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RESEARCH ARTICLE

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Seed Hydropriming Duration for Potential Seedling Vigour in Pigeon Pea [*Cajanus cajan* (L.) Millsp.], Sunflower (*Helianthus annuus* L.) and Rice (*Oryza sativa* L.)

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ABSTRACT

Background: In the climate change scenario, the increasing frequency of drought is the major constraint for crop yields. Under rainfed conditions, the most vulnerable stage of the crop cycle is seed germination and seedling establishment. One of the approaches to enhance the seedling establishment is hydropriming or seed hardening. Hydropriming is a recommended practice for rainfed crops to improve the appropriate crop stand and productivity. For effective hydropriming, the priming duration is crucial because a lesser duration of priming will be ineffective, a longer duration will damage the seed coat, embryonic axis and the seed will fail to germinate. The detailed investigation on optimal priming duration in major pulse (pigeon pea), oilseed (sunflower) and cereal (rice) is scanty.

Methods: Hence, the effect of priming duration on the imbibition rate and seedling establishment in major pulse (pigeon pea, cv. BRG-5), oilseed (sunflower hybrid, KBSH-78) and food crop (rice, cv. MTU-1001) was conducted in the laboratory, Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India during the year 2020. The experiment was laid out in a completely randomized design with 14 soaking period treatments and five replications for imbibition studies and three replications for seedling establishment in the field.

Result: The imbibition rate was rapid in the beginning hours and reached zero by 10 to 12 hours. The cumulative imbibition of 30-40% was attained in the first 2-3 hours of priming in pigeon pea and sunflower and 11 hours in rice. Similarly, 2-3 hours of hydropriming resulted in higher SVI-II (field establishment), but a 7-8 hour period of priming showed the highest seedling growth and seedling vigor index in all three crops. Therefore, 3-hour hydropriming is critical and can be extended to 7-8 hours for better seedling establishment in pigeon pea, sunflower and rice.

Key words: Imbibition, Pigeon pea, Rice, Seedling vigour, Sunflower.

INTRODUCTION

In the climate change scenario, drought situation is the predominant threat to crop production under rainfed conditions (Ocvirk *et al*., 2020) and drought is generally accompanied by heat (Krishna *et al*., 2021) leading to more than a 50% reduction in the yield of crops (Bukhari *et al*., 2019). The primary constraints for productivity under rainfed conditions are poor seed germination, seedling emergence and crop stand (Singh *et al*., 2015; Finch-Savage and Bassel, 2016; Fahad *et al*., 2017). The seedling stage is more sensitive to drought stress (Nanja Reddy *et al*., 2021). Hydropriming or seed hardening is one of the approaches to improve seed performance and offset the negative effects of abiotic stresses (Rajashekara *et al*., 1970; Hussain *et al*., 2017). Hydropriming is the rapid uptake of water that induces partial hydration under *in vitro* conditions to initiate the germination process but not the protuberance of radical (Fahad *et al*., 2017). The priming was reported to enhance the hydrolytic enzymes like alpha-amylase activity to increase the conversion of starch to glucose for the respiration process of seed germination in cereals (Zhao *et al*., 2018; Ren *et al*., 2023), proteases (pulses) and lipases (oil seeds). Hydropriming improves the metabolism and transport of proteins, free amino acids and sugars from the endosperm to the embryo for rapid seed germination (Hussain *et al*., 2017) and thus improves seedling emergence and crop establishment upon sowing under field conditions (Singh *et al*., 2015; Mishra *et al*., 2017).

However, the effect of hydropriming varies with crop (Sarvjeet *et al*., 2017 in rice, finger millet, chickpea), varieties within a crop (Carver *et al*., 2014; Nakao *et al*., 2020), seed density (Nakao *et al*., 2020), drought conditions (Carver *et al*., 2014) and period of hydropriming (Carver *et al*., 2014; Mishra *et al*., 2017). For effective hydropriming, the duration

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of the priming is crucial, but in general, overnight soaking is followed. Such a longer priming duration might lead to seed deterioration and a shorter priming duration will be ineffective in initiating the germination process. Although genotypic differences exist for the priming effect (Nakao *et al*., 2020), as a proof of concept, considering one popular variety for each one of the major crops could be meticulous. Therefore, the objective of the present investigation was to determine the critical and optimal priming duration in the predominant pulse (pigeon pea), oilseed (sunflower) and cereal (>45% area is upland rice) crops for better seedling growth and seedling vigour index.

MATERIALS AND METHODS

The experiments were conducted in the laboratory (temperature of 26°C and 65% RH) and the field facility of the Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bengaluru (May 2020 to August 2020). The fresh seeds of pigeon pea (cv. BRG-5), sunflower hybrid (KBSH-78) and rice (MTU-1001) were hydro-primed (10g in 100 ml water in a 200 ml beaker) in five replications in a completely randomized design. At every hour interval of priming, seeds were strained out of the beaker using a mesh strainer, gently wiped with tissue paper to remove the adhered water, took the fresh weight and placed back into the beaker. The rate of imbibition and the cumulative imbibition were calculated. In another set, seeds were hydro-primed for 0 to 12 h at hourly and at 24 h, the primed seeds were shade-dried to their original dry weight. Such seeds were sown in the field in three replications with protective irrigation during July 2020 for 15 days. At 15 days after sowing, the seedling length and weight were recorded to calculate the seedling vigour index. ANOVA was performed using OPSTAT statistical software (Sheoran *et al*., 1998).

Imbibition rate in 1st hour (%) =

$$\frac{\text{Fresh weight of imbibed seeds in } 1^{st}\text{ h - Initial weight of seeds}}{\text{Initial weight of seeds}} \times 100$$

Imbibition rate in 2nd hour (%) =

$$\frac{\text{Fresh weight of imbibed seeds in } 2^{nd}\text{ h - Fresh weight of imbibed seeds in } 1^{st}\text{ h}}{\text{Fresh weight of imbibed seeds in } 1^{st}\text{ h}} \times 100$$

Cumulative Imbibition in 1st hour (%) =

$$\frac{\text{Fresh weight of imbibed seeds in } 1^{st}\text{ h - Initial weight of seeds}}{\text{Initial weight of seeds}} \times 100$$

Cumulative Imbibition in 2nd h (%) =

$$\frac{\text{Fresh weight of imbibed seeds in } 2^{nd}\text{ h - Initial weight of seeds}}{\text{Initial weight of seeds}} \times 100$$

Seedling vigour index = Seedling length $\times$ Seedling dry weight (As the germination % was 100% in all the priming periods)

RESULTS AND DISCUSSION

Seed germination and seedling emergence are the prominent stages of crop establishment, plant population and productivity (Nakao *et al*., 2018). In the climate change scenario, frequent drought is one of the major threats to seed germination and crop establishment that depends on rainfall for seed germination in arid and semi-arid regions (Angadi and Entz, 2002). The predominant crops like pigeon pea, sunflower and upland rice are drought-prone under rainfed conditions. One of the approaches to mitigate the drought stress during the initial stage of the crop could be by adopting the high vigour seeds or improving the seedling vigour (Matsushima and Sakagami, 2013). Among these two, hydropriming or seed hardening is preferable to enhance the seedling vigour and drought tolerance during the seedling stage (Rajashekara *et al*., 1970). The advantage of seed priming is that the hydropriming can also be extended for low vigour seeds. The increase in seedling vigour due to priming depends on the rapid imbibition of the water by the seed for subsequent physiological processes to improve the seed germination (Singh *et al*., 2015). Imbibition increases the hydrolytic enzymes to break the complex molecules like sugars, proteins and fats into simpler sugars like glucose for increased respiration rate and seed germination (Woodstock, 1988).

Effect of hydropriming duration on imbibition percentage

The rate of imbibition in the first hour of water soaking was very high (130%) in pigeon pea and relatively slower in sunflower (40%) and rice (40%; Fig 1; Maqueo *et al*., 2020). Such higher imbibition rates could be due to the exceptionally lower water potential (matric potential) in the dry seeds at the initial stages, activation of protein synthesis and mitochondrial repairs (Vari and Dadlani, 2010). Also, the high imbibition rate in pigeon pea is due to the thin seed coat compared to sunflower and rice (Fig1; Woodstock, 1988). The imbibition rate was decreased to nearly 10% in sunflower and rice within 3 to 4 hours, whereas it was 5 hours in pigeon pea. The extended hour of imbibition in pigeon pea is due to higher protein and absorbs more water with higher swelling. The imbibition rate reached zero (saturation) by 11 hours (pigeon pea) and 12 hours (sunflower and rice). Once again, in the third phase of the germination process, a marginal increase in imbibition rate from 12 to 24h was observed in pigeon pea and sunflower, whereas it was very high (35%) in the case of rice. The increase in imbibition could create high turgor pressure to enable the rupture of the seed coat and protrusion of radical (Vari and Dadlani, 2010). These results demonstrate that the rate of water uptake will be high during the early hours of seed imbibition and remains similar in the rest of the phases in sunflower and pigeon pea. However, rice followed a rapid increase in imbibition rate during the third phase of germination for radical protrusion.

However, the seed germination depends on the cumulative critical imbibition percentage of 30-40%. It attained within 2-3 hours of priming in pigeon pea and

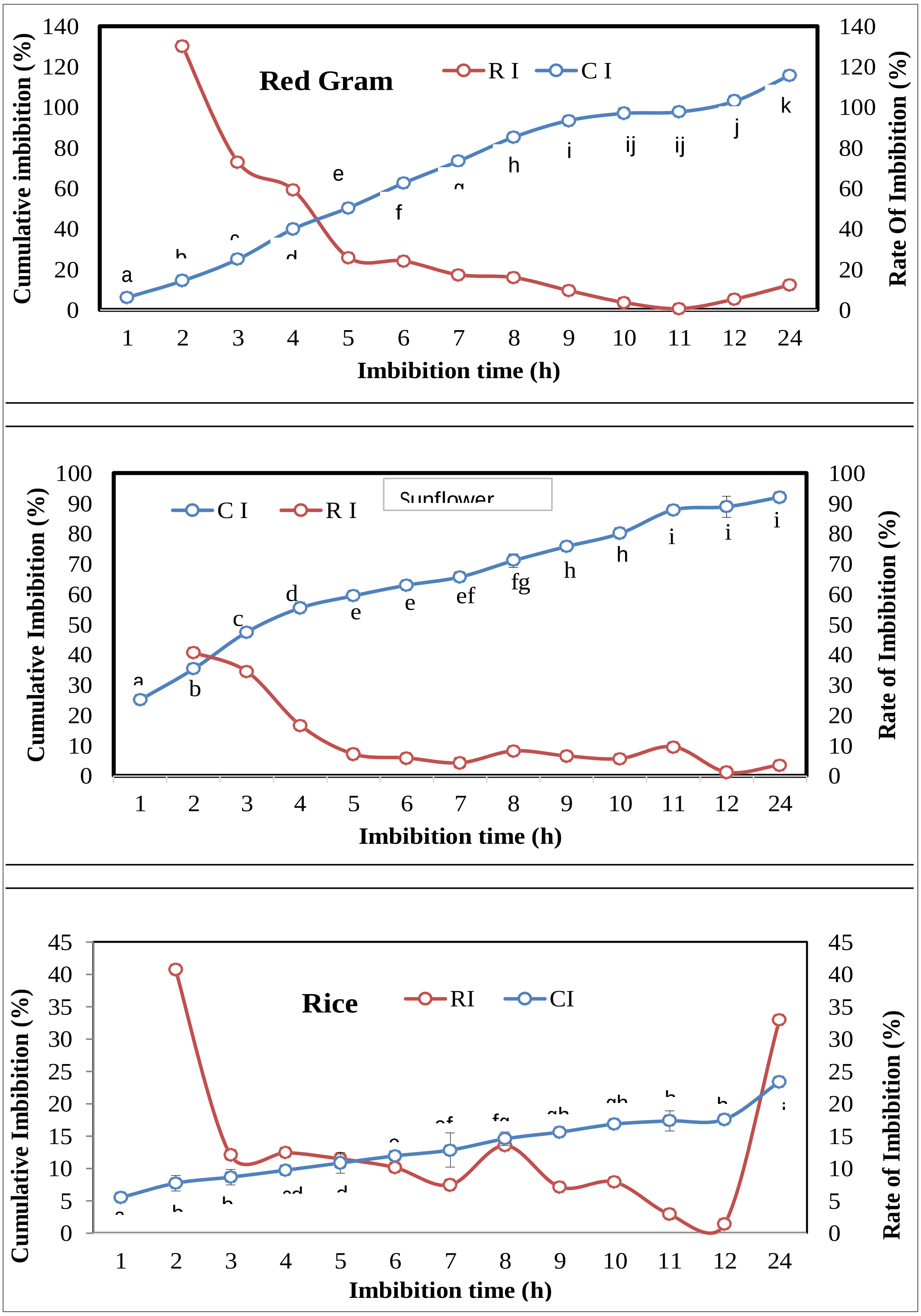

Fig 1: Effect of hydro-priming period on imbibition % of pigeon pea (cv. BRG-5), sunflower hybrid (KBSH-78) and rice (cv. MTU-1001) seeds.

sunflower (Table 1; Priya Reddy and Deshpande, 2020) and 24h in rice to reach 25% imbibition (Fig 1). As pigeon pea and sunflower non-endospermic seeds with cotyledons will have high protein content, thus rapid imbibition compared endospermic seed the rice (Zhao *et al*., 2018). The cumulative imbibition was increased gradually up to 24h priming and reached 120% (pigeon pea), 90% (sunflower) and 25% (rice) (Fig 1). Similar cumulative imbibition of 81.5% by 24h has been reported in sunflower (Priya Reddy and Deshpande, 2020). The higher cumulative imbibition in pigeon pea and sunflower could be due to higher protein content when compared to carbohydrate-rich rice (Zhao *et al*., 2018). Although the required critical imbibition (40%) reached in 2-3h in sunflower and pigeon pea, the long period of soaking period up to 7-8 hours could be apt, as the seeds will be shade dried back original seed moisture content, such priming will induce immediate absorption of water under field conditions. These results imply that the maximum priming period can be 7-8 hours and seeds (pigeon pea) with thinner seed coats and higher protein content will accumulate more water in the seed upon priming. In sunflower, it could be due to higher protein content and a large air space between the hull and kernel. The rice being a carbohydrate-rich seed with less protein, the maximum imbibition was less (25%).

Effect of hydropriming duration on seedling growth and vigour

In pigeon pea, 2-3h priming increased the seedling length, with the highest in the 8-hour priming (25.7 cm; Table 1; Fig 2). Similarly, 1 to 2 hours of hydropriming shown to increase the seed germination, seedling length and seedling vigour index (Sajjan *et al*., 2017; Ashok kumar *et al*., 2017) and also by 4 hours priming, however over-priming above 4h affected these seedling parameters (Carver *et al*., 2014). A 6h hydropriming has been shown to increase the seed germination, seedling length, seedling vigour, chlorophyll content and enzymes like nitrate reductase, nitrite reductase, catalase, peroxidase, superoxide dismutase leading to increased seedling vigour (Tiwari and Agarwal, 2021). In contrast, hydropriming of pigeon pea seeds for 8-12 hours was reported to increase the field emergence, with an increased nodule number and nodule weight at 40 to 90 days after sowing (Mishra *et al*., 2017). The present study confirms that the maximum seedling length and SVI could be attained by 7-8h of priming, beyond which it declines. The seedling fresh weight (931 mg/seedling), dry weight (165.5 mg/seedling) and SVI (4068) were also higher in 7h priming. The SVI was reduced by 8.2% and 5.7%, respectively in 12 and 24h of priming compared to 8h priming (Table 1). Therefore, the critical imbibition period could be 2-3 h and to a maximum of 7-8 hours could be appropriate for pigeon pea, beyond which the thin seed coat will be ruptured, causing damage to the embryo and endosperm.

In sunflower, hydropriming the seed for 8h significantly increased the seedling length (13.08 cm) compared to no-priming (11.95 cm) and significantly reduced by 24h of priming (11.38 cm, Table 2; Fig 2). The SVI was highest (3322) in 8h priming and reduced significantly to 2339 by 24h priming duration (Table 2). Similarly, priming for 6h positively influenced the seed germination and seedling length (Lekic *et al*., 2015). The increase in germination and SVI with priming was ascribed to rapid uptake of water, higher dehydrogenase activity, lower leakage of solutes (Shanthala *et al*., 2013); increased catalase, peroxidase, glutathione reductase, proline content, with a reduced MDA

Table 1: Effect of hydro-priming on field performance of pigeon pea (cv. BRG-5) seedlings at 15 days after sowing.

Priming period (h)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Shoot/root ratio	Fresh wt. (mg/seedling)	Dry wt. (mg/seedling)	SVI-II
0	12.9	9.1	22.0	1.42	668	123.3	2709
1	13.0	10.3	23.3	1.26	678	126.4	2955
2	12.6	10.0	22.6	1.28	751	141.6	3203
3	12.7	9.5	22.2	1.35	687	128.4	2857
4	12.1	9.5	21.5	1.28	672	124.3	2679
5	13.0	9.9	22.9	1.31	769	118.0	2732
6	12.6	9.9	22.4	1.28	743	120.5	2700
7	13.5	10.5	24.0	1.28	931	165.5	3985
8	14.3	11.5	25.7	1.25	911	158.2	4068
9	14.0	11.1	25.0	1.26	901	136.9	3420
10	13.6	10.2	23.8	1.34	833	127.7	3032
11	13.2	10.7	23.9	1.24	846	157.0	3747
12	14.3	9.5	23.8	1.52	870	156.9	3734
24	13.4	11.0	24.4	1.22	849	157.0	3837
SE (m) ±	0.39	0.39	0.56	0.06	37.5	10.3	274
C.D. (P = 0.05)	1.1	1.1	1.6	NS	109	30.0	799
C.V. (%)	5.1	6.7	4.1	8.5	8.2	12.8	14.6

(Ocvirk *et al*., 2020); and increased reducing sugars required for respiration (Bourioug *et al*., 2020). An 8h hydropriming was found to reduce the blooming duration with enhanced chlorophyll, carotenoids, stomatal conductance, transpiration, photosynthetic rate and soluble sugars and yield (Bourioug *et al*., 2020). Hydropriming for 12 h (Catiempo *et al*., 2021; Pavithramata *et al*., 2023) and 18h (Shanthala *et al*., 2013; Jovicic *et al*., 2022) was also found to increase the seedling length, dry weight and vigor index in sunflower. In contrast, no improvement in seedling parameters was observed by priming for 18 h (Catiempo *et al*., 2021), 12 h (Farahani *et al*., 2011) and 16h (Bourioug *et al*., 2020), rather yield was decreased by 16.7% with 16h priming (Bourioug *et al*., 2020). Therefore, priming for beyond 8h appears to be negative (Table 2) and 6-8h hydropriming duration could be appropriate for maximum SVI in sunflower. The critical imbibition period could be 2 hour and a maximum can be 8 hours for sunflower.

In rice, hydropriming did not increase the total seedling length significantly, but the root length was increased significantly in 6h priming compared to the no-priming (Table 3). The SVI was significantly higher with 3h of imbibition compared to the control, beyond which there was no significant improvement (Table 3). In this direction, 6h hydropriming was reported to enhance germination and vigour index, especially in drought-tolerant varieties

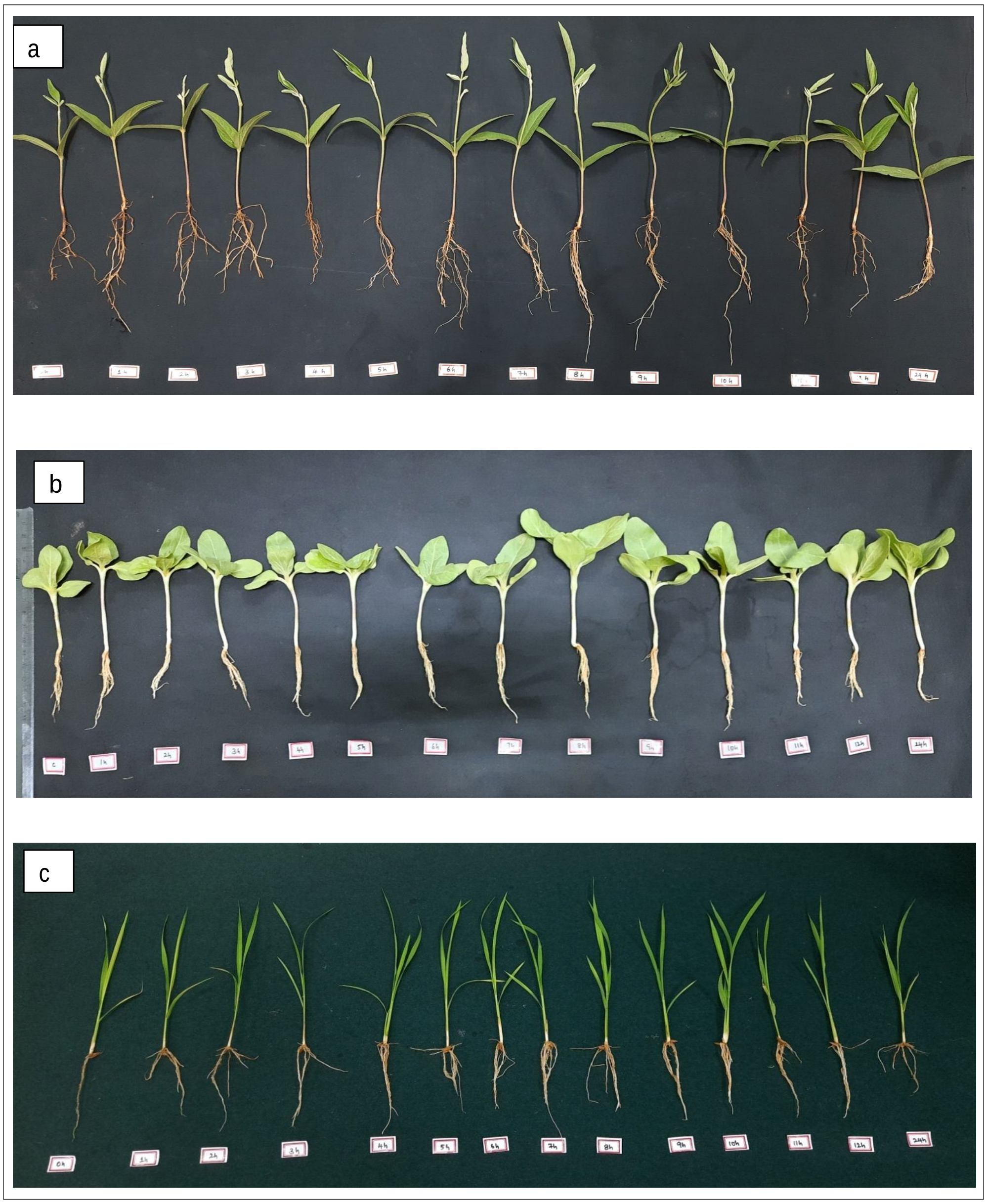

Fig 2: Effect of hydro-priming (0 to 12 h at hourly interval and at 24 h) on seedling performance of (a) pigeon pea (cv. BRG-5), (b) sunflower hybrid (KBSH-78) and (c) paddy (cv. MTU-1001), at 15 days after sowing under adequate moisture condition.

(Ranmeechai *et al*., 2022) and even 24h soaking (Ramesh and Singh, 2006). In the present study, priming for 12 or 24 h significantly reduced the fresh weight, dry weight of seedlings and the SVI evidenced by phenotypic expression (Fig 2). The prolonged priming was reported to lead to seed deterioration (Ren *et al*., 2023) which could be due to anaerobic conditions during imbibition thus reduced respiration and hence more than 7-8h hydropriming had no advantage. Furthermore, during re-drying of primed seed, the higher respiration might have used the available carbohydrates available and hence decreased growth in 24 soaking periods.

The shoot-to-root ratio was lesser in pigeon pea and sunflower compared to the rice (Table 1, 2, 3), suggesting the higher effect of hydropriming on root growth in pigeon pea and sunflower, the rainfed crops, where root growth is crucial for seedling survival in contrast to rice. The phenomenon is evidenced by a higher standardized range for root compared to the shoot in all three crops with the phenotypic expression (Fig 2). The priming could give a head start due to the stimulatory effect conserved during re-drying (Catiempo *et al*., 2021), that induces the de novo synthesis of hydrolases, breaking down food reserves and

Table 2: Effect of hydro-priming on field performance of sunflower hybrid (KBSH-78) seedlings at 15 days after sowing.

Priming period (h)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Shoot/root ratio	Fresh wt. (mg/seedling)	Dry wt. (mg/seedling)	SVI-II
0	6.13	5.83	11.95	1.05	1880	187	2232
1	5.95	6.39	12.34	0.93	2033	200	2468
2	6.04	6.16	12.20	0.98	2253	240	2927
3	6.03	5.89	11.92	1.03	2380	247	2943
4	6.14	6.01	12.15	1.02	2640	260	3155
5	5.93	5.26	11.19	1.13	2147	220	2456
6	6.26	5.98	12.24	1.05	2587	253	3095
7	6.26	5.90	12.16	1.06	2533	267	3245
8	6.91	6.17	13.08	1.12	2587	253	3322
9	6.50	6.29	12.79	1.04	2593	260	3322
10	6.30	6.95	13.26	0.91	2673	253	3359
11	6.45	6.79	13.23	0.95	2433	260	3441
12	6.16	6.28	12.44	0.98	2313	240	2986
24	6.25	5.14	11.38	1.22	1933	207	2339
SE (m)	0.17	0.26	0.37	0.04	210	14.3	184
C.D. (P<0.05)	0.51	0.76	1.06	0.12	NS	41.5	536
C.V. (%)	4.8	7.4	5.1	6.7	15.4	10.3	10.8

Table 3: Effect of hydro-priming on field performance of rice (cv. MTU-1001) seedlings at 15 days after sowing.

Priming period (h)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Shoot/root ratio	Fresh wt. (mg/seedling)	Dry wt. (mg/seedling)	SVI-II
0	14.0	6.58	20.6	2.15	183.5	41.3	859.8
1	13.1	6.23	19.3	2.10	197.5	44.2	837.5
2	13.9	6.81	20.7	2.05	225.9	47.9	992.6
3	14.3	6.82	21.1	2.10	237.3	51.4	1084.9
4	14.0	7.39	21.3	1.90	223.2	49.8	1062.8
5	13.9	7.06	20.9	1.97	205.4	44.2	924.9
6	13.3	7.60	20.9	1.75	213.6	46.2	964.3
7	13.1	6.87	20.0	1.91	191.1	39.9	795.3
8	13.7	7.27	21.0	1.90	213.4	48.8	1048.1
9	14.0	6.48	20.5	2.18	213.3	48.5	995.5
10	14.2	6.51	20.7	2.19	233.6	49.0	1005.5
11	13.7	6.00	19.8	2.25	197.1	41.4	815.3
12	13.8	5.77	19.5	2.39	186.9	38.5	764.9
24	13.9	5.59	19.5	2.52	182.4	40.0	772.4
SE (m)	0.24	0.32	0.4	0.11	7.5	1.9	50.2
C.D. (P<0.05)	0.7	0.93	1.2	0.31	22.0	5.4	146.8
C.V. (%)	3.04	8.36	3.4	8.73	6.3	7.1	9.4

efficiently translocation to the growing embryo (Hussain *et al*., 2017). Another hypothesis could be memory due to priming, for rapid imbibition of water upon sowing in the field (Chen and Arora, 2013). Although the imbibition period expected to vary with the genotype, considering the popular varieties, as a proof of concept, the critical imbibition period could be 3h and can be extended to 7-8h for maximizing the seedling vigour in pigeon pea, sunflower and rice.

CONCLUSION

Seedling establishment is a major impediment in rainfed crops like pigeon pea, sunflower and upland rice. Critical imbibition of 30-40% required for optimum seed germination will be attained within 3-4 hours of hydropriming, however, the seedling establishment could be better by 7-8 hours of hydropriming in pigeon pea, sunflower and rice. Therefore, hydropriming is a simple, inexpensive and easy technique to enhance the crop stand.

Conflict of interest

No conflict of interests regarding the publication.

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