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Effect of seed priming induced metabolic changes on germination and field emergence of chickpea

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Abstract

Aim : Seed priming improves vigour, however, different priming agents may vary in their effectiveness. Therefore, main aim of this study was to decipher the physiological mechanism of vigour improvement following seed priming using different agent.

Methodology : Chickpea cultivar Kripa was primed with different concentration of poly ethylene glycol, mannitol, salicylic acid, gibberellic acid and water for varying duration. Dry unprimed seed was used as control. *De novo* protein synthesis, sugar content, electrical conductivity of seed leachate, germination percent, time to 50% germination, uniformity of germination, germination energy, field emergence, hundred seed weight, seed yield were determined to understand the physiological basis of priming.

Results : Priming reduced time to 50% germination, mean germination time and improved germination energy, final germination, field emergence and seed yield over control. Overall, the favourable effect of seed priming were related to *de novo* protein synthesis, an improved membrane repair (revealed by low ion leakage) and germination substrates (increased sugar content) for vigorous and earlier production of seedlings.

Interpretation : Of the treatments, seeds primed with water for 17 hrs and PEG -0.5 / -1.0 Mpa for 24 hrs were most effective in enhancing field emergence and seed yield of chickpea and can be therefore recommended for increasing productivity in chickpea.

Introduction

High, rapid and uniform emergence is crucial for achieving an optimal crop stand and better productivity, since slow rate of germination exposes seedling to unfavourable environmental condition affecting their vigour (Osburn and Schroth, 1989). Germination begins with the imbibition of water by dry seed and completes with protrusion of radicle through seed coat (Bewley *et al.*, 2013), within these period the seed repairs all the deformities and prepares itself to produce a normal and healthy seedling. Seed vigor is a complex trait which is defined as the sum of those properties that determines the activity and performance of seed lots of acceptable germination in a wide range of environments (ISTA, 2011). Increased production of quality seed has become a need of an hour to meet the current demand of crop production. Seed priming is one of the approaches to enhance seed vigour (Farooq *et al.*, 2006; Catusse *et al.*, 2011). Seed priming method includes hydro-priming, osmo-priming, nutrient-priming, halo-priming, bio-priming, hormonal-priming, matrix-priming, drum-priming, magneto-priming and thermo-priming (Kubala *et al.*, 2015). Primed seeds are reported to performed better even under adverse field conditions like salinity (Kim *et al.*, 2006; Srivastava *et al.*, 2010a, b), temperature extremes (Wahid and Shabbir, 2005; Nawaz *et al.*, 2011), drought (Kaur *et al.*, 2005; Elkoca *et al.*, 2007). Thus, seed priming cannot be considered only as a mere germination acceleration process but as a stress mitigating process, since it prepares seed to perform better even in different biotic and abiotic stresses prevailing during seedling emergence and early seedling growth (Jisha *et al.*, 2013). The uniform, rapid and better germination of primed seed occurs due to changes in morphology, physiology, biochemistry and molecular level. Among them, alterations in metabolite levels are important events during seed invigoration or priming. Transcriptomic and proteomic studies have revealed up regulation or down regulation of a number of genes and proteins during seed priming (Gallardo *et al.*, 2001; Kubala, 2015; Szymon *et al.*, 2015). Advanced germination metabolism, increased activity of antioxidants, decreased level of malondialdehyde, altered ratio of saturated and unsaturated fatty acids and increased level of hydrolyzing enzymes are some of the changes associated with seed priming (Bailly *et al.*, 2000; Walters *et al.*, 2005; Yacoubi *et al.*, 2011).

Chickpea (*Cicer arietinum* L.) is an important legume and is a vital source of protein to majority of vegetarian population. India is the leading chickpea producing country with about 68% share in global chickpea production (FAO, 2014). Generally, two market types of chickpea, kabuli (non-pigmented and large seeded) and desi (pigmented and small seeded) are well reported. Seed coat colour is imparted by the presence of tannin in seed coat, which is known to have antifungal, antimicrobial properties and acts as a chemical defensive trait for plant or seed (Zhang *et al.*, 2016). In legumes, seed vigour is associated with seed coat pigmentation, pigmented seeds being more vigorous than unpigmented one (Kantar *et al.*, 1996; Peksen, 2007). Similarly, kabuli chickpea are less vigorous than desi type. Kabuli

chickpea recorded poor field emergence as compared to desi type even though their laboratory germination was high (Lamichaney *et al.*, 2016, 2017). As per an estimate, the use of quality seed, keeping other inputs constant, could enhanced the yield by more than 20%. Therefore, use of high quality seed is vital to produce healthy and vigorous seedlings that lead to better crop stand and higher yield under a wide range of environment. Reports showing benefits of priming on vigour enhancement are numerous, but those highlighting the biochemical and physiological mechanism of such changes, especially in kabuli chickpea are scarce. Each treatment varies in their effectiveness, therefore the effect of metabolic changes due to different seed priming agents are specific. With respect to this hypothesis, the physiological changes caused by the most effective priming agent (in terms of improved vigour) and their relationships to laboratory germination and field establishment was evaluated.

Materials and Methods

Seed materials : The experiments were conducted at ICAR-Indian Institute of Pulses Research, Kanpur. Seeds of chickpea cv. Kripa were used as an experimental material. Kripa is an extra large seeded kabuli chickpea having a hundred seed weight of ~55g.

Seed priming treatment : There were 9 treatments with 4 seed pre-treatment reagents, namely, Polyethylene glycol (-0.5, -1.0, -1.5 MPa), Mannitol (2, 4 %), Salicylic acid (50 nm), Gibberellic acid (50 ppm), hydro-priming and unprimed seed as control. Surface sterilization of seeds was carried out by immersing completely in 0.1% mercuric chloride for 2 min followed by washing with distill water. These surface sterilized seeds were placed in between two layer of filter paper pre moistened with the respective priming agents. Thereafter, the Petri-plates were kept at dark in incubator set to a temperature of 20°C for varying time depending upon the treatment. For PEG and mannitol, the priming duration was 24 hrs and for rest of the treatments the priming duration was 17 hrs. In any case, there was no evident cracking of seed coat or protrusion of radicle at the end of the priming period. After the respective priming duration, the seeds were thoroughly washed 2-3 times using distill water to remove osmoticum or chemicals from seed coat, followed by drying at ambient laboratory temperature for 3-4 days till the seeds had dried to their original moisture content. Dry unprimed seeds were used as control.

Laboratory germination studies : Total of 150 seeds (three replicates of 50 seeds each) were kept for germination in an incubator at 20°C in rolled germination towels (ISTA, 2011). Germination was observed three to four times a day. Seed was considered germinated when the radical had attained a length of 2 mm. After seven days, the final percentage germination, actual time to 50% emergence (t_{50}) and uniformity of germination ($t_{90/10}$) were calculated. Germination energy represents the ratio of germinated seeds over total seeds on 4th day of experiment. Daily germination performance of primed and unprimed seeds were

interpreted by germinator model of Joosen (2010) by the equation given by El-Kassaby *et al.* (2008).

Field experiment : The field evaluation of primed and unprimed control was done in New Research Campus, ICAR-Indian Institute of Pulses Research, Kanpur for two consecutive rabi season (2015-16 and 2016-17). The crop was sown in a plot of size 4 m length and 2 m width in Randomized Complete Block Design in three replicate. Each replication (plot) had four rows with 25 seeds placed in each row (total of 100 seeds per plot). Field emergence data was recorded after 21 days of sowing. At harvest, ten plants were randomly selected per replication to measure plant height (cm), number of pods and seeds per plant. The seeds obtained from each plot represents seed yield per plot.

Priming-induced metabolic changes : To extract total protein for SDS PAGE analysis, seed material (200 mg) was ground in a pre-chilled mortar pestle in chilled extraction buffer (0.1 M Tris (pH 7.5), 1% Triton X-100). The amount of total proteins was quantified using Lowry's method (Lowry *et al.*, 1951). For fractionation of proteins, 20 g of protein was mixed in appropriate volume of 4X loading buffer and resolved on 12% gel and electrophoresed at 80 V. The gel was stained in a staining solution (0.1% Coomassie brilliant blue R-250, 50% methanol and 10% glacial acetic acid) for overnight. After washing several times in de-staining solution (40% methanol and 10% glacial acetic acid), the gel was scanned, and molecular mass of the expressed peptides was ascertained by comparing with standard protein markers.

Electrical conductivity of seed leachate was estimated according to ISTA (2011). Total sugar contents were estimated by anthrone reagent (Yoshida *et al.*, 1976). Reducing sugar was estimated according to Miller (1959) and differences between total sugar and reducing sugar gave the content of non reducing sugar.

Statistical analysis : SPSS 16.0 software package was used for statistical analysis. Duncan Multiple Range Test (DMRT) was performed at $p=0.05$ to test the significance of differences, which were indicated by alphabets on data sets.

Results and Discussion

All seed treatments significantly improved laboratory germination percent, germination energy and subsequently reduced mean germination time and time to achieve 50% germination (t_{50}) over control. Also, the time interval between 10% and 90% of the seeds to germinate or uniformity of germination ($t_{90/10}$) was better in seeds primed by different chemicals/agents. Data on germination attributes of seeds revealed significant ($P=0.05$) differences among priming treatments (Table 1). Although all treatments were effective in curtailing the time to achieve 50% germination, hydro-priming, salicylic acid and 2% mannitol were the most effective. Similar increase in germination speed and synchrony of chickpea (Elkoca *et al.*, 2007), maize (Tian *et al.*, 2014), rice (Basra *et al.*, 2005), sunflower (Wahid *et al.*, 2008) have been reported through seed priming in previous studies. Rapid and synchronized germination after priming may be the result of dormancy breakdown. Increased metabolic activities and replication in root tips might have resulted into early and synchronized germination after priming (Basra *et al.*, 2002). Mean germination time was highest (29.55 hr) in control, but it was lowest (11.95 hr) in hydro-primed seeds. Seed priming greatly improved germination energy (potential of seed to germinate vigorously) as compared to control, being highest in PEG -1.5 Mpa, hydro-priming, salicylic acid and 2% mannitol followed by gibberellic acid and 4% mannitol. Likewise, laboratory germination percent was improved by all the priming treatments but most of all by PEG -1.5 Mpa and hydro-priming (Table 1, Fig. 1). Seed germination stage and seedling stage are most critical stages in crop cycle which affects crop yield both qualitatively and quantitatively. Seed germination comprises of three phases, it starts with water imbibition by dry seeds, followed by a lag phase (germination *stricto sensu*) and ends with radical protrusion through seed coat. Seed priming prolongs phase II that is lag phase, that allows seed to go through various pre-germination changes but prevents radical protrusion. Results indicated that seed priming significantly decreased mean germination time and time taken to achieve 50% germination. However, seed priming increased germination energy as well as final germination value in kabuli chickpea cv. Kripa. The favourable effect of seed

Table 1: Effect of priming treatments on germination and related attributes of chickpea

Treatments	T_{50} (hr)	$T_{90/10}$ (hr)	MGT (hr)	Germination energy	Germ (%)
Control	32.65 ^a	24.03 ^a	29.55 ^a	62.5 ^d	72.50 ^c
PEG -0.5 Mpa (24 hr)	28.85 ^b	20.77 ^{ab}	25.75 ^b	65.0 ^{cd}	90.00 ^{ab}
PEG -1.0 Mpa (24 hr)	26.20 ^{bc}	20.50 ^{ab}	26.40 ^{ab}	67.5 ^{bcd}	90.00 ^{ab}
PEG -1.5 Mpa (24 hr)	22.25 ^d	20.77 ^{ab}	21.90 ^c	92.5 ^a	95.00 ^a
Mannitol 2% (24 hr)	12.70 ^f	17.55 ^{ab}	13.70 ^{de}	87.5 ^a	87.50 ^{ab}
Mannitol 4% (24 hr)	23.30 ^{cd}	15.85 ^{ab}	23.00 ^{bc}	72.5 ^{bc}	85.00 ^b
Salicylic acid (17 hr)	11.30 ^f	19.90 ^{ab}	13.00 ^{de}	85.0 ^a	87.50 ^{ab}
Gibberellic acid (17 hr)	20.42 ^d	17.77 ^{ab}	20.16 ^c	73.5 ^b	86.33 ^b
Hydropriming (17 hr)	10.15 ^f	17.45 ^{ab}	11.95 ^e	85.0 ^a	95.00 ^a

Mean followed by similar alphabets indicates non-significant differences between the values at 0.05 level of significance

Table 2 : Effect of priming treatments on biochemical changes in chickpea seeds

Treatments	Total sugar (mg g ⁻¹ fresh seed)	Reducing sugar (mg g ⁻¹ fresh seed)	Non reducing sugar (mg g ⁻¹ fresh seed)
Control	6.66 ^f	3.61 ^e	3.05 ^a
PEG -0.5 Mpa	8.77 ^c	6.65 ^b	2.12 ^b
PEG-1.0 Mpa	11.16 ^a	8.22 ^a	2.94 ^a
PEG -1.5 Mpa	7.60 ^{de}	6.28 ^b	1.32 ^c
Mannitol 2%	7.60 ^{de}	6.32 ^b	1.28 ^c
Mannitol 4%	7.52 ^e	4.61 ^d	2.91 ^a
Salicylic acid	7.97 ^d	6.41 ^b	1.55 ^c
Gibberellic acid	9.44 ^b	6.55 ^b	2.89 ^a
Hydropriming	7.79 ^{de}	5.41 ^c	2.37 ^b

Mean followed by similar alphabets indicates non-significant differences between the values at 0.05 level of significance

Table 3 : Effect of priming treatment on emergence and yield attributes of chickpea

Treatments	Field emergence (%)	Plant height (cm)	No. of pods per plant	No. of seeds per plant	100-seed weight (g)	Yield (kg ha ⁻¹)
Control	47.83 ^b	40.18 ^a	17.73 ^a	16.89 ^a	47.74 ^{bc}	507.28 ^c
PEG (-0.5 Mpa)	68.17 ^a	40.10 ^a	17.94 ^a	17.55 ^a	53.79 ^a	733.09 ^a
PEG (-1.0 Mpa)	67.33 ^a	40.53 ^a	17.73 ^a	16.97 ^a	47.05 ^{bc}	713.00 ^{ab}
PEG (-1.5 Mpa)	62.33 ^{ab}	39.11 ^a	18.27 ^a	17.80 ^a	46.12 ^c	610.81 ^{abc}
Mannitol (2%)	67.67 ^a	38.42 ^a	19.47 ^a	19.63 ^a	53.13 ^a	607.18 ^{abc}
Mannitol (4%)	63.33 ^{ab}	36.94 ^a	16.15 ^a	16.90 ^a	54.56 ^a	552.58 ^{bc}
GA (50ppm)	59.50 ^{ab}	40.24 ^a	16.34 ^a	14.23 ^a	53.23 ^a	486.19 ^c
SA (50nm)	60.33 ^{ab}	40.36 ^a	16.43 ^a	15.20 ^a	52.63 ^a	579.39 ^{abc}
Hydropriming	68.67 ^a	41.15 ^a	17.94 ^a	16.80 ^a	49.52 ^b	717.98 ^a

Mean followed by similar alphabets indicates non-significant differences between the values at 0.05 level of significance

Fig. 1 : Effect of the priming treatments on germination curve of chickpea seeds. Bars are $\pm$ S.E.

Fig. 2 : Electrophoretic pattern and molecular weight of expressed protein following priming in chickpea

Fig. 3 : Effect of priming treatments on electrical conductivity of chickpea seed leachate. Bars with same alphabets were not significantly different ($p=0.05$).

priming observed may be attributed to repair mechanisms, enhanced protein synthesis, enhanced respiratory activity of seeds, breaking of dormancy, imbibition and enzymes activation.

Plant height, number of pods and seeds per plant were not affected by seed priming. However, seed priming significantly increased field emergence. Field emergence was lowest (47.83%) in control, whereas PEG -0.5 Mpa, PEG -1.0 Mpa, 2% mannitol and hydro-priming recorded highest (67-69%). Similarly, seed priming improved hundred seed weight over control, except for PEG -1.0 and PEG -1.5 Mpa. Osmo-priming with PEG -0.5 Mpa recorded highest yield

followed by PEG -1.0 Mpa and hydro-priming. Kaur *et al.* (2002), have reported improved performance of osmo-primed and hydro-primed chickpea crop under field with an increase in yield up to 39 to 64%. Favourable effect of seed priming treatments in terms of increasing field emergence may be attributed to *de novo* protein synthesis, membrane repair and better accumulation of reserves. These findings are in agreement with Tian *et al.* (2014); Harris *et al.* (2000, 2002), who reported better performance following pre-soaking and drying. The metabolic processes which are induced in seed by priming persist even after drying (Asgedom and Becker, 2001). Thus, upon sowing, dried primed seed as soon as

imbibes water revives the seed metabolism, resulting into faster and synchronous emergence.

SDS PAGE profiling indicated the differential expression of proteins with seed priming. Notably, protein of 89 kDa was expressed in all the priming treatments except for control. Similarly, proteins of 20 and 25 kDa were expressed in seeds primed with PEG, mannitol, salicylic acid and hydro-priming, while a 12 kDa protein was induced only by hydro-priming. In general, the band intensity as well as the number of proteins induced by seed priming increased over control (Fig. 2). Kubala *et al.* (2015), found positive association of improved germination in PEG-osmoprimed brassica seeds with protein synthesis and reported higher expression of proteins involved in water transport, modification in cell wall, cell division and management of oxidative stress after PEG-osmopriming. Proteins like tubulin, sub unit of 12S-seed storage protein, heat shock proteins, phosphoenolpyruvate carboxykinase, catalase increased upon hydropriming and/or PEG-osmopriming in *Arabidopsis* seed, all of which has very important role in germination or plant growth (Gallardo *et al.*, 2001). The sugar content of the seed (total, reducing and non-reducing) was significantly influenced by various seed priming treatments. Highest contents of total and reducing sugars were recorded in the seeds primed with PEG - 1.5 Mpa for 24 hrs (Table 2). Seed priming resulted in significantly higher contents of total as well as reducing sugars but reduced the content of non-reducing sugars over control. Our findings corroborated with the findings of Basra *et al.* (2005). They reported that the increase in alpha amylase activity following priming resulted into increased content of total and reducing sugar as alpha amylase hydrolyzed non reducing sugar to reducing and starch to sugar. Rapid, uniform and better germination of primed seed over control suggested that the effect of starch hydrolysis were not lost during re-drying process. Regarding electrical conductivity of seed leachate, unprimed control seeds recorded highest value and seeds primed by salicylic acid recorded lowest. However, electrical conductivity of seed leachate in salicylic acid did not differ with that of Mannitol (2%), Mannitol (4%), Gibberellic acid and hydro-priming (Fig. 3). It is evident that priming process leads to repair mechanism of membrane, enhancing its stability as primed seeds recorded lower electrical conductivity than unprimed control.

Seeds primed with water for 17 hrs and PEG -0.5/-1.0 Mpa for 24 hrs resulted into higher field emergence and seed yield, therefore, these treatments may be recommended for increasing productivity of chickpea. Enhanced germination, field emergence and seed yield of hydro and osmo-primed seeds were associated with *de novo* proteins synthesis, repair mechanisms and greater availability of substrate.

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