Pakistan Journal of Nematology (2019)37(1):83-89 ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk
http://dx.doi.org/10.18681/pjn.v37.i01.p83-89

Arbuscular mycorrhizal fungi as potential bioprotectant against *Meloidogyne incognita* on *Lagenaria siceraria*

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Abstract

The present investigation was carried out to determine the interrelationship of arbuscular mycorrhiza (AM) fungi with root-knot nematode *Meloidogyne incognita* and their effect on carbon and nitrogen metabolism of *Lagenaria siceraria* (Molina) Standley, a mycorrhizal plant of family Cucurbitaceae. Biochemical analysis including carbon and nitrogen profiles was taken into account.The estimation of carbon profile comprising of carbohydrate, glucose, sucrose and total soluble sugars and nitrogen profile comprising of proteins, amino acids protease and proline. Results showed that carbon profile in plant treated with AM fungi have high to low and varied amount of carbohydrates, sucrose, glucose and total soluble sugars in different parts of the plant; amino acids and proline in nitrogen profile found in higher amount in AM treated plants.

Keywords: Arbuscular mycorrhizal fungi, *Lagenaria siceraria*, *Meloidogyne incognita*

Arbuscular mycorrhizal fungi (AMF) do have the potential as biocontrol agent for nematode management when both groups of organisms occur simultaneously in the roots and rhizosphere of the same plant (Talavera *et al*., 2001). The plants heavily colonized with AM fungi are able to grow well in spite of the presence of damaging levels of nematodes, thus promoting tolerance to nematodes. The favorable effect of mycorrhizae in decreasing the disease intensity in nematode affected plants has been demonstrated in various crops (Shreenivasa *et al*., 2007).

Bioprotective effect of arbuscular mycorrhizal fungi (AMF) differed among species (Singh *et al*., 2000; Whipps, 2004). *Glomus intraradices* did not protect clover against nematode infection (Habte *et al*., 1999). Root colonization by *G. mosseae* protected against nematodes and *Phytophthora parasitica* infection (Habte *et al*., 1999; Pozo *et al*., 2002). The host-pathogen relationship is influenced indirectly through physiological alteration and competition for space and/or host resources. Through increased phosphate nutrition, AM fungi enhance root growth, expand the absorptive capacity and affect cellular processes in roots (Maia *et al*., 2006). Fungi supply nutrient to plant that would not normally be available to plants; improves plant material nutrient acquisition from soil especially immobile elements such as P, Zn and Cu but also mobile ions such as S, Ca, K, Fe, Mn, Cl, Br and N (Cooper & Tinker, 1978). In addition to phosphate, AM fungi enhance uptake of Ca 2+, Cu z+, $SO_4$2- and Zn z+ (Smith & Gianinazzi-Pearson, 1988). The objective of the present study was to evaluate the efficacy of AM fungi as biocontrol agent against root-knot infection on bottle gourd.

Materials and Methods

Plant material: Bottle gourd (*Lagenaria siceraria*) was used as plant material in this study. Seeds of bottle gourd were sterilized with $HgCl_2$ and then washed with distill water. The sterilized seeds were sown into pots filled with garden soil and irrigated after germination as per requirement of water.

Experimental design: Six sets of plants with six replicates were placed in earthen pots of 56

Published by Pakistan Society of Nematologists
Received:16 Sep, 2018
Accepted:22 Nov, 2018

cm diameter filled with sandy-clay loam soil. The pots were arranged in a complete randomized block design. After fifteen days treatments were applied as follows:

Treatments: i) C= Control; ii) T_1 = AM only; iii) T_2 = Nematodes only; iv) T_3 = AM + nematodes simultaneously; v) T_4= AM + nematodes (AM one week before nematode inoculation); vi) T_5 = Nematodes + AM (AM one week after nematode inoculation).

Root-knot nematodes: Females of root-knot nematodes were isolated from egg plants by Baermann funnel method (Jepson, 1987). Fifteen days old seedlings of bottle gourd were inoculated with the nematodes @ 1000 larvae/pot.

AM fungi: The AM fungal spores collected from different agricultural soils were isolated by the wet sieving and decanting technique (Gerdemann & Nicolson, 1963). These fungal spores were cultured in Sudan grass grown in earthen pots containing sterilized sandy-clay loam soil and was used as stock culture. 500 g soil (1000 spores) from soil based stock culture was inoculated into the seedlings.

Biochemical analysis: Samples of roots, shoots and leaves were collected separately for biochemical analysis by the following methods:

- **Carbon Profile:** i) Estimation of total carbohydrates (Yemm & Willis, 1954);
  ii) Estimation of glucose (Riazi *et al*., 1985);
  iii) Estimation of sucrose (Riazi *et al*., 1985);
  iv) Estimation of total soluble sugar (Riazi *et al*., 1985).

- **Nitrogen Profile:** i) Estimation of total protein (Lowry *et al*., 1951); ii) Estimation of amino acid (Moore & Stein, 1948); iii) Estimation of proline (Bates *et al*., 1973); iv) Estimation of protease (Ainous, 1970)

Statistical analysis: Statistical analysis was performed by the procedure followed by Gomez & Gomez (1984).

Results

Results of the present studies showed that carbon profile in plants treated with AM fungi only, had high to low and varied amount of carbohydrates (sucrose & glucose) and total soluble sugars in different parts of the plant. This indicates the hindrance of building up process by the infection of AM fungi. Amino acids and proline in nitrogen profile was found in higher amount in the AM-treated plants. Similar observations were found by Bansal & Mukerji (1994) and Harrison & Dixon (1993).

Carbon profile of *Lagenaria siceraria* (Table 1)

Carbohydrates: Amount of carbohydrates was more in roots than leaves and shoots in all treatments. Shoots had the least amount of carbohydrates due to transient zone for the translocation of the metabolites. Mycorrhizal plants showed high amount of carbohydrates in their roots as AM fungi utilize host photosynthates accumulating in roots and as a result, rate of photosynthesis increased and more carbohydrates were produced. AM fungi increased the uptake of nutrients which were incorporated into different primary and secondary metabolites, both macro and micro molecules. Nematode treated plants T_2 and in plants where AM were applied one week before nematodes and after one week of nematodes i.e., T_4 and T_5 had more or less equal amounts of carbohydrates in all three parts. Due to the nematode infection, there was perhaps a leaching of carbohydrates from roots, resulting in decrease in shoots and leaves, as nematodes affect the leaf pigments mainly chlorophyll and leaf area and reduce the rate of building of carbohydrates by the process of photosynthesis. While in plants where AM fungi and nematodes were applied simultaneously, the highest amounts of carbohydrates were found in their roots than all other treatments.

Glucose: Amount of glucose was higher than control in AM fungi roots and shoots but it decreased in AM fungi leaves. In T_2 nematode treated plants roots had the highest amount while

shoots had lower amount than control and AMF; plant leaves also had very low amount like AM plants. Plants where AM and nematodes applied simultaneously (T_3), highest amount of glucose was found in roots, which was more or less equal to roots of nematodes treated plants. Glucose was higher in shoot than control and nematode treated plants, but in leaves glucose was lower than control and higher than in all other treatments. In plants where AM were applied one week before and one week after nematodes; i.e., T_4 and T_5, very low amount of glucose was found in all three parts of plants.

Sucrose: Amount of sucrose was high in roots of nematode treated plants (T_2), followed by AM (T_1) and where treatment was simultaneous (T_3) while roots of T_4 and T_5 had lowest amount of sucrose. Shoots of T_1 and T_2 had more or less equal higher amount of sucrose than control while shoots of T_3 had the highest amount of sucrose.T_4 shoots had equal amount of sucrose to control; while shoots of T_5 had the lowest amount of sucrose. Control, T_1, T_3 and T_4 leaves had more or less equal amount of sucrose. T_2 had the highest amount in leaves while T_5 was at the least.

Total soluble sugars: Like the carbohydrates, TSS was found highest in T_3 roots followed by T_1 roots. Roots of T_4 and T_5 had more or less equal amounts of TSS. T_2 roots had lower amount of TSS than T_4 and T_5, but all treatments had higher amount of TSS than control. Amount of TSS in shoots had the same pattern as in roots. Amount of TSS was more or less equal in leaves of control T_1 and T_3. Likewise T_2, T_4 and T_5 also had equal amount of TSS in their leaves.

Nitrogen profile of *Lagenaria siceraria* (Table 2)

Protein: AM treated roots had highest amount of protein in their roots. T_2 plants had higher amount of protein than the control but it was lower thanT_1. Similarly T_3 plant roots had much higher amount of protein than control but it was lower than T_1 and T_2. In T_3 amount of protein was higher than control but it was lower than T_1, T_2 and T_4. T_5 plant roots also had somewhat higher amount of protein than control but it was lowest among all treatments. AM plant (T_1) shoots also had highest amount of protein, whileT_3, T_4 and T_5 had nearly equal amount of protein in their shoots. T_2 shoots had very little amount but yet higher than control. AM plant leaves had highest amount of protein, 2nd highest amount was in T_3 leaves; while T_2, T_4 and T_5 had lower amount of protein than control but among them T4 had the highest amount.

Amino acids: Control roots had lower amount of amino acids than T_1, T_3, T_4 and T_5, and it further decreased gradually in these treatments, respectively. T_2 roots had lower amount of amino acids than control. In contrast to root, control shoots had a very high amount of amino acids, indicating a drastic break down or lysis of protein. Leaves of control and T_1 had more or less equal amount of amino acids; similarly leaves of T_2, T_4 and T_5had equal amount of amino acids. Leaves of T_3 had the least amount of amino acids.

Protease: T_5 roots had the highest amount of protease followed by T_4 roots. T_1 roots had higher amount of protease than control while it was more or less equal inT_2 roots; amount of protease was the lowest in T_3 roots. Shoots of T_1 and T_3 had nearly equal amount of protease, it was lower in T_5 but all these treatments had amount of protease higher than control. Amount of enzyme was lower than control in T_4 and T_2, the lowest being in T_2 shoots. T_1 and T_4 leaves had nearly equal amount of enzyme but higher than control. T_5 leaves had highest amount of protease.T_2 and T_3 had more or less equal amount of protease as in control.

Proline: T_4 roots had highest amount of proline, T_2 and T_3 had nearly equal, similarly T_1 and T_5 equal amount of proline. Control roots had lowest amount of proline. Shoots of control, T_1 and T_2 had almost equal amount of proline, shoots of T_3 plants had highest amount of proline than in T_4 and T_5. In control shoots and leaves shared the lowest amount of proline. T_2 and T_3 leaves had more or less equal amount of proline, followed by T_4 and T_5 and lowest in T_1 leaves.

Table 1. Effect of RKN and AM fungi on carbon profile of *Lagenaria siceraria* (Molina) Standley.

Treatments	Carbohydrate (mg/g)			Glucose (mg/g)			Sucrose (mg/g)			Total soluble sugars (mg/g)		
	Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf
C	744.4e	528.6c	588.5d	1981.7c	1323.5c	1095.4a	2255.9c	1160.0c	688.8d	2569.0f	941.7f	5251.1c
T_1	1081.9b	744.4a	904.6a	2110.8c	1872.3a	536.6c	4886.7b	1785.6b	696.2c	11824.6b	5368.6b	5558.6b
T_2	836.4d	463.3d	666.6 c	3454.6a	1022.7d	437.3d	8579.9a	1723.9b	896.0a	4713.9e	3259.0c	2490.9e
T_3	1333.3a	539.0c	805.2b	3350.4b	1559.4b	842.9b	3094.0c	2857.8a	592.4e	13634.0a	8194.7a	5853.8a
T_4	888.8c	645.5b	566.6d	501.6e	271.5e	347.7e	1641.0d	1225.2c	825.7b	5349.6d	2008.4e	2915.3d
T_5	817.7d	513.3c	505.8e	628.0d	213.3e	377.3e	1081.9e	552.5d	462.1f	5734.2c	2511.5d	2877.9d

Mean with similar letters in each column are not significantly different at 0.05 probability level.

Table 2. Effect of RKN and AM fungi on nitrogen metabolism of *Lagenaria siceraria* (Molina) Standley.

Treatments	Protein (mg/g)			Amino acids (mg/g)			Protease (mg/g)			Proline(mg/g)		
	Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf	Root	Shoot	Leaf
C	1.17f	0.35 e	1.70c	920.2d	2925.4a	1355.4b	273.6d	440.9c	249.6e	2.5d	1.62d	1.64e
T_1	3.83a	1.49 a	3.61a	1958.2a	1919.5b	1434.7a	353.6c	565.2a	351.3b	3.5b	1.64d	2.28d
T_2	3.67b	0.50 d	0.78e	834.8d	880.0d	825.4c	255.2d	95.8e	281.1d	2.9c	1.58d	5.50a
T_3	2.14d	0.92 c	2.78b	1456.4a	374.8f	504.8e	126.2e	567.7a	255.7e	2.7c	5.05a	5.55a
T_4	2.80c	1.19 b	1.30d	1239.9c	812.2e	629.3c	592.7b	291.4d	329.2c	4.3a	3.24b	4.55b
T_5	1.51e	1.18 b	0.79e	1141.2c	984.7c	842.2c	753.7a	526.8b	397.8a	3.4b	2.59c	3.40c

Mean with similar letters in each column are not significantly different at 0.05 probability level.

Discussion

This investigation assessed biochemical aspects on plant tolerance to a nematode pest and AMF inoculation. Obtained results of the present studies showed that application of AM fungi significantly reduced infectivity of the root-knot nematode infection on bottle gourd. They increased the water and mineral nutrient uptake for their host plants and modify the plant metabolism. Significant difference was observed in mycorrhizal and non mycorrhizal plants in the nutrients. Mycorrhizal plants had more carbohydrates in their roots than shoot and leaves whereas total soluble sugars increased in all parts of the mycorrhizal plant (root, shoot and leaves) showing significant difference to other treatments and control. Mycorrhizal inoculated plant showed increased glucose and sucrose in shoots whereas these nutrients were more in non mycorrhizal roots as compared to other treatments. Our results correspond well with Smith & Read (2008) and Dubey & Trivedi (2012). Similarly protein and amino acids increased in all plant parts in mycorrhizal treated plants while protease and proline also increased in mycorrhizal treated plants as compared to other treatments. Similar observations were reported by Zhu *et al*., (2011) that protein concentration and protective enzyme activities were high in mycorrhizal as compared to non-mycorrhizal plants.

Our findings are in accordance with those who found that AM fungi has an important role in the protection of the crop plants by the attack of nematode pest and thereby enhance plant tolerance (Borowicz, 2001; Pozo & Anguilar, 2007; Natarajan & Kumutha, 2009; Smith & Smith, 2011; Baum *et al*., 2015). The protective effect of AMF against root-feeding nematodes has also been well documented by many researchers (Hol & Cook, 2005; Jothi *et al*., 2005; Shreenivasa *et al*., 2007; Elsen *et al*., 2008; Dubey & Trivedi, 2012; Li-Hui & Wu, 2016). In general, AMF provide improved plant nutrition and health (Smith & Read, 2008). So AMF could be considered as biological control agents or potential bio-protectors (Talavera *et al*., 2001).

Conclusion

In conclusion, results indicate that mycorrhizal plants may be beneficial for growth and nutrient uptake of bottle gourd by suppressing the development and reproduction of root-knot nematodes. AM fungi play a significant role in plant physiology because they enhance nutrient availability and modify plant metabolism which leads to a reduced response to stress and increased resistance to pathogen attacks. AM fungi as potential bio-control agent for crop improvement and bio-protectant against root-knot nematodes is very promising perspective. Therefore there is a need to further investigate and facilitate the research on AM fungi as bio-control agent and bio-protectant owing to their beneficial response in improving crop productivity.

Acknowledgements

This is the part of dissertation submitted by Hajra Nasreen to NNRC, University of Karachi in partial fulfillment of the requirements for the degree of Ph. D.

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