Plant growth stimulation of wheat (*Triticum aestivum* L.) by inoculation of salinity tolerant *Azotobacter* strains

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Abstract Five salinity tolerant *Azotobacter* strains i.e., ST3, ST6, ST9, ST17 and ST24 were obtained from saline soils. These *Azotobacter* strains were used as inoculant for wheat variety WH157 in earthen pots containing saline soil under pot house conditions, using three fertilizer treatment doses i.e., control (no fertilizer, no inoculation), 90 Kg N ha⁻¹ and 120 Kg N ha⁻¹. Inoculation with salinity tolerant *Azotobacter* strains caused significant increase in total nitrogen, biomass and grain yield of wheat. Maximum increase in plant growth parameters were obtained after inoculation with *Azotobacter* strain ST24 at fertilization dose of 120 kg N ha⁻¹ and its inoculation resulted in attaining 89.9 cms plant height, 6.1 g seed yield, 12.0 g shoot dry weight and 0.7 % total nitrogen. The survival of *Azotobacter* strain ST24 in the soil was also highest in all the treatments at 30, 60 and 90 days after sowing (DAS). However, the population of *Azotobacter* decreased on 90 DAS as compared to counts observed at 60 DAS at all the fertilization treatments.

Keywords *Azotobacter* · Wheat · Salinity tolerance · Plant height · Shoot dry weight · Seed yield

Introduction

Salinization, soil erosion and desertification are the most pressing ecological concerns affecting the degradation and loss of

productive agricultural land. In addition, several environmental stresses have been found to affect crop productivity (Jones 2009; Tiwari et al. 2011). Salinity is one of the serious environmental constraints and is increasing gradually particularly in arid and semiarid regions of the world due to mismanagement of agricultural practices. Nearly 40 % of world's surface has salinity problems (Jadhav et al. 2010). Salt stress negatively affects the establishment, growth and development of plant as well as biological diversity and stability of the ecosystems (Cordovilla et al. 1999; Moradi et al. 2011). The saline conditions may decrease seed germination either by creating osmotic potential or by toxic effects resulting from high concentrations of sodium ions in the soil (Hussain et al. 2003). Thus, the limitation of water absorption by seeds can reduce the rate of germination and may retard plant development (Poljakoff-Mayber et al. 1994).

Suitable biotechnological approaches could be used to improve crop productivity in salt affected areas (Zahir et al. 2004; Naz et al. 2012). For example, development of salt-resistant crop varieties (Flower et al. 1997) and use of salt-tolerant microbial strains associated with roots of different crops may improve plant resistance towards adverse salinity conditions (Yang et al. 2010; Tiwari et al. 2011). Moreover, salt-tolerant microorganisms may improve soil fertility through decomposition of organic matter and nutrient cycling, by fixation of atmospheric nitrogen or through production of growth hormones (Sturz and Nowak 2000; Magda et al. 2003; Wu et al. 2009; Sindhu et al. 2010). Recently, emphasis is also given on the application of trait-specific microbial inoculants that can be used for detoxification of toxicants (Weyens et al. 2009; de-Basan et al. 2012), disease suppression of pathogenic organisms (Sindhu et al. 2009) and stress tolerance in plants (Nadeem et al. 2007; Yang et al. 2008). Rashid et al. (2012) found that eight

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Pseudomonas spp. strains (out of 25 isolated strains) having highest level of salt tolerance, exhibited ACC deaminase activity and also showed several growth-promoting characteristics.

Inoculation effect of free-living *Azotobacter* species are largely associated with nitrogen fixation (Lakshminarayana 1993), formation of various physiologically active growth hormones (Gonzalez-Lopez et al. 1986), protection against root pathogens (Verma et al. 2001), stimulation of beneficial rhizospheric microorganisms and enhancement of plant yield (Sindhu et al. 1994; Lakshminarayana et al. 2000). Salinity and pH were found to reduce microbial population in the soil and rhizosphere, and also adversely affected beneficial activities (Ibekwe et al. 2010; Moradi et al. 2011). Inoculation of *Azotobacter chroococcum* and *Streptomyces niveus* on maize plants grown under different salinity levels were found to influence total soluble sugars, total free amino acids, proline and total soluble proteins, DNA and RNA in shoots and roots, which resulted in higher salt tolerance of the plants (Magda et al. 2003). Alikhan et al. (2007) reported that inoculation of three salt tolerant bacterial strains i.e., *A. chroococcum*, *A. vinelandii* and *A. beijerinckii* enhanced 75.8 % and 56.12 % root and shoot dry biomass in *Ceriops decandra* and *Avicennia marina*, respectively. Similarly, inoculation of *Azotobacter* in *Brassica oleracea* var. *italica* and wheat resulted in greater plant growth stimulation (Egamberdieva et al. 2008). Thus, considering the perspectives of crop production losses due to the severity of salinity stress, the use of salt-tolerant microbial inoculants has become more important. Therefore, the present investigation aims at application of salinity tolerant *Azotobacter* strains as biofertilizer for wheat crop under saline soil conditions.

Materials and methods

Soil sample collection and characteristics of *Azotobacter* isolates

Four soil samples were collected from saline soils of different electrical conductivity (EC) from different villages namely Dobhi, Shakhpura and Kirdhana of Haryana (India). The soils

pH and electrical conductivity was measured. Soil was enriched in Jensen's medium (JM) broth at 28 ± 2 °C for 2–5 days (Jensen 1951). Enriched samples were serially diluted and plated on JM plates for isolation of free living nitrogen-fixing bacteria *Azotobacter*. *Azotobacter* isolates were selected on the basis of their growth on Jensen's medium containing different sodium chloride concentrations viz, 2, 4, 6 and 8 % (0.342 to 1.36 M) and other beneficial characteristics such as carbon utilization, IAA production, ammonia excretion and total nitrogen (Chaudhary et al. 2011).

Inoculation of wheat seeds with *Azotobacter* strains

The effect of *Azotobacter* strains on the growth and yield of salt tolerant wheat (*Triticum aestivum*) var. WH157 was studied under pot house conditions. Salinity tolerant *Azotobacter* strains were inoculated in Jensen's broth and incubated on shaker at 28 ± 2 °C for 3 days. Wheat seeds were inoculated with *Azotobacter* strains (10^8 cfu ml⁻¹) and sown in pots containing 2.5 kg of unsterilized saline soil in triplicate. Experiment was done with three treatments using two doses of fertilizer nitrogen (urea) i.e., control (without fertilizer and without culture), 90 Kg N ha⁻¹ and 120 Kg N ha⁻¹. Phosphorus (P) and potassium (K) fertilizers were applied to soil at 100 % of the recommended doses i.e., P₆₀-K₃₀ha⁻¹, respectively. After germination, three plants were kept in each pot. Plant height, shoot dry weight, grain yield and total nitrogen in the plants were determined at 135 days after sowing (DAS).

Determination of microbial count

Soil samples collected from wheat grown pots were used for microbiological analysis and survival of *Azotobacter* strains was determined at different stages of plant growth i.e., 30, 60 and 90 days by dilution plate count method. Samples were serially diluted in 9 ml water blanks upto 10^{-4} . One ml of different diluted samples was pour plated on nutrient agar medium plates under aseptic conditions in front of Laminar flow chamber. The plates were incubated at 28 ± 2 °C for 2–3 days in a BOD incubator. Numbers of microbial colonies appeared on medium plates were counted.

Table 1 Soil samples used for the isolation of *Azotobacter* isolates

Sample source	Soil type	*E.C.	No. of soil isolates obtained	Colony forming units (CFU) g ⁻¹ soil
Shakhpura (Hansi)	Sandy loam	3.6	7	2.6×10^3
Kirdhana (Fatehabad)	Sandy loam	2.7	8	2.9×10^3
Dobhi (Hisar)	Sandy loam	1.7	5	3.2×10^3
Dobhi (Hisar)	Sandy loam	1.1	7	3.7×10^3

*E.C electrical conductivity

Table 2 Effect of salinity tolerant *Azotobacter* strains on plant growth of wheat at 135 days of plant growth

<i>Azotobacter</i> strains	Height (cm)			Shoot dry weight (g)			Seed yield (g)			Total % N		
	^a Kg N ha ⁻¹											
	0	90	120	0	90	120	0	90	120	0	90	120
Control	57.3	60.5	64.3	4.2	5.2	5.7	1.9	2.5	3.1	0.16	0.26	0.36
ST3	67.2	78.1	87.8	5.9	7.7	10.4	3.4	4.6	5.8	0.33	0.49	0.65
ST6	59.3	70.9	71.8	4.6	5.3	6.6	2.2	3.0	4.1	0.27	0.37	0.51
ST9	64.5	76.2	78.8	4.8	6.0	8.4	2.5	3.3	5.2	0.27	0.34	0.51
ST17	67.7	70.7	83.2	5.8	6.4	10.3	3.3	3.7	5.5	0.33	0.40	0.58
ST24	68.4	78.4	89.9	5.9	7.4	12.0	4.8	5.0	6.1	0.40	0.50	0.70
CD at 5 %	8.010			1.879			0.878			0.086		

Values given are the mean values of three replications

^a indicates the doses of nitrogen fertilizer applied

Results

Benefits of inoculation with effective biofertilizer strains depend upon their establishment in the rhizosphere of the plant. Thus, there is an urgent need to characterize bacterial inoculants like *Azotobacter* that can enhance crop production under different stress conditions for agriculture sustainability (Doran and Zeiss 2000). In this study, twenty seven bacterial isolates were obtained from four different saline soils (Figs. 1 and 2) having variation in the E.C from 1.1 to 3.6 (Table 1). Specific carbohydrate utilization tests indicated that soil isolates namely ST3, ST17 and ST24 belonged to *A. chroococcum* whereas isolates ST6 and ST9 were found *A. vinelandii*. Sodium chloride tolerance up to 8 % was observed with *Azotobacter* strains ST3, ST6 and ST24, whereas other strains ST9 and ST17 showed tolerance only up to 6 % NaCl concentration (Chaudhary et al. 2011).

Effect of seed inoculation on wheat growth

Salinity tolerant *Azotobacter* strains were used for inoculation studies on wheat crop (variety WH157) using saline soil under

pot house conditions. There was significant increase in total nitrogen, biomass and grain yield of wheat on inoculation with selected salinity tolerant *Azotobacter* strains. Maximum increase in plant parameters was observed with *Azotobacter* strain ST24 at fertilization dose of 120 kg N ha⁻¹ and its inoculation resulted in attaining 89.9 cms plant height, 6.1 g seed yield, 12.0 g shoot dry weight and 0.7 % total nitrogen at 120 kg N ha⁻¹ (Table 2). Another *Azotobacter* strain ST3 showed 87.8 cms plant height, 5.8 g seed yield, 10.4 g shoot dry weight and 0.65 % total nitrogen. *Azotobacter* strain ST17 also showed significant gain in all the growth parameters i.e., 83.2 cms plant height; 5.5 g seed yield; 10.3 g shoot dry weight and 0.58 % total nitrogen.

Survival and establishment of inoculated *Azotobacter* strains

Studies on survival count of different *Azotobacter* strains i.e., ST24, ST3, ST6, ST9 and ST17 showed that population of inoculated strains increased upto 60 days of sampling as compared to 30 days observation (Table 3). *Azotobacter* strain ST24

Table 3 Survival of selected salinity tolerant (ST) *Azotobacter* strains in wheat after 30, 60 and 90 days after sowing (cfu ml⁻¹ × 10⁴)

<i>Azotobacter</i> strains	30 DAS			60 DAS			90 DAS		
	^a Kg N ha ⁻¹								
	0	90	120	0	90	120	0	90	120
Control	1.02	1.15	1.27	1.39	1.48	1.67	1.92	2.05	2.36
ST3	7.15	7.57	8.01	6.58	6.62	7.23	5.58	6.18	6.78
ST6	7.13	7.20	7.40	6.33	6.35	6.51	5.86	6.17	6.67
ST9	6.60	6.80	7.23	5.60	5.76	6.37	5.07	5.53	6.10
ST17	5.53	6.10	6.40	4.53	5.32	5.37	3.97	4.33	4.87
ST24	7.95	8.23	8.50	6.64	7.63	7.87	6.20	6.70	7.50
CD at 5 %	0.736			0.569			0.258		

Values given are the mean values of 9 plants (three plants in each pot)

^a indicates the doses of nitrogen fertilizer applied

Fig. 1 Colonies of *Azotobacter* strains ST3 and ST24 on Jensen's medium

showed maximum bacterial counts (cfu ml⁻¹) at 0, 90 and 120 kg N ha⁻¹ as compared to other strains. The count of *Azotobacter* strain ST24 increased from 7.95×10^4 (without fertilization) to 8.5×10^4 at 120 kg N ha⁻¹ fertilizer dose. There was significant increase in the population of *Azotobacter* strain in comparison to counts observed in control treatment i.e., the population increased from 1.02×10^4 in uninoculated treatment to 7.95×10^4 in treatment inoculated with *Azotobacter* strain ST24 under unfertilized conditions. Similar increase in bacterial population was observed at 60 DAS. However, the bacterial count decreased on 90 DAS as compared to counts observed at 60 DAS at all the fertilization treatments.

Discussion

Inoculation with salinity tolerant *Azotobacter* strains significantly improved the plant biomass and grain yield of wheat. The inoculation with *Azotobacter* strain ST24 resulted in attaining 89.9 cms plant height, 6.1 g seed yield, 12.0 g shoot dry weight and 0.7 % total nitrogen at fertilization dose of 120 kg N ha⁻¹. Similar enhancement in grain yield (16.3 %) was reported by inoculation of *Azotobacter chroococcum* strain A103 in wheat variety WH291 (Lakshminarayana et al. 2000) and the inoculation with different analogue resistant mutants of *Azotobacter* i.e., Msx1, Msx27, Mal27, Mal30, Mac19 and Mac27 also resulted in increased grain yield varying from 10 to 30 % under field conditions. Ananthanaik et al. (2007) found that inoculation of *Azotobacter chroococcum* increased growth, biomass and nitrogen of *Adasathoda vasica*. Similar increases in plant growth parameters of wheat and maize were observed by application of microbial inoculants under saline conditions (Magda et al. 2003; Mahmoud and Mohamed 2008).

The success of seed-applied bacterial or fungal inoculants depends on their competition with the indigenous microbial strains and their colonization ability in the soil and rhizosphere (Goel et al. 1997). Usually, abiotic stress conditions and indigenous populations of microorganisms create a

hostile environment for the introduced microorganisms in the soil (van Veen et al. 1997; Sindhu and Dadarwal 2000). Moreover, responses of the inoculant strains to environmental variables also contribute to the ability of the introduced microorganisms to occupy a particular ecological niche and to perform the beneficial activity for the benefit of the host plant. In this study, survival of *Azotobacter* strain ST24 in the soil was found highest in all the treatments at 30, 60 and 90 DAS. Furthermore, enhancement of seed yield in some of the treatments in this study indicated that inoculated *Azotobacter* strains are competent enough to displace the resident microbial population leading to improvement in growth and yield of wheat under pot house conditions. These results indicated a high degree of adaptation between the wheat plant and the diazotrophic *Azotobacter* strains in saline soil, which in turn benefit the host.

The population of *Azotobacter* strains decreased on 90 DAS as compared to counts observed at 30 and 60 DAS at all the fertilization treatments. Narula et al. (2007) also found that population density of inoculant strains decreased with later stages of plant growth in the wheat rhizosphere. Singh and Lakshminarayana (1982) observed that survival and competitive ability of *Azotobacter chroococcum* strain A10 (with high nitrogenase activity and high ammonia excretion) was superior to strain A42 in sterile sandy loam soil and a decrease in cell numbers of approximately four log units was observed over a period of 4 weeks in all the three treatments. Recently, higher salinity level in the soil was found to reduce free-living diazotrophic and total heterotrophic bacterial populations (Moradi et al. 2011).

Thus, use of compatible wheat variety and appropriate management practices under different stress conditions such as salinity, sodicity and high temperature needs to be tested to ensure maximum benefit from bioinoculants. Research should also be focused on understanding the molecular mechanism of salt-tolerance in rhizobacteria for improvement of agricultural production, as the saline areas under agriculture is increasing every year all over the world. For realistic inoculation effects, salt-tolerant *Azotobacter* strains needs to be evaluated in the saline soil under the field conditions.

Fig. 2 Colonies of *Azotobacter* strain ST24 streaked on Jensen's medium

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