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RESEARCH ARTICLE

HPLC (High Performance Liquid Chromatography) based quantification of Indole-3 acetic acid production ability of lentil (*Lens esculenta*) under AM-inoculated and un-inoculated conditions

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ABSTRACT

Two lentil genotypes-Asha (having high root hairs) and L- 249 (without root hairs) were pot cultured for fifteen days under mycorrhiza inoculated and uninoculated conditions to see whether these two genotypes exhibit any variation in the level of Indole -3- acetic acid production in the root and shoot. Results indicated that under mycorrhiza inoculated and uninoculated conditions IAA production in shoot was detected in both genotypes but it was not detected in the roots of Asha. The level of IAA production in shoot was found more in Asha than L – 249 under mycorrhiza non-inoculated condition. It seemed that Asha had inherently higher IAA production capacity than L – 249.

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INTRODUCTION

Lentil is an important pulse crop grown in the Indian subcontinent and many semiarid regions of the tropics and subtropics. It ranks sixth and fifth in the world and India, respectively. It act as an important source of amino acids - proteins (globulin, cystine, methionine, lycine etc.) of high biological values,essential minerals (especially calcium and iron), vitamins and several compounds considered essential for health of vegetarian and non-vegetarian people. Their cultivation enriches soil fertility by scavenging atmospheric nitrogen, enhancing phosphorus availability, adding organic matter and improving the physical, chemical and biological properties of soil.

The significance of IAA in plant growth promotion is well known. Mycorrhiza can also produce hormones in plants and that may be one of the reasons for growth promotion in mycorrhizal plants. Plant hormone acts as signal molecules for proper establishment of mycorrhizal symbiosis and colonization process (Müller, 2000). Plant colonized by AM fungi is known to alter levels of auxins, cytokinin and gibberellins (Tagu and Barker, 2000). AM fungi are known to synthesize some plant hormones like IAA and cytokinins. At least a part of total hormone production is contributed by the endosymbiotic arbuscular mycorrhizal fungi and the rests by the plant itself (Barea and Azcon, 1982; Allen *et al.*, 1982).

Knowing the significance of IAA in plant growth promotion and keeping in mind the demand of lentil crop, two lentil genotype Asha and L-249 were challenged with and without AM-inoculation to see its effect on IAA production.

MATERIAL AND METHODS

The research was conducted in the year 2012. During the period of experimentation, several materials were used and different methods were followed. Detailed descriptions

of material used and methods followed are now described below.

Experimental soils and their physico-chemical properties :

Alluvial soil samples from experimental plots of Kalyani Simanta District Seed Farm were collected and used for physico-chemical analyses. Sand, silt, clay, pH, organic carbon per cent, total nitrogen per cent and available P (ppm) of experimental soils before the start of the experiment were analyzed and presented in (Table A).

Table A : Physico-chemical properties of alluvial soils

Soils	Mechanical composition (%)			pH	Organic carbon (%)	Total nitrogen (%)	Available P (ppm)
	Sand	Silt	Clay				
Alluvial	40.4	36.4	24.3	6.5	0.69	0.047	14.6

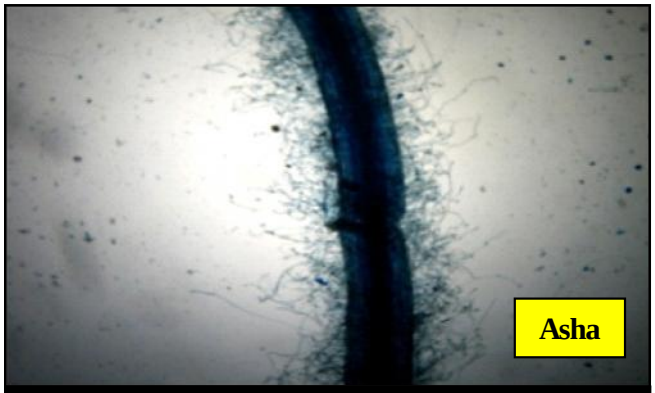

Plate 1(a) : Lenti genotypes Asha having high root hairs

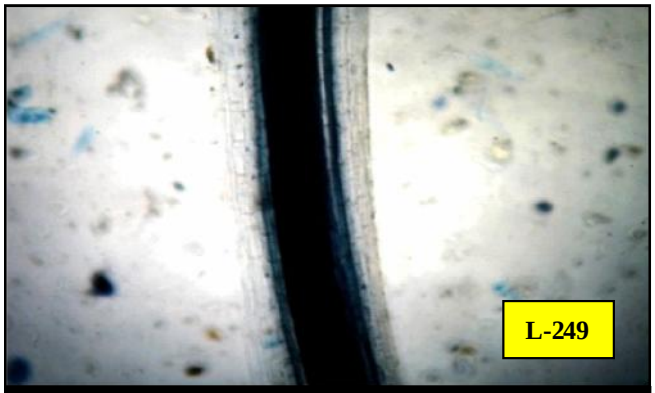

Plate 1(b) : L-249 without any root hair

Growing of two lentil genotypes in alluvial soil under polyhouse condition with or without mycorrhizal inoculation:

Experiments with two lentil genotypes–Asha having high root hairs (Plate 1(a) and L-249 without root hairs (Plate 1 (b), were conducted in pot culture with alluvial soil under polyhouse condition at Banana Research Centre, Mandouri, Nadia. For each lentil genotype, a total of six earthen flat pots, properly cleaned and sterilized, of 30 cm diameter and 8 cm height were taken and each pot was filled with 3 kg of sterilized soil mixed with 30g well decomposed sterilized farm yard manure. Experiment had only two treatment combinations consisting of sterile soil inoculated with (21 g live) or without (21 g heat- killed) arbuscular mycorrhiza consortia formulation per kg soil in three replications. One hundred fifty uniform, water-soaked seeds of each lentil germplasm were sown at one cm depth, covered with the soil of same treatment and then watered to maintain optimum moisture level. After emergence, one hundred seedlings were maintained in each pot after thinning. No other organic manure and fertilizers were used during the entire period of experimentation. Watering to the plants was done according to the needs. Plants were allowed to grow for 15 days. Total plants of each pot were harvest and immediately brought to the laboratory. Stems and roots were separated. Roots were properly cleaned and washed in tap water then followed by distilled water. Roots and stems were immediately processed for hormonal analysis.

Estimation of indole-3-acetic acid by (HPLC) method :

One gram freshly harvested plant sample was taken in a mortar and pastel and kept in ice bath. Ten ml of pre-chilled methanol (80%) as extractant was added to the sample. Then sample was thoroughly crushed, homogenized, stirred and kept overnight. Homogenized residue was extracted three times with 80 per cent pre-chilled methanol, filtered using Whatman no.1 filter paper and collected in 150 ml of conical flask. Volume of extract was reduced to $1/3^{th}$ by heating at 40^0C on hot water bath. After cooling, partitioning of the extract was done with equal volume of petroleum ether for 2-3 times and added 0.1 g of polyvinyl pyrrolidone and agitated for 30 minutes, adjusted extractant pH to 3.0 with the addition of 1.0 M HCl and then extractact was again partitioned with equal volume of ethyl acetate for three times and ethyl acetate extract was filtered with Na_2SO_4 and the filtrate was used for further analysis by dissolving in mobile phase solution.

HPLC condition :

The detector wavelength was set at 254 nm. A hypersil BDS C_{18} column (4.6 mm × 200 mm × 5 um) was used for separation. The mobile phase was prepared by mixing a mixture of methanol, water and acetic acid in the ratio of 45:54:1 per cent (pH 3), respectively. Flow rate was maintained at 0.8 ml/ minute. IAA standard of 1ppm and sample were injected 25µl each in HPLC. Considering the RT value and corresponding spectral area of IAA standard, spectral area of IAA in root and shoot samples was found out. Necessary calculation was made to determine IAA content of the samples taken.

RESULTS AND DISCUSSION

Two lentil genotypes - Asha and L-249 were pot cultured

for fifteen days under mycorrhiza inoculated and uninoculated conditions to see whether these two genotypes exhibit any variation in the level of Indole -3- acetic acid production in the root and shoot. Results indicated that under mycorrhiza inoculated and uninoculated conditions, IAA production in shoot was detected in both genotypes but it was not detected in the roots of Asha (Table 1). The level of IAA production in shoot was found more in Asha than L–249 under mycorrhiza non-inoculated condition. It seemed that Asha had inherently higher IAA production capacity than L–249. But on mycorrhizal inoculation, level of IAA in shoot was found 2.65 times and 4.39 times enhanced in Asha and L -249, respectively whereas the same in root of L -249 was found 3.46 times higher (Fig. 1).

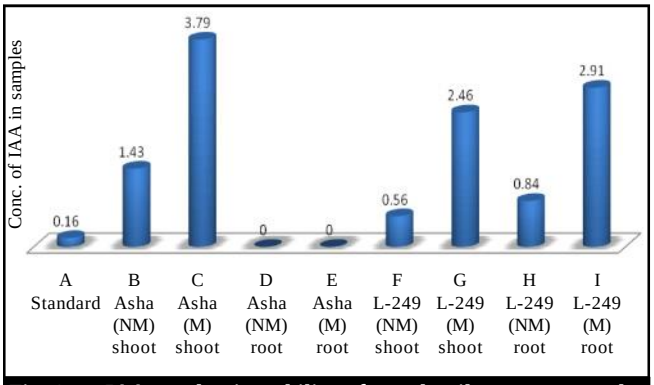

Fig. 1 : IAA production ability of two lentil genotypes under mycorrhiza inoculated and uninoculated conditions

It was evident from the results that two lentil genotypes differed in IAA production. Total IAA production was higher in Asha than L-249 but rate of IAA production was more in L-249 than Asha. Mycorrhiza inoculated plants enhanced IAA level than mycorrhiza uninoculated plants. Such augmentation of IAA level in mycorrhiza inoculated plants was observed by Tagu and Barker (2000). AM fungi are known to synthesize some plant hormones like IAA and cytokinins (Barea and Azcon, 1982). There are several examples of increased level of auxins in roots of maize (Müller *et al.*, 2005) or soybean (Vierheilig, 2005) after inoculation with AM fungi. In soybean, IAA levels were higher in AM roots than in controls (Vierheilig, 2005). Chakrabarti *et al.* (2010) reported that healthy root nodules of *Vigna mungo* appeared to contain higher amount of indole-acetic acid (IAA) than non-nodulated roots. It was recorded that the roots which were inoculated with *Glomus fasciculatum* exhibited greater amount of IAA production than the non-inoculated roots.

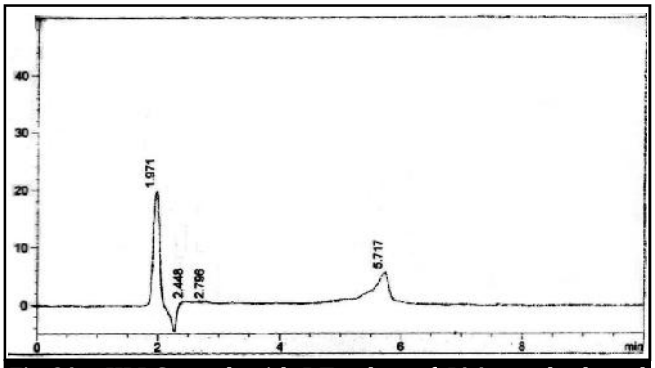

Fig. 2A : HPLC graph with RT values of IAA standards and others

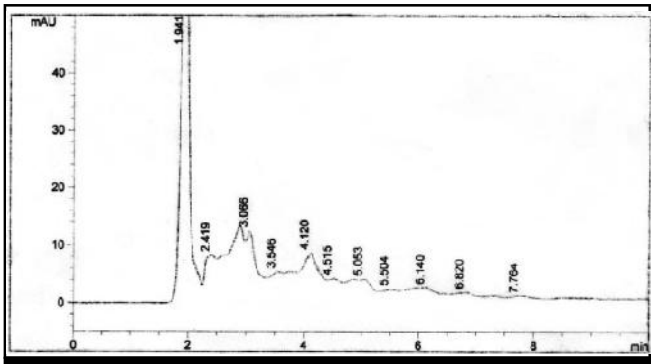

Fig. 2B : HPLC graph with RT values of IAA and other obtained from the shoot sample of Asha in AM fungi uninoculated plant

Table 1 : Indole-3 acetic acid concentration (ppm) in root-shoot of two lentil genotypes under mycorrhiza inoculated and non-inoculated conditions

Standard treatments	RT value for IAA	Area for IAA	Conc. of IAA in 20 µl	Volume of samples	Conc. (ppm) in samples
Standard (Fig:1A)	5.717	127.23	0.0079	20 ml	0.16
Asha(NM shoot) (Fig:1B)	5.504	40.35	0.3170	4.50	1.43
Asha (M shoot) (Fig: 1C)	5.525	91.007	0.7150	5.30	3.79
Asha (NM root) (Fig:1D)	0.00	0.00	0.00	0.00	0.00
Asha (M root) (Fig:1E)	0.00	0.00	0.00	0.00	0.00
L-249(NM shoot (Fig:1F)	5.428	11.48	0.0903	6.20	0.56
L-249 (M shoot) (Fig: 1G)	5.456	45.30	0.3560	6.90	2.46
L-249(NM root) (Fig:1H)	5.891	19.89	0.1560	5.40	0.84
L-249 (M root) (Fig:1I)	5.746	65.03	0.5110	5.70	2.91

*NM indicates Non-mycorrhizal and M indicates mycorrhizal

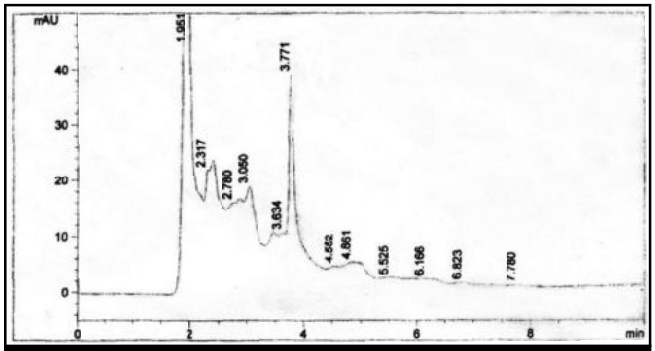

Fig. 2C : HPLC graph with RT values of IAA and others obtained from shoot sample of Asha in AM fungi inoculated plant

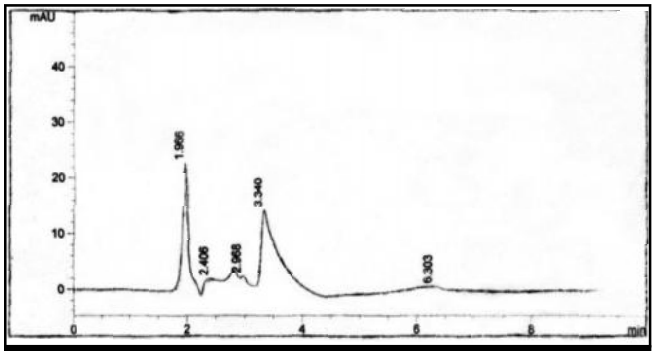

Fig. 2D : graph with RT values of IAA and others obtained from root sample of Asha in AM fungi uninoculated plant

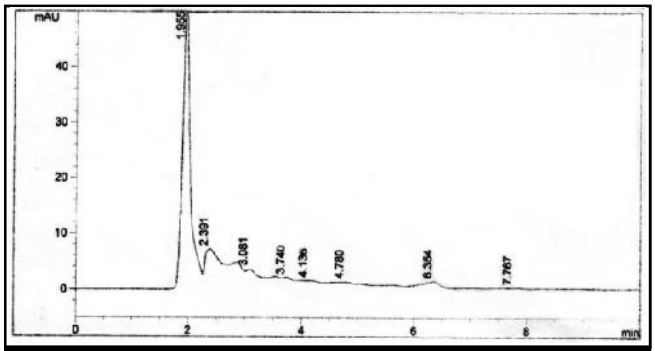

Fig. 2E : HPLC graph with RT values of IAA and others obtained from root sample of Asha in AM fungi inoculated plant

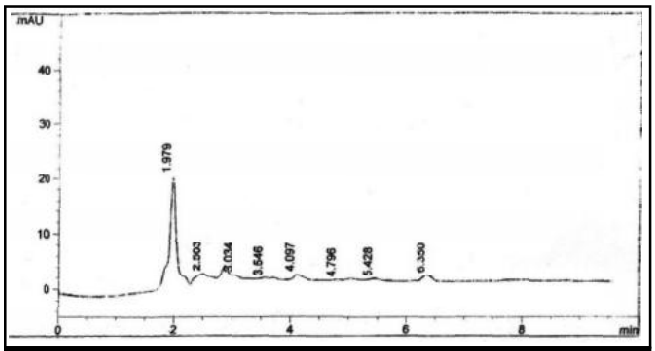

Fig. 2F : HPLC graph with RT values of IAA and others obtained from shoot sample of L-249 in AM fungi uninoculate plant

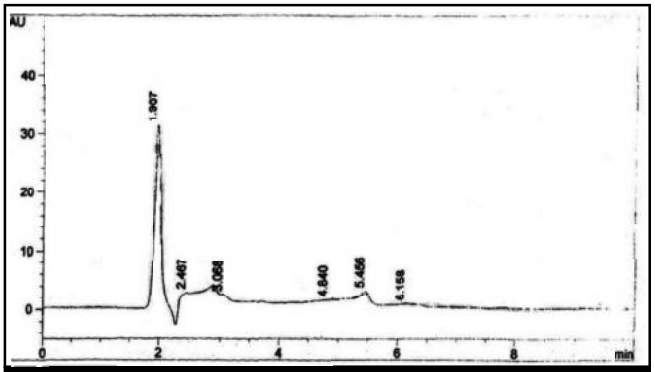

Fig. 2G : HPLC graph with RT values of IAA and others obtained from shoot sample of L-249 in AM fungi inoculated plant

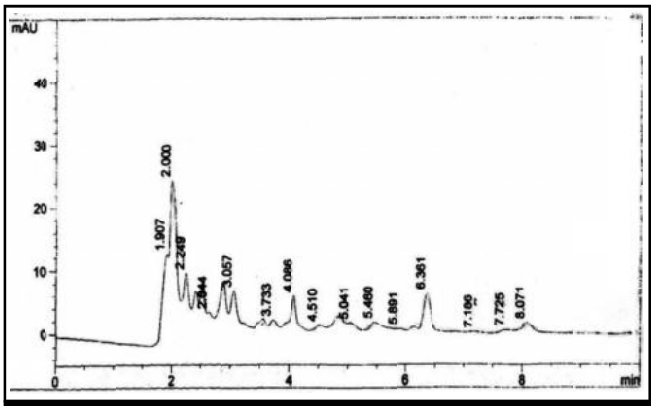

Fig. 2H : HPLC graph with RT values of IAA and others obtained from root sample of L-249 in AM fungi uninoculated plant

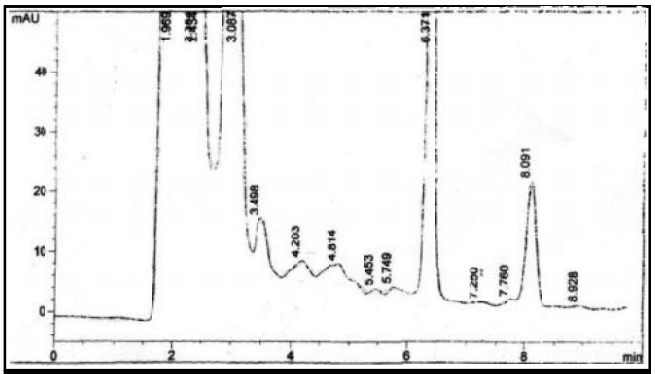

Fig. 2I : HPLC graph with RT values of IAA and others obtained from root sample of L-249 in AM fungi inoculated plant

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