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Effect of hydropriming on germination and seedling vigor of pigeonpea [*Cajanus cajan* (L.) Millsp.]

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ABSTRACT

The effect of hydropriming on germination and seedling vigor of pigeonpea was studied on four hybrids and six inbred lines at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Seeds of each genotype were soaked in distilled water at room temperature (25°C) for two, four, and six hours. Non-primed seeds were used as a control. Research revealed that the length of hydropriming had a significant effect on number of seedlings germinated in day three, days to 60% germination, plumule length on day eight, and the radicle length on day twenty. Hydropriming seeds for four hours led to faster germination and longer plumule (cm) and radicle lengths (cm). Soil type also significantly affected seedling growth, with larger seedlings growing in Alfisols (16.5 cm plumule, 11.9 cm radicle) as compared to Vertisols (14.6 cm plumule, 10.3 cm radicle). Highest seedling vigor index was exhibited in control (2693.79) with four hours soaking (2660.35) treatment. The results of this study showed that pigeonpea can be hydroprimed for four hours to induce faster germination. Hydropriming for four hours can be recommended in rainfed pigeonpea areas where there is lack of sufficient moisture in the soil as it maintain seedling vigor due to longer radicle length and probably expresses for high drought tolerance while over priming for six hours reduced germination rate and detrimental to seedlings.

Key words : *Genotype, Germination, Hydropriming, Pigeonpea, Seedling vigor.*

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important grain legume grown in over 4.63 million hectares in the tropics and subtropics.Of the 25 countries in Asia where pigeonpea is grown, India and Myanmar are the major producers. Although traditional varieties of pigeonpea take about 175 to 280 days to reach maturity (Mula and Saxena 2010), varieties recently developed by the International Crops Research Institute for the Semi-Arid Tropics mature in 90-140 days (short duration) (Saxena *et al.,* 2007).

Water deficit conditions have been reported as one of the major constraint to seed germination which affects seedling growth by adversely altering carbohydrate metabolism and the transport of sucrose in seedling (Gupta *et al.,* 1993). However, the adverse effects of water deficit can be alleviated by seed priming (Ashraf and Foolad 2005). Priming of seeds in water, also known as hydropriming, has been reported to be an economical, simple and safe technique for increasing the capacity of seeds to osmotic adjustment and enhancing seedling establishment and crop production under stressed conditions (Mc Donald, 2000).

The general purpose of seed priming is to take the seed to a point where germination processes start, but are not completed. Priming could lead to faster emergence of root and shoots, production of more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield under adverse conditions (Cayuela *et al.,* 1996 and Harris *et al.,* 2001). Although the priming of seeds had been reported to result in better seedling growth under water deficit stress conditions, little is known about the metabolic changes of seeds by seed priming (Kaur *et al.,* 2002). However, Jyotsna and Srivastava (1998) found out that in pigeonpea, hydropriming was uncovered to be very effective in the mobilization of compounds such as proteins, free amino acids, and soluble sugars from storage organs to growing embryonic tissues under salt stress.

The effect of hydropriming has been investigated in many

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different legumes and results were varied. In some legumes, such as chickpea, hydropriming induced faster germination but decreased germination percentage at certain temperatures (Elkoca *et al.,* 2007) while in other legumes like soybean, hydropriming for 12 hours decreased the speed of germination (Rouhi *et al.,* 2011). Abbasi *et al.* (2012) reported that hydropriming is a simple and useful technique. In pigeonpea, the slow seedling growth is considered to be a major yield-restricting factor when the growth period is shortened (Katayama *et al.,* 1998). To date, there is no information about the effects of hydropriming and priming duration on germination rates of pigeonpea. In this respect the use of seed with enhanced vigor can be a practicable strategy to obtain healthy seedling and a better crop stand under a range of environmental situation in real condition of pigeonpea fields. This study was taken up to examine the response of pigeonpea seeds through hydropriming in two different soil types.

MATERIALS AND METHODS

The present study was conducted under glass house condition at International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh from June 5 to 25, 2013. Four hybrids (ICPH 2671, ICPH 4431, ICPH 4182, ICPH 2740) and two in bred lines of early (ICPL 88039, ICPL 87091) and four inbred lines of medium (ICP 11811, ICP 7035, ICP 14304, ICP 13395) pigeonpea type were evaluated to study the effect of different time intervals of hydropriming on germination rate and initial seedling growth. The weight (g) of each genotype were taken at 100 seeds and treated with tetra methyl thiuramdi sulphide at 2.5 g/kg and were immersed in 100 ml distilled water at room temperature (25°C) following three treatments of two, four and six hours. Unprimed seeds were used as control.

Two sets of pigeonpea genotypes were sown in paper cups (250 ml) in two types of soils (Alfisols and Vertisols) consisting of five sample plants in each treatment with three replications. Observations on germination percentage were recorded at 24 hour intervals, starting from the time of sowing until all seeds had germinated. Plumule length was recorded at eight and twenty days after sowing while the radicle length was recorded twenty days after sowing. Seedling vigor index for each treatment was recorded at twenty days after sowing.

Data for germination percentage was analysed using analysis of variance (ANOVA) technique in a factorial experiment of hydropriming treatment, soil type and genotype in a completely randomized design (CRD). ANOVA was carried out using the GLM (Generalized Linear Model) procedure of the SAS software version 9.3 for Windows (SAS Institute Inc. 2011, Cary, NC). Interaction effect of three and two factor interactions was tested for significance and significance of main effects of hydropriming treatment, soil type and genotype was interpreted based on crossover/non-crossover three and two factor interaction. Least Square Means of individual factor levels were estimated and compared using least significant differences with the use of t-grouping.

RESULTS AND DISCUSSION

Effect of hydropriming

The study revealed that hydropriming had a significant effect on number of seedlings germinated on day three, days to 60% germination, plumule length on day eight, radicle length and seedling vigor index on day twenty (**Table 1**), which is in conformity with the findings of Ghassemi-Golezani *et al.* (2008). Hydropriming for four hours had the highest number of seedlings germinated on day three at 2.2 seedlings (**Table 2**). Seeds soaked for six hours and seeds not soaked had the lowest number of seedlings germinated on day three with 1.0 and 0.9, respectively. Moreover, seeds soaked for four hours took the least amount of time (3.6 days) to reach 60% germination, while seeds soaked for six hours and the control took the longest time to reach 60% germination at 4.2 and 4.3 days, respectively which is in conformity to the findings of Elkoca *et al.* (2007).

Table 1. Statistical significance of treatments on various germination related traits

Source	DF	Seedlings germinated (Day 3)	Days to 60% germination	Plumule length		Radicle length (Day 20)	Seedling vigor index
				Day 8	Day 20		
Hydropriming	3	22.544**	6.293**	1.992*	6.375ns	14.407*	2.16 ns
Soil type	1	30.817**	6.338**	10.965**	213.533**	148.712**	33.72**
Genotype	9	14.739**	2.421**	39.061**	383.745**	34.666**	35.96**
Hydropriming x Soil type	3	2.183ns	0.026ns	0.789ns	5.495ns	3.864ns	1.05 ns
Hydropriming x Genotype	27	1.671*	0.512*	0.596	3.205ns	2.695ns	0.74 ns
Soil type x Genotype	9	7.067**	1.588**	2.061**	51.581**	12.166**	4.08**
Hydropriming x Soil type x Genotype	27	0.853ns	0.548*	1.099**	3.312ns	4.158ns	0.92 ns
Error	160	0.937	0.317	0.526	4.463	3.756	196294.0

Note: **Highly significant; *significant; ns = non-significant

Table 2. Effect of hydropriming on seedling vigor of pigeonpea genotypes

Source	Seedlings germinated (Day 3)	Days to 60% germination	Plumule length		Radicle length (Day 20)	Seedling vigor index
			Day 8	Day 20		
No soaking	0.9 a	4.3 a	5.8 a	15.8 a	11.5 a	2693.79 a
2 hr soaking	1.7 b	3.8 b	5.9 ab	15.8 a	11.0 ab	2597.13 a
4 hr soaking	2.2 c	3.6 c	6.1 b	15.7 a	11.4 a	2660.35 a
6 hr soaking	1.0 a	4.2 a	5.7 a	15.1 a	10.4 b	2502.56 a

Note : Means followed by the same letter (*a-b*) in a column do not differ significantly at *P* = 0.05

On day eight, seeds hydroprimed for four hours showed the longest plumule length at 6.1 cm (**Table 2**) but not significantly different in seeds soaked for two hours, which is in conformity to the findings of Kaur *et al.* (2002). With regards to radicle length on day twenty, the longest radicles were found in seedlings in the control (11.5 cm), although it was not significantly different in hydropriming for two hours or four hours (Table 2). Likewise, soaking seeds for six hours negatively affected radicle length on day twenty, as these seedlings had significantly shorter radicles (10.4 cm) which is in accordance with the findings of Elkoca *et al.* (2007), stating that overpriming can be detrimental to seedlings.

There was no significant difference on the seedling vigor index among the treatments. However, the highest seedling vigor index was observed in control (2693.7) followed by four hours soaking (2660.35) (Table 2).

Effect of soil type

Soil type had a significant effect on seedling germination on day three, days to reach 60% germination, plumule length on day eight and day twenty, radicle length and seedling vigor index on day twenty (Table 1). **Table 3** showed that seedlings grown in Alfisols performed better than those grown in Vertisols, which is in accordance with the findings of Noriharu *et al.* (2011). With regards to number of seedlings germinated on day three, the Alfisols produced more seedlings (1.8) than Vertisols (1.1). In addition, seedlings in the Alfisols took less time to reach 60% germination. On day twenty, seedlings from the Alfisols had longerplumule length (1.9 cm) than the Vertisols (1.6 cm). However, on day eight, seedlings from the Vertisols had longer plumule length by 0.5 cm than in Alfisols.

Table 3. Effect of soil on pigeonpea seedlings related characters

Source	Seedlings germinated (Day 3)	Days to 60% germination	Plumule length		Radicle length (Day 20)	Seedling vigor index
			Day 8	Day 20		
lfisols	1.8 a	3.8 a	5.6 a	16.5 a	11.9 a	2779.52 a
Vertisols	1.1 b	4.1 b	6.1 b	14.6 b	10.3 a	2447.40 b

Note : Means followed by the same letter (*a-b*) in a column do not differ significantly at *P* = 0.05

Seedling vigor index was significantly higher in Alfisols (2779.52) than in Vertisols (2447.40).

Effect of genotype

Table 1 indicated that genotype showed highly significant effect on germination on day three, days to 60% germination, plumule length on day eight and day twenty, radicle length and seedling vigor index on day twenty. ICP 14304 and ICPL 87091 had the highest number of seedlings germinated in day three (2.9 and 2.5, respectively) and took the least amount of time to reach 60% germination (3.3 days and 3.6 days, respectively) as shown in **Table 4**.

The longest plumule lengths on day eight were observed in ICP 14304, ICP 11811, and ICP 7035 with 7.4 cm, 7.3 cm, and 7.0 cm, respectively with least observed in ICPH 2740 (4.0 cm) (Table 4). ICP 11811 and ICP 7035 had the longest plumule length on day twenty at 20.9 cm and 20.7 cm, respectively whileI CPH 2740 had the shortest plumule length (10.4 cm), but not significantly different with ICPH 4182 (10.0 cm).

With regards to radicle length on day twenty, ICP 11811 showed the longest (12.6 cm), although it was not significantly different with ICP 7035 (12.4 cm), ICPH 2671 (11.7 cm), and ICP 13395 (11.6 cm) (**Table 4**) while ICPH 4182 demonstrated the shortest mean radicle length of 9.0 cm, but did not differ significantly with ICPH 4431 (9.3 cm). ICP 7035 (3309.4) and ICP 11811 (3303.9) had exhibited highest seedling vigor index which was significantly different from the rest of genotypes.

Table 4. Effect of genotype on germination and seedling related traits

Source	Seedlings germinated (Day 3)	Days to 60% germination	Plumule length		Radicle length (Day 20)	Seedling vigor index
			Day 8	Day 20		
ICPH 2671	1.5 bcd	3.9 bc	5.1 e	14.6 e	11.7 abc	2560.8 c
ICPH 4431	1.4 bcd	4.0 c	4.7 ef	12.0 f	9.3 ef	1992.7 d
ICPH 4182	1.6 b	4.2 cd	4.5 f	10.0 g	9.0 f	1772.0 d
ICPH 2740	0.4 f	4.4 d	4.0 g	10.4 g	10.3 de	2014.5 d
ICPL 88039	1.5 bc	3.9 bc	5.5 d	14.4 e	11.5 bc	2561.6 c
ICPL 87091	2.5 a	3.6 ab	6.9 b	18.9 b	11.3 bcd	2984.5 b
ICP 11811	1.0 de	4.0 c	7.3 a	20.9 a	12.6 a	3303.9 a
ICP 7035	1.0 cde	4.0 c	7.0 ab	20.7 a	12.4 ab	3309.4 a
ICP 14304	2.9 a	3.3 a	7.4 a	17.6 c	11.2 cd	2876.5 b
ICP 13395	0.7 ef	4.4 d	6.4 c	16.3 d	11.6 abc	2758.9 bc

Note : Means followed by the same letter (*a-g*) in a column do not differ significantly at *P* = 0.05.

Interactive effect of hydropriming and soil type

There was no significant interactive effect of hydropriming and soil type on seedling germination on day three, days to reach 60% germination, plumule length on day eight and day twenty, radicle length on day twenty and seedling vigor index (Table 1).

Interactive effect of hydropriming and genotype

The number of seedlings germinated on day three and days to 60% germination was significantly affected by the interaction of hydropriming and genotype (Table 1). ICPH 4182, ICPH 2740, ICPL 88039, ICPL 87091, ICP 11811, ICP 7035, and ICP 14304 had more seedlings germinated on day three from the four hour hydropriming treatment (**Table 5**). However, for ICPH 2671 and ICP 13395, the two hour treatment had more number of seedlings than the other hydropriming treatments while for ICPH 4431, the six hour treatment had the most number of seedlings.

With regards to days to 60% germination, ICPH 4431, ICPH 4182, ICPH 2740, ICPL 88039, ICPL 87091, ICP 7035, ICP 14304, and ICP 13395 reached 60% germination the quickest when hydroprimed for four hours, which is in conformity with the findings of Nascimento and Souza de Aragão (2004) (Table 5).

Interactive effect of genotype and soil type

The interaction of genotype and soil type had a significant effect on the number of seedlings germinated on day three, days to 60% germination, plumule length on day eight and day twenty, radicle length and seedling vigor index on day twenty (Table 1).

Table 6 showed that the germination of the three hybrids, ICPH 2671, ICPH 4182, and ICPH 2740was higher in the Vertisolson day three while the other hybrid, ICPH 4431 and all other inbred lines had moreseedlings germinated in the Alfisols. In addition, all of the genotypes except for ICPH 4182 took less time to reach 60% germination in the Alfisols which conforms to the findings of Noriharu *et al.* (2011).

When considering plumule length on day eight, ICPH 2740 sown in Alfisols and Vertisols produced the shortest at 3.8 and 4.1 cm, respectively (Table 6). However, by day twenty, the plumule length of ICPH 4182 was smaller (8.9 cm)

Table 5. Interactive effect of genotype and hydropriming on pigeonpea seedlings germinated on day 3 and days to 60% germination

Genotypes	Seedlings germinated on day 3				Days to 60% germination			
	No soaking	2 hour soaking	4 hour soaking	6 hour soaking	No soaking	2 hour soaking	4 hour soaking	6 hour soaking
ICPH 2671	1.2	2.0	1.8	1.0	4.0	3.5	3.8	4.2
ICPH 4431	0.8	1.2	1.5	2.0	4.0	4.3	3.7	3.8
ICPH 4182	0.7	1.5	3.0	1.2	4.7	4.3	3.2	4.5
ICPH 2740	0.2	0.5	0.5	0.3	4.8	4.3	4.0	4.3
ICPL 88039	0.8	1.8	2.8	0.7	4.7	3.7	3.3	4.0
ICPL 87091	2.0	2.5	3.8	1.8	3.8	3.5	3.2	4.0
ICP 11811	0.5	1.2	1.5	0.7	4.7	3.7	3.8	4.0
ICP 7035	0.7	1.2	1.8	0.3	3.8	3.8	3.7	4.5
ICP 14304	1.7	4.0	4.3	1.7	3.7	3.0	3.0	3.7
ICP 13395	0.7	1.2	0.8	0.2	4.5	4.2	4.0	4.8

Table 6. Interactive effect of soil and genotype on germination and seedling vigor characters

Genotypes	Soil type											
	Seedlings germinated on day 3		Days to 60 % germination		Plumule length (cm)				Radicle length (cm) on day 20		Seedling vigor index	
					Day 8		Day 20					
	Alfisols	Vertisols	Alfisols	Vertisols	Alfisols	Vertisols	Alfisols	Vertisols	Alfisols	Vertisols	Alfisols	Vertisols
ICPH 2671	1.4	1.6	3.8	3.9	4.7	5.4	15.1	14.1	12.5	10.8	2488.8	2632.7
ICPH 4431	1.8	0.9	3.8	4.2	4.6	4.7	14.0	9.9	10.2	8.3	1695.4	2290.1
ICPH 4182	0.8	2.4	4.6	3.8	3.9	5.0	8.9	11.1	9.2	8.8	1840.0	1703.9
ICPH 2740	0.3	0.4	4.3	4.4	3.8	4.1	10.9	9.9	11.5	9.2	1831.9	2197.1
ICPL 88039	2.3	0.8	3.8	4.1	5.8	5.2	17.0	11.7	12.3	10.7	2233.0	2890.2
ICPL 87091	3.3	1.8	3.3	4.0	6.8	6.9	21.3	16.6	12.7	9.9	2615.0	3353.9
ICP 11811	1.9	0.0	3.6	4.5	7.4	7.2	23.4	18.3	12.7	12.5	3034.3	3573.4
ICP 7035	1.8	0.2	3.8	4.2	6.8	7.2	22.4	19.4	14.1	10.7	3008.5	3610.3
ICP 14304	3.3	2.6	3.3	3.4	6.7	8.0	16.5	18.6	10.6	11.8	3049.5	2703.5
ICP 13395	1.3	0.2	3.9	4.8	5.9	6.8	15.9	16.6	12.9	10.2	2677.5	2840.2

Table 7. Interactive effect of pigeonpea hydropriming, soil type & genotype on days to 60% germination & plumule length

Soil type	Genotype	Days to 60% germination				Plumule length (cm) on day 8			
		No soaking	2 hour soaking	4 hour soaking	6 hour soaking	No soaking	2 hour soaking	4 hour soaking	6 hour soaking
lfisols	ICPH 2671	4.0	3.3	3.7	4.3	5.3	4.5	5.1	4.1
	ICPH 4431	4.0	3.7	3.7	3.7	4.7	4.8	4.3	4.5
	ICPH 4182	5.0	5.7	3.3	4.3	3.9	3.3	4.3	4.1
	ICPH 2740	5.0	4.3	4.0	4.0	3.0	3.6	4.7	3.9
	ICPL 88039	4.7	3.3	3.0	4.0	5.4	5.9	6.3	5.4
	ICPL 87091	3.3	3.0	3.0	3.7	6.5	7.3	6.5	7.0
	ICP 11811	4.0	3.3	3.3	3.7	7.9	7.8	7.4	6.4
	ICP 7035	3.7	3.7	3.3	4.3	6.8	6.9	6.9	6.6
	ICP 14304	3.3	3.0	3.0	3.7	6.7	6.8	6.5	7.0
	ICP 13395	4.0	3.3	4.0	4.3	5.2	6.6	5.5	6.4
Vertisols	ICPH 2671	4.0	3.7	4.0	4.0	5.5	5.7	4.8	5.6
	ICPH 4431	4.0	5.0	3.7	4.0	4.6	3.4	6.1	4.9
	ICPH 4182	4.3	3.0	3.0	4.7	5.2	5.2	6.0	3.8
	ICPH 2740	4.7	4.3	4.0	4.7	4.0	3.9	4.7	4.0
	ICPL 88039	4.7	4.0	3.7	4.0	5.3	5.5	5.1	4.9
	ICPL 87091	4.3	4.0	3.3	4.3	6.5	6.6	7.6	7.0
	ICP 11811	5.3	4.0	4.3	4.3	6.5	7.4	7.3	7.5
	ICP 7035	4.0	4.0	4.0	4.7	7.7	7.6	7.0	6.6
	ICP 14304	4.0	3.0	3.0	3.7	7.9	7.9	8.5	7.8
	ICP 13395	5.0	5.0	4.0	5.3	6.6	6.6	7.7	6.4

in the Alfisols while ICPH 4431 (9.9 cm) and ICPH 2740 (9.9 cm) were the least in Vertisols. The radicle length of ICPH 4182 (9.2 cm) and ICPH 4431 (8.3 cm) were shortest in Alfisols and Vertisols, respectively. The study likewise revealed that inbreds have longer plumule and radicle length than hybrids during day twenty as shown in Table 6.

Seedling vigor index in Alfisols was recorded by ICP 14304 (3049.5) whereas in Vertisols by ICP 7035 (3610.3). The graph (**Fig. 1**) showed that ICPH 4182 and ICP 14304 had recorded significant differences for seedling vigor index in Alfisols and Vertisols (Table 6).

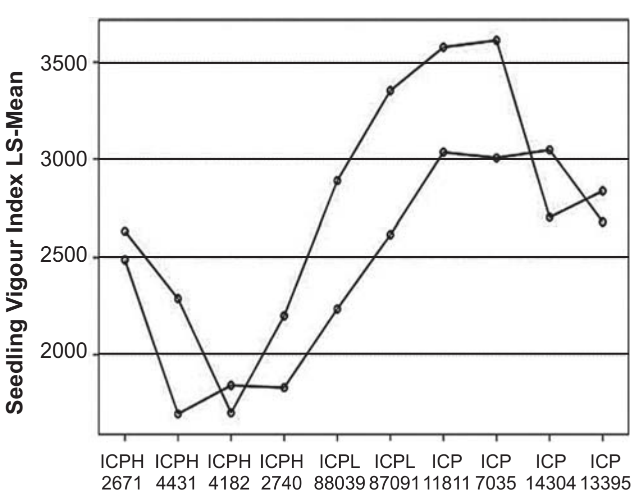

Fig. 1. Interactive effect of soil and genotype on seedling vigor index

Interactive effect of genotype, hydropriming and soil type

The interaction between genotype, hydropriming, and soil type had a significant effect on days to 60% germination and plumule length on day eight and non-significant effect on seedlings germinated on day three, plumule length on day twenty, radicle length and seedling vigor index on day twenty (Table 1). In Alfisols, ICPL 88039, ICPL 87091, and ICP 14304 when soaked for four hours and ICPL 87091 and ICP 14304 when soaked for two hours took the least amount of time to reach 60% germination at day three (Table 7) while in Vertisols, ICPH 4182 and ICP 14304 reached 60% germination the fastest (3.0 days) when soaked for two and four hours.

With regards to plumule length on day eight, the longest was ICP 14304 (8.5 cm) soaked for four hours in Vertisols while the shortest was in the control with genotype ICPH 2740 (3.0 cm) in Alfisols (**Table 7**).

CONCLUSION

The present study revealed that hydropriming has a significant effect on the germination and seedling vigor of pigeonpea. Soaking seeds in water for four hours showed the best results and led to the fastest germination and longest plumule and radicle lengths. Furthermore, longer radicles will lead to more drought-tolerant seedlingsas perceived by Thakare *et al.* (2013). In this regard, hydropriming pigeonpea seeds for four hours can be recommended as a simple technique to induce immediate germination and obtain seedlings that are most resistant to drought. This method can be of potential for pigeonpea in the rice-fallow cropping systems. It has been observed that in the rice-fallow cropping system, there is still available moisture in the soil that would last for 2-2.5 months after a rice crop has been harvested. In this regard, if hydropriming for four hours allows seeds to germinate faster, the seedlings can utilize the available moisture before it runs dry. Nevertheless, further research should be conducted to observe the effectiveness of hydropriming in a rice-fallow system.

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