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Effects of Seed Priming on the Antioxidant Enzymes Activity of Mungbean (*Vigna radiata*) Seedlings

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Abstract: In seed priming, seeds are partially hydrated to a point where germination processes commence but radical emergence does not occur. A lab experiment was conducted for assessment of antioxidant enzymes activity as affected by different seed priming treatments. The seeds were invigorated by traditional soaking (hydropriming), osmo-conditioning (soaking of seeds in aerated, low-water-potential solutions) using, potassium di-hydrogen phosphate, Mannitol, Polyethylene glycol, sodium molybdate dihydrate and hormonal priming by using salicylic acid. The ranges of osmotic potential for all the priming treatments were -0.5 to -1.2 M Pa. All the invigoration treatments significantly affected the activities of anti-oxidants i.e., Superoxide Dismutase (SOD), Peroxidase (POD), Polyphenoloxidase (PPO) and Catalase (CAT) activity. Osmopriming using P @ 0.60% applied in the form of KH_2PO_4 significantly improved the SOD and CAT activity while T10 showed improvement in PPO. All the seed priming treatments also enhanced the seedlings vigour in terms of germination and vigour index.

Key words: Osmo-priming, hydropriming, *vigna radiata*, seedling vigour, nodulation

INTRODUCTION

Seed priming describes the different germination enhancing pre-sowing treatments which do not result in radical emergence (Ghana and Schillinger, 2003). Seed priming can be found effective for legumes i.e., yields of Mungbean and Chickpea were increased substantially by priming seeds for 8 h before sowing (Harris *et al.*, 1999; Musa *et al.*, 2001; Rashid *et al.*, 2004).

Mungbean (*Vigna radiata* (L.) Wilczek) is grown on over 200,000 ha with production of more than 100,000 t under rainfed and irrigated conditions in Pakistan. Poor crop establishment is a major restraint for mungbean production (Naseem *et al.*, 1997; Rahmianna *et al.*, 2000) and high yields can be associated with early vigor (Kumar *et al.*, 1989).

Improved seed invigoration techniques are being used to reduce the germination time, to get synchronized germination, improve germination rate and improve seedling stand in many horticultural (Bradford *et al.*, 1990; Rudrapal and Nakamura, 1998) and field crops like wheat, maize (Aquilla and Tritto, 1991; Basra *et al.*, 2002) and more recently rice (Farooq *et al.*, 2004a). These invigoration techniques include hydropriming, osmoconditioning (Basra *et al.*, 2005), osmohardening (Farooq *et al.*, 2006a) and hardening (Farooq *et al.*, 2004b).

All the seed priming significantly improved the germination rate and vigour of the mungbean seedlings (Umair *et al.*, 2010). It is also reported that seed priming improve the antioxidant enzymes activity which decrease the adverse effects of Reactive Oxygen Species (ROS) (Del Ryo *et al.*, 2002).

There is little information available on the role of priming treatments in mungbean seeds and possible physiological processes that lead to the reported benefits of priming. The objective of this study was to determine if the improved vigour of mungbean seedlings also increased the antioxidant enzymes activity like SOD, POD, PPO and CAT.

MATERIALS AND METHODS

Seed material: Seeds of mungbean cultivar Chakwal Mung-97 (CM-97) were obtained from Barani Agricultural Research Institute (BARI), Chakwal. The seeds were sterilized by using 30% hypochlorite for five minutes and then washed three times with distilled water.

Seed treatments: The following seed priming treatments were applied:

Nutrient priming: The seeds were soaked in aerated solution of phosphorous (P @ 0.60 and 1.20%) and molybdate (Mo @ 0.02 and 0.04%). The sources for phosphorous and molybdenum were potassium dihydrogen phosphate (KH_2PO_4) and sodium molybdate ($Na_2MoO_4.2H_2O$), respectively.

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Osmopriming: The seeds were soaked in aerated solutions of mannitol (mannitol @ 2% and 4%) and polyethylene glycole (Polyethylene glycol @ 5% and 10%).

Hormonal priming: In hormonal priming the seeds were soaked in aerated solution of salicylic acid (SA @ 10 and 20 ppm).

Post treatment operations: After seed treatments the seeds were given surface washing three times by distilled water. Ten numbers of seeds were sown for each treatment in petri-dish having moist whatman 42 filter paper with distilled water. There were three replications for each treatment.

Fresh seedlings samples of five gram for each treatment was ground to powder using liquid N and homogenized in 3 mL ice-cold extraction buffer (50 mM potassium phosphate buffer, pH 7.8, containing, 5 mM DTT, 5 mM ascorbate, 5 mM EDTA 100 mM NaCl and 2% PVP-40). The supernatants were drawn from extracts at 0-4°C after centrifugation at 15,000 g for 15 min (Biemelt *et al*., 1998). The SOD Activity was assayed by using the NBT method as described by Dhindsa *et al.* (1980) which measure actually the photo-reduction of NBT at 560 nm with spectrophotometer.

The Complete Randomized Design (CRD) was used in pot experiment. Analysis of Variance (ANOVA) was used to compare treatment means.

RESULTS

The data showed that different seed priming treatments had significant ($p<0.05$) effect on anti oxidant enzyme activity (Fig. 1, 2, 3 or 4). All the seed priming treatments increased the superoxide dismutase activity. The highest SOD activity (6.46 U/mg protein) was observed in T5 (P @ 0.6%), which was 161% higher than control. The lowest SOD activity was observed in T1 which was at par with T4.

There was significant effect of seed priming on PPO activity (Fig. 3). All the seed priming treatments increased the PPO activity. The highest (0.89 U g^{-1} protein) PPO activity was observed in T10. The lowest PPO activity was observed in dried non-primed seeds.

There was also significant effect of seed priming treatments on the catalase activity of mung bean seedlings (Fig. 4). The highest activity of catalase was observed in T5, followed by T6 and T3. All the other treatments were showed similar results.

DISCUSSION

It is generally accepted that repair of seeds deteriorated by lipid peroxidation occurs during hydration, mainly via production of antioxidants and repair enzymes. In this connection, many authors (Aquilla and Tritto, 1991; Mcdonald, 2000) stated that membrane repair could be ascribed to evoked activities of free-radical scavenging enzymes.

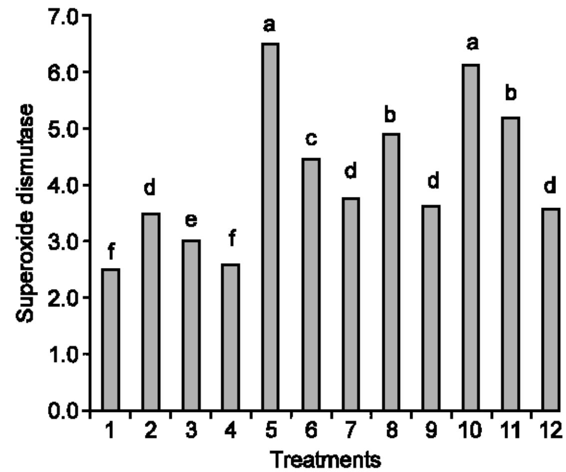

Fig. 1: Effects of seed priming on the superoxide dismutase (SOD) activity of mungbean (*Vigna radiata*) seedlings

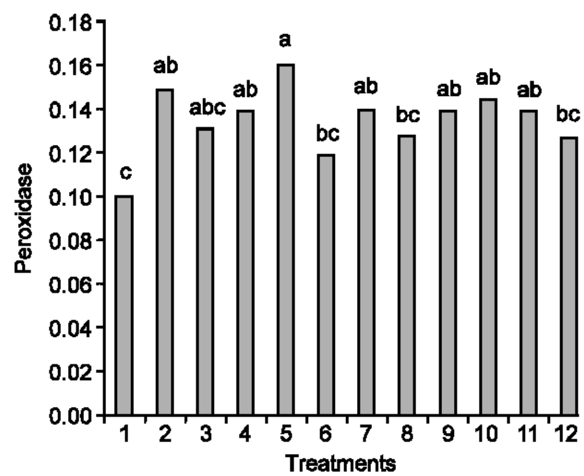

Fig. 2: Effects of seed priming on the peroxidise (POD) activity of mungbean (*Vigna radiata*) seedlings

Earlier and more uniform germination and emergence was observed in primed seeds as indicated by lower MET and E_{50} (Table 1). These findings support the prior work on canola (*Brassica compestris)* (Zheng *et al*., 1994), wheat (*Triticum aestivum*) (Nayyar *et al*., 1995) and rice (*Oryza sativa*) (Lee and Kim, 2000; Basra *et al*., 2003) who described improved germination rate and percentage in seeds subjected to hydropriming and seed hardening for 24 h (Farooq *et al*., 2006b).

This study revealed that osmo, nutrient and hormonal priming could invigorate mungbean seeds. One of the reasons for decreased MET is that during pre-sowing seed treatments the dormancy of the seed is broken and the seed bio-chemical processes commences, which lead to faster germination and emergence (Farooq *et al*., 2006c). Seed priming ensured the proper hydration, which resulted in enhanced activity of α-amylase that

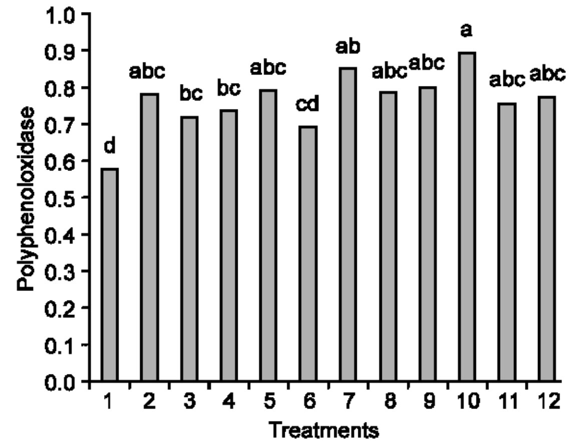

Fig. 3: Effects of seed priming on the polyphenoloxidase (PPO) activity of mungbean (*Vigna radiata*) seedlings

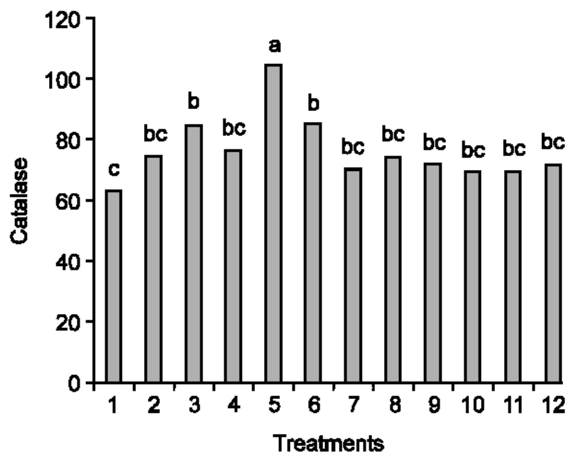

Fig. 4: Effects of seed priming on the catalase (CAT) activity of mungbean (*Vigna radiata*) seedlings

hydrolyzed the macro starch molecules in to smaller and simple sugars. The availability of instant food to the germinating seed gave a vigorous start as indicated by lower E_{50} and MET in treated seeds (Farooq *et al*., 2006d) during priming de novo synthesis of α-amylase is also documented (Lee and Kim, 2000). Early emergence as indicated by lower E_{50} and MET in treated seeds may be due to the faster production of germination metabolites (Saha *et al*., 1990; Lee and Kim, 2000; Basra *et al*., 2005) and better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins (Bray *et al*., 1989). Gray and Steckel (1983) also concluded that priming increased embryo length, which resulted in early initiation of germination in carrot seeds. The increased shoot and root length in primed plants can be due to metabolic repair of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and

Table 1: Impact of different seed priming treatments on Mean Emergence Time (MET), time to fifty percent seed to germinate (E-50)

Treatments	MET (days)	E-50 (days)
T1	5.52 a	4.44 a
T2	5.12 cd	4.00 cd
T3	5.37 abc	4.26 ab
T4	5.20 bcd	4.09 bcd
T5	4.51 e	3.41 f
T6	5.02 d	3.91 de
T7	5.38 ab	4.29 ab
T8	5.32 abc	4.18 bc
T9	5.05 d	3.72 e
T10	5.20 bcd	4.07 bcd
T11	5.27 bcd	4.23 abc
T12	5.24 abcd	4.23 abc
LCD	0.25	0.23

T1 = Control; T2 = Hydropriming; T3 = Mo @ 0.2 g/L; T4 = Mo @ 0.4 g/L; T5 = KH_2PO_4 @ 200 mM; T6 = KH_2PO_4 @ 400 mM; T7 = SA @ 10 ppm; T8 = SA @ 20 ppm; T9 = Manitol @ 20 g/L; T10 = Manitol @ 40 g/L; T11 = PEG @ 100 g/L; T12 = PEG @ 50 g/L

reduction of lag time between imbibition and radicle emergence (Bradford *et al*., 1990). Treated seeds had stronger embryos that were able to more easily emerge from seeds (Harris *et al*., 2005). These results are also in line with the findings of Sekiya and Yano (2009) who reported enhanced root and shoot length of seedlings obtained from P enriched seeds. To contribute to plant growth and development seed priming has been widely reported technique (Harris *et al*., 2005). Ajouri *et al.* (2004) reported a stimulation of P and Zn uptake, as well as an improved germination and seedling growth in barley after soaking seeds in water and in solutions containing 5-500 mM P.

It has been also reported invigorated seeds had higher vigour levels (Ruan *et al*., 2002), which resulted in earlier start of emergence as high vigour seed lots performed better than low vigour ones (Hampton and Tekrony, 1995). Yamauchi and Winn (1996) also reported positive correlation between seed vigour and field performance in rice.

Earlier, Zheng *et al.* (2002) reported earlier and uniform emergence in rice seeds osmoprimed with KCl and $CaCl_2$ and mixed salts under flooded conditions. Hydropriming improved the early and vigorous crop establishment in maize (Nagar *et al*., 1998) and *Helichrysum bracteatum* L. (Grzesik and Nowak, 1998). However, other studies resulted in poor emergence from hydroprimed Kentucky bluegrass seeds under field conditions (Pill and Necker, 2001). However Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research workers (Mwale *et al*., 2003; Giri and Schillinger, 2003).

From the present study it may be concluded that seed priming may enhance the seedling vigour of mungbean. Nutrient priming using phosphorous and osmopriming with mannitol were the most appropriate priming treatments for mungbean (*Vigna radiata*). In further research work biochemical basis for the enhanced phenology of mungbean may be evaluated.

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