Int. J. Biol. Res., 6(1): 43-48, 2018.

OSMOPRIMING EFFECTS ON GERMINATION, GROWTH AND ROOT ROT DISEASES

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ABSTRACT

Priming with sugar, water and salt is an interesting alternative method for improving plant quality. An experiment was designed in which various concentrations (0, 0.5, 1, 2%) of mannitol ($C_6H_{14}O_6$) and Sodium chloride (NaCl) were prepared and seeds of okra and lentil were primed for 5, 10 and 15 minutes. These primed seeds were sown and plants were grown for thirty days on screen house bench. Results indicated that seed priming with 1% mannitol and NaCl for 10 minutes increased shoot length, shoot weight, root length and root weight of okra and lentil while 2% mannitol for 10 and 15 minutes was helpful in reduction of root infecting fungi of lentil and okra plants. Hydropriming for 15 minutes also improved germination of okra but produced no effect on root infecting fungi.

KEYWORDS: Growth parameters, Mannitol and sodium chloride, Root rot fungi, Seed priming.

INTRODUCTION

Osmopriming is a seed priming technique in which seeds are hydrated in different solutions of inorganic substances (like NaCl, KNO_3, $CaCl_2$, and Mannitol etc.) and then the primed seeds dried to retain original moisture level. This process was helpful in seedling emergence evenly under unfavorable environmental conditions and germination of seeds and also can tolerate the higher range of temperatures with less sensitive to oxygen deprivation (Corbineau *et al*., 1993; Bray, 1995) than unprimed ones. Priming has a great impact on various, cellular, molecular and biochemical structures including enzyme activity, synthesis of DNA and proteins (Bewely and Black, 1994). It was recorded that during priming with salts, ions from potassium nitrate and sodium chloride solutions accumulate within the seeds, resulting with increment in absorption of water and reducing water potential (Parera and Cantliffe, 1994). Seed priming not only improves speed and uniformity of germination but also enhances different biochemical processes of seed helpful in breaking dormancy, mobilization or hydrolysis of seed reserves, enzyme activation, emergence of embryonic tissues (Catav *et al.,* 2012; Khalil *et al.,* 2010; Khan *et al.,* 2008).

Several valuable crops are sensitive to attack by various root rotting pathogenic fungi. Major losses in the yield occurs due to pathogenic fungi and root based fungi (Zamani *et al.,* 2004; Sharma and Muehlbauer, 2007). According to Ayyub *et al.* (2003), these root infecting fungi can live alive for more than six years even in the absence of its host. Root rot caused by *Aphanomyces euteiches, R.solani, Fusarium* spp., *Sclerotium rolfsii* are the most dangerous soil-borne diseases of pea, chickpea, lentil, fababean and lupine (Abou-

Zeid *et al*., 1997; Abdel-Kader *et al*., 2002; Infantine *et al*., 2006). Controlling diseases caused by root infecting fungi mainly depend upon fungicidal applications. However these applications are hazardous to human health and a cause of environmental pollution. Use of salt and sugars as priming agent seems to be one of the alternatives to decrease the use of fungicides for plant disease control (Tariq *et al*., 2016). Therefore present study was proposed to obtained good quality of crops by reducing the plant diseases caused by root infecting fungi.

MATERIALS AND METHODS

Sodium chloride (NaCl) and Mannitol ($C_6H_{14}O_6$) were obtained from laboratory of plant pathology, Department of Botany, University of Karachi. Seed samples of okra and lentil were bought from local market of Karachi, washed under running tap water followed by distilled water to remove the dust particles. NaCl and Mannitol with different concentrations of 0.5, 1 and 2% were prepared and seeds of lentil and okra were primed with NaCl and mannitol with respective concentration separately for 5, 10 and 15 minutes, respectively. Seeds after priming with respective solutions and concentrations were air dried.

Plastic pots of 8.1 cm diameter were set on screen house of Department of Botany, University of Karachi in a randomized manner. Primed seeds of okra and lentil (4 seeds per pot) were sown in each pot (11 treatments with 3 replicates of each host) and allow to germinate. Plants were watered on regular basis in order to provide sufficient amount of moisture. After a month of germination, plants were uprooted to observed growth parameters. Impact on root infecting fungi was observed by plating roots of each treatment separately on Potato Dextrose Agar poured Petri plates containing sufficient amount of antibiotics (penicillin and streptomycin) and plates were incubated at 25-30 ºC for 5-6 days. For colonization of root infecting fungi on roots following formula was used.

$$\text{Colonization \%} = \frac{\text{Number of root pieces colonized by a fungus}}{\text{Total number of root pieces}} \times 100$$

Data obtained on growth and root infecting fungi was statistically analysed (Sokal and Rohlf, 1995).

RESULTS

Priming of okra seeds using NaCl, water and mannitol with different concentrations showed prominent effect on growth of plant. Germination % was maximum (100%) when 0.5% mannitol was used for 10 minutes ($p<0.001$). However, seed priming with water (hydropriming) for 15 minutes also showed improved germination % compared to other treatments. Seed priming of okra with 1% mannitol for 10 minutes gave highest shoot length (53.3 cm) and shoot weight (6.26 g) followed by 0.5 % NaCl for 5 minutes (Table 1). Mannitol (1%) used for 10 and 15 minutes improved root length (53.66 cm) and weight (3.8 g) compared to other treatments (Table 1). Root infecting fungi was reduced when seeds of okra primed with NaCl and mannitol as compared to non-primed seeds. However, *R. solani* colonization (25%) was reduced significantly ($p<0.05$) when okra seeds were primed with 2 % NaCl and mannitol for 10, 15 minutes while colonization of *Fusarium*

species (40.33%) was reduced when seeds were primed with 0.5 and 2% NaCl, mannitol for 10, 15 minutes ($p<0.001$). NaCl (2%) for 5 minutes significantly ($p<0.001$) reduced colonization of *M. phaseolina* (16.66%) on okra roots (Table 1). It is noted that hydropriming donot have any effect on reduction of root infecting fungi.

In case of lentil plants, seed priming with 0.5% mannitol for 10 minutes significantly ($p<0.05$) increased germination % (100%) while 1% NaCl and mannitol for 10 minutes and hydropriming for 10 minutes gave significant ($p<0.01$) increase of shoot length (50 cm). However, shoot weight (6.04 g) was increased when seeds primed with 1 % mannitol for 10 minutes followed by 1% NaCl for 5 minutes. Furthermore, root length (51 cm) was better when lentil seeds primed with 1 % NaCl for 10 minutes and 1% mannitol for 10, 15 minutes ($P<0.05$) while 2 % mannitol and NaCl for 10 minutes improved root weight (3.75 g). Seed priming with 2% mannitol for 5 minutes reduced the colonization % of *Fusarium* species (15.31%) while *M. phaesolina* (10.91%) was significantly ($p<0.05$) reduced due to seed priming with 2% mannitol for 10 minutes. However, seed priming with 2% NaCl for 10 minutes, 1, 2% mannitol for 5 and 15 minutes reduced the colonization % of *R. solani* (Table 2).

Table 1. Effect of osmopriming with sodium chloride and mannitol in the control of root infecting fungi of okra plants.

Treatments		Growth parameters					Colonization% of root infecting fungi		
		G%	RL	RW	SL	SW	FS	RS	MP
Control		46.6	39.66	2.83	44.1	5.43	53.3	49.66	55
C + water 5min		26.6	38.66	2.43	41.9	6.13	40.33	41.66	43.33
C + water 10 min		62.3	40	2.5	46.8	5.06	41.66	51.66	25
C + water 15 min		96.66	39	2.8	40.3	5.6	46.33	49.33	20
0.5% NaCl, 5 min		63.33	40.66	2.13	40.5	5.26	63.33	50.33	22
0.5% NaCl, 10 min		93.66	40.33	2.5	41.9	5.23	40.33	48.33	30
0.5% NaCl, 15 min		96	44.33	2.86	48.6	5.06	50.33	63.33	18
1% NaCl, 5 min		90.2	40.66	2.5	41.7	6.23	45.66	51.66	30.33
1% NaCl,10 min		83.33	38.33	3.8	40.7	5.43	41.66	56.66	25.33
1% NaCl, 15 min		90	53.66	2.86	51.3	6.13	40.66	41.66	20
2% NaCl, 5 min		70	40.66	2.36	48.6	5.73	40.33	41.66	16.66
2% NaCl, 10 min		53.33	39.66	1.93	48.5	6.16	41.33	49.66	25
2% NaCl, 15 min		63.33	39.33	2.63	39.9	5.26	58.33	25	40.33
0.5% Mannitol, 5min		83.66	40.66	1.86	41.8	5.06	53.66	41	18
0.5% Mannitol,10min		100	44.3	2.5	40.5	5.86	63.66	48.33	30.33
0.5% Mannitol, 15, min		93.66	39.66	2.36	41.9	5.26	41.33	51.33	22
1% Mannitol, 5 min		86.66	39.66	1.93	48.5	5.43	51.33	25.66	18.33
1% Mannitol, 10min		96.66	53.66	2.83	53.4	6.26	55.66	49	30.33
1% Mannitol, 15min		93.33	44.33	2.36	40.6	5.76	40.66	48	43
2% Mannitol, 5 min		83.66	53.33	2.63	51.3	5.06	51.33	50.33	25
2% Mannitol, 10min		70.33	39.66	2.83	48.4	6.13	40.33	25	22.33
2% Mannitol, 15min		60	38.66	2.43	38.5	5.86	63.33	51.66	40
$LSD_{0.05}$	Time	49.771	1.669	0.271	30.96	0.253	4.426	43.66	4.396
	Conc.	64.254	2.154	0.350	39.97	0.326	5.714	5.680	5.676
	Treat.	40.638	1.362	0.221	25.28	0.206	3.613	3.592	3.590

G% = Germination percentage; RL = Root length (cm); RW = Root weight (g, FW); SL = Shoot length (cm); SW = Shoot weight (g, FW); FS = *Fusarium* species;
RS = *Rhizoctonia solani*; MP = *Macrophomina phaseolina;* Conc. = Concentration; Treat. = Treatments; Min = Minutes

Overall results showed that seed priming with 1% mannitol and NaCl for 10 minutes were effective in the increment of growth parameters of okra and lentil while 2% mannitol and NaCl was helpful in the reduction of root infecting fungi of lentil for10 and 5 minutes reduces root infecting fungi of okra and lentil plants.

Table 2. Effect of osmopriming with sodium chloride and mannitol in the control of root infecting fungi of lentil plants.

Treatments	Growth parameters					Colonization% of root infecting fungi		
	G%	RL	RW	SL	SW	FS	RS	MP
Control	46.6	37.66	1.86	43.66	5	40.66	25.33	40.66
C + water 5min	63.3	44.33	1.6	40.33	5.3	45.33	50.33	43.66
C + water 10 min	60	41.33	1.86	50.33	5.5	44.33	45.66	40.66
C + water 15 min	53.3	40.33	2	41.33	5	45.66	55.66	35.66
0.5% NaCl, 5 min	63.3	37.66	1.86	43.66	5.4	48.33	51	48.66
0.5% NaCl, 10 min	86	44.33	2.53	44.33	5.36	48.66	30.31	16.32
0.5% NaCl, 15 min	96	47.66	2.73	44.33	5.4	16.6	22	15.33
1% NaCl, 5 min	83.3	39.66	2.8	48.33	6.03	30.31	16.66	25.51
1% NaCl,10 min	90	51	2.6	50	5.9	20.33	15.66	22.33
1% NaCl, 15 min	93.3	50.66	3.66	48.33	5.6	25	11.31	20.66
2% NaCl, 5 min	70	40.66	3.2	44.33	5.66	16.1	12.91	40.31
2% NaCl, 10 min	50	41.66	3.7	47.66	5.13	25.66	11.11	41.66
2% NaCl, 15 min	66.6	39.33	2.5	43.66	5.73	18	25	16.21
0.5% Mannitol, 5min	70	44.33	1.78	41.66	5.41	30.66	42.33	11.31
0.5% Mannitol,10min	100	39.66	2.73	39.66	6.4	42.66	30.33	30.33
0.5% Mannitol, 15, min	86.6	40.66	2.8	48.33	5.14	15.31	16.66	22
1% Mannitol, 5 min	96.6	50.66	2.5	43.66	5.9	18.66	11.11	10.87
1% Mannitol, 10min	93.3	51	3.66	50	6.04	22	15.21	25.67
1% Mannitol, 15min	76.6	51.33	2.53	47.66	5.61	30.33	25.66	20.33
2% Mannitol, 5 min	87.3	47.66	1.86	42.33	4.39	15.21	30.33	11.67
2% Mannitol, 10min	53.3	41.33	3.75	48.66	5.82	18.31	26.31	10.91
2% Mannitol, 15min	87.3	39.66	2.73	47.66	5.71	16.62	11.11	22.33
$LSD_{0.05}$ 5.709	2.530	1.795	1.419	0.275	3.701	2.940	3.812	
7.371	3.267	2.317	1.832	0.355	4.877	4.688	4.922	
4.662	2.006	1.465	1.159	0.224	3.022	3.600	3.113	

G% = Germination percentage; RL = Root length (cm); RW = Root weight (g, FW); SL = Shoot length (cm); SW = Shoot weight (g, FW); FS = *Fusarium* species;
RS = *Rhizoctonia solani*; MP = *Macrophomina phaseolina;* Conc. = Concentration; Treat. = Treatments; Min = Minutes

DISCUSSION

Seed priming is used to partially hydrate the seed in which seeds were soaked in different solutions like water (hydropriming), soaking in inorganic salts (halopriming) and in organic (osmopriming) which were redried before use. These treatments may have different effects depending on plant species, stage of plant development, concentrations of priming agent and incubation period (Ashraf and Foolad, 2005). Present results showed that seed priming with 1 % mannitol and NaCl for 10 minutes were effective in the increment of growth parameters of okra and lentil. It was reported by Iqbal *et al.* (2006) that priming agents like $CaCl_2$, KCl and NaCl resulted in increment in grain yield, fresh and dry shoot biomass of spring wheat. Higher amount of NaCl causes less nitrogen fixation inhibition with a higher root shoot ratio, normalized nodule weight and shoot

K/Na ratio and reduced foliar accumulation of Na^+ in chickpea cultivar ILC1919 (Tejera *et al.*, 2006). Similarly Rafi *et al.* (2015) observed that seed priming using plant extracts like *Acacia nilotica* and *Sapindus mukorossi* leaves extract for 10 minutes showed pronounced effect on growth of sunflower, okra and peanut plants. Priming with water and mannitol (4%) showed increased number of seeds and seed yield per plant in chickpea crops with an enhanced acid invertase activity in the apical part of main stem and the portions below it which might result in increased availability of hexoses to the plant parts. This increment in supply of hexoses is responsible for increasing growth of plant, source of energy and biomass in primed plants (Kaur *et al.*, 2005).

Present study showed that seed priming with 2% mannitol and NaCl for 10, 15 minutes was helpful in reduction of root infecting fungi of lentil and okra plants. Abdel-Monaim *et al.* (2012) used ethephon, hydrogen peroxide, mannitol and salicylic acid (SA) as chemical inducers for significant reduction in root rot and wilt diseases in pot and field experiment. Of which maannitol and SA suggested to be helpful in highest reduction of root rot fungi of tomato plants. These chemicals due to priming might stimulate some defense mechanisms like phenolic compounds, oxidative enzymes and some metabolites. Saber *et al.* (2003) reported that mannitol used at 1mM reduced fruit rot caused by *Botrytis cinerea* in strawberry under green house and field conditions. Similarly Kanwal *et al.* (2015) reported that seeds of mash bean and chick pea primed with *Carica papaya* for different time periods and *C. papaya* seed extracts primed for 40 minutes time interval impressively reduced the colonization of root infecting fungi on mash bean plants.

Results of above study indicate that increase of okra and lentil growth due to priming might be due to modulation of enzymes of sucrose metabolism resulting in improved growth. Furthermore, seed priming could make the plant resistant to various pathogenic fungi resulting possibly in better growth.

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(Received January 2018; Accepted April 2018)