

The effects of seed priming techniques in improving germination and early seedling growth of *Aeluropus Macrostachys*

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ABSTRACT

The purpose of this study was to examine the evaluating the potential of seed priming techniques in improving germination and early seedling growth of *Aeluropus Macrostachys* under salinity stress condition. This study was performed at the Islamic Azad University in Baft city which is located in southeast of kerman province (2250 meters above the sea level, 92° 17 N latitude and 56° 36 E longitude, and 220 mm annual rainfall). This experiment was conducted a completely randomized design. The factors examined include four levels of seed priming, control (without priming) with NaCl and CaCl₂ KCl and five salinity levels (0, 4, 8, 12, 16 Desysimenz/m), respectively. In this experiment traits such as germination percentage, speed of germination, radicle length and shoot length were measured. Data were analyzed by using General Linear Models procedure of SAS software. In addition, Means were compared using Duncan's multiple range test. Results of this study showed that different seed priming treatments improved shoot and radicle length, germination percentage and speed of germination of *Aeluropus Macrostachys* specie.

Key words: *Aeluropus Macrostachys*, Seed priming, Germination, Salinity stress,

INTRODUCTION

Seed priming (osmoconditioning, osmopriming, osmotic priming) is a pre-sowing treatment that involves exposure of seeds to a low external water potential that limits hydration. Seed priming is a controlled hydration treatment in which seeds are allowed to imbibe before radical protrusion (Bradford, 1986) and improves the germination rate, uniformity of germination, and sometimes greater total germination percentage (Basra et al., 2002, 2003, 2004, 2005, 2006; Farooq et al., 2004, 2005, 2006). This hydration is sufficient to permit pregerminative metabolic events but insufficient to allow radicle protrusion through the seed coat (Heydecker et al., 1975). Priming allows some of the metabolic processes necessary for germination to occur without germination take place. In priming, seeds are soaked in different solutions with high osmotic potential (Ghobadi et al, 2012). This technique has become a common seed treatment that can increase rate, percentage and uniformity of germination or seedling emergence, mainly under unfavorable environmental conditions. Rapid seed germination and stand establishment are critical factors for crop production under stress conditions. In many crop species, seed germination and early seedling growth are the most sensitive stages to stresses. Seed priming is

known as the seed treatment which improves seed performance under environmental conditions (Ashraf and Foolad, 2005). Constraints to good cost abolishment include improper seedbed preparation (Joshi, 1987), low quality seed, untimely sowing (Van Oosterom et al, 1961), poor sowing techniques (Radford, 1983), inadequate soil moisture (Harris et al, 1999) and adverse soil conditions (Lee et al, 1998). It is reported that seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions. Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows seed to imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat. The most important priming treatments are halopriming and hydro priming. Halopriming is a pre-sowing soaking of seeds in salt solutions, which enhances germination and seedling emergence uniformly under adverse environmental conditions. Technology that progress seed germination and stand establishment would enable the parental plants to capture more soil moisture, nutrients, solar radiation, and help to attain high synchronization of the reproductive stages of each parents and mature before the occurrence of cool stress in fall (Subedi and Ma, 2005). Therefore, seed invigoration treatments have been developed to improve seed performance during germination and seedling early growth. The general purpose of seed priming is to hydrate partially the seed to a point where germination processes are initiated but not completed. Most priming treatments involve imbibing seed with restricted amounts of water to allow sufficient hydration and advance of metabolic processes but preventing the protrusion of the radicle. Treated seeds usually would exhibit rapid germination when absorb water under field conditions (Ashraf and Foolad, 2005). Earlier works showed that the success of seed priming is influenced by the complex interaction of factors including plant species, water potentiality of the priming agent, duration of priming, temperature, seed vigor and dehydration, and storage conditions of the primed seed (Moradi Dezfuli et al., 2008). Dell-Aquila and Tritto (1990) reported that primed seeds emerged 12h earlier than non primed seeds. This may be due to increase in activity of enzymes such as amylase, protease and lipase which have great role in break down of macromolecules for growth and development of embryo that ultimately resulted in early and higher seedling emergence. On-farm seed priming involves soaking the seed in water, surface drying and sowing the same day. The rational is that sowing soaked seed decrease the time needed for germination and may allow the seedling to escape from deteriorating of soil physical condition. Besides better establishment, farmers have reported that primed crops grew more vigorously, flowered earlier and yielded higher. In wheat, researchers have recorded mean yield increases in six large series of on-farm trials of 5% to 36% (Harris et al., 2001). Kant et al., (2006) reported that priming seed improves stand establishment, growth and yield of late sown wheat in rice-wheat systems. Poor stand establishment results in less tillers and ultimately reduced grain yield. Seed priming improves the germination rate, speed and uniformity even under less than optimum field condition (lee et al., 1998, kant et al., 2006)thus enabling the establishment of uniform and good crop stand establishment. Due to readily available food during germination (Farooq et al., 2006), primed seed are better able to complete the process of germination in a short time and cope with environmental stresses including low temperature (Kant et al., 2006, Farooq et al., 2007). Salt stress is known to perturb various growth processes including photosynthesis, ion regulation, water relations, etc. (Ashraf, 2004). Salt affected soils can be managed by reclamation, however the reduced availability of quality of water, low soil permeability and high costs of amendments make this approach not often feasible on a large scale. Saline agriculture technology is another approach for effective utilization of salt affected

soils, which involves the cultivation of salt tolerant plants (Qureshi et al. 1998). This approach is frequently not suitable for crops for which there are few salt tolerant cultivars. Screening of salt tolerant genotypes is another strategy to overcome this problem. However, the classical screening method for salt tolerance based on yield responses to salt (Belkhodja et al. 1994) is very expensive, space consuming and slow (Munns and James, 2003). Similarly, screening methods based on physiological mechanisms are often not feasible and can be inconsistent or entirely unsuitable (Munns and James, 2003). Improvement in salt tolerance of spring wheat is possible through selection and breeding (Ashraf and O'Leary, 1996), but progress in developing salt tolerant crop cultivars has been very slow because of our incomplete knowledge of the mechanisms of salt damage and the complex nature of the mechanisms of salt tolerance. Even different varieties of a particular crop species may exhibit different tolerance mechanisms (Ashraf, 1994). Moreover, lack of diverse germplasm for various crops including wheat is another barrier to success in breeding for salt tolerance. Typical responses to priming are faster and closer spread of times to germination and emergence over all seedbed environments and wider temperature range of germination, leading to better crop stands and hence improved yield and harvest quality, especially under sub-optimal and stress condition growing conditions in the field (Halmer, 2004). Normally priming is done either in low water potential solution (osmopriming) or in tap water (hydro-priming), however, incorporation of plant growth regulators during priming have improved seed germination, establishment and crop performance (Shafiei Abnavi and Ghobadi, 2012). In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing enough water for radicle protrusion, thus suspending the seeds in the lag phase (Taylor et al., 1998). Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence (Parera et al., 1994). Generally, priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions (Farooq et al., 2004). However, the effectiveness of different priming agents varies under different stresses as well as in different crop species. Therefore, the aim of this study were to evaluating the potential of seed priming techniques in improving germination and early seedling growth of *Aeluropus Macrostachys* under salinity stress condition.

MATERIALS AND METHODS

This study was performed at the Islamic Azad University in Baft city which is located in southeast of kerman province (2250 meters above the sea level, 92° 17 N latitude and 56° 36 E longitude, and 220 mm annual rainfall). Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried for 48h. All priming media were prepared in distilled water. Seeds were fully immersed in priming media at 20 °C under dark conditions. This experiment was conducted a completely randomized design. The factors examined include four levels of seed priming, control (without priming) with NaCl and CaCl₂ KCl and five salinity levels (0, 4, 8, 12, 16 Desysimenz/m), respectively. For doing this test 25 primed seed was placed on filter paper that wetted with 10 ml of respective test solutions and germinated in a germinator at 25°C and 16 hour light for 12 days. The filter papers were replaced every 2 days to prevent accumulation of salts (Rehman et al., 1996). Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was recorded every 24 h for 12 days. In this experiment traits such as germination percentage, speed of germination, radicle length and shoot length were

measured. Seeds were considered germinated when radicle protruded for 2 mm. Seed germination was recorded daily up to day 7 after the start of the experiment. Germination percentage (GP) was calculated based on equation (Ashraf and Foolad, 2005):

$$GP = (\text{Total germinated seed})/(\text{Total number of seed})$$

Then the mean germination rate was calculated according to the following equation (Ellis et al., 1987):

$$MGR = n / Dn$$

Where MGR is the mean germination rate, n is the number of seeds germinated on day and Dn is the number of days from the start of test. Data were analyzed by ANOVA using General Linear Models procedure of SAS software (Sas soft ware). Means were compared using Duncan's multiple range test. Level of significance used in all results was 0.05. Least square treatment means were compared if a significant F statistic (5% level of P) was detected by analysis of variance.

RESULTS AND DISCUSSION

The analysis of variance seed priming treatments on germination percentage of *Aeluropus Macrostachys* are presented in table 1. This table indicate that maximum germination percentage were observed when the seeds primed by S and KCL4 treatments. Results also showed that germination percentage between treatment was significant ($P < 0.05$). This result in agreements with observations of Mohamadi and Amiri (2003) who reported that seeds primed with KNO_3 was better germination parameters than those primed with distilled water. According to results of Mohamadi (1993) different seed treatments led to improved seed germination percentage, germination rate and seedling growth of soybean. Consistent with our results, similar finding were observed by Harris et al., (2001), Arif et al., (2003), Sung and Chang (1993), who reported improvement in seed germination, reduction in germination time and enhanced emergence in hydro primed seed. Ramezan et al., (2010) indicated that Potassium nitrate at 1% had a positive interaction with both time periods that in germination with result this study. It is possible that its positive effect might be due to its role in influencing the permeability of the membranes which ultimately leads to activation of enzymes involved in protein synthesis and carbohydrate metabolism (Preece and Read., 1993).

Table 1 : Analysis of variance seed priming treatments on germination percentage of *Aeluropus Macrostachys*

Source	DF	Sum of squares	Mean Squares	F, calculated	P-Value
Treat	12	9485.523	790.433	1121.39	0.0001
Error	26	18.326	0.704		
Total	38	9503.523			CV= 8.24

Salinity stress showed more delicacy of the aerial parts to salinity. Root with more length and consistency soil water absorption, will not face with the lack of water in critical stages of growth and prevents from decrease in yield under stressful conditions (Turner, 1986). Actually the seeds which are able to growing a higher length root under salinity stress are more successful in comparison with the seeds which do not have this capability

(Kayani et al., 1990). The reason of reduction germination percentage and rate with increased salinity is because of over-presenting of cation which not only are toxic but can decrease the water potential, in a way which the plant is unable to absorb water and has a kind of water deficit (Singh, 1995). The most effects of the stress of water its salinity, includes different patterns in proteins synthetise, make delay on emergence the basic tissues, and decrease in germination percentage and rate (Petruzzeli et al., 1991). The analysis of variance seed priming treatments on speed of germination of *Aeluropus Macrostachys* are presented in table 2. Maximum speed of germination was recorded in seeds primed with S and KCL4 treatments. Results showed that speed of germination between treatment was significant ($P < 0.05$).

Table 2 : Analysis of variance seed priming treatments on speed of germination of *Aeluropus Macrostachys*

Source	DF	Sum of squares	Mean Squares	F, calculated	P-Value
Treat	12	1913.347	159.445	3983.59	0.0001
Error	26	1.04	0.04		
Total	38	1914.388			CV= 4.27

It is revealed from the present study that different priming techniques can enhance the speed of germination of *Aeluropus Macrostachys*. Shahzad et al, (2003) reported that Shoot length was increased in hydroprimed and matricconditioned seeds for 12 or 24 h as compared to hydroprimed for 6 h and non-primed seeds. An increase in root length was recorded in matricconditioning treatment which might be the result of higher embryo-cell wall extensibility. Beckman et al. (1993) reported that solid matrix priming significantly increased adventitious roots than that of control in switch grass during green house experiment. Jett et al. (1996) also reported that root growth rates of matricprimed seeds were significantly higher than either osmotic or non-primed seedlings at most temperatures. The increase in root/shoot ratio with hydropriming treatments may be due to the fact that, priming induced nuclear replication in root tips of fresh seeds. These observations are in conformity with earlier work on wheat and pepper seeds (Stofella et al., 1992). The analysis of variance seed priming treatments on shoot length of *Aeluropus Macrostachys* are presented in table 3. Maximum shoot length was recorded in seeds primed with S and KCL4 treatments. Results showed that shoot length between treatment was significant ($P < 0.05$).

Table 3 : Analysis of variance seed priming treatments on shoot length of *Aeluropus Macrostachys*

Source	DF	Sum of squares	Mean Squares	F, calculated	P-Value
Treat	12	1069.025	89.085	48.93	0.0001
Error	26	47.333	1.820		
Total	38	1116.358			CV= 32.01

Similar to these results, Sadeghian and Yavari (2004) reported that seedling growth severely diminished with drought stress and genetic differences were found in sugar beet. Kaur et al., (2002) reported that hydropriming showed three to four more growth with respect to root and shoot length under comparison with seedling obtained from non-primed seeds. Basra et al., (2003) found that wheat seeds responded to different presowing seed treatments with

hydropriming showing the maximum invigoration followed by hydropriming 48 h. These findings support the other work where improved germination rate and percentage were observed following hydropriming for 48 h in wheat (Basra et al., 2003). Hydroprimed seeds could achieve earlier and more uniform germination, or by higher GI and heavier seedlings. Basra et al. (2006) also reported that hydroprimed seeds of sunflower and wheat could germinate faster and produced longer seedling under salinity stress, compared with untreated seeds. Salt deposit in the root growing medium is the main reason for physiological drought and subsequently reduced cell division and enlargement in the root growing region and ultimately reduced root growth (Godfery et al., 2004). Meanwhile these parameters in primed seeds at all salinity levels was higher than of non-primed seeds. Primed seeds had better efficiency for water absorption from growing media, and it is obvious that metabolic activities in seed during germination process commence much earlier than radicle and plumule appearance, i. e. emergence (Ascherman-Koch et al., 1992). Beneficial effects of hydropriming under normal and stress conditions could be due to earlier metabolic activities, faster and imbibitions, lesser mechanical restriction of seed coat as a result of softening of seed coat (Mc Donald, 2000). This research has shown that seed priming improved the *Aeluropus Macrostachys* germination traits in the laboratory. The analysis of variance seed priming treatments on radicle length of *Aeluropus Macrostachys* are presented in table 4. Maximum radicle length was recorded in seeds primed with KCL4 and KCL12 treatments. Results showed that shoot radicle length treatment was significant ($P < 0.05$).

Table 4 : Analysis of variance seed priming treatments on radicle length of *Aeluropus Macrostachys*

Source	DF	Sum of squares	Mean Squares	F, calculated	P-Value
Treat	12	754.769	62.897	11.63	0.0001
Error	26	140.666	5.410		
Total	38	859.435			CV= 22.67

Demir and Van De venter (1999) reported that drought and salinity may influence germination by decreasing the water uptake. Drought and salinity stress have adverse effects on germination while the effects of drought stress were more severe than salinity stress. Accordance to our results Janmohammadi et al. (2008) reported that both salinity and drought stress affected germination adversely while the effects of drought stress were more severe than salinity stress. Also they reported that compared to the control osmo and hydropriming showed enhanced performance under stress conditions. Hydropriming treatment may therefore be used to improved seed performance of *Aeluropus Macrostachys*. This treatment, using water alone, is a simple, cheap and environmentally friendly technique that does not need expensive chemical and sophisticated equipment. According to the results of this experiment, it seems that *Aeluropus Macrostachys* genotypes has a relatively tolerance against salinity, also are very sensitive in relation with drought stress. Hydropriming and osmopriming caused recovery on germination under non-stress conditions. It has to be mentioned that also the seed treatment does not make any improvement in germination percentage and rate in salinity and drought condition. But through increase and decrease in radical and plumule length will be maintained by the capability of more able in absorbing water and protecting the photosynthesis bodies (as an effective factor in yield) leads to the yield increase and probably increase in the resistance of drought in the *Aeluropus Macrostachys* plant.

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