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The role of MHB on Mycorrhiza Infected *Arachis hypogaea* L.

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The MHB concept is generic, It depends neither on the type of mycorrhizal symbiosis nor the on the taxonomy of the MHB strains. Synergetic interaction of mycorrhiza fungi and MHB leading to enhanced nodulation ,mineral uptake and plant growth have been studied .In this study ,the percentage of AM infection was correlated with chitin content of the *Arachis hypogaea* L. Plant root which was coinoculated with MHB and AMF.

Key words: MHB, Arbuscular Mycorrhizal Fungi (AMF), chitin, *Arachis hypogaea*, N_2 fixation.

Mycorrhiza helper bacteria (MHB),a term that was coined by Garbaye (1994). The *Pseudomonas sp*.and *Rhizobium sp*. had a positive effect on the germination and mycelial development of AMF in the soil has well as in root colonization .These bacteria were called mycorrhization helper bacteria (Garbaye *et al.,* 1989). It could soften the cell wall and middle lamella between the cell of the root cortex by producing specific enzyme and thus making the fungal penetration easier . A direct contact between MHB and plant roots may be required to the promotion of mycorrhizal symbiosis (Aspray *et al.,* 2006). MHB effectors that facilitate root colonization could be plant cell wall digesting enzymes which enhance penetration and spreading of fungus within the root tissues (Mossae & Hepper 1975). During the stage of this MHB interaction with VAM proposed mechanism a binding may occur often concerned by general physicochemical parameters suchas pH ,electroconductivity ,NPK level (Bidartonda *et al.,* 2002). Our objectives were to evaluvate the percentage of AM infection by chitin content of the plant root, measurement of plant growth and estimating the level of macroelements in the rhizosphere soil of groundnut with the association of MHB.

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MATERIAL AND METHODS

MHB1 isolation

1 gm of rhizosphere soil was serially diluted in saline up to 10^{-8}. The dilution 10^{-6} was taken to isolate MHB1. 1 ml of diluted soil sample was inoculated on nutrient agar. The plate was then incubated at 37°C for overnight. After the incubation, translucent, circular, greenish color colony was subcultured on king's B medium and performed the following biochemical tests to characterize MBH1. IMViC, TSI,oxidase,catalase tests were done (Bergey's manual of determinative bacteriology, 1994).

MHB2 isolation

Healthy pink color nodules were picked and thoroughly washed with tap water to remove adhering soil particles. Then, it was surface sterilized with 1% mercuric chloride solution and

immediately washed with clean water. It was then crushed with glass rod in the microfuge tubes. The extract was serially diluted in saline up to 10^{-7}. 1 ml diluted sample from 10^{-6} was transferred on YEMA medium. The plate was incubated at 37°C for 3 days.

AM cultivation

AM infected china grass roots were obtained from TNAU, Coimbatore. It was further revived by inoculated on onion plant root. The AM infected onion plant roots were stained with lactophenol cotton blue and the root segments were used as source of AMF inoculum.

Root staining

Hundred fine root segments ,one cm in length were exised ,washed ,cleaned and stained with 0.5%tryphan blue for assesment of mycorrhizal infection. (Phillips and Hayman,1970).The percentage of AM infection on test plant was calculated by the formula,

$$\text{Percentage of Mycorrhizal infection} = \frac{\text{No. of rootbits for AM infection}}{\text{No. of rootbits observed}}$$

Chitin assay

The samples (100 mg of dried plant material or 6 mg of pure chitin) were acid hydrolysed with 5ml of 6N HCl for 16 hrs at 80°C (Swift, 1973) . After adjustment of the pH of the hydrolysate to 7.5 by the addition of 2.5 ml Na acetate per 0.5 ml of the hydrolysate (Plassard *et al.,*1982), the glucosamine residues were assayed colorimetrically (Tsuji *et al.,* 1969).

Colorimetric assay

In order to measure the total absorbance (aldehydes+amino sugars) 1ml of 5%$KHSO_4$ (W/V) and 1ml $NaNO_2$ 5%(W/V)were added to 1ml solution containing glucosamine residues. The absorbance due to the aldehyde group was measured by adding 1ml of 5%$KHSO_4$and 1ml of water. After shaking, the mixture was allowed to stand for 15min (deamination time). Then, 1ml of 12%$H_2NSO_3NH_4$was added . After shaking for 5min, 1ml of 0.5% MBTH was added. The mixture was incubated at room temperature for 1 hr. Then, 1ml of 0.5% $FeCl_3$ was added. The color was allowed to develop for 30 min and the absorbance was measured at 653 nm against a reagent blank.

Pot culture

A.hypogaea.L. seeds were received from Tamilnadu Agricultural University(TNAU), Coimbatore and it allowed to germinate in sterile water. After two days the seeds were sprouted.

Table 1. The biochemical characterization of MHB1 and MHB2*

S.No	Tests	MHB1	MHB2*
1.	Gram 's reaction	Gram negative rod	Gram negative rod
2.	Motility	+	+
3.	Indole	_	
4.	Methyl red	_	
5.	Vogas praskauer test	_	
6.	Citrate utilization test	+	
7.	Oxidase	+	+
8.	Catalase	+	+
9.	Carbohydrate utilization	Only glucose;no gas	Wide range of sugars,no gas
10.	H_2S production	+	_
11.	Lipid hydrolysis	+	_
12.	Starch hydrolysis	_	_
13.	Caesin hydrolysis	_	_
14.	Gelatin liquefaction	+	_
15..	Selective media	King's B medium	YEMA medium
16.	Pigment production	+	_
17.	Optimum temperature for growth	35+/_2°c	25 -30°c
18.	Identification	*Pseudomonas sp*.	*Rhizobium sp*.

*only important biochemical tests were listed.

It was transplanted into experimental pots. Each pots contained approximately 5 kg of soil and clay. At transplanting, seedlings were inoculated with a suspension of MBH1 and MBH2 cells.(1 ml containing 10^6cell/ml). Half of the pots in each treatment were also inoculated with a suspension of chlamydospores of AMF and AM infected onion plant roots. The plants were grown under greenhouse condition .

Data collection

At each 30 days interval the height of the plant was measured and recorded. To determine the level of mycorrhizal infection the roots were removed ,cleansed and stained with lactophenol cottonblue. Then, the percentage of mycorrhizal infection was correlated with chitin content of test plant root tissue.

Nodule study

The degree of nodulation was estimated using an arbitary scale of 0-9 or by counting the nodules under a low power stereomicroscope. Dry weights were determined after air dry at 90°C.An appropriate correction were made for the sample of roots removed.

Soil analysis (Macroelement (N P K) level estimation)

Before seeding and at 90 DAI, the soil NPK level was estimated by chemical analysis and the data was recorded (Tamilnadu Agricultural Lab, Madurai).

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) with AMF and MHB treatment and time of harvest as independent factors.

RESULTS AND DISCUSSION

MHB1 and MHB2 were subjected to morphological, cultural, biochemical and physiological characterization (Table 1) as suggested by SAB(1952), Cappuccino *et al.,*

Table 2. Effect on percentage of AMF infection and chitin content by MHB1 and MHB2 inoculation on *Arachis hypogaea* L. at 90 DAI

Test plants	Percentage of AM infection	Chitin content (mg/g dry root)
Control	22[a]	0.216a
AMF	55	0.520
P_1	18[a]	0.203a
P_2	54*	0.510*
R_1	20[a]	0.211a
R_2	78**	0.612**

P1-MHB1only; P2-MHB1+AM; R1-MHB2 only; R2-MHB2+AM; AMF-Arbuscular mycorhhiza fungi.
a. AM uninoculated plants were undergo natural AM infection.
*,**-significant at the 0.05 and 0.001% levels respectively.
All values are means of at least of 3 replications.

Table 3. MHB1 and MHB2 and mycorrhiza effect on plant growth, nodule number, nodule fresh and dry weight and NPK level of *Arachis hypogaea* L.at 90DAI.

		Mean squares						
Test plants	Degree of freedom	Plant growth(cm)	Numbe r of Nodules	Nodules fresh wt(g)	Nodules dry wt(g)	N(mg/k g soil)	P(mg/k g soil)	K(mg/k g soil)
Control	2	33*	32*	0.160**	0.045**	8.4**	8.5**	3.5*
AMF	2	39**	42**	0.230**	0.054**	6.4**	9.3**	9.8**
P_1	4	36*	33*	0.180**	0.050**	6.3**	14.1**	10.7*
P_2	6	32**	38***	0.320***	0.070***	6.7***	12.2***	11.4***
R_1	4	37*	40***	0.210**	0.061**	7.0**	9.3**	4.7*
R_2	6	40***	49***	0.340***	0.112***	7.4*** (12.8)	10.4*** (18.9)	4.8*** (12.9)
Error	50	3.2	3.03	0.072	0.004	0.61	0.70	0.041

Test plants sample act as source of variables, degree of freedom and mean squares for plant growth, nodule number, nodule fresh and dry weight at 90 DAI.( ) – NPK level at 0 day.
*,**,***—significant at 0.05,0.001,0.0001 probability level respectively.

(1999) and Schaad *et al.*, (2001) for its identification. On the basis of result from these tests the isolate were identified as strain of *Pseudomonas sp* and *Rhizobium sp*. following Berge's manual of systematic bacteriology (1984) and Berge's manual of determinative bacteriology (1994).

Percentage of AM infection and chitin content

The percentage of root colonization of AMF significantly varied within the test plant and was as low as 30% in control plant and as high as 78%in R2. The percentage of AMF infection was positively correlated ($P<0.05$) with chitin content of root tissue. The investigation shows that chitin content was directly related to the AM infection level to the mycorrhizal plant of *Allium porrum* (Rosendhal *et al.,* 1998). In contrast Teuben and De jong (1983) found a negative relationship between infection level and the amount of glucosamine. So, the result present in this work support the former view of positive relationship between AMF and chitin content (Table 2). In case of P_2 the percentage of VAM infection is significant with chitin content.

The plant invest between 10 to 20% of the net photosynthetate production (Jakobsen *et al.,* 1990). According to our observation the maximum plant growth (Table 3) was significant ($P<0.001$) in R_2(40cm) and minimum in P_2(32cm) after 90 DAI. The bidirectional transport of nutrient s occurring at the arbuscules provide the fungi with carbohydrates from photosynthesis and provides the plant with mineral nutrients mainly P which are actively uptaken by the external mycelium (Rosendahl ,1988).

Plant growth, Nodule and NPK study

Rhizobium symbiosis is dependent on high concentration of P. It enhanced P nutrition arising from AM colonization can result in an increase nodulation and N2 fixation (Vazquaz and Barea , 2002). It supports our work, in which the high number of nodules were obtained in R2 (49) (Table3) and AMF42. The intermediate number of nodules were in P_2. The control and P_1 produced nearly equal number of nodules.

The nodule study was correlated with NPK level of plant. P content of the plant rhizosphere soil increase at 90DAI. It is positively correlated with nodule formation. NPK can alter the availabilities of other nutrients and may lead to inhibition of the uptake of some macroelement and micro elements by crop plants (Mulder, 1953 and Davidescu, 1974). However, the difficulties in the effect of organic manures and inorganic fertilizers on soil and crop nutrients balances are still something of a mystery.

CONCLUSION

We have concluded that the rhizobacteria isolated by us from the rhizosphere soil and root nodules groundnut are having mycorrhizal helper activicity. This is a primary work developed by us to improve mycorrhizal biofertilizer efficiency. Mycorrhizal biofertilizer along with helper bacteria improves soil health, Plant nodulation efficiency, and macroelements(NPK) uptake and plant growth rates. Mycorrhizal biofertilizer along with helper bacteria plays a very important role in developing sustainable agriculture.

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