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Effect of PGPR on Improving the Germination of Durum Wheat (*Triticum durum* Desf.) under pH Stress Condition

Bingiala Laloo*, Prashant Kumar Rai and Pramod W. Ramteke

Department of Genetics and Plant Breeding, Allahabad School of Agriculture, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad- 211007, Uttar Pradesh, India
**Corresponding author*

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A B S T R A C T

The experiment was conducted on 14 genotypes of durum wheat to determine the influence of Plant Growth Promoting Rhizobacteria on germination of wheat under pH stress. The genotypes used in this experiment are 1.AVKD-3 X RD1008 2. NIDW295 X HI8636 3. MPD-153 X MASA499 4. DBPY-02-03 X MASA499 5. NIDW-309 X MASA499 (SHIATS DW2) 6.DBPY-02-03XHI 86388 (SHIATS DW6) 7. NIDW295 X RD1008 (SHIATS DW3) 8. AVKD-2XMASA499 (SHIATS DW5) 9. AVKD-2XRD 1008 (SHIATS DW1) 10. HI 8653 11. RAJ 1535 12. DBP-01-11 13. RAJ 6560 and check variety HD 2009. The 14 genotypes were inoculated with 5 PGPR strains viz. 3AAB1, 3AAB7, 3BAB8, RBA6 and RBA8. The genotypes were first screened for their response to inoculation with PGPR. The genotypes that performed well were further selected for ph stress experiment. The results indicated that SHIATS DW3 responded well under pH stress condition and bacterial culture 3AAB1 showed the most positive outcome.

Introduction

Wheat is a commercially important crop belonging to poaceae family. At present India is the second largest producer of wheat after China. *Triticum durum Desf.* is the second most important crop after *Triticum aestivum*, and is the only tetraploid (2n=4x=28) species of wheat. Durum wheat has a tough horny endosperm, higher carotenoid pigments and better resistance to rusts and karnal bunt as compared to other wheat varieties. The use of micro-organisms in agriculture is at a low level despite the investment in scientific work. Microbial inoculants can be used as an alternative to chemical fertilizers in view of the damaging effect of pesticides and insecticides. Plant Growth Promoting Rhizobacteria (PGPR) are such groups of bacteria that colonize the rhizosphere and improve plant growth (Kloepper and Schroth 1978). When applied to seeds or crops, plant growth promoting rhizobacteria enhance the growth of the plant or reduce the damage from soil borne pathogens. Plant growth promoting rhizobacteria can produce plant growth promoting compounds including phytohormones, auxins, cytokinins and gibberellins (Dashti *et al.*, 2000). The use of PGPR can be used in the future to enhance agricultural production. PGPRs also play an important role in enhancing the root growth,

and act as efficient microbial competitors in the root zone. Significant effects have been observed in wheat. The use of PGPR reduces soil borne pathogens and thus enhances plant growth directly or indirectly.

The task of increasing wheat production has become overwhelming. There is an urgent need to meet the growing demands under constraints like depleting natural resources, environmental fluctuation and increased risk of epidemic outbreak. Salinity, alkalinity, nutrient deficiency and waterlogging are some of the major constraints affecting wheat cultivation. Low infiltration capacity of the soil, stagnation of water, reduction of soil biological activities, deficiency of bases have a negative impact on germination of wheat seed and soil productivity. An extremely high pH can occur in sodic soils and even saline soils after heavy rainfall. The effects of high pH include nutritional disorders such as phosphorous, iron, and zinc deficiencies. An effective approach to deal with the decline in soil health and environment quality must be meted out, and thus promote sustainable agriculture through greater use of biological potential of microbial species.

Materials and Methods

The experiment was carried out from September 2013-March 2014 at the Biochemistry and Microbiology (PGPR) laboratory Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. The fourteen genotypes of wheat used were 1.AVKD-3 X RD1008 2. NIDW295 X HI8636 3. MPD-153 X MASA499 4. DBPY-02-03 X MASA499 5. NIDW-309 X MASA499 (SHIATS DW2) 6.DBPY-02-03XHI 86388 (SHIATS DW6) 7. NIDW295 X RD1008 (SHIATS DW3) 8. AVKD-2XMASA499 (SHIATS DW5) 9. AVKD-2XRD 1008 (SHIATS DW1) 10. HI 8653 11. RAJ 1535 12. DBP-01-11 13. RAJ 6560 and check variety HD 2009. The varieties were treated with the bacterial strains of *Azobacter* (3AAB1, 3AAB7, 3BAB8) and *Rhizobium* (RBA6 and RBA8).

The nutrient agar was prepared by preparing 0.5gm Peptone, 0.5gm yeast extract, 0.1gm beef extract, 0.5g NaCl, 2g Agar and 100ml distilled water at pH 7.0. The prepared mixture was autoclaved at 121˚ C for 15 minutes. The mixture was allowed to cool down after which it was poured into petriplates. The bacterial strains were inoculated with the help of an inoculating needle into test tubes filled with 9ml of water. Each variety of wheat was treated with the five different bacterial strains. One test tube was left un-inoculated and treated as control. The test tubes were vigorously shaken to facilitate effective mixing of seeds with the bacterial culture. The test tubes were left standing for 30 minutes. The seeds were then transferred to petri plates lined with germination paper.

Test for pH stress

The genotypes that performed well with the bacterial strains were further selected to test their performance under varying pH stress. For screening the tolerance of the wheat genotypes and PGPR to different pH, the distilled water was adjusted to 5 different pH levels (pH 5, 6, 7, 8, 9). The acidity or alkalinity of the water was maintained by 1N HCl or 1M NaOH respectively. Each genotype was tested with the respective bacterial strain at different pH levels. A control experiment was maintained with neutral pH (pH 7). The observations were made on the 3rd, 5th, 7th and 9th days after inoculation. The inoculated plants were left at room temperature. The treatments were frequently watered to avoid drying. Care was taken to ensure that the plants were watered

with the suitable pH water. The observations were recorded on 3rd, 5th, 7th and 9th days after inoculation (DAI). The number of seeds used for germination percentage test was 25 and the number of seeds used for root and shoot length observation was 10 with 3 replications. The germination percentage was calculated according to the prescribed standards given by ISTA (1999).

Results and Discussion

The results obtained in the present investigation "Effect of PGPR on improving the germination of durum wheat (*Triticum durum* Desf.) under ph stress condition" was carried out from September 2013-March 2014 at the Biochemistry and Microbiology (PGPR) laboratory Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. The experiment was carried out with Completely Randomized Design One way ANOVA. From the table values obtained, the varieties and PGPR strains identified as best performing were as follows. The salient results of the experiment and the conclusion drawn from it are summarized here as follows:

The mean sum of squares due to the genotypes was significant for all the characters studied. The results showed significant difference was observed at 5% level of significance.

At pH 5, SHIATS DW3 and PGPR strain 3AAB1, showed maximum germination percentage (96%) and better performance over the germination percentage at neutral pH (76%).

At pH 6, SHIATS DW3 and PGPR strain 3AAB1 showed maximum germination percentage (92%) and better performance over germination percentage at neutral pH (76%), indicating significant increase in germination percentage.

Table.1 List of responsive wheat genotypes and PGPR strains

Genotype	PGPR Strain
i.) NIDW295XRD1008	3AAB1
ii.) AVKD-2XMASA499	3AAB1, RBA8
iii.) RAJ1535	3AAB1, RBA8
iv.) AVKD-2XRD1008	RBA8
v.) DBPY-02-03XHI8636	3AAB7, 3BAB8, RBA6
vi.) NIDW309XMASA499	3AAB7
vii.) DBPY-02-03XMASA499	3AAB1
viii.) HD2009	3AAB1,RBA8

The effect of the selected PGPR strains on the seedling growth parameters (germination) of the selected genotypes at varying pH stress was further tested.

Table.2 Effect of PGPR on seed germination of durum wheat under pH stress

Genotypes	Culture	Percent germination %				
		pH5	pH6	pH7	pH8	pH9
SHIATS DW3	Control	40	60	80	72	64
	3AAB1	96	92	76	76	92
SHIATS DW5	Control	92	84	95	60	60
	3AAB1	72	56	76	76	78
	RBA8	69	68	76	76	60
RAJ 1535	Control	68	68	44	48	48
	3AAB1	72	60	60	44	96
	RBA8	60	36	40	40	16
SHIATS DW1	Control	40	72	96	72	64
	RBA8	72	84	96	80	84
SHIATS DW6	Control	72	56	48	68	27
	3AAB7	80	60	76	76	72
	3BAB8	32	52	48	52	48
	RBA6	52	16	40	48	88
SHIATS DW2	Control	76	48	36	48	72
	3AAB7	40	72	60	56	80
DBPY-02-03XMASA499	Control	48	64	72	68	92
	3AAB1	76	80	96	68	96
HD2009	Control	60	52	65	60	48
	3AAB1	68	60	76	72	52
	RBA8	72	64	52	72	92
	Mean	64.76	62.09	67.19	63.42	68.047
Range	Max	96	92	96	80	96
	Min	32	16	36	40	16
	CV	1.544	1.61	1.488	1.577	1.47
	CD (5%)	1.654	1.654	1.654	1.654	1.654

Mean of 3 replicates

Table.3 Germination percentage of genotypes at neutral pH

Neutral pH	Number of genotypes	Number of genotypes with high germination percentage
pH 7	8	5 (62.5%)

Table.4 Germination percentage of genotypes at acidic stress condition

Acidic pH	Number of genotypes	Number of genotypes with high germination percentage
pH 5	8	5 (62.5%)
pH 6	8	3 (37.5%)

Table.5 Germination percentage of genotypes at alkaline stress conditions

Alkaline pH	Number of genotypes	Number of genotypes with high germination percentage
pH 8	8	2 (25%)
pH 9	8	5 (62.5%)

Fig.1 Germination percentage of SHIATS DW3 under pH stress

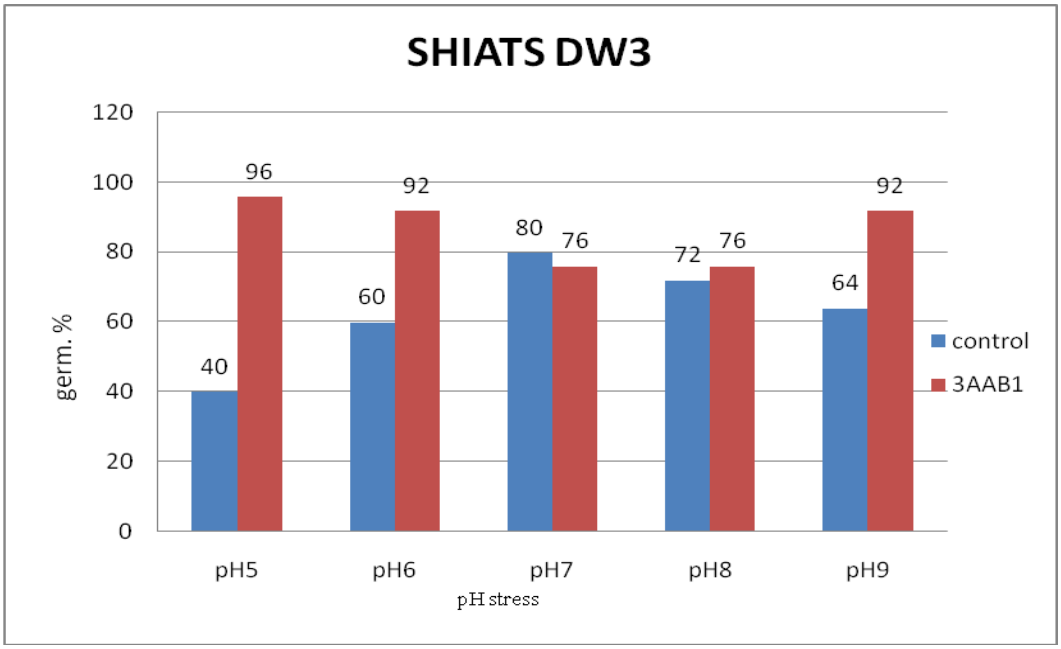


Fig.2 Germination percentage of SHIATS DW5 under pH stress

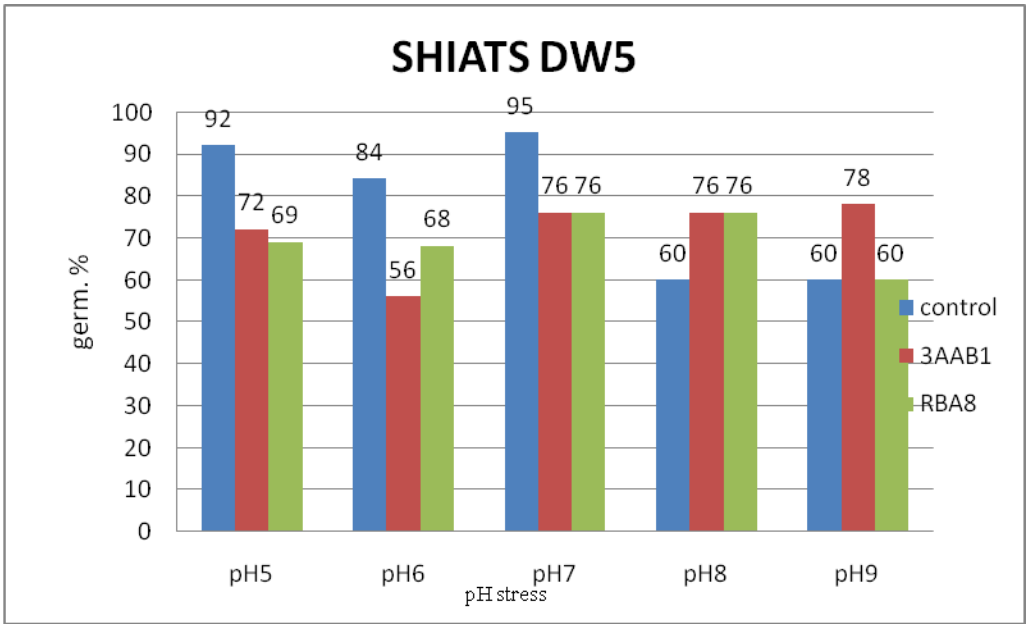


Fig.3 Germination percentage of RAJ 1555 under pH stress

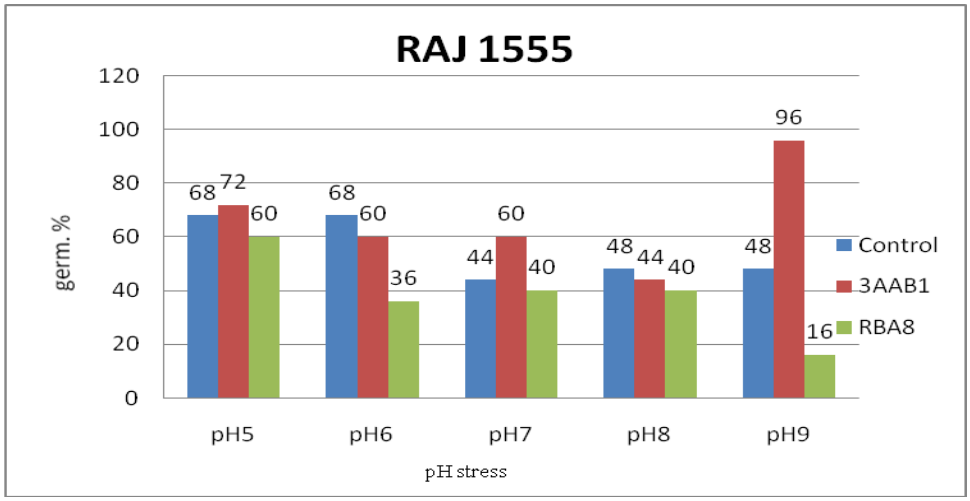


Fig.4 Germination percentage of SHIATS DW6 under pH stress

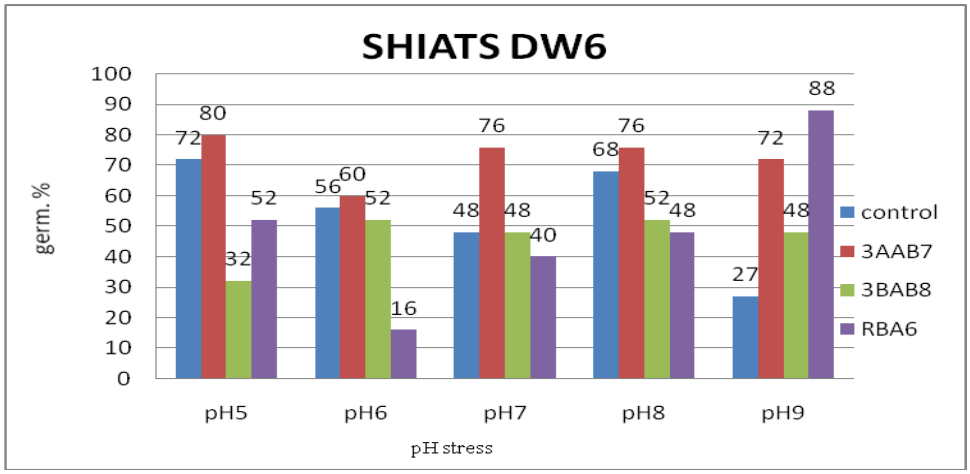


Fig.5 Germination percentage of SHIATS DW2 under pH stress

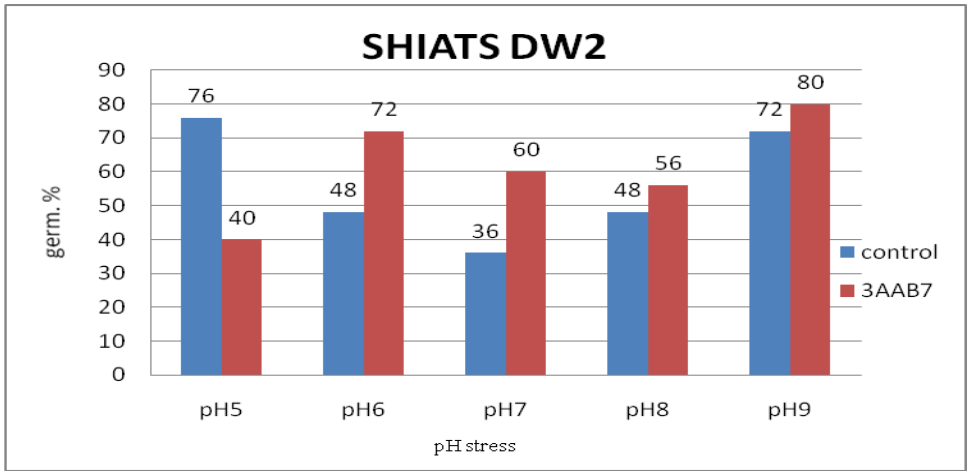


Fig.6 Germination percentage of DBPY-02-03 XMASA499 under pH stress

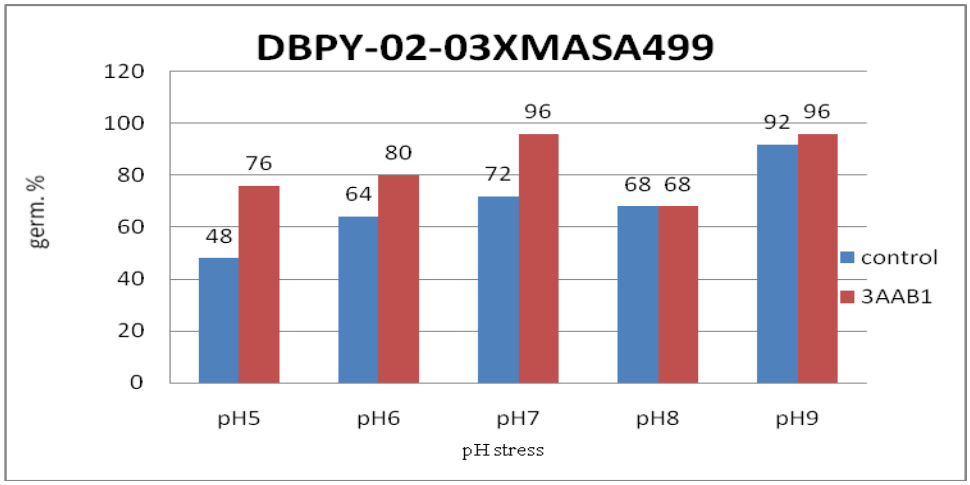


Fig.7 Germination percentage of SHIATS DW1 under pH stress

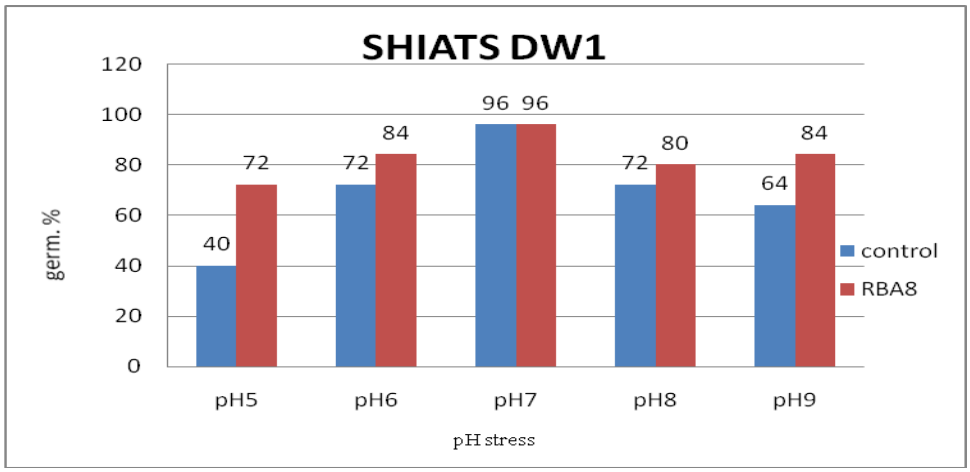
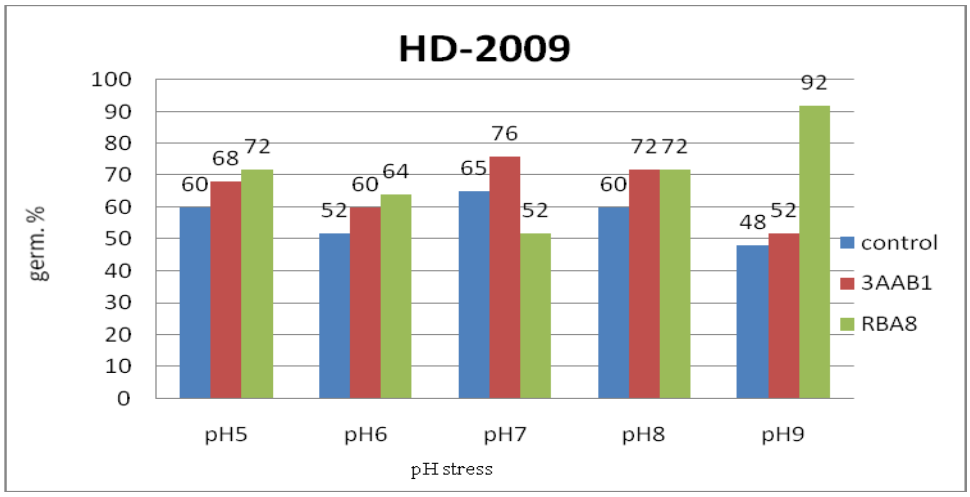


Fig.8 Germination percentage of HD2009 under pH stress



At pH 8, SHIATS DW6 with PGPR strain 3BAB8 showed a higher germination percentage (52%) as compared to the neutral pH (48%), indicating significant increase over the germination percentage.

At pH 9, RAJ1535 and PGPR strain 3AAB1, DBPY-02-03XMASA499 and 3AAB1 showed maximum germination percentage (96%) and better performance over the germination percentage at neutral pH (60%), indicating significant increase over the germination percentage.

62.5 % of the genotypes germinated under neutral pH condition. Under acidic stress condition, 62.5% (pH 5) and 37.5 % (pH 6) of genotypes showed higher germination percentage as compared to the neutral pH while 25 % (pH 8) ad 62.5 % (pH 9) genotypes showed higher germination percentage under alkaline condition as compared to performance at neutral pH (Table 3–5).

It was observed from table 1 that 62.5 % of the genotypes germinated under neutral pH condition. As indicated in table 2, under acidic stress condition, 62.5%(pH 5) and 37.5 % (pH 6) of genotypes showed higher germination percentage as compared to the neutral pH while 25 % (pH 8) and 62.5 % (pH 9) genotypes showed higher germination percentage under alkaline condition as compared to performance at neutral pH. Similar results were reported by Gholami *et al.*, 2009 in field grown maize inoculated with plant growth promoting rhizobacteria.

The figures 1–8 represent the response of durum wheat genotypes to PGPR strains.

It can be concluded from the present investigation that PGPR strains have a significant effect on the germination of durum wheat genotypes. The germination percentages of the genotypes at different levels of pH stress were significantly improved when compared with the control. Thus it can be derived that PGPR has a positive effect in improving the germination percentages of wheat genotypes under pH stress condition. Okon and Vanderleyden (1997) suggested that the secretion of plant growth-promoting substances by the bacteria could be responsible for the beneficial effects of PGPR. The germination parameters were observed to know the rapidity of germination which helped in reflecting the quality of seeds and response to PGPR inoculation. The results indicated that the germination percentage of the genotypes inoculated with PGPR showed better performance over the neutral pH. The germination percentage of wheat seeds inoculated with PGPR also showed better emergence over control (uninoculated genotypes). Similar results have been recorded by Ashrafuzzaman *et al.*, (2009) in rice seeds inoculated with PGPR whereby the rice seeds showed an increase in germination by 2.3 to 14.7% over control. Similar improvement in germination parameters have been reported in several crops such as sorghum and pearl millet by Raju *et al.*, (1999) and Niranjan *et al.*, (2003,2004). The germination percentage of SHIATS DW3 treated with 3AAB1 showed a germination percentage of 96 percent as compared to the control. Thus this particular treatment combination (SHIATS DW3 with 3AAB1) displayed the beneficial effects of inoculation with PGPR in durum wheat. The increase in the germination percentage of the wheat genotypes may be due to the increased synthesis of hormones like gibberellins which trigger the activity of certain enzymes that promote early germination.

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