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Does Hydro and Osmo-Priming Improve Fennel (*Foeniculum vulgare*) Seeds Germination and Seedlings Growth?

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Abstract

This experiment was conducted to investigate the effects of hydropriming and osmopriming on germination rate, percentage of, root–shoot length and root–shoot weight of fennel (*Foeniculum vulgare*) seeds. Priming was done by: hydropriming with distilled water, . osmopriming with NaCl at four levels (-0.3, -0.6, -0.9, -1.2 MPa), osmopriming with K_2SO_4 in four levels (-0.3, -0.6, -0.9, -1.2 MPa), priming with PEG6000 in four levels (-0.3, -0.6, -0.9, -1.2MPa) and 5. seeds with unprime control at treat. In this study, RCBD experimental design was used for the analysis of experimental factors. The results showed that priming significantly effected at all treatment methods. Maximum and minimum germination percentage were obtained with PEG (-0.9 MPa) applied, and in control. Maximum and minimum germination rates were obtained when K_2SO_4 (-0.3 MPa), NaCl (-0.3 MPa) were used. Maximum and minimum root length were obtained when NaCl [(-1.2 MPa), PEG (-0.3 MPa)], NaCl (-0.6 MPa) were used. Maximum and minimum shoot length were obtained when PEG (-0.3 MPa), NaCl (-0.6 MPa) were used. Maximum and minimum root weight, root/shoot length were obtained when NaCl (-1.2 MPa), NaCl (-0.6 MPa) were used. Maximum and minimum shoot weight were obtained when NaCl (-1.2 MPa), NaCl (-0.3 MPa) were used. Maximum and minimum root/shoot weight were obtained when [PEG (-0.3 MPa), K_2SO_4 (-0.3, -0.6, -0.9 MPa), NaCl (-0.6, -0.9 MPa), hydropriming] and [PEG (-0.6, -1.2 MPa), NaCl (-1.2 MPa)] were used.

Keywords: fennel, *Foeniculum vulgare*, NaCl, K2SO4, PEG, hydropriming, osmopriming, germination

Introduction

Fennel (*Foeniculum vulgare*) is an aromatic biennial plant with soft, feathery, almost hair-like foliage. Native to coastal areas in the Mediterranean region, and widely naturalized in Europe and North America (Christman., 2004). Similar to some other medicinal plants (Bannayan *et al.*, 2008; Khazaie *et al.*, 2008) supplemental irrigation would improve its production during dry periods. Fennel belongs to the Umbelliferae (Apiaceae) family, a medicinal plant used as anti-spasmodic, appetite stimulant, stomachic, diuretic, anti-inflammatory, anti-diarrheic, against colic and as a lactation promoter (Marotti *et al.*, 1993; Piccaglia and Marotti, 1993; Cavaleiro *et al.*, 1993). Several components of the essential oil of this plant show important applications, including, fenchone as counterirritant; limonene as solvent, resins, wetting and dispersing agent; trans-anethole, flavoring agent in perfumery, cosmetics, soap; metylchavicol or estragole is used in perfumeries and as flavor in foods and liquors; a-pinene α, used in manufacture of camphor, insecticides, solvents, perfume bases (Marotti *et al.*, 1993; Piccaglia and Marotti, 1993; Cavaleiro *et al.*, 1993). Seed germination is mostly an issue in medicinal plant seeds emergence (Nadjafi *et al.*, 2006). Good seedling establishment is an important constraint to such crop production (Harris *et al.*, 1999). Poor seedbed, low quality seed, environmental stresses such as high and low temperature and salinity constrains to good establishment include (Weaich *et al.*, 1992; Towned *et al.*, 1996). A robust seedling establishment enhances competitiveness against weeds, improves tolerance to environmental stresses and maximizes biological and grain yields (Ghiyasi *et al.*, 2008).

Several approaches including, hardening, seed priming, seed soaking and seed coating have been employed to precondition seeds to improve germination and seedling growth of various crops (Basra *et al.*, 2003). Seed priming treatments such as osmopriming, hydropriming, matricopriming, hormonal-priming have been employed to accelerate the germination, seedling growth and yield in most of the crops under normal and stress conditions (Basra *et al.*, 2003). Osmopriming is most common type of seed priming in which seeds are soaked in aerated low water potential solution (Farooq *et al.*, 2005). Although, the mechanism of seed priming treatments is not fully understood, it has been observed that physiological and biochemical changes take place during the seed treatments (Basra *et al.*, 2005; Ghiyasi *et al.*, 2008), which could allow seeds to begin the germination sequences before sowing.

Rapid germination and emergence is an important determinant of successful establishment (Harris *et al.*, 1991).

Germination is one of the most salt-sensitive plant growth stages and severely inhibited with increasing salinity both in glycophytes and halophytes (Sosa *et al.*, 2005). Seed priming accelerates seed germination and seedling establishment under both normal and stressful environments (Ashraf and Foolad., 2005). Although priming is one of the physiological methods, which improves seed performance and provides faster and synchronised germination (Sivritepe and Dourado., 1995) it has been shown that NaCl priming could be used as an adaptation method to improve salt tolerance of seeds (Wiebe and Muhyaddin., 1987; Cano *et al.*, 1991; Cayuela *et al.*, 1996). Successful results have been obtained for tomatoes, (Pill *et al.*, 1991) and asparagus (Passam and Kakouriotis., 1994) under saline conditions.

Hydropriming is the simplest approach to hydrate seeds and minimize the use of chemicals. However, if the seeds are not accurately hydrated, hydration rate cannot be exactly controlled. It was observed that hydropriming practically ensured rapid and uniform germination accompanied with low abnormal seedling percentage (Singh, 1995; Shivankar *et al.*, 2003). They uderline that hydropriming has high potential in improving field emergence and ensures early flowering and harvest under stress conditions especially in dry areas. Hydrated seeds with higher germination percentage under salt stress or micronutrient application increased tolerance of seeds to salt stress. In addition, reported protocol is simple, cheap and does not require expensive chemicals and sophisticated equipment. The protocol has practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions.

The objective of this study was to explore the effects of two different priming (hydropriming and osmopriming) treatments on germination of fennel seeds (*Foeniculum vulgare*).

Materials and methods

In order to determine the impact of different primes on germination of Fennel seeds, an experiment was conducted at Zabol University in 2007. Seeds were primed with various materials, including hydroprime as seeds were primed with distilled water, osmoprime seeds were treated with three different chemical as NaCl, K_2SO_4, polyethylene glycol 6000. All three different osmopriming provided osmotic potential of -0.3, -0.6, -0.9 and -1.2 MPa. Simulating various osmotic potential using PEG was according to Michel and Kaufmann (1973) and Money (1989).

Initially seeds were disinfected by sodium hypochlorite (NaOCl). Seeds were kept in sodium hypochlorite (1.5%) for one minute and then were washed with distilled water. After disinfecting, seeds were put in disinfected Petry dish. Each Petry dish contained 25 seeds. After 24 hours of priming seeds were washed with distilled water and then dried and kept in laboratory room at temperature of 25° C for two hours. Afterwards dried seeds were located in Petry dishes and treated with distilled water at temperature of 25°C for seven days. Statistical experimental design was randomized completely block, with three replications. The differences between the means were compared using Duncan test ($P < 0.01$).

Germination tests

Three replicates of 25 seeds were put between double layered rolled. The papers were replaced every 2 days to prevent accumulation of salts. The rolled paper with seeds was put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 25 ± 1°C for 7 days. Germination was considered when the radicles were 2 mm long. Germination percentage was recorded every 24 h for 7 days. Root length and shoot length were measured after the 7th day. The germination rate was calculated as follows (according to Bannayan *et al.*, 2006):

$$\text{Germination rate} = \sum_{n=1}^{45}$$

number germinating since n-1 / n where, n is the days of incubation.

Results and discussion

For NaCl primed seeds the germination rate increased at osmotic pressure of -0.6 and -0.9 MPa, but germination rate decreased with increasing the osmotic pressure to -1.2 MPa (Tab. 1). The germination percentage of NaCl primed seeds decreased by increasing the osmotic pressure to -0.6 MPa, but germination percentage increased by increasing the osmotic pressure to -0.9 and -1.2 MPa. However, the best results of of germination percentage in NaCl primed were obtained with -0.3 MPa pressure (Tab. 1). The root length of NaCl primed seeds decreased with increasing the osmotic pressure to -0.6 MPa, but root length increased

Tab. 1. Comparison of germination rate and germination percentage of fennel seeds treated with NaCl, K_2SO_4 and PEG

Osmotic level (Mpa)	Control		Hydropriming		NaCl		K_2SO4		PEG	
	G.Rate	G.Per	G.Rate	G.Per	G.Rate	G.Per	G.Rate	G.Per	G.Rate	G.Per
0	9.6 cd	4.3 g	9.3 cd	17.6bc						
-0.3					0.6 f	18.0b	12.0 a	17.6bc	9.7 cd	17.3b
-0.6					10.2cd	15.0f	9.1cde	17.6bc	11.9ab	16.6bcd
-0.9					9.5cd	16.0def	10.3bcd	17.3bcd	10.8abc	20.0a
-1.2					7.5e	17.3bcd	8.8de	15.6ef	9.3cd	16.3cdef

Tab. 2. Comparison of root length and shoot length of fennel seedling treated with NaCl, K_2SO_4 and PEG.

Osmotic level (Mpa)	Control		Hydropriming		NaCl		K_2SO4		PEG	
	R.length	S.length	R.length	S.length	R.length	S.length	R.length	S.length	R.length	S.length
0	5.7bc	2.9cd	6.3bc	3.4abcd						
-0.3					6.1bc	3.2abcd	6.2bc	3.9ab	8.2a	4.1a
-0.6					0.5d	0.3e	6.2bc	3.2abcd	5.0c	3.0bcd
-0.9					5.8bc	2.7d	5.2bc	2.7d	5.5bc	3.1bcd
-1.2					9.2a	3.9ab	6.5b	3.5abcd	6.2bc	3.6abc

at the osmotic pressure of -0.3, -0.9 and -1.2 MPa. Maximum root length was obtained at NaCl level providing -1.2 MPa osmotic pressure. This might be due to the fact that seedlings send their photosynthesis products to roots when they were exposed to salt stress in order to tolerate the imposed stress, and minimum root length was obtained at -0.6 MPa (Tab. 2). The shoot length at NaCl primed seeds decreased with increasing the osmotic pressure to -0.6 and -0.9 MPa, but shoot length increased as the osmotic pressure increased to -1.2 MPa (Tab. 2). Root weight and shoot weight of NaCl primed seeds increased with increasing the osmotic pressure, and maximum root weight and shoot weight was obtained at -1.2 MPa pressure (Tab. 3). Maximum and minimum root/shoot (R/S) length in NaCl primed seeds were obtained at -1.2 MPa and -0.6 MPa pressure, respectively. Maximum and minimum R/S weight in NaCl primed seeds were obtained at -0.9 MPa and -0.3 MPa pressure, respectively (Fig.1, 2).

The maximum germination rate of K_2SO_4 primed seeds was obtained at -0.3 MPa pressure and beyond that pressure the germination rate decreased (Tab. 1). The germinated percentage of K_2SO_4 primed seeds decreased with increasing the osmotic pressure (Tab. 1). The root length of K_2SO_4 primed seeds increased with increasing the osmotic pressure (Tab. 2). Both shoot length and root weight showed significant effects of priming with K_2SO_4 at all potentials, except -0.9 MPa (Tab. 2, 3). Shoot weight of K_2SO_4 primed seeds decreased with increasing the osmotic pressure (Tab. 3). Maximum R/S length and R/S weight of K_2SO_4 primed seeds were obtained at -0.9 Mpa pressure. Minimum R/S length and R/S weight in K_2SO_4 seeds primed were obtained at -0.3 MPa and -1.2 MPa pressures, respectively (Fig.1, 2).

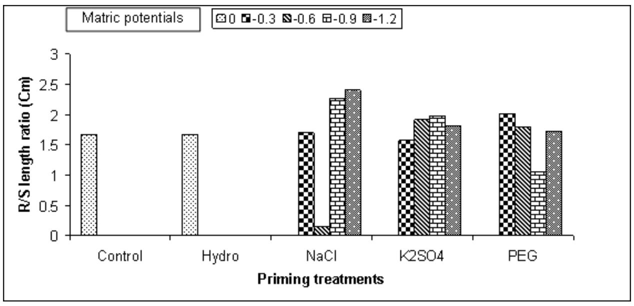

Fig. 1. Root/Shoot length (cm) of fennel seedling primed by hydropriming, NaCl, K_2SO_4, PEG and control

Using PEG priming, the germination rate increased at -0.6 and -0.9 MPa pressures (Tab. 1). The germination percentage of PEG primed seeds almost decreased by increasing solution concentration, except at -0.9 Mpa pressure. Maximum germination percentage of PEG primed seeds was obtained at -0.9 MPa pressure and all seeds in Petry dishes were germinated (Tab. 1). The root length and shoot length of PEG primed seeds decreased with increasing osmotic pressure, and maximum root and shoot length were obtained at -0.3 MPa (Tab. 2). The root weight in PEG primed seeds generally decreased, but in PEG -0.6 MPa increased (Tab. 3). The shoot weight of PEG primed seeds decreased, except -0.9 MPa pressure, when increased again (Tab. 3). Maximum R/S weight and R/S length were obtained at -0.3 Mpapressure, and minimum R/S weight and R/S length were obtained at -0.9 MPa pressure (Fig. 1, 2). Several authors described positive effects of seed priming with water alone (Harris *et al.*, 1999, 2002, 2004; Rashid *et al.*, 2002). Seedlings from seeds primed with water alone are known to emerge more quickly and grow more vigorously than those from non-primed seeds (Rashid *et al.*, 2002; Arif *et al.*, 2005; Miraj, 2005). Our data confirmed that simple seed priming with water is an effective way to increase all characteristics compared to control treatment (Tab. 1, 2, 3 and Fig 1, 2).

It is evident from the results that NaCl salinity caused growth inhibition in fennel seeds due to a decrease in total germination. These effects of salinity on fennel seeds supported by the previous findings (Franco *et al.*, 1993, 1997; Botia *et al.*, 1998; Carvajal *et al.*, 1998) studying on melon cultivars. Accumulation of Na ion changes ion balances such as Na:Ca and K:Na in plant cells under saline conditions. While the change in Na:Ca balance results in

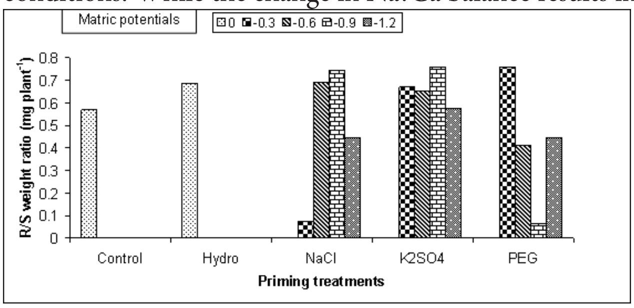

Fig. 2. Root/Shoot weight (mg plant-1) of fennel seedling primed by hydroprime, NaCl, K_2SO_4, PEG and control

Tab. 3. Root weight and shoot weight of fennel seedling treated with NaCl, K_2SO_4 and PEG

Osmotic level (Mpa)	Control		Hydropriming		NaCl		K_2SO4		PEG	
	R.weight	S.weight	R.weight	S.weight	R.weight	S.weight	R.weight	S.weight	R.weight	S.weight
0	0.6c	1.2abcd	0.8bc	1.0bcd						
-0.3					0.7bc	0.1e	0.7bc	1.1abcd	0.7bc	1.3abc
-0.6					0.1d	1.0cd	0.7bc	1.0cd	0.9ab	0.9d
-0.9					0.7bc	1.5ab	0.6c	1.0cd	0.5c	1.1abcd
-1.2					1.1a	1.5a	0.7bc	1.0bcd	0.7c	1.1bcd

increased cell permeability, the change in K:Na balance cause decreasing use of metabolic energy (Levitt, 1980).

Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells (McDonald, 1999). This process is important because allows the subsequent development of the embryo, especially in seeds characterised by a morphological dormancy (immature embryo), like *Chamaecyparis nootkatensis* seeds (Schimtz *et al.*, 2001). In tomato, priming improved the germination capacity by increasing endosperm volume (Dahal *et al.*, 1990).

The technique of seed priming is becoming familiar to farmers in several parts of the world, and has now been promoted there on a range of crops, for example wheat (Harris *et al.*, 2001), maize (Harris *et al.*, 2002), and mung bean (Rashid *et al.*, 2004), where similar responses to those reported here have been found. Equally encouraging results have been found for these crops in other countries, and for other crops, such as chickpea in India and Bangladesh (Harris *et al.*, 1999; Musa *et al.*, 2001), upland rice in India (Harris *et al.*, 1999, 2002), and finger millet in India (Kumar *et al.*, 2002).

In many coated seeds, germination and subsequent seedling growth can be inhibited by mechanical restriction exerted by the seed coat (Sung and Chiu., 1995). Priming may be helpful in reducing the risk of poor stand establishment under drought and salt stress and permit more uniform growth under conditions of irregular rainfall and drought on saline soils.

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