Pak. J. Bot., 50(5): 1669-1673, 2018.

EFFECTS OF HYDRO AND HORMONAL PRIMING ON QUINOA (*CHENOPODIUM QUINOA* WILLD.) SEED GERMINATION UNDER SALT AND DROUGHT STRESS

IHSANULLAH DAUR*

Department of Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia
**Corresponding author's email: iaslam@kau.edu.sa*

Abstract

An experiment was conducted to examine the effect of salinity and drought on germination of quinoa and to test the influence of priming treatments under these stresses. The stresses were built by different levels of polyethylene glycol (PEG) 6000 and NaCl, respectively. Treatments consisted of a control (non-primed seed) and seeds primed in water with or without the addition of plant growth regulator (40 mg. L^{-1} gibberellic acid; 80 mg. L^{-1} kinetin). The treated quinoa seeds were tested for germination and seedling growth under the drought and salinity stresses with similar water potentials of 0.0, −0.3, −0.6, −0.9, and −1.2 MPa. The study found that the application of both PEG and saline solutions delayed germination, whereas germination was enhanced by priming treatments. It was noted that at equivalent water potentials, the adverse effect of salinity was less than the PEG solution for mean germination time (MGT), germination percentage, abnormal germination percentage, and root and shoot lengths. Moreover, seeds germinated at all salinity levels, but no seed germinated at −1.2 MPa of the PEG treatment. These results indicate that germination was inhibited by osmotic effect rather than salt toxicity at equivalent water potentials of PEG and NaCl.

Key words: Germination, NaCl, PEG, Quinoa (*Chenopodium quinoa* Willd.), Seed priming.

Introduction

Among the most important crops, quinoa (*Chenopodium quinoa* Willd.) is suit for drought and saline conditions. Key issues of crop production in arid regions include drought and salinity stresses, which sometimes cause uneven seed germination that results in poor stand establishment (Panuccio *et al*., 2014; Eisa *et al*., 2017).

Drought and salinity stresses generally affect seed germination by creating an osmotic potential in the exterior of the seed and subsequently inhibiting water uptake, or they have toxic effects of Na+ and Cl− ions on the growing seedling (Castroluna *et al.,* 2014; Llanes *et al.,* 2016). Seed germination inhibition, delayed germination, and seedling establishment in arid regions are primarily caused by salt or osmotic stresses (Alshameri *et al.,* 2017). Gupta and Huang (2014) reported that drought and salinity stresses decrease water uptake but salt stress may cause ion toxicity.

Seed priming has been positively validated for the improvement of seed germination and subsequent crop growth, such as wheat, coriander, and quinoa (Jisha *et al*., 2013; Ceccato *et al*., 2015; Ibrahim, 2016; Tabassum *et al*., 2017). Mandal *et al*., (2015) demonstrated priming for better stand establishment. Moreover, sometimes conditioning solutions for seed priming are supplemented with the addition of plant growth regulators. Previous studies have examined this in pigeon pea (Sneideris *et al*., 2015), maize (Jiang *et al*., 2016), wheat (Ulfat *et al*., 2017), and Okra (Hussein *et al*., 2017).

The aim of the present study was to determine the causes related to the failure of quinoa seed germination in drought and saline conditions that are representative of osmotic barrier and/or ion toxicity. For this reason, seed germination was compared under various osmotic potentials of polyethylene glycol (PEG) and salinity. To alleviate the effects of salt and drought stresses, the study examined the potentials of hydro-priming or supplemented it with the addition of gibberellic acid and kinetin.

Materials and Methods

An experiment on quinoa seed germination and seedling growth (up to 7 d) conducted two times with four replications was carried out using completely randomized design with three factors. The first factor was four seed treatments: the control (unprimed) and soaking for 6 h in either distilled water, distilled water with 40 mg L^{-1} gibberellic acid, or distilled water with 80 mg L^{-1} kinetin. The second factor entailed the PEG and NaCl solutions, and the third factor was the five levels of osmotic potential (0, −0.3, −0.6, −0.9, and −1.2 MPa) of PEG 6000 (Michel and Kaufmann, 1973) or salinity (Coons *et al*., 1990). The electrical conductivity (EC) of saline water was 0, 6.5, 12.7, 18.4, and 23.5 dS m^{-1}, corresponding to the above five levels of potential.

Seed priming, germination, and growth: The seed priming treatments included a control (unprimed) and soaking for 6 h in either distilled water, distilled water with 40 mg L^{-1} gibberellic acid, or distilled water with 80 mg L^{-1} kinetin.

The seeds subjected to soaking were followed by surface drying and were then immediately sown in sterilized petri-dishes. Each treatment had four replicates of 25 seeds on top of three layers of Whatman No. 2 filter paper with 10 ml of the relevant experimental solutions. The petri dishes were covered to prevent moisture loss during incubation. Seeds were stored at 25 ± 1°C in a Seed Germination Incubator and allowed to germinate for 7 d. The seeds were observed every 24 h for 7 d, and the germination percentages were recorded. The seeds that had not germinated in the test solutions were moved to distilled water to examine the toxic effects of the solutions for 3 d (data not shown). Mean germination time (MGT) was determined according to Ellis and Roberts (1980). The sprouts with underdeveloped primary roots and undersized, thick, and twisted hypocotyls were recorded as abnormally germinated according to ISTA

(International Seed Testing Association) (2003). Root and shoot lengths and fresh weights of the seedling were measured after day seven.

Statistical analysis

The data for various parameters were statistically analysed using three-way ANOVA software of Statistix 8.1 (Tallahassee, FL, 32317, USA). The differences in means were calculated using LSD test (p<0.05).

Results

The data analysis indicated a significant (p<0.05.) three-way interaction for all parameters included in the present study. MGT was extended by reducing the osmotic potential of PEG and salinity, where PEG took more time than saline solution (Table 1). Priming was found to be effective in reducing the seed germination period of quinoa. However, the acceleration of seed germination due to priming was ranked equally for PEG and NaCl, particularly at lower osmotic potentials, as follows: gibberellic acid > kinetin > hydropriming > control.

While both osmotic and salt stresses inhibited seed germination, PEG influenced germination inhibition more strongly than NaCl (Table 2). No germination was observed for PEG at −1.2 MPa. Gibberellic acid was more effective for germination in NaCl solutions at all osmotic potentials, while the germination percentage decreased at osmotic stress −0.9 MPa of PEG. Among the seed treatments, gibberellic acid was generally recorded with higher germination percentages for both PEG and NaCl solutions. No toxic effect of PEG was observed, as 100% of the non-germinated seeds from all levels of osmotic potential were germinated in distilled water (data not shown). Gibberellic acid, kinetin, and hydro-priming were effective in reducing abnormal germination in NaCl but were ineffective in PEG. Moreover, in both solutions, abnormal germination increased as water stress increased (Table 3). Longer seedling root length in PEG was noted compared to NaCl up to −0.6 Mpa while beyond that reduced root length was observed for PEG compared to NaCl, although both osmotic and salt stresses inhibited this measurement (Table 4). The growth of the radicle was completely inhibited at −0.9 MPa shortly after primary root emergence.

Likewise, longer seedling shoot length in PEG was noted compared to NaCl up to −0.6 Mpa while beyond PEG caused a significant (p<0.05) decrease in shoot length compared to salinity, and no shoot growth was recorded in the control for PEG at −0.9 MPa (Table 5). However, priming in water, water with added gibberellic acid, and water with kinetin enhanced shoot growth and produced shoot growth for PEG, even at −0.9 MPa. Similarly, priming treatments showed shoot growth at all concentrations of salinity, and effectiveness was especially observed at −0.9 MPa and −1.2 MPa. Seedling fresh weight also decreased as a result of the decrease in root and shoot lengths at low osmotic potentials (Table 6). However, the osmotic stress due to PEG caused a greater reduction in the seedling fresh weights compared to salinity, particularly at −0.9 MPa and above.

Table 1. Days taken by germination of Quinoa seeds in PEG and salinity under various treatments [control (non-primed), primed in water, and in solution of plant growth regulator (Gibberellic acid and Kinetin)].

MPa	Seed treatments							
	Control [non-primed]		Primed in water (hydropriming)		Primed in GA		Primed in Kinetin	
	PEG	Salinity	PEG	Salinity	PEG	Salinity	PEG	Salinity
0	1.73	1.66	1.59	1.46	1.47	1.32	1.58	1.46
−0.3	1.91	1.85	1.59	1.64	1.48	1.40	1.64	1.50
−0.6	1.97	1.90	1.80	1.65	1.50	1.42	1.65	1.52
−0.9	1.98	1.92	1.83	1.77	1.59	1.52	1.70	1.59
−1.2	-	1.95	-	1.78	-	1.58	-	1.73

LSD = 0.18

Table 2. Quinoa seeds germination (%) in PEG and Salinity under various treatments [control (non-primed) and primed in water, and in solution of plant growth regulator (Gibberellic acid and Kinetin)].

MPa	Seed treatments							
	Control [non-primed]		Primed in water (hydropriming)		Primed in GA		Primed in Kinetin	
	PEG	Salinity	PEG	Salinity	PEG	Salinity	PEG	Salinity
0	88.2	93.0	95.2	97.8	95.2	97.9	94.9	97.7
−0.3	88.2	91.3	90.1	92.8	94.3	96.9	93.8	95.9
−0.6	87.3	89.8	88.4	90.4	93.8	96.6	91.9	95.1
−0.9	48.6	88.6	53.9	89.8	64.8	96.2	60.1	93.8
−1.2	-	84.1	-	87.5	-	93.8	-	91.9

LSD = 2.94

Table 3. Quinoa seeds abnormal germination (%) in PEG and Salinity under various treatments [control (non-primed) and primed in water, and in solution of plant growth regulator (Gibberellic acid and Kinetin)].

MPa	Seed treatments							
	Control [non-primed]		Primed in water (hydropriming)		Primed in GA		Primed in Kinetin	
	PEG	Salinity	PEG	Salinity	PEG	Salinity	PEG	Salinity
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
−0.3	21.35	2.04	19.1	1.84	15.54	-	15.13	1.64
−0.6	20.84	8.22	21.31	8.51	15.07	1.90	16.2	4.15
−0.9	25.72	9.92	20.56	8.86	14.14	3.04	19.03	5.30
−1.2	27.07	10.50	28.68	11.50	17.09	5.67	21.32	7.88

LSD = 1.98

Table 4. Quinoa seedlings root length (cm) in PEG and Salinity under various treatments [control (non-primed) and primed in water, and in solution of plant growth regulator (Gibberellic acid and Kinetin)].

MPa	Seed treatments							
	Control [non-primed]		Primed in water (hydropriming)		Primed in GA		Primed in Kinetin	
	PEG	Salinity	PEG	Salinity	PEG	Salinity	PEG	Salinity
0	3.56	2.78	4.04	3.51	3.43	3.93	3.62	3.83
−0.3	3.25	2.85	3.40	3.09	3.52	2.70	3.32	3.99
−0.6	3.40	2.01	2.15	2.47	2.82	2.21	2.17	2.88
−0.9	1.16	1.72	1.73	2.81	1.18	2.99	1.03	1.68
−1.2	-	0.94	-	1.99	-	1.99	-	1.19

LSD = 0.37

Table 5. Quinoa seedlings shoot length (cm) in PEG and Salinity under various treatments [control (non-primed) and primed in water, and in solution of plant growth regulator (Gibberellic acid and Kinetin)].

MPa	Seed treatments							
	Control [non-primed]		Primed in water (hydropriming)		Primed in GA		Primed in Kinetin	
	PEG	Salinity	PEG	Salinity	PEG	Salinity	PEG	Salinity
0	4.14	3.30	4.02	4.04	4.79	4.61	4.56	3.25
−0.3	3.66	3.35	4.38	3.69	4.19	3.03	4.06	3.45
−0.6	3.29	2.45	3.60	3.02	2.19	3.70	3.72	3.09
−0.9	-	1.30	2.13	3.13	2.57	2.44	2.53	3.09
−1.2	-	1.23	-	2.37	-	1.97	-	2.86

LSD = 0.52

Table 6. Fresh weight of Quinoa (mg plant^{-1}) in PEG and Salinity under various treatments [control (non-primed) and primed in water, and in solution of plant growth regulator (Gibberellic acid and Kinetin)].

MPa	Seed treatments							
	Control [non-primed]		Primed in water (hydropriming)		Primed in GA		Primed in Kinetin	
	PEG	Salinity	PEG	Salinity	PEG	Salinity	PEG	Salinity
0	14.45	11.91	15.43	14.31	15.97	14.89	15.43	13.08
−0.3	12.40	12.08	14.42	12.28	13.21	11.74	14.03	13.19
−0.6	9.00	10.97	11.76	12.58	10.02	10.66	14.32	12.53
−0.9	-	8.45	7.62	11.35	8.50	10.32	6.81	12.47
−1.2	-	8.45	-	10.61	-	9.34	-	10.03

LSD = 1.73

Discussion

The priming treatments enhanced the seed performance under both saline and osmotic stresses. The MGT was reduced by the priming treatments of seeds, while the stress conditions were observed to prolong the MGT. Additionally, the MGT was shorter for NaCl compared to PEG at equivalent osmotic potentials. The addition of hormones to hydro-priming-especially gibberellic acid-improved the MGT and rate of germination under both salt and drought stresses. Likewise, the priming treatments (with or without hormones) stimulated normal seed germination in quinoa. The results of the present study are consistent with the findings of Ulfat *et al.,* (2017), in which priming enhanced emergence and yield of wheat under normal and drought stress. *Jiang et al.,* (2016) indicated that, compared to the control, priming improved germination, relative water content, seedling vigour, root and shoot growth, and fresh and dry matter; priming additionally reduced emergence time and electrolyte leakage in saline conditions. It seems that priming encouraged germination by initiating pre-germination metabolic processes and it has been reported to activate antioxidants that promote seed emergence and growth (Ibrahim, 2016; Wojtyla *et al.,* 2016; Górnik and Lahuta, 2017). Fallah *et al.,* (2017) reported the favourable effects of priming with gibberellic acid and suggested an increase in antioxidants during the early stages of seedlings.

Petrović *et al.,* (2016) highlighted that stresses like drought and salinity, primarily influenced pea germination due to a reduction in water uptake. Likewise, germination percentages and seedling fresh weights are better under saline conditions than PEG, comparable reported by Khajeh-Demir and Mavi (2008) in pepper (*Capsicum annuum* L.) seed. This could be due to the presence of sodium and chlorine ions during germination, which allow more water to maintain a water potential gradient. Moreover, the lower germination percentages in PEG compared to saline conditions at equivalent water potentials could be attributed to the osmotic impact of PEG. Furthermore, no toxic effect was observed for PEG in which all seeds germinated when transferred from the PEG solution to fresh water (data not shown). Previous studies have indicated that molecules of PEG don't pass in the seed (Niemann *et al.,* 2013; Paparella *et al.,* 2015). Additionally, previous research has confirmed that PEG molecules have no toxicity (Khajeh-Hosseini *et al.,* 2003; Rasool *et al.,* 2016). Under salinity stress, the uptake of Na+ and Cl− may cause toxic effects of NaCl in the seed. However, in the current experiment, high salinity (23.5 dS m−1) did not cause a considerable decrease in the germination percentages, which may be due to salt tolerance in quinoa.

Nevertheless, the main objective of the present study was to increase seed germination and early seedling growth, in which the improvement of root and shoot growths and seedling fresh weights were observed with priming treatments. Moreover, it was observed that gibberellic acid solutions highly improved these parameters and yielded the highest root and shoot lengths and maximum seedling fresh weights. Shi *et al.,* (2015) reported that osmotic stress significantly decreased root and shoot growth in castor bean. Sharif *et al.,* (2017) indicated the inhibition of seedling growth in canola by salinity and PEG-induced drought stress; however, high inhibition was observed for PEG. Mohtashami *et al.,* (2016) suggested priming for increasing emergence and seedling growth in common beans.

Findings of the present study indicated that when PEG and salinity are at similar water potential, germination is inhibited because of osmotic effect and not caused by salinity. All the seed priming treatments were effective as it showed advantage over control (untreated) under both drought and salt stresses. Precisely, the efficiency of the treatments was in order of distilled water with 40 mg L−1 gibberellic acid > distilled water with 80 mg L−1 kinetin > soaking in distilled water > control (unprimed) for enhancing germination at low water potentials.

Acknowledgement

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under Grant No. (G-201-155-38). The author, therefore, acknowledges with thanks DSR for technical and financial support.

References

Alshameri, A., F.A.H.A.D. Al-Qurainy, S. Khan, M. Nadeem, A.R. Gaafar, M. Tarroum, A. Alameri, S. Alansi and M. Ashraf. 2017. Appraisal of guar [*Cyamopsis tetragonoloba* (L.) Taub.] accessions for forage purpose under the typical Saudi Arabian environmental conditions encompassing high temperature, salinity and drought. *Pak. J. Bot*., 49(4): 1405-1413.

Castroluna, A., O.M. Ruiz, A.M. Quiroga and H.E. Pedranzani. 2014. Effects of salinity and drought stress on germination, biomass and growth in three varieties of *Medicago sativa* L. *Avances en Investigación Agropecuaria*, 18(1).

Ceccato, D., J. Delatorre-Herrera, H. Burrieza, D. Bertero, E. Martínez, I. Delfino, S. Moncada, D. Bazile and M. Castellión. 2015. seed physiology and response to germination conditions. State of The Art Report On Quinoa, p.131.

Demir, I. and K. Mavi. 2008. Effect of salt and osmotic stresses on the germination of pepper seeds of different maturation stages. *Braz. Arch. Biol. Technol*., 51(5): 897-902.

Eisa, S.S., M.A. Eid, E.H. Abd El-Samad, S.A. Hussin, A.A. Abdel-Ati, N.E. El-Bordeny, S.H. Ali, H. Al-Sayed, M.E. Lotfy, M.A. Masoud and A.M. El-Naggar. 2017. 'Chenopodium quinoa' Willd. A new cash crop halophyte for saline regions of Egypt. *Aust. J. Crop Sci*., 11(3): 343.

Fallah, S., S. Malekzadeh and M. Pessarakli. 2017. Seed priming improves seedling emergence and reduces oxidative stress in Nigella sativa under soil moisture stress. *Plant Nutr*., 1-12.

Górnik, K. and L.B. Lahuta. 2017. Application of phytohormones during seed hydropriming and heat shock treatment on sunflower (*Helianthus annuus* L.) chilling resistance and changes in soluble carbohydrates. *Acta Physiol. Plant*., 39(5): 118.

Gupta, B. and B. Huang. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genomics*., 2014.

Hussein, H.J., 2017. The Effect of Seed Priming with Salicylic Acid and Ascorbic Acid on Viability of Local Okra (*Abelmoschus esculentus* L.) Seeds Stored for Three Years. *J. Global Pharma Technol.,* 9(8).

Ibrahim, E.A. 2016. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant physiology*, 192: 38-46.

Jiang, X., H. Li and X. Song. 2016. Seed priming with melatonin effects on seed germination and seedling growth in maize under salinity stress. *Pak. J. Bot.*, 48(4): 1345-1353.

Jisha, K.C., K. Vijayakumari and J.T. Puthur. 2013. Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum*, 35(5): 1381-1396.

Llanes, A., A. Andrade, O. Masciarelli, S. Alemano and V. Luna. 2016. Drought and salinity alter endogenous hormonal profiles at the seed germination phase. *Seed Sci. Res.*, 26(1): 1-13.

Mandal, A.B., R. Mondal and P.M.S. Dutta. 2015. Seed enhancement through priming, coating and pelleting for uniform crop stand and increased productivity. *J. Andaman Sci. Association*, 20(1): 26-33.

Mohtashami, M., A. Naderi, A.A. Ghanbari, M. Alavifazel and L.A.K. Shahram. 2016. Effect of seed pre-treatment with growth regulators on seed yield and yield components of common beans (*Phaseolus vulgaris* L.). *Turk. J. Field Crops*, 21(2): 313-317.

Niemann, S., M. Burghardt, C. Popp and M. Riederer. 2013. Aqueous pathways dominate permeation of solutes across Pisum sativum seed coats and mediate solute transport via diffusion and bulk flow of water. *Plant Cell Environ*., 36(5): 1027-1036.

Panuccio, M.R., S.E. Jacobsen, S.S. Akhtar and A. Muscolo. 2014. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB plants, 6, p.plu047.

Paparella, S., S.S. Araújo, G. Rossi, M. Wijayasinghe, D. Carbonera and A. Balestrazzi. 2015. Seed priming: state of the art and new perspectives. *Plant cell reports*, 34(8), pp.1281-1293.

Petrović, G., D. Jovičić, Z. Nikolić, G. Tamindžić, M. Ignjatov, D. Milošević and B. Milošević. 2016. Comparative study of drought and salt stress effects on germination and seedling growth of pea. *Genetika*, 48(1): 373-381.

Rasool, S.G., A. Hameed, M.Z. Ahmed, M.A. Khan and B. Gul. 2016. Comparison of seed germination and recovery responses of a salt marsh halophyte Halopeplis perfoliata to osmotic and ionic treatments. *Pak. J. Bot*, 48(4): 1335-1343.

Sharif, P., M. Seyedsalehi, O. Paladino, P. Van Damme, M. Sillanpää and A.A. Sharifi. 2017. Effect of drought and salinity stresses on morphological and physiological characteristics of canola. *Intl. J. Env.l Sci. and Technol.*, 1-8.

Shi, G., S. Xia, J. Ye, Y. Huang, C. Liu and Z. Zhang. 2015. PEG-simulated drought stress decreases cadmium accumulation in castor bean by altering root morphology. *Env. Experiment. Bot*., 111, pp.127-134.

Sneideris, L.C., M.A. Gavassi, M.L. Campos, V. D'Amico-Damiao and R.F. Carvalho. 2015. Effects of hormonal priming on seed germination of pigeon pea under cadmium stress. *Anais da Academia Brasileira de Ciências*, 87(3): 1847-1852.

Tabassum, T., M. Farooq, R. Ahmad, A. Zohaib and A. Wahid. 2017. Seed priming and transgenerational drought memory improves tolerance against salt stress in bread wheat. Plant Physiology and Biochemistry, 118, pp. 362-369.

Ulfat, A., S.A. Majid and A. Hameed. 2017. Hormonal seed priming improves wheat (*Triticum aestivum* L.) Field performance under drought and non-stress conditions. *Pak. J. Bot*, 49(4): 1239-1253.

Wojtyla, Ł., K. Lechowska, S. Kubala and M. Garnczarska. 2016. Molecular processes induced in primed seeds-increasing the potential to stabilize crop yields under drought conditions. *J. Plant Physiol*., 203: 116-126.

(Received for publication15 November 2017)