Journal of Environmental Biology

31 261-264 (**May** 2010)

Response in germination and seedling growth in *Phaseolus mungo* under salt and drought stress

Gunjan Garg*

School of Biotechnology, Gautam Buddha University, Gautam Buddha Nagar, Greater Noida (NCR) - 201 308, India

(Received: October 21, 2008; Revised received: February 17, 2009; Accepted: March 02, 2008)

Abstract: *The effect of salt and drought stress at the water potentials of -2, -4,-6 and -8 bars induced by NaCl and PEG 6000 (Polyethylene glycol 6000) each, on germination and early seedling growth, were investigated for two varieties (PU-19 and Type-9). Electrical conductivity (EC) value of the NaCl solutions were 4.5, 8.8, 12.7 and 16.3 dS m^{-1}. Germination percentage, root and shoot length, and seedling fresh and dry weight were measured in the study. The objective was to determine genotypic differences among P.mungo varieties in terms of salt and drought stress and to determine factors(salt toxicity or osmotic stress due to PEG) inhibiting seed germination. The germination results revealed that the genotypes significantly differed for salt and drought stress. PU-19 appeared to be more tolerant to salt and drought stress comparable to var. Type-9. Both NaCl and PEG inhibited germination and seedling growth in both the varieties, but the effects of NaCl compared to PEG was less on germination and seedling growth. All varieties were able to germinate at all NaCl levels without significant decrease in germination, while a drastic decrease in germination was recorded at -6 and -8 bars of PEG. It was concluded that inhibition in germination at equivalent water potential of NaCl and PEG was mainly due to an osmotic effect rather than salt toxicity.*

Key words: *Salt toxicity, Osmotic stress, Seed germination, Seedling growth*
PDF of full length paper is available online

Introduction

Salinity and drought are the major environmental constraints to crop productivity through out the arid and semi arid regions of the world. Between 30 and 40% of the world irrigated agricultural lands are prone to salinity (Foollad and Yin, 1997). *Phaseolus mungo* is an important legume for human nutrition in the world. Plant growth of *P. mungo* is sensitive to salinity and drought (Aouani *et al.*, 1998). The deleterious effects of salinity and drought on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors. In addition to the use of traditional breeding and plant genetic transformations, the use of plant growth-promoting micro-organisms may prove useful in developing strategies to facilitate plant growth in saline soil (Mayak *et al.*, 2004). Lal (1985) indicated that earlier growth stage were more sensitive to salinity than subsequent ones. Cerda *et al.* (1982) found useful variation in salt tolerance of pea cultivars whose half maximal yield was between 6 and 10 dS m^{-1}.

Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansourie *et al.*, 2001; Bhattacharjee, 2008). Crop establishment depend on an interaction between seedbed environment and seed quality (Brown *et al.*, 1989; Khajeh-Hosseini *et al.*, 2003). Factors adversely affecting seed germination may include sensitivity to drought stress (Wilson *et al.*,1985), and salt tolerance (Perry,1984; Sadeghian and Yavari, 2004; Ozdener and Kutbay, 2008). Germination and seedling growth are reduced in saline soils with varying responses for species and cultivars (Bliss *et al.*, 1986; Hampson and Simpson, 1990). Salinity may also affect the germination of seeds by creating an external osmotic potential that prevents water uptake or due to toxic effects of Na^+ and Cl^- ions on the germinating seed (Redmann, 1974; Murrillo-Amador *et al.*, 2002; Khajeh-Hosseini *et al.*, 2003).

The relative importance of the osmotic or toxic effects of NaCl and PEG on seed germination of *P. mungo* is not clear and this study was conducted to determine the effect of NaCl and PEG on seedling growth and germination and to determine factors responsible for failure in seed germination under saline conditions comparing various level of NaCl and PEG.

Materials and Methods

This study was carried out at the Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut (U.P). The seeds of *P. mungo* varieties PU-19 and Type-9 obtained from the certified National Seed Corporation (Ltd.) shop, were used as material and were treated with fungicide before planting. Drought stress was induced by polyethylene glycol (PEG 6000) treatment. Four drought stresses with different osmotic potentials of -2,-4,-6 and-8 bars were arranged as described by Michel and Kaufmann (1973).Salt concentrations that had the same osmotic potentials of -2, -4, -6 and -8 bars (electrical conductivities of the solutions were 4.5, 8.8, 12.7 and 16.3 dS m^{-1}, respectively) were adjusted using NaCl (Coons *et al.*, 1990). Distilled water served as a control.

Three replicates of 20 seeds of two varieties *i.e.* PU-19 and Type 9 were germinated in 2 rolled Whatman filter papers with 10 ml

*** Corresponding author:** garg29g@yahoo.co.in

of respective test solutions. The papers were replaced every 2 days to prevent accumulation of salts (Rehman *et al.*, 1996). In order to prevent evaporation, each rolled paper was put into a sealed plastic bag. Seeds were allowed to germinate at 20±1°C in the dark for 10 days. To determine the toxic effects of the solutions on germination, non-germinated seeds in each treatment were transferred to distilled water and counted for an additional 3 days. A seed was considered to have germinated when the emerging radicle elongated to 1mm. Germination percentage was recorded every 24 hr for 10 days. Root and shoot length (cm), and seedling fresh and dry weights (mg plant^{-1}) were measured on the 10th day. Dry weights were measured after drying samples at 70°C for 48 hr in an oven (Bohm, 1979).

The experimental design was 3 factorial, arranged in a completely randomized design with 3 replications and 20 seeds per replicate. The first factor was varieties (PU-19 and Type-9), the second was solutions (NaCl and PEG) and the third was solution levels (0, -2, -4, -6 and -8 bars). Significant effects of the treatments were analyzed by analysis of variance.

Results and Discussion

Germination percentage was not significantly decreased by NaCl solutions while it decline considerably with decreasing water potential of PEG. The maximum decrease in germination percentage was determined in PEG of Type-9 var. (5.2%) at -8 bars. The control (0 bars) showed differences among the varieties for germination percentage. The lowest germination percentage in control (76.7%) was obtained from Type-9 (Table 1). Transfer of non germinated seeds from PEG solution to the distilled water resulted 98% germination regardless of osmotic potential (data not shown). It is evident that PEG was not toxic. It was noted that germination time was delayed by decreasing water potential. Drought affected more adversely than did NaCl.

Increasing NaCl resulted in decrease in root length in var. PU-19, where as in var. Type-9, root length initially increase but latter it decline as the water potential of NaCl increased. If we compare the root length between the two varieties, PU-19 gave the high values at all water potential of NaCl (Fig. 1). No variety were able to grow roots at -6 and -8 bars of PEG. NaCl had a stimulating effect on the growth of Type-9 up to a certain limit of growth.

The highest shoot length was determined in PU-19 in all NaCl concentration. Shoot length was influenced by both salt and drought stress but inhibition was greater in PEG (Fig. 2). No shoot length was recorded in variety Type-9 at -2 bars or PU-19 at -4 bars. Water stresses depressed the shoot growth of the varieties rather than their root growth.

Decreasing water potential by NaCl and PEG caused a remarkable decrease in seedling fresh weight (Table 2). Differences determined among the varieties were significant. Although the varieties showed different responses to each NaCl and PEG concentration, the highest values were observed PU-19 in NaCl. NaCl reduced the seedling fresh weight of PU-19; however, it increased seedling fresh weight of Type-9.

Table - 1: Change in germination percentage of *P. mungo* varieties at different osmotic potentials of NaCl and PEG at 10 days

	Germination percentage (%)			
	Varieties			
	PU-19		Type-9	
Bars	**NaCl**	**PEG**	**NaCl**	**PEG**
0	100	100	76.7	76.7
-2	96.7	98.3	78.0	65.0
-4	96.7	81.7	69.0	13.3
-6	98.1	38.3	62.0	11.6
-8	95.0	17.7	55.0	5.2

Table - 2: Seedling fresh weight of *P. mungo* varieties at different osmotic potentials of NaCl and PEG at 10 days

	Seedling fresh weight (mg plant^{-1})			
	Varieties			
	PU-19		Type-9	
Bars	**NaCl**	**PEG**	**NaCl**	**PEG**
0	0.83	0.83	0.18	0.18
-2	0.62	0.11	0.29	0
-4	0.51	0	0.25	0
-6	0.39	0	0.26	0
-8	0.22	0	0.16	0

Table - 3: Seedling dry weight of *P. mungo* varieties at different osmotic potentials of NaCl and PEG at 10 days

	Seedling dry weight (mg plant^{-1})			
	Varieties			
	PU-19		Type-9	
Bars	**NaCl**	**PEG**	**NaCl**	**PEG**
0	0.065	0.065	0.011	0.011
-2	0.043	0.019	0.029	0
-4	0.037	0	0.022	0
-6	0.029	0	0.027	0
-8	0.024	0	0.020	0

Seedling dry weight showed a trend similar to that of fresh weight, and depending on the decline in seedling fresh weight in var. PU-19, dry weight of this variety (PU-19) decreased with increasing NaCl and PEG (Table 3).With in these two varieties, PU-19 appeared more tolerant to salt and drought stress compared to Type 9.

NaCl and PEG adversely affected the germination and seedling growth of *P.mungo* but PEG had a greater inhibitory effect than did NaCl. Our results agree with those given by Murillo-Amador *et al.* (2002), who observed that NaCl had a lesser effect on the germination and seedling growth of cowpea than did PEG and Sadeghian and Yavari (2004), who stated that seedling growth was severely diminished by water stress in sugar beet. More over, distinct genetic difference were found among the

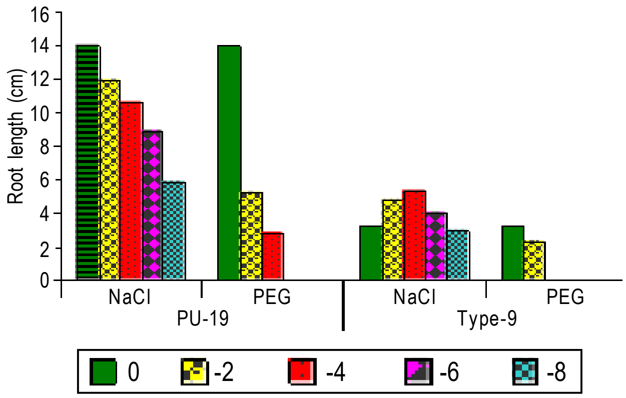

Fig. 1: Root length of *P. mungo* varieties at different osmotic potentials of NaCl and PEG at 10 days

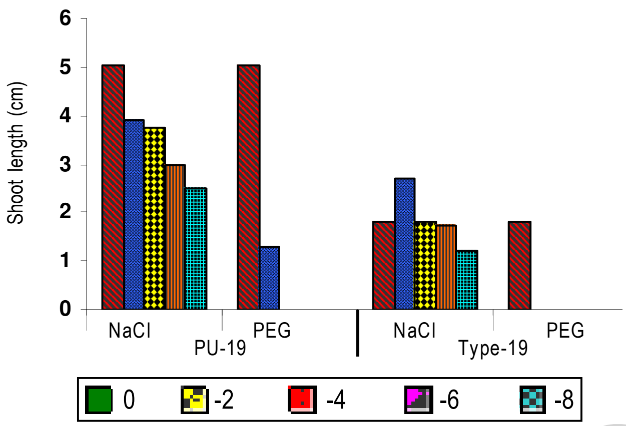

Fig. 2: Shoot length of *P. mungo* varieties at different osmotic potentials of NaCl and PEG at 10 days

varieties with respect to germination and seedling growth subjected to NaCl and PEG. Germination percentage of both the varieties varied with solutions and doses. Var.PU-19 showed better germination percentage compared to var. Type-9. At the same germination percentage is faster in NaCl than in PEG. Khajeh-Hosseini *et al.* (2003) found faster germination in NaCl in soyabean. Delgado *et al.* (1994), who studied the effects of salt stress on growth and nitrogen fixation by pea, faba bean, common bean and soya bean plants, indicated that pea was the most sensitive legume among them (Ellis and Robert, 1980). Root and shoot length and seedling fresh and dry-weight were decreased by increasing NaCl and PEG concentrations. Consequently, seedling growth was inhibited in *P.mungo*. Difference between NaCl and PEG were significant for all investigated characters. Inhibition of NaCl was less than that of PEG. Our results confirm the findings of Khajeh-Hosseini *et al.* (2003) in soyabean and those of Murillo-Amador *et al.* (2002) in cowpea. However, our findings showed that NaCl had greater inhibitory effects on seedling growth than on germination because no significant decrease in germination in both the varieties was observed. Higher final germination in NaCl than in PEG could be explained by more rapid water uptake in NaCl solutions and achievements of a moisture content that allowed germination. Khajeh-Hosseini *et al.* (2003) suggested that the achievement in NaCl solutions was due to rapid imbibition in soyabean seeds.

In the germination and early seedling growth stages, the investigated varieties showed different response to water and salt stress. Var.PU-19 was more tolerant than var.Type-9. Furthermore, germination failure due to NaCl resulted from an osmotic barrier induced by NaCl. However, seedling growth was more sensitive to salt stress than was germination.

Acknowledgments

The author is grateful to Prof. V.N. Sharma, Director of School of Biotechnology and Mr. Vishnu Sharan, Chairman, Meerut Institute of Engineering and Technology, Meerut (U.P. India) for allowing me to use his laboratory for this study.

References

Almansouri, M., J.M. Kinet and S. Lutts: Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant Soil,* **231**, 243-254 (2001).

Aouani, M.E., R. Mhamdi, M. Mars and R. Grir: Nodulation and growth of common bean under NaCl-stress. *Soil Biol. Biochem.*, **30**, 1473-1475 (1998).

Bhattacharjee, Soumen: Triadimefon pretreatment protects newly assembled membrane system and causes up-regulation of stress proteins in salinity stressed *Amaranthus lividus* L. during early germination. *J. Environ. Biol.,* **29**, 805-810 (2008).

Bliss, R.D., K.A. Platt-Aloia and W.W. Thomson: Osmotic sensitivity in relation to salt sensitivity in germinating barley seeds. *Plant Cell Environ.*, **9**, 721-725 (1986).

Bohm, W.: Methods of Studying Root Systems, *Springer-Verlag*, Berlin (1979).

Brown, S.C., P.J. Gregory, P.J.M. Cooper and J.D.H. Keatinge: Root and shoot growth and water use of chickpea (*Cicer arietinum*) grown in dry land conditions: Effects of sowing date and genotype. *J. Agric. Sci.,* **113**, 41-49 (1989).

Cerda, A., M. Caro and F.G. Fernandez: Salt tolerance of two pea cultivars. *Agronomy. J.,* **74**, 796-798 (1982).

Coons, M.J., R.O. Kuehl and N.R. Simons: Tolerance of ten lettuce cultivars to high temperature combined with NaCl during germination. *J. Americ. Soc. Hort. Sci.,* **115**, 1004-1007 (1990).

Delgado, M.J., F. Ligero and C. Lluch: Effects of salt stress on growth and nitrogen fixation by pea, faba bean, common bean and soybean plants. *Soil Biol. Biochem.*, **26**, 371-376 (1994).

Ellis, R.H. and E.H. Roberts: Towards a rational basis for testing seed quality. *In*: Seed production (*Eds.*: P.D. Hebblethwaite). Butterworths, London. pp. 605-635 (1980).

Foollad, M.R. and G.Y. Yin: Genetic potential for salt tolerance during germination in Lycopersicon species. *Horticulture Sci.,* **32**, 296-300 (1997).

Hampson, C.R. and G.M. Simpson: Effects of temperature, salt and osmotic potential on early growth of wheat (*Triticum aestivum,* L.) germination. *Can. J. Bot.*, **68**, 524-528 (1990).

Khajeh-Hosseini, M., A.A. Powell and I.J. Bingham: The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. Technol.,* **31**,715-725 (2003).

Lal, R.K.: Effect of salinity applied at different stages of growth on seed yield and its constituents in field peas (*Pisum sativum* L. var. arvensis). *Ind. J. Agron.,* **30**, 296-299 (1985).

Mayak, S., T. Tirosh and B.R. Glick: Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.,* **42**, 565-572 (2004).

Michel, B.E. and M.R. Kaufmann: The osmotic potential of polyethylene glycol 6000. *Plant Physiol.,* **51**, 914-916 (1973).

Murillo-Amador, B., R. Lopez-Aguilar, C. Kaya, J. Larrinaga-Mayoral and A. Flores-Hernandez: Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. *J. Agronomy Crop Sci.*, **188**, 235-247 (2002).

Ozdener, Y. and H. Guray Kutbay: Effect of salinity and temperature on the germination of *Spergularia marina* seeds and ameliorating effect of ascorbic and salicylic acids. *J. Environ. Biol.,* **29**, 959-964 (2008).

Perry, D.A.: Factors influencing the establishment of cereal crops. *Aspects of Applied Biol.*, **7**, 65-83 (1984).

Redmann, R.E.: Osmotic and specific ion effect on the germination of alfalfa. *Canadian J. Bot.*, **52**, 803-808 (1974).

Rehman, S., P.J.C. Harris, W.F. Bourne and J. Wilkin: The effect of sodium chloride on germination and the potassium and calcium content of acacia seeds. *Seed Sci. Technol.,* **25**, 45-57 (1996).

Sadeghian, S.Y. and N. Yavari: Effect of water-deficit stress on germination and early seedling growth in sugar beet. *J. Agron. Crop Sci.*, **190**, 138-144 (2004).

Wilson, D.R., P.D. Jamieson, W.A. Jermyn and R. Hanson: *In*: Models of growth and water use of field pea *(Pisum sativum* L.) (*Eds.*: P.D. Hebblethwaite, M.C. Heath and T.C.K. Dawkins). The Pea Crop. Butterworths London, U.K. (1985).