were used in this study.

2.2 Methods

2.2.1 Experimental Design

The evaluation was conducted from June to October 2014 in a greenhouse at Hue University of Agricultural and Forestry, Thua Thien Hue. The experiment was laid out in a random complete block design (RCBD) with three replications, as following Harika et al. designed for bottle gourd in 2012 [8] and Choudhary et al. designed for ridge gourd (*Luffa acutangula*) in India in 2014 [9]. Each accession in each replication was represented by six plants in a plot size of 6 m^2. The spacing was 100 cm between plants and 100 cm between rows. Sponge gourd was cultivated based on national technical regulations of QCVN 2013 DUS of Angel Loofah (*Luffa acutangula*) [10]. Seedlings with 2-3 fully expanded true leaves were transplanted into experimental field in plastic house. The basal fertilizing consisted of 20 tons manure, 120 kg superphosphate and 30 kg potassium per ha. Watering fertilizers were applied every three weeks with 37.5 g N:P:K (16:16:16), 24 g urea and 4 g K for each plot. Plastic film mulch was used to cover the bed. Setting frame stand for plant was before appearing tendrils.

2.2.2 Data collection

The data was collected from five randomly selected plants per replication. The time of growth periods had been measured since 50% number of plants of each accession had reached of requirements. Node height, stump diameter, leaf width and leaf length were measured to describe the ability of growth. Observations on leave were recorded in mature leaf emerging from nodes of the 15th to 20th. Fruit traits were recorded during harvesting period. Aroma trait was assessed by sense evaluation from 10 people. Total of 23 traits were evaluated during experiment.

2.2.3 Statistical analysis

Average values which collected from each plot were analyzed using analysis of variance (one-way ANOVA) by Statistix 9.0 version. Differences between means values were compared using Duncan's test at $P < 0.05$ [11].

3. Results and Discussions

3.1 Period of Growth and Development

Time from sowing to appearing the first male flower ranged from 46 d (A13) to 65 d (HN) (Table 1). Time from sowing to appearing the first female flower ranged from 46 d (A13) to 72 d (HN). Time which the first female flower opened was later than the first male flower in almost accessions. Choudhary et al. [9] reported that days to first female flower had negative and significant correlation with marketable fruit yield per plant. Thus, selection of genotypes producing female flowers early would increase yield of ridge gourd (*Luffa acutangula*). Choudhary et al. [12], Hanumegowda et al. [13] and Prasanna et al. [14] also reported similar trend in ridge gourd. Kumar et al. [15]

Table 1 Period of growth and development of promising sponge gourd accessions.

Accession	Time from sowing to…				
	Appearing the 1st male flower	Appearing the 1st female flower	Harvesting		
			1st time	2nd time	3rd time
A2	51	61	98	131	176
A6	49	56	98	131	176
A7	51	51	98	131	176
A13	46	46	98	131	176
A16	51	60	98	131	176
A17	57	60	98	131	176
B29	62	57	98	131	176
B30	60	65	98	131	176
HN	65	72	98	131	176
QN	61	70	98	131	176

Table 2 Ability of growth and development of sponge gourd accessions.

Accession	Node height (cm)	Stump diameter (mm)	Leaf width (cm)	Leaf length (cm)
A2	16.80^{abc}	54.53^{a}	26.00^{a}	19.70^{a}
A6	14.57^{bc}	56.00^{a}	21.76^{c}	17.56^{bc}
A7	17.38^{abc}	55.13^{a}	23.73^{abc}	17.41^{bc}
A13	17.46^{ab}	54.27^{a}	23.36^{abc}	18.56^{ab}
A16	17.87^{ab}	53.87^{a}	22.87^{bc}	18.15^{ab}
A17	18.81^{a}	59.00^{a}	22.75^{bc}	16.88^{bc}
B29	16.71^{abc}	54.33^{a}	23.53^{abc}	18.79^{ab}
B30	14.03^{c}	54.27^{a}	21.03^{c}	15.44^{c}
DC	17.70^{ab}	61.67^{a}	25.35^{ab}	18.08^{ab}
QN	17.72^{ab}	59.47 ^{a}	23.64^{abc}	17.73^{ab}
LSD $_{0.05}$	3.35	10.34	2.94	2.13

$^{a, b, c}$ Means in different letter (s) are significantly different at $P = 95\%$.

indicated that days to anthesis of the first male flower were positively correlated with total yield per vine in sponge gourd [15]. In this study, B29, B30, HN and QN had the first male flower at about 60 d and A13 had the earliest of first female flower (46 d). All of accessions had the same harvesting times.

3.2 Ability of Growth and Development of Promising Sponge Gourd Accessions

Ability of growth and development was presented in node height, stump diameter, leaf width and leaf length. Choudhary et al. [12], Chowdhury and Sharma [16], Karuppaiah et al. [17], and Singh et al. [18] have observed a wide variation in growth and flowering traits of ridge gourd. The height of node ranged from 14.03 cm to 18.81 cm (Table 2). Node height of A17 was the highest and the lowest one was B30. The difference was found significantly between them. These results were similar to internodal length (from 12.85 cm to 17.17 cm). This result is in agreement with result of Choudhary et al. (2008) [12]. The big stump diameter shows good growth ability. Stump diameter was obtained from 53.87 cm (A16) to 61.67 cm (DC), but there was no significant difference among accessions. Leaf width and leaf length are not only variety's feature, but also characteristic to affect photosynthesis capacity of plant. Leaf width and leaf length of A2 were found to be biggest, whereas B30 accession had the lowest leaf width (21.03 cm) and the lowest leaf length (15.44 cm). Leaf width and leaf length were significantly different among accessions.

3.3 Morphological Traits of Promising Sponge Gourd Accessions

Morphological traits depend on genetics and are different among accessions. Morphological traits of the sponge gourd accessions were recorded and presented in the Table 3. Leaf, fruit and seed were different in color and shape. Leaf shape of five accessions, such as A2, A7, A16, A17, B29 and QN was reniform, whereas orbicular leaf shape was observed in other accessions. A7, A16 and QN had dark green leaves; the remained accessions had green color. High leaf pubescence (hair on ventral surface) will have high ability of pest resistance. A13, A17, B30 and QN had high leaf pubescence.

Fruit shape was divided in two kinds, ellipse and oblong. The ellipse was observed in A2, A6 and A17, and the remains were oblong. Fruit color consisted of light green (A2, A7, A13 and A17), green (A6, A16 and B29) and dark green (B30, HN and QN).

3.4 Fruit Quality Traits

Fruit quality is an important criterion for sponge gourd production. Joshi et al. (2004) [19] reported that fruit length and fruit weigh were considered as primary traits for increasing fruit number. Fruit size determined by fruit length and fruit diameter was significant difference among accessions (Table 4). Fruit length was recorded from 29.3 cm to 43.7 cm. B29 had the shortest fruit (29.3 cm) and QN had the longest fruit (43.7 cm). There was significant difference among accessions. Fruit diameter ranged from 4.55 cm to 5.87 cm. B29 had the smallest fruit (4.55 cm), whereas A13 had the biggest fruit (5.87 cm). Davis and DeCourley [20] have reported that average of sponge gourd fruit length varied from 48 cm to 79 cm and the diameter from 7 cm to 11 cm, respectively. The longest fruit peduncle belonged to A13 (17.27 cm) and the shorted fruit peduncle belonged to QN (18.55 cm). The length of fruit peduncle of accession was significantly different among accessions.

Davis [21] emphasized that important characteristic of *Luffa* depends on the purpose of the use of sponge gourd. As vegetable for daily meal, almost consumers will choose good fruits as weight, sweetness and aroma. Thus, total soluble solid (brix) and aroma were evaluated. Brix ranged from 2.17 (B30) to 3.32 (B29). Almost accessions had similar brix value. Only B29 kept aroma after cooked. Stickiness help food more tasty. All of accessions were sticky.

3.5 Yield Components and Yield

Ratio of fruit setting, number fruit per plant and fruit weight constituted of yield. Ratio of fruit setting ranged from 16% (QN) to 45.17% (B29). Ratio of fruit setting of all accessions was significantly different (Table 5). This fruiting rate was higher than those in previous study [7].

Table 3 Morphological traits of promising sponge gourd accessions

Accession	Leaf shape	Leaf color	Leaf pubescence	Fruit shape	Fruit color
A2	R	G	M	E	LG
A6	O	G	M	E	G
A7	R	DG	M	OB	LG
A13	O	G	H	OB	LG
A16	R	DG	M	OB	G
A17	R	G	H	E	LG
B29	R	G	M	OB	G
B30	O	G	H	OB	DG
HN	O	G	M	OB	DG
QN	R	DG	H	OB	DG

R = reniform, O = orbicular, G = green, DG = dark green, LG = light green, M = medium, H = high, E = elliptical, OB = oblong blocky.

Table 4 Fruit quality of promising sponge gourd accessions.

Accession	Fruit length (cm)	Fruit diameter (cm)	Length of fruit peduncle (cm)	Brix	Aroma	
					Raw	After cooked
A2	38.89^{abc}	5.17^{abcd}	15.05^{abc}	2.55^{bcd}	Yes	No
A6	38.37^{abc}	5.69^{ab}	10.58^{d}	2.53^{bcd}	No	No
A7	35.06^{cd}	5.20^{abcd}	14.89^{abc}	2.37^{bcd}	No	No
A13	39.56^{abc}	5.87^{a}	17.27^{ab}	2.68^{bcd}	Yes	No
A16	41.80^{ab}	4.97^{bcd}	16.97^{ab}	2.92^{ab}	No	No
A17	30.83^{de}	5.64^{abc}	13.68^{bcd}	2.79^{abc}	Yes	No
B29	29.30^{e}	4.55^{d}	12.63^{cd}	3.32^{a}	Yes	Yes
B30	38.08^{bc}	4.86^{cd}	11.17^{cd}	2.17^{d}	No	No
HN	39.25^{abc}	5.00^{bcd}	17.11^{ab}	2.31^{cd}	No	No
QN	43.70^{a}	4.96^{bcd}	18.55^{a}	2.52^{bcd}	Yes	No
LSD $_{0,05}$	5.45	0.82	4.02	0.57		

[a, b, c] Means in different letter (s) are significantly different at $P = 95\%$.

Table 5 Yield components and yield of promising sponge gourd accessions.

Accession	Ratio of fruit setting (%)	Number of fruit/plant (fruit)	Fruit weigh (g)	Theory yield (ton/ha)	Yield (ton/ha)
A2	41.08^{ab}	3.33^{abc}	367.33^{abc}	60.05^{abcd}	46.61^{abc}
A6	37.50^{ab}	3.33^{abc}	416.00^{ab}	65.66^{abc}	51.73^{abc}
A7	39.42^{ab}	4.80^{a}	356.00^{bc}	82.83^{a}	83.25^{a}
A13	39.50^{ab}	3.47^{abc}	471.33^{a}	78.67^{ab}	62.56^{ab}
A16	43.42^{ab}	2.33^{cd}	372.00^{abc}	40.61^{abcd}	34.51^{bc}
A17	38.92^{ab}	4.60^{ab}	356.00^{bc}	79.55^{ab}	71.17^{ab}
B29	45.17^{a}	2.60^{bcd}	274.00^{c}	33.65^{cd}	29.95^{bc}
B30	29.17^{bc}	1.80^{cd}	439.33^{ab}	36.98^{bcd}	58.67^{ab}
HN	42.17^{ab}	1.90^{cd}	416.00^{ab}	39.39^{bcd}	34.51^{bc}
QN	16.00^{c}	0.87^{d}	440.11^{ab}	18.30^{d}	14.91^{c}
LSD $_{0,05}$	14.88	2.02	112.51	42.97	42.00

[a, b, c] Means in different letter (s) are significantly different at $P = 95\%$.

Number of fruit per plant ranged from 0.87 fruit to 4.8 fruits. QN had the lowest number of fruit per plant (0.87 fruit) and A7 had the highest number of fruits per plant (4.8 fruits), and the followings were A17 (4.6 fruits) and A13 (3.47 fruits). The accession A7 only had 2.6 fruits per plant reported in 2014 by Truong et al. [7]. While Davis and DeCourley [20] reported that number of fruits of gourds per plant were ranged from 3.5 to 20 fruits.

Fruit weight ranged from 274 g to 471.33 g. B29 had the smallest fruit weigh (274 g), whereas A13 had the biggest fruit (471.33 g). Some accessions had big fruit, such as QN (440.11 g) B30 (439.33 g), A6 and HN (416 g), but there was no significant difference. Kumar et al. [15] have reported that fruit number per vine ranged from 20.92 to 35.87 fruits and fruit weight ranged from 106.87 g to 216.20 g. While Choudhary [9] reported that fruit weight of ride gourd ranged from 74.04 g to 109.06 g. Thus, number of fruit, fruit length and average weight of fruit are important characters for increasing yield potential in *Luffa*.

Theory yield ranged from 18.3 ton/ha to 82.83 ton/ha. QN had the lowest theory yield (18.3 ton/ha), whereas A7 had the highest theory yield (82.83 ton/ha). This occurred in true yield too. A7 obtained the highest yield (83.25 ton/ha), following by A17 (71.17 ton/ha) and A13 (62.56 ton/ha). There was no significant difference among accessions. QN had the lowest yield (14.91 ton/ha), and next was B29 (29.95 ton/ha). These results were in agreement with results of Kumar et al. [15] that fruit number was positively correlated with total yield. Choudhary et al. [9] also

indicated that the marketable yield per plant had positive and highly significant correlation with fruit weight and number of marketable fruit per plant at phenotypic level [9].

4. Conclusions

All promising accessions can grow well under Thua Thien Hue conditions. Different varieties had different morphological traits and growth characteristics. Only fruit of accession B29 kept aroma after cooked, but fruits of all accessions were sticky after cooking. Accessions A7, A13 and A17 had higher yield than other accessions. Accessions B29, A7, A13 and A17 had high yield and good fruit quality (aroma, sticky), therefore should be used in breeding F1 Sponge gourd.

References

[1] Sastri, B. N. 1962. *Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products.* Vol. 6. New Delhi: CSIR.

[2] Bal, K. J., Hari, B. K. C., Radha, K. T., Madhusudan, G., Bhuwon, R. S., and Madhusudan, P. U. 2004. *Descriptors for Sponge Gourd (Luffa cylindrica (L.) Roem.)*. Nepal: NARC and LIBIRD, and Italy: IPGRI.

[3] Heiser, C. B. 1979. *The Gourd Book.* Norman: University of Oklahoma Press, 235.

[4] Du, Q., and Cui, H. 2007. "A New Flavones Glycoside from the Fruits of *Luffa cynlindrica*." *Fitoterapia* 78: 609-10.

[5] Ng, Y. M., Yang, Y. H., Sze, K. H., Zhang, X., Zheng, Y. T., and Shaw, P. C. 2011. "Structural Characterization and Anti-HIV-1 Activities of Arginine/Glutamate-Rich Polypeptide Luffin P1 from the Seeds of Sponge Gourd (*Luffa cylindrica*)." *Journal of Structural Biology* 174 (1): 164-72.

[6] Seki, Y., Sever, K., Erden, S., Sarikanat, M., Neser, G. K., and Ozes, C. 2012. "Characterization of *Luffa cylindrica* Fibers and the Effect of Water Aging on the Mechanical Properties of Its Composite with Polyester." *Journal of Applied Polymer Science* 123: 2330-7.

[7] Truong, H. T. H., Phan, T. T., Tran, N. B. T., Tran, T. V, and Nguyen, T. T. T. 2014. "Study on Sponge Gourd (*Luffa cylindrical*) in Spring-Summer Season 2014 at Gia Lam, Ha Noi." *Journal of Agriculture and Rural Development* 98 (10): 75-81.

[8] Harika, M., Gasti, V. D., Shantappa, T., Mulge, R., Shirol, A. M., Mastiholi, A. B., and Kulkarni, M. S. 2012. "Evaluation of Bottle Gourd Genotypes (*Lagenaria siceraria* (Mol.) Standl.) for Various Horticultural Characters." *Karnataka J. Agric.* Sci. 25 (2): 241-4.

[9] Choudhary, B. R., Kumar, S., and Sharma, S. K. 2014. "Evaluation and Correlation for Growth, Yield and Quality Traits of Ridge Gourd (*Luffa acutangula*) under Arid Conditions." *Indian Journal of Agricultural Sciences* 84 (4): 498-502.

[10] Ministry of Agriculture and Rural Development (MARD). 2013. *National Technical Regulation on Testing for Distinctness, Uniformity and Stability of Angled Luffa.* (in Vietnamese)

[11] Duncan, E. B. 1995. "Multiple Range Test and Multiple *F*-Tests." *Biometrics* 11 (1): 1-42.

[12] Choudhary, B. R., Pandey, S., Bhardwaj, D. R., Yadav, D. S., and Rai, M. 2008. "Component Analysis for Qualitative Traits in Ridge Gourd (*Luffa acutangula* (Roxb.) L.)." *Vegetable Science* 35 (2): 144-7.

[13] Hanumegowda, K., Shirol, A. M, Mulge, R., Shantappa, T., and Kumar, P. 2012. "Correlation Co-efficient Studies in Ridge Gourd (*Luffa acutangula* (L.) Roxb.)." *Karnataka Journal of Agricultural Sciences* 25 (1): 160-2.

[14] Prasanna, S. C., Krishnappa, K. S., and Reddy, N. S. 2002. "Correlation and Path Coefficient Analysis Studies in Ridge Gourd." *Current Research* 31: 150-2.

[15] Kumar, R., Ameta, K. D., Dubey R. B., and Pareek, S. 2013. "Genetic Variability, Correlation and Path Analysis in Sponge Gourd (*Luffa cylindrical* Roem.)." *African Journal of Biotechnology* 12 (6): 539-43.

[16] Chowdhury, D., and Sharma, K. C. 2002. "Studies on Variability, Heritability, Genetic Advance and Correlations in Ridge Gourd (*Luffa acutangula* (Roxb.) L.)." *Horticultural Journal* 15 (3): 53-8.

[17] Karuppaiah, P., Kavitha, R., and Senthilkumar, P. 2002. "Studies on Variability, Heritability and Genetic Advance in Ridge Gourd." *Indian Journal of Horticulture* 59 (3): 307-12.

[18] Singh, R. P., Mohan, J., and Singh, D. 2002. "Studies on Genetic Variability and Heritability in Ridge Gourd (*Luffa acutangula* L.)." *Agricultural Science Digest* 22 (4): 279-80.

[19] Joshi, B. K.; Tiwari, R. K., KC, H. B., Regmi, H. N., Adhikari, B. H., Ghale, M., Chaudhary, B., Gyawali, S., Upadhyay, M. P., and Sthapit, B. R. 2004. "Evaluation of Sponge Gourd (*Luffa cylindria* L.) Diversity for Vegetable Production." In *On-farm Conservation of Agricultural Biodiversity in Nepal.* Vol. 1. Nagarkot: International Plant Genetic Resources Institute (IPGRI), 122-8.

[20] Davis, J. M., and DeCourley, C. D. 1993. "Luffa Sponge

Gourds: A Potential Crop for Small Farms." In *New Crops,* edited by Janick, J., and Simon, J. E. New York: Wiley, 560-1.

[21] Davis, J. M. 1994. "Luffa Sponge Gourd Production Practices for Temperate Climates." *HortScience* 29 (4): 263-6.

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Effect of High Temperature on Fruit Productivity and Seed-Set of Sweet Pepper (*Capsicum annuum* L.) in the Field Condition

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Abstract: Chili pepper grow best and are likely to reach the maximum yield at temperature ranging from 21 °C to 33 °C. In the plastic house, the temperature increases to 42 °C in the summer. The fruit set and fruit growth were effected correlative with the high temperature condition. In this study, Shishito peppers were grown in plastic house two periods (in the early stage of April and the end stage of May) in 2012. The difference in temperature between two periods of planting was about 4 °C. In fruit set period of the 2nd planting, the weather condition is disadvantage for fruit growth. During temperature changed in the summer, the fruit weight and the number of seeds per fruit of both "Shishi-homare" and 105c-10 varieties were reduced about 0.5 and 2.8 times in the 2nd planting when compared with the 1st planting. The number of seeds per fruit reduced corresponding with the fruit size under the high temperature condition.

Key words: High temperature, seed-set, fruit productivity.

1. Introduction

The origin of pepper (*Capsicum annuum L*) is Mexico and the neighboring areas of Central America. Shishito is one of four popular sweet pepper cultivars (Hushimi-ama type, Shishito type, Bell type and F1 type) in Japan [1]. Fruit is small, green, and non-pungency. In Japan, The crop of sweet pepper begins in the last stage of spring and harvests from May to November [2]. During this time, the temperature increased strongly, when the extreme temperature of more than 40 °C sometimes was recorded in August [3]. Under plastic house in the field, the inside temperature will be higher. Temperature strongly influenced the development of fruit and flowers of chili pepper [4]. The optimum temperature for chilli pepper cultivation is 21-33 °C. Low and high temperature condition affect the size of fruit and seed germination ability [5, 6]. Under changing of weather, temperatures are higher than the optimum chili's growing, thus the plants are objected to disadvantageous temperature period [7, 8].

In general, high temperature influences many aspects of plant physiology and growth, which may lead to significant losses in crop productivity in many species due to limited vegetative, reproductive growth and seed yield [9]. According the survey of Erickson and Markhart [10], fruit set and productivity of pepper reduced during periods of high temperature. High temperature frequently occurs after anthesis of chilli pepper and strongly impacts the reproduction and yield. However, the rare investigation into the effect of high temperature during chili pepper crop has not been elucidated. Therefore, the present study aimed to study the impact of high temperature during summer period in Japan on the phenological and morphological character of fruits of Shishito pepper.

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2. Materials and Methods

The experiment was carried out in plastic house of Okayama University, Japan. Four varieties derived from Shishito (*Capsicum annuum L.*), viz., "Shishi-homare" (a favorite variety in Japan), "105c-10" (low-pungent mutation line derived from "Shishi-hormare"), "Kounou Shishito" and "Kairyo Shishito" were used in the current experiment.

The seeds were sown in sowing tray in both the planting times. The 1st time was on April 9th 2012 to August 16th 2012, "Shishi-homare" and "105c-10" were planted in this time; and the 2nd time was on May 30th 2012 to October 30th 2012, four varieties were planted and filled with commercial soil (300 mg/L N, 450 mg/L P and 370 mg/L K). During the 1st planting, the pots containing the seeds were kept in the growth chamber, which set at a daily 16/8 h (light/dark) with a temperature of 28/20 °C, respectively. After three weeks when seedlings were at 3-4 leaf stage, 30 seedlings of each variety were transplanted to plastic pot (10.5 cm, containing commercial soil) and keep in the plastic house, and distance between two pots is 15 cm. At anthesis stage, 10 plants of each one were transplanted again to the ground in plastic house. The distance between two plants was 50 cm and the distance between two rows was 1.2 m. The seedlings were trained with two main branches. Depending on the daily temperature, plants were watered once or twice per day. The plants were supplied with fertilizer of NPK fertilize (20 g/m^2), and Ca and Mg (80 g/m^2 each), respectively.

Temperature in plastic house was recorded with thermocouples connected to data-logger. The plant growth situation before the 2nd transplanting was recorded. All the individual flowers were labeled for finding the days after flowering (DAF). Fruits from flowers of 28-30 DAF were harvested with one fruit per branch weekly. The morphological characteristic of harvest fruits (fruit size, fruit diameter, fruit length) and number of seeds were recorded. Fruits from labeled flowers were harvested once per week from the last of June to the middle of August for the 1st planting and from middle of August to the end of October for the 2nd planting. The data obtained were analyzed following Tukey-test ($P \leq 0.05$).

3. Results and Discussion

The temperature in plastic house increased gradually from April 15th, and the highest temperature of 42 °C was recorded from the early August to the early September (Fig. 1). During the 1st planting period (Fig. 1a), the temperature averaged from 28 °C to 33.4 °C in day and 11.8 °C to 21.4 °C at night, which gradually increased and reach a peak of 42 °C in the early August. The temperature increased nearly 4 °C from the 1st planting to the 2nd planting.

The 1st flower in the 2nd planting appeared in the late July, when the maximum temperature is about 41.6/24.6 °C (day/night) (Fig. 1b). The difference in temperature between the 1st planting (the early stage of June) and the 2nd planting for the 1st flower opening was 7 °C.

No significant difference was observed between "Shishi-homare" and "105c-10" in the number of days to 1st flowering. When the temperature increased, the days to the 1st flower reduced, respectively (Table 1). Similar results were reported by Qumeret al. [11]. No significant difference was observed in the node number in all the varieties during both planting times. This might be because all the varieties belong to Shishito group, and thereby exert similar morphological characteristics.

The fruit size of harvested fruits, such as fruit length, fruit weight, fruit diameter and seed number per fruit were recorded in Table 2 and no difference was observed in the fruit weight, fruit length, fruit diameter and seed number per fruit between "Shishi-homare" and "105c-10" in the 1st planting. But when the temperature increases in the 2nd planting, the morphological characteristic of fruits in all varieties changed, fruit weight and number of seeds per fruit of both 'Shishi-homare' and "105c-10" were reduced about 0.5 times and 2.8 times, respectively.

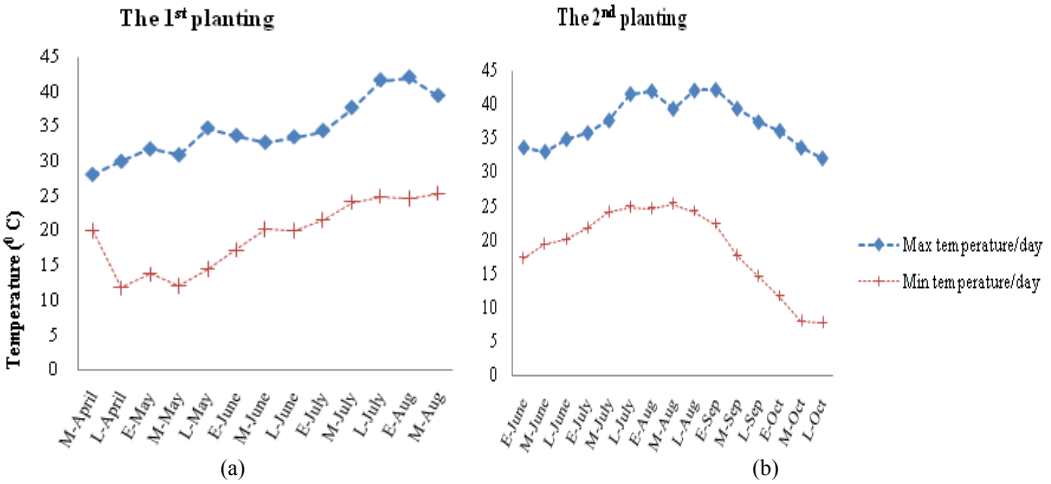

Fig. 1 The temperature condition during culture of two times planting.
E: the early stage of month (1st-10th), M: the middle stage of month (11th-20th), L: the last stage of month (21st-30th).

Table 1 The number of days to the 1st flowering, the node number of the 1st flower and the plant height in the 2nd transplanting.

Plant time	Variety	Days to the 1st flowering	The node number of the 1st flower	The height in the 2nd transplanting (cm)
1st planting	Shishi-homare	57.8 ± 0.9[a]	15.1 ± 0.7[b]	15.4 ± 0.8[c]
	105c-10	56.6 ± 0.7[a]	14.3 ± 0.6[b]	15.9 ± 0.62[c]
2nd planting	Shishi-homare	53.4 ± 1.7[b]	15.0 ± 0.8[b]	17.0 ± 1.0[c]
	105c-10	52.3 ± 1.1[b]	15.1 ± 0.7[b]	18.0 ± 0.7[bc]
	Kounou Shishito	53.2 ± 0.8[b]	16.9 ± 1.0[a]	21.8 ± 1.1[a]
	Kairyo-Shishito	52.6 ± 1.3[b]	15.1 ± 1.0[b]	19.0 ± 1.1[b]

[a-c]Different letters within same column indicate a significant different at 5% level by Tukey's test.

Table 2 The morphological characteristics of harvested fruits at 28-30 DAA.

Plant time	Variety	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	Numbers of seed/fruit
1st planting	Shishi-homare	8.9 ± 5.1[b]	1.6 ± 0.4[b]	6.8 ± 2.2[b]	51.8 ± 33.1[b]
	105c-10	8.9 ± 4.9[b]	1.7 ± 0.3[b]	6.8 ± 2.1[b]	54.1 ± 34.9[b]
2nd planting	Shishi-homare	4.3 ± 1.6[a]	1.2 ± 0.2[a]	5.0 ± 1.1[ab]	18.3 ± 23.58[a]
	105c-10	4.5 ± 1.2[a]	1.3 ± 0.2[a]	5.2 ± 1.5[ab]	19.9 ± 18.9[a]
	KounouShishito	3.9 ± 1.6[a]	1.3 ± 0.3[a]	4.6 ± 1.3[a]	25.3 ± 25.1[a]
	KairyoShishito	4.4 ± 2.1[a]	1.3 ± 0.3[a]	4.9 ± 1.9[ab]	27.0 ± 22.3[a]

[a and b]Different letters within same column indicate a significant different at 5% level by Tukey's test.

The chili peppers grow best and are likely to reach the maximum yields at temperature from 21 °C to 33 °C [3]. After the 1st flowering in the 2nd planting, the temperature increases fluctuating from 37 °C to 42 °C, and thereby affects the fruit weight. The fruit diameter and fruit length in the 2nd planting were smaller than these in the 1st planting. Among the varieties, fruits of "Kounou Shishito" are the smallest in shape. In 1982, Ali, A. M., and Kelly, W. C [12] reported that, fruit weigh, fruit length and fruit diameter of sweet pepper reduce in the high temperature conditions. In Shishito pepper, fruit weight reduced significantly and seeds per fruit was lowered by under high temperature (38/30 °C day/night) [13].

The morphological characteristics of fruit were affected strongly under high temperature (Fig. 2). The highest fruit weight and fruit length were recorded in

July 8th in harvested "Shishi-homare" fruits of the 1st planting (Fig. 2a). In both "Shishi-homare" and "105c-10" varieties in the 1st planting, fruit weight, fruit diameter and fruit length reduced rapidly after July 22th, 2012, which correlates with the increase temperature at this time. The fruit weight reduced strongly responding to high temperature in both "Shishi-homare" and "105c-10" varieties.

In the 2nd planting, the 1st flower appeared when the temperature stayed in high condition (37.8/24 °C,

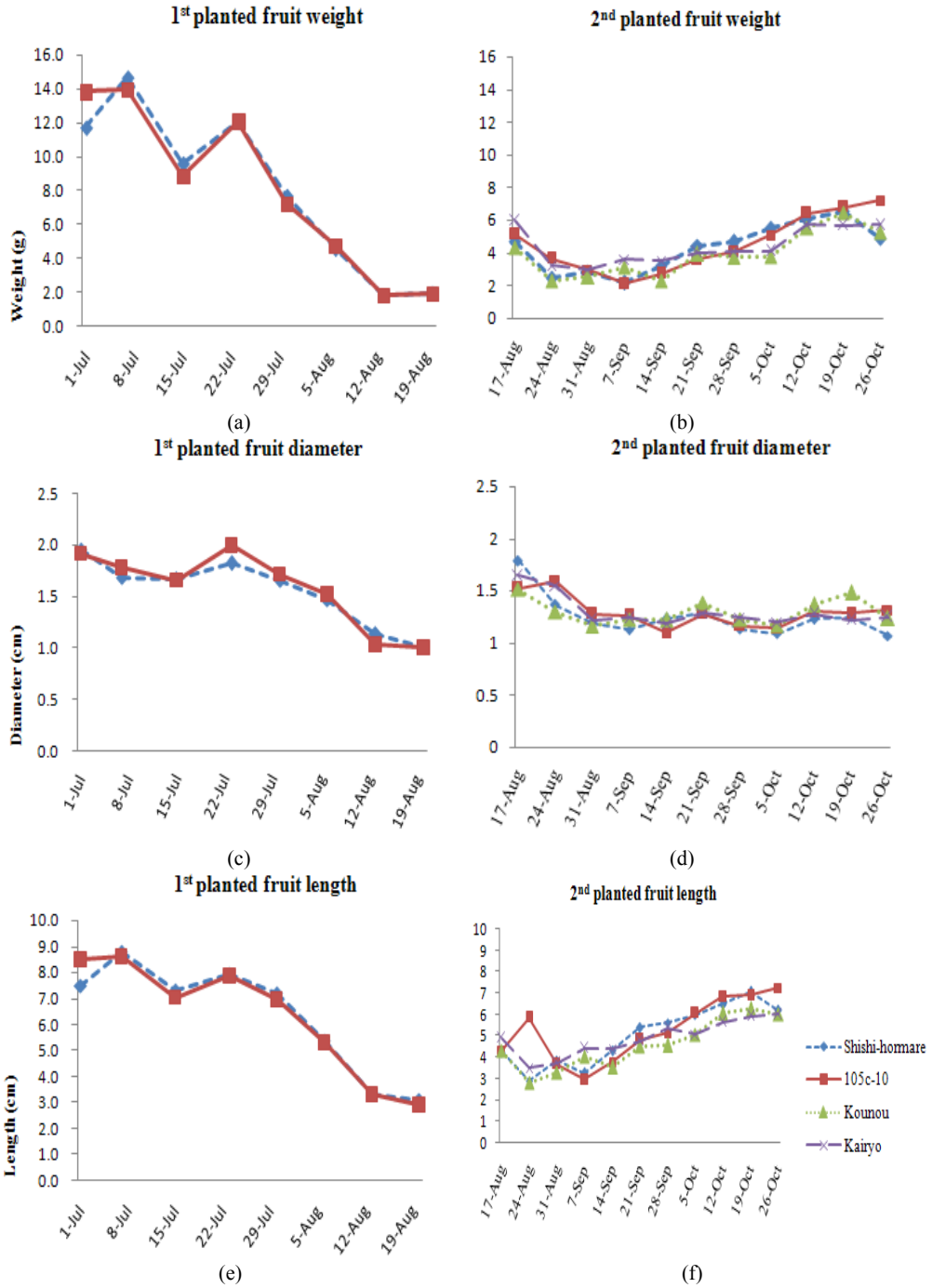

Fig. 2 The change of morphological fruit under two temperature regimes of two times of planting.

day/night), after that the temperature increased rapidly and remained a long time. It correlates with the development of four morphological fruit varieties when the fruit weight, fruit diameter and fruit length were small in the 1st harvested time and decreased gradually. After September 14th, fruit weight, fruit diameter and fruit length begun to increase, but it was still smaller than that in the 1st planting. The "Kounou Shishito" variety had the smallest fruit weight and fruit length (Fig. 2d and 2f) in almost harvested time.

During the 1st flower appearing in the 2nd planting, the temperature condition fluctuates in 33-42 °C, thus the weight of fruit was effected. When the weight of fruit decreases, the diameter and the length of fruit will reduce, correlatively. The diameter and the length fruit in the 2nd planting were smaller than that in the 1st planting. Thus, all of Shishito varieties in this experiment were impacted by high temperature.

In Japan, the annual temperature has been increasing at rate of 1.1 °C per century since 1898. As temperatures rise, the numbers of days with the minimum temperatures ≥ 25 °C and the maximum temperatures ≥ 35 °C are increasing, respectively [14]. In plastic house, the temperature is higher than outside. High temperature (≥ 35 °C) has kept in long time since the early of June. The highest temperature in plastic house was 42.05 °C, which was recorded by thermocouples. Shishito pepper grew best under the field and green house with temperature ranging from 21 °C to 33 °C [13, 3]. Field and controlled environment observations of pepper production indicate substantial abortion of floral buds occurs when day temperatures are ≥ 34 °C and/or night temperature are ≥ 21 °C. Thus, when the maximum temperature per day was ≥ 34 °C during the day and night temperature ≥ 21 °C in early stage of July (Fig. 1a), the fruit weight, fruit diameter, fruit length and number seed of fruit decreased rapidly until the lst harvest of the 1st planting and the almost harvest time of 2nd planting-fruit developed under over the suitable condition, therefore harvested fruits from the 2nd planting were smaller than that from the 1st planting (Fig. 2 and Table 2). Exposure to high temperature, from microspore mother cell meiosis of pepper flower to tetrad dissolution, in greatly reduces pollen viability and anther dehiscence [15]. High temperature inhibits the development of pollen grains during the period of final tetrad formation to tetrad dissolution. The reduction of pollen viability effectively reduces fruit size and fruit set [10]. After fertilization, the fruit size was determined by cell divisions and cell expansions. The Shishito pepper fruit width was the most sensitive to high temperature until 10 DAF and less effected from 30 DAF onwards [13]. Exposure to high temperature throughout fruit development significantly reduced Shishito pepper fruit weight. The reduction of fruit length and fruit diameter responds the reduction of fruit size in the high temperature condition in the 2nd planting stage.

The reduction of seed numbers per fruit may have been partly responsible for the reduction of fruit size. A positive correlation was observed between fruit weight and number of seeds per fruit of sweet pepper [10, 16]. In the 2nd planting, under the high temperature condition when the fruit weight decreased, the seeds/fruit reduces in the same way. Besides, under high temperature condition, the reduction in the number of viable pollen affect fertilization capacity, hence reducing number of seeds per fruits. After fertilization, abortion of seeds during the initial stages is probably due to failure in proper cell division, which might due to high temperature [13]. Thus, numbers of seed in all varieties reduced strongly in the 2nd planting compared to the 1st planting.

The results indicated that a significant response of Shishito pepper was observed under high temperature condition in the summer. Fruit quality of Shishito was reduced drastically. Thus, managing the time to sowing and harvesting is necessary for growing chilli pepper.

4. Conclusions

The morphological fruits (the fruit weight, the fruit diameter, the fruit length and number of seeds per fruit) under high temperature decreased strongly. The results indicated a significant difference in the response of Shishito when the temperature increased in the summer. Fruit quality of Shishito reduced clearly in bad condition for growing. Thus, managing the time to sowing and harvesting is so necessary when growing sweet pepper.

Reference

[1] Ishikawa, K., Sasaki, S., Matsufuji, H., and Nunomura, O. 2004. "High ß-Carotene and Capsaicinoid Contents in Seedless Fruits of 'Shishitoh' Pepper." *HortScience* 39 (1): 153-5.

[2] The Japanese Society for Horticultural Science. 2006. *Horticulture in Japan*. Japan: Shoukadoh Publishing of Nakanishi Printing Co., Ltd..

[3] Pagamas, P., and Nawata, E. 2007. "Effect of High Temperature during Seed Development on Quality and Chemical Composition of Chili Pepper Seeds." *Jp. J. Trop. Agr.* 51 (1): 22-9.

[4] Mansour-Gueddes, S. B., Tarchoun, N., Teixeira da Silva, J. A., and Saguem, S. 2010. "Agronomic and Chemical Evaluation of Seven Hot Pepper (*Capsicum annum* L.) Populations Grown in an Open Field." *Fruit, Vegetable and Cereal Science and Biotechnology* 4 (1): 93-7.

[5] Polowick, P. L., and Sawhney, V. K. 1985. "Temperature Effects on Male Fertility and Flower and Fruit Development in *Capsicum annuum* L.." *Scientia Horticulture* 25 (2): 117-27.

[6] Saha, S. R., Hossain M. M., Rahman M. M., Kuo, C. G., and Abdullah, S. 2010. "Effect of High Temperature Stress on the Performance of Twelve Sweet Pepper Genotypes." *Bangladesh J. Agril. Res.* 35 (3): 525-34.

[7] Khanduri, V. P., Shamar, C. M., and Singh, S. P. 2008. "The Effect of Climate Change on Plant Phenology." *Environmentalist* 28 (2): 143-7.

[8] Inthichack, P., Nishimura, Y., and Fukumoto, Y. 2013. "Diurnal Temperature Alternations on Plant Growth and Mineral Absorption in Eggplant, Sweet Pepper and Tomato." *Hort. Envir. Biotechnol.* 54 (1): 37-43.

[9] Cross, R. H., Mckay, S. A. B., Mchughen, A. G., and Bonham-Smith, P. C. 2003. "Heat Stress Effects on Reproduction and Seed Set in *Linum usitatissimum* L. (Flax)." *Plant Cell Envir.* 26 (7): 1013-20.

[10] Erickson, A. N., and Markhart, A. H. 2001. "Flower Production, Fruit Set and Physiology of Bell Pepper during Elevated Temperature and Vapor Pressure Deficit." *J. Amer. Soc. Hort. Sci.* 126 (6): 697-70.

[11] Qumer, I., Muhamad, A., Muhamad, R. A., Muhamad, A. A., and Riaz, A. 2009. "Vegetative and Reproductive Evaluation of Hot Peppers under Different Plastic Mulches in Ploy/Plastic Tunnel." *Pak. J. Agri. Sci.* 46 (2): 113-8.

[12] Ali, A. M., and Kelly, W. C. 1993. "Effect of Pre-anthesis Temperature on the Size and the Shape of Sweet Pepper (*Capsicum* spp. L.) Fruit." *Scientia Horticulturae* 54: 97-105.

[13] Pagamas, P., and Nawata, E. 2008. "Sensitive Stages of Fruit and Seed Development of Chili Pepper (*Capsicum annuum* L. var. Shishito) Expose to High Temperature Stress." *Scientia Horticulturae* 117 (1): 21-5.

[14] MEXT, JMA and MOE. 2009. *Climate Change and Its Impact in Japan.* Synthesis Report on Observations, Projections and Impact Assessments of Climate Change.

[15] Erickson, A. N., and Markhart, A. H. 2002. "Flower Development Stage and Organ Sensitivity of Bell Pepper (Capsicum annuum L.) to Evaluated Temperature" *Plant, Cell and Environment* 25: 123-30.

[16] Heuvelink. E., and Korner, O. 2001. "Parthenocarpic Fruit Growth Reduces Yield Fluctuation and Blossom-End Rot in Sweet Pepper." *Ann. Bot.* 88: 69-74.

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Photosynthetic Responses of Sweet Sorghum Cultivars to Cadmium Toxicity

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Abstract: Sweet sorghum is a C4 plant with high efficiency of photosynthesis and accumulation of photosynthetic products during growth stage. Until now, little is known about photosynthetic characteristics of sweet sorghum subjected to heavy metal contaminated soil. In this study, photosynthetic modification of sweet sorghum was measured in response to cadmium (Cd) under greenhouse conditions. The experiment was concentrated specifically on Keller (KE) and E-Tian (ET), two cultivars of sweet sorghum. The plants subjected to 100 mg/kg Cd in soil were compared with the control plants without Cd treatment. The results indicated that both cultivars of sweet sorghum display similar photosynthetic responses to Cd exposure. The high Cd concentration resulted in significant decline of photosynthesis net rate (Pn). Pn of ET declines more than Cd-treated KE plants. Moreover, Cd-treated plants were detected to have lower stomata conductance (gs) and intercellular CO_2 concentrations (Ci) than control plants. Chlorophyll content also displayed significant differences between control and Cd-treated plants. In ET, the reduction of both chlorophyll-a and chlorophyll-b was observed under Cd stress, compared with the controls. In contrast, chlorophyll content was significantly enhanced by Cd stress in KE. In conclusion, through the comparison between two sweet sorghum cultivars, it is demonstrated the photosynthetic activity of ET more sensitive to Cd stress than that of KE. Therefore, KE has a potential for biofuel production and phytoremediation of heavy metal in soil.

Key words: Sweet sorghum, photosynthesis, cadmium.

1. Introduction

Heavy metals are the main group of inorganic contaminants that seriously threaten food safety and public health. Nowadays, a large amount of land is contaminated by heavy metals due to industrial development and human activities, including fertilizers and emissions from municipal waste incinerators, car exhaust, residues from metalliferous mines and smelting industries, sludge or municipal compost and use of pesticides [1, 2]. Heavy metal contamination, such as cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and so on, has become a public concern in the world. Among them, Cd is one of the most toxic and non-essential heavy metal. Cd can be easily absorbed by plants and accumulated in agricultural products. Many methods and processes of preventing, removing and correcting the negative effects of pollutants released into the environments exist, but their application for this purpose has either been poorly implemented or not at all [3]. Thus, all major research studies ponder on the use of biological factors to deal and repair environment. Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless [4].

Sorghum bicolor originated from Africa is a pro-poor multipurpose crop that provides food, feed, fiber and fuel across a range of agro-ecosystems, especially in those with fragile conditions [5]. Sorghum is classified into the following four groups by application: grain sorghum, sweet sorghum, broom sorghum and grass sorghum. Sweet sorghum is an

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important bioenergy crop, because it can produce both food and fuel, with a high photosynthetic activity and high concentration of soluble sugars in stem. Sorghum is resistant to drought, high temperature and toxic pollutants. It is able to accumulate large quantities of Cd, Cu, Pb and Zn in shoots, and its biomass production was higher than other energy crops biomass production [6].

Moreover, sorghum plants were highly tolerant to metal pollution and capable of reaching high biomass values in the presence of metals [7]. The effect of heavy metals (Cd, Pb, Zn and Cu) on morphological and physiological characteristics of sorghum has been studied by Soudek et al. [6], Zhuang et al. [8] and Liu et al. [9]. They investigated the effect of Cd^{2+} on root activities, malondialdehyde contents in leaves, etc., at some stages of development, such as seedlings, elongation stage and so on. The results showed that low concentrations of Cd^{2+} stimulated the growth of sorghum plants, but high concentrations of Cd^{2+} (50 mg/kg and 100 mg/kg) significantly depressed the growth of sorghum plants. Previous studies have also focused on photosynthesis response of sorghum under different watering regimes [10].

Photosynthesis is the basic process of energy and biomass production in plants [11]. However, few studies have been performed to explore the photosynthesis characteristics of sweet sorghum under Cd stress. In this study, the photosynthesis characteristics of two sweet sorghum cultivars were assayed to demonstrate the effect of heavy metals on the photosynthesis process, especially during the flowering and seed production period. The results would provide a theoretical basis for using sweet sorghum to produce bioenergy combined with phytoremediation for heavy metal contaminated soils.

2. Materials and Methods

2.1 Plant Materials and Experimental Design

Two lines of sweet sorghum, Keller (KE) and E-Tian (ET), were chosen as plant materials. KE (GRIN access code PI 653617) is an elite sweet sorghum line developed by DM Broadhead at US Sugar Crops Field Station at Meridan, Mississippi in 1982, and has been grown globally and proven to have good performance across a range of environmental conditions. ET (literally meaning Russian sweet in Chinese) introduced to China in the early 1970s, is known to possess high Brix content in stem [5].

Seeds were immersed in distilled water and then germinated in the dark at 28 °C for 5 d. Subsequently, the seedlings were transferred to pots (diameter: 30 cm; height: 25 cm) with peat soil (2 kg soil for 2 seedlings/pot) and cultivated under glasshouse conditions (28-32 °C with 14-16 h light/22-26 °C with 8-10 h dark). $CdCl_2$ in solution was mixed with soil to increase Cd concentration to 100 ppm at the eight-leaf stage for Cd treatment. The plants without Cd treatment acted as the control. The measurement of photosynthesis parameters was conducted at 75 d after Cd exposure. There are 12 biological replicates for Cd treatment and control.

2.2 Photosynthesis Analysis

CIRAS2 portable photosynthesis system (PP System company, USA) was used for the measurement of photosynthesis parameters. Two fully expanded leaves under the flag leaf of each plant were assayed (the 2nd leaf and 3rd). The measurements (3 readings/leaf) were replicated on 12 plants every treatment. The net photosynthesis (Pn), Intercellular CO_2 concentration (Ci), transpiration rate (E) and stomatal conductance (gs) were determined under a light intensity of 1,200-1,600 μmol (photon)/m^2/s.

Chlorophyll pigments were extracted for measurement. Leaves were cut down and immediately put into ice. Then 0.15 g of the sample was weighed and extracted for chlorophyll according to the method of Lichtenthaler and Wellburn [12]. A spectrophotometer (Hitachi U-3000) was then used to measure the chlorophyll pigments.

2.3 Data Analysis

All the data were expressed as means ± standard deviation (SD). Comparison of means was performed by the least significant difference (LSD) test ($P \leq 0.05$) using Statistix software (version 10.0).

3. Results and Discussion

3.1 Effect of Cd^{2+} on Plant Photosynthesis Parameters

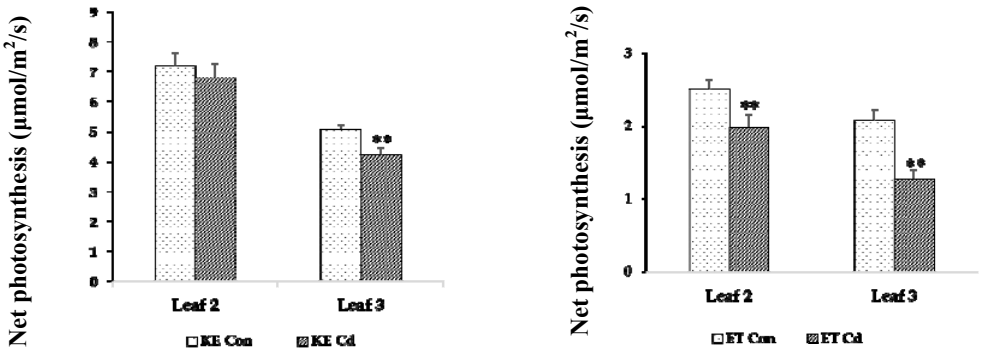
Photosynthesis is sensitive to changeable environmental conditions, and the way photosynthesis activity adapts to environments would be different in plants. Here, four parameters, including net photosysnthesis rate (Pn), intercellular CO2 concentration (Ci), Transpiration rate (E) and stomatal conductance (gs), were measured to characterize the Cd-induced photosynthetic changes.

In both KE and ET cultivars, Pn displayed significant differences between control and Cd-treated plants (Figs. 1a and 1b). Compared with control plants, the Pn of Cd-treated plants decreased by 5.29% to 16.7% and 27.3% to 29.4% in the 2nd leaf and the 3rd leaf of KE and ET, respectively. Especially in the 3rd leaf, Pn of KE in control reached 5.07 μmol/m^2/s, while only 4.2 μmol/m^2/s in Cd-treated KE plants. In ET, the inhibition of Cd on Pn was clearer. For both leaves, the net photosynthesis in the control plants was higher than the Cd-treated plants (Fig. 1).

Pn decreases with increasing concentration of Cd treatment, which has been reported by Liu et al. [9].

In addition, Pn of the 2nd leaf was higher than Pn of the 3rd leaf was observed in both cultivars (Figs. 1a and 1b), probably due to leaf senescence during growth. In this experiment, position of leaves was determined from top to bottom, therefore the 3rd leaf was older than the 2nd leaf. Senescence is a normal event in the life cycle of plants [13]. It is a sequence of complex degenerative processes that are initiated at full maturity and may ultimately lead to leaf death. The most remarkable events in leaf senescence are the loss of chlorophyll and the disassembly of the photosynthetic apparatus, which result in decreases in the photosynthetic energy conversion capacity and efficiency [14]. The result of Falqueto et al. [14] indicated that pigment content, photochemical efficiency of photosystem II and electron transport decreased significantly according to the position of leaves in two rice genotypes from the 1st to the 4th leaf.

Comparing the two varieties KE and ET, KE had higher Pn than ET, probably due to genotype and morphological characteristics of plant. In this experiment, we also found that the specific leaf area (SLA) of KE was higher than of ET.



(a) (b)

Fig. 1 Net photosynthesis (Pn, μmol/m^2/s) of KE (a) and ET (b) under Cd stress conditions.
Data with ** on the column mean significant difference at 0.05 level.

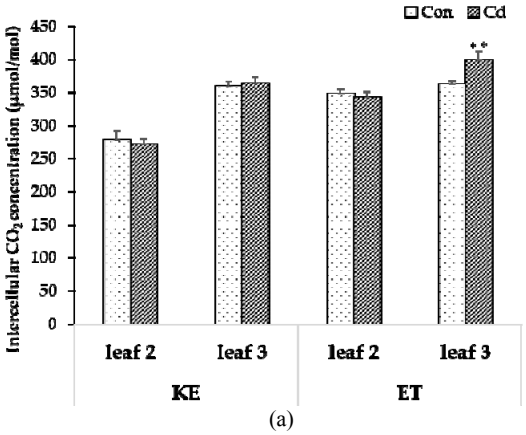
Previous result has suggested that the rate photosynthesis may be relative with the leaf area [15]. The species with higher SLA usually show high potential relative growth rates and higher Pn [16, 17].

Therefore, photosynthesis was inhibited by Cd exposure, it can be concluded that ET is more sensitive to net rate of photosynthesis changes than KE under Cd stress.

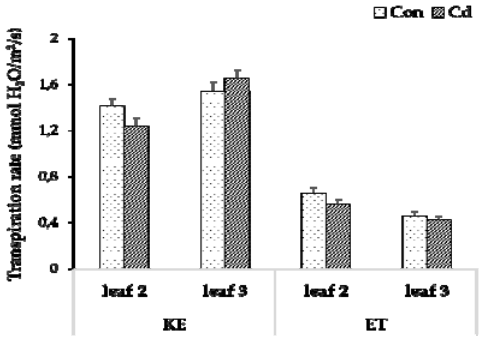
The rate of photosynthesis is an important physiological parameter, which governs the dry matter production and consequently the yield; intercellular CO_2, transpiration rate and stomatal conductance play an important role in assimilation of photosynthesis [18]. The results showed no significant differences in Ci of the two sweet sorghum cultivars. Intercellular CO_2 concentration decreased in the 2nd leaf of Cd plant, but was not statistically significant, in comparison to the 3rd leaf (Fig. 2a). The highest Ci (399.25 μmol/mol) was observed at the 3rd leaf of ET Cd-treated plant, while the lowest Ci (272.97 μmol/mol) was recorded at the 2nd leaf of KE Cd-treated plant (Fig. 2a). It suggests an ability of negative correlation between Pn and Ci in sweet sorghum under Cd treatment.

Moreover, transpiration rate (E) is an important physiological trait that facilitates gas exchange, and shows significant variations among plants. The results indicated that no significant differences in transpiration between control plant and Cd-treated plants in both cultivars. It ranged from 1.23 mmol $H_2O/m^2/s$ to 1.65 mmol $H_2O/m^2/s$ in KE, and from 0.42 mmol $H_2O/m^2/s$ to 0.66 mmol $H_2O/m^2/s$ in ET (Fig. 2b).

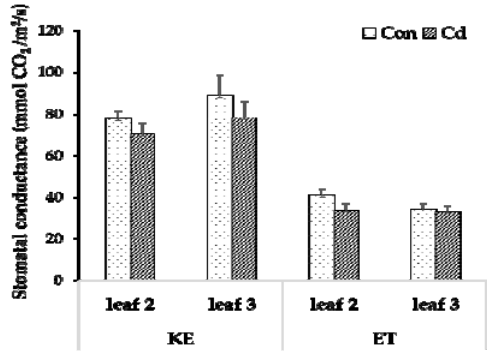
There was no significant difference between stomatal conductance of the two treatments of both cultivars. The highest value of gs (88.69 mmol $CO_2/m^2/s$) was observed in the leaf of KE control plant, while the lowest (33.08 mmol $CO_2/m^2/s$) was present at the leaf of ET Cd-treated plant (Fig. 2c). Hence, the stomata conductance was not sensitive to Cd. Although Cd had no significant effects on stomata conductance, it cause slight decline in the value of gs in all observed leaves. The decrease of stomata conductance in Cd-treated plants was accompanied by a decline of net photosynthesis rate. These results are consistent with the previous study [19].



(a)



(b)



(c)

Fig. 2 Intercellular CO_2 concentration (a), transpiration rate (b) and stomatal conductance (c) of KE and ET under the Cd stress condition.

Data with ** on the column mean significant difference at 0.05 level.

3.2 Photosynthesis Pigment: Chlorophyll

The results of this study showed that chlorophyll content in KE and ET were different in response to Cd stress. Chlorophyll content of KE was enhanced by Cd stress, but not in ET (Fig. 3). In ET, compare to the control, reductions of chlorophyll a and chlorophyll b were both detected under the Cd concentration of 100 mg/kg, although there was no significant difference. Chlorophyll content decreased from 5.74 mg/g fresh weight (FW) to 5.4 mg/g FW.

In contrast to that of ET, chlorophyll content of KE was significantly enhanced by Cd stress. An increase in the content of total chlorophyll, chlorophyll a and chlorophyll b were detected after treatment with Cd. Chlorophyll concentration increased from 5.08 mg/g FW in control plants to 6.43mg/g FW in Cd plants (Table 1).

In the case of ET, the results also suggest that the reduced rate of photosynthesis might be correlated with chlorophyll content. Similar with the previous studies by Zhu et al. [20], chlorophyll synthesis was affected by Cd stress. Chlorophyll a decreased greatly, while chlorophyll b did not, this indicate that the influence of Cd stress on chlorophyll synthesis is mainly manifested by the inhibition of chlorophyll a synthesis [20]. Cd stress on chlorophyll synthesis was positively correlated with Cd^{2+} concentration of 50 mg/kg and 100 mg/kg, and the chlorophyll contents significantly decreased ($P < 0.05$) [9].

In the case of KE, the result was also consistent with results of Kamelia et al [21]. There was a slightly increase in the chlorophyll a + b content after Cd treatment. The decrease of chlorophyll content in control plants was probably due to leaf senescence during growth. When chlorophyll content was determined, KE control plants were already at the hard dough stage, while the Cd-treated plants just finished the process of granulation and started the soft dough stage.

KE control plants displayed lower chlorophyll concentration and higher Pn, but KE Cd-treated plants inhibited the opposite phenotype (Fig. 3 and Table 1). In this case, the differences in Pn between the two treatments were not caused by the difference in chlorophyll, probably by light reaction system, photosynthesis efficiency or enzyme systems. Heavy metals could react with the photosynthetic apparatus at various levels of organization and display multiple effects on photosynthesis, including interaction with cytosolic enzymes and organics; alteration of the functions of chloroplast membranes, particularly on photosystem I and II, membrane acyl lipids and carrier proteins in vascular tissues; photosynthetic carbon reduction cycle enzymes, xanthophyll cycle and adenylates [22]. Hence, it is necessary to further study the mechanism of Cd toxic effect on chlorophyll content and photosynthesis response of KE on a cellular or molecular level.

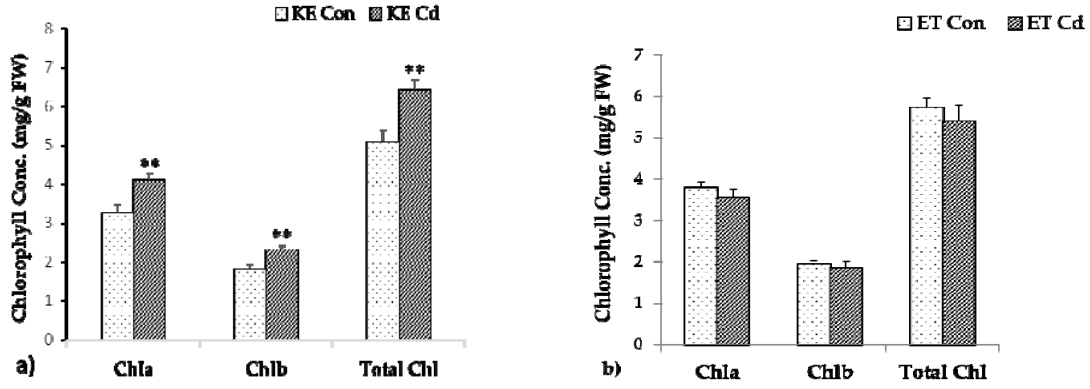


Fig. 3 Chlorophyll concentration in KE (a) and ET (b).

Data with ** on the column means significant difference at 0.05 level. FW: fresh weight

Table 1 Chlorophyll concentration (mg/g FW) of KE and ET under the Cd treatment condition.

Sorghum	KE		ET	
Chlorophyll	CK	Cd	CK	Cd
Chla	3.27 ± 0.20^{b}	4.11 ± 0.35^{a}	3.79 ± 0.35^{a}	3.55 ± 0.53^{a}
Chlb	1.81 ± 0.12^{b}	2.32 ± 0.19^{a}	1.95 ± 0.19^{a}	1.85 ± 0.39^{a}
Total Chl	5.08 ± 0.32^{b}	6.43 ± 0.54^{a}	5.74 ± 0.54^{a}	5.40 ± 0.89^{a}

Data with different letters in the same row of a cultivar means significant difference at 0.05 level.

4. Conclusions

According to the results, although there was not significantly different in a few parameters, but generally Cd damaged the photosynthetic activity of ET via the inhibition of chlorophyll content, intercellular CO_2 concentration, transpiration rate, stomatal conductance and especially the decline of photosynthetic rate. Hence, the resistance of KE in photosynthesis activity to Cd is greater than that of ET. KE with all the advantages could become a good candidate for combining phytoremediation and biofuel production.

Acknowledgments

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References

[1] Garbisu, C., and Alkorta, I. 2003. "Basic Concepts on Heavy Metal Soil Bioremediation." *Eur. J. Min. Proc. & Environ. Protect.* 3 (1): 58-66.

[2] Halim, M., Conte, P., and Piccolo, A. 2003. "Potential Availability of Heavy Metals to Phytoextraction from Contaminated Soils Induced by Exogenous Humic Substances." *Chemosphere* 52 (1): 265-75.

[3] Etim, E. E. 2012. "Phytoremediation and Its Mechanisms: A Review." *International Journal of Environment and Bioenergy* 2 (3): 120-36.

[4] Salt, D. E., Smith, R. D., and Raskin, I. 1998. "Phytoremediation." *Annu. Rev. of Plant Physiology and Plant Molecular Biology* 49: 643-68.

[5] Zheng, L. Y., Guo, X. S., He B., Sun, L. J., Peng, Y., Dong, S. S., Liu, T. F., Jiang, S. Y., Srinivasan, R. C., Liu, C. M., and Jing, H. C. 2011. "Genome-Wide Patterns of Genetic Variation in Sweet and Grain Sorghum (*Sorghum bicolor*)." *Genome Biology* 12: R114.

[6] Soudek, P., Jakub, N., Lukas, P., and Petrova, S. 2013. "The Sorghum Plants Utilization for Accumulation of Heavy Metals." In *Proceedings of the 3rd International Conference on Energy and Environmental Science (ICEES 2013)*.

[7] Pinto, A. P., Varennes, A., Goncalves, M. L. S., and Mota, A. M. 2006. "Sorghum Detoxification Mechanisms." *Journal Plant Nutrition* 29 (7): 1229-42.

[8] Zhuang, P., Shu, W. S., Liao, Z. L., Li, B., and Shao, J. S. 2009. "Removal of Metals by Sorghum Plants from Contaminated Land." *Journal of Environmental Sciences* 21 (10): 1432-7.

[9] Liu, D. L., Hu, K. Q, Ma, J. J, Qiu, W. W, Wang, X. P., and Zhang, S. P. 2011. "Effects of Cadmium on the Growth and Physiological Characteristics of Sorghum Plants." *African Journal of Biotechnology* 10 (70): 15770-6.

[10] Xie, T. T., Su, P. X., and Shan, L. S. 2010. "Photosynthetic Characteristics and Water Use Efficiency of Sweet Sorghum under Different Watering Regimes." *Pak. J. Bot.* 42(6): 3981-94.

[11] Sultana, N., Ikeda, T., and Kashem, M. A. 2001. "Effect of Foliar Spray of Nutrient Solutions on Photosynthesis, Dry Matter Accumulation and Yield in Seawater-Stressed Rice." *Environ. Exp. Bot.* 46 (2): 129-40.

[12] Lichtenthaler, H. K., and Wellburn, A. R. 1983. "Determination of Total Carotenoids and Chlorophylls a and b of Leaf in Different Solvents." *Biol. Soc. Trans.* 11 (5): 591-2.

[13] Borras, L., Maddonni, G. A., and Otegui, M. E. 2003. "Leaf Senescence in Maize Hybrids: Plant Population, Row Spacing and Kernel Set Effects." *Field Crops Research* 82 (1): 13-26.

[14] Falqueto, A. R., Casso, D., Junior, A. M., Oliveria, A. C., and Bacarin, M. A. 2009. "Physiological Analysis of Leaf Senescence of Two Rice Cultivars with Different Yield Potential." *Pesq. Agropec. Bras.* 44 (7): 695-700.

[15] Guo, D. P., Guo, Y. P., Zhao, J. P., Liu, H., Peng, Y., Wang, Q. M., Chen, J. S., and Rao, G. Z. 2005. "Photosynthetic Rate and Chlorophyll Fluorescence in Leaves of Stem Mustard (*Brassica juncea* var. *tsatsai*) after Turnip Mosaic Virus Infection." *Plant Science* 168 (1): 57-63.

[16] Gulias, J., Flexas, J., Mus, M., Cifre, J., Lefi, E., and

Medrano, H. 2003. "Relationship between Maximum Leaf Photosynthesis, Nitrogen Content and Specific Leaf Area in Balearic Endemic and Non-endemic Mediterranean Species." *Annals of Botany* 92 (2): 215-22.

[17] Reich, P. B., Ellsworth, D. S., and Walters, M. B. 1998. "Left Structure (Specific Leaf Area) Modulates Photosynthesis-Nitrogen Relations: Evidence from Within and Across Species and Functional Groups." *Functional Ecology* 12 (6): 948-58.

[18] Channappagoudar, B. B., Biradar, N. R., Patil, J. B., and Hiremath, S. M. 2007. "Study on Morpho-Physiological, Biophysical Characters and Alcohol Production in Sweet Sorghum Genotypes." *Karnataka J. Agric. Sci.* 20 (2): 234-7.

[19] Chen, X., Wang, J., Shi, Y., Zhao, M. Q., and Chi, G. Y. 2011. "Effects of Cadmium on Growth and Photosynthetic Activities in Pakchoi and Mustard." *Botanical Studies* 52: 41-6.

[20] Zhu, J. L., Xu, Z. F., Cao, H. L., Ye, W. H. 2008. "Effect of Cadmium on Photosynthetic Traits in Wedelia Trilobata." *Ecol. Environ.* 17 (2): 657-60.

[21] Miladinova, K., Markovska, Y., Tzvetkova, N., Geneva, M., and Georgieva, T. 2014. "Photosynthesis and Growth Response of Two *Paulownia* Hybrid Lines to Heavy Metals Cd, Pb and Zn." *Silva Balcanica* 15 (1): 83-99.

[22] Prasad, M. N. V., and Strzalka, K. 1999. "Impact of Heavy Metals on Photosynthesis." In *Heavy Metal Stress in Plants*. Heidelberg, Berlin: Springer Publisher, 117-38.

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Social Impact Assessment of the Benefit Sharing Mechanism Pilot in Co-management of Special-Use Forest in Vietnam: Case Study at Bach Ma National Park

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Abstract: To enhance local participation in forest protection and sustainable development in Bach Ma National Park, Thua Thien Hue province, Vietnam, a benefit sharing mechanism (BSM) based on principles of co-management has been piloted. This study aimed to evaluate socio-economic impacts of this pilot on people living in seven targeted villages of Thuong Nhat commune, Nam Dong district, Thua Thien Hue province after two years of implementation. Evaluation methods were household interview, in-depth interview, village meeting, focused group discussion and workshop with stakeholders at commune level. Results showed that: (1) the local community was strongly supported and involved in the BSM implementation, displaying a high level of demand for the measures; (2) local people's awareness of rights, responsibilities, forest protection and sustainable use of non timber forest products (NTFPs) has increased considerably; (3) the policy has also enhanced the awareness and participation of local people in cooperation with the park rangers to co-manage the forest. Additionally, this study provides a number of suggestions to improve the BSM, including (1) raising local people's awareness; (2) simplifying BSM procedures; (3) clarifying incentives and responsibilities of BSM actors to enhance co-patrolling and monitoring activities; (4) creating good incentives either based on the livelihood program for those who actively comply with the BSM regulations or direct payments to those who join forest-protection activities

Key words: Benefit sharing mechanism, Bach Ma National Park, special-use forest, co-management.

1. Introduction

Vietnam has more than 13.8 million ha of forest, including the system of special-use forests (SUFs), which comprises about 2 million ha distributed among 164 sites: 30 national parks, 58 nature reserves, 11 species-protected areas, 45 landscape-protected areas and 20 scientific practice and research forests [1]. According to the Law on Forest Protection and Development [2], the local people are not allowed to access and collect any natural resource in the SUFs. In most cases, the SUF management boards are staffed by officials assigned by the relevant provincial forest departments, but does not include representatives from other sectors or stakeholders from the local communities [3].

To create the legal framework for the co-management policy development, in February 2012, the Prime minister issued decision No. 126 on the pilot policy of benefit sharing mechanism (BSM) in management, protection and sustainable development in SUFs in Vietnam. According to this decision, the Bach Ma National Park and the Xuan Thuy National Park were the two SUFs chosen as pilot sites for implementing the BSM in Vietnam. The BSM pilot aimed at setting up a legal platform for benefits, rights and responsibilities-sharing mechanisms between the SUF management boards and local communities based on the co-management principles [4].

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As sharing benefits and responsibilities in co-management of SUFs was a relatively new issue in Vietnam, the BSM pilot would have both positive and negative impacts on the local people [5, 6]. Hence, the research objective was to assess the impacts of the BSM pilot implementation on the social factors affecting the local people living in the pilot area. The research also aimed to identify local perspectives on the BSM's achievements, failures and challenges, and to propose ways of improving the BSM development and implementation, with the ultimate goal to scale up the BSM policy for SUFs in Vietnam in the future.

2. Methods

2.1 Study Site Selection

The Thuong Nhat commune has been selected to research, because the BSM pilot took place in its communities (Fig. 1). Following the BSM plan approved by Ministry of Agriculture and Rural Development (MARD) in October 2012, the seven villages, including Ta Rin, Lap, Ta Lu, A Sach, A Tin, La Van and Hop Hoa, located in the Thuong Nhat commune would have the official rights and responsibilities to cooperate with the Bach Ma National Park management board to protect the forest and use sustainably non timber forest products (NTFPs) in the park.

2.2 Data Collection

In this study, both qualitative and quantitative data was collected. Firstly, the author gathered relevant documents of the BSM pilot policy, related research on BSM and co-management in Vietnam and the world wide, and open-access information on the study sites of the Bach Ma National Park and the Thuong Nhat commune. The information and research collected were reviewed to develop the research objectives and research questions. Then, the author held a group discussion with experts in June 2013 to get their advice on developing factors for impact assessment of the BSM pilot policy. The experts involved in this consultant group discussion included the two lecturers from the Hue University of

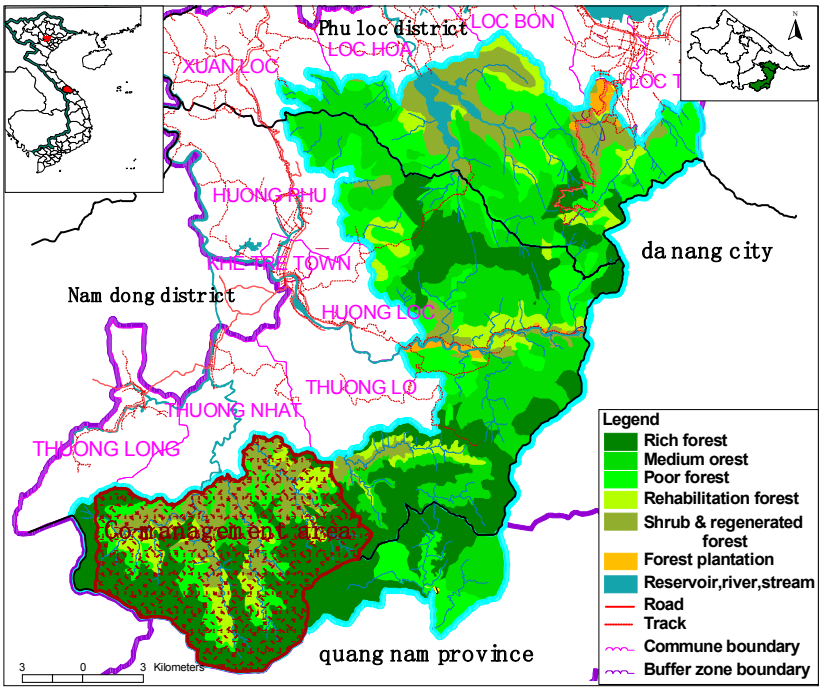

Fig. 1 Map of Bach Ma National Park and the co-management area under the BSM [7].

Agriculture and Forestry who undertook consultant work for developing the BSM plan, the BSM project coordinator, the president of Thuong Nhat People's Committee, the village head of Talu village in Thuong Nhat and the head of the Thuong Nhat forest-ranger station.

The discussion output was a framework of factors used to assess impacts of the BSM pilot policy and the policy implementation. Based on this framework, the open and closed questionnaires were designed, tested and used for household interview surveys. There were 112 semi-structured and structured household interviews undertaken during two periods in December 2013 and December 2014. The closed questions were used to measure the level of local support, demand and participation in the BSM implementation, the local people's awareness of their benefits, rights and responsibilities when participating in the BSM, and the BSM impacts on NTFPs dependence. The open and mixed (closed and open) questions were used to clarify advantages, disadvantages, conflicts, and inequity and risks of the BSM, as well as the local perspectives on the BSM implementation's achievements, failures and potentials. Further information was gathered by means of village meetings, group discussions, in-depth interviews, a consultant workshop, and village and forest transects. The summary of the primary data collection process is described in Table 1.

2.2.1 Household Interviews

One socio-economic baseline study of the Thuong Nhat commune in November 2012 showed that about 65% of the households (Hhs) were harvesting NTFPs in the Bach Ma National Park and 35% of them were not. The study revealed that 36% of the Hhs were poor and sub-poor, and 64% were non-poor [8]. To ensure the accurate representation of the Hhs in the interview survey, based on the participated household name lists of the seven villages, the authors randomly selected those to be interviewed according to the current ratio

Table 1 Summary of the primary data collection process.

Data collection process			Main issues
Methods	Quantity/description	Time/place	
Household interviews	40 randomly selected households (first collection)	7-12 Dec. 2013; interviewee's house	-Local awareness of the benefits, rights and responsibilities of BSM; -BSM advantages/disadvantages; -Conflicts, inequity and risks; -Local perspectives on the BSM's achievements, failures, potentials.
Village meetings	7 meetings × 20 participants/each village (7 villages) = 140 participants	15-17 Dec. 2013; village communal houses	-Level of local support, participation in the BSM policy; -Impacts of the BSM on social schemes; -Level of local dependence on NTFPs (number of NTFP users); - Issues and solutions of BSM.
Group discussions	3 group discussions × 6 NTFP-based villagers = 18 participants	19-20 Dec. 2013; village communal houses	-Failures and solutions when implementation of BSM; -BSM management and illegal cases' treatment status; -Social vulnerabilities: risks, inequities, conflicts.
In-depth interview	19 key informants: 7 village leaders, 2 local NTFPs traders, 2 rangers, 7 VFP team heads and 1 local official	26-28 Dec. 2013; Interviewee's houses or office	-Individual perspectives on the BSM failures, risks, conflicts and future potentials: reasons and suggestions for improvement; -Crosscheck of the BSM issues that arose in village meetings.
Consultant workshop	5 local agencies + 5 Bach Ma National Park + 22 BSM actors + 14 VFP team members + 35 villagers	Mar. 2014; Thuong Nhat communal house	-Awareness and feedback of the BSM from stakeholders; -Suggestions, solutions for BSM implementation.
Household interviews	72 randomly selected households (second collection)	16-25 Dec. 2014; interviewee's house	-Local awareness of the benefits, rights and responsibilities of BSM; - BSM advantages/disadvantages; - Conflicts, inequity and risks; -BSM impacts on NTFP dependence and the local people's participation in forest protection; - Local perspectives on the BSM's achievements, failures, potentials.

of Hhs types mentioned above. The interviewees were all above 18 years old and mostly gender balanced, as the author interviewed both spouses of the households. The profile of the Hhs interviewed is shown in Table 2.

Questionnaires were designed and pre-tested with five Hhs to verify and validate the Hhs survey instrument in Thuong Nhat villages on December 3-4, 2013 (for the first household interview survey) and December 10-11, 2014 (for the second household interview survey).

The first Hhs interview survey in December 2013 was implemented with the questionnaire, including 10 open (semi-structured), 18 closed multiple-choice and open (mixed) and 9 closed multiple-choice questions. The questionnaire for the second Hhs interview survey in December 2014 included 5 open (semi-structured), 24 closed multiple-choice and open (mixed), and 12 closed multiple-choice items.

The closed questions were used to measure the level of local support for and participation in the BSM implementation, the local awareness of their benefits, rights and responsibilities when participating in the BSM, and the BSM's impacts on the local income and NTFP dependence.

The open (and mixed/closed and open) questions were used to clarify advantages, disadvantages, conflicts, inequity and risks of the BSM and the local perspectives on the BSM's achievements, failures and future potential.

The questionnaire was used to interview the responders at home. The closed-questionnaire interviewees were asked about: (1) their support and participation level in the BSM, (2) their awareness of the benefits, rights and responsibilities when participating in BSM, (3) NTFP dependence of local people, (4) perception of forest-protection status and (5) perception of NTFP sustainable use.

For each factor, the response multi-choice options were based on the responder's agreement level, e.g., "strongly agree", "agree", "agree normally" "disagree", "strongly disagree" and "I don't know". The results were analyzed by creating simple graphs in Excel that presented the distribution of responses among the given response options.

The mixed questionnaire (between the closed and open questionnaires) was also used to ask the interviewees for their responses to the multiple-choice or Yes/No questions. Then the interviewer asked the individuals why they had selected the specific option. The answers to these questions and the open questions were classified and coded as "a", "b", "c", "d", etc., based on the category of the relevant answer for each question. The Countif formula of Microsoft Excel 2007 was used to calculate the percentage of each answer category.

The results of the Hhs interview surveys were often quantitative types, such as average number, total number and percentage. The calculated results of the first and second Hhs interview surveys were compared question by question to clarify the changes in local perceptions on relevant issues. The qualitative data collected from the focus group discussions or the in-depth interviews were used to crosscheck and triangulate the results of the Hhs interview surveys and the results of the village meetings.

Table 2 Interviewee profile of the household interview surveys.

Total number of Hhs interviewed	Age		Households' dependence on NTFPs		Household type	
	18-40	> 40	Harvesting	Non-harvesting	Poor/sub-poor	Non-poor
40 (9% total Hhs population) (December, 2013)	36	4	30	10	15	25
	90%	10%	75%	25%	37.5%	62.5%
72 (15% total Hhs population) (December, 2014)	62	10	46	26	26	46
	86%	14%	64%	36%	36%	64%

Source: Hhs interview survey in December 2013 and 2014.

2.2.2 Village Meetings

After finishing the first Hhs interview survey, the researcher, along with the village heads, conducted seven village meetings in the villages investigated in Thuong Nhat to gain information on levels of local support and participation in the BSM policy, impacts of the BSM on social schemes and levels of local dependence on NTFPs.

There were seven village meetings, each involving the participation of 20 villagers. The village meetings took place in the village communal houses from 15 to 17 December 2013. The questionnaire designed and used for village meetings included only open questions. At the village meetings, the researcher and the village head raised questions (if needed explained them to the participants). The frequency of each reply to each question was recorded on sheets of paper. The results of the village meetings were used to clarify the issues when interpreting the results of Hhs interview surveys.

2.2.3 Focus Group Discussions

After completion of the household surveys, the village meetings and focus group discussions were organized to clarify the issues of failures and solutions in the implementation of BSM and social vulnerabilities, such as risks, inequities and conflicts. There were three group discussions. Each group included six villagers, who were involved in various main types of NTFP exploitation (rattan group, lingzhi mushroom group and honey group). Issues were presented one by one to the participants for discussion, and both the issues and the answers were written in brief form on a notice board during the exchange of opinions. Notes were taken in regard to all answers in order to support the data analyzed from the household survey. The group discussions took place in the village communal houses from 19 to 20 December 2013. Each group session took about 2 h.

2.2.4 In-depth Interviews

The researcher also undertook in-depth interviews with the leaders of the seven villages investigated, two local NTFPs traders, two forest rangers (one of them is the head of the Thuong Nhat Forest Protection Station), seven VFP team heads and the president of Thuong Nhat people's committee (who is also the vice-head of the BSM management council). The purpose of the in-depth interviews was to crosscheck the information gathered from the group discussion and the household interview surveys. The in-depth interviews were conducted with 19 key informants from 26 to 28 December 2013. Based on the role of the interviewees, the interviewer asked specific questions to deepen the individual perspectives on the BSM failures, risks, conflicts and future potential. Notes of the responses were taken in order to support the data triangulation of the respective issues. Each in-depth interview took about 30 min.

2.2.5 Consultant Workshop

In order to share the study results, the researcher took part in a workshop to evaluate the BSM one year after its implementation. The workshop was held in the Thuong Nhat commune in March 2014. The workshop participants were from the provincial forest protection department (5 people), the Bach Ma National Park management board (3 people), the Thuong Nhat Forest Protection Station (2 people), the BSM management council members (12 people), the monitoring group (10 people), the village forest protection teams (14 people) and residents of seven villages (35 people). The author of this research attended the workshop to present the initial results of the research in order to get feedback from different stakeholders and to take notes of ideas or suggestions from the participants for data triangulation.

The researcher also joined and shared the study results at the workshop to evaluate the BSM after two years of implementation in Ha Noi in March 2015. The workshop participants were from the different governmental agencies of MARD, BSM implementation partners, such as local authorities and national parks, and the relevant non-governmental organizations (NGOs) working in Vietnam. The

workshop was held by the Vietnam Forestry Administration (VNFOREST) with the purpose of evaluating the BSM pilots and discussing the possibilities of extending the pilots and the co-management policy development for the SUFs in Vietnam.

3. Results and Discussion

3.1 Impacts of the BSM on the Rights and Responsibilities of Local People

Later on one year of BSM implementation, through the efforts of Bach Ma National Park and the local authority, the local awareness of people's rights and responsibilities increases considerably. Table 3 showed that local people have understood mostly the rights of "harvest NTFPs legally" (100%), "remind or chase away the outsiders when entering into the forest" (93%), "remind others of sustainable NTFP extraction" (73%) and "monitor the village fund" (81%), which were a good deal higher than the results surveyed one year ago.

Regarding local awareness on their responsibilities in the BSM implementation, the results showed that the awareness has increased considerably after two year of participation in the BSM (Table 4). This proved that the BSM or co-management was a learning process, and local people, especially the ethnic people of Catu, could learn by doing. Therefore, the protected area management board and the BSM management council need to do well the outreach program of BSM/co-management propaganda in order to let local people fully understand their rights and responsibilities not only through village meetings but also through facilitating their engagement and information sharing in the BSM.

3.2 Impacts of the BSM on the Local People's Participation in Forest Protection

The BSM pilot based on the co-management principles aimed to enhance the co-operation of local community in forest protection. The research clarified from the work record books of the Thuong Nhat Forest Protection Station that the number of logging cases decreased significantly after two years of BSM implementation (Table 5). Additionally, Table 5 showed that the number of local messages delivered to the park rangers to stop the illigal activities in the forest increased after two years of the BSM implementation (12 times in 2012, 19 times in 2013 and 25 times in 2014).

Table 3 Awareness of rights after two years of the BSM implementation.

Parameter	Year 2013 (%)	Year 2014 (%)
Harvest NTFPs legally	65.0	100.0
Remind or chase away the outsiders when encroaching the forest	62.5	93.0
Remind others of sustainable NTFP extraction	47.5	73.0
Monitor the village fund	52.5	81.0
Protect the forest	42.5	36.0

Source: empirical survey data, December 2013 and December 2014.

Table 4 Awareness of responsibilities after two years of BSM implementation.

Parameter	Year 2013 (%)	Year 2014 (%)
Apply for harvest permission paper	65.0	95.4
Follow of the NTFP extracting regulations	55.0	93.2
Deduction for the village fund after harvest	40.0	81.8
Deliver of messages for rangers	0.0	72.7
Village fund oversees	0.0	56.8
Sustainable NTFP extraction reminds	0.0	95.4

Source: empirical survey data, December 2013 and December 2014.

Table 5 Illegal logging status in the co-management area.

Category	Total of illegal logging cases	Total of wood confiscated (m^3)	Total of messages delivered by locals
Year 2012	14	14.06	12
Year 2013	6	11.59	19
Year 2014	8	5.737	25

Source: work record book of Thuong Nhat Forest Protection Station, 2012, 2013, 2014.

Table 6 Conflicts when implementation of the BSM.

Parameter	Agree (%)
No conflict when BSM implementation	83.3
Conflicts when BSM implementation:	16.7
NTFP exploitation zones	50.0
Social arguments among villagers and traders	16.7
Deduction for the village fund after harvest	16.7
Registered and non-registered harvesters	8.3
Procedures of application for harvest permission paper	8.3

Source: empirical survey data, December 2014.

It was also assessed by the rangers when doing the in-depth interviews that even the number of message delivers was not yet high, but much more than the years before the BSM implementation. They said that it was more important to have exact rather than many messages, and for the year 2013 and 2014, they felt happy and supportive for their work due to the right messages and the increasing number of messages they could get from the local people than before. This was a positive sign to prove that the BSM pilot policy has brought the more co-operation of the local people, when they aware of their rights, benefits and responsibilities from the BSM pilot.

The above results were similar to the research conclusion of Minh et al. (2014) [9], who reported that there was no encroachment on forestland in the co-managed area, which happened before in some places along the park boundary and the Thuong Nhat commune, and no significant impacts on the forest vegetation.

From the above analysis, we could say that thank to the BSM policy, the local awareness and actions in cooperation with the park rangers in forest co-management have increased annually after the BSM implementation. Nevertheless, it was necessary to deliver a clear mechanism for local people, when they delivered serious messages. In addition, it was also important to set up a good working mechanism, budget and training of necessary skills for the village forest protection (VFP) team members to be more active in implementing their full responsibilities as required.

3.3 Impacts of the BSM on the Social Vulnerability

During the BSM implementation, some social issues, such as conflicts and inequities happened, should be assessed in order to promote the co-management's effectiveness. The results of impacts of the BSM on conflicts are seen in the Table 6.

Table 6 indicates that only 16.7% of the interviewees said that there were conflicts regarding implementation of the BSM. Although this number is low, it needs to explore what these issues were, because they are the source of potential conflicts in future. The Hhs interview also showed that many conflicts (about 50% of total number) were on the boundary demarcation of the NTFP exploitation zones of each villages. This was because in some cases, villagers exploited NTFPs in places managed by other villages, especially in the case of honey and lingzhi mushroom collection. Conflicts between registered and non-registered harvesters only account for 8.3% of total conflicts. In addition, the research results of impacts of the BSM on inequity are also presented in

the Table 7.

According to results of Hhs interview in December 2014, 54% of interviewees said that the BSM implementation was equal, while 32% said that it was unequal. Most of the cases of inequality happened in the treatment methods of the registered and non-registered harvesters, which accounted for 61% of the total number of cases. The other inequality when implementation of the BSM was the "difference of harvested amount among harvesters", which accounted for 26%. Due to no regulation to balance the harvest amount among harvesters, some Hhs with stronger labour force, e.g. young male, could harvest a lot more of NTFPs, while other Hhs could not. Adding in to the statement of unequality, people who lived in other communes, such as Thuong Long, Huong Huu, Huong Giang, and who used to exploit the NTFPs in the co-management forests of Thuong Nhat commune at the Bach Ma National Park said that it is unfair, when the government had a decision to give only people from Thuong Nhat commune an official right to manage and harvest the NTFPs in the forests.

3.4 Local People's Perspectives on the BSM Continuation

The BSM pilot has encouraged local people to participate in forest protection and sustainable use of the NTFPs at Bach Ma National Park. Therefore, this pilot should be continued in the near future.

Results from the Hhs interview survey about potential impacts of the BSM implementation on livelihood and the forest protection in the coming years are summarized in the Table 8.

Table 8 showed that 96% of interviewees expected that the policy needed to be continued, because the BSM would give more benefits and income to local people (37.7%), provide long-term NTFPs exploitation (23%), and lead to better forest protection (13%).

Table 7 Inequity when implementation of the BSM.

Parameter	Agree (%)
Equity	54
Do not know	14
Inequity:	32
Treatment of registered and non-registered harvesters	61
Difference of harvest levels among the harvesters	26
Compliance of deduction for village fund after harvest	9
Classification of NTFPs exploitation areas	4

Source: empirical survey data, December 2014.

Table 8 Impacts of the BSM on livelihood and the forest protection for next years.

Agreement	Reasons to continue/not continue the BSM	Rate of agreement (%)
Continue to implement the BSM (96%)	Increase of local Hhs income	11.50
	Better forest protection	13.04
	Better forest protection	13.04
	Long-term NTFPs exploitation	23.19
	Give more benefits to local people	26.09
	Not so clear but need to continue the BSM	23.19
	Others	2.90
Not to continue the BSM (4%)	Rarely or not go to the forests	66.67
	Don not know	33.33

Source: empirical survey data, December 2014.

Table 9 Local perspectives on the better BSM implementation.

Activities need to do	Rate of agree (%)
Raise the local awareness and mobilize the locals to join the BSM	6.0
Support the seedlings and trainings to improve the livelihood	28.6
Simplify the procedures to register and apply for harvest permission paper	17.9
More allowance supports and incentive policies for the VFP teams and good participants of the BSM	11.9
More serious enforcement and monitoring of the rangers and the VFP teams under the clear mechanism	21.4
Don not know or other ideas	14.3
Total	100.0

Source: empirical survey data, December 2014.

3.5 Local People's Perspectives on the BSM Implementation Improvement

Local people suggested several ways to improve the BSM, if it continued in the future. From the Hhs interview survey results stated in Table 9, most of the interviewees mentioned that the BSM should pay more attention to the livelihood program to reduce dependence of local people on the forest resources (28.6%). They said that they needed to have good seedlings, animal breeds and trainings to improve their livelihoods. The interviewees also recommended enhancing the law enforcement and forest monitoring of the park rangers and the village forest protection teams under a clear mechanism (21.4%). As analyzed in the above sections, this activity was necessary to increase the local compliance with the law/regulations and the local autonomy in the BSM implementation.

Additionally, 17.9% of the interviewees suggested that the BSM management council should simplify the procedures of registration and application for NTFPs harvest. The village meetings also had similar ideas on this issue, because it was time consuming and complicated, when local NTFPs users have to pass three steps to get the harvest permission paper. They recommended that, instead of three steps, a two-step application process should be applied under the delegation of the BSM management council to the village heads and the ranger stations. On the other hand, 11.9% of the interviewees expected to have more allowance support and incentive policies for the VFP teams and the participants, if the BSM continues to operate.

4. Conclusions

The present research showed that the BSM pilot in the Bach Ma National Park produced various achievements, failures and challenges. The BSM pilot has achieved some definite successes, including (1) high support, demand and involvement by the local people during the BSM pilot-policy implementation; (2) improvement of the local people's awareness and practices in NTFP sustainable use and management; (3) increase of the local people's awareness and participation in forest protection; (4) improvement of natural-resource conservation in the co-management area during the BSM pilot. However, the process of the BSM implementation still faced a number of challenges, such as: (1) the complexity of NTFP harvest procedures; (2) the low local self-management (autonomy) in compliance with the BSM regulations; (3) the conflicts arisen between NTFPs users from village to village and between local traders and NTFP collectors in Thuong Nhat; (4) some inequities happened when implementation of the BSM pilot.

According to the local people's perspectives, the BSM pilot needed to be continued, because it would give more benefits and income to local people, provide long-term NTFPs exploitation, and lead to better forest protection. Additionally, for better implementation of the BSM, it needed to pay more attention to the livelihood programs, to enhance the law enforcement and forest monitoring under a clear mechanism, to simplify the procedures of registration and application for NTFPs harvest, and to secure more allowance support and incentive policies for the BSM

participants.

Furthermore, the research ascertained that the BSM implementation techniques and sustainability were considered as significant barriers to wide-spread adoption in the near future. As the case study in the Bach Ma National Park showed, it remained a challenge to be able to perfect the management system, to empower the BSM participants' capabilities and to create the autonomy of local communities to co-manage the SUF. Therefore, the BSM pilots need to be continued and adopted to well-managed SUFs under the MARD regulated guideline to have more assessment to develop the appropriate BSM policy for SUF co-management in the future.

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Refereences

[1] VNFOREST 2014. *Rural Development on the National Planning of the Special-Use Forests System until 2020.* Report of the Vietnam Administration of Forestry, the Ministry of Agriculture, Hanoi, Vietnam.

[2] Vietnam National Assembly. 1991. *Law on Forest Protection and Development of Vietnam.*

[3] ICEM 2003. *Vietnam National Report on Protected Areas and Development: Review of Protected Areas and Development in the Lower Mekong River Region.*

[4] Minister of Agriculture and Rural Development (MARD). 2012. *Decision No. 126/QD-TTg: The Prime Minister on the Benefit Sharing Mechanism Pilot Policy on Protection, Management and Sustainable Development of SUFs in Vietnam.*

[5] Loi, N. V., and Linh, N. V. 2013. "Study on the Status and Propose Solutions to Minimize the Risk in Implementing the Benefit-Sharing Mechanism of Water Rattan Species at Bach Ma National Park in Thuong Nhat Commune, Nam Dong District, Thua Thien Hue Province." *Vietnam Journal of Forest Science* 1: 2670-9.

[6] Loi, N. V. 2013. "Status and Propose Solutions to Minimize Conflict on Use of Natural Resources in Implementing the Benefit-Sharing Mechanism at Bach Ma National Park." *Science and Technology Journal of Agriculture and Rural Development* 23: 94-101.

[7] Minister of Agriculture and Rural Development (MARD). 2012. *Decision No. 2394/QD-BNN-TCLN: The Vietnam Administration of Forestry on the Approval of the Benefit Sharing Plan in Management, Protection and Sustainable Development of Bach Ma National Park in Thuong Nhat Commune, Nam Dong District, Thua Thien Hue Province, Vietnam.*

[8] Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ). 2012. *Report on the Baseline Study of Socio-Economic Status and Natural Resource Uses within the Seven Villages under the Decision No.126/QD-TTg in Bach Ma National Park and Buffer Zone.*

[9] Minh, H., Linh, N. V., and Loi, N. V. 2014. "Analysis of Normalized Difference Vegetation (NDVI) of Landsat 8 to Assess Impacts of the Benefit-Sharing Mechanism's Implementation to the Vegetation Cover in the Bach Ma National Park." *Journal of Forest and Environment, Hanoi: Science and Technology* 63: 7-11.

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Nutrient Mass Balances in Intensive Shrimp Ponds with a Sludge Removal Regime: A Case Study in the Tam Giang Lagoon, Central Vietnam

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Abstract: Understanding the sources and sinks of nutrients is of significant importance for better management of pond water quality and the environmental impact of aquaculture. The objective of the present study was to estimate the nutrient mass balances of four intensive shrimp (*Litopenaeus vannamei*) ponds in Tam Giang Lagoon, Central Vietnam, using a sludge management regime. The nutrient budgets were calculated based on the sources and sinks of nutrients in the ponds over a period of 49 d. The input sources of N and P were mainly shrimp feed, which accounted for more than 90%. Shrimp harvesting was the largest sink of N (37.5%), but not of P (18.3%). Almost a 30.4% N and 16.9% P of input were not accounted for the measured losses. While the smallest proportion of N (18.9%) was retained in sludge, the largest amount of P was accumulated in sludge (53.2%). The farm was operated without water exchange, so ponds gained only 1.9% N and 4.2% P from water intake. The pond lost about 13.2% N and 11.6% P from discharge water. Production of 1 kg shrimp needed 84.9 g N and 26.1 g P from total input sources and discharged 47.3 g N and 16.0 g P to the environment. Environmental losses of nutrients were lower or intermediate, when the loads were expressed in both kg/ha/cycle and kg of N or P per ton of shrimp produced. Furthermore, the environmental impacts of aquaculture are controlled from the system.

Key words: Shrimp pond, *L. vannamei*, nutrient mass balances, N, P.

1. Introduction

Rapid development of shrimp farming has generated considerable concerns about the effects of aquaculture pond effluents on nearby aquatic ecosystems due to increasing nutrient input [1]. Higher stocking densities of shrimp in ponds usually increases feeding rates with a concurrent increase in accumulation of sludge [2], which exacerbates problems of water quality and sediment deterioration. The water quality in ponds varies with the intensity of farming [3], and the deterioration of water quality in intensive culture ponds has been mainly related with organic matter, total nitrogen and inorganic phosphorus [4]. The feed ration is estimated from the shrimp biomass in ponds, and intensive culture requires more feed input. On the other hand, the growth of shrimp in an extensive system depends fully on natural food organisms, and in semi-intensive culture systems, both supplemental feed and natural food organisms are used [5].

According to International Union for Conservation of Nature (IUCN & IISD) [6], shrimp farms on sandy land utilize unproductive land and offer opportunities for development of methods of shrimp disease management. However, one of the biggest challenges for shrimp culture is how to simultaneously overcome environmental and economic concerns by implementing management strategies to reduce water contamination and sludge [7], because large amounts of nutrient inputs are lost to the environment [1, 8]. In response to this, enhancing nutrient recovery through

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the shrimp harvest and reducing environmental problems should be applied to intensive shrimp culture. Removing sludge accumulated at the bottom is considered to be an effective technique that can reduce up to 67% of N added as feed [9]. Furthermore, pond effluents and sludge disposal need much more attention for sustainable aquaculture development, because sludge removal takes materials out of the ponds before they are mineralized and release inorganic nutrients into the water column. Sludge removal coupled with water exchange methods is acceptable in reducing the nutrient concentration and risk of shrimp diseases. Previous works have presented methods to improve water quality and productivity of intensive shrimp ponds. So far, very few reports have described the nutrient mass balances in aquaculture on sandy lands that may have different pond bottom characteristics, especially in ponds with a sludge removal regime.

Nutrient mass balances that account for all inputs and outputs should be examined to assess environmental impacts of aquaculture [10, 11]. Many studies have calculated nutrient mass balances in extensive shrimp ponds [12], semi-intensive ponds [8, 10, 11] and intensive ponds [7, 13], while, the authors need to elucidate the nutrient budgets in the system for sustainable development. So, the objective of the present study was to estimate the nutrient mass balances for intensive shrimp culture in Tam Giang Lagoon, Central Vietnam, using a sludge management regime.

2. Materials and Methods

2.1 Study Area Description and Pond Management

Tam Giang Lagoon, located in Central Vietnam, is the largest lagoon in Southeast Asia with a total surface area of 22,000 ha [14]. Aquaculture in the lagoon has been developed since the 1970s, and shrimp culture began in the early 1990s. It quickly became a very important economic sector at the end of the 1990s and early 2000s. Farming of *L. vannamei* in Central Vietnam is more intensive (8%) than in other regions of the country [15]. The C.P. Group, belonging to the Charoen Pokphand Group of Thailand, has invested in *L. vannamei* shrimp farming on sandy land located in the North part of the lagoon (Fig. 1), Thua Thien Hue Province, Vietnam, since 2011. The shrimp farm has an area of 180 ha in total for industrial shrimp production. The first shrimp production cycle was operated in the spring from March to May 2013 (49 d). Before being transferred to grow-out ponds, post-larvae (PL) were reared with a density of 8,000 PLs/m^3 in a 300 m^2 pond in a greenhouse to the size of approximately 2,000 larvae/kg in order to minimize mortality and to reduce the grow-out culture period. Seawater was pumped directly from the sea into the pretreatment pond, and mixed with fresh groundwater to get a salinity of 28-30 ppt. Intake water was delivered to the ponds through a network of plastic pipes. *L. vannamei* shrimp were cultured at two sub-farms, A and B, consisting of 59 grow-out ponds; sub-farm C, rearing ponds in a greenhouse; and sub-farm D, under construction for grow-out ponds. Four grow-out ponds at sub-farm A were randomly selected to monitor water and sludge quality and calculate nutrient mass balances. An average stocking density of shrimp in four ponds was 70 PL/m^2. Characteristics of ponds used for mass balance estimation was described in Table 1. Shrimp were fed four times per day (07:30, 12:00, 17:00 and 22:00) with 40% crude protein commercial diet of C.P. shrimp feed [16]. The initial feeding rate was 9.5% of the biomass of each pond with adjustment according to apparent consumption through feeding trays. The daily rations were decreased to 1.5% of biomass at harvesting phase.

Fig. 2 shows the layout of the ponds used in the present study. Pond bottoms and dikes were lined with high-density polyethylene (HDPE) film to prevent water loss through seepage. Each pond had an average area of 0.43 ha, a depth of 1.4 m, and six electric aerators (12 horsepower (HP)) arranged to circulate the flow in the pond.

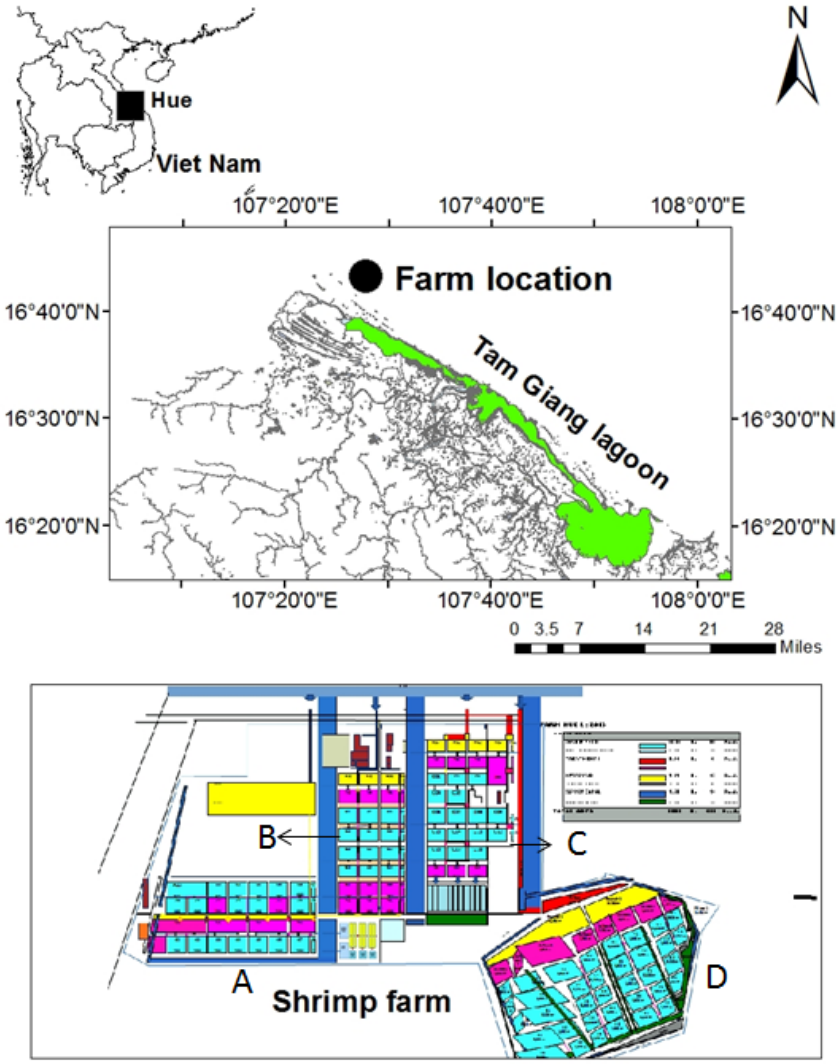

Fig. 1 Shrimp farm location.
A, B are sub-farms for grow out ponds, C is sub-farm in greenhouse for post-larvae rearing, and D is under construction for grow out.

Table 1 Characteristics of ponds for nutrients mass balance estimation.

Ponds	Density (No./m^2)	MBW (g)	Survival rate (%)	Yield (kg/ha/cycle)	FCR	Cultural period (days)	Feed used (kg/ha/cycle)
1	70.0	15.9	64	7,111.1	1.17	45.0	9,386.7
2	71.0	10.0	68	4,828.0	1.28	53.0	6,952.3
3	71.0	15.2	72	7,745.5	1.45	47.0	12,625.1
4	68.0	17.9	70	8,500.0	1.47	51.0	14,025.0
Mean	70.0 ± 1.4	16.2 ± 3.3	69.0 ± 3.4	7,046.1	1.34 ± 0.2	49.0 ± 3.7	10,747.3

MBW: mean body weight, FCR: feed conversion rate = dry weight of feed/wet weight of shrimp harvest.

In this way, sludge accumulation from uneaten feed, feces, detritus, suspended solids and dead organisms was gathered in the center of the ponds, where a drain was placed. Effluent and sludge were physically removed from the pond bottom by polyvinyl chloride (PVC) pipe lines underground connected the center of

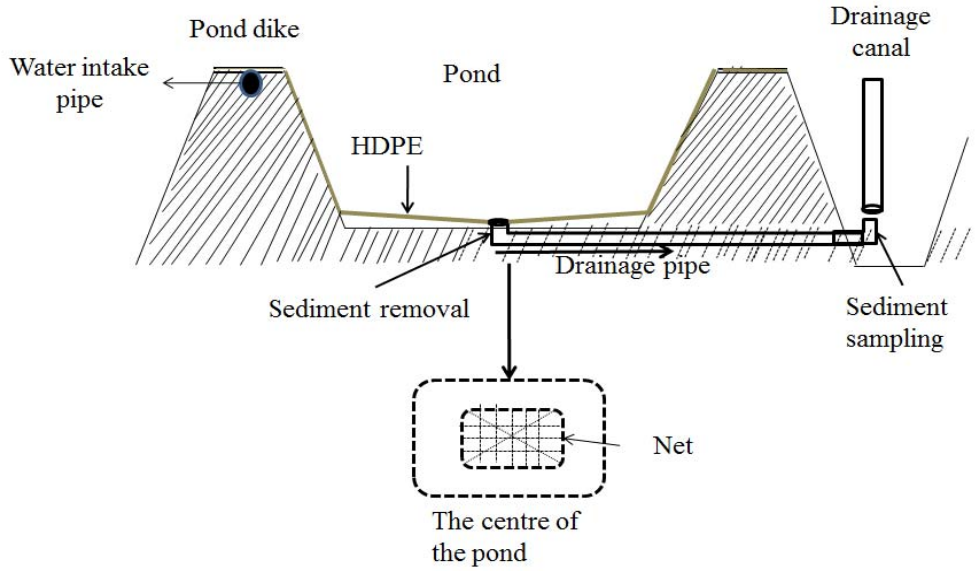

Fig. 2 Pond layout using sludge management regimes.

the ponds to an effluent canal at 20, 30, 40, and 49 d after larvae being stocked. To prevent shrimp leaving from the ponds during sludge removal, the central drain was equipped with a screen rack, an overflow mesh weir and a drain valve. This method has the advantage of allowing removal of the sludge and cleaning of the pond bottom flexibly throughout the culture period. An automatic feeding technique is used to deliver commercial feed to shrimp.

2.2 Sampling and Analytical Methods

Pond water samples were taken weekly from each pond at 20 cm below the water surface according to the method of Jackson et al. [7]. The intake water samples were collected before they entered the ponds, while the discharge samples were obtained inside the ponds before sludge was removed. The "initial" samples were those collected immediately before post-larvae stocking, and the samples collected during harvesting phase were designated as "final". Triplicate sludge samples were collected at the end of the pipe outside the pond in the drainage canal, and the water content of sludge was calculated after being dried for 24 h at 105 °C.

Water samples were filtered through a 0.2 μm filter (DISMIC-25AS, Advantec) to determine NH_4-N, NO_3-N, and PO_4-P concentrations. An unfiltered 10 mL water sample and 2 mL reagent of NaOH + $K_2S_2O_8$ for total nitrogen (TN) or 2 mL reagent of $K_2S_2O_8$ for total phosphorus (TP) were digested at 120 °C for 30 min, and the product (NO_3-N or PO_4-P) was analyzed for TN or TP [7]. The contents of TN in sludge samples and shrimp feed were analyzed by the dry combustion method using a CN Corder (MT-700, Yanaco, Japan). Approximately 1 g of air-dried sludge was digested with sulfuric acid and hydrogen peroxide at 300 °C for analysis of TP in the sludge. All water parameters were measured by the spectrophotometric method using a continuous flow auto-analyzer (QuAAtro 2-HR, Bltec, Japan). For more detail, environmental parameters and analytical methods are summarized in Table 2.

2.3 Nutrient Mass Balance Calculation

The general mass balance was calculated according to Teichert-Coddington et al. [10] as Eq. (1):

$$S_{in} + F_{in} + Fert_{in} + IPW_{in} + WI_{in} = S_{out} + PWH_{out} + WD_{out} + RS_{out} + UN \quad (1)$$

Table 2 Summary methods for analyses of parameters in water and sludge, shrimp and shrimp feed samples.

Sample parameters	Methods
Water	
Temperature (°C)	Digital thermometer *in situ*
Dissolved oxygen (mg/L)	Winkler method [1]
pH	Potentiometric method ([1]
Alkalinity (mg/L)	Indophenol blue method [17]
Salinity (ppt)	Electrical conductivity method
NH_4^+-N (mg/L)	Indophenol blue method [17]
NO_3-N (mg/L)	Colorimetric method [18]
Total nitrogen (TN) (mg/L)	Digestion method [19]
PO_4-P (mg/L)	Ascorbic acid method [18]
Total phosphorus (TP) (mg/L)	Digestion and ascorbic acid method [18]
Sludge, shrimp and shrimp feed	
TN	Digestion method [8]
TP	Digestion method [8]

where *S* = shrimp, F = feed, Fert = fertilizer, IPW = initial pond water nutrients, WI = intake water nutrients, PWH_{out} = nutrients remaining at harvest, WD_{out} = nutrients in discharged water, RS_{out} = nutrients in sludge removed (dry weight), and UN = unaccounted nutrients.

The total amount of commercial shrimp feed used, quantity of sludge removed and yields of each pond were recorded. Fertilizer was not used in this study. Water intake and discharge volumes were estimated based on the measurement of water levels inside each pond. No drainage occurred during the production, and the intake water was to compensate for water losses during sludge removal. Inputs of nutrients through atmospheric rainfall and nitrogen fixation by blue-green algae were considered negligible [1, 7, 8]. The study took place in the spring, and water loss by evaporation was negligible.

3. Results and Discussion

3.1 Water and Sludge Properties

Table 3 shows the water parameters, which were measured daily on site. The commercial pelleted feed contained 6.02% N, 1.47% P and 11% water. Shrimp comprised 29.3% dry matter, 10.88% TN and 1.33% TP in the whole body. The average volumes of water losses and compensation were 30% of total pond volume for 10 d or 2.45% daily over the period. Nitrogen (NH_4-N, NO_3-N, and TN) and phosphorus (PO_4-P and TP) concentrations in the intake and discharge water increased with increasing length of culture (Table 4). These data are in agreement with those of Ma et al. [4], who also showed that nutrient concentrations in ponds increased with increasing culture period. For example, TN concentrations increased from 0.20 mg/L to 0.51 mg/L, and TP showed the same trend from 0.25 mg/L to 0.34 mg/L in the first 20 d to final day, respectively. The concentrations of TN and TP were much higher in discharge water, from 2.5 mg/L to 3.2 mg/L and 0.38 mg/L to 1.47 mg/L, respectively. On average, total food added and shrimp harvested per hectare of shrimp pond per cycle were 10,747.3 kg and 7,046.1 kg, respectively. Farm staff removed an average of 12,709 (± 3050) kg/ha/cycle of sludge (dry weight) with concentrations of TN and TP of 8.9 g/kg and 6.2 g/kg, respectively.

3.2 Nutrient Mass Balances

The mass balances of TN and TP are shown in Table 5. The input source was mainly shrimp feed, which accounted for more than 90% of nutrient input. The present results may be comparable with other

Table 3 Water quality parameters in ponds (n = 4).

Temperature at 7:30/14:00 (°C)	28.9 ± 0.7/30.3 ± 0.6
Dissolved oxygen (DO) (mg L^{-1}) (at 7:30/14:00)	6.2 ± 0.1/6.5 ± 1.3
pH (at 7:30/14:00)	7.8 ± 0.2/8.0 ± 0.3
Alkalinity (mg/L)	143.6 ± 20.6
Salinity (ppt)	28.9 ± 1.9

Table 4 Nutrient concentrations of intake and discharge water and sludge samples.

Water samples	Intake water	Discharge water
NH_4^+-N (mg/L)		
20 d	0.15 ± 0.01	0.30 ± 0.02
30 d	0.14 ± 0.00	1.50 ± 0.32
40 d	0.33 ± 0.01	2.40 ± 0.02
49 d	0.33 ± 0.06	2.90 ± 0.12
NO_3-N (mg/L)		
20 d	0.025 ± 0.00	0.13 ± 0.01
30 d	0.021 ± 0.00	0.28 ± 0.01
40 d	0.036 ± 0.01	0.34 ± 0.01
49 d	0.030 ± 0.01	0.43 ± 0.00
TN (mg/L)		
20 d	0.20 ± 0.17	2.50 ± 0.53
30 d	0.24 ± 0.25	2.00 ± 0.70
40 d	0.40 ± 0.03	3.01 ± 0.12
49 d	0.51 ± 0.09	3.18 ± 0.78
PO_4-P (mg/L)		
20 d	0.03 ± 0.02	0.22 ± 0.07
30 d	0.04 ± 0.01	0.30 ± 0.02
40 d	0.08 ± 0.00	0.40 ± 0.07
49 d	0.07 ± 0.01	0.76 ± 0.01
TP (mg/L)		
20 d	0.25 ± 0.10	0.38 ± 0.07
30 d	0.28 ± 0.04	0.49 ± 0.05
40 d	0.21 ± 0.09	0.64 ± 0.01
49 d	0.34 ± 0.03	1.47 ± 0.05
Sludge samples	TN (mg/L)	TP (mg/L)
20 d	7.3 ± 0.28	4.74 ± 0.01
30 d	8.3 ± 0.28	5.06 ± 0.00
40 d	9.25 ± 0.07	7.37 ± 0.00
49 d	10.75 ± 0.35	7.66 ± 0.01

intensive shrimp ponds in Thailand [16, 20], where the majority source of the nutrient input in the pond (more than 90%) was from commercial pelleted feed. Similarly, Jackson et al. [7] reported nitrogen mass balance from intensive shrimp farm in Australia where 90% of N input was from shrimp feed. Post-larvae were reared in a greenhouse for one month before being transferred to grow-out ponds, where shrimp contributed up to 1.8% of N and 0.9% of P input. For the sinks of N in the ponds, harvested shrimp was the largest with 37.5% of total input, but not of P (18.3%). The assimilated shrimp harvest nutrients from the total inputs were higher than those in other studies of Refs. [16, 20]. In an intensive shrimp system, Xia et al. [21] reported that 32.9% N and 14.2% P from total inputs were recovered from harvested shrimp, just slightly

Table 5 Estimation of nutrient mass balances in shrimp ponds (n = 4).

Nutrients	TN (kg/ha/cycle)	%	TP (kg/ha/cycle)	%
Input				
S_{in}	11.0 ± 0.2	1.8	1.3 ± 0.3	0.9
$Fert_{in}$	0.0	0.0	0.0	0.0
F_{in}	575.8 ± 170.9	96.2	140.6 ± 41.7	94.9
IPW_{in} + WI_{in}	11.5 ± 0.0	1.9	6.2 ± 0.0	4.2
Total	598.4	100.0	148.2	100.0
Output				
PWH_{out} + WD_{out}	78.4 ± 5.5	13.2	17.1 ± 2.1	11.6
S_{out}	224.6 ± 50.5	37.5	27.2 ± 6.1	18.3
RS_{out}	113.1 ± 27.1	18.9	78.8 ± 18.9	53.2
Un-accounted	212.7 ± 6.2	30.4	25.0 ± 17.9	16.9
Total	598.4	100.0	148.2	100.0

TN: total nitrogen, TP: total phosphorus, S_{in}: nutrients in postlarvae shrimp, $Fert_{in}$: nutrients in fertilizer, F_{in}: nutrients in shrimp feed, IPW_{in}: nutrients in pond water before shrimp stocking, WI_{in}: nutrients in water intake, PWH_{out}: nutrients remaining in pond water after harvesting, WD_{out}: nutrients in drainage water, S_{out}: nutrients in shrimp harvest, RS_{out}: nutrients in removed sediment.

lower than those of the present study.

About 30.4% N and 16.9% P of input were not accounted for in measured losses. Nitrogen loss was attributed to denitrification and volatilization [1, 7, 10, 13]. While removed sludge retained the smallest proportion of N (18.9%), the largest quantity of P was trapped in removed sludge (53.2%). Funge-Smith and Briggs [20] showed that sludge retained 24% N and up to 84% P in intensive shrimp ponds in Thailand. Likewise, Hopkins et al. [9] reported 15%-24% N inputs for intensive ponds without sludge removal at the end of the season. Mariscal-Lagarda and Páez-Osuna [13] suggested that sludge accumulation was responsible for the sink of P in an integrated tank with shrimp and tomato.

In this study, the farm was operated without water exchange, so the pond gained only 1.9% N and 4.2% P from water intake. By contrast, approximately 13.2% N (78.4 kg/ha/cycle) and 11.6% P (17.1 kg ha/cycle) was lost from water discharge (during sludge removal). These values were in closed agreement with those from other literature, for instance, Funge-Smith and Briggs [20] reported 27% N and 10% P for the nutrient budget of discharged water. The proportions of N and P in discharge water of the present study are comparable to those of closed intensive systems, in which drainage water contained 14%-28% N and 12%-19% P [16].

Table 6 indicates that 333.2 kg N (47 kg N/ton shrimp) and 113.1 kg P N (16 kg P/ton shrimp) of the nutrients for a hectare per cycle were lost to the surrounding environment. In other words, the authors' results noted that for 1 kg shrimp production needed 84.9 g N and 26.1 g P from input sources, and 47.3 g N and 16.0 g P was discharged into the environment. Similarly, Mariscal-Lagarda and Páez-Osuna [13] estimated that environmental losses of 1 kg of product harvested were 57 g N and 7.1 g P for an integrated shrimp culture.

These results were different from those of semi-intensive and extensive shrimp systems, in which ponds gained nutrients mostly from water intake and fertilizers. Water exchange in these systems is greater (11% of total pond volume per day) [8] than in an intensive system (little or no water exchange) [22]. Using fertilizers augments natural food organisms within a water body as a supplemental food for shrimp and balances the environmental conditions in ponds, because extensive and semi-intensive ponds usually do not have aerators. Islam et al. [11] reported that intake water and fertilizers accounted for 55% and 29% of total nitrogen inputs, respectively. By contrast,

Table 6 Environmental losses of nutrients for different pond systems and management methods.

Systems	N		P		References
	In kg/ha/cycle	In kg/ton	In kg/ha/cycle	In kg/ton	
Intensive systems					
P. monodon	596	92	265	41	[23]
P. monodon	764	112	213	31	[24]
P. monodon	327	93.4	–	–	[7]
L. vannamei	457	116	57	14.6	[13]
L. vannamei	333.2	47	113.1	16	This study
Semi-intensive systems					
L. vannamei	66	36	22	12	[1]
L. vannamei	19	29	8	12	[10]
P. monodon	73	111	38	58	[11]
L. vannamei (FT)	235	71	43	12	[8]
L. vannamei (FD)	214	73	38	13	[8]

FT: feeding tray, FD: feed dispersal device.

the sink of N from the shrimp harvest was only 12% and up to 78% from discharge water. The high amount of N in effluent was different from that of a water exchange procedure. However, Casillas-Hernández et al. [8] noted that more than 70% N and 50% P inputs were from shrimp feed in semi-intensive shrimp ponds, respectively.

Table 6 showed that 40.3% TN and 19.5% TP were recovered by shrimp harvest from feed added. These values were much higher than those of semi-intensive systems. For example, shrimp harvest in semi-intensive ponds assimilated only 12% TN and 3.3% TP Islam et al. [11]. Likewise, Páez-Osuna et al. [1] revealed that 35.5% TN and 6.1% TP was recovered by shrimp harvest in semi-intensive ponds in Northwestern Mexico. These differences depended on nutrient sources in shrimp ponds. *L. vannamei* is an omnivorous species and it consumes diverse foods in a pond, including detritus and microorganisms. The distribution of natural foods in semi-intensive ponds is more available to shrimp, so the nutrients of inputs from other sources are converted to product (harvested shrimp).

The amounts of nutrients discharges from shrimp systems of this study are lower comparable with those in semi-intensive ponds reported by Islam et al. [11] (78 g N and 25 g P), but higher than the data of Teichert-Coddington et al. [10], who estimated approximately 16.8 g N and 2.3 g P discharge to the environment in a similar system. Environmental losses of nutrients were lower or intermediate, when the loads were expressed in both kg/ha/cycle and kg of N or P/ton shrimp. These values were lower than that reported for other intensive shrimp ponds (Table 6). However, environmental losses of nutrients in the present study were much higher than those in semi-intensive shrimp ponds when expressed as kg/ha/cycle, which indicated that higher intensity has a greater environmental impact of shrimp aquaculture.

Accordingly, the present study removed sludge periodically on days 20, 30, 40, and 49 of shrimp culture, reducing the impacts of sludge on the pond environment. Consequently, pond water was maintained in good condition for shrimp, because dissolved oxygen and ammonia gas concentrations were > 6mg/L and < 0.1 mg/L, respectively. Furthermore, economic efficiency can be improved significantly when productivity is more than 7 tons/ha/cycle. Sludge and effluent from intensive shrimp culture and the environmental impacts of aquaculture are controlled by sludge removal, especially prevention of ground water contamination by seepage through the HDPE film at the bottom.

4. Conclusions

The present study is the first mass balance of nutrients in intensive shrimp culture on sandy land using sludge management regime. The input source nutrient in the ponds was mainly from shrimp feed. While shrimp harvest was the largest sink of N, the largest amount of P was accumulated in sludge. The results suggest that the shrimp culture in the system removed sludge periodically can be improved pond water quality. Concurrently, the environmental losses of N and P are reduced to minimize the risk of eutrophication of receiving waters.

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References

[1] Páez-Osuna, F., Guerrero-Galván, S. R., Ruiz-Fernández, A. C., and Espinoza-Angulo, R. 1997. "Fluxes and Mass Balances of Nutrients in a Semi-intensive Shrimp Farm in Northwestern Mexico." *Marine Pollution Bulletin* 34 (5): 290-7.

[2] Velasco, M., Lawrence, A. L., and Castille, F. L. 1999. "Effect of Variations in Daily Feeding Frequency and Ration Size on Growth of Shrimp, *Litopenaeus vannamei* (Boone), in Zero-Water Exchange Culture Tanks." *Aquaculture* 179 (1-4): 141-8.

[3] Pillay, T. V. R. 2004. *Aquaculture and the Environment,* 2nd ed. Oxford, UK: Blackwell Publishing Ltd..

[4] Ma, Z., Song, X., Wan, R., and Gao, L. 2013. "A Modified Water Quality Index for Intensive Shrimp Ponds of *Litopenaeus vannamei*." *Ecological Indicators* 24: 287-93.

[5] Food and Agriculture Organization of the United Nations (FAO). 1986. *Shrimp Culture: Pond Design, Operation and Management*. NACA Training Manual Series No. 2, Aquaculture Department, Southeast Asian Fisheries Development Center.

[6] IUCN and IISD. 2003 *Expanding Shrimp Aquaculture on Sandy Land in Vietnam: Challenges and Opportunities.* Hanoi: IUCN and IISD.

[7] Jackson, C., Preston, N., Thompson, P. J., and Burford, M. 2003. "Nitrogen Budget and Effluent Nitrogen Components at an Intensive Shrimp Farm." *Aquaculture* 218 (1-4): 397-411.

[8] Casillas-Hernández, R., Magallón-Barajas, F., Portillo-Clarck, G., and Páez-Osuna, F. 2006 "Nutrient Mass Balances in Semi-intensive Shrimp Ponds from Sonora, Mexico Using Two Feeding Strategies: Trays and Mechanical Dispersal." *Aquaculture* 258 (1-4): 289-98.

[9] Hopkins, J. S., Sandifer, P. A., and Browdy, C. L. 1994 "Sludge Management in Intensive Pond Culture of Shrimp: Effect of Management Regime on Water Quality, Sludge Characteristics, Nitrogen Extinction and Shrimp Production." *Aquacultural Engineering* 13 (1): 11-30.

[10] Teichert-Coddington, D. R., Martinez, D., and Ramírez, E. 2000. "Partial Nutrient Budgets for Semi-Intensive Shrimp Farms in Honduras." *Aquaculture* 190 (1-2): 139-54.

[11] Islam, S., M., Sarker, J., Yamamoto, T., Wahab, A., and Tanaka, M. 2004. "Water and Sediment Quality, Partial Mass Budget and Effluent N Loading in Coastal Brackishwater Shrimp Farms in Bangladesh." *Marine Pollution Bulletin* 48 (5-6): 471-85.

[12] Wahab, M. A., Bergheim, A., and Braaten, B. 2003. "Water Quality and Partial Mass Budget in Extensive Shrimp Ponds in Bangladesh." *Aquaculture* 218 (1-4): 413-23.

[13] Mariscal-Lagarda, M. M., and Páez-Osuna, F. 2014. "Mass Balances of Nitrogen and Phosphorus in an Integrated Culture of Shrimp (*Litopenaeus vannamei*) and Tomato (*Lycopersicon esculentum* Mill) with Low Salinity Groundwater: A Short Communication." *Aquacultural Engineering* 58: 107-12.

[14] Frignani, M., Piazza, R., Bellucci, L. G., Cu, N. H., Zangrando, R., Albertazzi, S., Moret, I., Romano, S., and Gambaro, A. 2007. "Polychlorinated Biphenyls in Sediments of the Tam Giang-Cau Hai Lagoon, Central Vietnam." *Chemosphere* 67 (9): 1786-93.

[15] Hung, L. T., and Quy, O. M. 2013. *On-Farm Feeding and Feed Management in Whiteleg Shrimp (Litopenaeus vannamei) Farming in Vietnam.* FAO Fisheries and Aquaculture Technical Paper 583.

[16] Thakur, D. P., and Lin, C. K. 2003. "Water Quality and Nutrient Budget in Closed Shrimp (*Penaeus monodon*) Culture Systems." *Aquacultural Engineering* 27 (3): 159-76.

[17] Parsons, T. R., Maita, Y., and Lalli, C. M. 1992. *A Manual of Chemical and Biological Methods for Seawater Analysis*. New York, NY, USA: Pergamon Press, 173.

[18] American Public Health Association (APHA). 2005 *Standard Methods for the Examination of Water and Wastewater,* 21th ed.. Washington, D.C., USA: APHA.

[19] Grasshoff, K., Kremling, K., and Ehrhardt, M. 1999. *Methods of Seawater Analysis.* Verlag, Weinheim, Germany: Wiley-VCH.

[20] Funge-Smith, S. J., and Briggs, M. R. P. 1998. "Nutrient Budgets in Intensive Shrimp Ponds: Implications for Sustainability." *Aquaculture* 164: 117-33.

[21] Xia, L. Z., Yang, L. Z., and Yan, M. C. 2004. "Nitrogen and Phosphorus Cycling in Shrimp Ponds and the Measures for Sustainable Management." *Environmental Geochemistry and Health* 26 (2): 245-51.

[22] Ray, A. J., Dillon, K. S., and Lotz, J. M. 2011. "Water Quality Dynamics and Shrimp (*Litopenaeus vannamei*) Production in Intensive, Mesohaline Culture Systems with Two Levels of Biofloc Management." *Aquacultural Engineering* 45 (3): 127-36.

[23] Briggs, M. R. P., and Funge-Smith, S. J. 1994. "A Nutrient Budget of Some Intensive Marine Shrimp Ponds in Thailand." *Aquaculture and Fisheries Management* 25 (8): 789-811.

[24] Robertson, A. I., and Phillips, M. J. 1995. "Mangroves as Filters of Shrimp Pond Effluent: Predictions and Biogeochemical Research Needs." *Hydrobiologia* 295 (1): 311-21.

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Biological Features and Distribution of Giant Trevally (*Caranx ignobilis* Forsskal, 1775) in Tam Giang-Cau Hai Lagoon Systems, Vietnam

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Abstract: The study aimed to investigate biological characteristics and distribution of a new fish species Giant trevally (*Caranx iginoblis*) in Tam Giang-Cau Hai lagoon systems and preliminarily discover the reproductive abilities of a migration species from sea into lagoon. Results showed that the species appeared in the lagoon since 2005 and farmers start raising by cages at Tu Hien estuary and Loc Binh area for well-growing and a best market to consumers. There were distributed of the fingerlings from sea into lagoon from October to December though inlets, so fishermen can collect every year by bottom nets and enclosed net. Fish heads are growing a faster than other species, but not sustainable development of the culture model. Besides, wild fish also can be collected by fishmen at inlets with the bigger with averaged body weight 1.99 ± 1.22 kg and length 45.66 ± 17.56 cm. The species are big mouth and contained sharp teeth. There are flat body and head length, pectoral fin rays, dorsal and anal yellow, gray dorsal, and ventral surface is silver white. They adapted in water environmental conditions of pH (7.1-8.5), dissolved oxygen (DO) (2.7-4.5 mg/L), salinity (17.0‰-33.3‰) and temperatures (16.8-32.5°C). The reproductive performance of 50 fish samples, which were detected on mature female fish and male fish for eggs or spermatorrhoea, respectively, was investigated. Results of slices on gonads in stages I, II and III showed that the germplasm is a special source of valuable genes of the species and local aquatic resources.

Key words: Giant trevally, lagoon, biological features, distribution, characteristics.

1. Introduction

Tam Giang-Cau Hai has more than 22,000 ha area with many aquatic species and it is also good for fishing opportunities. Giant trevally (*Caranx iginoblis*) migrate and live in the lagoon, and fishing men can obtain the wild fish species from Cau Hai area and Tu Hien estuary to culture in the fish-cages and thus to have a good result and economic income. This fish is a natural source of a new species to live in the lagoon since 2005 through Tu Hien estuary, with a high commercial value of price and well-adapted to salinity changes from sea water to brackish water. The fish have a faster growth rate than other species at the fish cages. Giant trevally is the concern of many fishermen and communities to collect the fingerlings for aquaculture, but the seed source are limited and usually just drift in the estuaries in a short time (October to March) in rainy condition. Farmers and scientists would like to determine age and growth rate of this species, and they can have understand more biological characteristics, specially migrations, distributions and growth, mature in life cycle. The fish age study was conducted by scales, but forever soles at 20th century, the study about age and growth of new fish has many achievements recorded [1-3]. This was also the first case to have demonstrated in scientific and technological application for locations for backward water condition.

Due to requirement of scientific studies, the

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investigate trends on the biological characteristics and reproductive performance have been focused on, such as embryonic and gonad development, the level for gonad maturity, fecundity, hatchering and spawning conditions. Reproductive activity is closely related to various processes, such as growth, nutrition and feeding assurance. Study on the significant theoretical and practical aspects combination the same times, contributing to exploit, protect and use resources appropriately [4-6]. The research aimed at investigation of biological characteristics and distribution of a new fish species with potential for cage culture in Tam Giang-Cau Hai lagoon systems, as well as preliminarily discovering the reproductive abilities of a migration species from sea into lagoon.

2. Materials and Methods

2.1 Materials

All specimens of giant trevally (*Caranx ignobilis* Forsskal, 1775) (Fig. 1) were collected at Thua Thien Hue coastal region, especially in Tam Giang- Cau Hai lagoon system from 2014 to 2015.

2.2 Methods of Collecting Information and Samples

The survey collected information via questionnaires to fishermen and farmers at Loc Binh, Vinh Hien and Hai Duong, Tam Giang-Cau Hai lagoon systems. Samples have been collected in wild and cage conditions. Morphologically samples must be intact form and shape in formaldehyde 5% enclosed with the label, and the local name, date and place of sampling are post outside sample bottles.

2.3 Biological Variables

The study of biological characteristics of fish giant trevally includes a morphological characteristics, growth variables, nutritional feeding characterizes and reproductive characteristics.

2.3.1 Morphological Characteristics

Morphological characteristics can be discovered by observation in fields and labs and based on the classification criteria from Figs. 1 and 2.

2.3.2 Growth Variables

The intercept (a) and slope (b) of regression line were calculated by using the following Eq. (1), according to R.J. H. Berverton – Holt (1956) update by Ref. [3]:

$$W = a \times L^b \quad (1)$$

where, W = body weight (g) and L = body length (cm).

The age of fish was identified by scales. The fish Giant trevally with flakes are soaked in 4% NaOH solution to remove grease, dirt or pigment clinging on flakes. Cash observations of opaque concentric rings, contiguous rings throughout the fish on the scales and the boundary between the opaque regions allow us to identify individuals within the age in years. Directly observe using microscopy by eyes, and estimate fish age according to Nikolsky update by Ref [3].

2.3.3 Nutritional Feeding Characterizes

Feed was removed from the intestine and stomach for specimens and then observed under a microscope or magnifying glass binocular. Use keywords to classify low level plants, invertebrate aquatic, as “The kind of invertebrate” of cthe classification of marine planktonic algae silicon [3], Identification of the invertebrate groups freshwater common in Vietnam. The coefficient of fat fish was calculated using Eqs. (2) and (3) according to Fulton (1902) and Clark (1928), updated by Ref [3]:

$$Q = \frac{\mathrm{W}}{L^3} \times 100 \quad \text{(Fulton)} \quad (2)$$

$$Q = \frac{\mathrm{W}_0}{L^3} \times 100 \quad \text{(Clark)} \quad (3)$$

where, Q: fat parameter, W: body weight and L: body length.

Fat is accumulated in the fish and the fat fish can only be pinpointed with the fat accumulation into belly and fat layers. Additionally, when conducting anatomical fresh fish, the simple method can be used to determine fat under 5 levels by Prozovxkaia M. L, 1957, updated by Ref [3].

2.3.4 Preliminary Reproductive Characteristics

Fish samples were obtained at surgery tables and then determined the volume of the maturity stages and gonadal morphology under 6 ladder levels according by Kiselevits KA (1923), updated by Ref. [3] and then fixed in Bouin solution which composed of picric acid, acetic acid and formaldehyde in an aqueous solution.

3. Results

3.1 The Classification System of Giant Trevally

Giant trevally belongs to order Perciformes, family Carangidae, genus *Caranx*, species *Caranx ignobilis* (Forsskal, 1775). The English name is giant trevally and the local name is Vau.

3.2 Some Morphological Characteristics

The measurement criteria of morphological characteristics as proposed by Lowe-McConnell (1971) and Grant & Spain (1977), updated by Ref. [2] in biological research are presented in Table 1:

Giant trevally (*Caranx ignobilis*) has a big head, big mouth, mouth under thrown up on, inside contains sharp teeth, flat body, with a body length (TL) in 3.13 times body height (BH) and in 4.19 times head length (HL) (i.e., TL/BH = 3.13 and TL/HL = 4.19), head length in 4.14 times the diameter of the eye (ED) (i.e., HL/ED = 4.14). Big round eyes have fat layer covering the outside, with four nostrils and each side has two holes. Giant trevally fish often change color according to the environment; when there is the same size 10 cm and on the body with the black stripes, the adults have gray body white or yellow depending on species and the environment where they live, and the abdomen is white. There are two dorsal fins; the 2nd dorsal fin is gray and the outer edge is black, upper lobe of caudal fin is black outer edge, the lower lobe of the outer edge is pale yellow, and pelvic fins is trimmed white. The body covering is round and oval scale. The pectoral fins, dorsal and anal fins are yellow to fish and also called Giant trevally contract yellow fin.

3.2.1 Description of Morphology

Dorsal (D1 = VII; D2 = I, 20): include two dorsal with dorsal 1 has 7 spines; dorsal 2 has 1 spine and 21 soft rays has to branch.

Pectoral fin (P = 19) has 19 branched soft rays, crescent-shaped, longer than the first length.

Ventrals (V = I, 4) has 01 spine and 4 soft rays.

Anus (A1 = I - III, A2 = 17-19) have two spines anal forward split followed by a sequence with soft rays branching spines include 17-19 soft rays.

Tail fin (C = (19 – 23) +2). Tail lobe is divided into two clear, and each fin consists of 19-23 branched rays with soft spikes. A top of side edge lobes has yellow black colour, and the lower margin lobes has pale yellow colour.

The line has a distinct texture and moderately long, curved part intersecting with the straight part below the second dorsal fin lobe. Part of the road curved sides has 58-64 scales. Connections part includes one row connected continue, consisting of 31 layers (Fig. 2).

3.2.2 Biometric Indicators

The indicators of body (HL, ED, high body BH) in a correlation with TL are considered as an indicator of biometric (bio-metric index. In each index link between the target TL (HL/ED; TL/BH), the average value of the index will be determined biometric follow Fig. 3, according Bayagbona, 1963 updated Ref. [3], if in all size groups of fish research, biometric indicators of each individual characteristics indicate each odds

Fig. 1 ***Caranx ignobilis*** **Forsskal, 1775.**

Table 1 Morphological characteristics of giant trevally (n = 50).

No.	Morphological variables	Units	Mean	Min-Max	SD
1	Body weight (W)	g	1,993.954	2.2-4,500	1,223.98
2	Body length (TL)	cm	45.66196	5.15-70.0	17.56
3	Head length (HL)	cm	10.543	1.45-19.0	4.23
4	Front spines (PD)	cm	13.627	1.65-24.6	6.60
5	Eye length	cm	3.568	0.35-7.4	2.03
6	Space between two eyes	cm	4.512	0.5-8.1	2.08
7	Eye dimension (ED)	cm	2.611	0.4-4.9	1.18
8	Above mouth length	cm	5.0418	0.6-9.0	2.30
9	Below mouth length	cm	4.358	0.5-7.8	2.08
10	Anus length	cm	15.02	1.1-24.4	6.72
11	Body height (BH)	cm	14.05	1.9-22.3	5.55
12	Head height	cm	12.592	1.7-21.1	5.15
13	Mouth width	cm	3.493	0.4-6.2	1.51
14	Front dorsal spine width 1	cm	3.983	0.7-6.3	1.35
	Behind dorsal spine width 2	cm	7.425	0.6-13.4	3.32
15	Front dorsal spine width 1	cm	6.603	0.7-18.6	3.06
	Behind dorsal spine width 2	cm	13.644	1.5-20.9	5.21
16	Breast spine length	cm	12.098	1.1-20	5.21
17	Breast spine width	cm	2.476	0.3-5.25	1.21
18	Anus spine width	cm	7.182	0.7-12.3	3.12
19	Anus spine length	cm	12.662	1.4-24	4.83
20	Belly spine length	cm	5.444	0.6-9.6	2.38
1	Belly spine width	cm	1.5928	0.1-3.2	0.79
22	Mouth high	cm	8.308	0.9-15.2	3.72
23	Body high at dorsal	cm	13.657	1.9-21.9	5.50
24	Body high at chest	cm	12.841	1.8-20.4	5.00
25	Body high at anus	cm	13.529	2.0-20.8	5.15
26	Tail spine high	cm	13.186	1.2-21.2	5.59
27	Tail length	cm	6.807	0.5-13	3.30

decreased continuously, then characteristics survey shows a positive correlation (+) On the contrary, the characteristics of the survey was inversely correlated (-). If the index does not change biometric means the development of the survey indicators in relation to the correlation length and a peer [7]. Thus, based on Fig. 3, we find that the correlation between TL/BH, TL/HL and HL/ED compared to body length (TL) is a positive correlation.

3.3 Growth Characteristics

3.3.1 Age Structure of Fish

Table 2 and Fig. 4 showed the age distribution of the fish caught from sea and lagoon in four groups: < 1-year-old group (0 +), > 1-year-old group (1 +), > 2-year-old group (2 +), > 3-years-old group (3 +). The length of individuals under age group 0 + ranged from 51.5 mm to 395 mm with average 234.733 mm, and the weight ranged from 2.2 g to 1,230 g with average 429.11 g, respectively. The length of individuals aged 1 + ranged from 382 mm to 444 mm with average 419.730 mm, the corresponding volume ranged from 1,300 g to 2,200 g with an average of 1,608.5 g, respectively. The length of individuals aged 2 + ranged from 530 mm to 573 mm with average 557.257 mm, and the corresponding volume ranged from 2,300 g to 3,140 g in average 2,760.7 g, respectively. The length of individuals aged 3 + ranged from 563.3 mm to 673 mm with average 646.1 mm, and the corresponding amount ranged in 3,200 -4500 g, averaging 3,750 g.

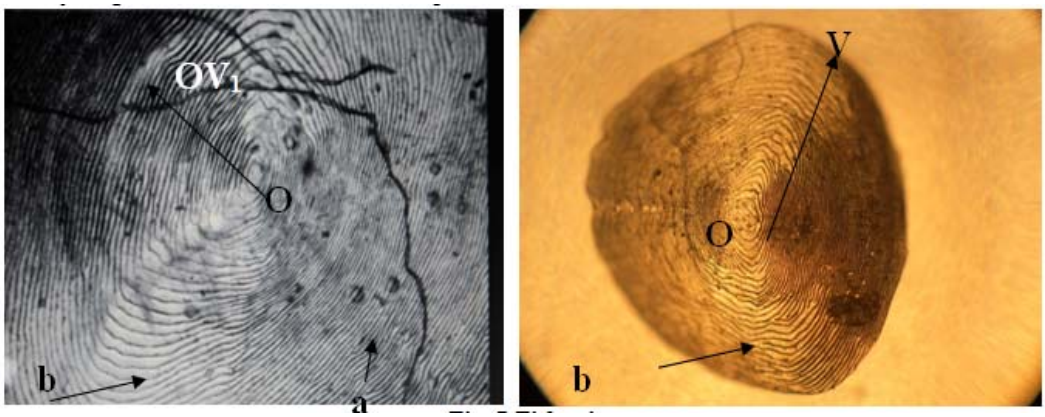

Fig. 2 Fish scales.

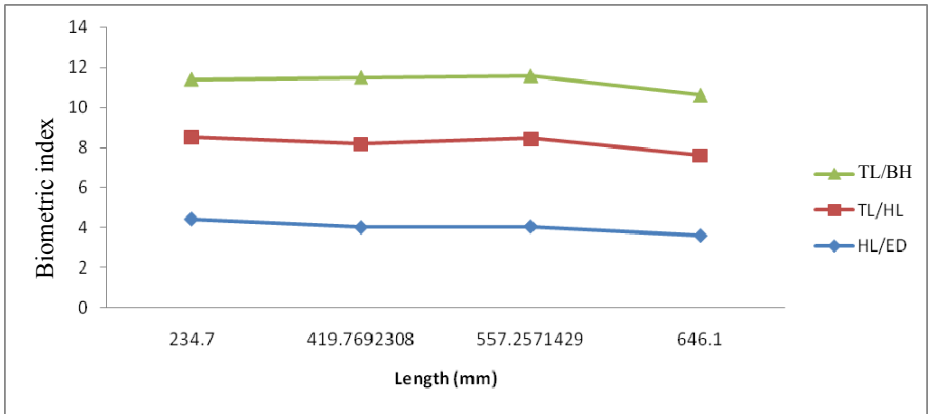

Fig. 3 The index of personal biometric giant trevally body length of each age group.

Table 2 Age and distribution composition.

Age	Length L (mm)			Weigh W (g)			Numbers	
	Mean	Min-Max	SD	Mean	Min-Max	SD	Frequency	%
0 +	234.733	51.5-395	139.711	429.11	2.2-1,230	466.61	15	30
1 +	419.730	382.0-444	15.000	1,608.5	1,300.0-2,200	258.03	13	26
2 +	557.257	530.0-573	16.734	2,760.7	2,300.0-3,140	240.55	14	28
3 +	646.100	563.3-673	36.661	3,750.0	3,200.0-4,500	396.41	8	16
Mean	438.958	51.5-673	172.533	1,919.93	2.2-4,500	1,253.47	50	100

3.3.2 Correlation between length and body weight of the Fish

In the process of growth and development of fish, the increase between length and weight has a relationship with each other. After analyzing 50 samples of fish with many sizes and different age groups showed that the correlation between the length and weight of fish are shown in Fig. 5.

Based on the formula Beverton-Holt (1956) updated by Ref. [3] and analyzing the research results, it was obtained that the correlation between length (L) and weight (W) of giant trevally is expressed as Eq. (4)

$$W = 0.025L^{2.8898} \quad (1)$$

From Fig. 5, the growth in length and weight of giant trevally close relationship with each other is clearly reflected in the correlation coefficient R^2 = 0.987, and this is positively correlated, meaning that when length increases, the volume of fish Vau also increased. However, the growth and length of giant

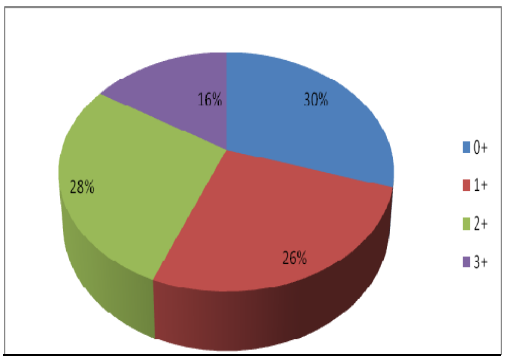

Fig. 4 Age structure of fish.

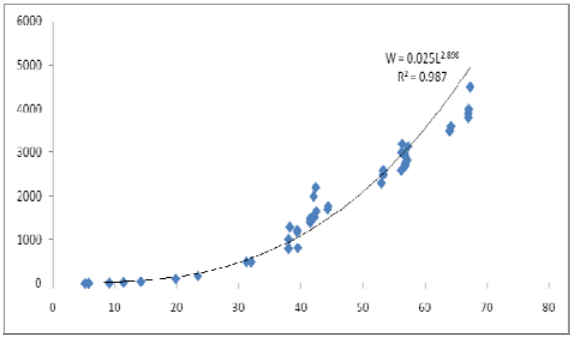

Fig. 5 Correlation between length and body weight of the fish.

Fig. 6 Teeth and stomach of giant trevally.

trevally was uneven shown in Fig. 5. Particularly, in the early stages (age 0 +), the length increased, the volume increases slowly. By the stage 1 +, 2 +, 3 +, giant trevally showed rapid growth in volumes and slow growth in length. Maybe at this stage, fish have increased in volume in relation with nutrient accumulation process, and the increase in size in early life is due to adaption in competition for food and animal data to ensure survival.

3.4 Nutritional Features

3.4.1 Anatomy of the Digestive Organs of Giant Trevally

The results observed showed that a giant trevally species have big heads, wide mouth, mouth under upwards, mouth width of 50 samples ranging in 0.4-6.2 cm and average3.493 + 1.51 cm; the upper jaw contained a series of sharp fangs outside with uneven distribution and the smaller teeth in innermost, while mandibular teeth have a conical line, and inside there are many teeth almost distributions as showed Fig. 6. Gill rakers are long and thin situated on bone sparse supply carries towards oropharyngeal cavity. On the supply carries, there are 18-24 carries combs, each side has the four leaf bearing and multi-motor bearing is surrounded by the gill cover. Esophagus is short, thick-walled, and many folds present in the esophagus should be so elastic lug that fish can swallow large prey. J-shaped stomach is large and thick wall capable of contraction. The fish has a short bowel, therefore it can be recognized that this fish can eat origin animal feed. They are carnivorous fish and in most of the habitat, they are known as independent predators. The research results are consistent with previous studies of some scientists, who believed that food of giant trevally was the primary prey of crustaceans, molluscs

and mollusks. The strategic large predatory fish like to ambush their prey. The diet is similar, mainly including fish eels, small squid, octopus, mantis shrimp, lobster and other crustaceans.

3.4.2 Correlations between Gut Length (Li) to Body Length (Lo)

According to Nikolsky (1963) updated Ref [3], the species of fish which are fond of animal feed would be valuable of Li/Lo ≤ 1, omnivorous fish have Li/Lo = 1-3 and fish with natural feeding about plants have Li/Lo ≥ 3. From collated results in Table 3, it was shown the coefficient RLG = 0468 and it can be concluded that giant trevally feeding inclined to animal due to Li/Lo = 0.468 ≤ 1.

3.4.3 Feeding Analysis by Occurrence Frequency

To determine the composition of giant trevally feeding, the food in the gastrointestinal tract with 50 fish samples were analyzed and divided according to the size based on the length of the largest and smallest fish by means of determining occurrence frequency of these foods in the digestive tract. The results about occurrence frequency of foods in the digestive tube lugs fish are presented in Table 4. It can be seen that the food of giant trevally feeding is mainly animal.

3.4.4 Natural Food of Fingerlings

Analyze food in the digestive tract of 15 specimens of giant trevally following different size group, where the smallest was 2.0 cm and the largest was 12.0 cm, to determine the natural spectrum of giant trevally fingerlings feed. The analysis results of giant trevally fingerlings are very diversified, including three groups of phytoplankton and animals (zooplankton and small size) and organic humus (decomposed food).

According Table 5, concrete phytoplankton group includes 14 species of four phylums. Among them, Chlorophyta phylum had the highest rate with six species and made up 42.8% of phytoplankton group; Bacillariophyta phylum with five species made up 35.7%; Cyanophyta phylum had two species and made up 14.2%; the least was Euglenophyta phylum with one species and made up 7.3%. The spices of animals consist of zooplankton and small size animal; it had 19 species on 7 classes. Among them, the Copepoda class had the highest rate with eight species and 42.1% of animal group, and the lowest rate was Gastropoda class with one species and 5.26% of animal group. In Osteichthyes class, it just can be identified in order and unidentified in species. In addition, the organic humus component wad also identified. It consists of algae and decomposing animal, accounting for 30% high present in the digestive tract.

3.4.5 Fat Level of Giant Trevally

According to Nikolsky 1963, updated by Ref. [3], the method of Fulton (1902) and Clark (1928) [3] was used to determine the difference of fat ratio, the level of individual nutrients accumulate of giant trevally.

According to the results of the study (Table 6), fat levels of individuals of giant trevally varied between age groups. The fat level III and IV appeared mostly in all age group. At the age group 2 + and 3 +, the fat

Table 3 The variation rate Li/Lo by size (*n* = 50).

Measurement indicators	Mean	Min	Max
Total length (mm)	438.958 ± 172.5	51.5	673.0
Gut length (mm)	205.780 ± 66.3	65.0	273.0
Correlations between gut length and total length (RLG)	0.468	0.03	1.00

Table 4 The results of feeding analysis by frequency of occurrence (*n* = 50).

Dietary composition	The number of caught times	Frequency of occurrence (%)
Crustacean	40	80
Fish	32	64
Ink	24	48
Mollusca	10	20

Table 5 The result in the digestive tract of giant trevally fingerlings (*n* = 15).

Category	Name of food	Size group (cm)	
		2-7	7.1-12.0
(1) Phytoplankton			
I Bacillariophyta			
1	*Cerataulina pelagica*	-	+
2	*Thalassiosira sutilis*	+	-
3	*Coscinodiscus* sp.	+	+
4	*Navicula* sp.	+	-
5	*Achnanthes* sp.	+	-
II Chlorophyta			
6	*Pediastrum* sp.	+	+
7	*Selenastrum* sp.	+	-
8	*Microsporaceae*	+	+
9	*Oophila amblystomatis*	+	-
10	*Chlorella* sp.	-	+
11	*Chlorococcaceae*	+	-
		2-7	7.1-12.9
III Euglenophyta			
12	*Phacus* sp.	+	+
IV Cyanophyta			
13	*Microcystis* sp.	-	+
14	*Chroococcus*	-	+
(2) Animal			
V Crustacean class			
15	*Scylla paramamosain* (Estampador, 1949)	-	+
16	*Penaeus monodon* Fabricius, 1798	-	+
VI Rotatoria			
Asplanchnidae order			
17	*Asplanchna priodonta* Gosse, 1850	+	+
VII Cladocera			
(a) Sididae order			
18	*Diaphanosoma sari* Richard, 1894	-	+
(b) Bosminidae order			
19	*Bosminopsis deitersi* Richard, 1895	+	+
VIII Copepoda			
(a) Pseudodiaptomidae order			
20	*Schmackeria dubia* Poppe & Richard, 1979	+	+
21	*Pseudodiaptomus incisus* Shen & Lee, 1963	-	+
(b) Acartidae order			
22	*Acartia clausi* Giesbrecht, 1889	+	+
23	*Acartiella sinensis* Shen & Lee, 1963	+	+
(c) Oithonidae order			
24	*Limnoithona sinensis* (Bruckhardt, 1912)	-	+
25	*Oithona nana* (Giesbrecht, 1892)	+	-
(d) Temoridae order			
26	*Temora turbinate* (Dana, 1849)	+	+
27	*T. discaudata* Giesbrecht, 1889	+	+

(Table 5 continued)

Category	Name of food	Size group (cm)	
		2-7	7.1-12.0
(1) Phytoplankton			
IX Polychaeta class			
(a)	Chrysopetalidae order	-	+
(b)	Hesionidae order	+	+
(c)	Nephthydidae order	+	+
(d)	Opheliidae order	+	+
XI Osteichthyes class			
28	*Stolephorus* sp.	+	+
29	Lish larval	+	+
30	Fish eggs	+	+
X Bivlavia class			
(a) Corbiculidae order			
31	*Corbicula* sp.	-	+
(b) Arcidae order			
32	*Anadara* sp.	+	+
(c) Placunidae order			
33	*Placuna* sp.	+	+
XI Gastropoda class			
(a) Potamididae order			
34	*Cerithidea sinensis* (Gmelin)	+	+
(3) Organic humus			
Disintegrate animals + plants		+	+

Table 6 The accumulation of fat degree by giant trevally age group.

Age group	Fat levels										Total	
	0		I		II		III		IV			
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
0 +	3	6	2	4	4	8	4	8	2	4	15	30
1 +	0	0	0	0	5	10	4	8	6	12	15	30
2 +	0	0	0	0	0	0	5	10	7	14	12	24
3 +	0	0	0	0	0	0	1	2	7	14	8	16
Total	3	6	2	4	9	18	14	28	22	44	50	100

Table 7 The fat ration of giant trevally.

Age group	Fulton ratio (1902)	Clark ratio (1928)	*N*	
			n	%
0 +	$3{,}317.7 \times 10^{-6}$	$2{,}843.23 \times 10^{-6}$	15	30
1 +	$2{,}175.2 \times 10^{-6}$	$2{,}027.66 \times 10^{-6}$	13	26
2 +	$1{,}595.3 \times 10^{-6}$	$1{,}526.18 \times 10^{-6}$	14	28
3 +	$1{,}390.4 \times 10^{-6}$	$1{,}341.16 \times 10^{-6}$	8	16

was the most 14% in level IV, and at this age there was not fat in stage 0, I, and II. In the age group 0 +, the fat was caught at all stages from 0-IV, and in group 1 +, the fat was not caught in stages 0 and I. Thus, it can be concluded that the fat levels of fish species with giant trevally is very high. Thus, it can be concluded that the fat levels of fish species with giant trevally is very high. Table 7 showed that there are relationships between ages and fat accumulation by Fulton ratio (1902) and Clark ratio (1928) with percentages of fish grow-up 3+, they can have mature soon gonads in culture stages in cages.

3.5 Reproductive Characteristics

Only a small number of fish species differ in outside appearance between males and fingerlings (stingrays, sharks), and some species have differences in spawning time (salmon males have a longer snout in female children during reproduction). Many fish species have distinctive characteristics of sex and can be identified through the external morphological characteristics, such as the level of micro-smooth chest in carp, big belly and protruding genital growth. However, for some wild fish species, identification of sex based on outside shape is difficult, especially for not sexually mature fish.

For individuals of giant trevally, some giant trevally fish have eggs with size > 3.5 kg/head. At the same time, a survey on fishing capture on lagoon and sea was done, but not found yet any fishes having eggs. Besides, the gonads of giant trevally fish was only identified in phase I, II, III of aquaculture farming with cages and was observed clearly, Fig. 7 obtained on phase III.

Phase I: For individuals with gonads in stage I, it can not distinguish between male and female gonad when observed with the naked eye (Figs. 7 and 8). The back of gonads has a lot of fat clinging to, if not carefully observe it very easy to mistake for a part of gonads, as it is the same color as the gonads and also vascular distribution. Gonads have the distribution of blood vessels, but small numbers, very small diameter and no ramifications. gonads has very small volume and cell attributes were observed at ovarian in phase I; under the microscope, the acolytes appeared in synthetic period and also gonad cells developed, so the next, the ratio between the gonad diameter and the body diameter of the fish species was compared with other fish species. Results showed that the ratio of gonad in Giant trevally was slowly and smaller.

Phase II: TSD has become larger, but still not goes to mature as expecting, when observed with the naked eye, except in cases of ovarian at late phase II by this time, some small eggs had formed as beads. Yellow is the first period of growth in nutrition which can be seen by naked eye. Blood vessels are distributed in more gonads, and small blood vessels branch run around gonad to make gonad white roses. Due to the increase of protoplasm, egg cells become larger and uneven due to the tension of the plasma membrane to the cell polygon-shaped eggs. The relationship between egg diameter and sex cell diameter was deflected to a corner.

Besides, there are interstitial spaces for many gonad cells still in synthetic period. The size and weight of the ovaries increased significantly, so that the naked eye can distinguish the males and females. Ovaries occupy two-thirds of the volume of the abdominal cavity. Ovaries have a lot of blood vessel distribution, along the ovaries are the major blood vessel diameter and they are divided into smaller blood vessels surrounding the ovary. Use the hand to stroke along belly, there is egg to flow out (Fig. 9).

Phase III: In ovary of giant trevally, a big difference of size and different development phases of the oocytes can be seen in the nutritional growth period.

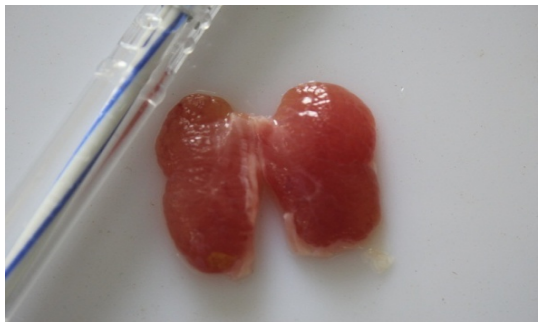

Fig. 7 The gonad of giant trevally.

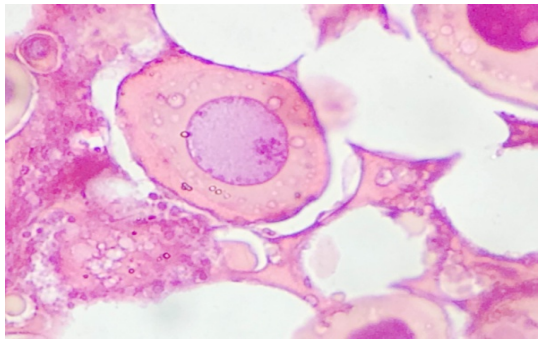

Fig. 8 Ovarian of fish at phase I.

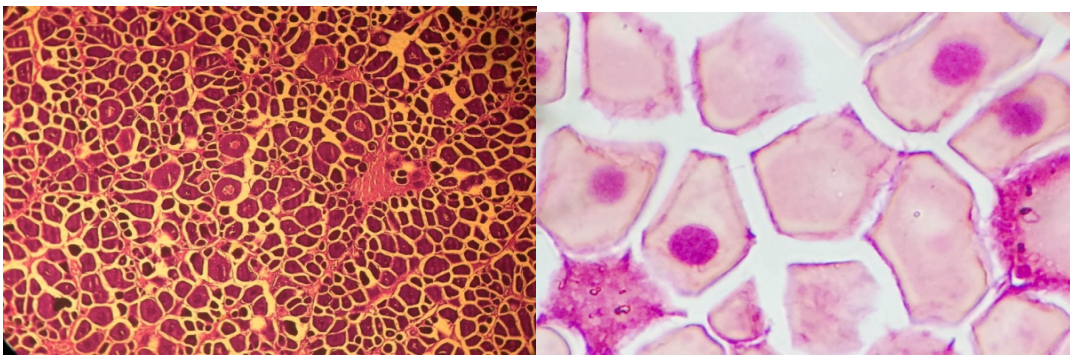

Fig. 9 Ovarian of fish at phase II.

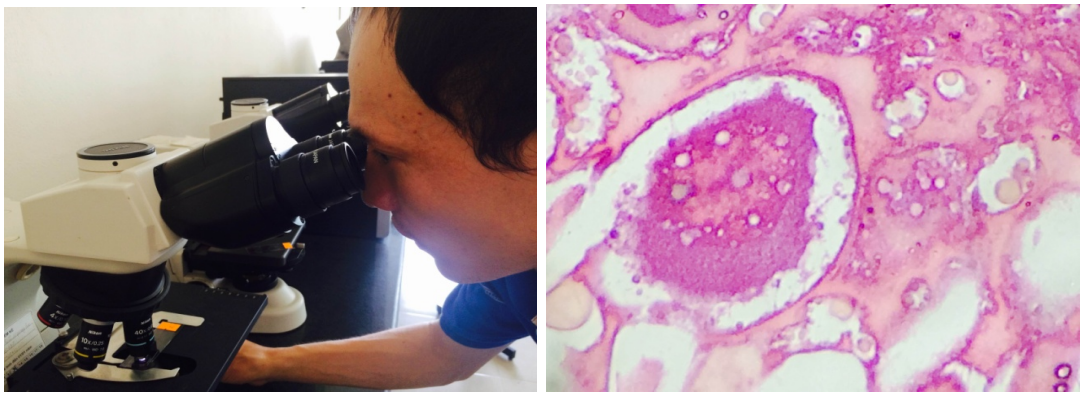

Fig. 10 Ovarian of fish at phase III.

There are larger cells inaccumulated yolk phase, t was smaller than the unsaturated cells that is storage stage the nutrition of oocytes for the next round of mature. However, the egg cells of small size are also different with each another (Fig. 10).

4. Discussion

Giant trevally fish is widely distributed in tropical seas and subtropics of the Indian Ocean and the Pacific, ranging along the coast of three continents and hundreds of small islands and archipelagos. In the Indian Ocean, the easternmost range of the species is the coast of the African continent, which is distributed from the Southern to South Africa, North along the East coast of Africa to the Red Sea and Persian Gulf. Its scope extends to the East along the coast of Asia, including Pakistan, India and Southeast Asia, Indonesian archipelago and Northern Australia. Elsewhere in the Indian Ocean, the species has been recorded from hundreds of small island groups, including the Maldives, Seychelles, Madagascar and the Cocos (Keeling) Islands. Sometimes, immature fish live in the estuaries, upstream river and coastal lakes in some places, including South Africa, Solomon Islands, Philippines, India, Taiwan, Thailand, North of Australia and Hawaii. Through surveys of households who exploited and lived around Tu Hien (Vinh Hien) and Thuan An estuaries (Thuan An town), it is found that hatch of fish (Giant trevally) is in sea, and larvae growing to fingerlings is under the water going into the lagoon through two estuaries (Thuan An and Tu Hien). Most Fingerlings are primarily concentrated in October to April every year, and every year just has one or two batches in raining seasons, often slipping into pre-season floods and storms. Every night, one bottom corral can catch hundreds of fingerlings, and sizes of fingerlings are around 3-10 cm of length according to Refs. [1, 7, 8].

Based on the morphological analysis of the body, results showed that there are some differences among the lower part on the lateral dorsal, lateral area of the body, the part near the tail fin fish scales, but generally fish scales are relatively large with oval, and bears some general characteristics of bone fish species. Giant trevally has thin scale, and is oval, central development, so the growth is flourishing in both the front and the back of the scales. In different areas of the body, fish scales, shapes and different sizes were observed in the study. The larger scales are in the lateral head of the body, and the front flounce of larger size is at rear area. Head part of fish has an abdomen paler, and contained less pigment than the body part, howere, the larger head is not clear; the growth rings close to the lateral middle have the largest distance, the color equivalent between belly parts of fish body is not explicit compared with the lateral body. Therefore, the length of scabs in the body on either side of the road was used to determine the age of fish. According to the results of some previous studies, the habitat of giant trevally has extensive salinity, from brackish to marine water environment, estuaries, bays and lagoons, while the immature live in the deeper reefs and offshore atolls. In the same period, the authors lived in a very low salinity waters, such as coastal regions and rivers upstream, and tended to prefer turbid waters, as research done by Smith and Parrish udated by Ref. [9].

Fingerlings were fed crustaceans and small fishes every day in the culture cages. For individuals collected and catching from lagoon and sea, it was showed their diets in table 4 with 80% of the stomach of fish and mostly they are small species combined, as shown in Refs. [5, 10-12]. Fig. 5 showed the growing rate of body weight and length with significant ($P <$ 0.05) correlation, and the body weight will be expected to 4-5 kg after three years feeding in lagoon conditions with dietary composition as Table 4. Many fish species have distinctive sex characteristics and can be identified through the external morphological characteristics. However, wild fish species are difficult to identify based on outside sex shape, especially for not sexually mature fish. For individuals of giant trevally collected from wild condition, they have eggs size weighing over than 3.5 kg/head. Meanwhile, individuals raised in culture condition showed mature sooner, inducing gonad stage, vascular distribution. At phase II, gonad becomes larger, but still not goes to mature as expecting, when observed with the naked eye, except in cases of ovarian at late phase II; and mature can reach at phase III with ovary of giant trevally for oocyte in the nutritional growth period. The larger ovarian cells are accumulated more yolk and stored the nutrition of oocytes for the next round of mature, [9].

5. Conclusions

The giant trevally (*Caranx ignobilis*) is large fish, big mouth, mouth under thrown up on and inside mouth contains sharp teeth, flat body, with a total body length in 3.13 times of body height and in 4.19 times of head length (TL/BH = 3.13, TL/HL = 4.19) and head length in 4.14 times of eye diameter (HL/ED = 4.14), pectoral fin rays, dorsal and anal yellow, gray dorsal, and ventral surface is silver white.

The research results showed correlation between length and weight of fish Vau is: $W = 0.0252*L^{2.898}$ and $R^2 = 0.987$, and this is correlated with age structure of giant trevally groups (0 +, 1 +, 2 +, 3 +).

Composition of food in the digestive tube lugs fish includes three groups: phytoplankton, animals (zooplankton and small size animals) and specific organic humus containing 14 species of phytoplankton and 19 species of animals. The average correlation coefficient between gut length and body length of giant trevally (RLG) is 0468 < 1, and it can be concluded that the feeding of giant trevally was inclined to animal.

Of the 50 samples studied, it could not distuinguish yet between sexes clearly, may be due to that the time of sampling does not coincide with the breeding

season of giant trevally in Tam Giang-Cau Hai lagoon system. However, only mature female fish for eggs and male fish for spermatorrhoea were detected. Results showed that in slice of fish samples, some gonads were identified in phase I, II, III and the sex cells and gonads were continuing to grow.

References

[1] IMOLA/FAO. 2007. *Report on the Survey and Inventory Activities and Aquaculture Operators.* Geographic Information System.

[2] Pham, T. L., and Tran D. T. 2004. *Curriculum Biological Research Methods of Fish.* Lecture Note from Can Tho University.

[3] Vo, V. P. 1979. "The Method of Biological Research of Tropical Fish Bones." *Russian Journal of Marine Science* 8: 135-45.

[4] Pravdin, I. F. 1973. *Guide to Study Fish.* Translated by Giang, P. T. M. Hanoi: Publishing House of Science and Technology.

[5] Mutia, T. M. 2011. "Induced Breeding of Malipulo *Caranx ignobilis*." NFRDI Fisheries Biolgical Research Centre, Butong, Taal, Batangas. Accessed November, 2015. https://repository.seafdec.org.ph/handle/10862/2792.

[6] Arreguin-Sanchez, F., Munro, J. L., Bolgos, M. C., and Pauly, D. 1996. "Biology, Fisheries and Culture of Tropical Groupers and Snappers." In *Proceedings of International Center for Living Aquatic Resources Management (ICLARM),* 448.

[7] Smith, G. C. 1992. "Occurrence and Diets of Juvenile *Caranx ignobilis* and *Caranx melampygus* (Teleostei: Carangidae) in the Hanalei River Estuary, North Kauai, Hawaii." *Pacific Science* 46 (1): 103.

[8] Murakami, K., James, S. A., Randall, J. E., and Suzumoto, A. Y. 2007. "Two Hybrids of Carangid Fishes of the Genus *Caranx*, *C. ignobilis* × *C. melampygus* and *C. melampygus* × *C. sexfasciatus*, from the Hawaiian Islands." *Zoological Studies* 46 (2): 186-93.

[9] Smith, G. C. 1991. "Abundance, Diet and Growth of *Caranx ignobilis* and *Caranx melampygus* (Carangidae) in the Hanalei River Estuary, North Kauai, Hawaii." M.Sc. thesis, University of Hawaii.

[10] Smith, G. C., and Parrish, J. D. 2002. "Estuaries as nurseries for the jacks *Caranx ignobilis* and *Caranx melampygus* (Carangidae) in Hawaii." *Estuarine, Coastal and Shelf Science* 55 (3): 347-59.

[11] Sudekum, A. E. 1982. "Notes on the Biology and Feeding Habits of *Caranx ignobilis* and *Caranx melampygus* in the Northwestern Hawaiian Islands." *Pacific Science* 36 (4): 515. (Abstract)

[12] Amarasinghe, U. S., Wickramaratne, I. U., and Wijeyaratne, M. J. S. 2011. "Hook Selectivity of Giant Trevally (*Caranx ignobilis*) and Nakedbreast Trevally (*Carangoides gymnostethus*) (Carangidae) Caught in the Hook-and-Line Fishery off Negombo, Sri Lanka." *Sri Lanka J. Aquat. Sci.* 16: 11-26.

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Nutritional Characteristics and Feeding of Rabbitfish (*Siganus guttatus*) in Tam Giang-Cau Hai Lagoon Systems

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Abstract: The research aimed to investigate for lagoon foodweb and dietary composition for rabbitfish in different stages (larvae, fingerlings and grower). Data were collected from two experiments using basic methods in field and laboratory. Experiments were structured on 2,000 larval head, 500 each tanks for nursery and 90 heads of three groups were collected in different seasons to laboratories for analysis of feeding intake and food compositions. Results showed that the number of omnivorous species, including animals, plants and organics, have been identified and grouped in 39 genera, 30 families, 23 sets, 8 classes and 6 branches. Animal groups have been identified as 18 breeds, 18 families, 13 lines, 3 classes and 3 animal species of industry animal. Plant foodweb, mainly Bacillariophyta, have accounted for 27 genera and 67.5% of total expenditure vegetation eaten by the rabbitfish. For the animal feed industry joint foot, Arthropoda have the highest number of 15 varieties, accounting for 83.3% of the animal's nutritional ratio. Organic residues as food for species have the highest prevalence in digestive tract (94.4%). The dominant food groups were determined as 15 genera and 5 animal breeds, particularly those large groups of algae.

Key words: Rabbitfish, nutrition, foodweb, characteristics, lagoon.

1. Introduction

Tam Giang-Cau Hai lagoon systems, a largest lagoon in Asian region, has more than 200,000 ha water covering areas, and there is a biodiversity of aquatic species and one of economic and potential food species, as rabbitfish (*Siganus guttatus*). There were studies and researches have done on this species to produce larvae, but not successful for the stage of nursery periods, and larvae are all dead after 7 d [1-3]. So, famers often collect the fingerlings from sea into lagoon through two inlets—Thuan An and Tu Hien, and numbers of the fingerlings are reducing in lagoon gradually [2-4]. For the problems for larvae death after 7 d of hatching by no food and starvation, Puvaneswari et al. [5] conducted a study to test problem of no food in digestion systems and thus dead. The questions were reminded which kinds and types of feedstuffs and foodweb that can be adapted for them in early growing stages. The hypothesis of the study can be pointed out and conducted to practice for microalgae or zooplankton supplied. In the discussion of hatchery the larval nauplii diets of *Brachionus*, *Artemia* and artificial food are also concentrated [6]. At larval stage, the fish grow very quickly within 24 h, the mouth of this species opens at 36 h after hatching, and learns to eat at 60 h after hatching [7, 8]. The larvae of *S. guttatus* starts to consume the yolk common and nutritious on day 2, and mortality happens very high in the 3rd and 4th day due to the lack of appropriate foods [9]. The diets required for larvae are less than 90 μm as size of *Brachionus*, 10-20 individuals/mL can be combined with other ingredients. The algae *Chlorella*, *Isochrysisla* and

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Tetraselmis are larval food source, but not used at the 4th day of *S. guttatus* larvae when exposes photophilic and flow properties as they reach full body length 2.6-2.7 mm, but photophobic properties disappear afterward at 9.2 mm size. Larvae size in the tank bottom runs stable in 19.6 mm size [9]. The eggs *of S. guttatus* hatched from the wild were often subjected to changes in salinity 24‰ and the lowest at 8‰. Tests for salinity tolerance on the different ages of *Discus* showed 0-day-old larvae resistant to low and high salinity concentration at 8‰ and 37‰, respectively, for a better larval age at days 14. From 21 to 35 old days, larvae can withstand sudden changes in salinity at 35‰ [4]. According to Ref. [9], there was not any significant difference observed in the survival rate of larvae reared at 20‰-32‰ salinity. The survival rate of larvae was enhanced at the temperature of around 22-26 °C.

There are more difficulties that the authors study the nutritional features and characteristics as a basis for producing artificial breeding discussion. The inherent natural food in the brackish lagoon with different salinity change creates diverse plankton and microalgae. The goals of study are to find suitable nutritional characteristics and feeding regime for different growth stages of rabbitfish, and identify the possibilities of using different types of food and foodweb. The alga *Chlorella*, *Isochrysis* and *Tetraselmis* are good sources of feedstuffs and foodweb to use for larvae on the starting four days period of feeding [4, 9]. The authors prefer to use zooplankton feed synthesis or *Isochrysis* or in combination with *Chlorella* with *Brachionus*. Crustacean nauplii larvae of copepod is suitable for the first stage of nutrition than *Brachionus*, but their biomass remains a problem which should be studied furthermore in the study and experiment practices.

2. Materials and Methods

2.1 Fish Samples

The fingerlings were collected from wildlife, started nursery at enclosed nets for 7 d for adapting period and designed into two experiments. Besides, these fish samples also were collected from wild condition for determination of the nutritional characteristics. All fish were cultured at Loc Binh and Hai Duong communes, Tam Giang-Cau Hai lagoon systems.

2.2 Experimental Design

2.2.1 Experiment 1

Collecting a total of 2,000 wildlife rabbitfish larvae with 1-2 cm length and allocated into the 16 composite tanks, then divided into four groups of 500 individuals per tank and observed the ability to catch food under the different food groups. Larvae were reared in composite tanks and maintained in the water temperature from 23 °C to 30 °C. Then they were allocated into four groups and larvae were fed Polychaeta for group 1, Artemia for group 2, Rotifers (*Brachionus plicatilis* and *Brachionus rotundiformis*) for group 3, and *Chlorella*, *Tetraselmis* and *Isochrysis* for group 4, respectively.

2.2.2 Experiment 2

Different sizes of rabbitfish are collected in the wild and then undergo surgery to remove the food from the gut to analysis of nutritional composition. There are three groups of fish were analyzed with feed ingredients in the gastrointestinal tract. The three groups were allocated as: group 1 with size of 4-12 cm (30 heads), group 2 with size of 12-20 cm (30 heads) and group 3 with size > 20 cm (30 heads), and they all were fed by commercial feedstuffs.

2.3 Methods

Fishes in every tank were fed after 1 h, and 24 individuals were randomly collected to test the ability to catch prey and not catch the food, then 3 h to continue catching 24 samples were randomly collected again for test in each nusery tank. Data were calculated according to the proportion of saturation and description of the ability that fish can catch baits. The first of full “0” means without food in the

gastrointestinal tract, the second level of full "1" means food appeared in the food pipe, the third level of full "2" means in the gastrointestinal tract have some food, but still many blanks gas, the fourth level of full "3" means in tube full of food digestion medium (no space to accommodate gas), but not tense, and the last level of full "4" means in tube filled with food digestion and swollen than normal.

Samples were surgical and laboratory analysis was done at the Center and Institute of Biotechnology, Hue University. The nutritional characteristics and functional characteristics of gastrointestinal tract were tested, and the ratio of the intestinal length compared to body length was observed. Gut and body lengths of the fish are observed and meanwhile the shape and structure are described to determine the length. Through that, we can be aware of its feeding habitat in the wild. Based on the research results in fish bone on the relationship between gut length (*Li*) with length (*L*), tie is known the spectrum as well as the food eaten of the whole disk [3]. Fresh fish samples were collected and put into a plastic box containing 10% formalin for conservation up to labs, and sampling was in May and September. The composition and species of flora and fauna, which they ate and were contained in the digestive tract, were determined by comparing morphological method. Group of plant material used by the authors in report of Ref. [10]; and zooplankton, the group of animals and bottom materials were used as reported in Refs. [11, 12].

2.4 Analysis and Determination

Identify the frequency of one type of food and record the number of times encountered such feed in the first paragraph and the gastrointestinal tract associated with the observed morphological anatomy of the digestive tube of *Discus* [13, 14]. Determination of full eating is based on the amount of food contained in the stomach and intestines of the fish ladder 5 steps, and the index are observed and evaluated by eye and microphoto. All of the samples were analyzed immediately or dying fish to avoid mistakes with 5-tier scales from 0 to 4. The first of full "0" means without food in the gastrointestinal tract, the second level of full "1" means food appeared in the food pipe, the third level of full "2" means in the gastrointestinal tract have some food, but still many blanks gas, the fourth level of full "3" means in tube full of food digestion medium (no space to accommodate gas), but not tense, and the last level of full "4" means in tube filled with food digestion and swollen than normal.

3. Results

3.1 The Ability to Catch Prey and not Catch the Baits

Table 1 showed that rabbitfish can get foods from feeding systems from nursering day 1, 2, 4 and 7. The results showed all group have no feed intake at day 1, and increase at days 2, days 4 and up to days 7 with 37.5%, 87.5% and 100% for group 1, and at day 7 eat at grade 1 of 33.3%, grade 2 of 25.0%, grade 3 of 25.0% and grade 4 of 16.7%, respectively. While for group 2, they have feed intake with 0, 54.2%; 91.7% and 100% at day 1, 2, 4, 7, respectively, and at day 7 they have different eating full grades, at grade 1 of 8.3%, grade 2 of 37.5%, grade 3 of 29.2% and grade 4 of 25%. For group 3 at day 1, 2, 4, 7, they have feed intake with 0, 66.7%, 95.8% and 100%, and at day 7 have different eating full grades with 4.2% for grade 1, 41.7% for grade 2, 29.2% for grade 3 and 25% for grade 4. For group 4 at day 1, 2, 4, 7, the number of having feed intake is 8.3%, 87.5%, 100% and 100% grades, respectively, and the number of at different eating grades levels at day 7 is 4.2%, 41.7%, 29.2% and 25%, respectively.

3.2 Nutritional Ecosystems

The rate of bowel length and body length is an important indicator to determine the spectrum of an object feed in any fish bone that biologists and aquaculturists expected. The ratio of intestine length and body length is shown in Table 2, and it shows that the percentage of intestinal length compared to body

Table 1 The eating full of larvae under four grades at nursering days.

Group	Days	Eating grades at different levels										Total	
		0		1		2		3		4			
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
I	1	24	100.0	-	-	-	-	-	-	-	-	-	0.0
	2	15	62.5	3	12.5	3	12.5	2	8.3	1	4.2	9	37.5
	4	3	12.5	8	33.3	6	25.0	4	16.7	3	12.5	21	87.5
	7	0	0.0	8	33.3	6	25.0	6	25.0	4	16.7	24	100.0
Total		42		19		15		12		8			
II	1	24	100.0	-	-	-	-	-	-	-	-	-	0.0
	2	11	45.8	6	25.0	4	16.7	2	8.3	1	4.2	13	54.2
	4	2	8.3	5	20.8	5	20.8	7	29.2	5	20.8	22	91.7
	7	0	0.0	2	8.3	9	37.5	7	29.2	6	25.0	24	100.0
Total		35		13		18		14		12			
III	1	24	100.0	-	-	-	-	-	-	-	-	-	0.0
	2	8	33.3	6	25.0	6	25.0	2	8.3	2	8.3	16	66.7
	4	1	4.2	8	33.3	7	29.2	4	16.7	4	16.7	23	95.8
	7	0		1	4.2	10	41.7	7	29.2	6	25.0	24	100.0
Total		35		15		23		13		12			
IV	1	22	91.7	2	8.3	-	-	-	-	-	-	2	8.3
	2	3	12.5	7	29.2	8	33.3	3	12.5	3	12.5	21	87.5
	4	0	0.0	6	25.0	10	41.7	5	20.8	3	12.5	24	100.0
	7	0	0.0	1	4.2	10	41.7	7	29.2	6	25.0	24	100.0
Total		25		16		28		15		12			

Table 2 Percentage of intestinal length compared to body length.

Groups	1	2	3
	Mean ± SD	Mean ± SD	Mean ± SD
Body length (*L*) (cm)	9.2 ± 1.7	16.1 ± 1.7	22.6 ± 1.4
Intestine length (*Li*) (cm)	20.5 ± 4.3	42.5 ± 9.4	56.4 ± 3.1
Rate (*Li*/*L*)	2.2	2.6	2.5

length varies according to individual group from 2.2 to 2.6. In different fish size groups, the rates that individuals catch sizes of feed are differences, the smaller sizes, they can get more diversity ingredients than greater groups; this indicates that the individual groups have different sizes with different food spectrum.

Table 3 confirms once again the characteristics of *Discus* food, including surface layer (zooplankton) and bottom benthics. However, the number of bottom animals breed is only four varieties which account for 22.2% of spending zoobenthos, the rest are 14 varieties of zooplankton which accounting for 77.8% of spending zoobenthos. Of the three identified animal, branch leg joints (Arthropoda) have spent the most amounts, with 15 genera, accounting for 83.3% of total expenditure natural resources for *Discus*. Meanwhile, there is only 11.1% and 5.6% of total zoobenthics and zooplankton for this species on the lagoon systems. It was found that organics are divided between three ecoregions of the lagoon (Tam Giang, Thuy Tu and Cau Hai) [15] (Table 4). And there is also plant genus expressed with a high frequency in all three regions, i.e., *Achnanthes* and *Nitzschia*. Meanwhile, there is the genus which dominates only in one ecoregion, for example, *Amphora*, *Licmophora* and *Polysiphonia* in the Tam Giang; *Merismopedia* in Thuy Tu region; *Chaetomorpha*, *Diatoma*, *Lyngbya*,

Table 3 Composition of zooplankton and bottom fauna in the gastrointestinal tract.

Zooplankton	Distribution areas and allocations of Tam Giang-Cau Hai		
	Tam Giang	Thuy Tu	Cau Hai
Arthropoda			
Crustacea			
Copepoda			
Calanoida			
Cartiidae family			
Acartia branch	x	x	x
Calanoida			
Calanidae family			
Canthocalanus branch			x
Centropagidae			
Centropages branch	X	x	x
Harpacticoida			
Clytemnestridae family			
Clytemnestra branch	x		
Cyclopoida			
Corycaeidae family			
Corycaeus branch	x	x	x
Ostracacoda			
Cypridinidae family			
Cypridina branch	x		x
Harpacticoida			
Tachidiidae family			
Euterpina genus			
Ectinosomidae family			
Microsetella branch	x	x	x
Cyclopida			
Oithonidae family			
Oithona branch		x	x
Oncaeidae family			
Oncaea branch	x		x
Calanoida			
Pontellidae family			
Pontellina branch			x
Psediodiaptomus family			
Chi *Pseudodiaptomus*		x	x
Branchiopoda			
Ostracoda			
Halocypridae			
Conchoecia branch		x	x
Cladocera			
Polyhemidae			
Evadne branch	x		
Zoobenthos			
Amphipoda			
Corophiidae family			
Corophium branch	x	x	x

(Table 3 continued)

Zooplankton	Distribution areas and allocations of Tam Giang-Cau Hai		
	Tam Giang	Thuy Tu	Cau Hai
Annelida			
Polychaeta			
Nepthydida			
Nephthydidae grube family			
Nephthys branch	x	x	x
Nereidae Johnston family			
Ceratonereis branch			x
Mollusca			
Bivalvia			
Corbiculida			
Corbiculidae family			
Corbicula branch	x		
Total of branches	11	9	14

X means presented in the Tam Giang-Cau Hai lagoon as food for *Siganus guattatus*.

Table 4 Composition of plants and organics in the gastrointestinal tract.

Classification system	Length and sizes (cm)		
	4-12	13-20	> 20
Cyanophyta			
Chrooccocus branch	x	x	
Merismopedia branch		x	x
Pseudanabaena branch			x
Spirulina branch			x
Oscillatoriaceae family			
Oscillatoria branch	x	x	x
Lyngbya branch			x
Silic-Bacillariophyta			
Melosira branch	x	x	x
Biddulphia branch			x
Chaetoceros branch			x
Diatoma branch	x		x
Synedra branch			x
Rhaphoneis branch			x
Licmophora branch	x	x	x
Grammatophora branch	x	x	x
Ardissonea branch			x
Petroneis branch			x
Cocconeis branch	x	x	x
Achnanthes branch	x	x	x
Pinnularia branch		x	
Diploneis branch	x	x	x
Navicula branch		x	x
Climaconeis branch			x
Pleurosigma branch	x	x	x
Gyrosigma branch	x		x
Amphora branch	x	x	x

(Table 4 continued)

Classification system	Length and sizes (cm)		
	4-12	13-20	> 20
Pseudonitzschia branch			x
Nitzschia branch	x	x	x
Cylindrotheca branch		x	
Tryblionella branch	x	x	x
Rhopalodia branch	x	x	x
Campylodiscus branch			x
Surirella branch			x
Psammodictyon branch	x		
Dinophyta			
Prorocentrum branch	x		x
Chlorophyta			
Enteromorpha branch		x	x
Cladophorales			
Cladophoraceae family			
Chaetomorpha branch		x	x
Gracilaria branch	x	x	
Ceramiales			
Rhodomelaceae family			
Chi *Polysiphonia* branch	x	x	x
Higher plants			
Organics	x	x	x
Total of branches	19	21	34

Table 5 Occurrence frequency of some dominant plant.

Plant branches (seaweed)	Thuan An	Thuy Tu	Cau Hai	Total	%
Achnanthes	60	47	52	159	58.9
Amphora	51	14	42	107	39.6
Chaetomorpha		6	51	57	21.1
Cocconeis	60	69	21	150	55.6
Diatoma	27		52	79	29.3
Gammatophora	46	56	35	137	50.7
Gracilaria	50	64		114	42.2
Licmophora	61	29	27	117	43.3
Lyngbya			45	45	16.7
Merismopedia		67	34	101	37.4
Nitzschia	60	72	63	195	72.2
Oscillatoria	21	24	52	97	35.9
Pleurosigma	12	27	57	96	35.6
Polysiphonia	61	11	39	111	41.1
Organics	82	86	87	255	94.4

Oscillatoria and *Pleurosigma* in Cau Hai. The identification that the genus of zooplanton and phytoplanton are popular food items is significant for *Discus* on production practices. On that basis, we can choose the type of natural plant feedstuffs suitable for the production the fingerlings and open the prospect of the future to actively feed biomass to make food for *Discus*. Also, we could choose the ecoregions which have the distribution of this species to the fish, especially local feed resources from plants and seaweed.

3.3 Organic Residues in the Gastrointestinal Tract

Table 5 shows that the organic humus is foods with the highest frequency of appearance in the *Discus* gastrointestinal tract (94.4%). Also *Discus* also uses a variety of macroalgae (seaweed), as *Gracilaria* (42.2%), *Polysiphonia* (41.1%), weed membrane, seaweed fiber, seaweed and algae. Feed intake contained in the digestive tract of fish was the highest *Nitzschia* (72.2%), followed by *Achnanthes* (58.9%) and *Cocconeis* (55.6%).

4. Discussions

Feeding system and feed intake for larval individuals of rabbitfish at conditional composite tanks showed that for the earlier stage of larvae, they can not eat anything from artificial conditional feeding and had no intake at day 1to day 7; this was also adapted with report from Refs. [16], while in this study, we have got larvae from artificial reproduction of center and started feeding at day 1 up to day 7. At day 1, due to change from natural habitat to a composite tank condition and artificial feeding by water in tanks, the eating behaviours of the larvae appeared around the pool and explores the bottom. At day 2, the fish began to catch prey and the zooplankton is seen as their hobby, and larvae in group 4 can catch more food compared other groups. Thus, the proportion ranged from group 1 to group 3, showing the larvae are omnivores (animals, plants and organics). Analytical results shows that plant foods and organic humus in the gastrointestinal tract are 270 samples and have been identified with 39 genera, 30 families, 23 sets, 8 classes and 6 Dinophyta and organics in Table 4. Results obtained from Tables 3 and 4 show that dietary components in the gastrointestinal tract are very diverse plant and animal species, as reported by Refs [17, 18] that the young and adults in the wild eat any algae that they can catch and digested. Analytical results from Table 5 also show the different levels and numbers of ingredients between three ecoregions (Tam Giang, Thuy Tu and Cau Hai). Among the 39 genera (72.2%) identified, there is only 15 genera (27.8%) have their frequency quite common, and the residual limb with a very low frequency of appearance and there were more common plant species in Table 4. The detailedly identified methods of plants in life cycle are quite diversified for different forms and living layers. Thereby, they are found that the fish likely eat the genus at both surface and bottom. Of the six species of algae identified, Bacillariophyta of diatoms branches appeared in almost ecoregions, with 27 genera and accounting for 67.5% of total expenditure vegetation eaten by rabbitfish. The mostly ecological environment is saltwater and brackish, diatoms have abundant life and they are distributed from the surface layer as live plankton, middle layer (epiphytic species of seaweed) and the mud floor (organics). Due to characteristics of diatoms, so they are commonly encountered in the gastrointestinal tract [19]. In particular, diatoms dominate 20 genera regarding the volume of vegetation predominated, and there are also a few animals vertebrate. However, in this case the analytical results show plant foods and organics in the gastrointestinal tract of rabbitfish occupied 270, which has been identified with 18 genera, 18 families, 13 orders, 3 classes and 3 sectors in Table 4.

5. Conclusions

Omnivorous species, including animals, plants and organics, have been identified with 39 genera, 30 families, 23 sets, 8 classes and 6 branches; animal groups have been identified with 18 breeds, 18 families, 13 lines, 3 classes and 3 species. Plant foodweb (Bacillariophyta) have occupied the most of 27 genera (67.5%) of total expenditure vegetation for rabbitfish and animal as joint foot (Arthropoda) have a highest number with 15 varieties (83.3%) of the animal species and feed. Humus is mainly organic residues with a highest prevalence of digestive tract of rabbitfish (94.4%). In the analysis, there are 15 genera and 5 species, which are the dominant food groups as

natural plants, particularly those large groups of algae. Rabbitfish of grouping size < 20 cm of body length can catch baits better than other groups, and most of fish can eat with grade "3" and "4" as mentioned in early parts.

References

[1] Linh Q. N. 2002. "Feeding Strategy and Nutrition for Improvement of Health Status and Productivity of Aquaculture in Tam Giang-Cau Hai Lagoon Systems." In *Proceedings of National Conference on Tam Giang-Cau Hai Lagoon Systems,* 231-41.

[2] Lavens, P., and Sorgeloos, P. 1996. *Manual on the Production and Use of Live Food for Aquaculture.* Rome, Italy: FAO.

[3] Truong N. A. 1993. *Silica Algae Classification in Vietnam Marine Science.* Hanoi: Housing Publisher for Science and Technology, 315.

[4] Le, D. N., Le, V. D., and Chau, T. T. H. 2007. "Study for Rabbit Fish Culture (*Siganus guttatus* Bloch, 1787) and Shrimp Farming (*Penaeus semisulcatus)* in Thua Thien Hue Lagoon Systems." No. B2004-08-06-TĐ, Research Project of Ministry of Education and Training of Vietnam, 2004-2007.

[5] Puvaneswari, S., Marimuthu, K., Karuppasamy, R., and Haniffa, M. A. 2009. "Early Embryonic and Larval Development of Indian Catfish, *Heteroneustes fossilis*." *EurAsian Journal of Bioscience* 3: 84-96.

[6] Southeast Asian Fisheries Development Center (SEAFDEC). 1988. *Reproductive Performance of Hatchery-Bred Siganus guttatus Broodstock Fed Different Levels of Fat.* Annual Report of Southeast Asian Fisheries Development Center.

[7] Soletchnik, P. 1984. *Aspects of Nutrition and Reproduction in Siganus guttatus with Emphasis on Application to Aquaculture.* Terminal Report to the Aquaculture Department, SEAFDEC, Iloilo.

[8] Duray, M. N. 1998. *Biology and Culture of Siganids.* Iloiloi, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC).

[9] Hara, S. 1987. "A Fundamental Study on the Seed Production of the Rabbitfish, *Siganus guttatus*." Doctoral thesis, University of Tokyo.

[10] Iwamoto, K., Chang, C. W., Takemura, A., and Imai, H. 2012. "Genetically Structured Population and Demographic History of the Goldlined Spinefoot *Siganus guttatus* in the Northwestern Pacific." *Journal of Fisheries Science* 78 (2): 249-57.

[11] Imajiama, M. 1967. "Errant Polychatous Annelids from Tsukumo Bay and Vicinicy of Noto Peninsula, Japan." *Bulletin of the National Science Museum Tokyo* 10 (4): 403-41.

[12] Juario, J. V., Duray, M. N., Duray, V. M., Nacario, J. F., and Almendras, J. M. E. 1985. "Breeding and Larval Rearing of the Rabbitfish, *Siganus guttatus*." *Aquaculture* 44: 91-101.

[13] Avila, E. M. 1986. "Evaluation of Practical Diets in the Culture of Rabbitfish, *Siganus guttatus* (Bloch) (Pisces: Siganidae) Using Liver Ultrastructural Methods." *Zoologischer Anzeiger* 217 (3-4): 178-91.

[14] Tabugo, S. R., Rose, M., Sendaydiego, J. P., and Requieron, E. A. 2012. "Embryonic Developmental Stage in Cultured Rabbitfish (*Siganus guttatus*, Bloch 1787)." *International Research Journal of Biological Sciences* 1 (8): 65-70.

[15] Linh, Q. N., Le, C. T., and Nguyen, M. 2002. "First Research Results of Zooplankton in Tam Giang-Cau Hai Lagoon Systems." *National Centre for Nature Science and Technology of Vietnam* 25 (3): 17-21.

[16] Ayson, F. G. 1989. "The Effect of Stress on Spawning of Brood Fish and Survival of Larvae of Rabbitfish, *Siganus guttatus* (Bloch)." *Aquaculture* 80 (3-4): 241-6.

[17] Gilbert, S. F. 2000. *Developmental Biology,* 6th ed.. Sunderland, Massachusetts: Sinauer Associates, Inc., Publishers.

[18] Phu, V. V. 2002. "Fishery and Aquaculture Diverse Systems in Tam Giang-Cau Hai Lagoon Systems." *Hue University Journal of Science* 15: 165-79.

[19] Hara, S., Marietta, N. D., Parazo, M., and Taki, Y. 1986. "Year-Round Spawning and Seed Production of the Rabbitfish, *Siganus guttatus*." *Aquaculture* 59 (3-4): 259-72.

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