DOI: 10.31276/VJSTE.65(1).47-53

Effect of arbuscular mycorrhizal fungi inoculation on revegetation at Nukui Dam site, Japan

Minh Thi Nguyen[1*], Kazuhira Yokoyama[2], Takuya Marumoto[3]

[1]Faculty of Environment, Vietnam National University of Agriculture, Trau Quy Town, Gia Lam District, Hanoi, Vietnam
[2]Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi, Japan
[3]Takino filter Inc., 2-904-16 Hayama, Kudamatsu, Yamaguchi, Japan

Received 3 December 2021; accepted 1 March 2022

Abstract:

The establishment of the surviving arbuscular mycorrhizal (AM) fungi, *Gigaspora margarita* Becker & Hall, inoculated in revegetation at the Nukui Dam was studied. The population of AM fungi and AM colonization of three plant species Japanese hill cherry (*Prunus jamasakura* Sieb. ex Koidz, yamazakura-inoculated plant), eulalia (*Miscanthus sinensis* Anderss, susuki), and mugwort (*Artemisia vulgaris* Pampan, yomogi) were investigated to compare inoculated and non-inoculated treatments. The AM colonisation was enhanced in inoculated plots although natural or accidental AM fungi were involved in non-inoculated plots and successfully colonised. Coverage of soil surface was more than 80% and even reached nearly 100% in summer regardless of the soil amendment type. The number of spores and the percentage of colonisation of investigated plant roots, as well as the density of typical AM structures such as arbuscules, auxiliary cells, vesicles, and coiling hyphae by AM fungi in inoculated plots were higher than those in non-inoculated ones. Some spores were *Gigaspora* and *Glomus*-inoculated species and were relatively rich in inoculated plots of Yamazakura. There was a significant increase in the number of AM spores in inoculated treatments at a 5% confidence level (ANOVA, $p<0.05$) in Susuki and Yamazakura. Much more spores of *Gigaspora* sp. occurred in the rhizosphere soil of Yamazakura and Susuki plants in inoculated plots than in non-inoculated plots (2.33 and 3.73 times, respectively). The colonisation intensity and spore number in the rhizosphere were positively correlated. Therefore, inoculation at an early establishment should be important for the successive development of AM-plant symbiosis formation.

Keywords: **arbuscular mycorrhizal (AM) fungi, revegetation, soil amendment.**

Classification numbers: **3.1, 3.4**

1. Introduction

AM, the most widespread symbioses on Earth [1], is receiving attention because of the increasing range of their application in diverse, practical fields such as sustainable agriculture, reforestation programs, and ecosystem management [2].

Some plant species almost completely depend on mycorrhizal association for nutrient uptake [3]. Improved growth and survival of such species will help plants to establish themselves in a diverse ecosystem, an important criterion for revegetation success. Plants that are normally mycorrhizal will be at a competitive disadvantage in revegetation if phosphorus is limited and if few effective mycorrhizal fungi exist in the soil. Therefore, the presence of effective mycorrhizal fungi will likely need to be re-established in such cases. In general, plants from mature ecosystems require the presence of mycorrhizae for their development [4].

It is well known that AM fungi species of the genus *Gigaspora* appear to favour fluxes of carbon compounds from plant to soil biota ultimately resulting in enhanced soil aggregation, while *Glomus spp.* tend to favour root colonization, plant growth and productivity through improved mineral nutrition [5]. The management of AM during revegetation and reforestation is well documented [6, 7]. However, little is still known about the establishment and effect of the long-term survival of

*Corresponding author: Email: nguyenminhvn@hotmail.com

AM after revegetation. Indeed, to the best of the authors' knowledge, there is no report on relationships among AMs, soil amendments, and soil properties. The overall objective of the work presented in this paper is closely linked to the project "Utilization and management of symbiosis associations - Mycorrhiza (AM and Ectomycorrhizae (EM) in revegetation program at Nukui Dam".

In the experiment layout, there were different plots receiving different kinds of soil amendments. In this study, therefore, we summarized annual reports of that project and the evaluation after revegetation, especially through the viewpoint of the changes in soil properties. Additionally, we analysed the effect of AM fungi inoculation as a surplus on the various effects of the soil amendments after revegetation at Nukui Dam in order to clarify the relationship between AM establishment and soil properties with different soil amendments at Nukui Dam.

2. Materials and methods

2.1. Description of the study site

The Nukui Dam is the second highest arch dam in Japan. It is located across a narrow section of the Takiyama river in the Hiroshima Prefecture and has hillside slopes and banks ranging from 45 to 60° with a riverbed width ranging from approximately 40 to 60 m.

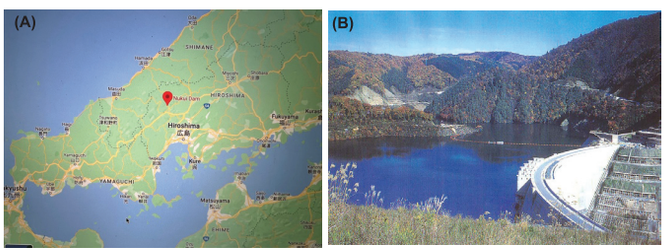

Fig. 1. (A) Location of Nukui Dam site on the map; (B) View of Nukui Dam. Red arrow indicates the study site on berm N°10.

The region extends from 34°36.6" N latitude to 132°19.2" E and from 270 to 386 m altitude with a catchment area of 1,700 km^2. The annual precipitation is approximately 2000 mm and the average annual temperature is roughly 13°C (cited from "Current activities on Dams in Japan, Japan commission on large dams, 2003").

The study site was berm N°10 - a part of the planting area at Nukui Dam, which was executed for a revegetation process in May 1998 (Fig. 1). The total area of this berm was 40.5 m^2 and was divided into 8 plots, each with an area of 3.0x1.5 m. Each plot was divided by plywood with a thickness of 2 cm.

The field sampling was carried out in September because the AM fungi spore populations are, in general, the greatest in the autumn in areas where there are marked warm/cold seasons [8, 9].

2.2. Revegetation program

Construction of the Nukui Dam began in July 1992. The dam's concrete placing commenced in May 1994, and the work was completed in December 1998. Regarding the preservation of the natural environment around the dam site, the research project of revegetation at Nukui Dam was done via cooperation between the dam construction office and Yamaguchi and Hiroshima Universities, Japan. The study on the N°10 berm started in 1998. Each plot (3x1.5 m) was filled with Masa soil (granite) with amendments shown in Table 1. Five seedlings of three tree species: Yamazakura (*P. jamasakura* Sieb. ex Koidz), Arakahi (*Quercus glauca* Thunb.), and Konara (*Quercus serrata* Thunb. Ex Muuray) were transplanted to every plot.

Table 1. Soil amendment in experimental plots in the N°10 berm at Nukui Dam site.

	Treatment							
	10-1	*10-2*	*10-3*	*10-4*	*10-5*	*10-6*	*10-7*	*10-8*
Masa soil (%)	60	54	60	54	59	35	60	54
Crushed sand (%)	24	23	24	23	20	20	24	23
Bark compost (%)	11		11		11		11	
Wheat straw compost (%)	5	10	5	10	10	15	5	10
Vermiculite (%)		5		5		15		5
Zeolite (%)		6		6		10		6
Bamboo charcoal (%)		2		2		5		2
Mycorrhizae fungi	+	+			+	+		
Fertilizer* (g.m^{-2})		90		90		90		90
Bonding agent (kg.m^{-3})								1.5

Fertilizer*: Green Map II (Sun Green Co. Ltd., Japan), N:P:K:Mg = 6:38:6:18.

Inoculation of AM fungi was carried out by mixing 15 g of an AM fungi spore-containing material, a Cerakinkong product (a commercial inoculum, Central Glass Co. Ltd, Tokyo) with soil around Yamazakura roots at transplanting in order to promote early rooting and initial growth of planted vegetation. The material contains at least spores of *Gigaspora margarita* Becker and Hall and *Glomus* sp. (ca. 2000 spores 100g^{-1}).

2.3. Soil and plant sampling

Soil was sampled along 8 plots of the N°10 berm, each with 5 cores (5x5x10 cm, each about 250 g in fresh weight) and composite giving pooled topsoil samples per plot. The soil samples were passed through a 2-mm sieve and then cautiously mixed before sub-sampling ca. 500 g of soil in every plot for physical, chemical, and biological analyses. A 100-g sub-sample of each plot was stored at 4°C for microflora analysis.

Additionally, randomly selected plants (containing rhizosphere and root zone soil) were gathered from 3 cores of each plot (3 plants/plot, separate for each plant) in order to estimate AM association and detect the survival of AM inoculated to the host plants with 3 replications. Three host plants were chosen for sampling: Japanese hill cherry (*P. jamasakura* Sieb. ex Koidz, Yamazakura-inoculated plant), Eulalia (*M. sinensis* Ander, Susuki), and Mugwort (*A. princes* Pampan, Yomogi). Susuki and Yomogi are popular herbaceous plants in Japan and were sampled to check the net amount of arbuscular mycorrhizae established from the inoculated plant. The hair roots were taken for Yamazakura because it is wood plant. After obtaining the root zone soil, the roots were washed gently under tap water to remove the adhering rhizosphere soil.

2.4. Quantification of the spore and AM colonization

The entire amount of rhizosphere and root zone soil from 3 plants of each species was processed to extract the spores, i.e., extramatricular chlamydospores, using the modified method of wet-sieving and decanting [10]. The rhizosphere soil amounted to about 500 g/plant.

Spores were counted under a dissecting microscope (Nikon) and collected individually using a pipette with a finely extruded tip and fine forceps and separated into different groups based on their morphology, colour, and type of hyphal attachment. After collecting, spores were sterilised by 2% Chloramine-T with 1200 mg/l streptomycin and stored at 4°C for further study.

The roots were cleared and stained by the modified method of P.P. Kormanik, A.C. McGraw (1982) [11]. The stained roots were estimated by a magnified intersection method [12], where roots are observed at 200x magnification. Each type of arbuscules, vesicles, or hyphae were quantified separately. For quantification and observation of AM structures inside the root, the glass slide method was used and root samples were mounted in lacto-glycerol solution on microscope slides. They were then observed under a compound microscope (Nikon) at 100-400x magnifications. After measuring the root length, 100 random root segments per sample were observed. To obtain an estimate of intensity, a morphometric technique [13] could be used where a grid of dots was placed over an image of squashed roots and colonized cortical cells were counted.

After examining roots for the presence or absence of AM mycorrhizal structures, the number of root segments colonised was counted and expressed as a percentage of total root segments in the sample. The frequency of arbuscules, vesicles, spores, auxiliary cells, and external and internal hyphae connected to arbuscules at each observed cross section was estimated. The AM infection was calculated as follows:

$$\text{Percentage of AM colonization } (\%) = \frac{\text{AM root length}}{\text{root length total}} x\ 100$$

$$\text{AM structure } (\%) = \frac{\text{AM structure}}{\text{AM root length}} x\ 100$$

2.5. Soil analysis

The soil's chemical, physical, and microbiological properties were determined according to general methods [14].

2.6. Statistical analyses

Results were tested with STATISTICA (1998) by the analysis of variance (Microsoft Office 98). Means and standard deviations (StDev) were produced in Microsoft Excel (Windows 97). Correlation within and between the soil parameters and the AM fungi data were initially determined by performing linear regressions in Microsoft Excel (Windows 97). A single ANOVA analysis was simultaneously carried out to prove the validity of the regression [15]. In one indicated case, the least significant difference (LSD) test was applied to reveal tendencies.

3. Results

3.1. Soil properties and microflora

At the beginning of the experiment, the soil amendments to every plot in berm 10 were recorded and are provided in Table 1. Soil amendments were different at every plot to test the different raw materials in Japan and their effect in Arbuscular mycorrhizae symbiosis.

According to the annual reports of the project, the changes in soil properties, growth of the seedlings, and invasion of naturally occurring plants from 1998 and in this study were summarized and are provided in Table 2.

Table 2. The soil properties at start of revegetation execution.

Parameter	Inoculated treatment (1,2,5,6 treatments)	Non-inoculated treatment (3,4,7,8 treatments)
Total carbon (%)	0.70-0.98	0.66-0.88
Total nitrogen (%)	0.034-0.043	0.028-0.042
Available P (µg P.g^{-1})	2.2-15.2	0.1-1.8
CEC (mol.kg^{-1})	5.1-10.1	4.2-11.6
Exchange Mg (cmol.kg^{-1})	0.7-0.83	0.59-0.88
Exchange K (cmol.kg^{-1})	0.06-0.82	0.01-0.80
Exchange Ca (cmol.kg^{-1})	2.77-3.98	2.37-4.40
Exchange (cmol.kg^{-1})	0.07-0.10	0.06-0.11
pH_{H_2O}	6.81-7.53	7.05-7.83
pH_{KCl}	5.93-6.00	5.93-6.14
Biomass C (µg C.g-^{1})	29.5-81.0	22.7-86.8
Biomass N (µg N.g-^{1})	5.6-14.4	4.6-18.7

Ranges of the soil's chemical properties at the start of the experiment are shown in Table 2 in combination with those determined in the present study. These were strongly affected by the composition of soil amendments and fluctuated annually. We were unable to find any evidence that AM inoculation affected soil properties and their fluctuation.

There was slightly increasing carbon content and microflora in every plot compared with the preliminary data from the team at Hiroshima University during the first revegetation project at Nukui Dam (data not shown). The fact that the total nitrogen was currently highest compared to the preliminary data and that the phosphate and exchangeable cations improved with time confirmed the increasing nutrient content after carrying out revegetation at Nukui. In both undisturbed and cultivable systems, potential productivity is directly related to soil organic matter concentration and turnover [16].

In general, mycorrhizal associations can also enhance N grain in ecosystems by increasing N-fixing associations [2, 17-19]. The overview of these changes suggests that soil properties in the experiment have improved over time and with vegetation development. Although we could not find a statistical difference in soil properties because of the remaining effect of soil amendments at start, a tendency for nutrient enrichment was slightly promoted in the inoculated plots.

Among the three wood species, Yamazakura markedly survived and grew such that the GoH value (an index of wood biomass, where Go and H mean a diameter at a soil surface or height in cm, respectively) of Yamazakura reached more than 70 to 90% of the total GoH of three species (data not shown). There was a large difference in the total GoH value among AM-inoculated plots (for example, 8.76 for the 10-1 treatment and 1.89 for 10-5 treatment), which suggests that AM-inoculation would positively affect the survival of Yamazakura but would be inefficient for their growth.

The number of plant species that invaded naturally and became established in the experimental plots was 33. The susuki (*M. sinensis* Ander.) and mugwort (*A. princes* Pampan.), popular Japanese weeds, were predominated in field.

Coverage of the soil surface was more than 80% and sometimes reached nearly 100% in summer regardless of the soil amendment type.

The soil properties of each plot at sampling were according to inoculation and non-inoculation treatment. The values of pH_{H2O}, maximum water holding capacity, and bulk density were similar in both inoculated and non-inoculated treatments. However, two plots gave an EC value significantly higher than other, namely, the 10-3 and 10-7 plots (Table 3), and those plots had the same soil components (Table 1). The total carbon and nitrogen content and exchange cations in inoculated plots were slightly higher than those in non-inoculated plots, but the differences were insignificant statistically.

Table 3. The physical and chemical properties and microflora of soil at the Nukui Dam.

Parameter	pH		EC	MWHC	Bulk density		Total C
	H_2O	KCl	(µS.cm^{-1})	(g.100g^{-1})	(g.100g^{-1})	(g.100ml^{-1})	%
Treatment					*Crude*	*Fine*	
Non-inoculation	5.53±0.38	4.31±0.28	108.95±53.16	33.47±3.85	102.63±3.02	159.81±3.09	1.03±0.32
Inoculation	6.03±0.59	4.73±0.16	72.25±8.13	35.12±1.84	95.83±11.25	153.48±9.31	1.16±0.29

Parameter	Total N (%)	Available P	Ca	K	Mg	Na	Fungi	Bacteria
		(cmol.kg^{-1})					(x10^6)	(x10^6)
Treatment							CFU.g^{-1}	
Non-inoculation	0.06±0.029	0.35±0.09	4.33±0.38	0.68±0.25	0.91±1.12	0.74±0.69	4.31±2.67	5.61±4.03
Inoculation	0.08±0.022	0.36±0.13	4.52±1.29	0.85±0.23	1.61±1.57	0.55±0.38	5.06±2.56	6.35±5.11

There was a slightly higher number of fungi and bacteria in the inoculated than in the non-inoculated treatments (Table 3), but not a significant difference in the experimental treatments (StDev, ANOVA). The

fungi quantity ranged from 4.31±2.67 to 5.06±2.56x10^4 CFU.g-^{1} in dry soil. The highest bacteria number was obtained at the 10-1 plot-inoculated treatment and the lowest in the non-inoculated treatment.

3.2. Spore number in rhizosphere soil of plant roots

Spores picked up from the Yamazakura and Susuki rhizosphere soil (250 cm^3) in the inoculated plots were mainly obtained from the sieved size range of 106-210 µm or 210-500 µm. There was a significant increase in the number of spores in inoculated treatments at 5% confidence level (ANOVA, $p<0.05$) in Susuki and Yamazakura (Fig. 2A).

Inoculation of AM fungi promoted the occurrence of spores and colonization to plant roots. The obtained results showed that the AM colonization rate in inoculated plots was higher than in non-inoculated plots. In addition, the density of typical structures (arbuscule, vesicle, coil hyphae, auxiliary cell) of AM was also much larger in inoculated treatments compared with non-inoculated treatments. This is a well-known response that has been widely reported in the literature [20].

Apart from AM efficiency on vegetation development, the rate of inoculated AM fungi at start should be discussed. There were some spores of *Gigaspora* sp. Many more

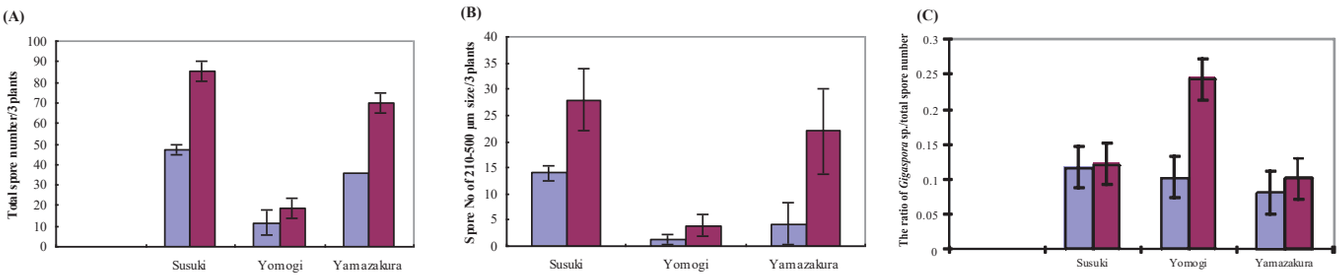

Fig. 2. (A) The total spore number in Rhizosphere soil; (B) Spore number in 210-500 µm sieved Rhizosphere soil; (C) The ratio of *Gigaspora* sp. to the total spore number. Legend: non-inoculated AM (■) and inoculated AM (■).

There was a 5.2 times difference in the number of spores of large size (>210 µm) between inoculated and non-inoculated treatments in Yamazakura. Clearly, plant hosts vary markedly in their ability to affect spore production by *Gigaspora* and *Glomus.* By observation of morphology and size of spores, the spores of *Gigaspora* sp. in inoculated plots were greater in number than in non-inoculated ones among all investigated plants (Fig. 2C).

In investigated plants, Yamazakura would receive the strongest benefits for their initial growth just after transplanting among three species. The primary effect of AM inoculation might be to hold AM-propagule levels higher in comparison to non-inoculated plots. This might help the establishment of invading weeds such as Susuki and Yomogi rather than Konera and Arakashi seedlings. The higher propagule level might support AM formation with Susuki (Fig. 2A) and Susuki could make abundant AM propagules in the next generation. This could result in AM activity in inoculated plots for long periods. The above advantages, however, do not show statistical differences among plant flora and biomass because the effect of soil amendment remains active.

Gigaspora sp. spores occurred in rhizosphere soil of Yamazakura and Susuki plant in inoculated plots than in non-inoculated plots (2.33 and 3.73 times, respectively). Further study is necessary to detect the inoculated strain, *Gigaspora margarita,* after revegetation process.

Although Yamazakura was an inoculated plant, the AM colonization was highest in Susuki. The reason might be that *Gigaspora* sp. had a greater effect on Susuki than other host plants, confirming the results of other workers in our laboratory.

3.3. The AM colonization in plant roots

In Yamazakura, high potential plots that showed a very high percentage of root infection were inoculated with treatments when ranges of infection were in the range of 72.16-84.0%. The minimum infection was found in non-inoculated treatment to be 38.33%. Moreover, the coiling hyphae of infection roots increased notably in inoculated treatments, twice higher compared to in non-inoculated ones. At the same time, there was a significant increase of arbuscules as well as vesicles and auxiliary cells in inoculated plots (Figs. 3A-E).

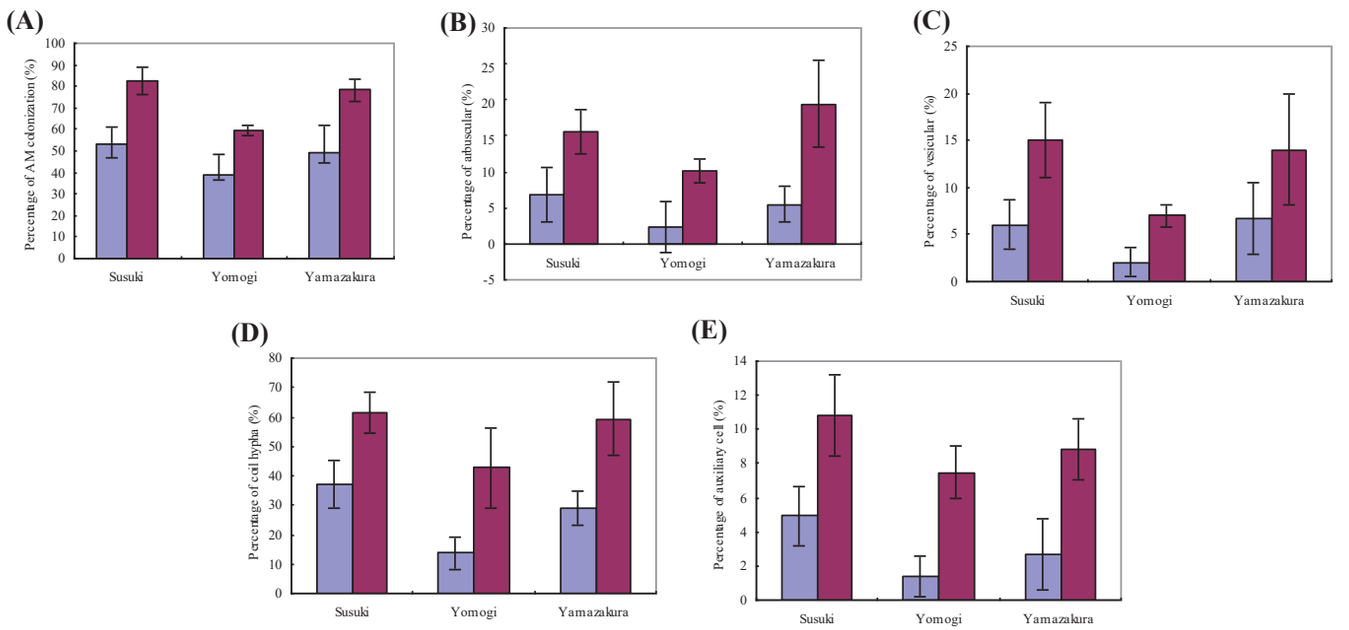

Fig. 3. AM colonization and structure in plant root. (A) Percentage of AM colonization (%); (B) Percentage of arbuscular; (C) Percentage of vesiclar; (D) Percentage of coil hypha; and (E) Percentage of auxiliary cells. Legend: Non-inoculated AM (■) and inoculated AM (■).

Similar results were obtained from Susuki roots on AM colonization. The AM colonization was high in both inoculated and non-inoculated treatments ranging from 47.06 to 88.18%. In inoculated plots, coil hyphae formed the main components of the AM colonisation. In non-inoculated treatments, a smaller proportion of infected root length (<10%) contained arbuscules, auxiliary cells, and vesicles. The AM colonization included infected root rate and AM structures in inoculated treatments given at higher frequencies than in non-inoculated ones (Figs. 3A-E).

A considerable proportion of root length (28.25-61.62%) was arbuscular mycorrhizae in infection rates of Yomogi roots with AM fungi. Colonization by AM fungi was highest in plot 10-6 with inoculated treatment. The level of colonisation in non-inoculated treatments was significantly lower than that of inoculated ones (ANOVA, $p<0.05$). There was a significant increase in typical AM structures in the inoculated treatments (Figs. 3A-E). On other hand, arbuscules, auxiliary cells, and vesicles were absent in some non-inoculated plots or very low in other ones.

The density of AM structures was increasing not only in arbuscules, coil hyphae, and auxiliary cells, but also in vesicles. Meanwhile, a did not form vesicles as part of its life cycle. The inoculum product, Cerakinkong, includes both *Gigaspora margarita* and *Glomus* sp. At the same time, the AM symbiosis of indigenous AM could strongly establish. Those AM strains might affect plant roots together.

Through observation of AM structures under a compound microscope, it was found that density of typical AM structures in inoculated treatments were higher than in non-inoculated ones. Indeed, there were many more arbuscules and auxiliary cells (Figs. 3B, E), which are typical structures of the Gigasporaceae family, in the roots of Yamazakura and Susuki plants in inoculated plots rather than in non-inoculated plots. The frequency of arbuscules and auxiliary cells were very low in inoculated plots. These findings suggest that there have been different kinds of host-AM fungi interaction and that Yamazakura - *G. margarita* containing material could be a better combination for the start of vegetation.

The spores of *Gigaspora* sp. in rhizosphere soil and arbuscules and auxiliary cells in roots formed much more often in inoculated plots than in non-inoculated plots at the Nukui Dam site. This indicates the possibility of survival and propagation of the inoculated AM fungi *G. margarita*. Regarding why AM inoculation enhances AM establishment even after several years, one hypothesis suggests that there is an interaction and competition among host plants. When pairs of plant species growth together with or without the addition of AM inoculum, the outcome of the interaction between them is known to be very different [21].

There was a linear correlation between spore numbers in rhizosphere soil and AM formation for each of the inoculated and non-inoculated plots (Fig. 4).

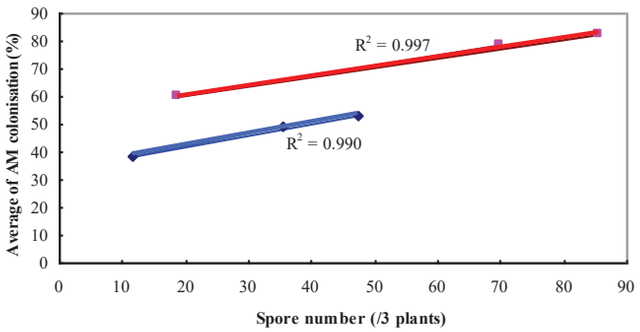

Fig. 4. The correlation between spore number and AM colonisation. Legend: (--■--) Non-inoculated AM; (--▲--) Inoculated AM.

The efficiency of AM fungi propagules to form symbiosis seemed to be higher in inoculated than non-inoculated plots. This, in turn, suggests the vegetation developed in inoculated plots depended on AM more strongly than those in non-inoculated plots.

4. Conclusions

Soil properties in the experiment have improved over time and with vegetation development and a tendency towards nutrient enrichment was slightly promoted in the AM-inoculated plots. Indeed, the coverage of the soil surface was more than 80% and sometimes reached nearly 100% in summer regardless of the soil amendment type.

There was a significant increase in the number of AM spores in inoculated treatments at 5% confidence level (ANOVA, $p<0.05$) in Susuki and Yamazakura. Many more spores of *Gigaspora* sp. occurred in rhizosphere soil of Yamazakura and Susuki plant in inoculated plots than in non-inoculated plots (2.33 and 3.73 times, respectively).

Inoculation of AM fungi promoted the occurrence of spores and colonization to plant roots. The spores of *Gigaspora* sp. in rhizosphere soil as well as arbuscules and auxiliary cells in root formed much more frequently in inoculated plots than in non-inoculated plots at the Nukui Dam site. This indicates the possibility of survival and propagation of inoculated the AM fungi *G. margarita*.

Clearly, AM inoculum improved the symbiosis between AM associations and plant communities and seems to be a key factor for revegetation success.

CRediT author statement

Minh Thi Nguyen: Analysis, Data curation, Writing-original draft manuscript, Sampling; Kazuhira Yokoyama: Medothology, Software; Takuya Marumoto: Conceptualization, Sampling, Reviewing.

ACKNOWLEDGEMENTS

The authors would like to express their deep gratitude to the Nukui Dam Office supported this work for sampling assistance.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] N.C. Johnson, J. Jansa (2017), "Mycorrhizas: At the interface of biological, soil, and earth sciences", *Mycorrhizal Mediation of Soil Fertility, Structure, and Carbon Storage*, chapter 1, Elsevier, DOI: 10.1016/C2015-0-01928-1.

[2] K.M. Rodrigues, B.F. Rodrigues (2014), "Arbuscular mycorrhizal (am) fungi and plant health", *Fungi in Biotechnology*, SIES College, 16pp.

[3] A. Bahadur, et al. (2019), "Arbuscular mycorrhizal fungi alter plant interspecific interaction under nitrogen fertilization", *European Journal of Soil Biology*, **93**, DOI: 10.1016/j.ejsobi.2019.103094.

[4] D.P. Janos (1980), "Mycorrhizae influence tropical succession", *Bio-Tropica*, **12(2)**, pp.56-64.

[5] R. Singh, N. Sharma (2019), "Application of arbuscular mycorrhizae in soil management", *Microbial Interventions in Agriculture and Environment*, Springer, DOI: 10.1007/978-981-32-9084-6_4.

[6] D.A. Jasper (1994), "Management of mycorrhizas in revegetation", *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*, Kluwer Academic Publishers, Dordrecht, Netherlands, 9pp.

[7] K. Marumoto, et al. (1996), "Application of symbiotic microorganisms to soil conservation and reforestation", *BioJapan '96 Symposium Proceedings*, pp.242-250.

[8] D.D. Douds, W.R. Chaney (1982), "Correlation of fungal morphology and development to host growth in a green ashmycorrhiza", *New Phytol.*, **92(4)**, pp.519-526.

[9] J.N. Klironomos, et al. (1993), "A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils", *Can. J. Bot.*, **71**, pp.1472-1480.

[10] J.W. Gerdeman, T.H. Nicolson (1963), "Spores of mycorrhizal Endogone species extracted from soil by wet-sieving and decanting", *Trans. Br. Mycol. Soc.*, **46(2)**, pp.235-244.

[11] P.P. Kormanik, A.C. McGraw (1982), "Quantification of vesicular arbuscular mycorrhizae in plant roots", *Method and Principles of Mycorrhizal Research*, American Phytopathological Society, pp.37-45.

[12] T.P. McGonigle, et al. (1990), "A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi", *New Phytol.*, **115(3)**, pp.495-501.

[13] R. Toth, D. Toth (1982), "Quantifying vesicular-arbuscular mycorrhizae using a morphometric technique", *Mycologia*, **74**, pp.182-187.

[14] https://www.waterboards.ca.gov/waterrights/water_issues/programs/bay_delta/california_waterfix/exhibits/docs/Islands/II_41.pdf

[15] J.H. Zar (1984), *Biostatistical Analysis*, 2nd Edition, Prentice-Hall, Inc., Englewood Cliffs, 718pp.

[16] F.B. Reeves, E.F. Redente (1991), "Importance of mutualism in succession", *Semiarid Lands and Deserts: Soil Resource and Reclamation*, Marcel Dekker, New York, pp.193-217.

[17] A. Varma (1979), "Vesicular-arbuscular mycorrhiza and nodulation in soybean", *Folia Microbiol.*, **24**, pp.501-503.

[18] K. Singh, et al. (1988a), "Mycorrhizal fungi stimulate legume growth and root nodulation in dry arid soils. I. Effect of dual infection of Rhizobium and VAM endomycorrhizal spores on tropical legume Bengal gram (*Cicer arietinum*)", *Proc. Mycorrhizal Worksh.*, Quebec, Canada, pp.356-371.

[19] K. Singh, et al. (1988b), "Mycorrhizal fungi stimulate legume growth and root nodulation in dry arid soils. II. Effect of dual infection of Rhizobium and VAM endomycorrhizal spores on soybean (*Glycine Max Merril*)", *Proc. Mycorrhizal Worksh.*, Quebec, Canada, pp.372-392.

[20] M.J. Daft, T.H. Nicolson (1969), "Effect of *Endogone* mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize", *New Phytol.*, **68(4)**, pp.575-578.

[21] R. Francis, D.J. Read (1994), "The contributions of mycorrhizal fungi to the determination of plant community structure", *Management of Mycorrhizas in Agriculture, Horticulture, and Forestry*, Kluwer Academic Publishers, Netherlands, 14pp.