INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 15, No. 3, p. 195-205, 2019

RESEARCH PAPER OPEN ACCESS

Effect of seed priming on seed germination, emergence and seedling growth of okra (*Abelmoschus esculentus* L.)

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Key words: Osmopriming, Hydropriming, Okra, Soaking durations.

http://dx.doi.org/10.12692/ijb/15.3.195-205 Article published on September 28, 2019

Abstract

Under normal conditions, low production of okra plants is due to uneven and slow germination of seeds. As the little information is available regarding the special effects of priming to increase the germination of okra seeds under normal conditions. Therefore with this interpretation, a lab experiment was performed to find out the effects of priming with various concentrations of PEG (a potential priming agent) along with soaking durations and distill wateron the germination, emergence, plant lengths and weights and seedling growth. Seeds of okra were pre-soaked in 4%, 8%, PEG solutions and distill water for 12 and 24 hrs. Results of the study showed that priming enhanced seeds germination, dry and fresh weight of seedlings, length of plants, number of leaves and seedling growth. All the characters showed the best results when wheat seeds treated with 8% PEG solution compared to nonprime and hydro primed seeds. Hydropriming also enhanced these parameters as compared to controls. Soaking durations were also found to be evident because optimal results were obtained after 24hrs of soaking in all treatments. Therefore it is suggested that seed priming had important role to improve the overall seedling growth as well as germination of seeds.

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Introduction

From many years different strategies related to improve the growth and development of plants have been investigated. Among these seed priming is universally used innovative technique to improve quality of seeds. It is a simple, cheap and efficient approach to enhance the growth of seedlings and yield under non-stressed and stressed conditions. In this process some physiological changes are produced in the seeds by using natural or synthetic compounds before germination, but it allow limited hydration of seeds to induce pre-germinative metabolic activities but germination is prevented. Appropriately conducted priming optimizes seed performance by enhancing germination percentage due to rapid and uniform germination, seedling growth, vigor and emergence of different crops by permitting seeds to germinate under a wider range of ecological conditions (Powell *et al*., 2000; McDonald, 2000). The effects of priming are attributed by the initiation of the biochemical process of mobilization of storage compounds and production of enzymes that catalyzes the decomposition activation of the antioxidant defense system (Di Girolamo and Barbanti 2012) as well as repair sub cellular and cellular damages of low vigor seeds that usually accumulated during development of seeds (Bray 1995). Depending on the plant species, seeds contains different proportions of triacylglycerols, proteins and starch as a source of energy. During germination these reserves are breakdown by specific enzymes i.e. lipases, proteases and amylases (Di Girolamo and Barbanti, 2012).

The effects of priming are additionally linked with enhanced production of proteins, the repairing of membranes and building up and repairing of nucleic acids McDonald (2000). He also exhibited that primed seeds get the ability to imbibe quickly and resuscitate the metabolism of seeds thus increases the rate of germination. In seeds priming treatment also increase the activities of anti-oxidative enzymes (Hsu *et al*., 2003; Wang *et al*., 2003). Different techniques are used for priming i.e. hydro-priming, osmopriming, halo-priming and bio-priming (Harris *et al*., 1999; Capron *et al*., 2000; Chiu *et al*., 2002; Wahid *et al*., 2008; Farooq*et al*., 2009). Osmopriming with PEG solution permits the preparation of metabolic activities for germination by water absorption as well as starting the repairing of membranes (Jisha *et al*. 2013). PEG had no toxic effect on seeds germination because these molecules do not enter into the seed (Mehra *et al*., 2003). Hydropriming and Osmopriming promote the proliferous and dynamic growth in roots (Carceller and Soriano, 1972) and also improved emergence and germination in wheat (Ashraf and Abu-Shakra, 1978). Many studies have shownpromotion in germination of seeds and seedlings growth by hydropriming under both stress and optimal conditions, in several crops i.e.wheat (Basra *et al*., 2002), canola (Omidi *et al*., 2009), Indian mustard (Srivastava *et al*., 2010), *capsicum annum* (Patade *et al*., 2012), rice (Goswami*et al*., 2013), *Helianthus annus* (Kaya *et al*., 2006), *Vignaradiata* (Posmyk and Janas, 2007) and *Triticumaestivum* (Fercha *et al*., 2013).

Abelmoschusesculentus is the solitary vegetable crop of economic importance in the Malvaceae family.It ischief summer vegetable crop in Pakistan (Shahid *et al*., 2013). In Pakistan 2.21 × 105 ha is under cultivation of okra and produced about 2.86 × 106 tons of it (Anwar *et al*., 2010) while 6 million tons/year of okra are produced worldwide (Akpan-Idiok *et al*., 2012). Due to various reasons it has low productivity so in the world it has fifth position. Among these factors that liable for less yield of okra, non-synchronous and poor germination is very important (Sharma *et al*., 2014).It is a cradle of carbohydrate, protein, fats, vitamins, minerals and fibers (Premsekhar and Rajashree, 2009) that are generally insufficient in the essential food. The soluble fibersare in the form of gums and pectin, which helps in reducing serum cholesterol and thus diminishes the risk of coronary heart disease while insoluble fibers aids in keeping the intestinal tract healthy and prevents the sensation of irritable bowel syndrome (Moyin-Jesu, 2007). The seeds are used as antispasmodic, stomachic, nervine and stimulant. On small scale seeds are consumed for production of oil (Anwar *et al*., 2010). The mucilaginous extract of okra

is often used to purify sugarcane juice from which brown sugar is produced. The stem of the plant provides non-digestible strong linear fiber, which distinguishes uses in the paper, packaging and textile industries (Sharma and Prasad, 2010). The nutritive significance of okra pod has stimulated attention in carrying the crop into profitable production (Moyin-Jesu, 2007).It had poor seedling emergence and vigor. In okra seeds the slow and uneven germination is the key hurdle in the early spring planting (Kaur *et al.*, 2015). The percentage of seeds germination of okra is relatively low, due toseed hardness (Mohammadi *et al.*, 2012).

There is little information on the role of seed priming in germination characteristics of okra under normal conditions, therefore the current study was intended with the objective to enhance the germination percentage (the maximum/uniform germination)of seeds, seedling emergence and growth of Okra by using priming agent and soaking durations for better crop production under normal conditions.

Materials and methods

Experiment was performed in the Horticulture Department, Bahauddin Zakariya University, Multan during 2018 to assess the effect of seed priming treatments and soaking durations on germination and seedling growth of okra (*Abelmoschusesculentus* L.). Seeds of Okra were obtained from NARC, Islamabad. The experiment was carried out in Completely Randomized Design with seven treatments and each treatment hadthree replicates. Priming treatments were applied to the seeds for different soaking durations (12, 24 hours). Seeds were air dried after the completion of treatment in tissue paper under laboratory conditions.

Osmopriming solution of 4% and 8% Polyethylene glycol was prepared by adding 4g and 8g PEG into 100 ml of distilled water respectively and mixing it on hot plate stirrer. Digital balance was used to measure PEG.A total of 60 seeds were used for each treatment (20 seeds/replication). For osmopriming 4% and 8% PEG treatment, seeds were fully immersed in 4% and 8% PEG solutions for soaking durations of 12 and 24 hours separately at 25°C in dark. For hydro priming, seeds were fully immersed in distilled water for soaking durations of 12 and 24 hours separately at 25°C in dark. In the priming solution a fungicide Captan (2 g/L) was added to avoid fungal growth during this process. Seeds were surface dried back to their original moisture content (8%) at room temperature for four hours. Dried untreated seeds were considered as control.

Pots having 9 inches diameter were used and filled with garden compost soil up to ¾ portion of pot. After that, pots were leveled by tapping them on floor. 20 seeds were placed in each pot randomly at equal distance and covered with fine layer of soil. Pots were watered immediately after the sowing with the help of sprinkler. Pots were arranged in completely randomized design. Plants were watered when necessary almost at one day interval. The number of germinated seeds were counted on daily basis till 18 days of sowing. When both radicle and plumule length were approximately 15 mm, the seeds were considered germinated. At the end of experiment plants were irrigated so that seedlings could be uprooted without root damage. After that seedlings were harvested and parted into shoots and roots. With the help of measuring tape length of shoots and roots were measured. Fresh weights of root and shoot were noted and then dried in oven at 70 °C for 7 days to measure dry weights after complete desiccation. Weight of seedling were recorded by 4 digit electrical balance.

From the above seedlings and from each replicate, number of leaves and number of shoots were counted and then average number of leaves and shoots were calculated.

Germination (%)

Number of germinated seeds was recorded on daily basis for seed germination rate. Germination percentage was calculated based on the following equation:

$$\text{Germination percentage} = \frac{\textit{Total number of germinated seeds}}{\textit{Total number of seeds sown}} \times 100$$

Emergence index

Seedling emergence index was calculated based on equation

EI = ∑ (Ni/ D)

Here, Ni = number of seeds germinated on D day

D = day of seed germination

Power of emergence

Power of emergence (PE) was calculated by following formula:

$$Power\ of\ E = \frac{4th\ day\ germination}{no.of\ total\ seeds} \times 100$$

Statistical analysis

Analysis of variance (ANOVA) was done with above data by using statistical package (CoStat v 6.3 CoHort, California, Berkely USA). By following the method of Snedecor and Cochran (1980) means were also compared and used the least significance difference (LSD) test at significance level of 5%.

Results and discussion

Germination percentage (%)

The seed primingand soaking durations had significant ($P< 0.005$) effects on the germination percentage (Table 2). All primed seeds either osmopriming or hydropriming had significantly higher germination percentage than control.

The similar results were observed by Basra *et al* (2003), Demir and Ermis (2003), Afzal *et al.* (2005), Kaya *et al.* (2006) and Yagmur and Kaydan (2008).

Table 1. Different seed priming treatments on Okra.

Treatment	Priming agents	Soaking duration
To	None	0 hour
T1	4% PEG	12 hours
T2	4% PEG	24 hours
T3	8% PEG	12 hours
T4	8% PEG	24 hours
T5	Distilled water	12 hours
T6	Distilled water	24 Hours

The concentration of different priming treatments showed different effects to germination percentage but the optimum seed germination was observed with 8% PEG after 24 hours of soaking during this study.

Osmo-priming with PEG enhanced the germination of seeds (Arif *et al.*, 2003; Kaur *et al.*, 2015). During seed priming extensive biochemical changes i.e. dormancy breakage, metabolism or hydrolysis of inhibitors, enzymes activation and imbibitionoccur that critical for seed germination (Ajouri *et al.*, 2004). Asgedom and Becker (2001) reported that priming process antedate the germination is improved by priming and preserved following the re-drying of the seeds. Additionally primed seed can quickly consume and revive the metabolism of seed, as a result physiological heterogeneity was triggered down and germination percentage was increased Rowse (1995). Priming also showed stimulating effects in the early phases of germination by the facilitation of cell division, in germinating seeds (Hassanpouraghdam *et al.*, 2009; Faijunnahar *et al.*, 2017).

Conditional to the priming duration the effects of seed priming showed variations on seed germination (Elcoka *et al.*, 2007). In this study soaking durations also showed differential responses. PEG solution with 24 hrs soaking time had shown better germination rate as compared to 12 hrs (Table 3). So 24 hrs soaking time is safe for seeds of *Abelmoschus*. Similar results were observed in rice, chickpea, beans and maize by Elcoka *et al* (2007) and Ghassemi-Golezani *et al* (2010) while in pea 24 hrs soaking was proved to be detrimental (Elkoca 2014).

Table 2. Analysis of variance (ANOVA) of plant fresh weight, plant dry weight, shoot length, root length, number of leaves, number of shoots, germination percentage, emergence index, power of emergence and seedling lengths after different priming treatments on okra.

Source	DF	Plant fresh weight	Plant dry weight	Shoot length	Root length	No. of leaves	No. of shoots	Germination %	Emergence index	Power of emergence	Seedling length
Treatment	6	0.1414	0.0228	0.000***	0.000***	0.000***	0.939ns	0.000***	0.043**	0.000***	0.000***

In this study hydropriming also showed greater effect on percentage of seed germination and reached to optimum level (Table 3). Hydropriming considerably boost germination rate of seeds Ghassemi-Golezani *et al.* (2008) and is a useful technique for elevation of overall seeds germination Maiti *et al.* (2013). According to Ashraf and Foolad (2005) these valuable effects of hydropriming have been credited to the mobilization of enzyme activities in the embryonic tissues of seeds that are essential for germination and other compounds i.e.soluble sugars, proteins and free amino acids from storage organs.

Table 3. Effect of different seed priming treatments on germination (%) and emergence index of Okra.

Treatment	Germination%	Emergence Index	Power of Emergence	Seedling length
T0	63.333^{d}	8.8680^{c}	11.667^{d}	14.833^{d}
T1	90.000^{abc}	17.113^{abc}	36.667^{c}	17.067^{c}
T2	98.333^{a}	22.802^{ab}	35.000^{c}	18.467^{b}
T3	81.667^{c}	20.030^{ab}	43.333^{bc}	18.267^{bc}
T4	100.00^{a}	19.993^{ab}	63.333^{a}	22.567^{a}
T5	86.667^{bc}	14.257^{bc}	26.667^{cd}	17.933^{bc}
T6	96.667^{ab}	24.213 c	60.000^{ab}	22.200^{a}

Emergence index

The analysis of variance revealed that significant difference in emergence index owing to the treatments was evident (Table 2). Seeds primed with water showed higher seedling emergence index than that of primed with PEG and unprimed seeds. Thus seed priming with distilled water enhanced seedling emergence index to maximum after 24 hrs of soaking. Emergence index of germinated seeds have deepimpact on the establishment of seedlings and yield of various crops. According to Kaur *et al.* (2015) emergence index was increased by priming due to the increase in the competence ability of plants for basic needs i.e. water, nutrients and light. In thepresent investigation seedling emergence index from nonprimed seeds was low but after osmopriming with 4% and 8% PEG it was increased at both soaking durations (Table 3).

However, these did not differ significantly among themselves. These results reveal the benefit of priming on seeds emergence index a lot. Bittencourt *et al.* (2005) and Ghassemi *et al.* (2008) stated that emergence index was increased in Asparagus and Lentil seedlings.

Table 4. Effect of different seed priming treatments on fresh and dry weights, shoot and root lengths, number of leaves and shoots of Okra seedlings.

Treatments	Plant fresh weight	Plant dry weight	Shoot length	Root length	No. of leaves	No. of shoots
To	1.1333^{bc}	0.2533^{c}	6.6000^{c}	8.2000^{d}	4.0667^{f}	4.0000^{a}
T1	1.3000^{abc}	0.3300^{bc}	7.9667^{b}	9.1333^{c}	4.8667^{e}	3.6667^{a}
T2	1.2333^{abc}	0.3100^{bc}	8.6000^{b}	9.8333^{b}	5.6667^{bc}	3.0000^{a}
T3	1.3000^{abc}	0.3300^{bc}	8.0000^{b}	10.267^{b}	5.2000^{d}	3.6667^{a}
T4	1.6000^{a}	0.4267^{ab}	10.667^{a}	11.900^{a}	6.0000^{a}	3.6667^{a}
T5	1.0333^{c}	0.2700^{c}	7.8667^{b}	10.033^{b}	5.4667^{cd}	3.6667^{a}
T6	1.5333^{ab}	0.4833^{a}	10.200^{a}	11.967^{a}	5.8667^{ab}	3.3333^{a}

Shoot and root length (cm)

The growth of seedlings from primed and unprimed seeds was measured in terms of root and shoot lengths. Different priming treatments and soaking durations significantly affected the root and shoot length of okra seedlings (Tables 2). Maximum shoot length and root length were noted in osmopriming with 8% PEG and hydroprimimg with 24 hrs soaking duration as compared to unprimed control seedlings (Table 3) that did not differ between themselves.

These result aresimilar with the results of several workers (Jisha *et al.*, 2013; Baque *et al.*, 2016; Faijunnahar *et al.*, 2017). Rennick and Tiernan (1978) and described that there was a fast and extra elongation of coleoptile and aggrandize vigorous root growth occurred in primed seeds than un primed seeds. The root and shoot lengths was significantly lower in untreated control seedlings in the present investigation. 4% PEG solution also promoted the shoot and root lengths of seedlings but these were lower than 8% PEG solution. In the primed seeds, the noteworthy increase in root and shoot length may be due to meristematic growth, cell division or its involvement in cell elongation (Khan *et al.*, 2006;Kaur *et al.*, 2015).As far as the duration of soaking is concerned the maximum shoot length and root length was recorded in which seeds were soaked for 24 hrs both for osmopriming and hydropriming (Table 4).Soaking durations improved the metabolic activities of seed that results in expanded root and shoots in the primed seeds than non-primed seed (Lee and Kim, 2000).

Power of emergence

Primeand nonprime seedlings showed significant differences in their power of emergence (Table 2). Maximum power of emergence was observed by osmopriming as compared to hydropriming and control. Nonprime seeds showed lowest power of emergence. In osmopriming 8% PEG showed more promising results than the 4% PEG.Baque *et al.* (2016) also observed that maximum power of emergence when the seed primed with PEG solution. Minimization of seed coat adherence during the emergence increased the power of emergence (Nascimento and West, 1998). This is due to the mobilization of reserve food material, synthesis and activation of some enzymes, RNA and DNA synthesis during osmopriming (Sadeghi *et al.*, 2011). Hydropriming also enhanced the power of emergence significantly as compared to controls (Table 3). This results are similar with the findings of Baque *et al.* (2016), Maiti *et al.* (2011) and Maiti *et al.* (2013) who reported that seed priming increases the power of emergence of seedlings. Soaking durations also affect the power of emergence significantly in both hydro and osmopriming. 24 hrs soaking duration was found to be more appropriate as compared to 12 hrs in promotion of power of emergence.

Number of leaves and shoots

The analysis of variance revealed significant difference in number of leaves owing to the priming treatments (Table 2). The maximum number of leaves were found in seedlings treated with 8% PEG solution. Hydropriming also produced more leaves as

compared to control, however soaking duration of a particular treatment did not differ between themselves. The order of number of leaves was T4>T6>T2>T5>T3>T1>T0. Statistically no significant differences were observed in number of shoots (Table 4).

Plant fresh weight (g)

The analysis of variance detected non-significant difference in fresh weight of plants owing to the different priming treatments. However 8% PEG and hydropriming had some positive effects on plant fresh weight of okra.

Plant dry weight (g)

Analysis of variance had detected the significant differences in dry weights of seedlings germinated from primed and unprimed seeds (Table 2). The results of the present investigation showed that the highest plant dry weight was scored by PEG treatment whereas the minimum plant dry weight was recorded from controls (non-primed seed).

These result are also supported by previous researchers Baque *et al.* (2016), Ghassemi-Golezani *et al.* (2008) and Sarwar *et al.* (2006). According to Ruan *et al.* (2002) priming probably repaired the damaged membrane due to the deterioration and employed improved level of germination and increase the vigor level as compared to non- primed. It also minimize the adherence of seed coat during emergence of seeds (Nascimento and West, 1998).The improvement in vigor and germination of seeds might be due to re-synthesis of some enzymes, active transportation of food material during osmotic priming RNA and DNA synthesis also starts. Removal of hindrance accelerate the seed germination eventually formed the strong shoot and thus shoot dry weight increased Basra *et al.* (2005). In this study 8% PEG showed higher plant dry weight than the rest significantly (Table 4). The maximum dry weight of plant was recorded from hydro priming after 24 hrs of soaking. Similar results were predicted by Ghassemi-Golezani *et al.* (2008) and Sarwar *et al.* (2006) that hydro-priming significantly enhanced shoot weights.

Conclusion

From the present work it may be concluded that PEG has a positive effect on germination, emergence, and plant height, fresh and dry weight of plant and seedling growth on okra seeds. Among different treatments 8% PEG performed best for germination percentage, plant height, fresh and dry weight of plant and seedling growth under normal conditions. Hydropriming also showed better results for all the parameters. However, non-primed seeds showed consistently poor performance. It can be concluded that seed treated with Polyethylene Glycol (PEG) helps to enhance the seeds germination, emergence, plant height, fresh and dry weight of plant and seedling growth under normal conditions. So, use of PEG is recommended for uniform germination of seeds of okra. However, keeping in view the seed priming with distilled water for 24 hours can also be successfully used by the farmers because it is cheaper than PEG.

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