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Effect of Duration of Hydration on Germination and Vigour of Aged Seed of Chickpea

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ABSTRACT

A study was conducted to investigate the effect of different durations of hydropriming and standardize the best duration of hydropriming for aged seed of chickpea, variety NBeG-3. The aged seed subjected to hydration for different durations ranging from 2 hours to 24 hours with an equal increment of 2 hours along with unhydrated seed was tested for germination and seedling traits using between paper method and sand method. Highly significant variation was observed among the durations of hydration for all the seed quality parameters except root / shoot ratio in both the methods of testing. The variation in root / shoot ratio was significant at 5 % level of significance in sand method only. Maximum germination (%), root length, shoot length, seedling length, root / shoot ratio and seedling vigour index were recorded with 8 hours of hydration and beyond that the seed quality declined gradually upto 24 hours.

Key words: *Hydropriming, Chickpea, Duration, Germination, Seedling quality*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a rainfed, low input, winter leguminous crop. It is a rich source of highly digestible dietary protein (18-22%), carbohydrate (61-62%) and fat (4.5%). It is also rich in calcium, iron, niacin, vitamin B and vitamin C[13].

Despite the high yielding potential and various advantages of chickpea, the yield per unit area of the crop is low in India. Low seed quality and lack of sufficient soil moisture are the major constraints for better crop establishment[4]. Seed priming involves controlled hydration of seed to enhance the metabolic activity within the seed but preventing radicle emergence so that all the seeds reached to the same stage of germination before sowing, so that subsequent germination after sowing will be rapid and synchronous. Priming duration affects the germination because seed of each species need specific amount of water to get into lag phase of germination in which all the pre-germinative metabolic processes occur[5].

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So, if priming is done for short period then seed would not get enough water that is required for getting seed into lag phase of germination. Priming for prolong period will allow water that may exceed the quantity required for the initiation of lag phase of germination and radicle protrusion due to which seed lose its desiccation tolerance thereby resulting in loss of seed viability[9]. Hence the present study was conducted with the main aim of investigating the effect of duration of hydropriming on germination and vigour of aged seed and to identify the best duration of hydropriming the aged seed of chickpea variety NBeG-3.

MATERIAL AND METHODS

The present investigation was carried out during 2017-18 in completely randomized design with four replications in the Department of Seed Science and Technology, Advanced Post Graduate Centre, Acharya N. G. Ranga Agricultural University, Lam, Guntur, Andhra Pradesh. Foundation seed of chickpea variety NBeG-3 harvested during *rabi*, 2015-16 was obtained from Regional Agricultural Research Station, Nandyal.

For evaluating the best hydropriming duration, the aged seed of chickpea variety NBeG-3 was soaked in distilled water with 1:5 seed weight to water volume (w/v) ratio for 12 durations ranging from 2 to 24 hours with an equal increment of 2 hours at ambient conditions and then air dried to 9 % seed moisture content. The hydrated aged seed was tested for germination along with unhydrated aged seed as control using between paper method and sand method.

In between paper method, four replicates of 100 seed from each treatment were placed at uniform spacing in between two wetted germination paper towels. The paper towels were rolled, secured with rubber bands on both the sides and kept in plastic trays in upright position and the trays were incubated in germinator at 25 ± 2 °C and 95 % RH for 8 days. For sand method also four replicates of 100 seed in each treatment were placed over moist sand with uniform spacing in plastic trays and covered with another layer of moist sand and the trays were placed in germinator at 25 ± 2 °C and 95 % RH for 8 days.

Data on germination and other seed quality parameters were recorded after 8 days of test period as detailed below:

The number of normal seedlings were counted and expressed as germination (%) as per the formula:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seed sown}} \times 100$$

The root length, shoot length and seedling length were determined by randomly selecting ten normal seedlings in each treatment and each replication at the end of the germination count and expressed in centimeters. The root length was measured from the tip of the primary root to the base of the hypocotyls. Shoot length was measured from the tip of the primary leaf to the base of the hypocotyl. Seedling length was calculated by adding root and shoot length. The root / shoot ratio of the 10 seedlings was computed and their mean was expressed as root / shoot ratio.

Seedling vigour index was computed by adopting the following formula as suggested by Abdul-Baki and Anderson[1] and was expressed in whole number:

Seedling Vigour index = Germination (%) x Seedling length (cm)

Statistical analysis: The data were subjected to Analysis of Variance (ANOVA) using SPSS software (version 16.0) at 1 % and 5 % level of significance. The treatmental means were compared using Duncan's Multiple Range test ($P<0.05$).

RESULTS AND DISCUSSION

The trend of variation observed in chickpea seed subjected to different durations of hydropriming was almost similar in both the methods of testing.

The analysis of variance clearly indicated that germination, root length, shoot length, seedling length and seedling vigour index were significantly affected by the durations of hydration in both the methods. The root / shoot ratio showed significant variation only at 5% level of significance in sand method (Table 1). Gradual improvement in germination, root length, shoot length, seedling length and seedling vigour index was noticed upto 8 hours of hydration of aged seed. Further increase in duration of hydration exhibited a decreasing trend in the above characters in both the methods of testing.

Germination (%): The highest and lowest germination were recorded with 8 hours and 24 hours of soaking (92.25 % and 78.00 % in between paper method and 92.50 % and 77.75 % in sand method), respectively (Table 2 & 3). Soaking of chickpea seed beyond 20 hours recorded lesser germination than that observed in untreated aged seed in both the methods of testing. The percent increment in germination with 8 hours of soaking over unhydrated aged seed was found to be 12.84 % and 12.46 % in between paper and sand methods, respectively (Figure 1). These results are in accordance with earlier reports in chickpea[3] where the germination reached maximum upto 8 hours of soaking and then declined with further increase in soaking period upto 24 hours. The possible reason for early and enhanced germination at a certain point of seed priming may be due to completion of pre-germinative metabolic processes which gives the primed seed a head start over the un-primed seed making the seed ready for radicle protrusion[14]. Decrease in germination due to prolonged duration of soaking for 24 hours was also reported earlier in chickpea[2]. This was supported by Murray, who concluded that overpriming may cause oxygen deficiency and the build-up of inhibitors[8].

Root length (cm): The root length increased with the increase in duration of hydration from 0 hour to 8 hours in both the methods of testing (Table 2 & 3). Among the hydration treatments, maximum and minimum root lengths were observed with 8 hours and 24 hours of hydration (15.92 cm and 10.50 cm in between paper method and 16.36 cm and 11.91 cm in sand method), respectively. Hydration for 24 hours showed slight reduction in the root length than that in unhydrated control in between paper method. The increase in root length with hydration was earlier reported in soybean[11]. Seed priming might stimulate the formation of enzymes which are important in the early phases of germination that helps for a fast radicle protrusion and hypocotyl elongation.

Shoot length (cm): There was an improvement in shoot length with increased duration of hydration upto 8 hours in both the methods of testing (Table 2 & 3). Among various durations, hydration for 8 hours and 24 hours recorded maximum and minimum shoot length (19.88 cm and 15.37 cm in between paper method and 19.89 cm and 16.57 cm in sand method), respectively. Shoot length recorded with 24 hours of hydration was found to be slightly lesser but on par with that of unhydrated aged seed in both the methods of testing. Similar findings were earlier reported in soybean[6]. The increase in shoot length with hydration might be due to the fact that the seed soaked in water had rapid translocation of nutrients after hydrolysis of the cotyledonary reserves to the growing seedling[12].

Seedling length (cm): Seedling length showed similar trend of variation that was noticed for root and shoot lengths. Among various durations of hydration, highest and lowest seedling lengths were recorded with 8 hours and 24 hours of soaking (35.80 cm and 25.88 cm in between paper method and 36.25 cm and 28.48 cm in sand method), respectively (Table 2 & 3). The increment in seedling length with 8 hours of soaking over unhydrated control was found to be 34.59 % and 32.64 % in between paper and sand methods, respectively (Figure 2). Earlier

studies in soybean[6] revealed that the seedling length was increased upto 24 hours of hydration and later decreased with further increase in the duration upto 48 hours.

Root / shoot ratio: The variation in root / shoot ratio was significant only at 5% level of probability in sand method while it was nonsignificant in between paper method (Table 1). The highest root / shoot ratio (0.83) in sand method was recorded with 8 hours of hydration. Increase in root / shoot ratio by priming was reported earlier in wheat[7].

Seedling vigour index: The highest and lowest seedling vigour indices were observed with 8 hours and 24 hours of hydration (3303 and 2020 in between paper method and 3353 and 2215 in sand method), respectively among different durations of hydration. Hydration beyond 22 hours showed slight decline in seedling vigour index than that noticed in unhydrated aged seed. Seedling vigour index with 8 hours of soaking was improved by 51.88 % and 49.27 % over that in control in between paper method and sand method, respectively (Figure 3). Earlier studies in soybean[6] also indicated that seedling vigour index increased upto 24 hours of hydration and depleted beyond that point. Improved seed performance due to hydration of seed may be due to changes in membrane integrity, breakdown of seed reserves and production of bioactive substances and other metabolites[10]. Reduction in vigour index due to prolonged soaking in water may result from the leakage of the essential soluble constituents of seed into the leachate[10].

Table 1: Mean squares for germination and seed quality traits in aged seed of chickpea as affected by durations of hydration

Source	DF	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root / shoot ratio	Seedling vigour index
Between paper method							
Treatment	12	43.296**	9.324**	8.487**	34.385**	0.001^{NS}	546541**
Error	39	2.622	0.743	1.148	1.820	0.001	18810
CV (%)		2.38	6.39	5.99	4.31	2.03	5.10
Sand method							
Treatment	12	40.980**	9.239**	4.434**	25.914**	0.001*	450536**
Error	39	2.364	0.744	1.069	1.783	0.001	15658
CV %		2.25	6.33	5.72	4.21	1.81	4.58

* significant difference at 5% probability level
** significant difference at 1% probability level
NS: Non significant

Table 2: Mean comparisons for seed quality traits in aged seed of chickpea as affected by duration of hydration by using Duncan's Multiple Range Test (Between paper method)

Soaking period	Germination (%)		Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root / shoot ratio		Seedling vigour index
0 h	81.75	$(64.69)^{*d}$	10.86^{f}	15.75^{e}	26.60^{h}	0.69	$(1.30)^{**a}$	2174^{gh}
2 h	87.00	$(68.95)^{bc}$	13.79^{bcde}	18.42^{ab}	32.20^{cde}	0.75	$(1.32)^{a}$	2801^{cd}
4 h	87.75	$(69.52)^{bc}$	13.99^{bcd}	18.24^{abc}	32.23^{cde}	0.77	$(1.33)^{a}$	2827^{cd}
6 h	89.50	$(71.09)^{b}$	14.48^{bcd}	18.84^{ab}	33.33^{bcd}	0.77	$(1.33)^{a}$	2982^{bc}
8 h	92.25	$(73.83)^{a}$	15.92^{a}	19.88^{a}	35.80^{a}	0.80	$(1.34)^{a}$	3303^{a}
10 h	89.50	$(71.07)^{b}$	15.01^{ab}	19.34^{a}	34.35^{ab}	0.78	$(1.33)^{a}$	3073^{b}
12 h	88.25	$(69.94)^{bc}$	14.67^{abc}	19.29^{a}	33.95^{abc}	0.76	$(1.33)^{a}$	2995^{bc}
14 h	85.50	$(67.60)^{c}$	13.23^{de}	18.55^{ab}	31.78^{def}	0.71	$(1.31)^{a}$	2717^{d}
16 h	85.50	$(67.66)^{c}$	13.66^{bcde}	17.41^{bd}	31.08^{ef}	0.79	$(1.34)^{a}$	2658^{d}
18 h	85.25	$(67.42)^{c}$	13.47^{cde}	17.46^{bcd}	30.93^{ef}	0.79	$(1.34)^{a}$	2636^{de}
20 h	82.00	$(64.90)^{d}$	13.21^{de}	16.59^{cde}	29.80^{fg}	0.80	$(1.34)^{a}$	2444^{ef}
22 h	81.25	$(64.33)^{de}$	12.50 e	16.23^{de}	28.73^{g}	0.77	$(1.33)^{a}$	2331^{fg}
24 h	78.00	$(62.07)^{e}$	10.50 f	15.37^{e}	25.88^{h}	0.69	$(1.30)^{a}$	2020^{h}
Mean	85.65	(67.93)	13.49	17.80	31.28	0.76	(1.33)	2690
CD (5%)	2.31		1.23	1.52	1.93	NS		196.09
S Em ±	0.81		0.43	0.53	0.67	0.01		68.58
CV (%)	2.38		6.39	5.99	4.31	2.03		5.10

*Values in the parenthesis indicate arc-sine transformed values
**Values in the parenthesis indicate square root transformed values
NS: Non significant at 5 % level of probability
Values in the same column with the same alphabetical letter are not significantly different (P<0.05).

Table 3: Mean comparisons for seed quality traits in aged seed of chickpea as affected by duration of hydration by using Duncan's Multiple Range Test (Sand method)

Soaking period	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root / shoot ratio	Seedling vigour index
0 h	82.25 (65.07)*^{f}	10.80^{g}	16.53^{e}	27.33^{g}	0.65 (1.29)**^{c}	2246^{h}
2 h	87.00 (68.94)^{bcd}	13.78^{cde}	18.42^{abc}	32.20^{bcd}	0.75 (1.32)^{abc}	2801^{cde}
4 h	88.25 (69.94)^{bcd}	14.30^{bcd}	18.33^{abcd}	32.63^{bcd}	0.78 (1.33)^{ab}	2878^{bcd}
6 h	89.25 (70.84)^{b}	14.72^{bc}	18.73^{abc}	33.45^{bc}	0.79 (1.34)^{ab}	2985^{bc}
8 h	92.50 (74.08)^{a}	16.36^{a}	19.89^{a}	36.25^{a}	0.83 (1.35)^{a}	3353^{a}
10 h	89.00 (70.63)^{bc}	15.46^{ab}	18.34^{abc}	34.30^{b}	0.82 (1.35)^{ab}	3053^{b}
12 h	88.50 (70.17)^{bcd}	14.73^{bc}	19.45^{ab}	34.18^{b}	0.76 (1.33)^{ab}	3025^{b}
14 h	86.50 (68.48)^{bcd}	13.68^{cde}	18.35^{abcd}	32.03^{cd}	0.75 (1.32)^{abc}	2770de
16 h	86.25 (68.23)^{cd}	13.25^{efg}	17.98^{bcde}	31.23^{d}	0.74 (1.32)^{abc}	2692^{def}
18 h	85.75 (67.81)^{de}	12.97^{efg}	17.71^{cde}	30.68^{de}	0.73 (1.32)^{abc}	2631^{ef}
20 h	83.25 (65.85)^{ef}	12.84^{ef}	17.63^{cde}	30.48^{def}	0.73 (1.32)^{bc}	2538^{fg}
22 h	81.75 (64.70)^{f}	12.27^{f}	16.69^{de}	28.95^{efg}	0.74 (1.32)^{abc}	2367^{gh}
24 h	77.75 (61.88)^{g}	11.91^{fg}	16.57^{e}	28.48^{fg}	0.72 (1.31)^{bc}	2215^{h}
Mean	86.00 (68.20)	13.66	18.05	31.70	0.76 (1.32)	2735
CD (5%)	2.20	1.23	1.48	1.91	0.03	178.91
S Em ±	0.77	0.43	0.52	0.67	0.01	62.57
CV (%)	2.25	6.33	5.72	4.21	1.81	4.58

*Values in the parenthesis indicate arc-sine transformed values

**Values in the parenthesis indicate square root transformed values

Values in the same column with the same alphabetical letter are not significantly different (P<0.05)

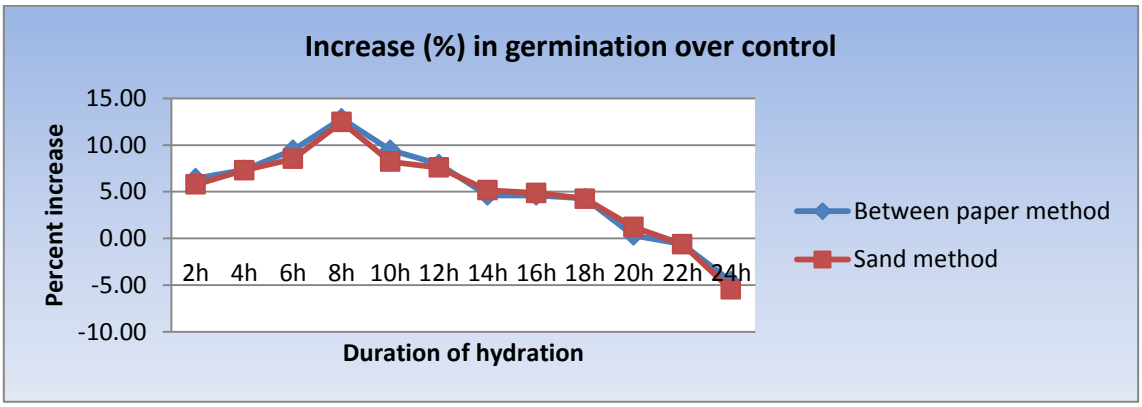

Figure 1: Percent increase in germination by hydration in aged seed of chickpea over untreated aged seed as tested by different methods

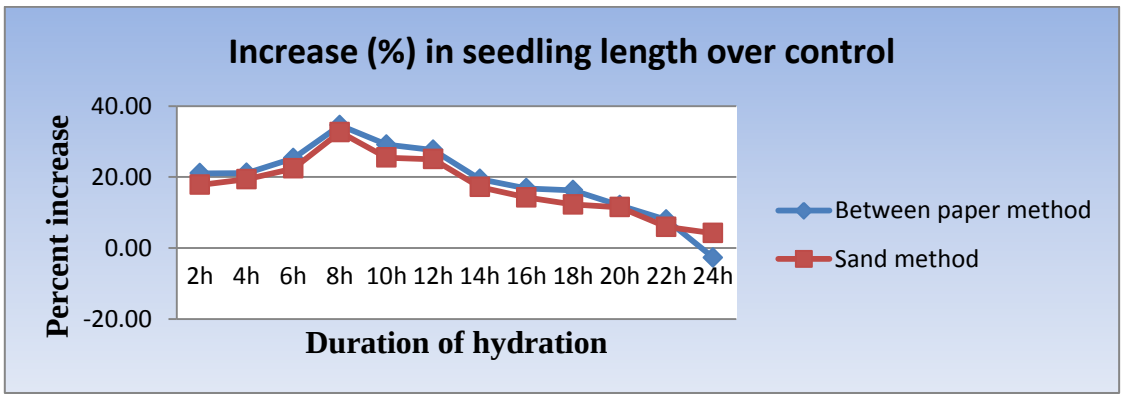

Figure 2: Percent increase in seedling length by hydration in aged seed of chickpea over untreated aged seed as tested by different methods

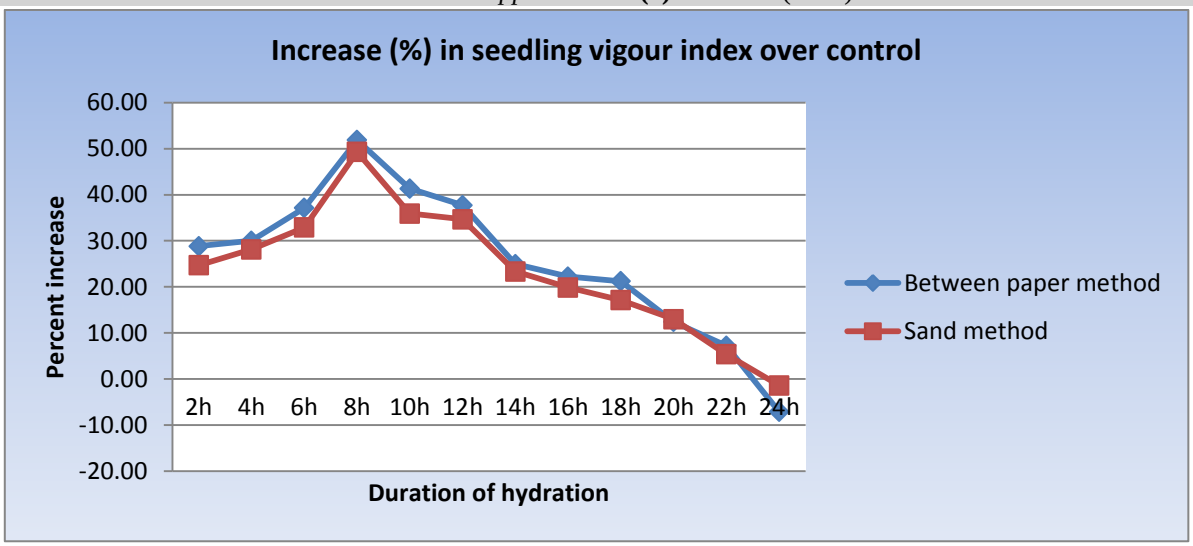

Figure 3: Percent increase in seedling vigour index by hydration in aged seed of chickpea over untreated aged seed as tested by different methods

CONCLUSION

The present study revealed that hydropriming is an effective tool for enhancement of seed germination and seedling vigour of aged seed of chickpea. The duration of hydration is important to achieve proper vigour enhancement. Hydropriming aged seed of for 8 hours is found to be the best for chickpea, while prolonged duration of priming resulted in degradation of seed quality.

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