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EVALUATION OF ARBUSCULAR MYCORRHIZAL COLONIZATION OF *CASSIA TORA* LINN. ROOTS COLLECTED FROM DIFFERENT LOCATIONS IN BANGLADESH

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Abstract

The arbuscular mycorrhizal (AM) fungal colonization in roots of *Cassia tora* L. under field conditions in 12 locations of Bangladesh and suitability of *C. tora* plant as a host for inoculum production was studied. The root and soil samples were collected from different districts of Bangladesh. A significant variation of root colonization was found among the locations and all types of AM fungal structures were detected during the observation. The range of total colonization was 61 - 88 per cent. A diverse type of mycorrhizal fungi recorded from the rhizosphere soils indicated that *C. tora* under field condition not only has high mycorrhizal colonization but also harbored different types of AM fungi in their mycotrophic nature. The results of the inoculum production under greenhouse conditions (25 - 30^{o}C and relative humidity above 90%) revealed that the high AM colonization indicated that the plants may be utilized as trap crop for large scale inoculum production.

Cassia tora L. belonging to Caesalpiniaceae is an annual weed that grows throughout the tropical and subtropical regions of the world and is very common in Bangladesh and abundantly found mainly in roadsides and wastelands. The plant is native to south-eastern Asia, Fiji, northern Australia, Africa, and Latin America and it also grows well in Japan, Malaysia, Burma, Bangladesh and Vietnam. The leaves and seeds of *C. tora* are used in Ayurvedic medicine for the treatment of many human diseases (Maity*et al.* 1998) and it has pesticidal and fungicidal activity (Kim *et al.* 2004) and also has allelopathic potential (Prasad *et al.* 2006). Chakravarty and Mishra (2008) reported the influence of endotrophic mycorrhizae on the Fusarial wilt of *C. tora.*

Because of over use of chemical fertilizers and application of pesticides, AM fungi cannot survive properly and are not available or not active in all agro-ecosystems, thus application of mycorrhizal inoculum could be critical for sustainable crop production. The application of AM fungi for plant growth under field conditions is limited due to lack of suitable inoculum production technology (Ijdo *et al.* 2011). Different types of host plants (e.g. onion, leek, maize, bahia grass, sorghum etc.) are commonly used for the large-scale inoculum production of AM fungi. *C. tora* has more or less same characteristics as was reported with other indicator plants and a few researchers have reported its mycotrophic nature from different habitats (Sawant *et al.* 2011, Sharma and Jha 2012). The present research work has dealt with the status of AM fungi infections in the *C. tora* roots collected from 12 locations of Bangladesh and mycorrhizal fungi in the rhizosphere soils of the plants and to evaluate for AM fungal inoculum production efficiency.

Roots along with rhizosphere soils (0 - 30 cm depth) of *C. tora* plants were collected from 12 locations *viz.*, Barisal, Bogra, Chittagong, Comilla, Dhaka, Dinajpur, Khulna, Mymensingh, Pabna, Rangpur, Rajshai and Sylhet of Bangladesh. In the laboratory, roots were separated from the soil samples, washed to get free of soil and debris and preserved in 50% ethyl alcohol.

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Preserved root segments (1 cm length) were stained following the methods of Phillips and Hayman (1970). The stained root segments (30 segments/slide for three replications in each sample) were mounted in lactoglycerol solution on glass slides for observation of different AM fungal structures. Percentage of mycorrhizal colonization, intensity of AM structural colonization was estimated in the stained roots for total infection. The intensity of colonization was measured as poor, moderate and abundant (Al-Qarawi *et al.* 2012) types of colonization. The intensity of infection of AM fungi was estimated as: poor (if only mycelium were present); moderate (if mycelium and vesicles or arbuscules were present) and abundant (if mycelium, vesicles and arbuscules were present). Mycelial colonization was regarded as total AM colonization. Per cent colonization was calculated by the following formula (Al-Qarawi *et al.* 2012).

$$\% \text{ colonization} = \frac{\text{Total number of AM positives segments}}{\text{Total number of segments studied}} \times 100$$

Soils were assessed following wet sieving and decanting (Gerdemann and Nicolson 1963) method with little modifications (Al-Qarawi *et al.* 2012) to enumerate the total spore population and diversity of AMF spore in the soils. Morphologically similar spores were picked up by using stereo-binocular microscope and examined under compound microscope (40x) after mounting them in polyvinyl alcohol in lactoglycerol (PVLG) and Melzer's reagent (1 : 1 v/v) and were identified by following Redecker *et al.* (2013) and Schenck and Perez (1990).

The efficiency of *C. tora* as an indicator plants or trap crop for inoculum production of AM fungi under greenhouse conditions was evaluated by using sandy loam soils with pH (6.9), OM (4.56%) EC (3.4 d/Sm), N (267 ppm) and P (421 ppm). Three replicated pots were used for setting out the experiment. Seeds collected from field were sown (ten seeds in each pot) in plastic pots containing 2 kg of sterilized soils. Soils were sterilized by autoclaving (121ºC with 15 psi for 30 min) three times. The soils were inoculated with 20 g of soil inoculum (containing infected root segments, fragments of AM fugal hyphae and 250 - 300 mixed spores of different *Glomus* spp.) developed in our ongoing experiments. The AM fungal inoculum was mixed at the 10 cm upper surface of the soil. Hoagland's solution without P was used before sowing of seeds once to fertilize the soil. Regular watering and other intercultural operation were done as and when necessary for the requirement of the experiment. After three months of growth, the plants were harvested and root infections were assessed by following the methods mentioned above to determine the efficiency of inoculum production by *C. tora*.

The mean values were computed from three replications with standard deviation (± sd). The percentage infection with the AM fungi in the roots of *C. tora* collected from 12 locations of Bangladesh varied independently (Table 1). The infection with mycelium was regarded as the total infection. The range of variation of total infection was 61 - 88%. The overall highest infection was recorded in the sample of Dhaka which was followed by Chittagong, Pabna, Bogra, Mymensingh and the lowest infection was found in the samples of Khulna. The total percentage infection of mycelium was not reflected with the infection of vesicles and arbuscules in individual location. The range of infection with vesicles was 31 - 59 % with maximum occurrence from Dhaka and the minimum was from Rangpur. In case of total infection with arbuscules the highest percentage of infection was recorded from Dhaka (77) and the lowest from Khulna (45).

The intensity of infection also varied independently in an individual location and was not always comparable to the percentage infection with different structures of the AMF (Table 1). In case of intensity of infection with mycelium, the maximum infection as poor, moderate and abundant types was recorded from Chittagong, Mymensingh and Chittagong, respectively and the

Table 1. Intensity and total structural colonization (%) of AM fungi in *Cassia tora* roots and total number of spore per100 g soil collected from 12 locations of Bangladesh.

Locations	Intensity			Total	Intensity			Total	Intensity			Total	No. spore/ 100 g soil
	Mycelium				Vesicles				Arbuscules				
	P	M	A		P	M	A		P	M	A		
Barisal	10 ± 1.6	20 ± 3.3	34 ± 3.3	64	7 ± 1.6	15 ± 1.6	8 ± 1.6	30	13 ± 1.6	13 ± 0.8	20 ± 3.3	46	390 ± 28.6
Bogra	16 ± 1.6	24 ± 3.3	40 ± 4.1	80	18 ± 1.6	10 ± 4.1	9 ± 1.6	37	16 ±1.6	16 ± 1.6	24 ± 1.6	56	420 ± 18.0
Chittagong	21 ± 3.3	16 ± 3.3	49 ± 4.9	86	10 ± 2.4	20 ± 2.4	12 ± 2.4	42	20 ± 3.3	11 ± 2.5	32 ± 3.3	63	597 ± 24.5
Comilla	19 ± 4.9	26 ± 1.6	31 ± 1.6	76	10 ± 2.1	18 ± 1.6	12 ± 2.4	40	18 ± 0.8	16 ± 3.3	30 ± 4.1	64	521 ± 33.5
Dhaka	20 ± 1.6	23 ± 3.3	45 ± 3.3	88	20 ± 1.6	19 ± 3.3	18 ± 6.5	57	14 ± 3.3	17 ± 2.4	19 ± 0.8	50	539 ± 16.3
Dinajpur	14 ± 3.3	20 ± 3.3	30 ± 0.8	64	12 ± 1.6	12 ± 0.8	15 ± 4.1	39	22 ± 4.1	27 ± 3.3	28 ± 1.6	77	397 ± 26.1
Khulna	15 ± 4.1	19 ± 2.5	28 ± 2.4	62	11 ± 4.1	15 ± 4.9	13 ± 3.3	39	12 ± 1.6	15 ± 3.3	25 ± 5.7	52	431 ± 25.3
Mymensingh	13 ± 2.4	31 ± 4.9	34 ± 3.3	78	9 ± 4.1	14 ± 3.3	12 ± 1.6	35	14 ± 3.3	16 ± 4.9	27 ± 2.4	57	549 ± 9.8
Pabna	20 ± 1.6	21 ± 4.9	42 ± 4.9	83	16 ± 3.3	14 ± 2.4	22 ± 3.3	52	15 ± 0.8	14 ± 2.4	36 ± 3.3	65	679 ± 16.3
Rangpur	18 ± 1.6	20 ± 4.1	30 ± 4.9	68	8 ± 2.4	13 ± 2.4	10 ± 2.4	31	20 ± 0.8	10 ± 3.3	17 ± 1.6	47	360 ± 26.1
Rajshahi	16 ± 3.3	19 ± 3.3	43 ± 2.4	78	10 ± 2.4	15 ± 3.3	20 ± 3.3	45	23 ± 2.4	16 ± 4.1	18 ± 5.7	57	454 ± 18.9
Sylhet	15 ± 4.9	21± 4.9	40 ± 5.7	76	12 ± 4.1	12 ± 4.9	18 ± 4.9	42	20 ± 0.8	18 ± 5.7	15 ± 6.5	53	326 ± 24.5

P = Poor; M = Moderate; A = Abundant, ± = Standard deviation.

minimum was recorded from Barisal, Chittagong and Khulna, respectively. In the same way, the intensity of infection with vesicles, the highest percentage of poor, moderate and abundant types was recorded from Dhaka, Chittagong and Pabna, respectively and the lowest per cent of poor type of infection was recorded from Barisal; both moderate and abundant type of infection was found from Bogra and Barisal, respectively. The intensity of infection with arbuscules was higher in comparison to mycelium and vesicles. Most of the samples were found to have abundant arbuscular presence in their root systems. Poor type of highest per cent infection was recorded from Rajshahi; moderate type was found in Dinajpur and the abundant type was detected from the sample of Pabna. On the other hand, the minimum poor, moderate and abundant type of infection was found in the samples collected from Khulna, Rangpur and Sylhet, respectively.

Spore population varied from 326 - 679/100 g dry soils, among the locations (Table 1). The highest spore population was recorded from Pabna which was followed by Chittagong, Mymensingh, Dhaka and the lowest number was found from Sylhet. Out of the recorded species of AMF, *Funneliformis mosseae, Glomus etunicatum, G. intraradices, G. fasciculatum, Acaulospora* spp. were identified and a few spores remained unidentified. *F. mosseae* and *G. etunicatum* were very common and was recorded from most of the soil samples studied.

In inoculum production experiment with *C. tora* plants inoculated with AM fungi under green house conditions showed 80% roots segments (each 1 cm length) infected with different types of AM fungal structures including the intraradical spores. The rhizosphere soils have also good number of AM fungal spores available.

The results obtained in root colonization study suggest that *C. tora* plants collected from different locations in Bangladesh exhibit high levels of mycorrhizal association. However, the degree of per cent root colonization and presence of arbuscules and vesicles varies from one location to another. It is evident from the results that there is no correlation between these factors (per cent root colonization and presence or absence of arbuscular and vesicular structures). Hence, it can be deduced that each location has its own preference towards mycorrhization, because of edaphic conditions and availability of inoculum. Several environmental factors such as soil moisture, temperature, pH, nutrients, organic matter, and changes in plant community can influence the composition of AM fungal species (Jansa *et al.* 2003). More or less similar results about colonization of *C. tora* were reported (Sharma and Jha 2012) in the present study. The incidence of AM fungal infection in different weeds including *C. tora* was reported by Sawant *et al.* (2011). The occurrence of large number of different species of AM fungi from various locations indicated that *C. tora* has low host specificity of infection by various AM fungi. This characteristic of a trap crop is useful for large scale inoculum production in association with different types of AM fungi. Therefore, it may be concluded that *C. tora* can be used for AM fungi inoculum production under greenhouse environments in Bangladesh.

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