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Alleviation of saline salt stress through pre-sowing biofertilizer seed treatment on crop growth and seed yield in green gram CV ADT3

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Abstract

A field trial was conducted to study the influence of presowing biofertilizer seed treatment using various bio-fertilizers on crop growth and seed yield parameters in green gram under saline stress condition. Freshly harvested, cleaned and graded seeds of green gram cv. ADT3 was imposed with the following seed treatments *viz.*, T_0 – Control (Untreated dry seed), T_1 – Rhizobium @ 600 gm/ha, T_2 – Phosphate solubilizing bacteria @ 600 gm/ha, T_3 – VAM @ 600 gm/ha, T_4 – T_1 + T_2, T_5 – T_1 + T_3, T_6 – T_2 + T_3, T_7 – T_1 + T_2+ T_3. From the results, it was concluded that seed treated with triple inoculation of biofertilizer such as rhizobium, phosphate solubilizing bacteria and VAM @ 600 gm/ha recorded the higher values for crop growth and seed yield parameters under saline stress condition.

Keywords: Greengram, biofertilizer, seed treatment, PSB, VAM, seed yield, saline

Introduction

Green gram is the third most important pulse crop of India in terms of cultivated area and production next to black gram and pigeon pea. A well-drained loamy to sandy loam soils are considered to be the best soils for mungbean seed production. The crop does not grow well on saline, acidic and alkaline soil or waterlogged soils. Salinity is a major environmental stress and is a substantial constant to crop productivity during their growth and development. This is attributed to the fact that Na^+ compete with K^+ for binding soils essential for cellular function (Tester and Davenport, 2003). Move over for over 6% of the world total land area is affected by salinity and sodicity (Munns, 2005). About 20% of the world cultivated land and approximately half of all irrigated land is affected by salinity (Zhu, 2001) which will increase to the tune of land loss up to 30% within next 25 years. In order to alleviate the effect of salinity during crop production, several methods are adopted and one of the method is externally supplying Ca^{2+} ions which reduces the toxic effect of NaCl presumably by facilitating higher K^+/Na^+ selectivity (Munns and Tester,2008). This can be achieved by increasing the Ca^{2+} availability in the rhizosphere which can done by using presowing bioinoculant seed treatment with biofertilizer. Biofertilizer like VAM can increase the availability of Ca^{2+}, P and micronutrient which helps to mitigate to salt stress.

Biofertilizers are microbial inoculants of selective microorganisms like bacteria, algae, fungi already existing in nature. They may help in improving soil fertility by the way of accelerating biological nitrogen fixation from atmosphere, solubilization of the insoluble nutrients already present in soil, decomposing plant residues and stimulating plant growth and production. Nitrogen requirements of pulses are much greater than the cereals or oil seed crops, for producing the equal amount of grain. High economic importance of greengram is reflected in many ways as ability of cultivation in arid and semi-arid areas, growth on marginal lands as well as the improvement of soil quality due to ability of symbiotic nitrogen (N) fixation (SNF). The ability of rhizobial bacteria to fix atmospheric N_2 in symbiosis with legumes, huge natural source of N from the air can be taken up from this symbiotic association leads to decrease or absence of N mineral fertilizer application in the field (Rahman *et al.*, 2008; Abbas *et al.*, 2011). Amount of N mineral fertilizer applied depends on abiotic and biotic factors. The application of bio-fertilizer is very essential because the insoluble phosphate which is not directly available to plants usually comprises around 95-99 per cent of the total soil phosphorous (Upadhyay *et al*., 1999).

Soils containing free calcium carbonate and clays saturated with calcium ions are capable of retaining greater amount of phosphorus. So, the most of the applied phosphorus is fixed in the soil. Several phosphate solubilizing microorganisms have the consistent capacity to increase the availability of phosphate to plants not only by mineralizing organic phosphorus compound but also by rendering inorganic phosphorus compound more available to plants (Arora and Gaur, 1979). Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* and fungi belonging to the genera *Penicillium* and *Aspergillus* posses the ability to bring insoluble phosphates in soil in to soluble forms by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. These acids lower the pH and bring about the dissolution of bound forms of phosphate (Gaur and Ostwal, 1972). With this background, an experiment was conducted to study the effect of presowing bioinoculant seed treatment using various bio-fertilizer on crop growth and seed yield attributing characters under saline stress condition.

Materials and Method:

Genetically and physically pure seeds of Greengram (*Vigna radiata* L.) cv. ADT3 obtained from Tamilnadu Rice Research Institute, Aduthurai, Tamilnadu formed the base material for the present investigation. The bulk seeds were manually cleaned to remove unwanted material from the lot and was graded using BSS 8 x 8 sieve for uniformity. Field experiment was conducted at the University farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Tamilnadu. After cleaning and grading, seed inoculations were done by pelleting the seed with bio fertilizer like *Rhizobium*, *Azospirillum and VAM* @ 600 gm/kg of seeds and in dual and triple inoculation with the bio fertilizers. Pelleting was done by using gum Arabica @ 10% as adhesive. After pelleting, seeds were dried under shade separately to bring back to their original moisture content and used for sowing.

Treatment details were as follows

T_0 – Control (Untreated dry seed)
T_1 – *Rhizobium* @ 600 gm/ha
T_2 – Phosphate solubilizing bacteria @ 600 gm/ha
T_3 – VAM @ 600 gm/ha
T_4 – $T_1 + T_2$
T_5 – $T_1 + T_3$
T_6 – $T_2 + T_3$
T_7 – $T_1 + T_2 + T_3$

Field trial was conducted to study the effect of presowing biofertilizer seed treatment using various biofertilizer on crop growth and yield parameters in greengram cv ADT3 adopting randomized block design with three replications. The plot size was 4m x 5m. The crop was raised with the spacing of 30cm x 10cm and the recommended packages of practices for seed production were followed. The following were the observations recorded on growth and yield parameters viz., plant height, number of branches, number of leaves, days to 50% flowering, number of clusters, number of pods per plant, pod's length, number of seeds per pod, 100 seed weight, seed yield per plant, seed yield per plot, seed yield per hectare and some physiological parameters were recorded.

After harvest, the resultant seeds were pooled, cleaned, dried to a moisture content of 8% and graded using BSS 8 × 8 sieve for uniformity. The randomly selected sample of seeds from each treatment was evaluated for their seed quality characters such as Germination per cent (ISTA Rules,2013), Root Length (cm), Shoot Length (cm), Dry matter production (mg/10 seedlings), Vigour index length (Abdul-Baki and Anderson,1973) and Vigour index mass(Abdul-Baki and Anderson,1973). The data were statistically analysed using ANOVA. Wherever necessary, the percentage values were transformed to angular (arc sine) values, before carrying out the statistical analysis. The critical difference (CD) was worked out of 5 per cent (P = 0.05) level.

Result and Discussion:

High quality seed is the key to successful agriculture. Modern agriculture with its bias for technology and precision, demands that each and every seed should readily germinate and produce a vigorous seedlings ensuring high yield. Uniformity of growth and synchrony in development are highly desirable characters for mechanized cultural operations. As such, only seed of high quality, generally pure and morphologically, pathogenically and physiologically sound is needed to increase the productivity.

Seeds are exposed to changing and often adverse environments in the soil for a considerably long period beginning with sowing and ending with emergence. The period of imbibition is extremely sensitive to changes in the environment and slight and sudden changes appear to profoundly affect the seedling emergence. Numerous efforts have been directed, therefore, to decrease the period between sowing and emergence on the assumption that quick germination or emergence will spare the seed, the 'agony' of prolonged exposure to hostile environment during imbibition and seedling establishment and thus improve crop stands.

In general, beneficial microorganisms in biofertilizer accelerate and improve the plant growth and protect the plant from pest and disease. The plant growth characters like plant height, number of branches/plant, number of leaves/plant and leaf area showed higher value in treatment T_7 under saline condition. Because of the combined inoculation treatment, salinity condition shows only 20% reduction in plant height, 27% reduction in number of branches per plant, 27% reduction in number of leaves per plant and 31% reduction in leaf area. This may be due to the combined effect of biofertilizer produced considerable amount of plant growth promoting substances (*e.g.* IAA and siderophores) which stimulates growth and may also contribute to the suppression of pathogens (Khan *et al.*, 2002; Khan *et al.*, 2009).Combining an improved plant nutrient supply with nitrogen (*Rhizobium*) phosphorus (PSM) and VAM (improved uptake of water, phosphorus and other macro and micronutrient, Zandavalli *et al.*, 2004) with plant growth promotion appears to have additive and possibly even multiplicative effects. The beneficial effects of N_2 fixing bacteria and PSM nodulation was reported by Zaidi *et al.* (2004).

Increase in plant growth through the production of growth promoting substances *e.g.* Auxins and gibberellin have been reported (Ponmurugan and Gopi, 2006). An increased availability of P can enhance nitrogen fixation and the supply of nutrients like phosphorus, sulphur, iron and copper (Mcmillan, 2007; Cakmakci *et al.,* 2006). VAM fungi in combination treatment along *Rhizobium* and PSB accumulate lower Na^+ content in their shoot and protect the leaf metabolism as reported by Rabie and Al-Humiany (2004). Salt stress inhibited cell division and cell expansion and subsequently leaf expansion (Hernandez *et al.,* 2003). The similar result on plant growth parameter was reported by Salahedinmoradi *et al.* (2013). Reduction in leaf area in case of control under salinity may be due to the death of older leaves caused by ion-toxicity which may prevent the supply of nutrients and hormone to young leaves (Munns, 1993, Tammam 2003) leading to reduced chlorophyll content.

The physiological characters like chlorophyll 'a', chlorophyll 'b', and total chlorophyll content were more in T_7 followed by T_5 under salinity condition. The slight reduction was observed under salinity condition for these traits compared to normal condition. This may be due to the microorganism which reduces the toxicity of salinity which leads to reduction in Na uptake together with a concomitant increase in P, N and Mg absorption and high chlorophyll content may be important salt-alleviating mechanisms for plants growing in saline soil. P may be helpful in mitigating salt stress by overcoming the P binding capacity of the soil. Salt interferes with chlorophyll synthesis more in control. The observed decrease of chlorophyll may be attributed to both of the increased degradation and inhibited synthesis of that pigment (Garsia-Sanchez *et al.,* 2002). It might be that Na has an antagonistic effect on Mg absorption (Alam, 1994). A higher concentration of Mg reduced the antagonistic effect of Na. VAM fungi are effective in the absorption of Mg and suppression of Na under salt stress condition (Rao and Tak, 2002). Reduced Na may help to increases chlorophyll content and N, S and Ca helps for formation of chlorophyll and Mg is an important constituent of chlorophyll (Pandey and Sinha, 1999) may be the reason for increased chlorophyll in T_7 and T_5 and also due to the positive influence on osmotic relations between the soil and roots.

The yield attributing parameters such as number of clusters per plant, number of pods per plant, number of seeds per pod, 100 seed weight, seed yield per plant, seed yield per plot and seed yield per ha showed increased trends in case of treated seeds when compared to control under salinity condition. The highest yield attributing parameters such as number of clusters, number of pods, number of seeds and seed yield/plant, seed yield/plot and seed yield/ha was recorded in T_7 followed by T_5. The yield reduction under salinity may be due to the effects of salt ions around the root zone. Salinity affects the seed yield as it has pronounced adverse effect on reproductive growth as the number of pods per plant decreases with increasing salinity (Elahi *et al.,* 2004). Salinity decrease the number of nodules per plant but increase the size of nodules. The negative effect of salt stress on nitrogen fixation by greengram may be due to three different responses (*i.e.*) effect on activity of rhizobia for infection of legumes effect on the growth and development of nodules and finally, direct effect on activity on nodules for nitrogen fixation (Bouhmouch *et al.,* 2005). Reduced nodule formation of greengram at low levels of salinity could be due to adverse effects on the process of nodule initiation; this process has been reported to be very sensitive to osmotic stress.

Salinity increases Na^+ and Cl^- concentration which not only reduces Mg, Ca_2^+ and K^+ availability but also decreases their mobility and transport to the actively growing plant parts thus affecting the quality of both vegetative and reproductive organs which directly affects the seed yield. Metabolic toxicity of Na^+ in plants is mainly due to its ability to compete with K^+ for essential binding sites for cellular function. Thus, high levels of Na^+ or lower K^+ to Na^+ ratio can disturb various enzymatic processes in plants. Thus salt stress affects the morphology anatomy, ultra-structure and metabolism of plant species (Prat and Fathi-Ettai, 1990), results in overall reduction in plant growth and yield.

Under salinity condition, increase in yield attributing character might also be due to the combined activity of microorganisms. Salinity affects the spore germination of VAM but *rhizobium* may affect VAM fungi and their host plants through a variety of mechanisms including effects on the receptivity of the root, root fungus recognition, fungal growth, modification of the chemistry of the rhizospheric soil thereby osmotic adjustment and uptake of Na^+. Further *Rhizobium* promotes VAM establishment through improved spore germination. PSB also enhance the biological nitrogen fixation in plants (Kennedy *et al.,* 2004). PSB enhance the yield by increasing solubilization and uptake of nutrients, production of plant growth regulators, suppression of pathogen and producing metal binding molecules called siderophores. Thus in the present study, *Rhizobium*, PSB and VAM were found to be good competitors due to their excellent synergistic effect, fixation of N_2 and other micronutrient uptake which leads to better growth and yield characters of the greengram. This findings are in confirmity with the results of Zaidi *et al.* (2003) in chickpea and Zaidi and Khan (2006) in greengram.

Tripartite cultures in general were effective when compared to other treatments. From our experiment, we observed that less reduction in RPG values in T_7 when compared to all other treatments. The maximum reduction was observed in T_0. The same effect was reflected in the salt tolerance also. The explanation is the VAM could be stimulated in quantity, efficiency and longevity by metabolic product released from the inoculated bacteria. Moreover, root exudation and plastically might have been changed by PSM inoculation, which could also affect VAM development (Poi *et al.,* 1989). The resulting inhibitory effect of dual inoculation of PSB and VAM could be due to the release of inhibitory metabolites in the growing environment, which adversely affected the plant growth (Zaidi *et al.,* 2004). This may be due to negative interaction occurred between PSB and VAM which induced to release more organic acids by PSB. Increased acidity might have decreased the colonization of VAM fungus in the greengram (Venkateswarlu *et al.,* 1984).

Thus from this study, the superior performance of the T_7 (treated with the mixed inoculation of N_2 fixing bacteria, PSB and VAM fungus) improved the seed quality, plant growth, vigour, nutrient uptake and dramatically increased the yield of greengram than other single (or) dual inoculation treatment which could be ascribed to carryover mechanism of the resultant seed with better membrane stability. This may be due to the presence of higher amount of metabolites (better translocation of sugars from source to sink), which helps in resumption of embryonic growth during germination and due to accumulation of higher quantity of seed constituents like protein (structurally stable compounds) and carbohydrates which inturn results in higher germination and vigour of the seeds. This results are in concurrence with the findings of the previous work by Zaidi *et al.* (2003) in chickpea and Zaidi and Khan (2006) in greengram; Ashrafuzaman *et al.* (2009) in rice.

Table 1: Effect of pre-sowing seed bioinoculant treatment using various biofertilizer on crop growth parameters of greengram under saline stress condition.

Treatment	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Chlorophyll a content	Chlorophyll b content	Total chlorophyll content
T_0	29.73	20.63	404.28	0.5640	0.2630	0.8270
T_1	41.47	25.10	478.09	0.6953	0.3087	1.0040
T_2	37.97	23.43	461.87	0.6247	0.2830	0.9077
T_3	39.93	23.93	465.78	0.6653	0.2923	0.9577
T_4	41.30	24.20	478.73	0.7067	0.3180	1.0247
T_5	42.63	25.20	471.52	0.7293	0.3340	1.0633
T_6	39.33	23.97	463.17	0.6920	0.3027	0.9947
T_7	44.532	26.80	509.11	0.7493	0.3673	1.1167
Mean	39.61	24.16	466.57	0.6783	0.3086	0.9870
SED	0.5299	0.4681	8.2185	0.0065	0.0045	0.0085
CD (P=0.05)	1.0811	0.9549	16.7657	0.0133	0.0092	0.0173

Table 2: Effect of pre-sowing seed bioinoculant treatment using various biofertilizer on yield attributing characteristics of greengram under saline stress condition.

Treatment	Number of Clusters per plant	Number of pods per plant	Number of seeds per pod	Seed yield per plant	Seed yield per plot	Seed yield per ha
T_0	4.33	12.77	4.33	0.6717	0.4343	217.45
T_1	7.33	16.63	5.99	2.0463	0.6187	309.34
T_2	5.33	13.73	5.05	1.0867	0.4966	248.32
T_3	6.67	14.93	5.66	1.6443	0.5579	278.95
T_4	6.33	14.37	6.20	1.9790	0.6573	328.65
T_5	7.67	17.23	6.18	2.5467	0.7590	379.50
T_6	6.00	13.83	5.60	1.2413	0.5753	287.65
T_7	8.00	17.83	7.29	3.0267	0.8590	429.50
Mean	6.46	15.17	5.79	1.7803	0.6198	309.92
SED	0.5288	0.2114	0.1369	0.0625	0.0250	6.4694
CD (P=0.05)	NS	0.4312	0.2792	0.1274	0.0510	13.1977

Table 3: Effect of pre-sowing seed bioinoculant treatment using various biofertilizer on seed qualities of resultant seeds of greengram under saline stress condition.

Treatment	Germination (%)	Root length(cm)	Shoot length(cm)	Dry matter production (g / 10 seedlings)	Seedling vigour Index Length	Seedling vigour Index Mass
T_0	89(70.69)	10.09	12.65	0.1100	2012.84	9.79
T_1	92(73.63)	12.70	17.85	0.1495	2810.10	13.75
T_2	90(71.65)	11.72	15.65	0.1300	2688.08	11.70
T_3	93(74.70)	12.35	16.30	0.1360	2650.05	12.58
T_4	92(73.63)	12.90	17.10	0.1520	2744.85	13.91
T_5	93(74.70)	13.64	18.20	0.1725	2961.23	16.04
T_6	91(72.61)	12.06	16.75	0.1445	2621.23	13.90
T_7	93(74.70	13.84	19.55	0.1825	3105.29	16.98
Mean	92(73.63)	12.41	16.76	0.1471	2699.21	13.58
SED	0.8574 (0.8807)	0.1316	0.1116	0.0019	56.5992	0.2694
CD (P=0.05)	1.7233 (1.7702)	0.2645	0.2243	0.0038	113.7644	0.5414

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