

# Mitigation of the influence of PEG-6000 imposed water stress on germination of halo primed rice seeds

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## Abstract

In the present investigation studies were made to see the effect of water stress, induced with the help of different concentrations (150 to 225 g/kg PEG-6000 equivalent to osmotic potential of -0.30 to -0.60MPa) of PEG-6000 in germinating rice varieties viz., HUR-3022 and Sahabhazi dhan. The results showed that osmotic potential from -0.30 to -0.49MPa, the inhibition in germination percentage was started as compared to control set but at -0.60MPa osmotic potential no germination was observed. When the  $Mg(NO_3)_2$  and  $K_2SO_4$  primed seeds were used with the osmotic potential of -0.30 to -0.49MPa the inhibitory effect of stress was found to overcome by the germinating seeds of rice. In the primed seeds the percentage of germination, vigor index, germination index and absolute water content (%) were increased in presence of imposed stress in respect to non primed seeds germinating under the same situation except mean time germination.

## Highlights

- Water stress induced with PEG-6000 decreased germination percentage in rice varieties viz., HUR-3022 and Sahabhazi dhan and also delayed germination time at higher concentrations.

**Keywords:** Germination percentage, vigor index, polyethylene glycol (PEG-6000), priming.

Seed germination is an essential process in plant development to attain optimal seedling number that produce high seed yield. Germination and seedling growth reduce with several abiotic factors such as salt and water stress which are possibly two of the main vital soil abiotic stresses that limits, number of germinating seeds/seedling and their development (Anaytullah *et al.* 2007, Ashraf *et al.* 1992, Almansori *et al.* 2001, Kaya *et al.* 2006, Atak *et al.* 2006, Agnihotri *et al.* 2006 and 2007). Water deficient conditions induced with PEG, decreased germination percentage and also delayed germination time at higher concentrations due to water stress. Therefore, the decrease in water potential gradient between seeds and their surrounding media by the effect of PEG 6000

adversely affects seed germination. Higher water deficient condition reduces germination due to limited water uptake by the seeds (Dodd and Donovan 1999, Kaydam and Yagmur 2008). The seed germination and emergence are reduced at a small negative osmotic potential (Taylor *et al.* 1982) still the specific potential inhibiting germination varied considerably. Moreover, it has been found that initiation of cell elongation itself during germination is differentially sensitive to water stress. The effects of water stress can be managed by plant improvement and selection, seed priming, plant growth regulators, and the use of osmo-protectants, with others. The embryo within the dry seed is dormant and highly tolerant to desiccation. It loses its tolerance upon germination and emergence.



Constitutive traits, such as seed size, may influence seedling establishment under water stress (Mian and Nafziger 1994). Drought stress induces reduction in plant growth and development of rice (Tripathy *et al.* 2000; Manikavelu *et al.* 2006). The cell growth is severely impaired due to reduction in turgor pressure under stress condition (Taiz and Zeiger 2006). Drought affects both elongation as well as expansion growth (Shao *et al.* 2008) and inhibits cell enlargement more than cell division (Jaleel *et al.* 2009). It impairs the germination of rice seedlings (Jiang and Lafitte 2007, Swain *et al.* 2014). By considering all the points, in the present investigation  $Mg(NO_3)_2$  and  $K_2SO_4$  primed seeds were used to see whether priming can mitigate the effect of induced water stress, imposed during germination of rice crop using various concentration of PEG-6000, presenting -0.30, -0.39, -0.49 and -0.60 MPa osmotic potential.

## Materials and methods

The present study was carried out in Seed Physiology Laboratory and Seed Priming Laboratory at Department of Plant Physiology, I.Ag.Sc., BHU, India, in the year 2012-13 in a completely randomized design (CRD) with 3 replication. Seeds were procured from the Genetics and Plant Breeding Department of the same institute. This experiment was conducted in the presence of diffused light condition at an average temperature of about 25-30°C during kharif season. Healthy bold seeds were surface sterilized with 0.1%  $HgCl_2$  (Mercuric chloride) solution for 2 min then washed thoroughly with distilled water for 5-6 times. For hardening treatment the seeds were either kept in beaker having different concentrations of  $Mg(NO_3)_2$  and  $K_2SO_4$  [5 and 7.5 mM ( $C_1$  and  $C_2$ )] for 20 h. After that the seeds were taken out and then dried back to its initial weight at the room temperature by placing them under fan. After that these seeds were packed in paper bags and were used as per requirement but within one month of treatment. The seeds without any treatment referred as control ( $C_0$ ). The osmotic potential of -0.30( $P_1$ ), -0.39( $P_2$ ), -0.49 ( $P_3$ ) and -0.60 ( $P_4$ ) MPa was induce to the rice seeds up to 96 h during germination by using concentrations of 150, 175, 200 and 225 g kg<sup>-1</sup> PEG-6000.

PEG solutions were prepared according to the method described by Michel and Kaufmann (1973).

$$\text{Osmotic Potential (OP)} = (-1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times C \times T + (8.39 \times 10^{-7}) \times C^2 T$$

where, C= PEG concentration, T= Temperature (25°C)

Petri dishes were placed at room temperature in the laboratory. A control was prepared for each set by using distilled water for germinating seeds.

Germination percentage, vigor index, mean time germination, germination index and absolute water content, were calculated by using these formulas:

$$\text{Germination Percentage} = \frac{\text{no. of seeds germinated}}{\text{total no. of seeds shown}} \times 100$$

$$\text{Vigor index} = \text{germination \%} \times \text{Dry wt.}$$

$$\text{Absolute water content} = \frac{\text{fresh weight-dry weight}}{\text{Dry weight}} \times 100$$

(Fresh and dry weight of seedlings were taken at 96 hour of germination. Dry weights of seedling were obtained by drying at 60-70°C till constant weight was achieved.)

$$\text{MTG (Mean Time Germination)} = \frac{\sum(nd)}{\sum n}$$

Where

- n: The number of germination during d days
- d: The number of days, since the beginning of germination

$$\sum n = \text{Total number of germinated seeds}$$

$$\text{GI (Germination Index)} = \frac{\text{Number germinated seeds/ days of first count} + \dots + \text{Number germinated seeds/ Days of final count}}{\text{Number germinated seeds/ Days of final count}}$$

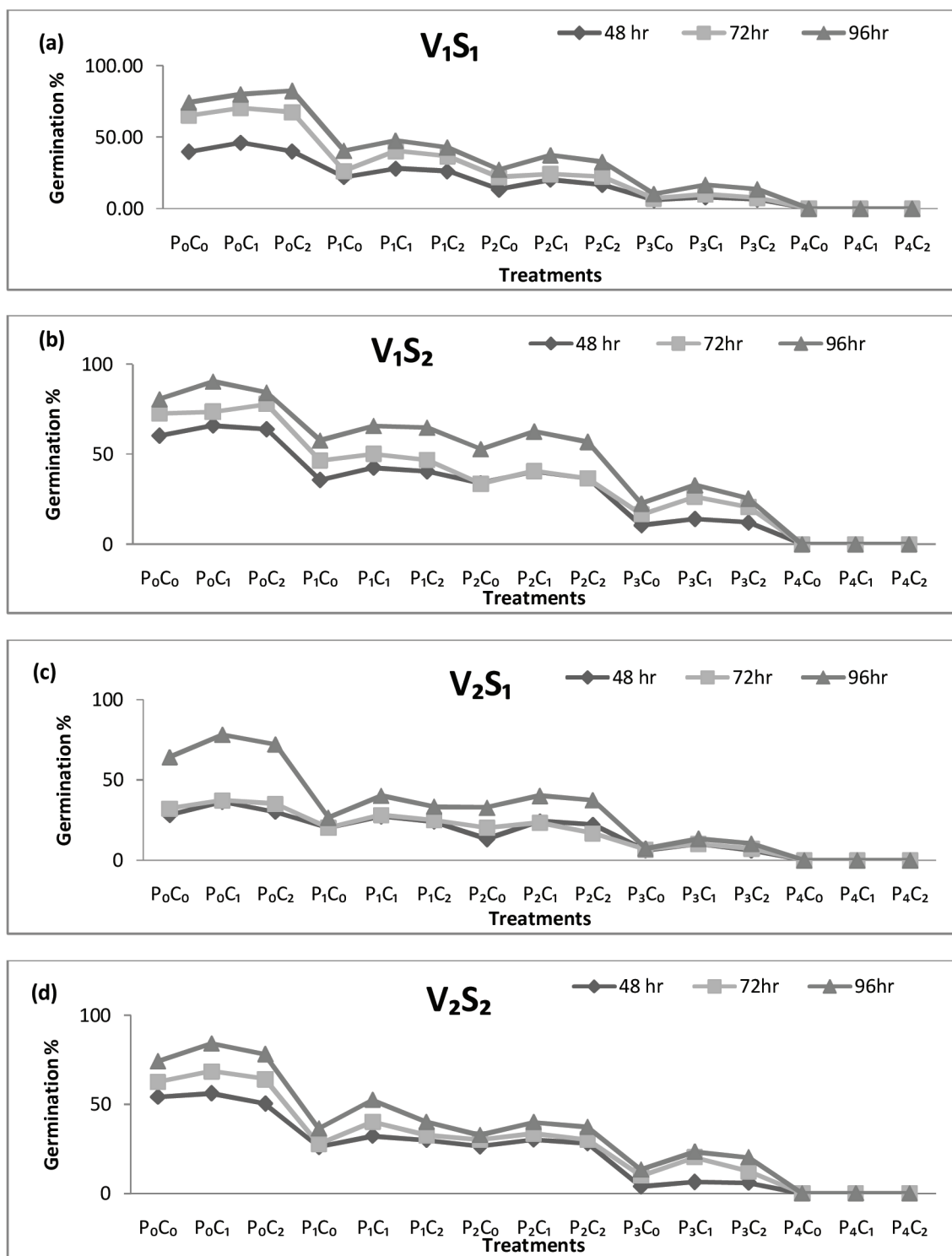
## Results and discussion

The data presented in figure 1(a, b, c and d) and Table 1 showed that as period of germination increased percent germination and vigor index were also increased for both the varieties. The observations regarding imposed water stress, non stressed control ( $P_0$ ) represented maximum germination percentage in  $C_2$  concentration (82.33%) of salt  $S_1$  while in  $S_2$  salt  $C_1$  concentration (78%) showed best result for variety  $V_1$ . In case of variety  $V_2$ ,  $C_1$  concentration [90.27% for  $S_1$  and 84.10% for  $S_2$ ] of both the salts showed better performance at 96 h of germination. The vigor index of seedlings was also recorded higher in  $C_1$  concentration of both the

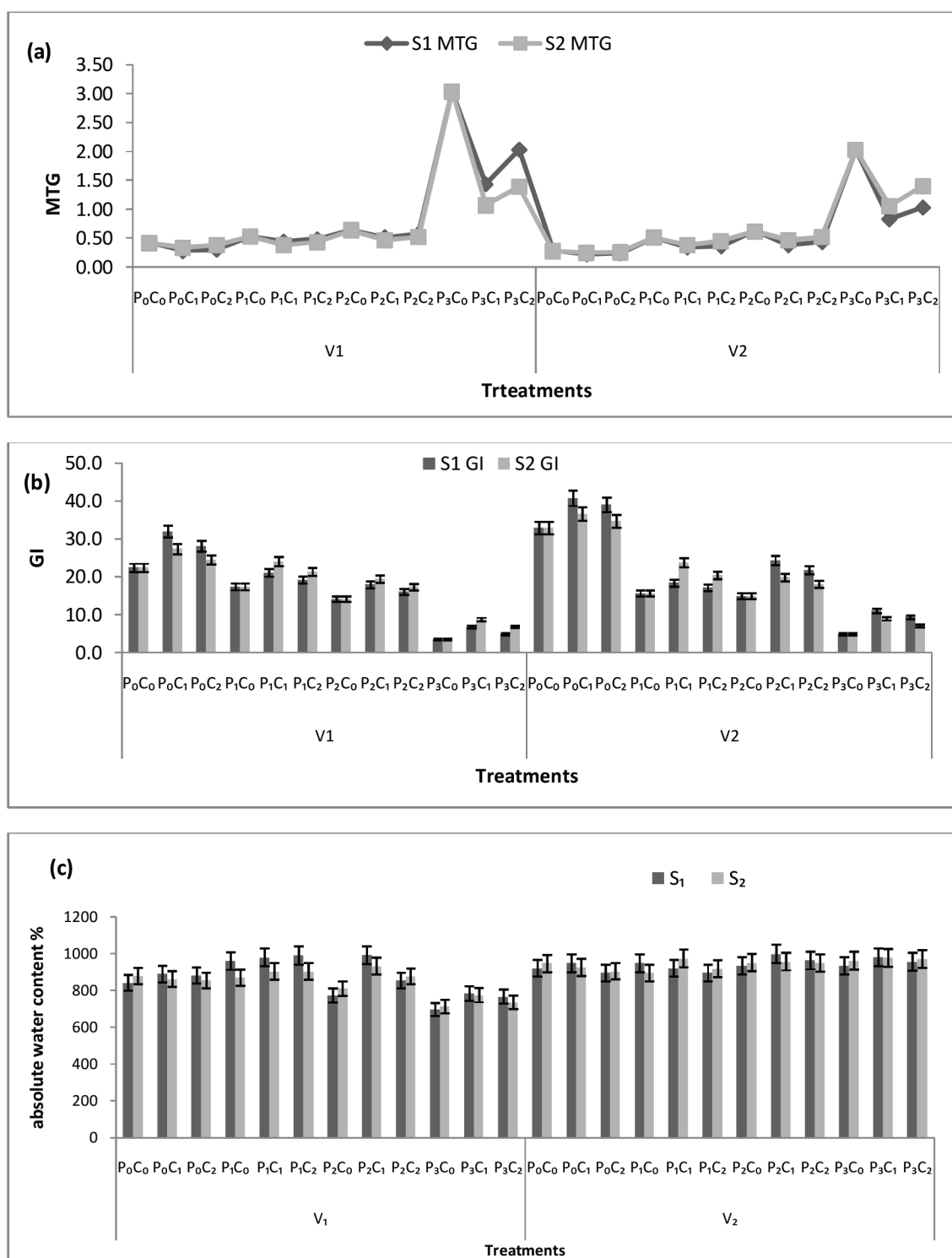
**Table 1:** Effect of water stress imposed by using PEG 6000 on vigor index of rice seeds [Var. V<sub>1</sub> (HUR3022), V<sub>2</sub> (Sahbhagidhan)] primed with different salts

variety	treatments		S <sub>1</sub>				S <sub>2</sub>					
			48 hr	72hr	96hr	Mean	48 hr	72hr	96hr	Mean		
V <sub>1</sub>	P <sub>0</sub> C <sub>0</sub>		903.74		1503.10		1749.84	1385.56	607.57	675.11	1367.25	883.31
	P <sub>0</sub> C <sub>1</sub>		1093.35		1779.18		2211.21	1694.58	878.37	883.35	1960.13	1240.62
	P <sub>0</sub> C <sub>2</sub>		942.66		1681.64		1927.71	1517.34	711.13	741.67	1790.57	1081.12
	P <sub>1</sub> C <sub>0</sub>		427.79		510.69		820.78	586.42	417.49	390.60	589.64	465.91
	P <sub>1</sub> C <sub>1</sub>		579.62		807.06		1035.64	807.44	640.16	701.36	942.36	761.29
	P <sub>1</sub> C <sub>2</sub>		510.28		728.93		900.97	713.39	547.81	588.26	749.51	628.53
	P <sub>2</sub> C <sub>0</sub>		246.51		409.15		548.09	401.25	274.86	387.19	892.54	518.20
	P <sub>2</sub> C <sub>1</sub>		398.57		469.55		794.50	554.21	539.66	560.34	1197.68	765.89
	P <sub>2</sub> C <sub>2</sub>		311.05		427.48		680.13	472.89	475.85	334.12	1020.97	610.31
	P <sub>3</sub> C <sub>0</sub>		119.16		128.79		190.52	146.16	109.80	122.55	147.51	126.62
	P <sub>3</sub> C <sub>1</sub>		152.72		192.90		324.22	223.28	211.78	233.71	291.65	245.71
	P <sub>3</sub> C <sub>2</sub>		115.34		142.24		261.40	172.99	117.77	143.74	217.35	159.62
V <sub>2</sub>	P <sub>0</sub> C <sub>0</sub>		1427.85		1551.55		1803.22	1594.21	1257.68	1448.46	1786.37	1497.50
	P <sub>0</sub> C <sub>1</sub>		1622.04		1779.18		2211.21	1870.81	1353.76	1650.06	2152.67	1718.83
	P <sub>0</sub> C <sub>2</sub>		1528.88		1848.94		1994.72	1790.85	1175.30	1492.95	1938.80	1535.68
	P <sub>1</sub> C <sub>0</sub>		707.72		928.05		1224.70	953.49	593.28	625.99	862.31	693.86
	P <sub>1</sub> C <sub>1</sub>		947.73		1154.91		1540.84	1214.49	768.83	955.96	1308.09	1010.96
	P <sub>1</sub> C <sub>2</sub>		866.98		1015.92		1449.21	1110.70	691.55	751.56	958.40	800.50
	P <sub>2</sub> C <sub>0</sub>		656.39		683.53		1103.23	814.38	558.47	649.02	703.03	636.84
	P <sub>2</sub> C <sub>1</sub>		878.69		879.57		1337.45	1031.90	690.76	749.18	911.26	783.73
	P <sub>2</sub> C <sub>2</sub>		784.56		752.04		1199.33	911.98	608.30	655.27	837.01	700.19
	P <sub>3</sub> C <sub>0</sub>		191.67		302.79		425.32	306.59	79.17	209.41	273.05	187.21
	P <sub>3</sub> C <sub>1</sub>		269.65		522.58		644.98	479.07	140.26	462.92	509.48	370.89
	P <sub>3</sub> C <sub>2</sub>		224.95		392.53		485.98	367.82	129.39	281.07	427.63	279.36
	48 hr		72hr		96hr		48 hr		72hr		96hr	
	SEm±	CD%	SEm±	CD%	SEm±	CD%	SEm±	CD%	SEm±	CD%	SEm±	CD%
P	2.38	6.77	3.56	10.12	3.64	10.34	2.24	6.37	4.97	14.12	2.77	7.89
S	2.06	5.86	3.08	8.77	3.15	8.95	1.94	5.52	4.30	12.23	2.40	6.83
V	1.68	4.79	2.52	7.16	2.57	7.31	1.58	4.51	3.51	9.99	1.96	5.58
P×S	4.12	NS	6.17	NS	6.30	NS	3.88	NS	8.60	NS	4.80	13.66
P×V	3.37	9.58	5.03	14.31	5.14	14.62	3.17	9.01	7.02	19.97	3.92	11.15
S×V	2.92	NS	4.36	NS	4.45	NS	2.75	7.81	6.08	NS	3.40	NS
P×S×V	5.83	NS	8.72	NS	8.91	NS	5.49	NS	12.17	NS	6.79	NS

P<sub>0</sub> = Control, P<sub>1</sub> = PEG treatment (-0.30MPa), P<sub>2</sub> = PEG treatment (-0.39 MPa), P<sub>3</sub> = PEG treatment (-0.49MPa), S<sub>1</sub> = Mg(NO<sub>3</sub>)<sub>2</sub>, S<sub>2</sub> = K<sub>2</sub>SO<sub>4</sub>, C<sub>0</sub> = Non primed, C<sub>1</sub> = 5mM, C<sub>2</sub> = 7.5mM, V<sub>1</sub> = HUR3022, V<sub>2</sub> = Sahbhagidhan, Values represented the mean of three biological replicates ±SD. Data was significant at P ≤ 0.05.



**Fig. 1:** Effect of water stress imposed by using PEG 6000 on germination % of rice seeds [Var. V<sub>1</sub> (HUR3022), V<sub>2</sub> (Sahbhagidhan)] primed with different salts. where: P<sub>0</sub>= Control, P<sub>1</sub>=PEG treatment (-0.30MPa), P<sub>2</sub>=PEG treatment (-0.39MPa), P<sub>3</sub>=PEG treatment (-0.49MPa), P<sub>4</sub>=PEG treatment (-0.60MPa), C<sub>0</sub>= Non primed, C<sub>1</sub>= 5mM, C<sub>2</sub>= 7.5mM, (a) (Mg(NO<sub>3</sub>)<sub>2</sub>:S<sub>1</sub>, HUR3022:V<sub>1</sub>), (b) (K<sub>2</sub>SO<sub>4</sub>:S<sub>2</sub>, HUR3022:V<sub>1</sub>), (c) (Mg(NO<sub>3</sub>)<sub>2</sub>:S<sub>1</sub>, Sahbhagi dhan:V<sub>2</sub>) and (d) (K<sub>2</sub>SO<sub>4</sub>:S<sub>2</sub>, Sahbhagi dhan:V<sub>2</sub>). Values represented the mean of three biological replicates  $\pm$ SD. Data was significant at  $P \leq 0.05$ .



**Fig. 2:** Effect of water stress imposed by using PEG 6000 on mean time germination (MTG) (a), germination index (GI)(b) and absolute water content(%)(c) of rice seeds [Var. V<sub>1</sub> (HUR3022), V<sub>2</sub> (Sahbhagidhan)] primed with different salts, where: P<sub>0</sub>= Control, P<sub>1</sub>=PEG treatment (-0.30MPa), P<sub>2</sub>=PEG treatment (-0.39 MPa), P<sub>3</sub>=PEG treatment (-0.49 MPa), S<sub>1</sub>= Mg(NO<sub>3</sub>)<sub>2</sub>, S<sub>2</sub>= K<sub>2</sub>SO<sub>4</sub>, C<sub>0</sub>= Non primed, C<sub>1</sub>= 5mM, C<sub>2</sub>= 7.5mM, V<sub>1</sub>= HUR3022, V<sub>2</sub>= Sahbhagidhan, Values represented the mean of three biological replicates ±SD. Data was significant at P≤ 0.05.





salts. As stress increased, the germination percentage and vigor index declined in respect to non stressed control ( $P_0$ ) for both the varieties but Sahbhagi dhan performed well in comparison to HUR-3022. At  $P_4$  stress regime there was no germination took place in non primed as well as in primed seeds of both the varieties so this concentration was discarded for subsequent experimental parameters.

According to the results listed in figure 2 (a and b) it can be seen that during control condition,  $C_1$  concentration of both the salts, showed minimum value for MTG and higher value for GI which was followed by  $C_2$  concentration in respect to non primed one. The data showed that as stress level increased MTG was also increased and GI was declined for both the varieties. The priming treatments showed its significance during stress condition. At  $P_1$  and  $P_2$  stress level  $C_1$  concentration of both the salts showed minimum value for MTG and maximum for GI which was followed by  $C_2$  concentration in respect to  $C_0$ . At  $P_3$  stress level MTG was recorded higher in respect to  $P_1$  and  $P_2$  but when comparing priming treatments of only  $P_3$  stress level the minimum value of MTG was recorded for  $V_1$  variety (1.43 for  $S_1$  and 1.07 for  $S_2$ ) and for  $V_2$  variety (0.83 for  $S_1$  and 1.06 for  $S_2$ ) at  $C_1$  concentration of both the salts respectively and the maximum value of GI for  $V_1$  (6.75 for  $S_1$  and 8.75 for  $S_2$ ) and for  $V_2$  (11.0 for  $S_1$  and 8.92 for  $S_2$ ) was also observed at  $C_1$  concentration at  $P_3$  stress level. The absolute water content (AWC) of the seedlings was decreased with increasing concentration of PEG-6000 (Figure 2c). In non stressed sets non primed seedling showed higher AWC but as stress was introduced primed sets showed better performance.  $Mg(NO_3)_2$  primed sets showed higher AWC in comparison to  $K_2SO_4$  primed sets for both the varieties. Similar findings were recorded by Haq *et al.* (2010) that the increase of PEG concentrations lower water uptake by seeds resulting in decrease of germination. Therefore, the decrease in water potential gradient between seeds and their surrounding media by the effect of PEG 6000 adversely affects seed germination. Pradhan *et al.* (2015) also reported that different osmotic potential and priming duration had significant effect on germination percentage, vigor index and dry weight of seedlings. Seed-priming of different types have been observed to improve seed germination rate and seedling emergence in a number of studies (Pant and Bose 2015, Srivastava *et al.* 2010, Chen and

Arora 2011). Priming usually exerts strong influence on germination rate and uniformity in emergence of seedlings under stressful conditions (Wahid *et al.* 2007). However soaking of mustard seeds with sulphate and nitrate ion containing salts were found to improve seedling growth, nitrate reductase activity and yield attributes (Bose and Mishra 1999); they suggested that sulphate might facilitate the use of other nutrients and finally may improve the yield parameters of mustard as reported by Chatterjee *et al.* (1985) and Singh *et al.* (1988). As per nitrate ion is concern Anaytullah and Bose (2007) reported that Nitrate-hardened seeds of wheat showed an increased rate of germination, seedling length and fresh and dry weight of roots and shoots in comparison to the non hardened seeds. Higher water deficient condition reduces germination due to limited water uptake by the seeds (Dodd and Donovan 1999, Kaydam and Yagmur 2008). The explanation of the higher inhibitory effects of PEG lies in ion or solute entry into the seed (Alam *et al.* 2002). The effects of priming on the subsequent growth of the seedlings were most likely due to the effect of potassium on increased water uptake and on other metabolic reactions (Owino and Ouma, 2011). Mg the another ion used in the present investigation in form of  $Mg(NO_3)_2$  in which the Mg ions may enter during priming (during soaking of seeds) and improve the respiratory metabolism (Bose and Mishra 1999) and also its carried over effects improves the seedling growth via increasing photosynthetic activity as Mg is the part of chlorophyll content (Anaytullah *et al.* 2012).

## Conclusion

Water deficient conditions induced with PEG-6000 decreased germination percentage and also delayed germination time at higher concentrations due to water stress which can be mitigated with the use of  $Mg(NO_3)_2$  and  $K_2SO_4$  halo primed seeds.

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