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Effect of tillage and crop sequences on arbuscular mycorrhizal symbiosis and soil enzyme activities in soybean (*Glycine max*) rhizosphere

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ABSTRACT

The aim of present study was to evaluate the impact of tillage practices and crop sequences on AM fungal propagules, infectivity potential and soil enzyme activities in the soybean rhizosphere of a long-term field trial maintained since 2001. Rhizosphere soil and root samples of soybean were drawn in *kharif* 2008 from three tillage systems (conventional-conventional (C-C), conventional-reduced (C-R) and reduced-reduced (R-R) and four soybean-based crop rotations (soybean–wheat (S–W), soybean–wheat–maize–wheat (S–W–M–W), soybean–wheat–soybean–wheat–maize–wheat (S–W–S–W–M–W) and soybean+ maize-wheat (S+M–W) which are being maintained in split plot design for the past seven years. On completion of six cropping seasons, significantly higher mycorrhizal spore count (17.0/g soil) and infectivity potential (IP) (4.58 IP/g soil) were observed in soybean grown under S–W–M–W rotation under C-R tillage system. However, the per cent root length colonized by AMF was found highest (12.66%) in S+M–W rotation under C-R tillage system. In general, the S+M–W or S–W–M–W rotations under R-R tillage system showed higher soil dehydrogenase activity (3.96 pKat/g soil) and fluorescein diacetate hydrolytic activity (110.76 pKat/g soil) when compared to other combinations. The inclusion of maize in the rotation irrespective of tillage systems showed comparatively higher phosphatase activities. Higher soybean grain yield (3 008 kg/ha) although not significantly higher was recorded in S+M–W rotation under C-C tillage, followed by same rotation (2 814 kg/ha) under C-R tillage system when compared to all other combinations. Moreover, IP of resident AM fungi in soybean rotation involving maize in conservation tillage was found to be highly correlated (r=0.96 to 0.99) with grain yield of soybean and maintaining higher organic carbon which indicates the functioning of resident AM fungi in enhancing the soybean yield.

Key words: *Glycine max,* Mycorrhizal symbiosis, Soil enzymes, Crop rotation, Tillage systems

Soybean [*Glycine max* (L.) Merrill], in terms of production has emerged as the most important oilseed crop of India. The production of soybean in 2010 was 10.47 million tonnes and from 9.3 million ha (AICRP-Soybean 2011). About 80% of national hectarage under soybean is in part of central India covering Madhya Pradesh, Bundelkhand region of Uttar Pradesh, southern parts of Rajasthan, Gujarat and northern and western parts of Maharashtra. By and large, soybean is mainly cultivated in rotations with crops like wheat, chickpea, mustard, potato, safflower, pigeonpea, sorghum, cotton, etc. Among all, soybean–wheat (S–W) rotation is the most predominant.

The arbuscular mycorrhizal symbiosis is the most commonly occurring underground symbiosis in plants. It can be found in a large majority of terrestrial plants and in almost a quarter of a million plant species (Gadkar *et al*. 2001). The symbiosis enhances plant growth through the ability of extrametrical fungal hyphae to take up water, supplying low-diffusing nutrients such as P from soil and improving N fixation by supplying ATP molecules required during *Rhizobium*-legume symbiosis (Ruiz-Lozano *et al.* 2001).

Soil enzyme activities are sensitive to the impact of agricultural management practices, any management practice, that influences microbial communities in soil may be expected to produce changes in soil enzymatic activities. Soil enzyme activities are important and should be measured because they are related to soil microbiological activities and thus, may be used as an index of soil functioning together with other mycorrhizal symbiosis as potential biochemical properties (Nannipieri *et al.* 2003). For example, hydrolytic enzyme seems to be involved in penetration and development of AM fungi in plant roots (Garcia-Garrido *et al.* 2000). Higher mycorrhizal population and colonization was observed in soybean–sweet potato sequence than fallow–mustard (Usuki and Yamamoto 2003). The mycorrhizal distribution

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and abundance, and mycorrhizal composition and community may change due to change in crop sequences (Mathimaran *et al.* 2007). Besides crop rotations, the other agricultural management practices (Jansa *et al*. 2003) also influence the mycorrhizal functioning which in turn hampers the plant growth in lack of beneficial AM symbiosis. The soil tillage affects the resident AM formation most significantly. AMF spore communities and diversity composition has been found to be affected due to tillage operations (Kabir 2005, Mathimaran *et al.* 2007). Recently, Curaqueo *et al.* (2011) suggested the active role of AM fungi and related soil quality parameters in long-term managed no-till soils provided with one moderate ploughing. Although, it is common to study the impact of single factors on mycorrhizae formation, interactions of agricultural management factors should also be examined since most of plant species harbours indigenous AM fungi hence managing these fungi appears to be a better option than inoculation due to their greater resilience to changing micro-climate and edapho-climatic conditions.

To sustain the soybean productivity and to draw maximum benefit from indigenous AM fungi, it is imperative to study the quantitative and qualitative changes being taken place in the rhizosphere and on the formation of indigenous mycorrhizal symbiosis attributed to change in crop sequences vis-à-vis soil tillage practices as a whole. In the present study, mycorrhizal symbiosis along with associated soil enzymatic activities were investigated in the rhizosphere of soybean cultivated under long-term field trial encompassing four soybean-based crop rotations and three tillage systems.

MATERIALS AND METHODS

Experimental site, treatment details and sampling

The long-term field trial was conducted in black soil (Vertisols) at Research Farm, Directorate of Soybean Research, Indore, since 2001. The field trial laid out in 3 × 4 split-plot randomized block design (3 blocks/replications) having 12 treatments comprising of three tillage systems (conventional-conventional (C-C), conventional-reduced (C-R) and reduced-reduced (R-R)) and four soybean based crop rotations (soybean-wheat (S–W, 1-year rotation), soybean–wheat–maize–wheat (S–W–M–W, 2-year rotation), soybean–wheat–soybean–wheat–maize–wheat (S–W–S–W–M–W, 3-year rotation) and soybean+ maize–wheat (S+M–W)). The conventional tillage [ploughing (1 time) + cultivator (two times) + planking (one time)] while reduced tillage [cultivators (two times) + planking (one time)] operations were performed before sowing. The initial soil properties in 2001 was soil *p*H 7.8; EC 0.2 dS/m; organic carbon (OC) 0.46%; available N 170 kg/ha; available P 8.2 kg/ha; exchangeable K 290 kg/ha. The soil and root samples of soybean rhizosphere were collected (0-20 cm depth) using 5 cm diameter core sampler at R3 reproductive stage (post flowering/pod initiation) and were used for various parameters ,viz, enumerating AM fungal spores, root colonization, inoculum potential, soil enzymes activities etc. in the present study.

Quantification of AMF propagules

AMF spores from soil were extracted by the method of wet sieving and decanting method and quantification was done by using the filter paper technique. Healthy and filled spores were counted from an aliquot poured over the filter paper and expressed as spore number/g soil. Mycorrhizal colonization in the roots was achieved by clearing and staining of roots, and the colonization percentage in roots by AM fungi was determined using the method of Biermann and Linderman (1981). The infectivity potential (of resident AM fungi) bioassay was done (Gaur *et al.* 1998) to determine the number of infective propagules present in soil samples drawn from soybean rhizosphere of different treatment.

Soil chemical analysis and soil enzyme assay

Olsen-P, organic carbon and available nitrogen in soil determined by standard methods (Olsen *et al.* 1954, Walkley 1935, Jackson 1973), The exchangeable potassium (K) was determined by the ammonium acetate method (Hanway and Heidel 1952). The soil dehydrogenases activity was determined by the method of Cassida (1977). The fluorescein diacetate (FDA) activity in soil was determined by the method of Aseri and Tarafdar (2006). Acid and alkaline phosphatases activity in soil was determined by the method of Tabatabai and Bremner 1969.

Grain yield and statistical analyses

The grain yield of soybean from each treatment plot was recorded at harvest after threshing. All the data were analyzed using the analysis of variance (SAS Institute Inc., 1991). The least significant difference (LSD) of Duncan's Multiple Range Test (DMRT) was used to separate the treatment means using Costat statistical software.

RESULTS AND DISCUSSION

Mycorrhizal symbiosis

It was recorded that the conservation tillage and crop rotation systems have a positive effect on AMF population when compared to conventional tillage (Jansa *et al.* 2003, Mäder *et al.* 2000) in a long-term field trial. After six cropping seasons, significantly higher AMF spore count (17.0 /g soil) was observed in the soybean grown under soybean–wheat–maize–wheat (S–W–M–W) rotation and conventional-reduced (C-R) tillage system (Table 1). The spore density under S–W rotation was at par in both C-R and C-C tillage systems but overall, it was significantly higher than R-R tillage system. The two way ANOVA analyses revealed that the interaction effect of crop rotation and tillage systems on spore density of soybean was found to be significant. Irrespective of tillage type higher spore count (12.7/g soil)

Table 1 Effect of tillage and crop rotation on the mycorrhizal spore density in rhizosphere soil and per cent mycorrhizal colonization in roots of soybean observed during grain-filling stage in long-term field trial (after undergoing six cropping seasons)

Crop rotation	Tillage systems							
	Conventional-conventional		Conventional-reduced		Reduced-reduced		Mean	
	Spore density (no./g soil)z	Per cent AM root colonization	Spore density (no./g soil)z	Per cent AM root colonization	Spore density (no./g soil)z	Per cent AM root colonization	Spore density (no./g soil)z	Per cent AM root colonization
S–W	13.0 bc	9.1bc	14.0ab	10.11abc	6.0g	7.11c	11.0ab	8.77b
S–W–M–W	10.33bcdef	9.66abc	17.0a	12.66ab	11.0bcde	10.66abc	12.7a	10.99a
S–W–S–W–M–W	7.33efg	8.33c	12.33bcd	9.66abc	6.33fg	10.22abc	8.66c	9.38ab
S+M–W	8.67defg	10.88abc	9.33cdefg	12.99a	9.33cdefg	9.44abc	9.1bc	11.10a
Mean	9.82b	9.48b	13.16a	11.3a	8.16b	9.35b		
One-way ANOVA LSD (P = 0.05)							3.77	3.28
Two-way ANOVA								
Interaction effect								
Tillage system							***	*
Crop rotation							**	*
CR × tillage							*	ns

[Z] data are average of three replicates; the treatment means followed by same letter did not differ significantly by DMRT test (ANOVA) (P=0.05); LSD, least significant difference; ns, non significant; +S–W, soybean–wheat; S–W–M–W, soybean–wheat–maize–wheat; S–W– S–W–M–W, soybean–wheat– soybean–wheat–maize–wheat; S+M–W, soybean+maize–wheat; ns, non significant; *,** significant at 5% level; *** significance at 1and 5% level

was observed in S–W–M–W rotation. When the effect of tillage system irrespective of crop rotation was observed, C-R system showed significantly higher spore density compared to R-R tillage system. Similar results were obtained by Curaqueo *et al.* (2011) where they suggested to maintain higher AM fungal colonization in no-tilled systems with one moderate ploughing (reduced tillage) soils reduced tillage and higher AMF spore densities were higher under maize than soybean (Troeh and Loynachan 2003). It is also expected that since more compactation in Vertisols might have not allowed much more growth and sporulation of AM fungi and therefore less sporulation takes place.

The per cent root length colonized by AMF observed during grain-filling stage in soybean followed the similar trend as did in spore count. Highest root length colonization (12.66%) was observed in S+M–W rotation under C-R tillage system. Whereas, AM colonization in roots of plants of continuous S-W rotation was less than 9%. The interaction effect due to crop rotation and tillage for AMF colonization in roots was found to be non-significant. Irrespective of crop rotations, significantly higher colonization in soybean was observed in C-R tillage when compared to other two tillage systems. In this study comparatively higher colonization in soybean was found in crop rotation plots where maize was included under C-R tillage system. Further reduction in tillage (R-R) did not enhance AM colonization. It is also expected that since more compaction in Vertisols might have not allowed much more growth and sporulation of AM fungi and therefore less sporulation takes place.

Crop rotation effects on mycorrhizal functioning have repeatedly been observed. It is known that mycorrhizal colonization of soybean increases if the preceding crop was maize. There is reduction in mycorrhizal colonization even after one year cropping with a non-mycorrhizal crop (Monreal *et al.* 2011). It is evident from this study that inclusion of maize in the soybean-based systems is favouring and enhancing the background level of native AMF and colonizing the successive crop. Mozafar *et al*. (2000) reported that maize roots were colonized to a greater extent by mycorrhizal fungi with NT than with CP or CT treatments in a long-term 3-year rotation trial of maize (*Zea mays* L.), winter wheat (*Triticum aestivum* L. emend. Fiori & Paol.), and canola (*Brassica napus* L.) under three tillage treatments (conventional, CT; chisel plow, CP; and no-tillage, NT).

The infectivity potential (IP) caused due to resident AM fungi (Fig1) in soybean rhizosphere soil revealed significantly higher IP (4.58/g soil) in the plots of S–W–M–W rotation under C-R tillage systems. Overall, higher IP was analyzed in the plots of rotation where maize was included. The interaction effect of crop rotation and tillage on IP production in soybean rhizosphere was found to be highly significant. Irrespective of tillage systems, higher IP value (4.11 IP/g soil) was found under S–W–M–W rotation. Similarly, irrespective of crop rotations, C-R tillage did produce significantly higher IP over rest of two tillage systems. The lower IP in C-C tillage could be attributed due to breakup of AMF networks during ploughing. Since, the infectivity potential is the expression of soil in terms of colonizing

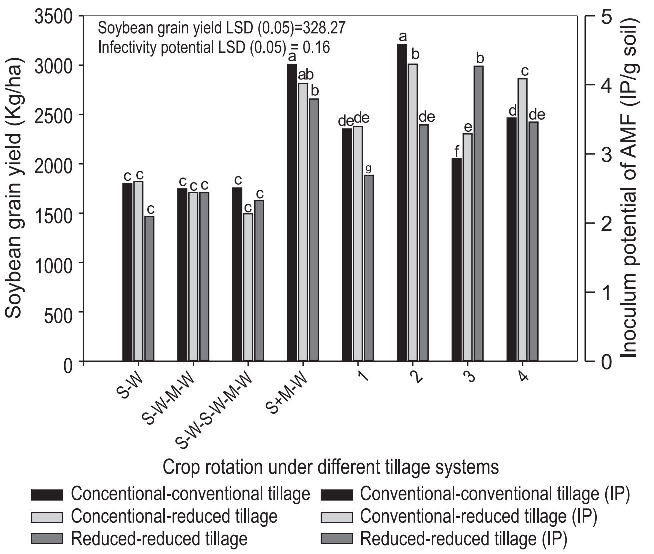

Fig. 1 Effect of tillage and crop rotation treatments on grain yield of soybean and infectivity potential of resident AM fungi in the soybean rhizosphere (observed during grain-filling stage) in long-term field trial (after undergoing six cropping seasons) [Z] data are average of three replicates; the treatment bars, followed by same letter did not differ significantly by DMRT (*P*=0.05); ns, non-significant
LSD, Least significant difference; [+]S–W, soybean-wheat (1); S–W–M–W, soybean–wheat–maize–wheat (2); S–W– S–W–M–W, soybean–wheat–soybean–wheat–maize–wheat (3); S+M–W, soybean+maize–wheat (4)

ability of native and background AMF population caused due to AMF networks like hyphae, spores and infected root bits while interacting with the soil environment.

Soil biochemical properties and grain yield

The analyses of results of soil dehydrogenases (DHA), fluorescein diacetate (FDA) activity, acid and alkaline phosphatases activity (ACP/ALP) in soybean rhizosphere are presented in Table 2. The plots cultivated with S+M–W or S–W–S–W–M–W under R-R tillage system showed significantly higher soil DHA (3.5 to 3.9 pKat/g soil) compared to other combinations. The least activity (1.52 pKat/g soil) was recorded on S–W–S–W–M–W and C-C tillage combinations. The two-way ANOVA results showed that comparatively higher DHA was observed in the plots where maize was included in the rotation. Fluorescein diacetate (FDA) activity in soybean was significantly higher 126.48 and 110.76 pKat/g soil in S–W and S–W–M–W rotation, respectively, under C-C tillage system. The interaction effect of crop rotation and tillage on FDA activity in soybean rhizosphere was found to be non-significant. Higher activity, irrespective of tillage systems, was recorded in S–W and S–W–S–M rotations. The acid and alkaline phosphatases (ACP/ALP) activity in soybean rhizosphere was influenced (although non-significantly) both by the crop rotation and tillage systems.

Table 2 Effect of tillage and crop rotation on the dehydrogenase, fluorescein diacetate analysis (FDA) and acid and alkaline phosphatase activity in the rhizosphere soils of soybean (observed during grain-filling stage) in a long-term field trial (after undergoing six cropping seasons)

Crop rotation	Tillage systems															
	Conventional-conventional				Conventional-reduced				Reduced-reduced				Mean			
	FDA	DHA	ALP	ACP	FDA	DHA	ALP	ACP	FDA	DHA	ALP	ACP	FDA	DHA	ALP	ACP
S–W	126.4a	2.9bc	56.25a	25.3ab	85.6bcd	2.9bc	39.2a	15.75b	82.4bcd	2.5bc	37.6a	32.08a	98.1a	2.78a	44.37a	24.36a
S–W–M–W	110.7ab	2.5bc	51.95a	25.0ab	79.8bcd	2.8bc	46.2a	23.72ab	66.2cd	2.7bc	40.1a	28.46a	85.5ab	2.68a	46.1a	25.72a
S–W–S–W; M–W	93.9bc	1.52d	56.2a	26.6ab	76.7cd	2.9bc	42.3a	25.45ab	60.7d	3.9a	49.0a	23.5ab	77.1b	2.79a	49.1a	25.16a
S+M–W	84.8bcd	2.6bc	40.75a	20.0ab	71.3cd	2.5c	47.5a	27.26ab	75.3cd	3.5ab	39.3a	23.2ab	77.15b	2.84a	42.5a	23.5a
Mean	104.0a	2.39b	51.3a	24.24a	78.3b	2.78ab	43.7ab	23.04a	71.1b	3.15a	41.5b	26.8a				
One-way ANOVA LSD (*P*=0.05)													27.70	0.85	17.36	10.61
Interaction effects																
Tillage system													***	**	ns	ns
Crop rotation													*	ns	ns	ns
CR× tillage													ns	**	ns	ns

[Z] data are average of three replicates; the treatment means followed by same letter did not differ significantly by DMRT (*P*=0.05); ns, non significant LSD, least significant difference; [+]S-W, soybean-wheat; S-W-M-W, soybean-wheat-maize-wheat; S-W- S-W-M-W,

Soybean-wheat- soybean-wheat-maize-wheat; S+M-W, soybean+maize-wheat; DHA, Soil dehydrogenases (pKat /g soil); FDA, Fluorescein diacetate hydrolytic activity (pKat/g soil); ALP, Alkaline phosphatases (nKat /100 g soil); ACP, Acid phosphatases (nKat /100 g soil); ns, non significant; *,** significant at 5% level; *** significance at 1and 5% level

The S–W–M–W rotation under C-R and R-R tillage systems showed comparatively higher activities. Balota *et al.* (2004) investigated enzymatic activities in a split-plot experiment where tillage (no till and conventional) was in the main plot and crop rotation (soybean/wheat, S/W; maize/wheat, M/W or cotton/wheat, C/W) was in the sub-plots. The 0-5 cm layer under NT system showed increases up to 68% for amylase, 90% for cellulase, 219% for arylsulfatase, 46% for acid phosphatase, and 61% for alkaline phosphatase. There were significant correlations of soil enzyme activities with total organic C, and C and N microbial biomass. They suggested that NT increased microbial activity and soil enzyme activity which is a sensitive indicator of alteration in soil quality of agricultural management.

There was no significant change in organic carbon (OC) and available P was observed due to tillage systems and crop rotations (Table 3). However, higher values of OC were found in the soil drawn from plots of S+M–W rotation under reduced tillage systems. This may be attributed to higher biomass of AMF such as glomalin secreted by these fungi (Schindler *et al*. 2007). In general, less removal (higher P status in soil, except in S–W) was evident in the plots managed with S–W+M rotation which indicates the role of native AM fungi in mobilizing P into available form to the plant.

The soybean yield was influenced both by tillage and crop rotation treatments (Fig 1). Higher soybean yield (3 008 kg/ha) was recorded in the plots of S+M–W rotation under C-C tillage, followed by same rotation (2814 and 2657 kg/ha) under both C-R and R-R tillage systems when compared to all other combinations of rotation and tillage systems. When data was subjected to two-way ANOVA analysis, both crop rotation and tillage system influenced the yield however the interaction of rotation and tillage did not influence the grain yield significantly. Irrespective of tillage systems, higher grain yield was obtained in S+M–W rotation over other rotations. Whereas, irrespective of crop rotations, yield of plots managed with C-R was higher but found at par with C-C plots. However, S+M–W rotation which produced higher grain yield did have comparatively higher values of organic carbon content particularly in C-R plots. It is generally viewed that switching from conventional ploughing to reduced tillage farming promotes restoration in soil organic carbon (Benhua *et al.* 2011), It is also suggested to understand the relationship between cropping systems and AM fungi to integrate native mycorrhizae into crop models without relying on predicted models rather long-term experimentations will ease us to predict the consequences of different combinations of cropping practices under varied agro-climatic conditions.

The current study indicates that the involving maize in the crop rotation under conservation tillage (C-R) played a greater role in enhancing the yield and maintaining higher AM inoculum load. This may be attributed to interactive effects of greater functioning of resident AM fungi, maintaining higher load of resident AM fungi and soil enzyme activities found in the plots of S+W–W or S–W–M–W in C-R tillage system.

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Table 3 Effect of tillage and crop rotation on the organic carbon and available phosphorus status in soils drawn after soybean harvest in a long-term field trial (after undergoing six cropping seasons)

Crop rotation	Tillage system							
	Conventional-conventional		Conventional-reduced		Reduced-reduced		Mean	
	Organic carbon (%)[z]	Available phosphorus (ppm)[z]	Organic carbon (%)[z]	Available phosphorus (ppm)[z]	Organic carbon (%)[z]	Available phosphorus (ppm)[z]	Organic carbon (%)[z]	Available phosphorus (ppm)[z]
S–W	0.48a	4.37a	0.54a	3.16abc	0.49a	2.28bc	0.48a	3.27a
S–W–M–W	0.40a	1.96abc	0.48a	3.47abc	0.45a	2.05bc	0.44a	2.49ab
S–W–S–W–M–W	0.35a	3.2abc	0.47a	2.0bc	0.54a	1.78c	0.45a	2.32b
S+M–W	0.41a	3.49ab	0.62a	1.82bc	0.60a	2.80abc	0.54a	2.70ab
Mean	0.41a	3.24a	0.51a	2.61ab	0.52a	2.23a		
One-way ANOVA LSD (*P*=0.05)							0.24	1.47
Two-way ANOVA Interaction effects								
Tillage system							ns	ns
Crop rotation							ns	*
CR × tillage							ns	*

[Z] Data are average of three replicates; the treatment means, followed by same letter did not differ significantly by DMRT Test (ANOVA) (*P*=0.05); LSD, least significant difference; ns, non significant; +S–W, soybean–wheat; S–W–M–W, soybean–wheat–maize–wheat; S–W– S–W–M–W, soybean–wheat– soybean–wheat–maize–wheat; S+M–W, soybean+maize–wheat; ns, non significant; *, significant at 5% level

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