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Augmenting Nitrogen and Phosphorus Content of Mungbean Seeds through Salt Tolerant Bacteria

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Abstract: *A total of 75 salt affected soil samples were collected from the saline rhizospheric soils of Tungabhadra command area and are used for the isolation of salt tolerant bacteria. A total of 450 salt tolerant bacterial isolates were isolated on nutrient agar medium supplemented with dissimilar salt (NaCl) concentration viz.,1, 3, 6, 9 and 12 per cent and all the isolates could also able to grow at 12 per cent salt. Finally, 20 salt tolerant bacteria were selected and subjected for morphological, biochemical and PGPR activity tests, in which GP-21, GS-36 and MP-63 emerged as an efficient P- solubilizer, nitrogen fixer and Indole acetic acid producer, respectively. Combined inoculation of GP-21 + GS-36 + MP-63 significantly enhanced nitrogen (3.11g kg^{-1}) and phosphorus (0.31 g kg^{-1}) content of mungbean seeds, compared to all other treatment and uninoculated control (1.91 and 0.12 g kg^{-1}) and resulted in the higher seed yield (1.58 g $plant^{-1}$) compared to uninoculated control (0.33 g $plant^{-1}$).*

Keywords: Salt tolerant, nutrient agar, nitrogen and phosphorus content

1. Introduction

Soil salinization is a serious problem, particularly in arid and semi-arid regions of the world, caused due to the accumulation of salts *viz.*, sodium, potassium, magnesium, calcium, chloride, sulphate, carbonate and bicarbonate (1).Most abundant salt found in the environment is NaCl. Na^+ induces osmotic stress and ionic toxicity in many plants (2), (3) and (4). Mungbean (*Vigna radiata* L.) is a pulse crop of special importance and provides an inexpensive source of proteins rich in leucine, phenylalanine, lysine, valine, isoleucine and certain vitamins (5). Presence of higher salt concentration in soil drastically reduces growth and development of mungbean (6). Many salt tolerant plant growth promoting (PGP) rhizobacteria like *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* capable producing Indole acetic acid (IAA) and gibberellins, fixation of nitrogen, solubilisation of phosphorus, have been reported from saline areas of India capable of improving the plant health under salt stress conditions (7), (8) and (9)

2. Materials and Methods

Seventy five salt affected soil samples were collected from rhizospheric soils of paddy, sorghum and cotton of Tungabhadra command area. Salt tolerant bacteria were isolated on nutrient agar (NA) medium supplemented with 1, 3, 6, 9 and 12 per cent salt (NaCl). The pure cultures were stored in refrigerator at 4°C and subsequent sub culturing was carried on NA broth and medium supplemented with 12 per cent salt. Shape, size, structure of colonies, pigmentation and Gram reactions of all the isolates were recorded. Biochemical tests *viz.*,starch hydrolysis, catalase, indole production, H_2S production, urease, gelatinase, protease tests, citrate utilization and nitrate reduction tests for all the 20 isolates were carried out. Indole acetic acid (IAA) production by salt tolerant bacterial isolates was estimated by the method prescribed by Tien *et al*., (10) NH_3 production (11), *In vitro* N_2 fixation (12), HCN production (13), Siderophore production by using Chrome azurol assay (CAS) developed by (14) and Phosphate solubilisation by using Pikovskaya's agar plates supplemented with 12 per cent salt (15).

3. Pot Culture Experiment

Efficient salt tolerant plant growth promoting (PGP) bacteria *viz.*, GP-21 (P-solubilising), GS-36 (nitrogen fixing) and MP-63 (IAA producing) were selected for *in vivo* investigation involving pot culture experiment under green house condition. Artificial salt stress was induced to the plants by adding salt in to the soils of inoculated and uninoculated control pots, three times a week in the form of aqueous solution of NaCl (200 mM) starting from 15 DAS and continued till the harvesting of the crop (60 DAS). Influence of these local salt tolerant PGP bacterial isolates on the nitrogen and phosphorus content of mungbean seeds under salt stress was evaluated.

4. Result and Discussion

Inoculation of salt tolerant bacteria significantly enhanced nitrogen and phosphorus content of the seeds under the elevated salt level, when analysed at 60 DAS. Individual inoculation involving GS-36 significantly enhanced the seed nitrogen content (2.84 g kg^{-1}) followed by PGP- USB- ED7 (reference) (2.74 g kg^{-1}), GP-21 (2.62 g kg^{-1}) and MP-63 (2.33 g kg^{-1}) compared to uninoculated control (1.91 g kg^{-1}) and control (2.39 g kg^{-1}). However inoculations of these strain in combination had maximum effect, as combined inoculation (GP-21+ GS-36+ MP-63) significantly increased the seed nitrogen content (3.11 g kg^{-1}) compared to all other treatment (Table 1 and Figure 1).

Table 1: Influence of salt tolerant PGP bacteria on seed nitrogen and phosphorus

Treatments	Seed N content (g kg^{-1})	Seed P content (g kg^{-1})	Seed yield (g plant^{-1})
T_1 - Control	2.39^d	0.18^d	0.94^b
T_2 - Uninoculated control	1.91^e	0.12^f	0.33^f
T_3 - GP-21	2.62^c	0.25^b	0.63^e
T_4 - GS-36	2.84^b	0.15^e	0.82^c
T_5 - MP-63	2.33^d	0.13^f	0.68^d
T_6 - GP-21+ GS-36+ MP-63	3.11^a	0.31^a	1.58^a
T_7 - PGP- USB- ED7 (Reference)	2.74^{bc}	0.24^c	0.92^b
S.Em±	0.06	0.01	0.01
CD at 1%	0.24	0.02	0.01

Note: Mean values are average of three replications

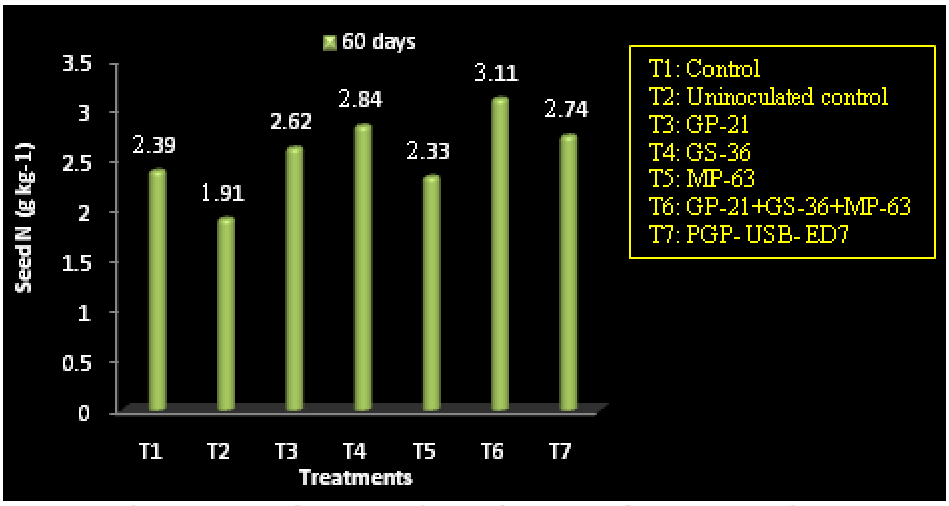

Figure 1: Influence of salt tolerant bacterial isolate on seed nitrogen content

Less nitrogen content in the seeds of uninoculated control treatment was mainly attributed to malnutrition and stress induced by the accumulation of Na^+ and Cl^- disrupting photosynthesis and other metabolic process (2), (3) and (4). But the plants inoculated with combined inoculum GP-21+ GS-36+ MP-63 were highly salt tolerant, alleviated the plants from salt stress and related osmotic challenges, improved the plant nutrition status by facilitating uptake of phosphorus, nitrogen and IAA, respectively, resulting in higher seed nitrogen content. Similarly, phosphorus content of seeds of all the treatments were analysed. Combined inoculation (GP-21+ GS-36+ MP-63) significantly increased the seed phosphorus content (0.31 g kg^{-1}) compared to all other treatment, followed by individual inoculation of GP-21 (0.25 g kg^{-1}), PGP- USB- ED7 (0.24 g kg^{-1}), GS-36 (0.15 g kg^{-1}), MP-63 (0.13 g kg^{-1}), compared to uninoculated control (0.12 g kg^{-1}) and control (0.18 g kg^{-1}) (Table 1 Figure. 2).

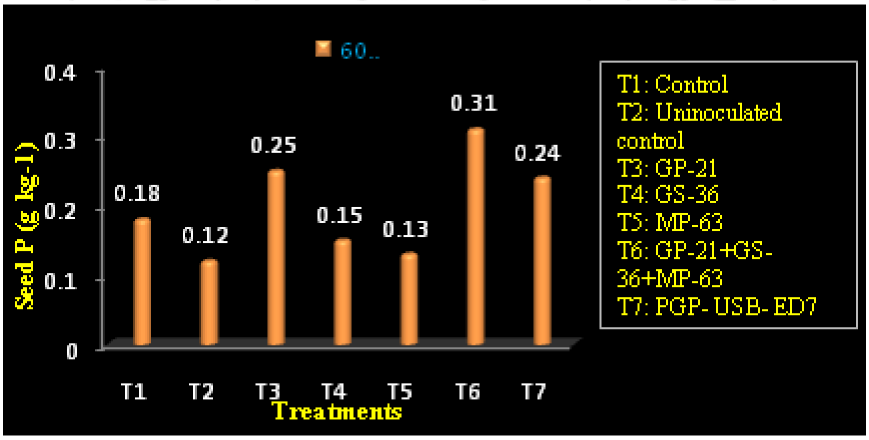

Figure 2: Influence of salt tolerant bacterial isolate on seed phosphorus content

In this investigation level of seed nitrogen and phosphorus content were more pronounced due to the combined inoculation of GP-21+ GS-36+ MP-63, which enhanced the root growth and development through enhanced nitrogen fixation by GS-36, IAA production by MP-63 and greater phosphorus solubilisation by GP-21 leading to higher accumulation of nitrogen and phosphorus in seeds, where as individual inoculants GS-36 and GP-21 also enhanced the seed nitrogen and phosphorus content (Table 1). Similar effect of increase in the seed nitrogen and phosphorus content was recorded after inoculating plants with halotolerant bacteria capable of fixing nitrogen, solubilising phosphorus and producing plant growth promotional substance under the elevated salt stress condition (9), (16), (17), (18) and (19).Mungbean inoculated with salt tolerant ACC-deaminase producing rhizobacteria grown under saline condition improved the nitrogen and phosphorus content of the seeds, compared to uninoculated control (6).

5. Conclusion

Salt affected soil is regarded as a major hurdle for agriculture in the arid and semi arid parts of the world resulting in the crop failure and drastic reduction in the yield. Identification, isolation and characterization of such salt tolerant bacteria from saline environment capable of discharging plant growth promotional substances could render ecofriendly service to the nature and farmers by alleviating crop plants from rhizospheric salt stress and producing a healthy and economic yield.

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