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Diversity of arbuscular mycorrhizal fungi in *Populus deltoides* agroforestry systems at Kurukshetra in Northern India

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Abstract

This study was carried out in Populus deltoides agroforestry systems located at Gulabgarh, Kurukshetra (29°58'N, 76°57'E, 250 m above mean sea level), northern India. The aim was to analyze arbuscular mycorrhizal (AM) fungal diversity, the spatial distribution of AM fungal spore density in the soil and AM root colonization in sugarcane and wheat crops integrated with trees in Populus deltoides agroforestry systems. A total of 47 species of AM fungi belonging to five genera, i.e., Acaulospora, Entrophospora, Glomus, Gigaspora and Sclerocystis were isolated from the rhizosphere soils of wheat and sugarcane in the studied agro forestry systems. There was significant effect of soil depth and crop growth stage on the AM fungal spore density and AM fungal root colonization. The AM fungal spore density was greatest in the Populus + sugarcane system as compared to that of the Populus + wheat systems. The density of AM spores was found to attain its peak at crop maturity of the wheat crop; a significant decrease in AM spore density occurred with increase in soil depth. The colonization of sugarcane roots by AM fungi was greatest at 15 to 30 cm soil depth under 1 to 2 yr old Populus + sugarcane agro forestry systems. In the case of wheat roots (5-7 yr old Populus + wheat agroforestry systems), the AMF root colonization was greatest at 7.5 to 15 cm soil depth as wheat roots were concentrated up to 15 cm soil depth.

Keywords: Arbuscular mycorrhizal (AM) fungi, AM fungal diversity, spore density, AMF root colonization.

Introduction

Soil biodiversity refers to the variety of life existing in the soil and play an important role in various ecosystem functions such as decomposition of organic matter, nutrient cycling and formation and stabilization of soil structure. Arbuscular mycorrhizal (AM) fungi help in maintaining soil structure, soil quality and various ecological interactions in soil. There are two phases of AM fungi, i.e., arbuscules, hyphae, and vesicles occurring within the cortical cells of plant roots, and the extra-cellular mycelium along with hyphae, arbuscules and vesicles present in the soil matrix. The extra-cellular mycelium forms spores for exploring new area for colonization within the soil, and serves as a useful measure for assessing percent root colonization by AM fungi[1,2]. AM fungal symbiosis has been reported to play an important role in the functioning of various soil processes such as nutrient cycling, bioremediation, adapting to salt stress, and soil restoration[3,4]. The structure and diversity in the natural and agricultural ecosystems have been found to be highly influenced by the AM fungal diversity[5-7]. The AM fungi associated with plant species may enhance the growth of both trees and crops in agroforestry systems.

It is important to have a greater understanding of soil biodiversity, particularly AM fungi, for improving the efficiency of nutrient cycling in agroforestry systems. Some reports have indicated that agroforestry practices could play a key role in maintaining the AM fungal inoculum potential in soils[8,9]. In nutrient poor tropical soils, AM fungi enhance the uptake of relatively immobile nutrients, such as phosphorus and zinc. AM spore density and diversity were found to be significantly higher in organic farming as compared to conventional farming as affected by management practices[10]. In small holder agroforestry systems in Ethiopia, a significant decline in AM spore densities with soil depth has been reported[9]. Arbuscular mycorrhizal fungi help in nutrient uptake especially phosphorus, regulating water relations, maintaining plant productivity and serving as biocontrol agent[11-13]. In the soil-plant system, AM fungi have an important role in carbon cycling and soil carbon sequestration[14].

In agroforestry systems, AM fungi have been reported to increase crop productivity[15,16]. Maize yield were found to be increase after mycorrhizal inoculation[17]. Arbuscular mycorrhizal fungal association in agroforestry system has been found to assist in maintaining AM propagules in the soil, which facilitate root colonization in the emerging crop seedlings[4]. It has been found that maize crop grown in soil taken from close to the *Senna siamea* tree showed high AM fungal colonization as compared to that grown in the soil taken from 2-m away from the tree[18]. There was a positive effect of presence of tree species on AM fungal root colonization in crop plants as trees may act as stocks of mycorrhizal fungi, thereby having beneficial effect on crop growth[19]. In agroforestry systems on the low fertile soil, AM fungi have a great potential improve the plant growth due to improved nutrient retention, control soil borne diseases, water

balance, and reducing drought stress[20]. Soil disturbances could have a negative effect on the diversity and AM root colonization due to destruction of extra-radical hyphae in the soil[21].

The aim of this study was to analyze the effect of crop type on AM fungal diversity, and soil depth on AM fungal spore density and root colonization in sugarcane and wheat crops integrated with trees in *Populus deltoides* agroforestry systems at Kurukshetra in northern India.

Materials and methods

Study site: The study site is located at Gulabgarh (located at Kurukshetra 29°58'N, 76°57'E, 250 m above mean sea level). The study was carried out in *Populus deltoides* + sugarcane (1 to 2 year old systems) and 3 to 7-year old *Populus deltoides* + wheat agroforestry systems (Figure-1).

Figure-1: A general view of (A) *Populus deltoides* + sugarcane and (B) *Populus deltoides* + wheat agroforestry systems at Gulabgarh, Kurukshetra. Photo: Reena Saini and SR Gupta.

Climate: The climate of the study area is tropical monsoonal[22], the year shows three seasons, i.e. warm-wet rainy season (June to September), a cool dry winter season (October to February) and a hot dry summer (March to May), the average annual rainfall of the area is 800 mm[22]. During the study period from January 2004 to December 2006, the average annual rainfall was 286mm to 634mm. About 80% of the total annual rainfall was received during the rainy season. During the study period from January 2004 to December 2006 for the agroforestry systems at Gulabgarh (Kurukshetra), the mean maximum and minimum temperature varied from 12.43°C to 40.00°C and from 3.0° C to 28.23° C, respectively. The soil of the study area is old alluvium which is reported to be sandy loam in texture[23]. The water holding capacity of the soil varied from 45.92 to 48.50%. The soil pH showed slightly alkaline soil reaction (soil pH was 7.6 to 8.2 at 7.5cm soil depth and 7.42 to 8.10 at 7.5-15cm soil depth). In different agroforestry systems selected for the study, soil organic carbon varied from 0.40 to 0.81% and total nitrogen was 0.036 to 0.092%. Bulk density of the soil in the experimental plots varied from 1.21 to 1.40 g cm^{-3}. The organic carbon (0.61 to 0.81%) indicated a C:N ratio of 8.34 to 11.96.

Methodology: Analysis of mycorrhizal spore diversity: AMF spore extraction was done by the method given by Gerdemann and Nicolsan[24]. Spore density was calculated spatially across the different soil depth up to 30cm soil depth and seasonally during the wheat growing season in different agroforestry systems. The AM spores were isolated from the soil collected from the experimental plots, the spores were individually picked and mounted in Polyvinyl-lactoglycerol (PVLG) and covered under a glass cover slip for the identification of spores of each category. The spores were examined using a light microscope at 100x and 400x magnification and identified by taxonomic criteria as given by Morton and Benny[25], as well as using the taxonomic criteria described by the International Collection of Vesicular Arbuscular Mycorrhizal Fungi. The AM fungal spore density was represented in terms of spores per 10g soil.

Analysis of AM fungal root colonization: For studying AM fungal root colonization in wheat and sugarcane, root samples were collected from the experimental plots demarcated within *Populus deltoides* + sugarcane (1 to 2 year old system) and *Populus deltoides* + wheat agroforestry systems (3 to 7 year old systems). Soil cores of 12x12 cm at depth interval of 0-7.5 cm, 7.5-15 cm and 15-30 cm were excavated during wheat and sugarcane growing season in different agroforestry systems. The fine roots of wheat and sugarcane were collected during the respective growing seasons from the three depth segments of soil cores. The fine roots were washed on 250 µm sieve under a fine jet of de-ionised water and cut into pieces of 1 to 2 cm. The freshly collected root segments were cleared with 10% KOH for 12 to 24 hours under laboratory conditions and then washed with 1% hydrochloric acid. Then, the roots were stained in lactic acid glycerol Trypan blue (Phillips and Hayman)[26]. The stained root segments of wheat and sugarcane were carefully mounted on glass slides and were observed under the microscope. The percent root colonization was calculated by using the formula given by Gerdeman and Nicolson[24].

Results and discussion

Arbuscular mycorrizal fungal diversity: A total of 47 species of AM fungi belonging to five genera including *Acaulospora, Entrophospora, Glomus*, *Gigaspora* and *Sclerocystis* were isolated from the rhizosphere soils of wheat and sugarcane in different *Populus deltoides* agroforestry systems (Table-1).

In the *Populus* + sugarcane system (1 to 2 year old), the number of mycorrhizal fungi were greater comprised of 47 species belonging to five genera (Table-1). The diversity of AM fungi

in the *Populus deltoides* + wheat systems was comparatively lower and represented by 35 species belonging to *Acaulospora, Entrophospora, Glomus* and *Gigaspora* (Table-1). In the studied system agroforestry systems, twenty species of *Glomus,* twelve species of *Acaulospora*, three species of *Gigaspora*, two unidentified species of *Entrophospora* and one species of *Sclerocystis* were recorded. Three unidentified species of *Acaulospora*, four *Gigaspora spp* and two *Glomus spp* were also recorded in soils from different agroforestry systems.

Arbuscular mycorrizal spore density: It was found that soil depth had a significant effect on the AM fungal spore density in different agroforestry systems. The total number of AM fungal spore was found higher at 7.5 cm soil depth. With increase in soil depth, there was a significant decrease in the number of spores in the soil. The number of spores in the sugarcane system varied from 90.2 to 97.4 per 10g soil. The number of AM fungal spores ($10g^{-1}$ soil) across the soil depths was: 79.0 to 97.4 at 0 to7.5cm soil depth; 52.6 to 78.6 at 7.5 to15cm soil depth and 36.6 to 53.2 at 15 to30cm soil depth (Table-2). There were significant differences in the number of spores across the soil depth ($F=25.22$ to 44.02; $df=2, 12$; $P<0.01$).

The variations in the number of spores at different growth periods of wheat in 3 year and 5 year old agroforestry systems are given in Table-3. During wheat growing season, the spore density was found to be greatest at crop maturity during the month of April in 3 to 5 year old agroforestry system. In wheat growing season, during January 2005 to April 2005, the numbers of AM fungal spores were slightly higher in 5 year old agroforestry system as compared to 3 year old system. There was significant decrease in the number of mycorrhizal spore with soil depth in the different agroforestry system. The number of spores at crop maturity in 3 year old *Populus deltoides* + wheat system at different soil depths (spore density per 10g soil) was: 82.0 (0 to7.5cm); 59.0 (7.5 to 15cm); 17.2 (15 to 30cm). In the 5 year old agroforestry system, the number of spores was 88 to 19.0 spores per 10g soil at different soil depths (Table-3). There was significant effect of sampling dates on the number of spores during the course of wheat growing period ($F= 6.58$ to 14.50; $df=3, 16$; $P<0.01$) and soil depth ($F=55.02$ to 156.12; $df= 2, 12$; $P<0.01$) (Table-3).

The total number of spores was low during the month of January and varied from 55.6 to 56.4 per 10g soil at 7.5 cm soil depth in 3 to 5 year old system. The spore density decrease with soil depth and varied from 12.6 to 14.8 at 15-30cm soil depth (Table-3). In the 6 and 7 year old agroforestry system the same trend in spore density was observed, the numbers of spores were higher at the stage of crop maturity (Table-4). The maximum number of spores during January 2005 to April 2006 in 6 and 7 year agroforestry system at 0-7.5cm soil depth was (spore density per 10g soil) 94.8 (6 year) and 92.2 (7 year). The number of spores increased with the crop growth and varied from 72.2 to 94.8 (6 year) and from 75.2 to 92.2 (7 year) at 0-7.5cm soil depth (Table-4). From the results given in Table-4, it is clear that the effect of sampling dates was found significant for 7.5 to 15 cm soil depths in 6 year old system. ($F=7.72$; $df= 3, 16$; $P<0.05$). In 7 year old *Populus deltoides* + wheat system there was no significant effect of sampling dates on the spore density. For the 6 and 7year old agroforestry system, there was significant effect of soil depth on spore density across the sampling dates ($F=28.95$ to 80.75; $df =2, 12$; $P<0.01$) (Table-4).

AM fungal root colonization: The AM fungal root colonization of sugarcane roots, wheat roots and *Populus* roots have been studied in 1 to 7 year old *Populus deltoides* agroforestry system. The AM root colonization in sugarcane was studied during the initial 2 year of agroforestry system and it was observed that root colonization increased down the soil depth (Table-5). The AM root colonization in sugarcane showed maximum value of AM fungal root colonization at 15-30cm soil depth (91.0 to 96.2%) in 1 and 2 year *Populus deltoides* agroforestry system. Arbuscular mycorrhizal infection in sugarcane roots is shown in Figure-2 a-b, which shows formation of arbuscules with mycorrhizal hyphae in root cortical cells; formation of round and globose vesicles with attached hyphae. The AM fungal colonization in sugarcane roots varied from 72.0 to 96.2% during the initial 2 year. Soil depth have been found to have significant effect on the AM fungal root colonization ($P<0.01$). In the 3 to 6 year old agroforestry system, the maximum AMF root colonization in wheat were observed at 7.5 to15cm soil depth as compared to that of 15 to 30cm soil depth. The maximum percentage of root colonization were observed in 6 year old system (32.4 to 92.2%) than in 5 year (24.6 to 78.6%) and 3 year old agroforestry system (20.6 to 77.8%) upto 30cm soil depth (Table-6).

The maximum colonization of wheat root was observed at 7.5-15cm soil depth, this is explained by the fact that in wheat maximum root were concentrated at 7.5-15cm soil depth. Arbuscular mycorrhizal infection in wheat roots is shown in Figure-3 (a-b). The arbuscular mycorrhizal fungal root colonization of wheat and *Populus deltoides* was studied during November 2004 to April 2006 (Table-7). During the initial stages of wheat crop growth (November), the AMF root colonization in wheat was found to be less (36 to 37.6%). In the rice-wheat cropping systems, maximum root colonization by AM fungi in wheat has been found corresponding to flowering period and crop maturity[27].

The AMF root colonization increased from 80.0 to 97.2% during February to April in 6 and 7 year old agroforestry system. It is also observed that in *Populus deltoides* the AM fungal root colonization increased with the crop growth and increased from 68.6 to 83.6% (Table-7). In the studied root samples of wheat and sugacane, it is evident that H and Y types fungal mycelium were present in the cortical cells, arbuscules are very conspicuous in both wheat and sugarcane roots; the vesicles were elliptical, globose and round types (Figure-2&3).

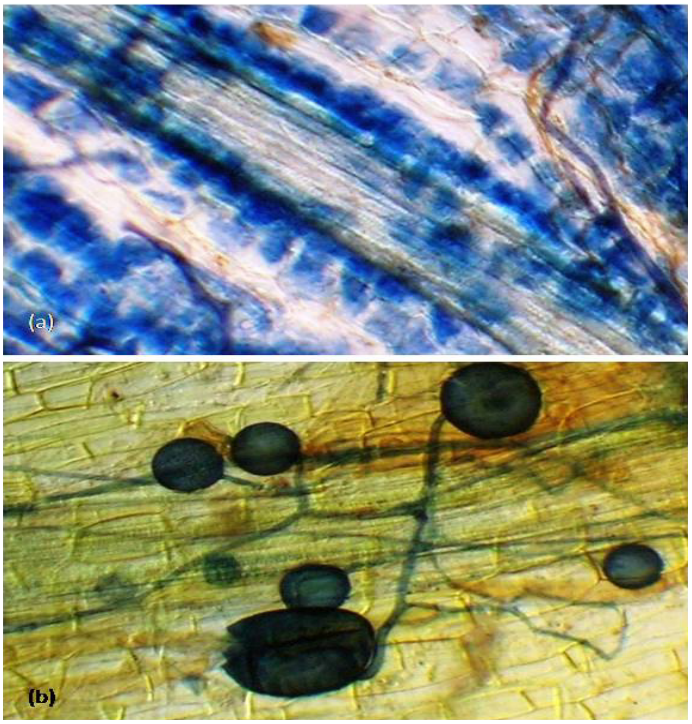

Figure-2: Arbuscular mycorrhizal infection in sugarcane roots. Showing (a) formation of arbuscules with mycorrhizal hyphae in root cortical cells; (b) formation of round and globose vesicles with attached hyphae (100 x).

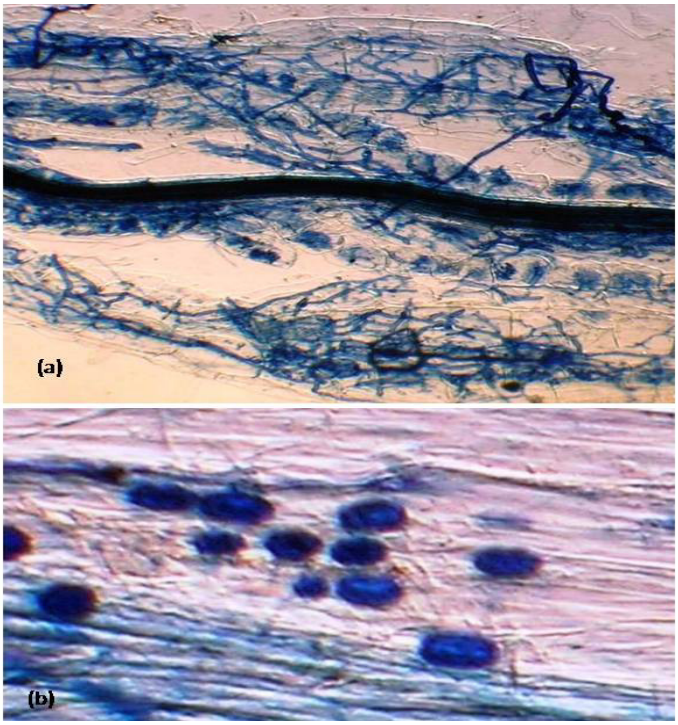

Figure-3: Arbuscular mycorrhizal infection in wheat roots under agroforestry system. Showing (a) formation of arbuscules with mycorrhizal hyphae in root cortical cells; (b) formation of globose vesicles with attached hyphae (100 x).

Table-1: The diversity of AM fungal species in *Populus deltoides* agroforestry systems at Kurukshetra.

AM fungal species	AM fungal species under sugarcane	AM fungal species under wheat
Acaulospora bireticulata	+	+
Acaulospora elegans	+	+
Acaulospora gerdemannii	+	+
Acaulospora lacunosa	+	+
Acaulospora laevis	+	+
Acaulospora mellea	+	+
Acaulospora nocolsonii	+	+
Acaulospora dendiculata	+	-
Acaulospora rehmii	+	+
Acaulospora scrobiculata	+	+
Acaulospora sp.I	+	+
Acaulospora sp.II	+	-
Acaulospora spinosa	+	+
Acaulospora trappei	+	+
Entrophospora sp.I	+	+
Entrophospora sp.II	+	+
Gigaspora albida	+	+
Gigaspora calospora	+	+
Gigaspora gigantea	+	+
Gigaspora sp.I	+	+
Gigaspora sp.II	+	+
Gigaspora sp.III	+	-
Glomus aggregatum	+	+
Glomus arborense	+	-
Glomus constrictum	+	+
Glomus deserticola	+	-
Glomus diaphanum	+	+
Glomus etunicatus	+	-
Glomus fasciculatum	+	+
Glomus fuegianum	+	-
Glomus fulvum	+	+
Glomus geosporum	+	+
Glomus gerdemanii	+	-
Glomus indica	+	+
Glomus macroaggregatum	+	-
Glomus macrocarpum	+	+
Glomus monosporum	+	+
Glomus mosseae	+	+
Glomus multicaulis	+	+
Glomus pulvinatum	+	+
Glomus reticulatam	+	-
Glomus sacrobiculata	+	-
Glomus sp.I	+	+
Glomus velum	+	-
Sclerocystis sp.I	+	+
Sclerocystis sp.II	+	+
Scutellospora sp. I	+	+

Table-2: AM fungal spore density ($10g^{-1}$ soil) in agroforestry systems.

	Spore density ($10g^{-1}$ soil)		
	0-7.5 cm	7.5-15 cm	15-30 cm
P.deltoides + sugarcane crop			
1 yr	90.2±4.61	72.2±2.69	45.4±2.48
2 yr	97.4±3.69	76.0±5.02	48.8±2.74
P.deltoides + wheat crop			
3 yr	79.0±4.8	52.6±2.78	36.6±3.37
5 yr	83.2±4.40	60.8±2.67	46.2±3.85
6 yr	91.2±3.92	78.6±3.84	53.2±2.44

Table-3: AM fungal spore density ($10g^{-1}$ soil) at different soil depths in 3 and 5 year old *Populus deltoides* + wheat systems during January 2005 to April 2005.

Soil Depth (cm)		AM fungal spore density (10 g^{-1} soil)			
		24 January	28 February	30 March	26 April
3 yr old system	0-7.5	55.6±2.77	68.6± 4.04	72.4±3.54	82.0±5.92
	7.5-15	32.6±2.71	49.2±4.02	53.2±2.67	59.0±3.45
	15-30	12.6±1.50	16.4±2.34	22.4±3.44	17.2±1.16
5 yr old system	0-7.5	56.4±3.20	71.0±4.35	72.4±2.58	88.0±4.74
	7.5-15	37.4±2.86	49.8±3.48	55.2±2.60	62.2±1.80
	15-30	14.8±1.02	17.8±2.06	18.2±1.16	19.0±1.00

Table-4: AM fungal spore density ($10g^{-1}$ soil) at different soil depths in 6 and 7 year old *Populus deltoides*±wheat systems during January to April 2006.

Soil Depth (cm)		AM spore density ($10g^{-1}$ soil)			
		18 January	20 February	25 March	21 April
6 yr	0-7.5	72.2±7.38	82.8± 4.75	88.4±5.22	94.8±6.78
	7.5-15	41.2±4.4	53.0±3.94	62.6±4.70	66.8±3.15
	15-30	17.4±2.04	21.2±3.71	23.4±2.98	20.6±2.67
7 yr	0-7.5	75.2±4.08	84.2±4.70	92.0±7.44	92.2±6.26
	7.5-15	43.0±3.27	53.4±5.03	69.0±4.49	56.6±4.34
	15-30	16.6±2.16	22.8±2.83	25.8±2.69	17.4±2.09

Table-5: AM fungal root colonization in sugarcane roots in 1 and 2 year old *Populus deltoids* ± *Sugarcane* agroforestry system.

Soil Depth (cm)	AM Root colonization (%)	
	1 year	2 year
0-7.5	72.0±3.37	76.0±3.74
7.5-15	85.0±3.58	88.4±3.86
15-30	91.0±1.95	96.2±1.43

Table-6: AM fungal root colonization in wheat in 3 and 6 year old *Populus deltoides* ± *wheat* agroforestry systems.

Soil Depth (cm)	AM Root colonization (%)		
	3 year	5 year	6 year
0-7.5	70.8±3.57	72.4±3.23	75.0±3.0
7.5-15	77.8±2.37	78.6±2.16	92.2±2.29
15-30	20.6±1.60	24.6±2.09	32.4±2.13

Table-7: AM fungal root colonization in wheat and *Populus* during November 2004 to April 2005 (I year) and from November 2005 to April 2006 (II year) in 6 and 7 year old *Populus deltoides* agroforestry system.

Sampling Dates		AM root colonization(%)	
		Wheat roots	*Populus* fine roots
I-year	November 2004	36±1.70	71.8±2.15
	February 2005	80.0±1.87	75.2±2.42.
	April 2005	97.2±0.80	81.2±1.85
II-year	November 2005	37.6±1.50	68.6±1.78
	February 2006	82.0±1.50	76.2±2.18
	April 2006	95.6±1.47	83.6±1.72

Conclusion

A total of 47 species of AM fungi belonging to five genera, i.e, *Acaulospora, Entrophospora, Glomus, Gigaspora* and *Sclerocystis* were isolated from the rhizosphere soils of wheat and sugarcane grown under the *Populus* trees. There was significant effect of crop type on AM fungal diversity, being greater in *Populus deltoides* ± sugarcane systems (47 species) as compared to that of *Populus deltoides* ± wheat systems (35 species). The AM fungal communities in the studied agroforestry systems were predominantly composed of species of *Glomus* and *Acaulospora.* The AM spore density was found to be greatest at 7.5 cm soil depth corresponding to the time of crop maturity of wheat during the month of April. The AM fungal spore density increased with the increase in the age of the agroforestry systems. In the sugarcane roots, the AMF root colonization was found to be greatest at 15-30cm soil depth.

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