Original Research Paper

Excess soil water impact on colonization and extraradical mycelium biomass production of arbuscular mycorrhizal fungi in soybean field

**Rintaro Hattori,
Atsushi Matsumura*,
Kenji Yamawaki, Arata
Tarui, and Hiroyuki
Daimon**

Graduate School of Life and
Environmental Sciences, Osaka
Prefecture University, Naka, Sakai,
Osaka 599-5831, Japan.

*Corresponding Author
E-mail: matsu@plant.osakafu-u.ac.jp
Tel/Fax: +81-72-254-9521

In Japan, soybean plants at early growth stage, mainly cultivated in upland fields converted from paddy, are often stressed by excess soil water. Although, symbiotic relationships between soybean and arbuscular mycorrhizal (AM) fungi play a crucial role in increasing nutrient uptake, few studies have considered the occurrence and roles of AM fungi in soybean under these conditions. In the present study, we established different soil water plots (control plot and wet plot), and assessed the effect of excess soil water during early growth stage of soybean on AM colonization, AM extraradical mycelium (ERM) biomass production and soybean growth in an upland field converted from paddy through two years field experiment. Our results showed that the excess water treatment negatively affected soybean growth ($P < 0.05$), and reduced AM colonization ($P < 0.05$). The ERM biomass at vegetative stage of soybean in wet plot was much lower ($P < 0.05$) compared with that in control plots in both years. However, the ERM biomass in wet plot recovered similar to that in control plots at the reproductive stage of soybean. These results indicate that the production and spread of ERM are severely suppressed by excess water conditions. It may be pointed out that one of the reasons for the growth inhibition of soybean cultivated in upland fields converted from paddy is the negative effects on the AM symbiotic relationship by excess soil water.

Key words: fatty acid, *Glycine max* (L.) Merrill., root length density, soil water content

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi can increase host plant fitness under stressful environments, by improving nutrient uptake, mitigating drought stress, providing disease resistance, and improving the soil structure (Smith and Read, 2008). Many studies have shown that symbiotic relationships with AM fungi play an important role in increasing the uptake of nutrients particularly P, Zn, and N in many crops (Li et al., 1991a, b; Smith and Read, 2008). In soybean and AM fungi association, AM fungi generally have a stimulating effect on soybean growth by increasing shoot, root and seed weights as well as pod and seed numbers (Carling and Brown, 1980; Porcel and Ruiz-Lozano, 2004).

In Japan, soybean plants are mainly cultivated in upland fields converted from paddy. In such fields, poor drainage conditions frequently result in excess soil water accumulation during the rainy season, usually from mid June to late July. Such excess soil water can lead to a marked reduction in soybean yield (Matsuzaki et al., 2006). For example, the yield of soybean subjected to wet conditions at the vegetative growth stage may be reduced by 17–43% (Oosterhuis et al., 1990; Scott et al., 1990). Yield losses arising because of excess water accumulation are thought to be derived from reduced root growth, decreased nodulation, impaired N fixation, reduced photosynthesis, inhibition of seed germination and plant death through disease (Schmitthenner, 1985; Sallam and Scott, 1987; Oosterhuis et al., 1990; Scott el al., 1990). In addition to these causes, inhibition of AM fungal symbiosis may involve

in reduction of soybean growth under excess water conditions. AM fungi require oxygen to thrive (Cooke and Lefor, 1998), and therefore anaerobic environments caused by excess soil water accumulation may be detrimental for their survival and infectivity. Solaiman and Hirata (1995) reported that AM colonization and sporulation were significantly lower in rice grown under flooded conditions, in pot and field experiments. Thus, one reason given for growth reduction of soybean by excess water may be the inhibition of AM fungal development.

Most previous investigations of the relationship between soybean and AM fungi have focused on evaluating AM colonization in roots. There is a lack of data regarding AM fungal biomass, including extraradical mycelium (ERM). The ERM of AM fungi consists mainly of fungal hyphae and fungal spores. The ERM proliferates in the soil and provides the surface area for uptake of soil nutrients (Li et al., 1991a, b; Chen et al., 2003; Jansa et al., 2003), which are then transferred to the host plant via the fungal mycelium. Recent studies indicate that extension of the AM fungal mycelium within the soil plays an important role in determining plant performance under drought stress (Augé et al., 2003, 2007). Nevertheless, studies on the ERM, especially in agricultural fields, are limited. Fatty acid methyl ester ($C16:1\omega5$) occurs widely in AM species (Olsson et al., 2005; Trépanier et al., 2005; Huang et al., 2009), and is therefore used as a biomarker for AM fungi in root systems (Graham et al., 1995; Olsson and Johansen, 2000) and soils (Olsson et al., 1999). We hypothesized that excess soil water negatively affects not only AM colonization but also the ERM biomass production. In the present study, we assessed the effect of excess soil water treatment during the early growth stage of soybean on AM fungal colonization and ERM biomass production in upland field converted from paddy.

MATERIALS AND METHODS

Experimental site

We conducted our field study during 2011–2012 in an upland field converted from paddy, located at the Experimental Farm of Osaka Prefecture University, Sakai, Osaka, Japan (latitude 34.5°N, longitude 135.5°E). The study site comprised gray lowland soil (pH 5.6: EC, 0.08 dS m^{-1}: available P (Truog-P), 425 mg kg^{-1} P). The cultivation history was rice (2008), green manure (sesbania)-wheat (2009), and soybean (2010). We used a two-factorial experimental design (control plot and wet plot). Each plot measured 8.8 m × 8.4 m and were amended with mineral fertilizer (N:P_2O_5:K_2O = 3:10:10 g m^{-2}). On June 15, 2011 and June 18, 2012, soybean seeds (*Glycine max* (L.) Merrill. cv. Fukuyutaka), obtained from Japan Agricultural Cooperatives, Sakai, Japan, were sown at a spacing of 70 cm × 20 cm. To establish the excess soil water plot (wet plot),

the open ditch along the edge was filled with water (furrow irrigation) after confirming the germination of all seedlings (9 days after sowing in 2011 and 2012). The excess water treatment in wet plot was continued for 28 days, then furrow irrigation was stopped and soybean plants in both plots were grown with conventional practices. Irrigation, tillage, and chemical insecticide treatment were conducted as needed.

Soybean growth, nutrient status and yield

On July 21, 2011 and July 25, 2012 (at the vegetative stage), we randomly selected eight plants from each plot, and measured the plant length, node number of the main stem, number of branches, leaf area index (LAI). For evaluation of the amount of chlorophyll present in leaves, SPAD value was measured. LAI and SPAD values were measured with a plant canopy analyzer (LAI-2000, LI-COR Inc., USA) and chlorophyll meter (SPAD-502, Minolta Co. Ltd., Japan), respectively. The dry weight of the shoots was measured after drying at 70°C for 48 h. The shoots were subsequently ground, and subsamples were used for N and P analysis. Shoot N was determined by using vario Max (Elementar, Germany). Shoot P was measured by ashing samples in a muffle furnace at 550°C and using the vanado molybdate colorimetric method. On September 6, 2011 and 2012 (at the reproductive stage), eight plants were sampled again and measured as mentioned above. On November 18, 2011 and November 16, 2012, we randomly selected eight plants from each plot for measurement of yield attributes such as number of pods, pods weight, number of seeds, and 100-seed weight.

Soil core sampling

To evaluate the soybean root length density (RLD), AM fungal colonization, and ERM biomass, soil samples were collected two times (vegetative stage and reproductive stage) per year during 2011 and 2012. In each plot, three soil samples adjacent to the plants were sampled at a depth of 0−20 cm by using a 5-cm diameter soil core (DIK-110, Daiki Rika Kogyo Co. Ltd.). The roots were removed from each soil sample, and the RLD and AM fungal colonization were evaluated. The remaining soil samples were stored at −80 °C prior to ERM biomass analysis.

Root length density (RLD) and arbuscular mycorrhizal (AM) colonization

The total root length in each soil core was measured by using an image analysis system (Win RHIZO, Regent Instruments Inc., Canada). After measurement of the total root length, more than 10 fresh root segments was randomly taken and cut into approximately 2 cm pieces to estimate the AM colonization ratio. AM fungal colonization was visualized by clarifying the roots with 10% (w/v) KOH and

staining with 0.05% trypan blue (Brundrett et al., 1984). The stained root segments were arranged lengthwise on a thin layer of lactoglycerol (14:1:1 = lactic acid, glycerol and water) mounted on a microscope slide. Total about 200 intersections were observed and AM fungal structures including intraradical mycelium (arbuscules, vesicles and hyphae) at each intersections were calculated by observation at 100x magnification (McGonigle et al., 1990).

Extraradical mycelium (ERM) biomass evaluation by fatty acid analysis

Phospholipid fatty acids (PLFAs) were extracted by using the following procedure. First, 1 g of each soil sample was recovered and saponified in 1.2 mL of NaOH (3.25 M) dissolved in methanol and water (1:1, v/v). An internal standard of 0.1 mg of C19:0 dissolved in 100 µL of methanol was used. The suspension was vortexed and heated in boiling water for 30 min, methylated by the addition of 1.8 mL of HCl (3.25 M) dissolved in methanol, vortexed, heated to 80 °C in boiling water for 10 min, and cooled under running tap water. Next, 3 mL of methyl *tert*-butyl ether and hexane were added, and the suspension was shaken on a reciprocal shaker for 10 min. The suspension was then centrifuged for 3 min at 3000 rpm. The organic phase was transferred to new vials, 0.6 mL of NaOH (0.3 M) was added, and the suspension was shaken on a reciprocal shaker for 10 min. The sample was removed and placed into vials, and the organic phase was dried in a vacuum. Finally, 500 µL of a mixture of methyl *tert*-butyl ether and hexane (1:1, v/v) was added.

Fatty acid methyl esters were identified by comparison of the chromatographic retention time with a calibration standard mixture, ranging from C9 to C20 (MIDI Inc., Delaware, USA). The analysis was performed by using an Agilent 6890A gas chromatograph with Sherlock system software (MIDI Inc., Delaware, USA), equipped with an HP-ULTRA 2 capillary column (length 25 m, diameter 0.2mm, film thickness 0.33 µm). The signature fatty acid C16:1ω5 was used as a biomarker for AM fungi.

Statistical analysis

Soybean growth data were analyzed by two-way analysis of variance (ANOVA). Simple comparison of the means and SE of RLD, AM fungal colonization, and ERM biomass data were performed using Student's *t*-test. The AM colonization data were arcsine-transformed prior to analysis. Statistical analysis was performed by using Excel Tokei 2008 software version 1.05 (SSRI Co. Ltd, Tokyo, Japan).

RESULTS

Soybean growth, nutrient uptake and yield

At the vegetative stage, such as length of main stem, node number of the main stem, LAI of soybean plants treated with excess water was significantly lower ($P < 0.01$) than those of the control plants (Table 1). Number of branches was also reduced by excess water treatment ($P < 0.05$) in both years. However, the degrees of these reductions were significantly greater in 2011 than in 2012. The excess water treatment (furrow irrigation) in early growth stage affected the soybean growth even at the reproductive stage, that is, the length of main stem, node number of the main stem and number of branches of plants treated with excess water were significantly lower ($P < 0.01$) than those were of the control plants in both years. Although there was no significant difference in SPAD value, soybean plants treated with excess water became etiolated more rapidly than control plants.

Shoot dry weight, shoot N and P contents of soybean plants treated with excess water were significantly decreased ($P < 0.01$ in shoot dry weight and shoot P content, $P < 0.05$ in shoot N content) by excess water treatment both at vegetative stage and reproductive stage (Table 2). At vegetative stage, the degrees of these reductions were significantly greater ($P < 0.01$) in 2011 compared with 2012. Although there were no significant differences, all yield parameters (number of pods, pods weight, number of seeds and 100-seed weight) showed lower values in wet plot except for number of pods in 2011 (Table 3). In 2011, the typhoon at the beginning of September caused severe lodging of the soybean plants.

Root length density (RLD)

In 2011, the root length density (RLD) was influenced by excess water treatment (Figure 1A), that is, the RLD values of soybean plants treated with excess water were lower than those of the control plants both at vegetative and reproductive stages. By contrast, in 2012, the RLD values were not influenced by excess water treatment, at either the vegetative or reproductive stages. Although there was no significance, the RLD values of plants treated with excess water showed higher than those of control plants (+11% at the vegetative stage and +3% at the reproductive stage, respectively) in 2012.

Arbuscular mycorrhizal (AM) colonization and extraradical mycelium (ERM) biomass production

AM colonization was reduced by furrow irrigation in both years (Figure 1B). AM colonization increased at the reproductive stage compared with that at the vegetative stage. Overall, AM colonization of control plot was higher in 2012 than in 2011. In 2011, although AM colonization was lower in the wet plot compared to the control, there were no significant differences between treatments. By contrast, in 2012, AM colonization of plants in wet plot was significantly lower than those of control plants at the vegetative and reproductive stage.

Table 1. Influence of excess soil water during early growth stage of soybean on length of main stem, node number of main stem, number of branches, LAI and SPAD value

Sampling period	Year	Treatment	Length of main stem (cm)			Node number of main stem (g plant⁻¹)			Number of branches (plant⁻¹)			LAI			SPAD value		
Vegetative stage	2011	Control plot	35.5	±	1.2	7.4	±	0.2	2.8	±	0.4	1.5	±	0.1	34.6	±	0.8
		Wet plot	27.2	±	1.3	5.8	±	0.4	0.9	±	0.5	0.9	±	0.1	33.1	±	1.8
	2012	Control plot	27.2	±	0.6	9.1	±	0.3	3.1	±	0.5	2.1	±	0.2	37.0	±	1.2
		Wet plot	22.5	±	1.5	8.3	±	0.5	2.4	±	0.8	1.2	±	0.1	34.3	±	1.0
	ANOVA Plot (A)		**			**			*			**			ns		
	Year (B)		**			**			*			**			ns		
	A × B		ns			ns			ns			ns			ns		
Reproductive stage	2011	Control plot	71.4	±	2.7	18.7	±	0.4	14.1	±	0.4	6.6	±	0.6	46.9	±	3.8
		Wet plot	49.6	±	2.6	16.3	±	0.5	10.4	±	1.0	5.8	±	0.4	48.9	±	0.8
	2012	Control plot	70.2	±	1.5	18.6	±	0.3	7.3	±	0.7	6.1	±	0.4	45.5	±	0.5
		Wet plot	46.2	±	2.9	14.7	±	1.0	5.9	±	1.3	5.7	±	0.4	42.6	±	0.7
	ANOVA Plot (A)		**			**			*			ns			ns		
	Year (B)		ns			ns			**			ns			ns		
	A × B		ns			ns			ns			ns			ns		

Values are the means ± S.E. of 8 replications. *, ** = Significant at 5 and 1% probability levels respectively; ns = not significant. Vegetative stage: July 21 in 2011 and July 25 in 2012, Reproductive stage: Sep 6 in 2011 and 2012. LAI: leaf area index, SPAD value: index of the amount of chlorophyll present in leaves

Table 2. Influence of excess soil water during early growth stage of soybean on shoot dry weight, and shoot N and P contents

Sampling period	Year	Treatment	Shoot dry weight (g plant⁻¹)			Shoot N content (g plant⁻¹)			Shoot P content (mg plant⁻¹)		
Vegetative stage	2011	Control plot	8.8	±	0.6	0.2	±	0.01	65.9	±	5.0
		Wet plot	3.9	±	0.7	0.1	±	0.02	25.4	±	4.7
	2012	Control plot	14.9	±	1.1	0.4	±	0.07	88.3	±	6.8
		Wet plot	7.9	±	1.4	0.2	±	0.05	56.9	±	9.0
	ANOVA Plot (A)		**			**			**		
	Year (B)		**			**			**		
	A × B		ns			*			ns		
Reproductive stage	2011	Control plot	104.9	±	7.1	2.3	±	0.25	622.6	±	63.0
		Wet plot	66.4	±	8.1	1.5	±	0.26	375.3	±	46.7
	2012	Control plot	101.9	±	2.9	1.7	±	0.23	511.8	±	119.1
		Wet plot	81.3	±	6.4	1.8	±	0.20	369.8	±	54.8
	ANOVA Plot (A)		**			*			**		
	Year (B)		ns			ns			ns		
	A × B		ns			*			ns		

Values are the means ± S.E. of 8 replications. *, ** = Significant at 5 and 1% probability levels respectively; ns = not significant. Vegetative stage: July 21 in 2011 and July 25 in 2012, Reproductive stage: Sep 6 in 2011 and 2012

Table 3. Influence of excess soil water during early growth stage of yield attributes in soybean

Year	Treatment	Number of pods (plant⁻¹)			Pods weight (g plant⁻¹)			Number of seeds (plant⁻¹)			100-seed weight (g)		
2011	Control plot	133.9	±	13.3	1.4	±	0.5	47.7	±	8.3	30.0	±	0.3
	Wet plot	151.9	±	6.7	1.0	±	0.2	43.3	±	7.2	29.2	±	0.5
2012	Control plot	159.0	±	17.4	1.3	±	0.2	57.7	±	9.2	30.2	±	0.3
	Wet plot	110.9	±	10.8	1.1	±	0.1	36.3	±	5.9	29.0	±	0.5
	ANOVA												
	Plot (A)	ns			ns			ns			ns		
	Year (B)	ns			ns			ns			ns		
	A × B	*			ns			ns			ns		

Values are the means ± S.E. of 8 replications. * = Significant at 5% probability level; ns = not significant
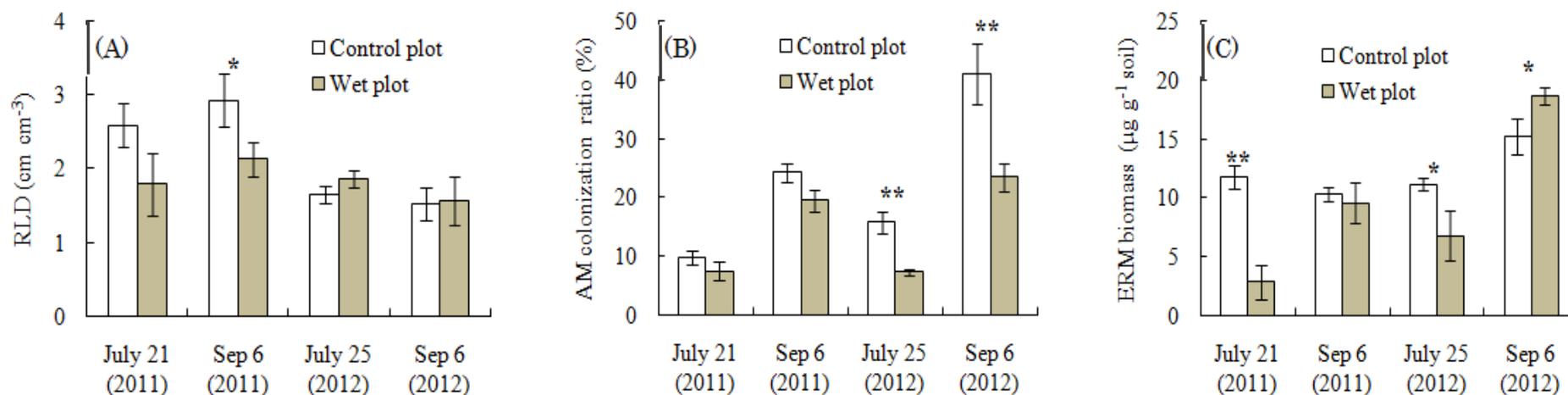

Figure 1. Influence of excess soil water during early growth stage of soybean on (A) RLD (root length density), (B) AM colonization ratio, and (C) ERM biomass. Values are means of three replicates and the vertical bars represent the standard errors. * and ** indicate significance at 5% and 1% level respectively (t-test). July 21 in 2011 and July 25 in 2012: vegetative stage, Sep 6 in 2011 and 2012: reproductive stage.

At the vegetative stage, the ERM biomass was markedly reduced by furrow irrigation in 2011 and 2012 (Figure 1C). In 2011, the AM fungal biomass of soil in the wet plot was 76% lower than that of soil in the control plot. On the other hand, the difference between treatments was reduced in 2012. The AM fungal biomass of soil in the wet plot was 39% lower than that of soil in the control plot. At the reproductive stage, the ERM of the wet plot was similar to that of the control plot (8.1 µg g⁻¹ and 10.5 µg g⁻¹ in 2011: 19.2 µg g⁻¹ and 16.1 µg g⁻¹ in 2012). With the exception of control plants at the vegetative

stage, the ERM biomass was higher in 2012 than in 2011.

DISCUSSION

Soybean damage under wet conditions has previously been attributed to a lack of oxygen to support root respiration. In Japan, soybean plants are usually sown during the rainy season from mid June to late July. Therefore, in converted paddy fields, early growth of soybean plants tends to be suppressed by excess soil water. In the present study, shoot growth of soybean plants treated with excess water was significantly lower than that of control plants.

In addition to soybean growth, AM fungal colonization and ERM biomass are also influenced by soil water status. The AM colonization of soybean plants treated with excess water was significantly lower than that of control plants. Moreover, excess soil water treatment led to a significant decrease in the ERM biomass. It is suggested that water saturation alone does not cause a reduction in the AM spore numbers, because AM fungi possess propagules that enable long-term survival in soils (Smith and Read, 2008). However, spore germination and hyphal elongation may be suppressed in the presence of low oxygen concentrations. Le Tacon et al. (1983) reported that anaerobic conditions inhibited germination of *Glomus mosseae* spores. Therefore, we propose that the reduction of AM fungal colonization and the ERM biomass production by excess water treatment may have been caused by the inhibition of AM fungal spore germination and hyphal growth. A decreased supply of photoassimilates from soybean to AM fungi might also reduce AM colonization and ERM biomass production because of growth inhibition by excess water. There is a possibility of the relationship between phytohormonal change of soybean roots by soil water conditins and AM colonization. Benschop et al. (2005) have reported that a decrease in abscisic acid (ABA) biosynthesis in the response to flooding in *Rumex* species. Since ABA is necessary in order to complete the arbuscle formation process and its functionality (Herrera-Mediba et al., 2007), ABA deficiency in soybean roots under excess soil water conditions might induce the reduction of AM colonization and ERM biomass production.

In comparison with control plants, plants treated with excess water showed significantly lower shoot biomass values but similar RLD values, especially in 2012. This may indicate an adaptive response of soybean roots to excess soil water (Walk et al., 2006). Wet conditions markedly increase the development of adventitious roots, which may absorb sufficient soil nutrients to sustain plant growth. Koide and Li (1991) reported that plant species with low RLD values were more dependent on AM colonization for resource acquisition than the species with high RLD values. Therefore, soybean plants cultivated in the wet plot may have been less dependent on AM fungi.

After excess water treatment (stop furrow irrigation), the AM fungal status (especially the ERM biomass) of plants in wet plot recovered to similar levels of plants in the control plot, that is, the ERM obtained from soil of the wet plot was approximately 50% lower than that of the control plot at vegetative stage, but was not affected at the reproductive stage. These results suggest that soil aeration is an important factor for ERM biomass production. Furthermore, the change of soil available P by wet to normal soil conditions through fixation by soil particles may have influenced AM fugal status of soybean. In Australia, crops such as maize, sorghum, and sunflower (sown in summer after the rice harvest) may suffer from P deficiency, because of P absorption capacity of soil when dried after flooding for rice (Muirhead and Humphreys, 1996). Therefore, the change of available P in wet plot soil by cessation of furrow irrigation might accelerate the development of ERM biomass production. But the relationship between AM colonization and ERM biomass remains to be elucidated.

The AM fungal status (as measured by AM colonization and ERM biomass) at the reproductive stage was higher in 2012 than in 2011. AM spores, infected root fragments, and ERM biomass are the main soil sources of inoculum that contribute to infectivity of plants. Carvalho et al. (2004) have reported that ERM biomass and root fragments are more important than spores during initiation of plant colonization. Soybean generally appears to have a high capability for proliferating AM fungi in soil, therefore the previous soybean planting (2011) may increase the quantity of AM fungal propagules in soil.

The results of our present study indicate that excess soil water may be responsible for the negative effect on AM fungal development (as measured by AM fungal colonization and the ERM biomass). But certain species of AM fungi may have been adapted to the fluctuation of soil water status in our experimental site. It has been suggested that some AM fungi are able to persist in flooded soils (Turner et al., 2000; Landwehr et al., 2002). For example, *Glomus geosporum* is usually dominant in European salt marshes (Landwehr et al., 2002; Carvalho et al., 2004). Recently, Isobe et al. (2011) identified AM fungi in soybean fields converted from paddy. These fungi represent promising AM fungal inoculum candidates for soybean fields converted from paddy. Since it is not possible to distinguish species of AM fungi by using PLFA methods, further detailed analysis is required to identify species of AM fungi suitable for the alleviation of flood damage of soybean. On the other hand, it is also important to focus on soybean cultivars. Some cultivars possess extensive aerenchymatous tissue (Shimamura et al., 2003; Thomas et al., 2005) and/or adventitious roots, thereby leading to a higher percentage of air within the roots. Soybean plants that develop highly secondary aerenchymatous tissue and adventitious roots may readily form symbiotic relationships with AM fungi, even under conditions of excess water. Further studies are required to clarify the

variation of AM status among soybean cultivars in fields converted from paddy.

REFERENCES

Augé RM, Moore JL, Stutz JC, Sylvia DM, Al-Agely A, Saxton AM (2003). Relating dehydration resistance of mycorrhizal *Phaseolus vulgaris* to soil and root colonization by hyphae. J. Plant Physiol., 160(10):1147-1156.

Augé RM, Toler HD, Moore JL, Cho K, Saxton AM (2007). Comparing contributions of soil versus root colonization to variations in stomatal behaviour and soil drying in mycorrhizal *Sorghum bicolor* and *Cucurbita pepo*. J. Plant Physiol., 164(10):1289-1299.

Benschop JJ, Jackson MB, Gühl K., Vreeburg RA, Croker SJ, Peeters AJ, Voesenek LA (2005). Contrasting interactions between ethylene and abscisic acid in *Rumex* species differing in submergence tolerance. Plant J., 44(5):756-768.

Brundrett MC, Pich Y, Peterson RL (1984). A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot., 62(10):2128-2134.

Busse MD, Ellis JR (1985). Vesicular arbuscular mycorrhizal *(Glomus fasciculatum)* influence on soybean drought tolerance in high phosphorus soil. Can. J. Bot., 63(12):2290-2294.

Carling DE, Brown MF (1980). Relative effect of vesicular-arbuscular mycorrhizal fungi on growth and yield of soybeans. Soil Sci. Soc. Ame. J., 44(3):528-532.

Carvalho LM, Correia PM, Martins-Loucao MA (2004). Arbuscular mycorrhizal fungal propagules in a salt marsh. Mycorrhiza, 14(3):165-170.

Chen BD, Li X-L, Tao HQ, Christie P, Wong MH (2003). The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere, 50(6):839-846.

Cooke JC, Lefor MW (1990). Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a costal salt marsh in Clinton, Connecticut, USA. J. Environ. Manage., 14(1): 131-137.

Graham JH, Hodge NC, Morton JB (1995). Fatty acid methyl ester profiles for characterisation of Glomalean fungi and their endomycorrhizae*. Appl. Environ. Microbiol., 61(1): 58-64.

Herrera-Medina MJ, Steinkellner S, Vierheilig HO, Bote JA, García Garrido JM (2007). Abscisic acid determines arbuscule development and functionality in the tomato arbuscular mycorrhiza. New Phytol., 175(3):554-564.

Huang H, Zhang S, Wu N, Luo L, Christie P (2009). Influence of *Glomus etunicatum / Zea mays* mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure. Soil Biol. Biochem., 41(4):726–734 .

Isobe K, Maruyama K, Nagai S, Higo M, Maekawa T, Mizonobe G, Drijber RA, Ishii R (2011). Arbuscular mycorrhizal fungal community structure in soybean Roots: Comparison between Kanagawa and Hokkaido, Japan. Adv. Microbiol., 1(1):13-22.

Jansa J, Mozafar A, Frossard E (2003). Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. Agronomie, 23(5-6): 481-488.

Koide RT, Li M (1991). Mycorrhizal fungi and the nutrient ecology of three old field annual plant species. Oecologia, 85: 403-412.

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Toth T, Biro B, Bothe H (2002). The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza, 12(4):199-211.

Le Tacon F, Skinner FA, Mosse B (1983). Spore germination and hyphal growth of a vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Gerdemann and Trappe) under decreased oxygen and increased carbon dioxide concentrations. Can. J. Microbiol., 29: 1280-1285.

Li X-L, George E, Marschner H (1991a). Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant Soil, 136: 41-48.

Li X-L, Marschner H, George E (1991b). Acquisition of phosphorus and copper by VA mycorrhizal hyphae and root-to-shoot transport in white clover. Plant Soil, 136: 49-57.

Matsuzaki M, Takahashi T, Hosokawa H (2006). Effects of wet condition and application of coated urea on yield and nitrogen accumulation in soybean growth in the field converted from heavy clay paddy in Hokuriku area. Natl. Agr. Res. Jpn. J. Crop. Sci., 75: 13-22.

McGonigle TP, Miller MH, Evan DG, Fairchild GL, Swan JA (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol., 115(3):495–501.

Muirhead WA, Humphreys E (1996). Rice-based cropping systems in Australia: Constraints to non-rice crops. In Kirchhof G, So HB (Eds.). Management of Clay Soils for Rainfed Lowland Rice-based Cropping Systems, Canberra: ACIAR. (pp. 181-185).

Olsson PA, Johansen A (2000). Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol. Res., 104(4):429-434.

Olsson PA, Thingstrup I, Jakobsen I, Bååth E (1999).

Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biol. Biochem., 31(13):1879–1887.

Olsson PA, van Aarle IM, Gavito ME, Bengtson P, Bengtsson G (2005). ^{13}C incorporation into signature fatty acids as an assay for carbon allocation in arbuscular mycorrhiza. Appl. Environ. Microbiol., 71(5): 2592–2599.

Oosterhuis DM, Scott HD, Hampton RE, Wullschleger SD (1990). Physiological response of two soybean (*Glycine max* L. Merr.) cultivars to short-term flooding. Environ. Exp. Bot., 30:(1)85–92.

Porcel R, Ruiz-Lozano JM (2004). Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J. Exp. Bot., 55(403):1743-1750.

Ross JP (1971). Effect of phosphate fertilization on yield of mycorrhizal and nonmycorrhizal soybeans. Phytopathology, 61: 1400-1403.

Sallam A, Scott HD (1987). Effects of prolonged flooding on soybeans during early vegetative growth. Soil Sci., 144: 61-66.

Schmitthenner AF (1985). Problems and progress in control of *Phytophthora* root rot of soybean. Plant Dis., 69(4):362-368.

Scott HD, DeAngulo J, Wood LS, Pitts DJ (1990). Influence of temporary flooding at three growth stages on soybean growth on a clayey soil. J. Plant Nutr., 13(8): 1045-1071.

Shimamura S, Mochizuki T, Nada Y, Fukuyama M (2003). Formation and function of secondary aerenchyma in hypocotyl, roots, and nodules of soybean (*Glycine max*) under flooded conditions. Plant Soil, 251: 351-359.

Smith SE, Read DJ (2008). Mycorrhizal Symbiosis. (3rd ed.). New York: Academic Press.

Solaiman MZ, Hirata H (1995). Effects of indigenous arbuscular mycorrhizal fungi in paddy fields on rice growth and N, P, K nutrition under different water regimes. Soil Sci. Plant Nutr., 41: 505-514.

Thomas AL, Guerreiro SMC, Sodek L (2005). Aerenchyma formation and recovery from hypoxia of the flooded root system of nodulated soybean. Ann. Bot., 96(7):1191-1198.

Trépanier M, Bécard G, Moutoglis P, Willemot C, Gagné S, Avis TJ, Rioux JA (2005). Dependence of arbuscular-mycorrhizal fungi on their plant host for palmitic acid synthesis. Appl. Environ. Microbiol., 71(9):5341-5347.

Turner ST, Amon JP, Schneble RM, Friese CF (2000). Mycorrhizal fungi associated with plants in ground-water fed wetlands. Wetlands, 20(1): 200-204.

Walk TC, Jaramillo R, Lynch JP (2006). Architectural tradeoffs between adventitious and basal roots for phosphorus acquisition. Plant Soil, 279(1-2):347-366