

Full Length Research Paper

Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl

Digdem Kaydan and Mehmet Yagmur*

Yüzüncü Yıl University, Faculty of Agric., Department of Field Crops, 65080 VAN-Turkey.

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Effect of seed size (small, medium and large) on germination and seedling growth of triticale (*xTriticosecale* Witm. cv. Presto) at the different osmotic potential of NaCl and PEG solutions were determined in the present study. Germination tests were conducted under five osmotic potential levels (-0.45, -0.77, -1.03, -1.44 MPa, and Control) of PEG 6000 and NaCl. Germination percentage (%) at 4 and 8th days and also seedling growth traits such as root and shoot length (mm), dry root and shoot weight (mg), root : shoot length (R:S) ratio, and relative water content of shoot (RWC, %) were investigated in this study. The results indicated that decreases in the osmotic potentials caused a reduction in germination percentage and seedling growth. It was seen that drought created by PEG 6000 had more negative effects on germination and seedling growth than that of NaCl. In consequence, the total germinability and seedling growth were higher in large seeds rather than in small seeds in control solution and under osmotic stress. In addition, it was observed that seedlings obtained from larger seeds survived even at the lower osmotic potential of PEG and NaCl; whereas, seedling obtained from small seeds did not survive in the intensive stress.

Key words: *xTriticosecale* Witm, seed size, drought, germination, seedling growth.

INDRODUCTION

Seed germination is an essential process in plant development to obtain optimal seedling numbers that results in higher seed yield. Germination and seedling growth declined with many abiotic factors such as salt and drought stress that are perhaps two of the most important grounded abiotic stresses that limit number of seedling and seedling growth (Ashraf et al., 1992; Almansouri et al., 2001, Kaya et al., 2006, Atak et al. 2006). There are many studies such as the selecting plant species or the seed treatments that are helpful for alleviating the negative effects of drought and salt on plants (Ashraf et al., 1992; Almansouri et al., 2001; Okçu et al. 2005; Kaya et al., 2006; Iqbal and Ashraf, 2007). Although seed size is an important parameter for plant growth and yield (Al-Karaki, 1998), the knowledge of the effects of seed size on plant growth in drought and salt

stress is little in triticale. Moreover, many studies are on the effects of seed size on yield of other crops and triticale, but they are mainly conducted in normal condition, in late sowing time or in different plant densities. These studies explained that seed size highly influenced seed yield and seed yield components such as plant stand, plant height, seed weight, and number of seeds per spike (Singh and Kailasanathan, 1976; Nietzsche et al., 2004; Stougaard and Xue, 2004; Royo et al., 2006). Similarly, Weimarck (1975) reported that large seeds germinated better than medium and small seeds, and seedlings from large seeds had a higher survival rate than smaller seeds under field conditions. Moreover, seed size is positively correlated with seed vigor, and larger seeds tend to produce more vigorous seedlings (Ries and Everson, 1973). Little work was conducted to determine the effects of seed size on germination and seedling growth in water deficient condition. This work pointed out that larger seeds produced vigorous seedlings, taller plants with greater tillering and higher levels of dry matter under water deficient condition (Manga and

*Corresponding author. E-mail: myagmur@yyu.edu.tr.

Yadav, 1995; Lopez et al., 1996; Westoby et al., 1996). However, seedlings from large seeds had greater survival rate in tree species (Khurana and Singh, 2004) and faster early growth in wheat than those from smaller seeds under water stress may be advantageous in establishing plants under dry soil conditions (Mian and Nafziger, 1994). Besides, large seeds with larger root systems of seedlings may provide an advantage by allowing access to soil moisture at deeper levels (Leishman and Westoby, 1994). Moreover, Tosun (1995) reported that there were positive and significant relationships between 1000 kernel weight and germination and 1000 kernel weight and emergence rate of seeds.

The studies of the effects of seed size on germination and plant growth under drought and salinity stress of field condition have difficulties. Therefore, NaCl and polyethylene glycol (PEG) compounds have been used to simulate osmotic stress effects in Petri dishes to maintain uniform water potential throughout the experimental period for plants (Kulkarni and Deshpande, 2007).

Generally, triticales are grown in marginal fields with higher stress condition in Turkey. Therefore, it is aimed at obtaining higher seedling growth and optimum number of seedling per area in triticales in stress conditions. The main objective of the present study was to assess to which seed size could alleviate the effect of different osmotic potential of salt and drought stress on germination and seedling growth of triticales.

MATERIAL AND METHODS

The experiment was conducted in the laboratory of the Department of Field Crops and Department of Plant Protection, Faculty of Agriculture, University of Yüzüncü Yıl in 2007. Seed of the recently developed triticales (*xTriticosecale* Witm.) cultivar (Presto) was used as a test material in the present study. Seeds were obtained from the Anatolian Agricultural Research Institute, Eskişehir, Turkey. Seed of this variety was grown in Van condition in 2005 - 2006 growing seasons to obtain newly harvested seed.

Seeds of Presto variety were divided into three based on their sizes (small, medium, and large). Seeds were initially treated with a 1.0% solution of sodium hypochlorite for 3 min for surface sterilization (McGee, 1988). Residual chlorine was eliminated by thorough washing of seeds with distilled water.

Four drought stresses with diverse osmotic potentials of -0.45, -0.77, -1.03, and -1.44 MPa were arranged as described by Michel and Kaufmann (1973). Salt concentrations having the same osmotic potentials of -0.45, -0.77, -1.03, -1.44 MPa and control were adjusted using NaCl. The osmotic potential of the control solution (distilled water) was -0.20 MPa. The osmotic potentials of two osmotic agents (PEG 6000 and NaCl) and control were read with a Wescor Vapour Pressure Osmometer- 5520.

Different size of seeds were then transferred to Petri dishes (20 seeds in a Petri dish) containing two layers of Whatman No 2 filter paper moistened with 10 ml of half-strength control solution or the same solution added with NaCl and PEG-6000. In order to avoid water losses, edges of Petri dishes were tightly sealed with an impermeable colorless parafilm. Petri dishes placed in a germination chamber to obtain the germination in the dark condition for 72 h

at 22±2°C. Then the Petri dishes were transferred into the germination chamber at 22±2°C under a 12 h daylight photoperiod (Almansouri et al., 2001). Petri dishes were controlled in one day intervals for solutions content. Germination percentages were obtained on the 4th day and on the 8th day using radicle extrusion (2 mm long) (ISTA, 1996).

The relative water content (*RWC*) was estimated according to Turner (1981) and was evaluated from the equation given below:

$$RWC = [(FW - DW)/(TW - DW)] \times 100$$

where *FW* is the fresh weight of the shoots, *TW* is the weight at full turgid, measured after floating the shoots for 24 h in distilled water in the light at room temperature and *DW* is the weight estimated after drying the shoots at 70°C until a constant weight is achieved.

Root and shoot length (mm), and root and shoot of dry weights (mg plant⁻¹) were measured on the 8th day (Bray, 1963). Root to shoot length ratio were estimated by dividing root length to shoot length. Dry weights of seedling parts were measured after drying samples at 70°C in an oven until a constant weight is achieved.

The experimental design was arranged in a completely randomized design with 3 factorials with 4 replications. The first factor was osmotic agents and the second was seed size and the third was solution levels. The data were statistically analyzed by MSTATc computer program.

RESULTS

In present study, there was a significant three-way interaction (osmotic agents, seed size and solution levels) ($P < 0.01$) for all investigated characters. The results of the study are given below.

Germination

Seed germination time delayed with the increasing of PEG and NaCl concentration. Therefore, germination process started at different times in various PEG and NaCl concentrations and in various seed size. Seed germination mostly completed in all seed sizes in control solution at the 4th day. However, the increasing of water stress with PEG and NaCl declined seed germination percentage at the 4th day (Figure 1A). Seed germination percentage (%) was significantly low at the highest NaCl concentration (-1.44 MPa) even in -1.03 MPa of PEG. However, at -1.44 MPa of PEG, no seed germination recorded in large, medium and small seeds sizes at the 4th day (Figure 1A).

Seed germination completed in all seed size under control solution and at -0.45 MPa of NaCl at the 8th day (Figure 1B). Although, medium and small seeds had low germination percentage at the -0.77 MPa of NaCl, all large seeds germinated at the equivalent osmotic potential. However, subsequent low osmotic potentials of NaCl decreased germination percentage. Therefore, low germination percentage recorded at the highest NaCl concentration in all seed size. Nevertheless, large seeds

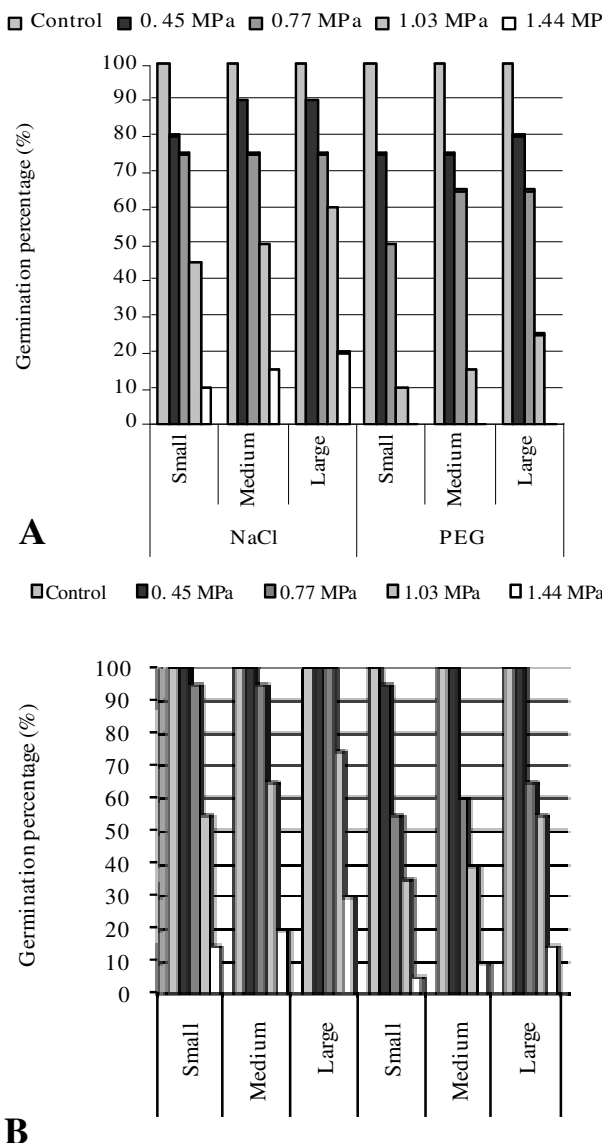


Figure 1. Effects of different seed sizes on germination percentage at the 4th day (A) and germination percentage at the 8th day in the different osmotic potential of PEG and NaCl.

had more germination percentage at the highest concentration (Figure 1B).

Drought created with using of PEG decreased seed germination percentage in response to increasing of PEG concentrations at the 8th day. Although PEG and NaCl decreased germination percentage; decreases in germination percentage with the negative effects of PEG were more than those of NaCl. Furthermore, seed sizes extremely influenced germination percentage depends on various PEG concentrations. Seed germination percentage did not complete at -0.45 MPa of PEG in small seeds, but medium and large seeds had 100% seed

germination percentage at -0.45 MPa of PEG. Besides, large seeds had higher germination percentage at higher PEG concentrations (Figure 1 B).

Seedling growth

The root length of the Presto differed at the different osmotic potentials of PEG and NaCl concentrations as shown in Table 1. The decreasing of osmotic potential of different solutions such as -0.45 MPa of PEG and NaCl increased root length compared to control solutions. The subsequent low osmotic potential such as -0.77 and 1.03 MPa of NaCl and PEG decreased root length. This reduction in root length was higher in PEG solution compared to that in NaCl solution. Moreover, root length differed by seed size depends on various NaCl and PEG concentrations. In normal condition, large and medium seeds had higher root length compared to small seeds. However, root length increased at the osmotic potential of -0.45 MPa of NaCl in medium and large seeds. However, in small seed, root length decreased insignificantly with increasing of NaCl concentration (-0.45 MPa).

Other water deficient conditions created with using PEG decreased seedling growth with higher negative effects such as low seed germination in all seed size recorded at -1.44 MPa and in consequence no root growth was obtained at -1.44 MPa of PEG. In addition, small seeds size had no root growth at -1.03 MPa of PEG. At the all concentrations of PEG and NaCl, large and medium seeds had higher root length compared to small seeds. However, large seeds had higher root length than medium seeds with the increase of stress levels.

The shoot length of the Presto differed under the different osmotic potentials of PEG and NaCl levels and also shoot length differed with seed size depending on stress conditions and stress levels (Table 1). In normal condition, large and medium seeds had higher shoot length compared to small seeds size. However, with increasing concentration of the PEG 6000 and NaCl, decline in shoot length occurred. The highest negative effect on shoot length of small seeds was obtained at the highest NaCl concentration (-1.44 MPa and -1.03 MPa). Although seed germination was recorded at -1.44 MPa, -1.03 MPa of -0.77 MPa of NaCl, shoot growth stopped after emergence of primary shoots from small seeds. In contrast shoot growth was recorded at -0.77 MPa and -1.03 MPa of NaCl in medium and large seeds.

As is the case in root length, low seed germination in small, medium and large seeds was recorded at -1.44 MPa and -1.03 MPa of PEG and in consequence no shoot growth was obtained. Although seed germination was recorded at -0.77 MPa of PEG in small seeds, shoot growth stopped after emergence of plumula from seeds. Small seeds had root growths up to -0.77 MPa of PEG, whereas at the same osmotic potential no shoot growth

Table 1. The effects of seed size on root length (mm) and shoot length (mm) of Presto triticale variety under osmotic stress of PEG and NaCl.

Osmotic stress	Seed Size	Osmotic potentials (-MPa)										
		Root length (mm)					Shoot length (mm)					
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44	
NaCl	Small	69.7	67.0	42.8	39.2	-	97.0	63.5	24.5	-	-	
	Medium	78.8	83.0	47.8	44.3	-	116.8	88.8	33.5	19.5	-	
	Large	78.3	85.5	61.0	50.8	-	118.3	73.8	37.8	23.0	-	
PEG	Small	69.7	76.3	33.7	-	-	97.0	49.5	-	-	-	
	Medium	78.8	88.5	53.5	29.5	-	116.8	54.6	24.5	-	-	
	Large	78.3	78.3	71.5	40.0	-	118.3	54.8	26.8	-	-	
LSD (P<0.05) Int.							11.69					

Table 2. The effects of seed size on dry weight of root (mg) and dry weight of shoot (mg) of Presto triticale variety under osmotic stress of PEG and NaCl.

Osmotic stress	Seed Size	Osmotic potentials (-MPa)										
		Dry weight of root (mg plant ⁻¹)					Dry weight of shoot (mg plant ⁻¹)					
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44	
NaCl	Small	5.0	4.6	5.0	2.0	-	7.0	5.0	3.5	-	-	
	Medium	5.3	5.5	4.8	4.3	-	11.6	7.0	4.5	2.0	-	
	Large	6.5	6.8	6.8	6.0	-	13.0	7.0	5.8	3.3	-	
PEG	Small	5.0	5.0	2.5	-	-	7.0	3.3	-	-	-	
	Medium	5.3	7.0	5.3	3.0	-	11.6	3.1	2.3	-	-	
	Large	6.5	8.3	5.5	3.5	-	13.0	5.3	2.4	-	-	
LSD (P<0.05) Int.							0.93					

was recorded.

Dry weights of root differed with seed sizes under osmotic stress levels and osmotic agents. In control condition, dry weights of root obtained in larger seeds size were significantly higher than those of medium and small seeds. Besides, the experimental results for root weight indicated that large seeds had higher root dry weight than those of medium and small seeds under various NaCl conditions. However, dry root matters from medium seeds were similar with those of small seeds under in low and in intermediate NaCl concentrations, but it is higher than those of small seeds in intensive NaCl concentration (Table 2). Although, increases in PEG concentration decreased dry root materials in all seed size; comparatively dry root materials were increased with the low concentration of PEG (-0.45 MPa) in large seeds and medium seeds than in those of small seeds. Generally larger seeds had higher dry root weights than that of other seed sizes under drought created by PEG in the present study.

It is important that drought resistance is characterized by small reduction of shoot growth in drought stressed conditions. PEG and NaCl caused a greater reduction in dry weight of shoot at higher concentrations compared to control condition. Besides, the decrease in dry shoot weight was significantly higher in PEG concentration than

in NaCl. Furthermore, shoot growth differed with seed size. At normal condition, larger seeds size had higher shoot growth than those of smaller seeds (Table 2). Additionally, larger seeds had higher dry shoot weights at the various PEG and NaCl concentrations.

Root to shoot length ratio

The results indicated that root to shoot length ratio increased with the increasing of PEG and NaCl concentration. But greater increase in root to shoot length ratio was in the increase of PEG concentration (Table 3). Small seeds had insignificantly higher root to shoot length ratio compared to medium and large seeds in normal condition and in water stress of PEG and NaCl. Therefore higher root growth in small seeds compared to shoot growth was obtained in normal condition and in stress. Moreover, this result showed that shoot growth of smaller seeds was more depressed with the stress conditions compared to those of larger seeds.

Relative water content (%)

PEG and NaCl significantly reduced relative water content of shoot compared to control (Table 3). Depending

Table 3. The effects of seed size on root to shoot length ratio (%) and RWC of Presto triticale variety under osmotic stress of PEG and NaCl.

Osmotic stress	Seed Size	Osmotic potentials (-MPa)											
		Root to shoot ratio (R:S)					RWC (%)						
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44		
NaCl	Small	0.71	1.05	1.73	-	-	74.6	65.0	57.0	-	-		
	Medium	0.67	0.93	1.41	2.25	-	82.8	73.8	54.5	52.5	-		
	Large	0.66	1.15	1.61	2.23	-	89.5	75.5	65.5	62.3	-		
PEG	Small	0.71	1.54	-	-	-	74.6	49.2	-	-	-		
	Medium	0.67	1.62	2.91	-	-	82.8	55.7	53.8	-	-		
	Large	0.66	1.42	2.71	-	-	89.5	57.5	55.5	-	-		
LSD (P<0.05) Int.							0.28						5.72

on decrease in shoot growth, RWC (%) gradually declined with the increasing of concentration of PEG and NaCl. Moreover, greater reduction in RWC (%) was obtained in PEG concentration compared to NaCl concentrations. RWC (%) in larger seeds were higher than those of smaller seeds under all osmotic potentials in water stress created with NaCl and PEG. In contrast, at the osmotic stress with PEG, shoot from large seeds had insignificantly higher RWC (%) than those of shoot obtained from medium seed.

DISCUSSION

Water deficient conditions induced with PEG and NaCl decreased germination percentage and also delayed germination time at the highest concentrations due to water stress. This was because the increase of NaCl and PEG concentrations lower water uptake by seed resulting in decreases of germination. Therefore, the decrease in water potential gradient between seeds and their surrounding media by the effects of PEG 6000 and NaCl adversely affects seed germination. Dodd and Donovan (1999) reported that conditions with higher NaCl contents and water deficient condition reduces germination due to limited water uptake by the seeds. The reduction in seed germination percentage was higher in PEG compared to NaCl, that is, at the equivalents of osmotic potential, seed germinated is better in NaCl than in PEG. Moreover, at the same osmotic potentials, germination starts earlier and higher germination percentage is obtained with NaCl (Figure 1). The explanation of the higher inhibitory effects of PEG than NaCl lies in ion or solute entry into the seed (Alam et al., 2002). Especially, the accumulation of Na⁺ by the imbibing seed embryo functions to promote a water potential gradient between the embryo and substrate, and maintain water uptake during seed germination (Eddleman and Romo, 1987; Dod and Donovan, 1999). Seed germination was only delayed with the low concentration of NaCl (Figure 1A), whereas germination percentage was not different with control at -0.45 and -0.77 MPa of NaCl (Figure 1B). Lower osmotic potentials such as -1.03 and -1.44 MPa of NaCl delayed germination

and also decreased germination percentage compared to control solution. Similar results were obtained in germination process in drought created with using PEG. The findings of Almansouri et al. (2001) confirmed our results and reported that moderate stress intensities only delayed germination, whereas the highest concentration of NaCl and PEG reduced final germination percentages.

There were differences in germination percentage of seed sizes depending on stress intensity. Larger seeds germinated earlier than those of medium and small seeds in the treatments of control. Although, larger seeds had higher germination performance than those of smaller seeds sizes at the low concentrations of PEG and NaCl solutions, they also have higher germination percentage at the highest concentration of solutions. Larger seeds with higher germination percentage in normal and stress condition may be related to privileged water uptakes. Similar results were reported by Al-Karaki (1994) that large lentil seeds had higher water potential compared to smaller seeds in low water potentials. Under normal condition and in moderate stress condition, higher germination percentage in large seeds may have little advantage compared to other seed sizes due to little differences in germination percentage. Under extreme stress conditions, larger seeds in triticale may have higher benefits in germination compared to smaller seeds. Therefore, higher germination percentage from larger seeds may be beneficial in establishing plants under dry soil conditions (Mian and Nafziger, 1994). Just like in the present study, total germination in all cereals was lower in small seeds and higher in large seeds in normal condition (Drena, 2004). Willenborg et al. (2005) reported that large oat seeds had greater final germination that resulted in better stand establishment, particularly where low spring soil moisture limits stand establishment than that of small seeds.

Root length is an important trait against drought stress in plant varieties; in general, variety with longer root growth has resistant ability for drought (Leishman and Westoby, 1994). In the present study, decreases in the external osmotic potential caused a reduction in seedling growth of Presto. Although, the increasing concentrations

induced water stress leading to decrease in root length and shoot length, this reduction in root and shoot length was lower in larger seeds than those of smaller seeds under control and stress conditions. Similar results were reported by Al-Karaki (1998) that lentil seedlings from large seeds had higher root lengths than those from small seeds at intermediate soil water potential. Therefore, larger seeds had an advantage of seedling establishment in low soil moisture condition due to larger root system (Leishman and Westoby, 1994). Roots play an important role in plant survival during periods of drought (Hoogenboom et al., 1987) and also drought resistance is characterized by an extensive root growth and small reduction of shoot growth in drought stressed conditions (Guoxiong et al., 2002). Moreover, Westoby et al. (1996) reported that seedlings of larger-seeded species were better able to survive drought.

PEG and NaCl solutions caused a growth reduction in shoots of triticale seedling; however, the root dry matter increased with rising osmotic stress (-0.45 MPa) (Leinhos et al., 1996; Atak et al., 2006). Similarly, Baalbaki et al. (1999) reported that root and shoot weights of all wheat cultivars declined when osmotic potential was decreased, but the extent of reduction in root growth was less than that for shoots. Although the seedling weights decreased with the effects of NaCl and PEG, large seeds had higher dry root weights, even in NaCl and PEG increased concentrations. Al-Karaki (1998) reported that lentil seedlings from large seeds had higher root dry matter than those from small seeds at intermediate soil water potential. Therefore, heavier root weights from large seeds protected seedlings from drought injuries. This is because heavier and longer roots reach to moisture at deeper levels in soil. Camacho and Caraballo (1994) reported that root dry weight was identified as the major criterion for selection of maize genotypes under drought conditions. As in the case in root growth, larger seeds produced higher dry shoot weights in control and also under various osmotic potentials of NaCl and PEG concentration. Therefore, large seeds may alleviate the negative effects of drought stress on seedling. Al-Karaki (1998) reported that lentil seedlings from large seeds had higher shoot dry matter than those from small seeds. Moreover, large kernel weight was considered as a possible characteristic that may improve the drought resistance of short-duration pigeonpea in another study (Lopez et al., 1996).

Root to shoot length ratio (R:S) increased with increasing PEG and NaCl concentrations (Bajji et al., 2000). But increase in root to shoot length ratio was higher in the increasing PEG concentrations in comparisons to NaCl (Table 3). Small seeds had insignificantly higher root to shoot length ratio compared to medium and large seeds in normal condition and in water stress created by PEG and NaCl. This result indicated that shoot growths from small seeds were more depressed in stress condition.

Okçu et al. (2005) reported that water stresses depressed the shoot growths of the cultivars rather than their root growths in pea.

Relative water content (RWC) was used as a measure of drought. This index may be useful for determining the plant leaf water status (McCaig and Romagosa, 1991). Drought induced with PEG and NaCl decreased shoot water status in the present study (Bajji, 2000; Guoxiong et al., 2002). But seedlings of large seeds had higher RWC than that of medium and small seeds. This result indicated that large seeds having longer root lengths had more water uptake abilities resulting in higher RWC of shoot.

Consequently, the selection of larger seeds to sow under drought and salt stress may be helpful in reducing the risk of poor stand establishment and permit more uniform growth under conditions of irregular rainfall and saline soils. Therefore, it is important that seed materials for sowing should be prepared by discarding the smaller seeds, or the seed density could be increased because small seeds may cause lower seedling growth and lower emergence in normal or in stress condition.

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