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Seed Germination Studies On *Viciafaba* L. Under Salt Strees

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Abstract

The present work has been conducted on the effect of different concentration of sodium chloride (Nacl) ranging from 100 ppm to 500 ppm at three different time duration (6, 12 and 24 hr) in two different Broad Bean (*Viciafaba*) varieties.The maximum germination percentage has observed at 100 ppm at 6 hr seed soaking beyond this level germination has reduced gradually, in the same way time diffraction and concentration has also reduced gradually and 6 hr duration has shown maximum germination in both. The shoot length, root length and total length also increase upto 100 ppm in 6 hr when it is in NaCl and there after it gradually reduced. Mitotic index percentage was observed highest at 100 ppm and less disturbed, sticky and breakage of chromosome in both varieties.

Key words: Viciafaba, NaCl, seed germination, Salinization

Introduction

Viciafaba L., also known as the broad bean, faba bean, field bean, is a species of bean (Fabaceae) native to North Africa, southwest and south Asia, and extensively cultivated elsewhere. It is generally accepted that the geographic origin of *Viciafaba* L. was the Near East and that the subspecies *V. fabapaucijuga*, presently found from Afghanistan to India, is a primitive form. Viciafaba is known to have been cultivated from early Neolithic times. The broad bean has high plant hardiness; it can withstand rough climates, and in this case, cold ones. Unlike most legumes, the broad bean can be grown in soils with high salinity, as well as in clay soil. However, it does prefer to grow in rich loams. It is very low in saturated fat, cholesterol and sodium. It is also a good source of dietary fiber, protein, phosphorus, copper and manganese, and a very good source of folate.

Salinity is a significant environmental stress for influencing crop growth around the world. Soil Salinisation may arise from intrinsic soil components. Soluble salts in saline soils which impair soil productivity are chlorides, sulfates, carbonates and bicarbonates of calcium, magnesium, sodium and potassium. These salts cause “physiological drought” and reduce the availability of phosphorus and micronutrients such as iron, manganese, zinc, copper and cobalt to crop plants.Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 1993; Munns, 2002). Furthermore, it decreases plant growth and yield, depending on the plant species, salinity levels, and ionic composition of the salts (Yadav *et al.,* 2010). Saline stress is one of the main factors limiting legume productivity in arid

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and semi-arid regions (Lluch*et al.,* 2007), and salinity has direct harmful effects on numerous plant species (Greenway and Munns, 1980; Keck *et al*., 1984; Cordovilla*et al.*, 1994). Salinity adversely affects the plant growth and development, hindering seed germination (Dash and Panda, 2001). Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansourie*et al*., 2001; Bhattacharjee, 2008). However, Bayuelos*et al*. (2002) reported that increase in salinity from 0 to 180 mM of NaCl decreases germination by 50% in species of the genus *Phaseolus.*

Salinity in the soil is a major detrimental factor for crop production in arid and semi-arid regions of the world. Epestein (1978), Massouncl (1974) examined the distribution of the 13.5 million hector of saline soils throughout the world. Soil salinity has caused heavy loss of natural resources in India. Out of 329 million hectares of land in the country, about 175 million hectares (53%) are suffering from degradation in some form or the other and out of which, 7.65 million hectares is salt affected (Anon., 2006). The salinity effects on soil can be of chemical, physical and biological. Chemical effects are certain exchanges and interactions among the salts, whereas, the major physical effect is soil permeability (Farooqi*et al*., 2005). Biological effects are the changes in osmotic pressure and alteration of protoplasm and cell membrane permeability (Abd El-Samad*et al*., 2004). Sharma (1983) and Balaji *et al*., (2002) have examined the mechanism of salt injury during germination and early seedling growth of wheat. Salt causes stress and damage on the plant during the vegetative period from germination, through growth, developing and harvesting time (Larcher, 1995). Salinity in general has inhibitory effects on germination of seeds of many crops (Kumar *et al*., 1988; Mondal 1988; Sharma and Yamadagni. 1989 and Yaseen *et al*.. 1989).

Salinity decreases the germination (Bernard *et al*., 2000). The effect of salinity on germination of seeds can be either by creating osmotic potential which prevent water uptake or by toxic effects of ion on embryo viability of the seeds (Houle *et al*., 2001). In addition to these, shoot growth is also reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (Kaymakanova, 2009). It is thought that depressive effect of salinity on germination could be related to declining in endogenous levels of hormone (Debez*et al*., 2001). However, pre-sowing seed treatments have been shown to enhance the vigour and synchronized stand established under stress conditions. Further pre-soaking seeds with optimal concentration ofphytohormones has been shown to be beneficial to growth and yield of some crop speciesgrown under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation (Singh and Darra, 1971).Helal and Mengel (1981) suggested that NaCl salinity depressed growth and restricted protein formation, CO2 assimilation, and especially the incorporation of photosynthates into the lipid fraction. Conversion of photosynthates in leaves was much more affected by salinity than was photosynthate turnover in roots.Ullah*et al.* (1993) reported that Grain as well as straw yields of Faba beans were decreased significantly by artificial salinity. At the highest stress level (60 mM salt) grain yields were reduced by 85%,while straw yields decreased by 43 % at 60 mM.

Therefore, in order to study the effect and mechanism of action of salinity on Bean seeds were taken for the following objectives:

1. To study the effect of salinity on physiology of germination/seedling emergence.
2. To study the cytological changes during salt stress condition in seedling stage.

Material and Methods

The *Viciafaba* varieties Primo (V_1) and Windsor (V_2) seeds were received from Department of Seed Technology, R.B.S. College, Agra (U.P.). The seeds were surface sterilized for2 min. in 0.05% mercuric chloride ($HgCl_2$) solution. The surface sterilized seeds were thoroughly washed with tap water 2-3 times and followed by distilled water. Then the seeds were soaked in distilled water up to 24h. The seeds were arranged equispecially on the periphery of sterilized petri dish lined with filter paper. Each Petri plate allotted with 40 seeds and treated with various concentrations & time level of sodium chloride ranging from 100,300and500 ppm (6, 12 and 24 hr). The controlled seeds were treated-with distilled water. The experimental work is maintained in the laboratory condition (25^0C). The following observations were recorded:

Morpho-physiological observations

1. Germination percentage:

The germinated seeds were counted daily according to the seedling evaluation procedure described in the Handbook of Association of Official Seed Analysts. The number of germinated seeds was recorded every 24 h (AOSA, 1990). Ten days after germination, the germination percentage (GP) was obtained by dividing the number of germinated seeds in any Petri dishes by the total number of seeds, multiplied by 100 (Tanveer*et al*., 2010; Cokkizgin and Cokkizgin, 2010)Ya Bedial Acaibi, Bil $ayrir Hamni Ýla Yevmiddin Ya Bedial Acaibi, Bil $ayrir Hamni Ýla Yevmiddin.

2. Seedvigour index:

Seed vigor index (SVI) was calculated according to Baki and Anderson (1973) as follows:

$$SVI = [\text{Seedling length (cm) x GP (\%)}]$$

3. Mean germination time:

The seedlings' stunted primary roots were considered as abnormally germinated (ISTA, 2011). A bean seed was considered to have germinated when the radicle reached a length of 10 mm (Goertz and Coons, 1989). The mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981) as follows:

$$MGT = \frac{\sum (Dn)}{\sum n}$$

Where,*n*is the number of seeds germinated on each day and *D* is the day of counting.

Cotyledons were not included in fresh and dry weight comparisons.

4. Germination index:

Germination Index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA, 1983) as,

$$GI = \sum \left(\frac{GT}{Tt}\right)$$

Or

$$GI = \left[\frac{\textit{Number} \text{ of germinated seeds in first count}}{\text{Days of first count}}\right] + \ldots + \left[\frac{\textit{Number} \text{ of germinated seeds in final count}}{\text{Days of final count}}\right]$$

5. Time taken to 50% Germination (G_{50})

E_{50} was calculated according to the following formulae of Coolbear*et al*. (1984).

$$G_{50} = t_i + \left[\frac{\frac{N}{2} - n_i}{n_j - n_i}\right](t_j - t_i)$$

Where, N is the number of final germination count and n_i, njcumulative number of seeds germinated at adjacent days t_i and $t_j$when n_i< (N+1)/2 <n_j.

6. Coefficient of velocity of germination:

Coefficient of velocity of germination (CVG) was evaluated according to Maguire (1962) as follows:

$$CVG = \frac{(G_1 + G_2 + \ldots + G_n)}{(1xG_1 + 2xG_2 + \ldots + nxG_n)}$$

Where,*G* is the number of germinated seeds and *n* is the last day of germination.

Cytological studies: cytological studies were evaluated to Brase and brase (1978) as fellows:

1. Mitotic Index: mitotic index was determined in percent by the following formula:

$$\textbf{M.I. (\%)} = \frac{\text{No. of dividing cells}}{\text{No. of observed cells}} \times 100$$

2. Disturbed chromosome: disturbed chromosome was determine in percent by the following formula:

$$\textbf{D.C. (\%)} = \frac{\text{No. disturbed chromosomes}}{\text{Total no.of observed cells}} \times 100$$

3. Sticky chromosome: sticky chromosome was determined in percentage by the following formula:

$$\text{S.C.(\%)} = \frac{\text{No. of sticky chromosomes}}{\text{Total no of observed cells}} \times 100$$

4. Breakage chromosome: breakage chromosome was determined in percent by the following formula.

$$\text{B.C.(\%)} = \frac{\text{No. of breakage chromosomes}}{\text{Total no. of observed cells}} \times 100$$

Results and Discussion:

1. Germination percentage:

The germination percentage of Viciafaba as affected at different concentrations of NaCl. The germination percentage has found to be maximum at 100 ppm and this has increased to 76.67 and 80.6% during 6-24 hr respectively. Minimum percentage of germination has recorded at 500 ppm of NaCl at 24 hr seed soaking in table-1.

Germination percentage decreased with higher concentrations of NaCI. Reduction in germination percentage of Broad bean at higher concentration may be attributed to the interference of sodium ions. Similar inhibition of germination at higher concentration of metal ions was observed by Jaya Kumar *et al..*(2006) with cobalt.

2. Seed Vigour Index:

Seed vigour index (SVI) decreased with increase in NaCl concentration. Highest reduction was found in 500ppm in both varieties. Same pattern was followed by time of seed soaking. With the increase of soaking time, gradual reduction was observed inSVI. Uniyal and Nautiyal (1998) also reported decrease in seedling vigourindex under salinity stress in *Ougeiniadalbergioides.*Similar observations were observed in some species of *Vignamungo* by *Jenci et al.,* (2006), Chidambaram *et al.,* (2006).In terms of evolution the ability of halophyte seeds to

3. Mean Germination Time:

In present experiment mean germination time was found to be increased with the increase of concentration and seed soaking time. Maximum mean germination time was found in 500ppm with 24hrs seed soaking time in both varieties. These results are supported by previous experimenters viz. Sivasankaramoorthy *et al*. (2010) in *Excoecariaagallocha*.Similar results were found in *Cicerarietinum* (Deepak, 2011).

4. Germination Index:

Germination Index was also found to be reduced with the increase in concentration and duration of seed soaking in both varieties in same pattern. Maximum decrease in germination index with respect to control was found in 500ppm with 24 hrs seed soaking.

5. Time taken to 50% germination (G_{50}):

Positive correlation was found in time taken to 50% germination with sodium chloride concentration and

Table: 1 Seed germination studies on *Viciafaba* L. under salt stress.

Tretment		MGT	G.I.	Germ.%	S.V.I.	G 50	C.V.G.
Concentration (mM)	Duration of soaking(Hrs.)						
Variety-! Control		5.76	4.3	84	514.08	4.88	0.174
100	6	6.05	3.72	80	444	5.33	0.165
	12	6.21	3.42	76	399	5.17	0.161
	24	6.17	3.19	72	345.6	5.5	0.162
300	6	6.28	3.14	72	365.76	5.6	0.159
	12	5.94	3.09	68	329.8	5.38	0.168
	24	6.29	2.97	68	276.08	5.83	0.159
500	6	6.5	2.73	64	286.72	6	0.154
	12	6.67	2.43	60	276	6.17	0.15
	24	6.77	2.04	52	187.2	6.17	0.148
Variety-2 Control		5.64	4.63	88	602.8	4.75	0.177
100	6	6	3.95	84	520.8	5.17	0.167
	12	5.84	3.63	76	414.2	5.13	0.172
	24	6.28	3.2	72	374.4	5.5	0.159
300	6	6.11	3.32	72	378	5.33	0.164
	12	6.18	3.12	68	350.2	5.5	0.162
	24	6.25	2.84	64	307.2	5.5	0.16
500	6	6.44	2.77	64	305.92	6	0.155
	12	6.38	2.79	64	300.8	5.67	0.157
	24	6.93	2.17	56	212.8	6.5	0.144

survive under hyper saline conditions can be considered as a selective advantage. The experiment shows the reduction in the growth of the seeds above 100ppm which coincides with the result of earlier findings of Marschner*et al.,* (1981) who reported that salinity effects reduces the growth in sugar beet and Thriupathi*et al.,* (2011) reported that salinity effects also reduce the growth in brinjal.

seed soaking period. Maximum time for 50% germination was taken by the 500 ppm with 24 hrs seed soaking treatment in both varieties.

6. Coefficient of velocity of germination:

Velocity of germination was decreased with the increase in NaCl concentration and time of seed soaking in the solution in both varieties.

Cytological studies:The mitotic index, disturbed chromosome, sticky chromosome and breakage chromosome of Viciafaba as affected at different concentrations of NaCl. The mitotic index percentage has found to be maximum at 100 ppm and this has increased to 40 and 50% during 6-24 hr respectively in both variety. Minimum percentage of mitotic index has recorded at 500 ppm of NaCl at 24 hr seed soaking and less chromosome disturbed and breakage at 100ppm concentration and more at 500ppm concentration in table no.2.

Mitotic index percentage decreased and highly disturbed, breakage and sticky of chromosome with higher concentrations of NaCl. Reduction in mitotic index percent of Broad bean at higher concentration may be attributed to the interference of sodium ions. Similar inhibition of cytology at higher concentration of metal ions was observed by Jaya Kumar *et al.*. (2006) with cobalt andshoot grwoth is also reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (Kaymakanova, 2009).

Conclusion

The investigation concludes that seeds can adapt to saline conditions themselves, but there have been suggestions of external to counteract the salinity. The mitotic index percentage was observed,less sticky, disturbed and breakage of chromosome in both varieties at 100ppm solution is due to an increase in tissue water content, which is due to faster accumulation of ions, minerals and water in the tissues. However, the growth has stunted at higher concentrations and the results showed a reduction in germination percentage, root length, shoot length, total length and mitotic index percentage beyond 100ppm NaCl at time duration. On the basis germination and cytological study we found that germination percentage, seed vigour index, germination index, coefficient of velocity of germination, mitotic index, chromosome breakage, sticky chromosome and disturbance of chromosome was decreased under salinity stress in both varieties, but the variety Windsor (V_2) was found comparatively more resistant to salinity.

Table: 2 Cytological studies on *Viciafaba* L. seeds Under Salt stress

		Mitotic Index (M.I.) (%)										
			V1					V2				
Concentration ppm	Hours	Total no. of cells	No of dividing cell	M.I. %	Disturbed chromo %	Sticky chromo %	Breakage chromo %	No of dividing cell	M.I. %	Disturbed chromo %	Sticky chromo %	Breakage chromo %
Control	-	80	42	52.50	2.5	-	-	44	55	2.5	-	-
100	6	80	36	45.00	2.5	-	-	40	50	-	-	-
	12	80	34	42.50	3.75	3.75	-	38	47.5	-	-	-
	24	80	32	40.00	2.5	3.75	3.75	37	46.25	1.25	-	-
300	6	80	28	3.50	7.5	6.25	3.75	32	40	6.25	-	1.25
	12	80	26	32.50	7.5	7.5	3.75	30	37.5	5.0	5.0	2.50
	24	80	24	30.00	8.75	7.5	5	28	35	7.5	6.25	3.75
500	6	80	20	25.00	8.75	7.5	5	25	31.25	7.5	3.75	2.50
	12	80	19	23.75	7.5	8.75	5	2.4	30	7.5	7.5	3.75
	24	80	18	22.50	10.0	8.75	6.25	22	27.5	8.75	7.5	3.75

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