



Influence of Mannitol Priming on Maize Seeds Under Induced Water Stress

P. Dhakal (Corresponding Author)

Institute of Agriculture and Animal Science, Lamjung Campus, Nepal

Email: pragvadhakal45@gmail.com

R. Subedi

Institute of Agriculture and Animal Science, Lamjung Campus, Nepal

Article History

Received: February 15, 2020


Revised: March 8, 2020

Accepted: March 15, 2020

Published: March 17, 2020

Copyright © 2020 ARPG & Author

This work is licensed under the Creative Commons Attribution International

 CC BY: Creative Commons Attribution License 4.0

Abstract

Drought stress is seen as the major abiotic stress in the modern day agriculture and hinders crop germination and seedling establishment and maize suffers the problem more as a summer season crop. Priming is a physiological method to overcome such deleterious effect of water stress with the main aim of increasing the germination of seed. A lab experiment was therefore performed with maize seed priming using Mannitol @ 0%, 2%, 4%, 6% and 8% (w/v) concentrations subjected to germination under induced drought of 0 Mpa, 0.15 Mpa, 0.5 MPa, 1.0 MPa and 1.7 MPa using NaCl. The experiment was laid in completely randomized design (CRD) with three replications. Priming with mannitol reduced the Mean Germination Time (MGT); the best result obtained in seeds primed with 2% mannitol. However, the final germination count, Relative Water Content (RWC) and root and shoot length remained unaltered. Germination activities reduced with increasing moisture stress. The study indicated that priming with mannitol could improve the speed of germination in maize seeds.

Keywords: Drought stress; Germination; Maize; Mannitol; Priming.

1. Introduction

Water stress due to drought is probably the most significant abiotic factor limiting plant and also crop growth and development [1]. Seed germination and early seedling growth are potentially the most critical stages for water stress. Uneven or poor germination and subsequently uneven seedlings growth can lead to great financial losses, by reducing crop production and lower prices of uneven plant batches. Being drought sensitive crop, maize is affected at each and every stage of growth and development by lesser moisture availability. Maize suffers this problem more as a summer crop. The germination and establishment of seedlings to drought stress is very sensitive, in such a huge extent that drought stress decreases seed germination and seedling has a non-uniform establishment [2]. Maize grain size is greater than other cereals so water requirement is greater for maintenance of osmotic potential and conversion of stored food into consumable form for proper germination. Water absorption, imbibition and metabolic enzymatic activation are hindered under limited water availability which reduces the maize grain germination.

Various seed enhancing technique are widely used to overcome such deleterious effect of drought stress with the main aim of increasing the germination of seed. Priming is one of the most important physiological method which boosts up the germination process of seed right up to the radicle emergence stage ensuring the germination of the seed [3]. Seed priming is soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radical emergence [4]. It has been reported that seed priming in osmoticum such as mannitol, polyethylene glycol (PEG), and Sodium chloride increases the yield of chickpea, maize and wheat under semiarid condition because of the rapid seed germination and seedling emergence. Also priming of seed improves speeds, synchrony and percentage of seed germination. These beneficial effects of priming on seed germination rate are related to the repair and build-up of nucleic acid, enhanced synthesis of RNA and proteins, repair of membranes and some age-induced damage [5].

The objective of this study was to evaluate the effect of seed priming by mannitol on germination characteristics of maize in relation to various concentrations of mannitol and different level of water stress. The ultimate aim was to identify the priming conditions that optimise seed performance in water stress environment.

2. Materials and Methods

2.1. Experimental Site and Materials

The above research was conducted in the laboratory of Agronomy -Institute of Agriculture and Animal Science, Lamjung campus. Seeds of maize (*Zea mays*) of variety NEW 940 were used.

2.2. Experimental Design and Procedure

The experiment was carried out in Two Factorial Complete Randomized Design (CRD) having 3 replications and 25 treatments.

Table-1. Treatment details

	Factor A (Water Stress level)		Factor B (Seed Priming)
T1	Control (0 Mpa)	M ₁	Control (0%)
T2	0.15 Mpa	M ₂	2% Mannitol
T3	0.5 Mpa	M ₃	4% Mannitol
T4	1.0 Mpa	M ₄	6% Mannitol
T5	1.7 Mpa	M ₅	8% Mannitol

Mannitol @ 2%, 4%, 6% and 8% (w/v) concentrations were used for seed priming. The unprimed dry seeds were taken as control. Seed priming was done for 12 hrs. Following the priming, seeds were dried back to their original moisture content at room temperature for 24 hours. Twenty five seeds each for each treatment were placed in Petri dishes. Water stress condition was created by using different concentration of NaCl solutions viz. 0.15 Mpa, 0.5 MPa, 1 MPa and 1.7 MPa and a treatment with tap water was taken as control. Seeds were incubated in a germination chamber at 24°C for 6 days, with regular supervision.

2.3. Data Collection and Parameters

Seeds were considered germinated when there was a visible coleoptile protrusion of more than 5mm in length through the seed coat. The germinated seeds were counted at an interval of 12 hours for 6 days.

2.3.1. Germination Percentage (G %)

Percentage of germination was measured according to formula of [Ahammad, et al. \[6\]](#).

$$\text{Germination percentage} = \frac{\text{no. of seeds germinated}}{\text{Total no. of seeds used}} * 100$$

2.3.2. Mean Germination Time (MGT)

Mean germination time was calculated using formula [7]

$$\text{MGT} = \frac{\text{Sum of Dn}}{\text{Sum n}}$$

Where, Dn = the no. of days counted from beginning of germination

n= the no. of different seed lots

2.3.3. Root and Shoot Length

On the seventh day 10 samples from each petridish were taken in random and root length and shoot height of the sample seeds were measured manually with a ruler.

2.3.4. Relative Water Content (RWC)

Fresh weight of the seeds along with the shoot and root were measured. Then the petridishes with the maize seedlings were filled with water and kept for 12 hours. After 12 hours the turgid weight of the seeds along with the shoot and root were measured by removing the extra water of the dish with the help of tissue paper. Then the seedlings were wrapped in the envelope and kept in hot air oven. The dry weights of shoot and root were determined after drying at 72°C for 24 h. Following formula was used [8].

$$\text{RWC}\% = \frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}} \times 100 \quad \text{where, FW, DW and TW are Fresh wt., Dry Wt. and Turgid wt.}$$

2.4. Statistical Analysis

The statistical analysis of data was done using computer software RStudio Version 1.1.463 applying 5% level of significance.

3. Results and Discussion

3.1. Germination Percentage

Mannitol priming at different concentrations had no effect on G% while, G % decreased with increasing level of water stress. No significant difference was seen when level of drought was elevated from 0 Mpa to 0.5 Mpa whereas, sharp decrease in G% was obtained with increasing drought from 0.5 Mpa to 1.7 Mpa. Similar results were obtained by [Elocka \[9\]](#) in pea where priming treatments with 1%, 2% and 3% mannitol had no significant effect on germination percentage. Present results are in line with [Partheeban, et al. \[10\]](#) reported that there was a decrease in germination percentage of three different varieties of maize with increase in the water stress levels. with [Jorenush and Rajabi \[11\]](#) who reported that germination rate decreased with increasing drought in artichoke where highest

germination rate was found in control treatment and the lowest was zero in highest level of stress induced by 25% PEG. Ahmad, *et al.* [12], also reported that drought stress has an inhibitory effect on sunflower seed germination. According to Ayaz, *et al.* [13], decrease in seed germination under stress conditions is due to some metabolic disorders. Germination, in strongly negative water potentials, especially at the beginning of the imbibition could influence water absorption by the seeds, and this event could reduce the viability of germination process [14].

3.2. Mean Germination Time (MGT)

Primed seeds showed decrease in MGT as compared to control with lowest MGT seen at 2% and 4% of mannitol with no significant difference between these two concentrations. As concentration of Mannitol was increased from 6% to 8% significant increase in MGT was observed. Similar results were obtained by Rahman [15] in mungbean where MGT decreased with mannitol priming @ 2% and 4% and increase in MGT was observed with increasing concentration of mannitol to 6% and 8%. The finding is also in line with Rahman, *et al.* [16] who reported reduced MGT by priming in okra seeds. The reduction in MGT by priming can be explained by the fact that priming activate and synthesize hydrolytic enzymes e.g. lipases, amylases and proteases which mobilize storage materials in seed and on rehydration quick emergence take place because all pregerminative processes had already taken place [17]. According to Pukackas and Ratajczak [18], priming activates antioxidant enzymes which lower the per oxidation in seed thereby maintaining seed vigour which may result in quick germination. Bray, *et al.* [5] reported protein synthesis in seeds whereas Burgass and Powell [19] and Varierf, *et al.* [17] reported that priming cause repair of damaged DNA, due to protein synthesis and repair in seed during priming seed become invigorated which result in reduced MGT. The probable reason for early emergence of the primed seed may be due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed [20]. Yamauchi and Winn [21], indicated that seed priming may help in dormancy breakdown possibly by embryo development and leaching of emergence inhibitors which resulted in an earlier start of emergence.

MGT increased gradually with increase in the stress levels. However, no significant differences in MGT at control and stress level of 0.15 Mpa was observed and MGT increased thereafter with increase in stress levels. According to Tesche [22], water stress conditions results delay in completion of germination because seeds require more time to absorb sufficient amount of water, which is vital for the act of initiation of germination. Gul and Allan [23], also reported that germination time increased to 8 folds in 93 lines of wheat, when water potential of seed was reduced to -14.4 bars.

Table-2. G%, CVG, MGT, RWC, Root length (RL) and Shoot length (SL) of Maize seeds exposed to different concentrations of priming and water stresses

Treatments	G%	MGT	RL	SL	RWC
A. Water Stress					
0 Mpa	99.46a	2.56d	9.24a	3.07a	84.95a
0.15 Mpa	99.73a	2.68d	7.40b	2.60a	77.65b
0.5 Mpa	98.73a	3.10c	4.30c	1.12b	78.64b
1.5 Mpa	87.46b	3.73b	2.48d	0.70bc	83.91a
1.7 Mpa	64.26c	5.37a	1.02e	0.55c	87.27a
F-Test	***	***	***	***	***
B. Mannitol					
0%	87.46	3.78a	4.94	1.53	83.17
2%	93.6	3.32c	5.17	1.7	81.52
4%	92.8	3.34c	4.91	1.71	83.15
6%	86.4	3.41bc	4.93	1.73	83.12
8%	89.6	3.58ab	4.48	1.36	81.45
F-Test	NS	**	NS	NS	NS
Interaction (A*B)	NS	NS	NS	NS	NS
LSD	7.55	0.65	0.52	0.24	4.29
CV%	11.44	18.06	43.81	9.66	7.09
Grand Mean	89.97	4.89	1.61	3.49	82.48

Means in the column followed by same letter(s) are not significantly different. *** Highly significant ($p \leq 1\%$), ** Significant ($p \leq 5\%$) and NS (Non Significant) CV= Coefficient variation, LSD= Least Significant Difference

3.3. Relative Water Content (RWC)

No significant results were obtained in RWC as seeds were induced to priming. However, RWC decreased as level of stress increased from 0 Mpa to 0.5 Mpa and again significant increase in RWC was observed with increasing stress from 0.5 Mpa to 1.7 Mpa. The results are somewhat in contrast with other findings. El-Tayeb [24], reported that drought caused a decrease in RWC in *Vicia faba* cultivars. Decrease in RWC with the increasing stress has been related to low water uptake by germinating seed [25]. Decrease in RWC in drought can be explained by the decrease in plant vigour [26] increase in cell penetrability and decrease in sustainability [27] and cleavage in the cell membrane and sedimentation of cytoplasm content observed through microscopic investigations of dehydrated cells [28].

3.4. Root and Shoot Length (RL and SL)

The root and shoot length were not significantly affected by mannitol priming. Similar result was obtained by Canak, *et al.* [29] who reported that effects of priming treatments of maize seeds on root length were insignificant.

However, both the lengths decreased with increasing level of water stress. RL was seen to be more affected by stress than the SL. RL decreased gradually with increasing stress. Shoot length at control and 0.15 Mpa were not significantly different while significant decrease was observed with elevated stress from 0.15 to 0.5 and 1.0 Mpa. Further decrease in SL was obtained as seeds were exposed to higher level of stress of 1.7 Mpa. Similar results were obtained by Batool, *et al.* [30]; Achakzai [31]. This decrease in RL and SL can be explained by the fact that water deficit causes decline in the cellular expansion leading to growth reduction [32]. The cellular elongation process and the carbohydrates wall synthesis were very susceptible to water deficit [33] and the decrease in growth was a consequence of the turgescence laying down of those cells [34]. Batool, *et al.* [30], reported that shoot cells growth depends upon water availability and when cell was exposed to water shortage as result shoot growth decrease. Takel [35] reported reduction or non-transfer of nutrients from seed storage tissues to the embryo as one of the causes of SL reduction in drought stress conditions. In addition, decrease in water absorption by seed under water stress causes decrease in enzymes and hormones secretion resulting into impaired root and shoot growth. Asghari [36]. Achakzai [31] explained that the rate of growth of plant cells and the efficiency of their physiological processes are highest when the cells are at higher turgidity. The turgor pressure of cell in plants subjected to water stress has lower than the maximum value. Cells are highly sensitive to water stress, because cell expansion is caused by the action of turgor pressure upon cell walls [37, 38]. They further revealed that even in mild water stress conditions i.e. when turgor pressure is reduced by only few bars, a significant decrease in growth could be observed.

4. Conclusion

The study indicated that priming with mannitol could improve the speed of germination in maize seeds. Mannitol @ 2% is found to be best concentration among 2%, 4%, 6% and 8%. The parameters representing the speed of germination i.e. CVG and MGT were found to be decreasing and increasing respectively with higher concentrations of mannitol although they showed better results than control. However, the final germination percentage, RWC, RL and SL remained unaltered. Germination activities reduced with increasing water stress.

References

- [1] Khalili, M., Naghavi, M. R., Aboughadareh, A. P., and Rad, H. N., 2013. "Effects of drought stress on yield and yield components in maize cultivars (*Zea mays* L.)." *Int. J. of Agronomy and Plant Production*, vol. 4, pp. 809-812.
- [2] Soltani, A., Gholopoor, M., and Zeinali, E., 2006. "Seed reserve utilization and seedling growth of wheat as affected by drought and salinity." *Environ. Exp. Bot.*, vol. 55, pp. 195-200.
- [3] Harris, D., Joshi, A., Khan, P. A., Gothakar, P., and Sodhi, P. S., 1999. "On-farm seed priming in semi-arid agriculture: Development and evaluation in corn, rice and chickpea in India using participatory methods." *Exp. Agric.*, vol. 35, pp. 15-29.
- [4] McDonald, M. B., 2000. *Seed technology and its biological basis*. Sheffield, UK: Sheffield Academic Press Ltd., pp. 287-325.
- [5] Bray, C. M., Davison, P. A., Ashraf, M., and Taylor, R. M., 1989. "Biochemical changes during priming of leek seeds." *Annals of Botany*, vol. 63, pp. 185-193.
- [6] Ahammad, K. U., Rahman, M. M., and Ali, M. R., 2014. "Effect of hydropriming method in maize (*Zea mays*) seedling emergence." *Agril. Res.*, vol. 39, pp. 143-150.
- [7] Bewley, J. D., Bradford, K. J., Hillhorst, H. W. M., and Nonogaki, H., 2013. *Seeds physiology of development, germination and dormancy*. 3rd ed. New York: Springer.
- [8] Weatherley, P., 1950. "Studies in the water relations of the cotton plant. I. The field measurements of water deficits in leaves." *New Phytologist.*, vol. 49, pp. 81-97.
- [9] Elocka, E., 2014. "Osmo- and hydropriming enhance germination rate and reduce thermal time requirement of pea (*Pisum sativum* L. CV. Winner) seeds." *Akademik Ziraat Dergisi.*, vol. 3, pp. 1-12.
- [10] Partheeban, C., Chandrasekhar, C. N., Jeyakumar, P., Ravikesavan, R., and Gnanam, R., 2017. "Effect of peg induced drought stress on seed germination and seedling characters of maize *zea mays* l. genotypes." *Int. J. Curr. Microbiol. App. Sci.*, vol. 6, pp. 1095-1104.
- [11] Jorenush, M. H. and Rajabi, M., 2015. "Effect of drought and salinity tensions on germination and seedling growth of artichoke *cynara scolymus* L." *Int. J. Adv. Biol. Biom.*, vol. 3, pp. 297-302.
- [12] Ahmad, S. R., Ahmad, M. Y., Ashraf, M., Ashraf, and Waraich, E. A., 2009. "Sunflower (*Helianthus annuus* L.) response to drought stress at germination and growth stages." *Pak. J. Bot.*, vol. 41, pp. 647-654.
- [13] Ayaz, F. A., Kadioglu, A., and Urgut, R. T., 2001. "Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ciananthe setosa*." *Canadian J. Plant Sci.*, vol. 80, pp. 373-378.
- [14] Bansal, R. P., Bhati, P. R., and Sem, D. N., 1980. "Differential specificity in water inhibition of Indian arid zone." *Biologia Plantarum*, vol. 22, pp. 327-331.
- [15] Rahman, A., 2015. *Enhancement of drought tolerance in mungbean through osmo and hydro priming Msc. Thesis*. Dhaka, Bangladesh: Sher-e-Bangla Agricultural University.

- [16] Rahman, Shamsher, A., Alam, M., Adnan, M., Ullah, H., Malik, M. F. A., Shah, A. S., Ibrahim, M., and Basir, A., 2016. "Effect of seed priming on germination performance and yield of Okra." *Pakistan J. Agric. Res.*, vol. 29, pp. 253-262.
- [17] Varierf, A., Vari, A. K., and Dadlani, M., 2010. "The subcellular basis of seed priming." *Current Science*, vol. 99, pp. 450-456.
- [18] Pukackas and Ratajczak, E., 2005. "Production and scavenging of reactive oxygen species in *Fagus sylvatica* seeds during storage at varied temperature and humidity." *J. Plant Physiol.*, vol. 162, pp. 873-885.
- [19] Burgass, W. R. and Powell, A. A., 1984. "Evidence for repair processes in the invigoration of seeds by hydration." *Ann. Bot.*, vol. 53, pp. 753-757.
- [20] Arif, M., 2005. *Seed priming improves emergence, yield and storability of soybean. PhD thesis in Agronomy.* Pakistan: NWFP Agricultural University Peshawar.
- [21] Yamauchi and Winn, T., 1996. "Rice seed vigor and seedling establishment in anaerobic soil." *Crop Sci.*, vol. 36, pp. 680-686.
- [22] Tesche, M., 1975. "Germination of conifer seeds under the conditions of simulated drought stress in polyethylene glycol (Lutrol)." *J. Phys. Biochem.*, vol. 176, pp. 577-584.
- [23] Gul, A. and Allan, R. E., 1976. "Interrelationships of seedling vigor criteria of wheat under different field situations and soil water potentials." *Crop Science*, vol. 16, pp. 615- 618.
- [24] El-Tayeb, 2006. "Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity." *Acta Agron. Hung.*, vol. 54, pp. 25-37.
- [25] Kayden, D. and Yagmur, M., 2008. "Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl." *African Journal of Biotechnology*, vol. 7, pp. 2862-2868.
- [26] Liu, Y., Fiskum, G., and Schubert, D., 2002. "Generation of reactive oxygen species by the mitochondrial electron transport Chain." *J. Neurochem.*, vol. 80, pp. 780-787.
- [27] Blokhina, O., Virolainen, E., and Fagerstedt, K. V., 2003. "Anti-oxidative damage and oxygen deprivation stress." *Annual botany*, vol. 19, pp. 179-194.
- [28] Blackman, S. A., Obendorf, R. L., and Leopold, A. C., 1995. "Desiccation tolerance in developing soybean seeds: The role of stress proteins." *Plant Physiol.*, vol. 93, pp. 630-638.
- [29] Canak, P., Mirosvlejevic, M., Cricic, M., Keselji, J., Vujosevic, B., Stanisavljevic, D., and Mitrovic, B., 2016. "Effect of seed priming on seed vigour and early seedling growth in maize under optimal and suboptimal temperature conditions. Selekcija I Semennarstvo." vol. 12, pp. 17-25.
- [30] Batool, A., Ziaf, K., and Amjad, M., 2015. "Effect of halo-priming on germination and vigor index of cabbage *Brassica oleracea* var. capitata." *J. Env. and Agri. Sci.*, vol. 2, pp. 1-8.
- [31] Achakzai, A. K. K., 2009. "Effect of water stress on imbibition, germination and seedling growth of maize cultivars." *Sarhad J.Agri.*, vol. 25, pp. 165-172.
- [32] Kramer, P. J., 1974. "Fifty years of progress in water relations research." *Plant Physiology*, vol. 54, pp. 463-71.
- [33] Wenkert, W., Lemon, E. R., and Sinclair, T. R., 1978. "Leaf elongation and turgor pressure in field grown soybean." *Agron. J.*, vol. 70, pp. 761-764.
- [34] Shalhevet, J., Huck, M. G., and Schoeder, B. P., 1995. "Root and shoot growth responses to salinity in maize and soybean." *Agronomy Journal.*, vol. 87, pp. 512-517.
- [35] Takel, A., 2000. "Seedling emergence and growth of sorghum genotypes under variable soil moisture deficit." *Agronomy Journal.*, vol. 48, pp. 95-102.
- [36] Asghari, M., 2002. "Ethylene effect on osmotic adjustment and growth of axial and cotyledon tissues of sunflower seeds under drought stress." *Industry Journal of Agricultural Science*, vol. 7, pp. 137-145.
- [37] Greacen, E. L. and Oh, J. S., 1972. "Physics of root growth." *New Biol.*, vol. 235, pp. 24-25.
- [38] Burstrom, H. G., 1975. "Growth and water conditions in etiolated *Pisum* stems." *Z. Pflanzen-Physiol.*, vol. 75, pp. 419-427.