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SHORT COMMUNICATION

Plant Emergence and t_{50} Responses of Two Chickpea Cultivar Differing in Seed Coat Colour to PEG-Osmopriming at Sub-optimal Temperature

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Abstract The effect of polyethylene glycol (PEG) priming on seedling emergence was assessed in two cultivars of chickpea (*Cicer arietinum* L.) differing in testa colour (Kripa-light coloured; DCP 92-3-yellowish brown) at optimal (20 °C) and sub optimal (14 and 30 °C) temperatures using sterilized sand and soil as substrate. PEG primed seeds performed better in sand. In soil, primed Kripa seeds failed to emerge at sub optimal temperatures while unprimed seeds emerged at all temperatures. DCP 92-3 was able to maintain the emergence as that of sand at all temperature but reduced significantly in terms of final emergence and time to reach 50% germination (t_{50}) following osmopriming. Such contrasting performance of osmoprimed seeds in sand and soil may be attributed to decrease in seed tannin content following PEG osmopriming. Kripa and DCP 92-3 had a seed tannin content of 206.4 ± 2.2 and 550 ± 2.2 mg/100 g seed fresh weight respectively, which was reduced to 101.6 ± 2.6 and 394.7 ± 6.1 mg/100 g seed fresh weight following priming. The result suggests using of cultivars with high tannin content when sowing has to be done at sub optimal temperatures.

Keywords PEG-osmopriming · Plant emergence · Chickpea · Sub optimal temperature · Tannin

Rapid and uniform emergence determines the successful plant establishment. Seed germination and early plant emergence are complex phenomenon which is dependent on the interaction of temperature, moisture, soil properties and photoperiod. In addition to water and oxygen, temperature plays an important role in seed germination and seedling establishment [1]. Low temperature is considered a major problem affecting stand establishment for autumn sown chickpea [2]. In countries like Canada, Russia early sown chickpea are subjected to low temperature during germination resulting in poor field stand and poor seedling vigour due to low temperature soaking injury [3]. However, in tropical countries like India, Pakistan, Bangladesh chickpea is majorly grown as a rainfed crops. Therefore, to avoid the dry spell at least during the initial stages of growing season, planting early on the residual moisture of the monsoon rains provides an opportunity. But, such early planting exposes the seeds to higher temperature during germination and seedling growth stages affecting the crop stand [4].

Osmopriming is a technique whereby the seeds are exposed to osmoticum like PEG, mannitol, glycerol etc. which allows controlled uptake of water by seeds, enough for the cellular repairs and to go through the initial phases of germination but not radicle protrusion. PEG is often considered as a better osmoticum as it is non toxic. However, PEG in a medium reduces availability of the oxygen due to its viscosity which can be overcome by aerating the PEG containing medium. There are reports on the effectiveness of PEG osmopriming in enhancing the germination and field emergence of many crops [5, 6]. PEG osmopriming enhances the germination and vigour of the chickpea seeds at sub-optimal temperatures [7]. However, the test was conducted in a sterile medium (Petri-dishes). [8, 9] found that PEG affected negatively the emergence of pigmented sorghum as the primed pigmented seeds performed worse than unprimed seeds at low temperature.

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Pigmentation to the seeds is imparted by the presence of tannins in the seed coat. Tannins are the complex polyphenols which are known to have antimicrobial, antifungal properties, also protect seeds from precocious germination and susceptibility to insect pests. Vigour differences in many crops have been reported to be associated with seed coat pigmentation [9–12]. Absence of tannin makes the seeds prone to fungal or mould attack prior to germination, thus, the use of high tannin pigmented seeds may help in preventing fungus attack and increase field stand especially at sub optimal conditions. Present investigation was undertaken to find the effectiveness of PEG osmopriming on plant emergence of chickpea cultivars differing in seed tannin content under different temperature regimes.

Present investigation included following factors: seed treatment-2; genotype-2; germination temperature-3 and germination substrate-2. Chickpea varieties, Kripa with white testa and DCP 92-3 having yellowish brown testa were used. Both the varieties had the laboratory germination of more than 90% at 20 °C as assessed in between paper [13]. For osmopriming seeds were fully immersed in aerated PEG 6000 osmotic solution having − 0.5 MPa water potential at 25 °C [14] for 24 h. The PEG treatment was chosen based on the previous research findings on chickpea seed germination [7]. After 24 h, the seeds were rinsed with distilled water, dried on blotting paper at room temperature for 24 h. Germination trial was conducted in a dark incubator at three different temperatures (14, 20 and 30 °C), 20 °C being considered ideal for seed germination of chickpea [13]. Sterile sand and soil collected from the field of Indian Institute of Pulses Research was used as germination substrate. Total of 120 seeds (three replicates of 40 seeds each) were placed in aluminium seedling trays (one seed per hole) filled with either sand or soil, at 2 cm of depth. The substrate was initially wetted with distilled water (30 ml) and was replenished as needed. The experiment was conducted in a randomized complete block design. Seedling emergence was counted daily. The seeds were considered emerged when seedling reached approximately 1 cm length. At the end of the experiment, the final percentage emergence and actual time to 50% emergence (t_{50}) were calculated.

Tannin content was estimated from the seeds before and after osmopriming. 400 mg of ground material was extracted in 20 ml of 70% acetone in electric shaker for 2 h followed by filtering it through Whatmaan filter paper No. 1. Folin–Ciocalteu method was used for determination of tannin content which includes spectrophotometric reading at 725 nm (Shimadzu). 1 ml of sample extract, 75 ml distill water, 5 ml Folin–Ciocalteu reagent and 10 ml of sodium carbonate solution was mixed and absorbance was measured after 30 min [15].

Daily germination performance of primed and unprimed seeds across the temperatures were interpreted by "Germinator curve-fitting1.29.xls" microsoft excel model" [16] using the following equation [17]

$$\mathrm{Y} = Yo + \frac{\mathrm{a}\mathrm{X}^{b}}{C^{b} + X^{b}}$$

where y is cumulative emergence percentage at time x, y0 is intercept on the y axis, a is maximum emergence percent, b is steepness of the curve and c is time taken by 50% of seeds to germinate (t_{50}). The arcsine transformed emergence data and t_{50} data were statistically analysed by completely randomized two-way ANOVA using SAS software version 9.3 separately for cultivar and each temperature. A one-way ANOVA was used for percent emergence and when germination occurred in one substrate only and for t_{50} data when 50% plant emergence was not achieved in one of the substrate [9].

Kripa (white coloured) and DCP 92-3 (pigmented) had a tannin content of 206.4 ± 2.2 and 550.0 ± 2.2 mg TA/100 g seed fresh weight respectively. Upon PEG priming, seed tannin content in Kripa reduced to 101.6 ± 1.6 and in DCP 92-3 to 394.7 ± 6.1 mg TA/100 g seed fresh weight.

The cumulative germination curve of primed and unprimed seeds of both the cultivars in sand and soil across the temperatures is given in Fig. 1. In sterilized sand, sub optimal temperature of 14 and 30 °C either reduced (as percentage) or delayed (t_{50}) or both the final emergence of unprimed seeds (Tables 1, 2). Kripa attained final plant emergence of 73% (t_{50} = 8.3 days) at optimum temperature (20 °C) which decreased to 45 and 20% at 14 and 30 °C (sub optimal temperature), respectively. In DCP 92-3, final plant emergence of unprimed seeds in sand at 20 °C was 75% which reduced to 66.7% at 14 °C and 58.3% at 30 °C. PEG osmopriming was very effective in enhancing the final emergence and in reducing the t_{50} in both the cultivars especially at sub optimal temperatures. In Kripa, plant emergence increased by 29.5% at 14 °C and by 91.5% at 30 °C upon priming. In DCP 92-3, osmopriming enhanced seed germination, whereby an increase of about 23% was achieved at 30 °C. In this cultivar, osmopriming reduced the t_{50} from 5.5 days to 4.3 days at 30 °C. [7, 8] had also reported the increased performance of PEG primed seeds at sub optimal temperature.

However, the effect induced by PEG osmopriming in sand tend to lost at non-sterilized soil as the unprimed seeds outperformed the primed seeds in terms of final emergence and in t_{50}. In soil, primed Kripa seeds failed to germinate at sub optimal (14 and 30 °C) temperature, whereas the unprimed seeds were able to germinate at all temperatures, though significantly slower and lesser than in sand. At 20 °C, the emergence was reduced by 41.7% following priming treatment. Tannin possesses antifungal

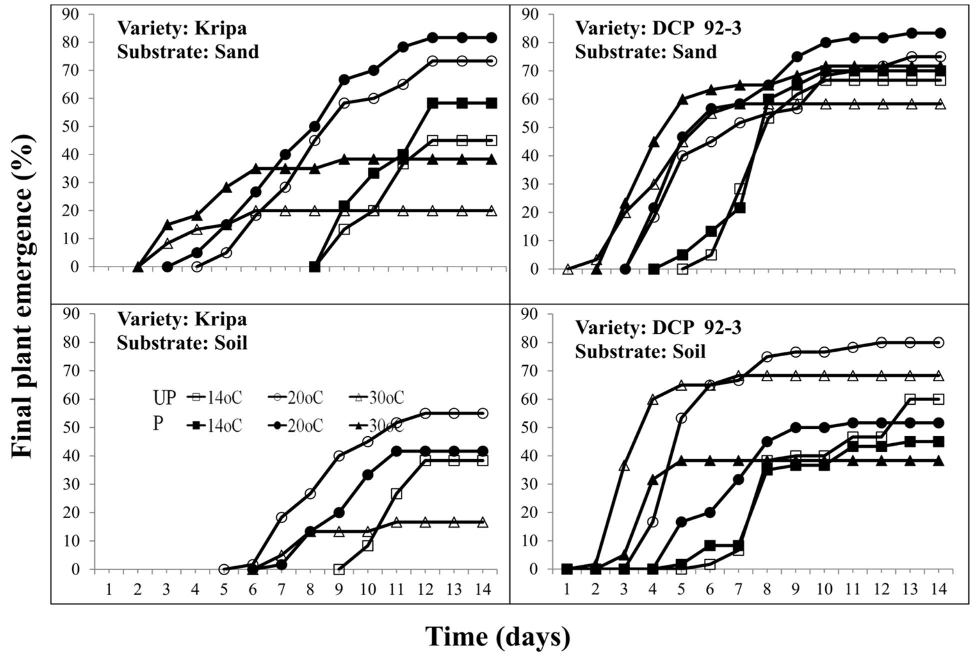

Fig. 1 Cumulative emergence curve of primed and unprimed seeds of chickpea at 14, 20 and 30 °C in sand and in soil

Table 1 Effect of seed osmopriming and germination substrate on seedling emergence and t_{50} in chickpea variety Kripa at optimal (20 °C) and sub optimal (14 and 30 °C) temperature

Germination substrate (S)	Seed treatment (P)	Seedling emergence (%)			t_{50} (days)		
		Temperature (°C)			Temperature (°C)		
		14	20	30	14	20	30
Sand	UP	45.0 (42.1)	73.3 (58.9)^B	20.0 (26.6)^A	–	8.3	–
	P_{PEG}	58.3 (49.8)	81.7 (64.6)^A	38.3 (38.2)^B	10.8	7.7	–
Soil	UP	38.3 (38.2)	55.0 (47.9)^C	16.7 (24.1)	–	10.4	–
	P_{PEG}	–	41.7 (40.2)^D	–	–	–	–
Mean of the main factors							
Germination substrate (S)	Sand	51.7 (46.0)	77.5 (61.7)^A	29.2 (32.7)	–	8.0	–
	Soil	–	48.4 (44.1)^B	–	–	–	–
Seed treatment (P)	UP	41.6 (40.1)	64.2 (53.2)	23.4 (28.9)	–	9.4	–
	P_{PEG}	–	61.7 (51.7)	–	–	–	–
Significance	S	–	NS	–	–	–	–
	P	NS	***	***		NS	
	S × P	–	***	–	–	–	–

Values within brackets are arcsine transformed values

Different letters within a temperature indicates significant difference at $P \leq 0.05$ level of significance

UP, unprimed; P_{PEG}, PEG primed; S, germination substrate; P, seed treatment; NS, non significant

*** Significant at $P \leq 0.05$ level of significance

and anti microbial properties [18]. Kripa seed contains low seed tannin which was further reduced upon priming. Also, it showed a delayed germination at 14 °C. Delayed emergence accompanied by reduced tannin content make the seed more vulnerable to fungal attack. White mycelia fungal growth was observed in Kripa seeds sown in soil especially at sub optimal temperatures. Hence, primed Kripa seeds failed to germinate in soil at sub optimal temperatures.

Seeds of DCP 92-3 were able to maintain similar if not less the germination percent at soil as that of sand. It clearly indicates the seed vigour differences between

Table 2 Effect of seed priming and germination substrate on seedling emergence and t_{50} in chickpea variety DCP 92-3 at optimal (20 °C) and sub optimal (14 and 30 °C) temperature

Germination substrate (S)	Seed treatment (P)	Seedling emergence (%)			t_{50} (days)		
		Temperature (°C)			Temperature (°C)		
		14	20	30	14	20	30
Sand	UP	66.7 (54.7)	75.0 (60.0)^{A}	58.3 (49.8)^{A}	8.4	6.7^{AB}	5.5^{A}
	P_{PEG}	70.0 (56.8)	83.3 (65.9)^{A}	71.7 (57.8)^{A}	8.3	5.8^{B}	4.3^{B}
Soil	UP	60.0 (50.7)	80.0 (63.4)^{A}	68.3 (55.7)^{A}	9.4	5.1^{B}	3.5
	P_{PEG}	45.0 (42.1)	51.7 (46.0)^{B}	38.3 (38.2)^{B}	–	8.0^{A}	–
Mean of the main factors							
Germination substrate (S)	Sand	68.4 (55.8)	79.2 (62.9)^{A}	65.0 (53.7)	8.4	6.3	4.9
	Soil	52.5 (46.4)	65.9 (54.2)^{B}	53.3 (46.9)	–	6.6	–
Seed treatment (P)	UP	63.4 (52.8)	77.5 (61.7)	63.3 (52.7)	8.9	5.9	4.5
	P_{PEG}	57.5 (49.3)	67.5 (55.2)	54.7 (47.7)	–	6.9	
Significance	S	NS	***	NS	–	NS	–
	P	NS	NS	NS	NS	NS	***
	S × P	NS	***	***	–	***	–

Values within brackets are arcsine transformed values

Different letters within a temperature indicates significant difference at $P \leq 0.05$ level of significance

UP, unprimed; P_{PEG}, PEG primed; *S*, germination substrate; P, seed treatment; NS, non significant

*** Significant at $P \leq 0.05$ level of significance

pigmented and non pigmented cultivars. Vigour differences in many crops are reported to be associated with seed coat pigmentation [9–12]. Primed and unprimed seeds of DCP 92-3 emerged at all temperatures though priming negatively affects the final emergence percentage and t_{50}. PEG priming reduced the final emergence by 33.3, 54.7 and 78.3% at 14, 20 and 30 °C, respectively.

According to Singh et al. [19], soaking in water significantly reduced the tannin content in chickpea seeds. The loss of tannin during osmopriming may be due to their leaching in the medium as was seen by browning colour of the medium in DCP 92-3. Also, PEG is a tannin binding polymer [20] that binds tannins irreversibly, and it reduces the formation of protein-tannin complexes [21]. Thus, PEG is supplemented with the feed to alleviate the negative effects of tannins on livestock [22]. Tannin is mostly present in testa imparting colour to the seed. Therefore, during osmopriming, tannin present in the testa binds with the PEG. As the molecular weight of PEG is high it does not enter into the seed [23]. Thus, the PEG bound tannins are removed when the seeds are washed at the end of the priming treatment. The tannin content of DCP 92-3 after priming was similar to unprimed Kripa seeds and was able to germinate in soil across the temperature so as unprimed Kripa seeds. But the tannin content of Kripa seeds after priming was significantly reduced hence may be the reason that they do not germinate at sub optimal temperature.

Tannins are anti nutritional factors and are considered undesirable. Breeding objectives are directed towards lowering the tannin content from the seeds. However, tannins are an important protectant for seeds survival in sub optimal conditions as it protects them from different biotic stress. Therefore, our breeding strategies should be formulated keeping in view the importance of such compounds for seed survival. Clear differences were observed between the cultivars on their behavior at soil, therefore cultivar with high tannin content may be used for sowing at sub optimal conditions. PEG osmopriming was not effective in improving the emergence of chickpea in non sterilized substrate suggesting adopting other osmoticum than PEG.

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