

Optimization of Hydro- and Osmo-priming in Different Seed Size of Sainfoin (*Onobrychis viciifolia* Scop)

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Abstract: Sainfoin is a legume crop that use for dry forage and grazing in irrigation and dryland conditions. Morphological structure and inhibitor substrates of pod and variety of seed (pod) size are effective on sainfoin germination. Farmers use high seed (pod) rate in sowing time in some regions of Iran. In order to determine hydro and osmopriming effects on seed germination of sainfoin in two seed (pod) size categories, priming agents (PEG 6000, KNO₃ and CaCl₂) were examined in different osmotic potentials -0.45 to 0 Mpa ranges for 12 to 24 hours. Results showed that the most germination of treated pods was obtained in the hydroprimed seeds (0 Mpa = hydropriming), but germination trend in the osmoprimed treatments reduced in -0.15 Mpa toward -0.45 Mpa. Otherwise, the least germination among different osmopriming agents obtained in -0.45 Mpa. Maximum and minimum germination in priming agents belonged to PEG6000 and KNO₃ respectively. Germination in primed pods at 24 h were more than other priming periods. Seed germination in the large seed was higher in compared with small size, but performance of priming were greater in small seed. Increasing of vigor and reducing of pod effects caused germination improvement for some of primed treatments particularly in hydroprimed for 24 h. Therefore hydropriming for 24 h can be employ to improve germination sainfoin in compare to other treatments.

Key words: Sainfoin, Hydropriming, Osmopriming, Germination.

INTRODUCTION

Sainfoin (*Onobrychis viciifolia* Scop.) is a leguminous forage crop grown in Europe, western North America and Asia (Frame, 2005). In Iran sainfoin generally is planted in irrigated condition and is a favorable forage crop for grazing or harvested forage particularly in early of spring. It commonly is grown based on sole crop, but the farmers use high rate of seed (pod) for confidence of obtaining crop density in sowing time. Non-uniformity in the pods maturity stage and earlier harvest for prevent of shattering pods, existence of pod and different pod size may be main reasons of this application.

Each sainfoin pod consists on a single seed, that formed indehiscent fruit (Peel *et al.*, 2004). In fact in sainfoin the fruit (pod) represents the seed in agricultural terms. The pod is a mechanical restriction for radical protrusion, also in the germination stage from the pod, the radicle can be injured and infected by fungi pathogens (Ditterline and Cooper, 1975). Additionally, the pods slow water imbibition and contain water-soluble inhibitors that slow germination (Carleton *et al.*, 1968). Removal of pod is possible but injury to seed is available irrespective of cost.

Seed size is important in seedling establishment and vigor of forage legumes (Singh *et al.*, 2009). Seedling vigor has been correlated to seed size in sainfoin and some legume crops (Carleton and Cooper, 1972, Franssen and Cooper 1976; Beveridge and Wilsie, 1959; Singh *et al.*, 2009). The size of a legume seed may be the result of either genetic or environmental factors or combination of both (Carleton and Cooper, 1972; Cash and Ditterline, 1996). The seed size indicated the amount of reserve food supply for seedlings. Small and shriveled seeds do not contain as much food to give the plant a vigorous start as the bold and non-shriveled seeds (Singh *et al.*, 2009). Seed variety and seed size greatly influence seedling establishment and vigor of legumes. However, breeding to increase seed size has had variable effects (Lafond and Baker, 1986; Li *et al.*, 2005; Mian and Nafziger, 1992; Singh *et al.*, 2009; Townsend, 1992). This reveals that increasing seed size is not enough to

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enhance the vigor of the seedlings, since there would be other factors involved in vigor expression (Nelson *et al.*, 1994). Alternative strategies are therefore needed to improve seed quality in sainfoin.

Various prehydration or priming treatments have been employed to increase the rate and synchrony of seed germination (Bradford, 1986). Two of common priming techniques include osmopriming and hydropriming or soaking seed in water (Ashraf and Foolad, 2006). The technique of osmopriming also known osmoconditioning involves the imbibitions of seed in salt or polyethyleneglycol (PEG) osmoticum (Abernethy, 1987). Soaking seed in varying concentrations of inorganic salts is defined also as halopriming (Ashraf and Foolad, 2006). Inorganic salts such as MgSO₄, NaCl, KMO₃ and others have been used for this method (Bittencourt *et al.*, 2004). Generally priming would cause an effective invigoration of the dry seed which is the inception of metabolic processes that normally occur during imbibitions and which are subsequently fixed by drying the seed (Hanson, 1973).

The advantageous effects of seed priming have been attributed pre-enlargement of the embryo (Austin *et al.*, 1969), biochemical changes and enzyme activation (Amooaghaie *et al.*, 2010, Lee and Kim, 1999, 2000; Basra *et al.*, 2005), more availability to ATP (Mazor *et al.*, 1984), repair of deteriorated seed parts (Shah *et al.*, 1990), reduced leakage of metabolites (Styer and Contcliffe, 1983) and improvement of germination rate particularly in old seeds (Lee and Kim, 2000). Hardening (repeated hydration-dehydration) treatment of fine rice for 24 h proved to be the best for vigor enhancement (Basra *et al.*, 2003; 2005).

Artola *et al.*, 2003 reported that hydropriming resulted to be a valid physiological treatment that significantly improves the vigor of birdsfoot trefoil (*Lotus corniculatus* L) seeds, since it allowed increase germination rate and uniformity, as well as soil emergence of seedling. The potential of using seed hydropriming technology to achieve rapid germination and enhanced establishment was demonstrated for three out of four annual cover crop species tested (Snapp *et al.* 2009). Abernethy (1987) reported that some germination enhancement of deteriorated cicer milkvetch (*Astragalus cicer* L) seed may be possible with osmoconditioning, it might be useful for maintaining germplasm lines which had deteriorated in storage and were in limited supply. Seed osmopriming especially with salt solutions for three herbage grasses, significantly increased germination percentage of *Festuca arundinacea* and *Dactylis glomerata* at low temperature, where the germination of untreated seeds was greatly reduced or inhibited (Mauromicale and Cavallaro, 1996). Priming treatments with NaCl, KNO₃ and PEG significantly advanced germination rate and percentage of white clover at 15 °C, but produced minimal or negative effects on alfalfa (Tiryaki *et al.* 2009). Moradi and younesi, (2009) demonstrated that both osmo and hydropriming improve the quality of sorghum seeds.

However for our knowledge until now attempts to improve seed germination performance of sainfoin using seed osmopriming and hydropriming and compare these in respect to seed size haven't been done. Therefore, the objective our study were to evaluate the impact of seed hydropriming and osmopriming with organic and inorganic solutions at different periods in two seed (pod) size categories for germination performance of sainfoin.

MATERIALS AND METHODS

Seed Preparation:

The experiment was performed on two seed (pod) ecotypes of sainfoin, ecotype B (Boldaji) and ecotype G (Ghazvin) collected from two regions of Iran (these ecotypes selected of earlier experiment). The pods were divided into two groups: large (pods retained on >4.5 mm screen) and small (pods retained on < 4.5 mm screen). Mean 1000 un-hulled (pod) and hulled seeds weight taken for ecotype B were 23.4, 17.7 and 19.6, 14.9 g for large and small seeds and for ecotype G were 22.2, 16.0 and 17.8, 13.5 g for large and small seeds, respectively.

Experiment Treatments:

Treatments formed from 3 priming agents (PEG 6000, KNO₃ and CaCl₂), 4 solution osmotic potentials (0, -0.15, -0.3, -0.45 Mpa), 3 priming period (12, 18, 24 hours) 2 ecotypes (Eco B and Eco G) and 2 pod size (>4.5 mm and < 4.5 mm) components . The osmotic potentials of priming agents were determined according to Michel and Kaufmann (1973) for PEG6000 and Braga *et al.* (2009) for KNO₃ and CaCl₂.

Hydropriming Method:

Pods with similar size rinsed with tap water for 5 minutes and then 6 h earlier each priming period allocated to soaking in distilled water for reduce germination inhibitors of pods. The pods immediate soaked in different osmotic potentials of priming agents in aerated condition at 25±1°C. In the end of each period, pods rinsed with distilled water for 3 minutes, followed by air-drying under shade. The pods were dried in 3 d at ambient room conditions (25 ± 1°C) in order to bring the pods to their approximate original moisture content.

Germination Tests:

Different primed pod categories were tested for germination potential. All the pods were treated with Rovral TS 1 g kg⁻¹ to retard saprophytic fungi. Four replications of 30 pods for each pod size category were

placed in covered Petri dishes containing one layer of Whatman 1 filter papers moistened with 4 ml distilled water. Plates were placed in a germination chamber at 25°C with 12 h fluorescent light and 12 h dark. Pods were identified as germinated when the radicle protruded. The Petri dishes were arranged in a completely randomized design (CRD). Counts of germinating seeds were made daily, starting on the first day of imbibition and terminated when maximum germination was obtained. Normal seedlings were recorded for calculating germination percentage (GP) at last count.

Statistical Analysis:

For statistical analysis, a factorial experiment ($3 \times 4 \times 3 \times 2 \times 2 = 144$) with completely randomized design (CRD) with four replications was used. Variance analysis was based on the ANOVA procedure by software SAS. Differences among treatments means were estimated using the Duncan's multiple range test at the 5% probability level. In this test usage of non primed pods were impossible but were evaluated in a separate test, results of these GP presented as a column in each chart.

RESULTS AND DISCUSSIONS

Statistical analysis showed that the effect of priming agent, priming time, osmotic potential, ecotype and seed size and many interaction effects for GP were significant ($p < 0.01$). In the among priming agents, maximum and minimum GP was achieved in primed seeds with PEG and KNO₃ respectively (Fig. 1). Optimal potential of priming solutions for sainfoin seed GP were obtained with 0 Mpa (distilled water = hydropriming) and -0.15 Mpa (Fig2). In generally GP trend reduced with increasing osmotic potential PEG, CaCl₂ and KNO₃ (Fig. 3). Results of preliminary priming tests with -0.6, -0.9 and -1.2 Mpa solutions of these agents showed that GP were less than this test and decreased trend with increasing osmotic potential, particularly in KNO₃ (data not present). Farooq *et al.*, (2006) used hardening (with water) and osmohardening with CaCl₂, KNO₃, KCl, and NaCl in similar potential (1.25 Mpa) for rice seed. In both coarse and fine rice, CaCl₂ osmohardening performed in compare with others osmohardening in respect to GP. Basra *et al.*, (2006) reported that delayed and weak germination in seeds rice subjected to osmoconditioning for 24 and 48 h in KNO₃ was probably due to toxicity. KNO₃ toxicity results in injury to cellular organelles and membranes of wheat (Singh and Gill 1988).

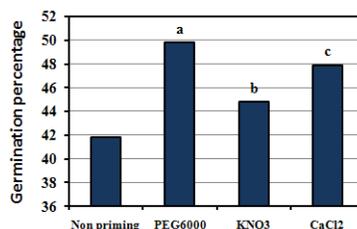


Fig. 1: Germination percentage of sainfoin primed seeds as affected by priming agent treatments irrespective of osmotic potential, priming period, seed ecotype and size. Values in columns marked with the different letter are significantly difference at $p < 0.05$.

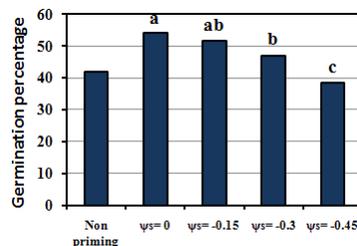


Fig. 2: Germination percentage of sainfoin primed seeds as affected by osmotic potential treatments (ψ_s Mpa) irrespective of priming agents, priming period, seed ecotype and size. Values in columns marked with the same letter are insignificantly different at $p < 0.05$

Ismaiel *et al* (2005), showed that an increase in the GP was obtained in primed tomato seed treatments with PEG solutions in compare with KNO₃ and control. Performance of PEG in osmopriming tomato seed agrees with other literature. McDonald (2000), stated that PEG would be the preferred osmoticum because it is inert

and large molecular size precludes it from being taken up by the embryo. The positive effect of inorganic salt osmotic in seeds such as tomato is related to the presence of selectively permeable tissue layer surrounding the embryo, which allows the uptake of water but prevents the diffusion of solutes into the seeds. Seeds without this tissue layer (e.g. sainfoin) probably absorb the salts and become damaged. However other research data are available where salts are in some cases superior to seed priming in compare with PEG for some plants including tomato (McDonald ,2000), muskmelon (Nerson and Govers, 1986) and pepper (Rivas *et al.*, 1984). Various responses of each species seed to priming agents is associated with water uptake, metabolic activities and structures of seeds.

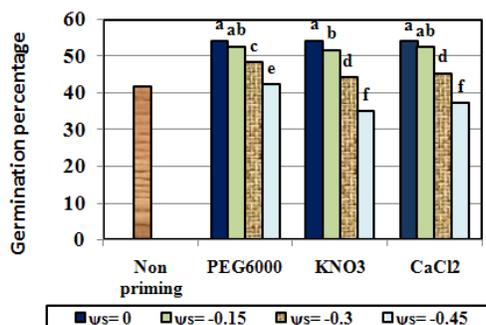


Fig. 3: Germination percentage of sainfoin primed seeds as affected by osmotic potential with priming agent treatments (ψ_s Mpa) irrespective of priming period, seed ecotype and size. Values in columns marked with the same letter are insignificantly different at $p < 0.05$.

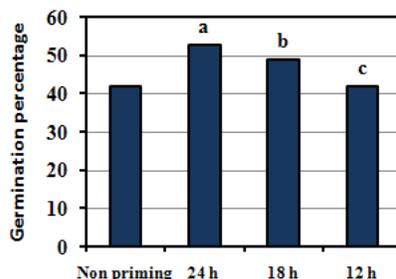


Fig. 4: Germination percentage of sainfoin primed seeds as affected by priming period (hours) treatments irrespective of osmotic potential, priming agent, seed ecotype and size. Values in columns marked with the different letter are significantly difference at $p < 0.05$

Seed structure of sainfoin consists to pod and seed. The single seed was born in a seed pod (Peel *et al.*, 2004). The pods use at planting time. Hydropriming (0 Mpa) may be enhanced water uptake and exit inhibitors as well as reduced pod mechanical restriction in soaking period in compare to others osmotic treatments (Fig. 3). Although the pods of all osmotic treatments at first imbibed in distilled water for 6 h but exit of inhibitors continued with low concentration in osmotic solutions in priming periods. Excitant of the inhibitors in concomitant with salts or osmotic components in priming period may be induced negative effects on the metabolic activities of seed germination. Smith (1976) stated that water-soluble inhibitors in the pods are nitrogen components, therefore hydropriming increase leaching inhibitors in priming period. Chen *et al* (2010) stated that improved germination of primed spinach seeds at supraoptimal temperature may be associated with leaching germination inhibitors of pericarp during priming. In addition to hydropriming may be improved water uptake and completed swelling of sainfoin seeds. In this state seed created a force toward pod walls and caused pod dehiscence in ventral or dorsal suture. Dehiscence of the pod is stable after drying and improved germination. We observed, seed imbibition for 24 h caused dehiscence of the pods about 20 percents, the most dehiscence obtained in small pods. Leopold (1983), reported that soybean seed volume increases in CaCl₂ and KNO₃ solutions were less than distilled water, however swelling of soybean seeds in KNO₃ was more than CaCl₂. This case may be a reason for more KNO₃ uptake and probably its toxicity in sainfoin seed in compare with CaCl₂.

The most germination in priming periods obtained of 24 h regardless of others factors (Fig. 4). In this period, germination in KNO₃ and CaCl₂ were less than PEG (Fig. 5). Soaking seed in high osmotic salts solutions for long hours may be increased uptake of the salts and caused negative effect on metabolic activities

of the seed germination. In the among interaction effects of priming period with osmotic potentials, the maximum and minimum germination obtained of 24 h with 0 Mpa and 24 h with -0.45 Mpa, respectively (Fig. 6). Adequate water absorption and performance of metabolic and biochemical process in phases I and II germination may be reasons of germination improvement in 24 h. Chen, (2010) stated an improper of seed priming may cause in negative effects on germination. Capron *et al.*, (2000, cited in chen *et al.*, 2010) suggested that improper control of seed partial hydration may result degradation of protective proteins (such as LEA), and render the primed seeds desiccation-intolerant. In preliminary tests we observed a slightly radicle emergence in the pods that soaked more than 24 h in distilled water (0 Mpa) in 25°C. Germination consists three distinctive stages (I, II and III), initial water uptake and metabolic events occur in phases I, II and radicle protrusion obtains in phase III (Ghana *et al.*, 2003), therefore the best hydropriming period for sainfoin pods was 24 h when that radicle protrusion didn't observed and probably completed phases I and II germination.

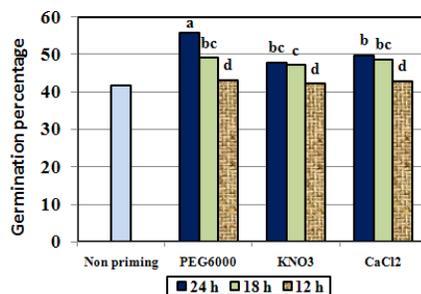


Fig. 5: Germination percentage of sainfoin primed seeds as affected by priming agent with priming period treatments irrespective of osmotic potential, seed ecotype and size. Values in columns marked with the same letter are insignificantly different at $p < 0.05$.

GP in Eco B was more than Eco G (Fig. 7). The difference between Eco B and Eco G was possibly due to environmental differences during seed development, however could also have been due to genetic shift. Additionally seed weight in ECO B was higher than ECO G. These data indicate that inherent differences in seed weight between the two seed ecotypes affect utilization ability of substrate reserves and germination. In present study germination in the large pods was greater in compare to the small pods (Fig. 8). Seedling vigor has been correlated to seed size in sainfoin and some legumes seed (Carleton and Cooper, 1972; Fransen and Cooper 1976; Thowesend, 1992; Singh *et al.*, 2009). Large seed in sainfoin was associated with increased embryo axis length, cotyledon area (seed reserve) and rapid seedling growth (Fransen and Cooper 1976). The size of a legume seed may be the result of either genetic or environmental factors or combination of both (Carleton and Cooper, 1972; Cash and Ditterline, 1996). Content of small pods were about 20 percents in two ecotypes. In generally small pods form on racemes with late flowering or on the end of earlier racemes and therefore physiological maturation may be uncompleted for some these seeds at harvesting time. Germination of the immature seeds is less than mature seeds.

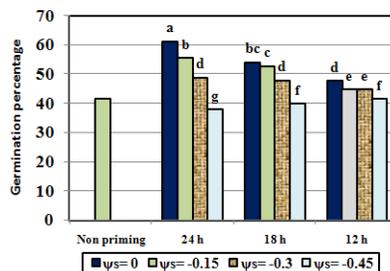


Fig. 6: Germination percentage of sainfoin primed seeds as affected by priming period with osmotic potential (ψ_s Mpa) treatments irrespective of priming agent, seed ecotype and size. Values in columns marked with the same letter are insignificantly different at $p < 0.05$.

Interaction effect of priming period with seed size was significant ($p < 0.01$). Small pods revealed more responses to priming period (Fig. 9). Germination difference between large and small pods were 7.6 % in 12 h, but 3 % in 24 h. A proper hydration and completed metabolic activities in phases I and II of germination may be alleviate negative effect of the small seed in 24 h in compare to large seed. In interaction effect of osmotic potential with seed size, minimum germination obtained of -0.45 Mpa in small pods (Fig. 10). Germination

difference between large and small pods were 11.1 % in -0.45 Mpa, but 2.9 % in 0 Mpa or hydropriming. In the small seeds may be water uptake more quickly, also may absorb more salt in high osmotic potential solutions and therefore affect on metabolic process of germination in compare to large seeds. Kaya *et al* (2008) reported that in pea small seeds water absorption was faster than large seed.

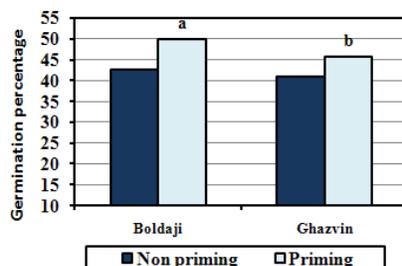


Fig. 7: Germination percentage of sainfoin primed seeds as affected by seed ecotype treatments irrespective of priming agent, osmotic potential, priming period, and size. Values in columns marked with the different letter are significantly difference at $p < 0.05$.

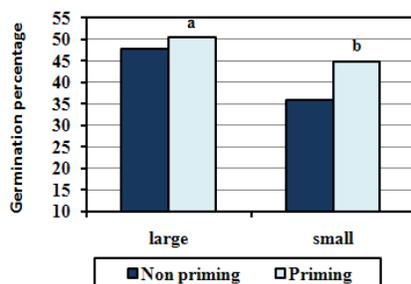


Fig. 8: Germination percentage of sainfoin primed seeds as affected by seed size treatments irrespective of priming agent, osmotic potential, priming period, and seed ecotype. Values in columns marked with the different letter are significantly difference at $p < 0.05$

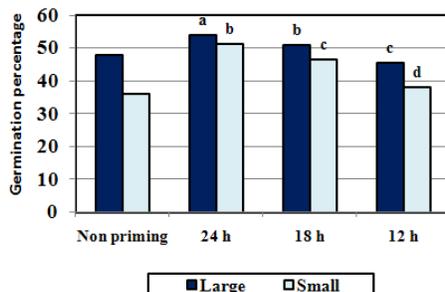


Fig. 9: Germination percentage of sainfoin primed seeds as affected by priming period with seed size treatments irrespective of priming agent, osmotic potential and seed ecotype. Values in columns marked with the different letter are significantly difference at $p < 0.05$.

Germination percentage trend in all priming treatments revealed that GP improved with reducing osmotic potential (toward 0 Mpa) and up to 24 h. The most GP obtained of 0 Mpa (hydropriming) in 24 h for large seeds of Eco B and Eco G with 64.2 and 63.2, respectively, that had no significant differences with -0.15 Mpa of PEG in 24 h for large seeds of these ecotypes. The maximum GP for CaCl₂ and KNO₃ treatments were less than mentioned treatments. Hydropriming had the most performance in among treatments in compared to non priming seeds. Similar results were stated for seed hydroprimed of some legumes seeds by Artola *et al* (2003) and Snapp *et al* (2008).

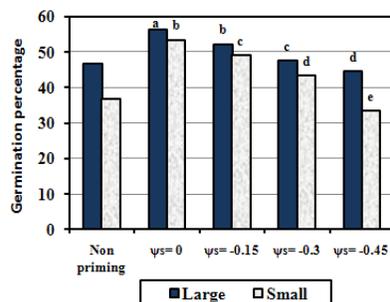


Fig. 10: Germination percentage of sainfoin primed seeds as affected by osmotic potential (ψ_s Mpa) with seed size treatments irrespective of priming agent, priming period and seed ecotype. Values in columns marked with the different letter are significantly difference at $p < 0.05$.

Conclusion:

Based on results hydropriming was more effective than osmopriming of PEG6000, KNO₃ and CaCl₂ solutions. It may be enhanced water uptake, exit inhibitors from pods, reduced mechanical restriction of the large and small pods and therefore elevated germination and vigor indexes. Biochemical parameters in relation to germination in hydroprimed seed were more than osmoprimed and non primed seeds (data not present). The best priming period obtained from 24 h. In this period, phases I and II of germination may be complete but not allow radicle protrusion. Germination of the large seed was more than the small seed. These data confirm the hypothesis that large seeds of forage legume have superior performance to small seeds. Results also suggest that use seed screen for separate large seeds size in the seed lot. Benefit of priming for the small seeds was greater than large seeds, probably, priming reduced the negative effects of environmental conditions during seed development in small seeds but could not completely alleviate inheritance differences between seeds size.

Results of this study suggest that hydropriming in 24 h is effective method for germination improvement of large and small seed (pod) of sainfoin in compare to other priming agents.

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