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Full Length Research Paper

The Effects of water and salt stresses on germination in two bread wheat genotypes

Roza Gholamin and Majid Khayatnezhad

Young Researchers Club, Ardabil Branch, Islamic Azad University, Ardabil, Iran.

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This study was performed to evaluating the effect of osmotic stress at the water potential levels induced by polyethylene glycol 6000 and NaCl solutions at germination stage of bread wheat genotypes (Cascogne and saisonez) at laboratory, in Iran in 2011 as factorial experiment under complete randomized design with four replications. Daily and final germination as well as germination and seedling emergence rate, and seedling fresh and dry weight were measured in the study under controlled conditions. Seedling growth was reduced by both stresses. But NaCl usually caused less damage than PEG to durum wheat seedlings, suggesting that NaCl and PEG acted through different mechanisms. Results of analysis of variance showed significant difference of all factors on all traits mostly at 0.01 percentage level. Results showed those germination rate was delayed by both solutions in both varieties, with differences between genotypes among growth stages, given that Saisonez genotype showed a higher germination rate than Cascogne genotype in NaCl. NaCl had a lesser effect on genotypes in terms of germination rate and the final germination than did PEG. This conclusively proves that the adverse effect of PEG on germination and early seedling growth was due to the osmotic effect rather than the specific ion. This difference in cultivar's behavior according to the growth conditions is discussed. It was concluded that inhibition in germination at equivalent water potential levels of NaCl and PEG was mainly due to an osmotic effect rather than did salt toxicity. Finally Saisonez genotype showed that resistance to both NaCl and PEG stress than Cascogne genotype. So Sisonez is tolerant that Cascogne genotype at germination stage.

Key words: Bread wheat, germination, NaCl, PEG (polyethylene glycol)

INTRODUCTION

Wheat (Triticum aestivum L.) is the core commodity of the Iranian food and agriculture systems, grown on nearly half of the country's rainfed areas and one-third of the irrigated area. As such, the rain-fed wheat crop covers nearly 4.5 million hectares, while the irrigated wheat crop covers approximately 2.2 million hectares. The average yield for irrigated wheat is approximately 3.0 ton/ha, compare to 0.95 ton/ha for rain-fed wheat. Most of the rain-fed wheat crop is located in the western provinces of Kermanshah, Kurdistan, and Azerbaijan, with a larger share of the irrigated wheat crops located in the east (Adasi et al., 2010). One of the major problems in agriculture is abiotic stress which prevents plants from realizing their full genetic potential and limits food production. In arid and semiarid regions with Mediterranean climate, wheat crops usually encounter drought during the grain filling period, which reduces grain yield, dramatically (Ehdaie and Waines, 1996). Stalinization is the scourge of intensive agriculture (khayatnezhad et al., 2011). High concentration of salts has detrimental effects on germination of seeds (Rahman et al., 2000; Kayani and Rahman, 1987), and plant growth (Pandey and Thakrar, 1997).

Many investigators have reported retardation of germination and growth of seedlings at high salinity (Bernstein, 1991). However plant species differ in their sensitivity or tolerance to salts (Torech and Thompson, 1993). Drought and soil salinity are major abiotic con-

*Corresponding author. E-mail: Gholamein@yahoo.com.

straints on crop production and food security, and adversely impact the socio-economic fabric of many developing countries. Water scarcity, declining water quality for irrigation, and soil salinity are problems which are becoming more acute (Flowers, 2004). It is estimated that 20% of all cultivated land and nearly half of irrigated land is affected by salt, greatly reducing the yield of crops to well below their genetic potential (Flowers, 2004; Munns et al., 2006; Jones, 2007). Achieving genetic increases in yield under these stresses has always been a difficult challenge for plant breeders (Khayatnezhad et al., 2010). High concentrations of salts in the soil solution impair cell metabolism and photosynthesis but impose an osmotic stress on cell water relations by increasing the toxicity of sodium in the cytosol (sayar et al., 2010). Under stresses conditions, the germination of seeds is affected by creating an external osmotic potential that prevents water uptake due to the toxic effects of Na^+ and Cl^- ions both during imbibition and seedling establishment (Murillo-Amador et al., 2002). The accumulation of soluble salts in soil leads to an increase in osmotic pressure of the soil solution, which may limit the absorption of water by the seeds or plant roots. Salt damage to plants is attributed to reduction in water availability, toxicity or specific ions, and nutritional imbalance caused by such ions (James et al., 2006). Polyethylene glycol (PEG) widely used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly (Kawasaki et al., 1983). In contrast, Na+ and Cl^- penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm for sensitive cultivars (Genc et al., 2007). The screening of salt tolerant lines/cultivars has been attempted by many researchers on various species at seedling growth stage (Khayatnezhad et al., 2010). The relation of various seedling growth parameters to seed yield and yield component under saline conditions are important for the development of salt tolerant cultivar for production under saline conditions. Powerful new molecular tools for manipulating genetic resources are becoming available (Munns, 2005). The same authors have reported that this approach identified several markers linked to a gene at a QTL designated Nax1 (Na^+ exclusion). Munns et al. (2006) has mentioned also that a region on the long arm of chromosome has been shown to contain a quantitative trait locus (QTL) for Na^+ exclusion and K^+/Na^+ discrimination. Major increases in salt tolerance of plants would be possible by introducing new genes either by crossing with new donor germplasm or by transformation with single genes. The study presented here deals with the response of two cultivars of wheat to NaCl and water stress at germination and early seedling growth stage.

MATERIALS AND METHODS

This study was carried out at a laboratory in Ardabil, located in North-western Iran, using two Bread wheat genotypes, namely: Saisonez and Cascogne, which appeared to be drought susceptible and tolerant, respectively (Gholamin et al., 2010). On the basis of the preliminary results of a field trial, 22 Durum wheat varieties were selected for this study. This study was performed in a factorial experiment based on completely randomized design with four replications. Germination and early seedling growth (ten days) of these varieties were studied in two experiments using distilled water (control) and osmotic potentials (-2, -4, -6 and -8 MPa), which were prepared by adding NaCl or PEG-6000 to the distilled water according to Van't Hoff's equation (Lang, 1967), to have the same osmotic potential in both NaCl and PEG-6000 solutions. Calcium hypochloride solution contained 5% active chlorine for 5 min was used. The seeds were then washed three times with sterilized distilled water. All the experiments were conducted in a Petri plate (12 cm) on filter paper beds in growth chambers. 20 grains were sown in 12 cm diameter Petri plate on filter paper beds irrigated with 5 ml solution of the respective treatment and incubated at 25°C. The dishes were moistened with equal amounts of the desired osmotic solutions (NaCl or PEG-6000 solutions, and osmotic potentials of 0, -2, -4, -6 and -8 MPa). The number of germinated seeds was recorded daily and the final germination percentage was determined after ten days. The germination rate was calculated using Maguire's equation (1962): $M = n_1/t_1 + n2/t_2 + \ldots + n_7/t_7$; where $n_1, n_2, \ldots, n_7$ are the number of germinated seeds at times t1, t2,…, t7 (in days).

Five seedlings were taken randomly and the seedling growth on the height and leaf area was measured by the fresh and dry weights of the different parts of the seedling on the tenth day after emergence. Dry weight was determined for each plant after drying the samples in a forced-air dryer at 80°C for 48 h. The experimental design and statistical analyses were similar to those used for the germination test. However, the data used were subjected to statistical analysis using ANOVA (a statistical package available from Spss v.16 and MStatc).

RESULTS

The results of ANOVA for germination rate and germination percent showed that genotypes, Solutions and interaction between genotype and osmotic potential were significant for both traits in 0.01 percentage level. Osmotic potential, Interaction between genotype and Solutions and Three-way interaction was significant at 0.05 percentage level for both traits, and interaction between solution and osmotic potentials was significant at 0.05 percentage level only for germination percentage (Table 1).

The germination rate decreased with a decrease in osmotic potential in both NaCl and PEG solutions but the inhibition was greater under PEG for both genotypes (Figure 1). In NaCl solution, Cascogne was more affected, although it increased the germination rate in -0.2 and again decreased in -0.6 and -0.8 MPa (Figure 2), while the rate in Saisonez did not decrease proportionally as osmotic potential increased under NaCl solution. Saisonez genotype decreased germination rate at PEG solution, but Cascogne genotype increased in -0.2 and then decreased for other levels.

However, the effect of osmotic agent (NaCl, PEG) was found. Both genotypes showed different germination (expressed as cumulative percentage) in all osmotic

Table 1. Effects of NaCl and PEG on the germination rate and germination percentage on Bread wheat Genotypes.

Source of variation (SOV)	df	Mean squares (MS)	
		Germination rate (GR)	Germination percentage (GP)
Genotypes (G)	1	**	**
Solutions (S)	1	**	**
Osmotic potentials (O)	4	*	*
GS	1	*	*
GO	4	**	**
SO	4		*
GSO	4	*	*
Error	54	0.84	12.58

** and * significant at the 0.01 and 0.05 levels, respectively.

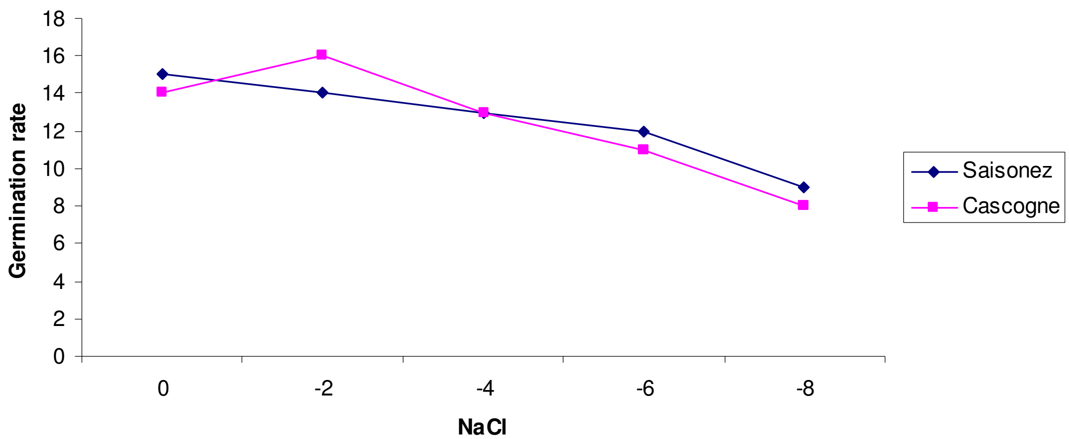

Figure 1. Germination rate of two Breas wheat genotypes under decreasing external osmotic potential levels induced by NaCl solution.

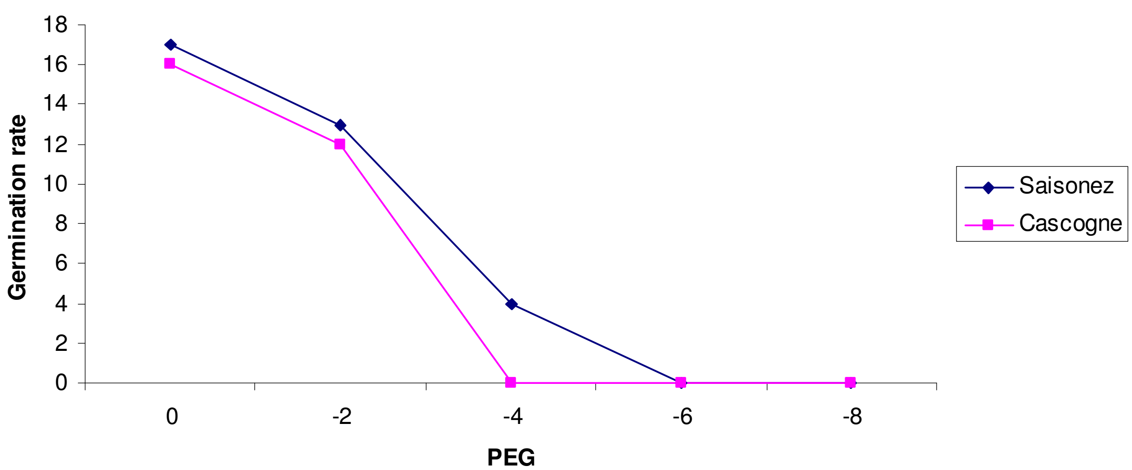

Figure 2. Germination rate of two Breas wheat genotypes under decreasing external osmotic potential levels induced by PEG solution.

potential levels of NaCl solution (Figures 3, 4), and their final germination percentage decreased, and delayed as the osmotic potential increased at PEG solutions (Figures 5, 6). Between genotypes, Saisonez was better than Cascogne at -0.8 levels of Nacl and PEG.

The analysis of variance showed significant differences between genotypes, solutions and osmotic potential for fresh weight shoot height and dry weight at 0.01 percentage levels (Table 2). In these traits also, the interactions between the examined factors were mostly significant. In leaf area and shoot height, however, the effects of osmotic potential and Three-way interaction were not significant but the effect of different solutions (different osmotic potential levels of NaCl or PEG) was highly significant.

PEG significantly reduced the seedling fresh-weight of both cultivars (Table 3), but Saisonez fresh weight in control levels of NaCl was higher than PEG control levels. The seedling dry-weight showed that both genotypes dry-weight were less affected than PEG solutions (Table 3). Seedling height was higher in PEG than in NaCl solution in both genotypes. Saisonez showed higher seedling height in NaCl than Cascogne but in PEG both genotypes decreased as osmotic potential increased (Table 3) Leaf area was more affected by PEG than NaCl. Saisonez was better than Cascogne in both solutions and all osmotic potential levels (Table 3). In both genotypes, leaf area decreased linearly as osmotic potential levels in PEG increased. In general, PEG at isoosmotic concentration was more harmful to seedling growth than NaCl. The higher inhibition of all growth variables in PEG treated plants than in NaCl shows osmotic dehydration in the factor affecting seedling growth.

DISCUSSION

Water and salt stresses, due to drought, are probably the most significant abiotic factor limiting plant and also crop growth and development (Hartmann et al., 2005). Drought stresses are physiologically related, because the induced osmotic stress and most of the metabolic responses of the affected plants are similar to some extent (Djibril et al., 2005). Water deficit affects the germination of seed and the growth of seedlings negatively (Van Den Berg and Zeng, 2006). Due to the fact that germination is one of the most important traits in the early stage of growth in most plants, it seems that golden west in drought stress condition have more resistance and yield potential than other cultivars. According to the results of this study, it is suggested that more experiments should be carried out on similar cultivars and further investigation should be done on golden west. The results of this study are in agreement with those obtained by Kalefetoglu et al. (2009) on wheat.

In this study, two Bread wheat genotypes with regards to their drought/salt resistance were compared with an imposed water stress in controlled conditions during germination and the early seedling growth stage. The results show that although the two genotypes were planted under the same conditions, they displayed distinct responses to salinity and drought stress. In this sense, genetic variability within a species offers a valuable tool for studying the different mechanisms of salt and drought tolerance. One of these mechanisms depends on the capacity for osmotic adjustment, which allows growth to continue under saline conditions. This is basically true for water stress, although osmotic adjustment is not achieved in the same way under both stresses. Under salt stress, this process is accomplished by uptake and accumulation of inorganic ion, mainly Na^+ and Cl^-; whereas under water stress, it is achieved by synthesis and accumulation of organic compatible solutes (Alian et al., 2000). Significant differences were observed between the examined genotypes, solutions, osmotic potentials and their interactions with regard to germination rate and final germination percentage (Table 1). Salinity (NaCl) may also affect germination by facilitating the intake of toxic ions, which may change certain enzymatic or hormonal activities of the seed (Smith and Comb, 1991). These physicochemical effects upon the seed seem to result in a slower and/or lower rate of germination or emergence. Both osmotic and toxic effects of salts have been implicated in the inhibition of seed germination (El-Hendawy et al., 2005). The cultivar Saisonez showed a higher germination rate in NaCl solution, but both cultivars decreased in PEG as osmotic potential increased (Figures 1 and 2). Since seed germination is more sensitive to salinity and drought stress than the emer-gence or growth of the established seedling (Freeman, 1973), the greater tolerance to salinity and drought of Durum wheat during emergence would be an adaptive feature of this species to saline or drought environments. Previous research, according to Levitt (1980), indicated that the germination tests were usually not good indicators of the differences in salt or drought tolerance among cultivars. Rhoades (1990) reported that some plants are relatively tolerant during germination, but become more sensitive later. In the same way, germi-nation and seedling emergence from laboratory results does not necessarily represent germination and seedling emergence from field soils. Still, the most important agronomic question is whether or not the observed differences in salt tolerance, during early stages, are representative of the salt tolerance of the cultivars during the whole growth cycle. The fresh and dry weights, seedling height and leaf area of the seedlings ten days after imbibitions, show that growth was inhibited by both NaCl and PEG. Apparently, the presence of NaCl or PEG in the germination and emergence medium reduced the uptake of water by the seedlings and inhibited the mobilization of the seed reserves to the growing embryonic axis. These data are

Table 2. Effects of NaCl and PEG on the fresh and dry weight, seeding height and leaf area of Bread wheat.

Source of variation (SOV)	df	Mean squares (MS)			
		Fresh weight	Dry weight	Plant height	Leaf area
Genotypes (G)	1	**	**	**	**
Solutions (S)	1	**	**	**	**
Osmotic potentials (O)	4	**	**	**	**
GS	1	**	**	**	**
GO	4	**	**	*	**
SO	4	**	**	**	**
GSO	4	**	**	**	**
Error	54	0.098	0.154	1.59	2.87

**: significant at the 0.01 level, respectively.

Table 3. Effects of NaCl and PEG on the growth of 10-days-old seedlings examined for fresh weight (FW. g/plant), dry weight (DW. g/plant), Seeding height (SH. cm) and leaf area (LA.cm²) in two Bread wheat genotypes; the bars represent the standard error of the mean (P < 0.05).

Osmotic Potential	Cascogne								Saisonez							
	NaCl				PEG				NaCl				PEG			
	LA	SH	DW	FW	LA	SH	DW	FW	LA	SH	DW	FW	LA	SH	DW	FW
0	12	14	0.1	0.28	9	13	0.11	0.28	16	18	0.12	0.35	13	17	0.14	0.32
-2	11	14	0.08	0.16	4	12	0.1	0.20	14	17	0.1	0.3	8	16	0.12	0.21
-4	10	13	0.07	0.14	3	10	0.08	0.14	10	16	0.08	0.27	6	15	0.11	0.18
-6	6	10	0.75	0.14	2	8	0.06	0.14	8	15	0.06	0.18	6	15	0.10	0.18
-8	6	10	0.75	0.10	1	7	0.07	0.8	8	13	0.06	0.16	6	13	0.11	0.18

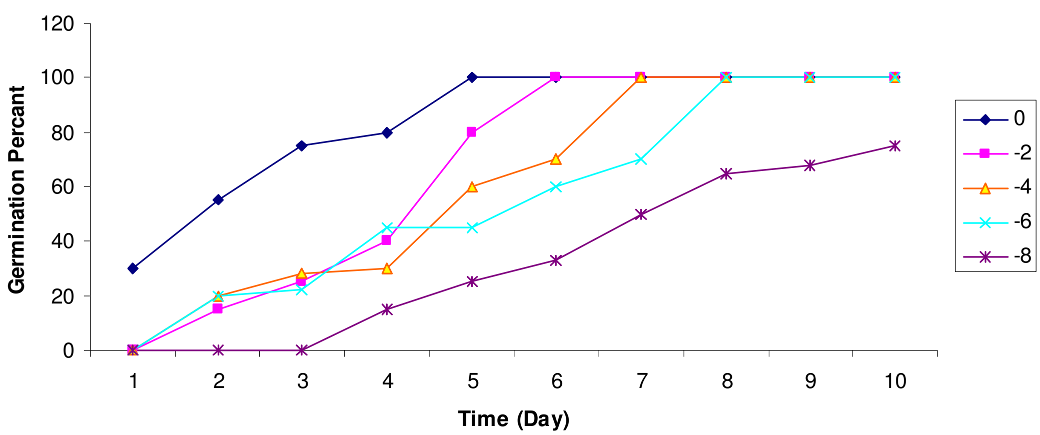

Figure 3. Germination of Saisonez seeds at NaCl osmotic potential.

in agreement with those of other studies on germination in the presence of NaCl or non ionic osmotic solutions, such as Mannitol or PEG (Kawasaki et al., 1983; Sayar et al., 2010). The results obtained in this study reveal that dry weights were less affected than fresh weight and that they are in agreement with the results obtained by Murillo-Amador et al. (2002) in cowpea. These results are also consistent with those found by Sayar et al. (2010) in wheat, who affirmed that growth medium salinity or drought may affect seed germination by decreasing the ease with which the seeds take up water because the activity and events normally associated with germination

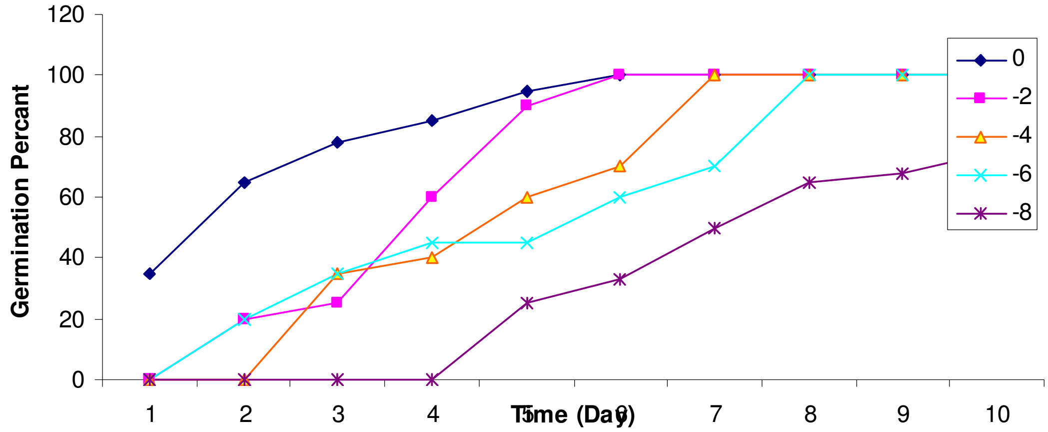

Figure 4. Germination of Cascogne wheat seeds at NaCl osmotic potential.

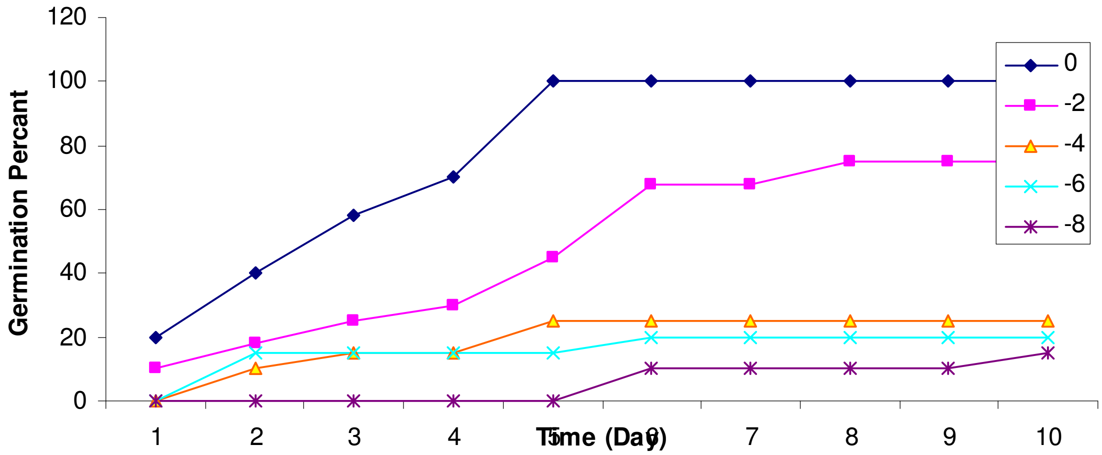

Figure 5. Germination of Saisonez seeds at PEG osmotic potential .

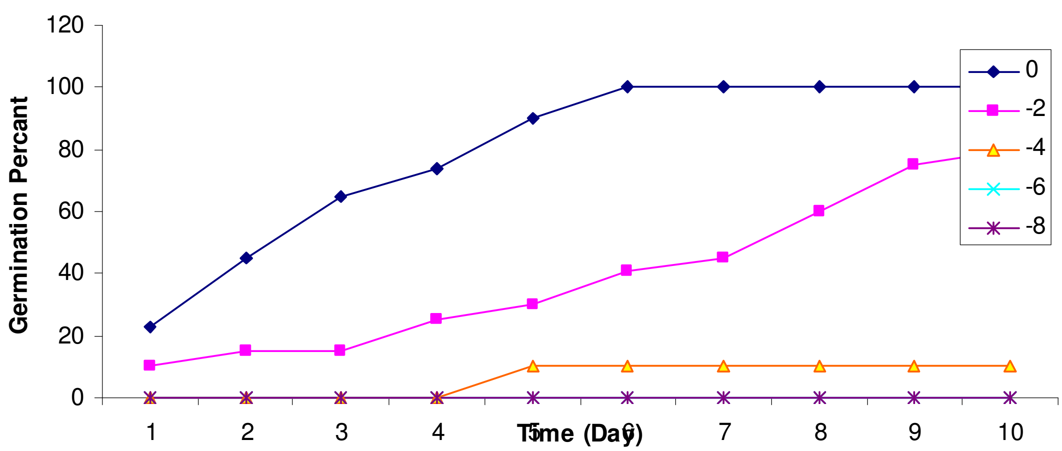

Figure 6. Germination of Cascogne wheat seeds at PEG osmotic potential.

get either delayed and/or proceed at a reduced rate. The effect of PEG on the leaf area of ten-days-old plants was greater than that for the fresh weight, dry weights and plant height (Table 3). The decline in leaf growth is the earliest response of glycophytes exposed to salt or drought stress (Shabala et al., 2006). Khyatnezhad and Gholamin (2011) in study on the two durum wheat genotypes reported similar results about PEG and NaCl stresses. Finally Saisonez genotype showed that resistance to both NaCl and PEG stress that Cascogne. So can say this genotype is better in germination stage for these stresses.

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