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Interaction Effect of am Fungi, *Rhizobium* and Drought Stress on the Nutritional Efficacy of Cowpea Grown Under Green House Condition

KEYWORDS: *Rhizobium*, AM fungi, Drought and macronutrients.

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ABSTRACT *Ubiquitous occurrence and importance of AM fungi for plant growth is now a well established fact. It is interesting to note that interaction of AM fungi with Rhizobium increases the growth of plants by enhancing the uptake of mineral nutrients. The dual soil application of AM fungi and Rhizobium showed the synergistic effect on protein content of leaves and seed, macro nutrients (NPK) and physiological tolerance of cowpea plants under green house condition with few days period of drought. The dual inoculam was significantly more effective than single species inoculam. It is suggested that biofertilizer application is possible to consider substantially reducing the chemical fertilizers & pesticides in agriculture field and at the same time they increases soil fertility and crop yield.*

Introduction

A mycorrhiza (fungus root) is a symbiotic association of a fungus and the roots of a vascular plant. In this association, the fungus colonizes the host plant's roots, either intracellular as in arbuscular mycorrhizal fungi or extracellularly as in ectomycorrhizal fungi. They are an important component of soil life and soil chemistry. They are commonly divided into ectomycorrhiza and endomycorrhiza. Arbuscular mycorrhizal (AM) fungi are ubiquitous in soil habitats and form beneficial symbiosis with the roots of angiosperms and other plants (Gerdemann, 1968). The AM forming genera of the family includes *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora.*

The wide spread presence of AM fungal symbiosis in nodulated legumes and the role of AM fungi in improving nodulation and rhizobial activity within the nodules, are both universally recognized processes (Barea *et al*., 2005b). The dual soil application of *Rhizobium* and VAM showed synergistic effect on all mung bean cultivars. Among farm cultivars tested variety, "Vaibhav" was found host responsive to root nodulation, growth parameters and grain yield (Manke *et al*., 2008).

Rhizobium inoculation is a promising fertilizer because it is cheap, easy to handle and improves chickpea plant growth and seed quality under pot experiment (Nishita and Joshi, 2010). This study was initiated to know the interaction of *Rhizobium*, AM Fungi and drought stress on the growth of cow pea (*Vigna unguiculata*, L. Walp.,). It was an ecofriendly beneficial to all in some way with the following objective: study the nutrient efficiency in terms of protein content of leaves and seed, macro nutrients namely nitrogen, phosphorus and potassium and physiological tolerance in AM fungi and *Rhizobium* inoculated cow pea plants under green house condition with few days period of drought stress.

Materials and methods

Cow pea (*Vigna unguiculata*, L. Walp.) plants were grown under green house condition. The pots were assigned for the following treatments :C -Control (without biofertilizer), T1-*Rhizobium*, T2-AM fungi, T3-AM fungi and *Rhizobium* treated. The selective AM fungal inoculam was inoculated over the lower layer of soil in each AM labeled pots. The selective *Rhizobium* inoculam was mixed with seeds sown in *Rhizobium* labelled pots. Drought stress was given by withholding water supply from 31st day to 35th day for 5 days.

Determination of nutritional parameters

The legume plant's nutritional and physiological analysis was measured by the following estimations at regular interval of 15 days. The total protein content of leaf and dried seeds (Lowry *et al*., 1951),free proline content (Bates *et al*., 1973), total nitrogen content (Umbreit *et al* ., 1972), total phosphorus content (Bartlett, 1954) and total potassium content was estimated. The data collected in this study was subjected to analysis of variance (ANOVA) and means comparison has done using Duncan's multiple range test (DMRT) (Little and hills, 1978).

Result and discussion

Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes; they include mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms (Goel *et al*., 1999). AM Fungi have been shown to differentially colonize plant roots, causing a variety of effects on plant growth, biomass and photosynthesis. There have been many reports on the effect of vesicular arbuscular mycorrhiza (*Glomus mosseae*) on the growth and productivity of legumes.

The present study showed that leaf protein content was increased gradually in cowpea of both control and AM fungi and *Rhizobium* treated plants as on plant age. This content was declined during drought stress. After drought stress recovery, it was observed maximum (8.96±0.04 μg) as in dual inoculation of *Rhizobium* and AM fungi treated plants and was medium level (6.66±0.26μg and 7.01±0.26 μg) in *Rhizobium* and AM fungi alone treated plants compared to control (4.78±0.05 μg) plants (Table: 1). After harvesting, the seed protein content of cowpea, the AM fungi in individual inoculation and dual inoculation with *Rhizobium* treated plant showed maximum protein content (27% and 30%) and it was minimum in both control and *Rhizobium* alone (20% and 23%) inoculated plants (Fig: 1).

Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline is one of the most common compatible osmolytes in drought stressed plants. For, example, the proline content increased under

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drought stress in Pea (Sanchez *et al*., 1998; Alexieva *et al*., 2001).

The significant ($P \leq 0.05$) rise of proline content was observed as maximum as in T1 (3.97±0.19 mg), T2 (3.84±0.45 mg) and T3 (3.94±0.37 mg) plants and minimum in control (3.16±0.44 mg) plants of cowpea during drought stress. After this stress, the proline levels declined sharply in both control and biofertilizers inoculated plants of cowpea (Table: 1). But it was half or one folds higher during drought stress in both control and biofertilizers inoculated plants. (Fig: 2). This proline accumulation in legume plants was stimulated by the biofertilizers under mild drought conditions. Further, the diffusion of proline after rehydration of legumes might be taken to indicate that proline served as a storage compound during stress.

Collectively, the biofertilizers inoculated cowpea plants showed significant ($P \leq 0.05$) increase in NPK at all stages of growth (Table: 2) than the control (Fig: 3). Drought stress did not affect the nitrogen accumulation in *Rhizobium* and AM fungi at both individual and dual inoculated plants. The results indicated that the *Rhizobium* involved in the nitrogen fixation and AM fungi involved in stimulation of uptake of nitrogen, phosphorus and potassium nutrients by leguminous plants.

Phosphate solubilizing microorganisms play a key role in the plant metabolism and crop productivity. They have been reported to increase the availability and uptake of native soil phosphorus by converting insoluble phosphorus to soluble forms by producing various organic acids (Raja *et al*., 2002). Chen *et al*., (2005) reported that colonization of plants roots by AM fungi greatly increased the plant uptake of both phosphorus and nitrogen. Ruiz- Lozano (2006) reported that AM fungi improved the uptake of nutrients by extra radical mycorrhizal hyphae. Lakshman and Kadam (2011) reported the influence of AM Fungi and *Rhizobium* on the growth and nutrient uptake in *Lens esculenta*. They observed that the inoculated with both the organisms contained higher amounts of nitrogen and phosphorus in their roots compared to the plants inoculated separately with either *Rhizobium* or *Glomus fasciculatum*.

Conclusion

The present study experimentally reported that the dual inoculation of AM fungi and *Rhizobium* could help the growth and yield of cowpea plants. It is suggested that such dual application of biofertilizer in agricultural fields of economically important crops can yield higher quantity with quality of seeds and also such plants showed mild tolerance against drought stress. Thus they are considered as environment friendly fertilizers and do not cause the pollution of any sort.

Table:1
Interaction effect of biofertilizers and drought stress on the leaf protein (µg/g fresh leaf) and proline (mg/g fresh leaf) contents of cowpea

Treatments	Nutrient content	15D	30D	35D	45D	60D
C	Leaf protein (µg)	$3.64^a±0.31$	$3.47^a±0.04$	$3.41^a±0.28$	$3.60^a±0.10$	$4.78^a±0.05$
	Leaf proline (mg)	$0.68^a±0.09$	$1.22^b±0.18$	$3.16^d±0.44$	$2.06^c±0.18$	$1.88^c±0.09$
T1	Leaf protein (µg)	$4.81^b±0.14$	$4.81^b±0.13$	$4.84^b±0.10$	$5.40^b±0.24$	$6.66^b±0.26$
	Leaf proline (mg)	$0.88^a±0.10$	$1.30^b±0.14$	$3.97^e±0.19$	$2.79^d±0.21$	$1.71^c±0.12$
T2	Leaf protein (µg)	$4.87^b±0.25$	$5.03^c±0.11$	$5.00^b±0.15$	$5.34^b±0.11$	$7.01^c±0.26$
	Leaf proline (mg)	$0.80^a±0.07$	$1.20^b±0.08$	$3.84^e±0.45$	$2.60^d±0.34$	$1.88^c±0.03$
T3	Leaf protein (µg)	$6.22^c±0.16$	$7.17^d±0.11$	$6.75^c±0.12$	$7.86^c±0.04$	$8.96^d±0.04$
	Leaf proline (mg)	$0.77^a±0.12$	$1.24^b±0.08$	$3.94^e±0.37$	$2.52^d±0.36$	$1.61^c±0.26$

Values are mean of five replicates ± SD The mean difference is significant at the 0.05

D- Days; Drought stress period- 31 to 35 Days

C- Control T1-*Rhizobium* T2-AM fungi T3- *Rhizobium* + AM fungi

Table:2 Interaction effect of biofertilizers and drought stress on the nitrogen, phosphorus and potassium content of cowpea

Treatments	Nutrient content (mg/g dry weight)	15D	30D	35D	45D	60D
C	Nitrogen	$1.04^a±0.12$	$1.87^b±0.12$	$1.74^b±0.14$	$2.18^c±0.15$	$2.48^d±0.28$
	Phosphorus	$0.79^a±0.10$	$1.16^c±0.15$	$1.01^b±0.14$	$1.30^d±0.06$	$1.32^d±0.12$
	Potassium	$0.94^a±0.03$	$1.34^c±0.02$	$1.29^b±0.04$	$1.35^c±0.07$	$1.43^d±0.02$
T1	Nitrogen	$1.32^a±0.09$	$2.47^b±0.29$	$2.64^b±0.30$	$3.08^c±0.17$	$3.82^d±0.15$
	Phosphorus	$0.89^a±0.08$	$1.16^b±0.11$	$1.20^b±0.16$	$1.78^c±0.09$	$2.03^d±0.12$
	Potassium	$1.22^a±0.01$	$1.45^b±0.03$	$1.58^b±0.02$	$1.87^c±0.02$	$2.32^d±0.10$
T2	Nitrogen	$0.99^a±0.08$	$1.85^b±0.13$	$2.17^c±0.22$	$2.69^d±0.24$	$2.85^d±0.10$
	Phosphorus	$1.07^a±0.10$	$1.58^b±0.22$	$1.72^b±0.16$	$2.08^c±0.15$	$2.96^d±0.21$
	Potassium	$1.26^a±0.02$	$1.56^b±0.11$	$1.97^c±0.01$	$2.21^c±0.05$	$2.54^d±0.02$
T3	Nitrogen	$1.07^a±0.10$	$1.83^b±0.27$	$2.14^b±0.42$	$3.06^c±0.12$	$3.72^d±0.40$
	Phosphorus	$1.20^a±0.12$	$1.68^b±0.19$	$2.16^c±0.18$	$2.37^c±0.31$	$3.48^d±0.40$
	Potassium	$1.32^a±0.12$	$1.78^b±0.01$	$2.10^c±0.03$	$2.56^d±0.03$	$3.32^e±0.03$

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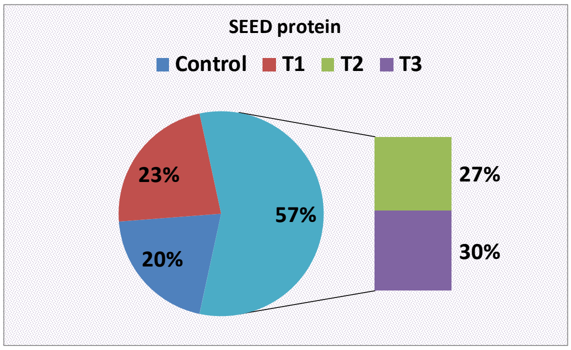
Values are mean of five replicates ± SD The mean difference is significant at the 0.05

D- Days; Drought stress period- 31 to 35 Days;

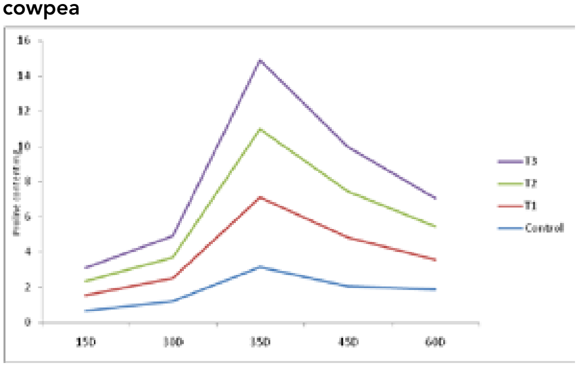
C- Control T1-*Rhizobium* T2-AM fungi
T3- *Rhizobium* + AM fungi

Fig: 1 Interaction effect of biofertilizers and drought stress on the seed protein content (μg/g seed) of cowpea



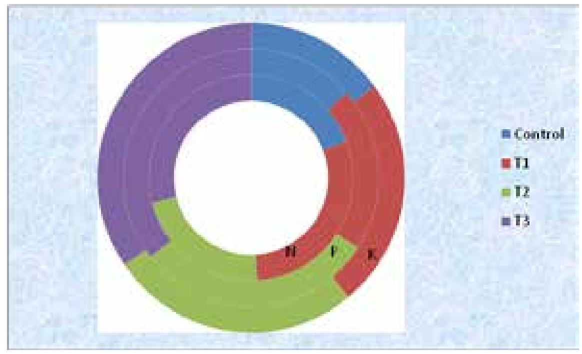
C- Control T1-*Rhizobium* T2-AM fungi
T3- *Rhizobium* + AM fungi

Fig: 2 Interaction effect of biofertilizers and drought stress on the proline content (mg/g. fresh leaves) of cowpea



C- Control T1-*Rhizobium* T2-AM fungi
T3- *Rhizobium* + AM fungi

Fig: 3 Interaction effect of biofertilizers and drought stress on the nitrogen, phosphorus and potassium content (mg/g.dry weight) of cowpea



C- Control T1-*Rhizobium* T2-AM fungi
T3- *Rhizobium* + AM fungi

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