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EFFECT OF ISO-OSMOTIC WATER AND SALT STRESS ON GERMINATION AND EARLY SEEDLING GROWTH OF ISABGOL (*PLANTAGO OVATA* FORSK.)

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The germination and early seedling growth of Isabgol cv. HI-5 under iso-osmotic water and salt stress was assessed in Petri-dishes at various stress levels 0, -0.2, -0.4, -0.6, -0.8, -1.0 and –1.2 MPa. Osmotic effect was mainly responsible for the inhibition of seed germination. Osmotic (water) stress induced by PEG was relatively more inhibitory to germination than that caused by salt. The effects of chloride and sulphate salts were not distinct at iso-osmotic stress levels. Increase of osmotic stress significantly reduced the speed of seed germination (Maguire Index). Maximum delay in germination was found under PEG induced water stress followed by sulphate salt and chloride salt. Root length of seedlings was promoted by NaCl and PEG. A general reduction in cotyledonary leaf length was recorded under water stress. Maximum reduction was observed under PEG induced water stress followed by NaCl and Na_2SO_4. Seedling vigour was increased only by low osmotic stress levels of PEG and NaCl. More than 50 per cent decline in seedling vigour occured at –0.8 MPa osmotic potential of NaCl and PEG while at –0.6 MPa of Na_2SO_4. The iso-osmotic water and salt stress in general caused reduction in dry weight of root as well as cotyledonary leaves of seedlings, Na_2SO_4 was found more inimical in this respect than NaCl and PEG.

Keywords: Isabgol; Salt stress, Seed germination; Seedling vigour; Water stress.

Introduction

Isabgol (*Plantago ovata* Forsk) has gained importance as a cash crop because of its medicinal values. In this plant, seeds have medicinal value and the husk which is rosy white membranous covering of the seeds constitutes the drug. It is considered a safe laxative and is mainly used to cure habitual constipation, chronic diarrhoea, dysentery, inflammation of mucous membrane of gastro-intestinal and gastro-urinary tracts, duodenal ulcers and piles[1]. The seed husk is also used in calico printing, gum and jelly making, ice cream industry as stabilizer and as binder in tablets[1]. Isabgol is an important cash crop of India. It is mainly cultivated in Mehsana and Banskanth districts of Gujarat and to some extent in some districts of Madhya Pradesh, Haryana and Rajasthan[2] during winter as a Rabi crop. In the tropical arid or semi-arid climate of the above growing areas, soil salinity is one of the limiting factor to the agricultural production. The Isabgol crop also suffers from salt stress in such areas. Germination and seedling growth being crucial stages of crop development, therefore, need investigation under salt stress. Salts in the soil impose osmotic as well as ionic effects on the crop. The present investigation, therefore, has been aimed to study the effects of iso-osmotic water and salt stress on germination and the early seedling growth of the Isabgol cv. HI-5 which is grown in different parts of Haryana and neighbouring states.

Materials and Methods

Effect of iso-osmotic water and salt stress on germination and early seedling growth of Isabgol cv. HI-5 was studied in Petri-dishes. Iso-osmotic solutions of polyethylene glycol (PEG-6000), NaCl and Na_2SO_4 were prepared according to Micheal and Kaufman[3] and USDA Handbook-60 with various stress levels *viz.* 0 (control), -0.2, -0.4, -0.6, -0.8, -1.0 and –1.2 MPa. PEG was used as an inert osmoticum to induce water (osmotic) stress. Twenty healthy seeds of uniform size were sown in each petridish on filter paper beds (Whatman paper) soaked with 7 ml solution of respective stress level. Each stress treatment was replicated three times. Distilled water served as the control. The experiment was performed under controlled conditions i.e. 25±1°C and 16h light and 8 h dark period. Germination counts were made periodically at an interval of 24 h upto seven days and germination per cent was calculated for each treatment on seventh day. The criterion for seed germination was the emergence of radicle (more than 1 mm length). Speed of germination in various stress treatments was determined in terms of Maguire Index (MI) that was calculated by the following formula[4]:

$$MI = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\ldots \frac{(X_n - X_{n-1})}{Y_n}$$

Where,

X_n = Number of seeds germinated on n^{th} counting date

Y_n = Number of days after sowing to the n^{th} count.

On seventh day of germination the length and dry weight of root as well as cotyledonary leaf were recorded is all stress treatments. Vigour Index (VI) was also determined to evaluate the seedling vigour by the formula[5] given as under :

VI = Per cent germination × seedling length (cm)

Completely randomized design (CRD) was used in the experiment. Statistical analysis was done for this two factor asymmetrical factorial experiment.

Results and Discussion

Isabgol seeds showed 96.67 per cent germination at 0 MPa (Control). A progressive and significant decline in per cent germination of seeds was observed with the increasing levels of stress resulting from lowering of osmotic potential of culture solutions (Table 1). The inhibition of germination was mainly due to osmotic effects. Among the iso-osmotic PEG, NaCl and Na_2SO_4 treatments, PEG induced osmotic stress led to complete inhibition of the germination of seeds at the osmotic potential of –1.0 MPa while the chloride and sulphate salts of sodium caused germination failure at –1.2 MPa stress level. The PEG induced osmotic stress was relatively more inhibitory to germination than salt induced osmotic and ionic effects. These results were similar to those of Heikel *et al.*[6] who found decrease in the rate of seed germination and final germination percentage with rise of osmotic stress level irrespective of the stress agent used. They also obtained more reduction under PEG-6000 than NaCl. The inhibition of seed germination with increasing salinity gradient was also reported by other workers[7-11]. Likewise, More[12] observed a decline in per cent germination of pea plant.

The speed of germination as indicated by Maguire Index (MI) also declined significantly with decrease in osmotic potential of the culture solutions (Table 1). Among NaCl, Na_2SO_4 and PEG stress treatments, PEG induced osmotic stress caused maximum reduction in MI i.e. delay in per cent germination. Between chloride and sulphate salts of sodium, sulphate delayed germination more than chloride. The results corroborate the finding of Varshney and Rajkumar[13].

Root length of seedlings was promoted by NaCl and PEG induced osmotic stress. Elongation of root was relatively more under PEG treatment at every stress level. This shows that root elongation is induced more by water stress than salt stress. NaCl promoted root length upto –0.6 MPa osmotic potential and reduced thereafter (Table 2). The root length, however, declined with the increase of NaCl induced osmotic stress beyond –0.2 MPa. Under PEG treatment root length increased with the decrease of osmotic potential upto –0.4 MPa and declined thereafter but it remained more than the control. Na_2SO_4 did not favour root elongation. It was rather found deleterious to root growth as it significantly reduced root length beyond –0.4 MPa stress level. The ionic effects prevailed over osmotic effects in reducing root length under salt stress. Increase in root length of seedlings under PEG induced osmotic stress was an adaptation in seedlings for increasing the surface area for absorption of water under physiologically dry environment.

Almost contrary effects were observed on the cotyledonary leaf length. Growth of cotyledonary leaf was suppressed due to osmotic effects. This became evident from the reduction in length of cotyledonary leaves with the decreasing osmotic potentials of the culture solutions under PEG, NaCl and Na_2SO_4 stress treatment (Table 2). Maximum reduction in this parameter was recorded under PEG from –0.2 MPa to –0.4 MPa osmotic potentials. Between NaCl and Na_2SO_4, the reduction in cotyledonary leaf length was more in NaCl upto –0.4 MPa but beyond this level, the reduction was more in Na_2SO_4 stress treatment.

Salt stress in general caused significant reduction in dry weight of root of seedlings (Table 3). Reduction was more pronounced under Na_2SO_4 treatment than NaCl or PEG treatments. Na_2SO_4 was more inimical in this respect than NaCl and PEG. Decline in root dry weight of seedlings was insignificant upto –0.2 MPa stress level under PEG treatment while it was insignificant upto –0.4 MPa stress level under NaCl treatment.

Significant decline in dry weight of cotyledonary leaf was evident under all stress treatments. The reduction was more conspicuous under water stress (PEG treatment) than under salt stress treatments. Between the two sodium salts, Na_2SO_4 was relatively more deleterious to seedlings as this reduced the dry weight of cotyledonary leaf more than NaCl at every stress level. Reduction in dry weight of root and cotyledonary leaf of seedlings under iso-osmotic salt and water stress was also reported in many crops[14-18].

Table 1. Germination per cent (GP) and maguire index (MI) of Isabgol seeds under iso-osmotic water and salt stress.

Stress level (SL)	Germination percentage (GP) Stress treatment (ST)				Maguire index (MI) Stress treatment (ST)			
OP (MPa)	NaCl	Na_2SO_4	PEG	Mean	NaCl	Na_2SO_4	PEG	Mean
0.0	96.67 (80.43)	96.67 (80.43)	96.67 (80.43)	96.67 (80.43)	78.75	78.75	78.75	78.75
-0.2	93.33 (75.24)	93.33 (75.24)	93.33 (75.24)	93.33 (75.24)	64.45	60.82	61.25	62.17
-0.4	88.33 (70.10)	86.67 (68.85)	85.00 (67.40)	86.67 (68.85)	62.02	55.40	41.21	52.88
-0.6	85.00 (67.40)	78.33 (62.29)	76.67 (61.22)	80.00 (63.64)	54.15	45.40	40.08	46.54
-0.8	66.67 (54.78)	65.00 (53.63)	61.66 (51.81)	64.44 (53.41)	40.00	39.15	32.90	37.35
-1.0*	61.66 (51.62)	56.67 (48.84)	0.00 (2.87)	39.44 (39.44)	30.00	29.00	-	-
-1.2*	0.00 (2.87)	0.00 (2.87)	0.00 (2.87)	0.00 (2.87)	-	-	-	-
Mean	70.24 (57.71)	68.10 (56.02)	59.05 (48.84)	-	59.88	55.91	50.84	-

CD at 5% for GP
ST-2.46, SL=3.76, ST×SL=6.52

CD at 5% for MI
ST=2.15, SL=2.78, ST×SL=4.81

* Not included in statistical analysis (In case of MI)
Values in parentheses represent arc-sine transformation data

Table 2. Root length (RL) in cm and cotyledonary leaf length (CL) in cm of Isabgol seedling under iso-osmotic water and salt stress.

Stress level (SL)	Root length (RL) Stress treatment (ST)				Cotyledonary leaf (CL) Stress treatment (ST)			
OP (MPa)	NaCl	Na_2SO_4	PEG	Mean	NaCl	Na_2SO_4	PEG	Mean
0.0	1.37	1.37	1.37	1.37	1.93	1.93	1.93	1.93
-0.2	2.03	1.40	2.80	2.08	1.63	1.77	1.63	1.68
-0.4	1.70	1.33	3.73	2.26	1.30	1.50	0.73	1.18
-0.6	1.50	0.53	2.80	1.61	1.20	1.23	0.67	1.03
-0.8	0.93	0.23	2.13	1.10	0.77	0.70	0.20	0.56
-1.0*	0.63	0.10	-	-	0.57	0.47	-	-
Mean	1.51	0.97	2.57	-	1.37	1.44	1.03	-

CD at 5% for RL
ST=0.14, SL=0.18; ST×SL=0.30

CD at 5% for CL
ST=0.08; SL=0.10; ST×SL=0.17

*Not included in statistical analysis

Table 3. Dry weight (mg/20 seedlings) of root (R) and cotyledonary leaf (CL) of Isabgol seedlings under iso-osmotic water and salt stress.

Stress level (SL)	Root (R) Stress treatment (ST)				Cotyledonary leaf (CL) Stress treatment (ST)			
OP (MPa)	NaCl	Na_2SO_4	PEG	Mean	NaCl	Na_2SO_4	PEG	Mean
0.0	5.00	5.00	5.00	5.00	9.47	9.47	9.47	9.47
-0.2	4.87	4.77	4.97	4.87	9.17	9.00	8.97	9.04
-0.4	4.90	3.77	3.66	4.11	9.10	9.07	8.77	8.98
-0.6	4.67	2.70	2.97	3.44	8.97	8.77	8.57	8.77
-0.8	2.97	1.16	1.26	1.80	8.56	8.27	5.36	7.40
-1.0*	1.80	-	-	-	6.13	-	-	-
Mean	4.48	3.48	3.57	-	9.05	8.91	8.23	-

CD at 5% for R
ST=0.04; SL=0.06; ST×SL=0.10
*Not included in statistical analysis

CD at 5% for CL
ST=0.04; SL=0.05; ST×SL=0.09

Table 4. Vigour Index of Isabgol seedlings under iso-osmotic water and salt stress.

Stress level (SL)	Stress treatment (ST)			
OP (MPa)	NaCl	Na_2SO_4	PEG	Mean
0.0	329.33	329.33	329.33	329.33
-0.2	342.67	296.00	413.17	350.61
-0.4	264.83	259.67	380.33	301.61
-0.6	229.17	138.00	264.67	210.61
-0.8	116.00	65.33	141.50	107.61
-1.0*	74.17	32.17	-	-
Mean	256.40	217.67	305.80	-

CD at 5%
ST=16.46; SL=21.26; ST×SL=36.82
*Not included in statistical analysis

Seedling vigour measured in terms of vigour index declined with increasing stress levels (Table 4). PEG and NaCl stress treatments favoured increase of vigour index only at low stess levels. Na_2SO_4 reduced vigour index more than NaCl at every iso-osmotic stress level. More than 50 per cent decline in seedling vigour occurred at –0.8 MPa osmotic potential of NaCl and PEG. Same decline was observed at –0.6 MPa osmotic potential of Na_2SO_4. These results corroborate the findings of Varshney and Rajkumar[13]. The experimental investigation revealed that in general PEG induced water stress was most inhibitory to seed germination while Na_2SO_4 salt stress was most inimical to seedlings.

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