

Selecting Drought Resistant Sweet Corn Cultivars Based on Germination Percentage and Seedling Proline Content under Osmotic Potentials Stress

ALI SHAHRIARI^{1,3*}, ADAM B PUTEH¹, GHIZAN B SALEH¹, ANUAR B ABDUL RAHIM²

1- Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, Selangor, Malaysia

2- Department of Land Management, Faculty of Agriculture, University Putra Malaysia, Selangor, Malaysia

3- Agriculture and Natural Resources Researches Center of Hormozgan, Bandar Abbas, Iran

* Corresponding Author Email: ali_shahriary_1346@yahoo.com

Received: 10 March 2013

Accepted: 12 November 2013

ABSTRACT

Water stress can affect germination by decreasing the percentage of germination. A study was undertaken to evaluate the influence of different osmotic potentials (MPa) on proline content and percentage seed germination of corn. The experiment was conducted in factorial with a randomized complete block design (RCBD) with three replications. Seeds of two open pollinated varieties (Masmadu and Thai super sweet) and three Hybrids (968,969 and 926) sweet corn were germinated at 0, -0.2, -0.5, -0.7, -1.2 and -1.4 MPa osmotic potentials. Results showed the percentage of germination and coefficient of velocity decreased with decrease in osmotic potential while proline content and mean germination time increased. Polyethylene glycol increased root length and length per volume at low osmotic potential (-0.2 MPa) but decreased at more than -0.7 MPa. Seedling proline content appears not to be related to percentage germination but appears to be related to the decline in osmotic potential in germination media. Seed germination test at -0.7 to -1.2 MPa has the potential to be used as a vigor test in sweet corn.

Keywords: Osmotic potential, Germination, Polyethylene glycol, Corn, Proline content

INTRODUCTION

Most of the agriculture land of the world is considered semi-arid, and the main limiting factor is water. Studies have shown that germination of seed is decreased and delayed under

water stress (Hao and De Jong, 1988; Rao and Dao, 1987). Germination percentage decreased in low osmotic potential of PEG (Almansouri *et al.*, 2001; Yagmur and Kaydan, 2008).

Roots are very important in plant growth as they absorb soil moisture and nutrients. Drought stress affects root weight of the plant during water stress conditions (Yang *et al.*, 2006). Root length is often directly related to absorbed water from soil (Hamblin and Tennant, 1987). Root growth is an important drought tolerance mechanism in beans for drought avoidance and absorbing water from depth of soil, but root growth decreases in drying soil (Sponchiado *et al.*, 1989). Root elongation and root diameter decreases at low water potentials in maize (Sharp *et al.*, 1988, 2004). Root elongation decreases under drought stress by more than 88% in millet (Radhouane, 2007). Gholami *et al.* (2009) reported drought stress decreased root length in corn. Inverse root length is reduced under low osmotic potentials in triticale (Yagmur and Kaydan, 2008).

Proline content accumulation is a common metabolic response of higher plants to water deficits and salinity stress and substantially increase in both young and old leaves during a dry period (Aziz and Khan, 2003). Little information is available on the response of seed proline content when seeds are sown under water stress field conditions. The ability of the plant to accumulate proline is important for the plant and may be an adaptation process of the plant over short periods to resist water stress (Jager and Meyer, 1977).

Therefore, the objective of this study was to evaluate the effects of different osmotic potential on the proline content and influence on sweet corn seed germination.

MATERIALS AND METHODS

Experiments were conducted in the laboratories of Faculty of Agriculture and Department of Crop Science, University Putra Malaysia (UPM). The design of the experiment was Factorial base on randomized completely block design (RCBD) with three replications. Seeds of three sweet corn hybrids (968,969 and 926) and two open pollinated varieties (Masmadu and Thai super sweet) were used. Twenty five seeds of any cultivar were selected and sterilized in sodium hypochlorite (5%) and then washed twice in water. Seeds were germinated at osmotic potentials of 0.0, -0.2, -0.5, -0.7, -1.2 and -1.4 MPa of polyethylene glycol 6000 (Cony and Trione, 1998) for nine days in Petri dishes on moist Whatman filter papers at 26 °C. The germination data were recorded daily. Percentage germination, mean germination time and coefficient velocity of germination were calculated by the equations 1, 2 and 3, respectively.

$$1) \text{ Germination Percentage (\%)} = \text{Final number of seeds germinated} \times 100 \text{ (Scott } et al., 1984).$$

$$2) \text{ Mean Germination Time (MGT) (day)} = \frac{\sum Dn}{\sum n}$$

Where, n is the number of seeds, which were germinated on day (D) and D is the number of counted days from the beginning of germination.

$$3) \text{ Coefficient of Velocity of Germination (CVG)} = \frac{N_1 + N_2 + \dots + N_x}{100} \times \frac{1}{N_1 T_1 + \dots + N_x T_x}$$

Where, N is the number of seeds germinated each day, T is the number of days from seeding corresponding to N (Jones and Sanders, 1987).

The seedling roots were scanned by using a root scanner WinRHIZO system (WinRHIZO, Regent Instrument, Inc.) on the ninth day of the germination period. Proline content in the nine-day seedlings was measured by Bates *et al.* (1973) method. The data were analyzed using SAS software (SAS Institute Inc, Cary, NC, USA, SAS (r) Proprietary Software 9.2 (TS1M0)). Treatment means were compared using least significant test (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

Results of these experiments showed significant effects of osmotic potential and variety on percentage germination, mean germination time (MGT) and coefficient of velocity of germination (CVG) (Table 1).

Table 1. Analysis of variance for maximum germination (GP), Mean Germination Time (MGT), Coefficient Velocity of Germination (CVG)

Source	GP(%)	MGT	CVG
Block	0.45 ns	0.43ns	0.28ns
Variety	12.72**	15.83**	12.5**
Water stress	205.87**	132.91**	106.24**
Variety × stress	3.01**	5.7**	4.75**
Error	45.03	0.131	2.72

*, ** significant differences at $p < 0.05$ and 0.01 , respectively; ns, not significant

Percentage germination for open pollinated varieties, hybrid 926 and hybrid 968 declined to <80% as osmotic potential decreased to -1.2 MPa. The high percentage germination in all varieties was at -0.5 MPa (Fig 1). Ghiyasi *et al.* (2008) reported the highest percentage germination and shorter time to 50% germination (T_{50}) when seeds were germinated at -0.5 MPa. Germination percentage decreased in low osmotic potential of PEG (Almansouri *et al.*, 2001; Yagmur and Kaydan, 2008). Percentage germination decreased with increased PEG 6000 concentration which is due to the reduction in water potential gradient between the seed and surrounding media (Dodd and Donovan, 1999).

Coefficient of velocity of germination (CVG) decreased progressively as osmotic potential decreased to -1.4 MPa, while mean germination time (MGT) increased progressively from 3-4 days to >5 days as osmotic potential decreased to -1.4 MP (Fig 2 and 3). MGT is an accurate measure of the time taken for a lot to germinate, but this does not correlate well with the time spread or uniformity of germination. It focuses instead on the day when most germination events occur. CVG does not focus on the final percentage of germination, but places emphasis on the time required for reaching it (Kader, 2005).

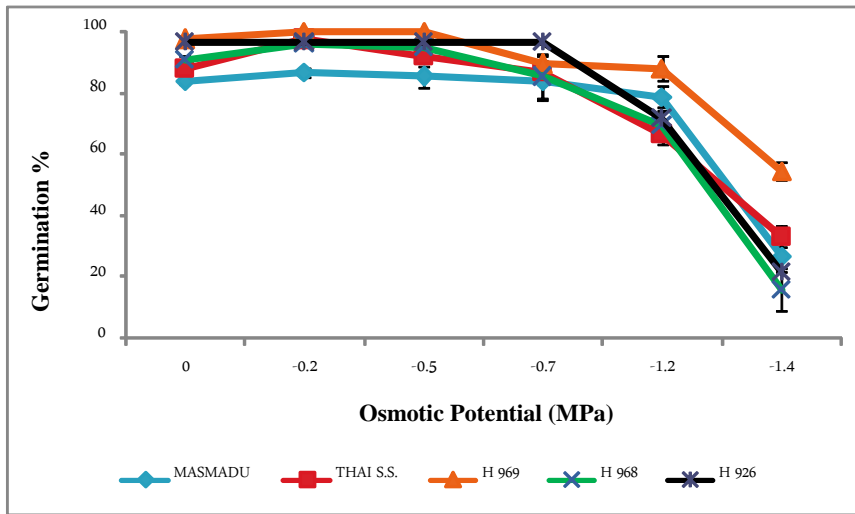


Figure 1. Interactive effects of osmotic potential and variety of sweet corns on percentage germination. Vertical bar represents \pm SE

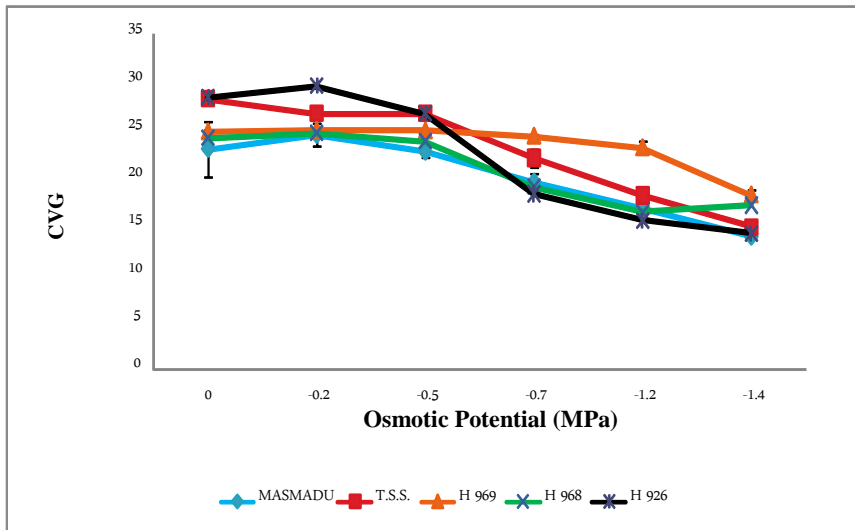


Figure 2. Interactive effects of osmotic potential and variety of sweet corns on coefficient of velocity of germination (CVG). Vertical bar represents \pm SE

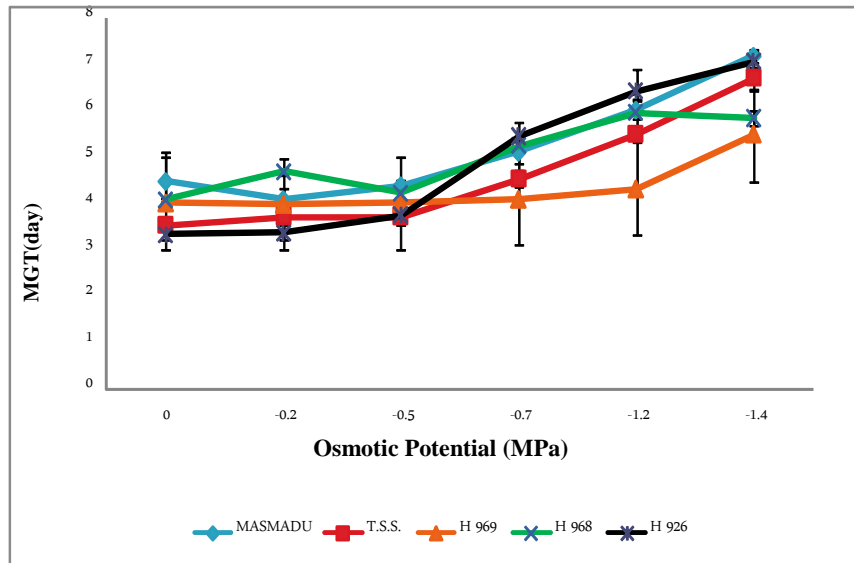


Figure 3. Interactive effects of osmotic potential and variety of sweet corns on mean germination time (MGT). Vertical bar represents \pm SE

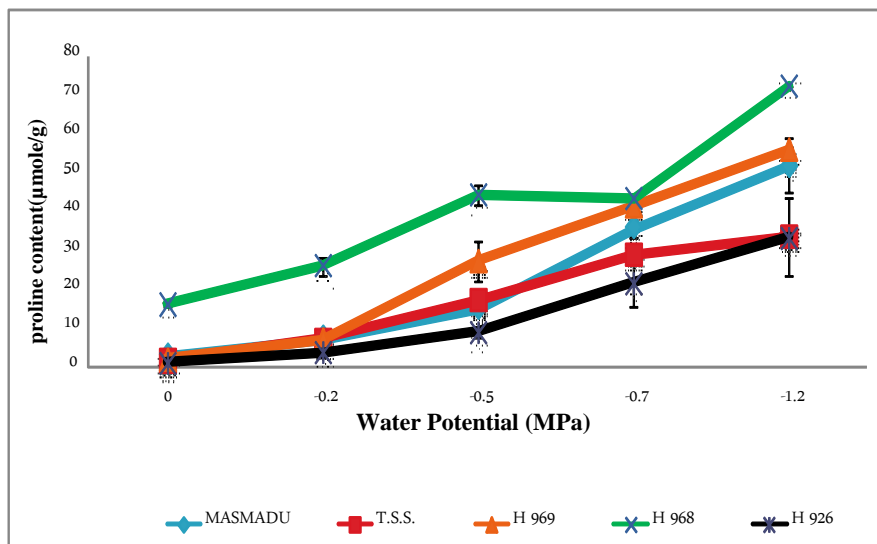


Figure 4. Interactive effects of osmotic potential and variety of sweet corns on seedling proline content. Vertical bar represents \pm SE

Seedling Proline content increased progressively with decrease in osmotic potential during germination with the highest proline content observed in hybrid 968 (Fig 4). The present results are in accordance with studies reporting that osmotic stress increased biosynthesis rate of proline content (Boggess *et al.*, 1976; Fattahi Neisiani *et al.*, 2009). The ability of the plant to accumulate proline is important for the plant and may be an adaptation process of the plant to resist short periods of water stress (Jager and Meyer, 1977). Results showed that proline content in the seedling increased with decreased water osmotic potential. This is because decrease in water osmotic potential of the media reduces the water potential gradient between the seedling and surrounding media, resulting in lower water uptake by the seedling, which may induce water stress in the seedling. Girousse *et al.* (1996) reported that water deficit increased the proline concentration of the phloem sap in alfalfa.

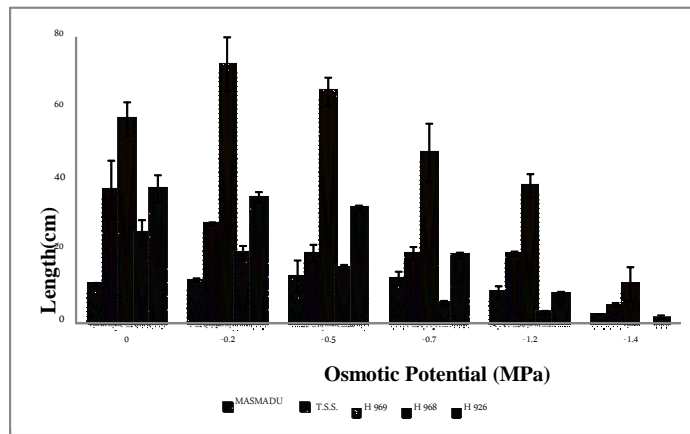


Figure 5. Interactive effects of osmotic potential and variety of sweet corns on root length were determined by root scanner WinRHIZO (WinRHIZO, Regent Instrument, Inc.). Vertical bar represents \pm SE

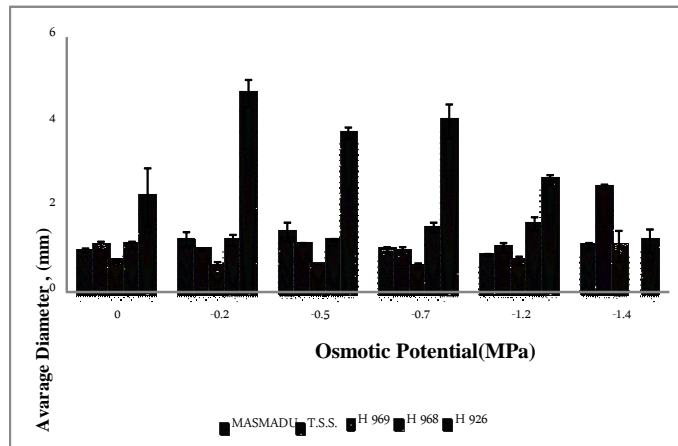


Figure 6. Interactive effects of osmotic potential and variety of sweet corns on root diameter were determined by root scanner WinRHIZO (WinRHIZO, Regent Instrument, Inc.). Vertical bar represents \pm SE

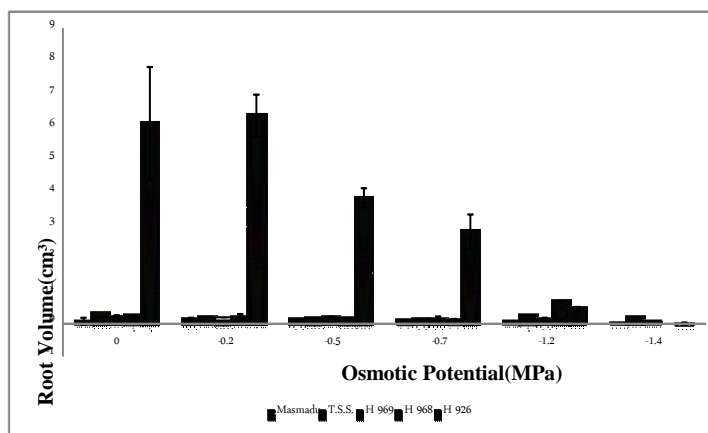


Figure 7. Interactive effects of osmotic potential and variety of sweet corns on root volume were determined by root scanner WinRHIZO (WinRHIZO, Regent Instrument, Inc.). Vertical bar represents \pm SE

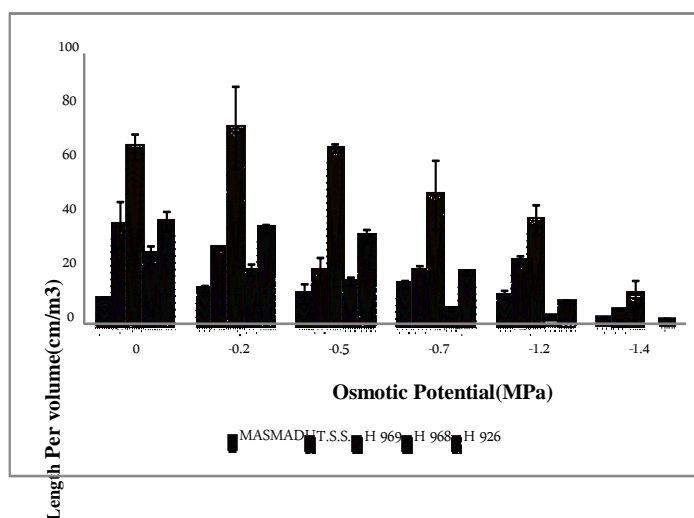


Figure 8. Interactive effects of osmotic potential and variety of sweet corns on root length per volume were determined by root scanner WinRHIZO (WinRHIZO, Regent Instrument, Inc.). Vertical bar represents \pm SE

Seeds of all Verities except hybrid 969 showed $>80\%$ germination when imbibed at up to - 1.2 MPa (Figure 1). Percentage germination of Thai Super Sweet ,926 and 968 hybrids declined to $< 70\%$ when osmotic potential of germination media was < -0.7 MPa (Figure 1).The present results are in accordance with studies by Thakur and Sharma (2005) who exported that a strong negative correlation between germination capacity and proline accumulation under stress. In potato plants there was reported increased proline content accumulation under water stress (Knipp and Honermeier, 2006).

The results of analysis of variance for root scan parameters are shown in Table 2. Results of these experiments show that the effects of osmotic potentials and variety on Root Length (RL), Average Diameter (AD), Length per Volume (LPV) and Root Volume (RV) are significant (Table 2). Also, interaction affect of osmotic potential \times variety was significant (Table 2).

Table 2. Analysis of variance for root scan including: root length (RL), length per volume (LPV), root volume (RV) and root diameter (RD).

	RL	LPV	RV	RD
source	(cm)	(cm/m ³)	(cm ³)	(mm)
Block	1.09ns	1.15ns	0.31ns	0.54ns
Variety	106.48**	65.92**	88.77**	160.03**
Water stress	55.43**	29.02**	13.36**	2.64*
Varity \times stress	4.51**	3.34**	11.07**	13.51**
Error	34.13	65.94	0.4	0.12

*, ** significant differences at $p < 0.05$ and 0.01 , respectively; ns, not significant

The highest root length (RL) and length per volume (LPV) at all levels of water osmotic potential were observed in Hybrid 96 9 (Figures 5 and 6). The present results are in accordance with many studies that reported root length decreasing under water deficit (Bilgin *et al.*, 2008; Radhouane, 2007). PEG increased root length at low concentration but decreased sharply at high osmotic potential (Radhouane, 2007; Yagmur and Kaydan, 2008). The average highest Root volume (RV) and Average Diameter (AD) were observed in hybrid 926 (Figures 7 and 8). Root growth is affected by adequate plant water supply during water deficit (O'Toole and Bland, 1987; Sponchiado *et al.*, 1989). Plant growth is generally decreased in decreased osmotic potential and root growth is often less inhibited than shoot growth (Blum *et al.*, 1983; Sharp *et al.*, 1988, 2004). Results showed that elongation of primary root in corn is very sensitive to decreased water potential of the growth medium.

Results showed that polyethylene glycol 6000 increased root length and length per volume at low concentration (-0.2 MPa) but decreased at high concentration (-0.7 MPa). A variety of high root length is capable of absorbing more water under water stress conditions compared to control. The present results are in accordance with the study of Farsiani and Ghobadi (2009) which indicated that the percentage of germination, germination rate, length of radicle and length root decreased under water stress condition. Corinc (2000) reported that in *Arabidopsis thaliana* root diameter and elongation rate reduced under water deficit.

CONCLUSIONS

The results of this study show that the increase in seedling proline content in the seedling during germination is due to the decrease in osmotic potential of germination media. High

seedling proline content in all levels was observed in hybrid 968. The results did not show a relationship between seedling proline content and percentage germination. Seed germination at -0.7 to -1.2 MPa appears to be a good selection criteria for hybrid and open pollinate varieties to be planted in arid or semi-arid areas.

ACKNOWLEDGEMENTS

This work was supported by University Putra Malaysia (UPM).

REFERENCES

- Almansouri M, Kinet JM, Lutts S. 2001 . Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum Desf.*). Plant and Soil, 231:243-254.
- Aziz I, Khan MA. 2003. Proline and water status of some desert shrubs before and after rains. Pakistan Journal. Botany, 35:902-906.
- Bates LS, Waldren RP, Teare ID.1973. Rapid determination of free proline for water-stress studies. Plant and Soil, 39:205-207.
- Bilgin O, Baser I, Korkut KZ, Balkan A, Saglami N. 2008. The impacts on seedling root growth of water and salinity stress in maize (*Zea mays indentata Sturt.*). Bulg. J. Agric. Sci., 14:313-320.
- Blum A, Mayer J, Gozlan G. 1983. Associations between plant production and some physiological components of drought resistance in corn. Plant, Cell and Environment, 6:219-225.
- Boguess S., D. Aspinall and L. Paleg. 1976. Stress metabolism. IX. The significance of end-Product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. Functional Plant Biology, 3:513-525.
- Cony MA, Trione SO. 1998. Inter-and intraspecific variability in *Prosopis flexuosa* and *P. chilensis*: seed germination under salt and moisture stress. Journal of Arid Environments, 40:307-317.
- Cornic G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture - not by affecting ATP synthesis. Trends in Plant Science, 5(5): 187-188.
- Dodd GL, Donovan LA. 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. American Journal of Botany, 86:1146-1153.
- Farsiani A, Ghobadi ME.2009. Effects of PEG and nacl stress on two cultivars of corn (*Zea mays L.*) at germination and early seedling stages. World Academy of Science, Engineering and Technology, 57:382-385.
- Fattahi Neisiani F, Modarres Sanavy SAM, Ghanati F, Dolatabadian A. 2009. Effect of Foliar Application of Pyridoxine on Antioxidant Enzyme Activity, Proline Accumulation and Lipid Peroxidation of Maize (*Zea mays L.*) under Water Deficit. Notulae Botanicae Horti AgrobotaniciCluj-Napoca, 37:116-121.
- Ghiyasi M, Zardoshty MR, Mogadam AF, Tajbakhsh M, Amirnia R. 2008. Effect of osmopriming on germination and seedling growth of corn (*Zea mays L.*) Seeds Research Journal of Biological Sciences, 3:779-782.
- Gholami A, Sharafi S, Sharafi A, Ghasemi S. 2009. Germination of different seed size of pinto bean cultivars as affected by salinity and drought stress. Journal of Food, Agriculture and Environment, 7:555-558.
- Girousse C, Bournoville R, Bonnemain J.1996. Water Deficit-Induced Changes in Concentrations in Proline and Some Other Amino Acids in the Phloem Sap of Alfalfa. Plant Physiology, 111:109-113.

- Hamblin A, Tennant D.1987. Root length density and water uptake in cereals and grain legumes: how well are they correlated. *Australian Journal of Agricultural Research*, 38:513-527.
- Hao X, De Jong E. 1988. Effect of matric and osmotic suction on the emergence of corn and barley. *Canadian Journal of plant science*, 68:207-209.
- Jager HJ, Meyer HR. 1977. Effect of water stress on growth and proline metabolism of *Phaseolus vulgaris* L. *Oecologia*, 30:83-96.
- Jones KW, Sanders DC.1987. The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. *Journal of Seed Technology (USA)*, 11:97-102.
- Kader MA. 2005. A Comparison of Seed Germination Calculation Formula and the Associated Interpretation of Resulting Data. *Journal and Proceedings of the Royal Society of New South Wales*, 138:65–75.
- Knipp G. and B. Honermeier. 2006. Effect of water stress on proline accumulation of genetically modified potatoes (*Solanum tuberosum* L.) generating fructans. *Journal of Plant Physiology*, 163:392-397.
- O'Toole JC, Bland WL.1987. Genotypic Variation in Crop Plant Root Systems, in: N. C. Brady (Ed.), *Advances in Agronomy*, Academic Press, pp. 91-145.
- Radhouane L. 2007. Response of Tunisian autochthonous pearl millet (*Pennisetum glaucum* L.) to drought stress induced by polyethylene glycol (PEG) 6000. *African Journal of Biotechnology*, 6:1102-1105.
- Rao SC, Dao TH. 1987. Soil water effects on low-temperature seedling emergence of five Brassica cultivars. *Agronomy Journal*, 79:517-519.
- Scott SJ, Jones RA, Williams WA. 1984. Review of data analysis methods for seed germination. *Crop Science*, 24:1192-1199.
- Sharp RE, Silk WK, Hsiao TC. 1988. Growth of the maize primary root at low water potentials. *Plant Physiol*, 87:50-57.
- Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT. 2004. Root growth maintenance during water deficits: physiology to functional genomics. *Journal of Experimental Botany*, 55:2343-2351.
- Sponchiado BN, White JW, Castillo JA, Jones PG. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil Types. *Experimental Agriculture*, 25:249-257.
- Thakur M, Sharma AD. 2005. Salt-stress-induced proline accumulation in germinating embryos: Evidence suggesting a role of proline in seed germination. *Journal of Arid Environments*, 62:517-523.
- Yagmur M, Kaydan D. 2008. Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. *African Journal of Biotechnology*, 7:2156-2162.
- Yang G, Luo Y, Li B, Liu X. 2006. The response of winter wheat root to the period and the after-effect of soil water stress. *Agricultural Sciences in China*, 5:284-290.