The Bioscan 9(2): 763-766, 2014 (Supplement on Genetics and Plant Breeding)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INFLUENCE OF LENTIL (*LENS ESCULENTA* M.) GENOTYPES ON ARBUSCULAR MYCORRHIZAL COLONIZATION INTENSITY AND POPULATION DENSITY

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KEYWORDS
Lentil
Lens esculenta
Arbuscular mycorrhizal fungi
Colonization
Spore population

Received on :
20.01.2014

Accepted on :
27.05.2014

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ABSTRACT
Forty lentil genotypes were evaluated under field condition for two consecutive seasons to see the range of AM fungal colonization and spore population density and to group into high medium and low categoties. Results showed that spore population density and root colonization intensity of these genotypes were found to vary from 22 - 81 (per 30 g dry soil) and 55.1 - 96.2% respectively. Spore number was found highest with the genotype L-338 (81 spore/30 gm of soil) followed by L- 245 (77 spore/30 gm of soil) and others whereas it was observed lowest with L-317 (22 spore/30 gm of soil) . Root colonization intensity was found highest with Hull - 57 (96.2 %), remained at par with L- 311 (94.9 %), L- 330 (94.1 %) followed by L- 225 (91.4 %) and others whereas it was observed lowest with L - 223 (55.1%). Based on population density, genotypes placed under low (22 - 35), medium (36 - 65) and high (66 - 81) [per 30 g dry soil]. Based on root colonization intensity, genotypes placed under low (0 - 30%), medium (31 - 60%) and high (61 - 100%) categories. Lentil genotypes grown under field condition cause differential stimulation of AMF spore numbers, exhibit variations in their level of colonization and be arbitrarily grouped into low, medium and high categories .So, for increasing lentil produvtivity suitable genotypes can be picked up from the cafeteria of lentil genotypes having high value of spore population density and root colonization intensity.

INTRODUCTION

Lentil, an important pulse crop ranking fifth and sixth in India and world respectively in production, is considered as important sources of proteins, essential minerals (especially calcium and iron), vitamins and several compounds considered essential for health. Besides, its cultivation enriches soil fertility, adding organic matter and improving the physical, chemical and biological properties of soil. At present, India is producing 0.9 million tons of lentil annually. In spite of that 2-3 millions tons of pulses are imported annually to meet the domestic consumption requirement. To increase production and to mitigate such escalated demand, non-traditional, nutrient poor-, stress- and marginal- lands are being brought under cultivation along with simultaneous introduction of high yielding cultivars/ genotypes. Application of arbuscular mycorrhizal (AM) fungal technology may be ideal option under such situation to boost further in lentil production, minimize and save the use of some costly and energy intensive inputs, check the threatening impacts of some agricultural inputs on environmental and health issues and to improve soil health and microbial properties of the soil.

AM fungi are considered essential for increasing sustainability of agricultural system, play a vital role in increasing plant productivity, species composition, diversity, dynamics and succession (Van der Heijden *et al.*, 1998), promoting plant fitness to stress environment through a range of mechanisms including protecting the host from pathogens, improving soil structure, enhancing water and nutrient uptake .Although AMF do not have any specificity, still they have preferentiality towards host. Crops may vary both inter - and intra - specifically or even within varieties in the degree of colonization, sporulation, mycotrophy, dependency and responsiveness (Yang *et al.*, 2010). Degree of stimulation in plant performance by AM fungi depends on the genetic makeup of host which determines its susceptibility towards the AM fungal colonization. AM fungal colonization in turn is dependent on its population density in the rhizosphere. For extending AM fungal benefit to the varieties/ genotypes of a crop, determination of susceptibility of crop varieties to and assessment of spore population density of AM fungi is the foremost pre-requisite.

Knowing the significances of pulses as a whole and lentil in particular in the nutritional, economical, soil physical and microbial management, realizing the significances and potentialities of AMF in extending growth, nutritional, hormonal and other symbiotic benefits to the crops. An experiment was conducted with forty lentil genotypes in field condition for assessing the status of AM fungi for specific location in terms of colonization intensity, spore population and to identify the genotypes having higher degree of mycotrophy and also for the selection of lentil genotypes suitable as parents for hybridization program.

MATERIALS AND METHODS

Table 1: AMF spore population density and root colonization intensity of lentil genotypes

Sl. No.	Genotypes	% root colonization	Spore count/ 30 gm of soil	Sl. No.	Genotypes	% root colonization	Spore count/ 30 gm of soil.
1	L-221	81.5(63.36*)	45	21	L-250	84.2 (68.95)	43
2	L-222	85.0 (63.94)	47	22	L-311	94.9 (79.06)	67
3	L-223	55.1 (45.00)	43	23	L-312	80.1 (64.01)	42
4	L-225	91.4 (73.15)	62	24	L-313	82.1 (66.11)	43
5	L-226	82.6 (64.30)	56	25	L-315	89.8 (69.91)	28
6	L-227	85.6 (66.42)	31	26	L-317	86.3 (60.60)	22
7	L-230	68.7 (56.54)	42	27	L-319	78.0 (58.12)	42
8	L-231	82.2 (65.35)	32	28	L-320	86.5 (70.91)	45
9	L-235	80.1 (62.17)	41	29	L-322	88.5 (70.81)	34
10	L-236	86.6 (70.36)	71	30	L-325	87.8 (67.86)	43
11	L-238	83.1 (66.74)	32	31	L-326	83.1 (62.94)	52
12	L-239	88.3 (67.62)	30	32	L-330	94.1 (75.46)	53
13	L-240	87.3 (67.94)	23	33	L-331	82.8 (68.61)	49
14	L-242	79.7 (61.75)	39	34	L-334	86.0 (70.09)	44
15	L-244	93.0 (72.34)	33	35	L-335	92.4 (73.15)	29
16	L-245	88.3 (66.34)	77	36	L-338	92.4 (73.57)	81
17	L-246	86.8 (71.47)	61	37	L-339	72.5 (58.50)	61
18	L-247	79.1 (62.17)	41	38	L-340	96.2 (68.87)	71
19	L-248	94.2 (78.17)	75	39	Asha	86.1 (72.34)	63
20	L-249	88.1 (70.81)	55	40	Hull-57	96.2 (78.61)	48

For % root colonization SEm ± = 1.6, C.D .05 = 4.5; for spore count SEm ± = 4.4, C.D .05 = 12.3; * Figure within parenthesis indicates arch- sine transformed value

Table 2A: Categorization of lentil genotypes based on AMF spore number

AMF spore number					
Low (22 - 35)		Medium (36 - 65)		High (66 - 81)	
Genotypes	No	Genotypes	No.	Genotypes	No.
L-227,L-231,L-238	10	L-221,L-222,L-223,L-225,L-226,L 230,	24	L-236	6
L-239,L-240,L-244		L-235,L-242,L-246,L-247, L-249,L-250,L-312,	L-245		
L-315,L-317,L-322		L-313,L-319, L-320,L-325,L-326,L-330,L-331		L-248	
L-335		,L-334, L-339, Asha,Hull-57		L-311	
				L-338	
				L-340	

Experiment with forty lentil genotypes was conducted in Gangetic alluvial soil [sand(40.4%), silt (36.4%), clay (24.3%), pH (6.5), organic carbon (0.69%), total nitrogen (0.047%) and available phosphorus (14.6ppm)] under field condition following Completely Randomized Block Design in three replications at Kalyani Simanta District Seed Farm ((Longitude 88°20" and Latitude 22°57") of Bidhan Chandra Krishi Viswavidyalaya, Kalyani Simanta, Nadia, West Bengal, India. Genotypes were sown 4 m x 1.5 m plots. Seed rates, spacing, fertilizer application, weeding, thinning, watering and other intercultural operations during crop growing and maintenance were done following standard and recommended agronomic practices. Soil and root sample of 45 days old lentil genotypes were collected and bulked replication wise. Root samples were washed replication-wise thoroughly, made 1 cm pieces and preserved in Forma-acetic alcohol (Formalin: acetic acid: alcohol: 5 mL: 5mL: 70% 90mL) for future use. Soil sample were kept in polyethylene packets separately, brought to the laboratory, air dried and kept in polyethylene packets. Root samples collected from field were preserved in FAA and stained following slight modification of original method proposed by Philips and Hayman (1970). Alkaline hydrolysis of root samples with 10% potassium hydroxide was done at 15 psi steam pressure either in pressure cooker or autoclave for 5-8 minutes depending upon the thickness of roots. Clearing of root cortex and subsequent stain penetration were better by this method. But allowing the boiling time above the specified period may disintegrate the roots. Ammoniacal hydrogen peroxide prepared by adding 3 ml of ammonium hydroxide/ ammonia solution and 30 ml of 10% hydrogen peroxide in 567mL of distilled water was used to bleach the coloured root pieces. Roots were then washed in several changes of water, treated with 1 (N) hydrochloric acid (HCl) for 15-20 minutes and ultimately stained by 0.05% trypan blue for about 15-20 minutes. Keeping the samples in hot air oven at constant temperature of 60ºC for 15-20 minutes after the addition of HCl and trypan blue are useful for better clearing and penetration of acid as well as stain respectively in roots. Percent length colonization was determined by estimating the length of root showing AM fungal hyphae, abuscules, vesicles *etc.* by microscopic measurements with ocular micrometer of at least 30x1 cm. root pieces of each replication. AM fungal spores were extracted from the soil following wet sieving and decanting method proposed by Gerdemann and Nicolson (1963) i.e AMF spores were isolated by using 500, 250, 150 and 45μ sieves. The isolated spores were suspended in thin layer of water in Petridish and counted under low power magnification by stereo-binocular microscope. For the convenience of spore counting, one sq. cm grids marked with black/ red/ blue ink of permanent marker were prepared on transparent sheet

Table 2B: Categorization of lentil genotypes based on intensity of AM fungal colonization

AM fungal colonization					
Low (1 – 30%)		Medium (31 – 60%)		High (61 – 100%)	
Genotypes	No.	Genotypes	No.	Genotypes	No.
Nil	0	L-223	1	L-225,L-239,L-244,L-245,L-249,L-311,L-315,L-322,L-330, L-335,L-338,L-340,L-230,L-221,L-222 L-226,L-227,L-231,L-235,L-236,L-238,L-240,L-242,L-246, L-247,L-248,L-250,L-312,L-313,L-317 L-319,L-320,L-325,L-326,L-331,L-334,L-339. Asha,Hul-57	39

or white paper and then the marked sheet / paper was placed below the spore containing Petridish. All grids were examined, spores were counted and totaled. The total spores so obtained indicated the population of AMF in unit quantity of soil.

RESULTS AND DISCUSSION

Soil and root samples were collected from the rhizosphere of forty lentil genotypes grown at Kalyani Simanta District Seed farm, Kalyani, Nadia under field condition for studying the AMF spore population density and root colonization intensity. Results presented (Table 1) on those two parameters revealed that lentil genotypes caused differential stimulation of AMF spore numbers 22-81 per 30g dry soil and exhibited variations in their level of colonization (55.1-96.2%).

It was evident from the results that spore number was the highest with L- 338 followed by L- 245 and L- 248 and others whereas it was the lowest with L-317 and closely followed by L- 240. Root colonization intensity was found highest with Hull-57, remained *at par* with L- 311, L- 330, L- 244, L- 335, L- 338 followed by L- 225 and others whereas it was observed lowest with L - 223.

The population density and root colonization intensity of forty lentil genotypes were categorized arbitrarily in three groups *viz.* low, medium and high (Table 2A, 2B). Based on population density, the number of genotypes placed under low, medium and high categories was 10, 24 and 6 respectively. Based on root colonization intensity, the number of genotypes placed under low, medium and high categories was 0, 1 and 39 respectively. . Spore number recorded here in different lentil genotypes were within the range that obtained by Germida and Talukdar (1995) in lentil variety grown in different sites. They found variations in AM fungal spore number in soil collected from the rhizosphere of spring wheat and lentil at 11 sites across four soil zones. The number of AM spores detected in field soils was observed to range from 78-272 per 100 g soil. But variation in stimulation of spore numbers by different genotypes of a crop has also been recorded by several workers. Panja *et al.* (2007) reported that when they conducted an experiment with thirty three banana genotypes belonged to five genomic groups (AAA, AAB, ABB, AABB and AABB), they observed that AMF root colonization intensity of banana genotypes and their spore densities varied from 10 – 100% and 245-590 per 25 g of dry rhizosphere soil.

There were six genotypes having 10 - 20%, eight having 21 - 50%, ten having 51 - 75% and nine having 76 - 100% root colonization. Out of five genomic groups of banana, the ABB genomic group had the highest root colonization (63.7%) and spore density (459) followed by AAB, AAA and others. It was revealed from the results that different genomic groups of banana and even the genotypes under the same genomic group varied with respect to their AMF susceptibilities and spore population densities. From the results of experiment Yang *et al.* (2010) hinted that plant genotype showed a significant influence on the percentage of root length colonized and abundance of arbuscules and vesicles, and there was much greater colonization of naked oat genotypes than of husk oat. During the studies on natural incidence of arbuscular mycorrhizas in 10 cowpea varieties Kumari and Nair (1989) reported that the average mycorrhizal index differed between varieties, with the maximum infection in the variety C 152 followed by Ptb 2 and New Era. Besides, there are several reports on crops that vary both inter - and intra - specifically in the degree to which they form mycorrhizae. Cultivars of wheat (Azcon and Ocampo, 1981), corn (Toth *et al.*, 1984), millet (Krishna *et al.*, 1985), oat (Yang *et al.*, 2010), tropical forage crops (Saif, 1986), soybean (Heckman and Angle, 1987), groundnut (Rao *et al.*, 1990), yam (Dare *et al.*, 2008) and tea (Routray and Gupta, 2010) have been shown to vary in their levels of colonization by AMF fungi. Further studies show that the degree to which cultivars are colonized by, and benefit from, mycorrhizae is a heritable trait selectable through plant breeding (Krishna *et al.*, 1985; Rao *et al.*, 1990).Variation in lentil genotypes was also reported by Joshi *et al.*,2013 by using ISSR marker. Besisides there are several reports on variation among genotypes *i.e.* germplasms of rice (Kumar *et al* 2013), Safflower (Jhajharia *et al.,* 2013), Cowpea (Binola and Kumar, 2013) and Green gram (Garje *et al.*, 2103) . It can be concluded that lentil genotypes grown under field condition cause differential stimulation of AMF spore numbers, exhibit variations in their level of colonization thus lentil genotypes having high value of spore number and AMF root colonization could be picked up from the cafeteria of lentil genotypes for increasing lentil productivity and these characters can also be used for the choice of parents for hybridization program.

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