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P Suma Varshini
Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Acharya N.G. Ranga Agricultural University, Guntur, Andhra Pradesh, India

Dr. K Bayyapu Reddy
Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Acharya N.G. Ranga Agricultural University, Guntur, Andhara Pradesh, India

Dr. K Radhika
Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Acharya N.G. Ranga Agricultural University, Guntur, Andhra Pradesh, India

Dr. V Saida Naik
Agricultural Research Station, Jangamaheswara Puram, Acharya N.G. Ranga Agricultural University, Guntur, Andhra Pradesh, India

Correspondence
P Suma Varshini
Department of Seed Science and Technology, Advanced Post Graduate Centre, Lam, Acharya N.G. Ranga Agricdultural University, Guntur, Andhra Pradesh, India

Influence of seed invigoration treatments on germination and vigor of chickpea

P Suma Varshini, Dr. K Bayyapu Reddy, Dr. K Radhika and Dr. V Saida Naik

Abstract
The present investigation was carried out to study the effect of invigoration treatments on seed germination, seedling growth and vigor of aged seed of chickpea, by subjecting one year old seed of chickpea to various invigoration treatments *viz*., hydration, hydration followed by seed treatment with thiram, seed treatment with 50 ppm GA_3, 2% KH_2PO_4, 2% $CaCl_2$ and 2% KNO_3 for 8 hours and osmo-conditioning with -0.5 MPa PEG for 6 hours followed by shade drying to 9% moisture content. The invigorated aged seed along with untreated aged seed and fresh seed (check) was tested for germination and seedling quality. Among the invigoration treatments, seed treated with $CaCl_2$ and osmo-conditioning with PEG showed significantly higher germination and other seed quality traits over untreated aged seed. The germination and seedling vigor index in $CaCl_2$ treated seed were significantly higher than that in fresh seed also.

Keywords: Calcium chloride, chickpea**,** invigoration, seed quality

Introduction
Chickpea (*Cicer arietinum* L.) is a rainfed, low input and winter leguminous crop used in various foods by several developing countries, particularly in India. It plays an important role in human nutrition as a source of protein, energy, fiber, vitamins and minerals for large population sectors in the developing world. However, the productivity is low and unstable as most of the area under chickpea cultivation is rainfed.
Poor germination and low seed viability are the serious problems limiting the production of chickpea. These conditions result in poor emergence that may subsequently cause sparse plant stands in the field. Seed is usually produced in excess as a precaution against germination failure and also against failure of crop in the subsequent crop growth period. Such left over seed when stored for sowing in subsequent season through the hot and humid monsoon periods looses viability and vigour. Seed priming is one of the simple and suitable methods which can improve seedling vigor and establishment and consequently crop performance in the field (McDonald, 2000) [1].
Seed priming involves controlled hydration of seed to enhance the metabolic activity within the seed but preventing radicle emergence. Pre-sowing soaking treatment of seed leads to redistribution of nutrients, increased tissue hydration, enhanced respiratory activity and stimulation of seedling growth and development. The product of these changes persists following desiccation and is available on re-imbibition of water during seed sowing, enabling completion of seed germination rapidly. Seed being a living entity, deterioration in its quality occurs with the advancement in ageing which is irreversible and continuous process. Under invigoration, metabolic repair occur in the deteriorated seed before the onset of germination process. The efficiency of different priming agents varies under different stresses and in different crop species (Ashraf and Foolad, 2005) [2]. Keeping this in view, the present study was conducted to study the impact of different seed invigoration treatments on seed germination and seedling vigor in aged seed of chickpea.

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. The fresh (*Rabi*, 2016-17 harvested) and aged (*Rabi*, 2015-16 harvested) seed of chickpea variety, NBeG-3, was obtained from the Regional Agricultural Research Station, Nandyal.
Aged (*Rabi*, 2015-16 harvested) seed of chickpea variety, NBeG-3, having initial germination of 81.75% was subjected to various seed invigoration treatments *viz*., hydration, hydration followed by seed treatment with thiram, seed treatment with 50 ppm GA_3, 2% KH_2PO_4, 2%

$CaCl_2$ and 2% KNO_3 for 8 hours and osmo-conditioning with -0.5 MPa PEG for 6 hours and shade dried to 9% moisture content.

50 ppm GA_3 was prepared by dissolving 50 mg of GA_3 in 5 mL of ethyl alcohol and making up the final volume to 1 litre using distilled water. 2% KH_2PO_4, 2% $CaCl_2$ and 2% KNO_3 solutions were prepared by dissolving 20 g of respective chemicals in 1 litre of distilled water. The aged seed were invigorated by soaking them in respective chemical solutions using 1:5 seed weight to solution volume (w/v) ratio for 8 hours. Polyethylene glycol solution of -0.5 MPa osmotic potential was prepared by dissolving 212.6 g PEG L^{-1}. Osmo-conditioning was done for 6 hours by keeping the seed in petriplates with two discs of blotter paper moistened with -0.5 MPa PEG solution. All the invigorated seed was dried back to 9% moisture content under shade at room temperature.

The invigorated seed along with the untreated aged seed (control) and untreated fresh seed (check) were used for evaluation of seed quality by germination test. 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. 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 hypocotyl. 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 lengths. 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 (1973) [3] 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 analysis of variance of the data showed significant variation in germination, root length, seedling length and seedling vigor index. Shoot length showed significant variation only at 5% level of significance. The variation in root / shoot ratio due seed invigoration was non-significant (Table 1).

Germination (%)

There was a significant difference in the initial germination of aged seed (*Rabi*, 2015-16 harvested seed) (84.50%) and that of fresh seed (*Rabi*, 2016-17 harvested seed) (88.00%). Among the seed invigoration treatments, the germination ranged from 81.00% to 96.50%. Highest germination (96.50%) was recorded in aged seed treated with $CaCl_2$. Osmo-conditioning of seed with PEG 6000 and hydration followed by seed treatment with thiram recorded same germination (89.50%) which was more than that of fresh seed (88.00%) and statistically superior over that of untreated aged seed (84.50%). Seed treated with KH_2PO_4 recorded lowest germination (81.00%) followed by GA_3 (83.00%) which was noticed to be below that of untreated aged seed. The germination in hydration treatment (86.00%) and KNO_3 (85.50%) treatment were slightly lower but statistically on par with that of fresh seed (Table 2).

Such enhancement in germination with $CaCl_2$ seed treatment was earlier reported in rice (Kata *et al*., 2014) [4]. Christiansen and Foy (1979) [5] reported that seed calcium concentration and germination were positively correlated which suggested the role of calcium as an important component in membrane stabilization and as an enzyme co-factor. Enhancement in germination due to osmo-priming with polyethylene glycol was earlier reported in wheat (Faijunnahar *et al*., 2017) [6]. Agawane and Parhe (2015) [7] also reported an increase in germination by seed priming with KNO_3 and hydro priming. Coolbear *et al*. (1979) [8] suggested that priming improves rRNA integrity, which supports protein synthesis to permit subsequent seed germination. Kata *et al*. (2014) [4] observed significantly higher □-amylase activity in primed seed compared to control which may be the probable reason for improvement of germination and related parameters.

Root Length (cm)

Aged seed recorded lesser root length (11.72 cm) than fresh seed (13.29 cm). Among the seed invigoration treatments, the root length varied from 10.87 cm to 14.78 cm. The highest root length was recorded by seed treatment with $CaCl_2$ (14.78 cm) followed by osmo-conditioning with PEG (14.07 cm) which were statistically superior over untreated aged seed but on par with fresh seed. The seed treated with KH_2PO_4 recorded lowest root length (10.87 cm) which was lower than that in untreated aged seed. The root length in the remaining treatments was higher than that in untreated aged seed (Table 2).

These results are in agreement with earlier findings of Janmohammadi *et al*. (2013) [9] in chickpea who reported an increase in root length due to treatment with $CaCl_2$, KNO_3 and hydration. Faijunahar *et al*. (2017) [6] observed an increase in root length by osmopriming with PEG in wheat. The increase in root length was attributed to increased rate of cell division in the root tips (Bose and Mishra, 1992) [10]. K^+ and Ca^{2+} improve cell water status, and also act as cofactors in the activities of numerous enzymes which are active when reserve mobilization and radical protrusion are in progress (Farooq *et al*., 2006) [11].

Shoot Length (cm)

The shoot length recorded in aged seed (12.32 cm) was lower than that in fresh seed (14.06 cm). Among the seed invigoration treatments, the seed treated with $CaCl_2$ recorded highest shoot length (14.54 cm) followed by PEG (14.41 cm) which were statistically superior over untreated aged seed and on par with fresh seed. All the remaining treatments in the present investigation except that with KH_2PO_4 (11.97 cm) recorded higher shoot length over untreated aged seed (Table 2).

Vishwas *et al*. (2017) [12] recorded higher shoot length in $CaCl_2$ treated chickpea seed over control. Sathish and

Sundareswaran (2010) [13] observed increased shoot length in maize by priming with KH_2PO_4, KNO_3 and $CaCl_2$. Improved shoot length might be caused by increased cell division within the apical meristem (Farooq *et al*., 2008) [14] or due to early emergence induced by the priming treatment (Vishwas *et al*., 2017) [12].

Seedling Length (cm)

The seedling length obtained with fresh seed (27.35 cm) was statistically superior to that of untreated aged seed (24.04 cm). Among the seed invigoration treatments, treating of aged seed with $CaCl_2$ recorded highest seedling length (29.32 cm) followed by osmo-conditioning with PEG 6000 (28.48 cm) which were statistically superior over untreated aged seed and on par with that of fresh seed (Table 2). All the invigoration treatments except seed treatment with KH_2PO_4 (22.84 cm) showed an improvement in seedling length of aged seed.

The improvement in seedling length with $CaCl_2$ was reported earlier in wheat (Amin *et al*., 2016) [15]. Faijunnahar *et al*. (2017) [6] obtained similar results with PEG in wheat. Increased □-amylase activity makes more reducing sugars available for utilization in the production of embryonic structures and contributes to the improvement in subsequent seedling growth (Farooq *et al*., 2010) [16].

Root / Shoot Ratio

The root / shoot ratio ranged from 0.90 in seed treated with KNO_3 to 1.04 in seed treated with GA_3 indicating non-significant influence of seed invigoration treatments on this trait (Table 2).

Seedling Vigor Index

The seedling vigor index of fresh seed (2405) was statistically superior to that of untreated aged seed (2030). Among the invigoration treatments, treating of aged seed with $CaCl_2$ recorded highest seedling vigor index (2829) and statistically superior to all the other treatments including untreated aged seed and fresh seed. Osmo-conditioning with PEG 6000 (2550), hydration followed by seed treatment with thiram (2379) and hydration (2249) treatments were statistically superior over untreated aged seed (2030) and on par with fresh seed (2405). Seed treated with GA_3 (2161) and KNO_3 (2149) recorded slightly higher seedling vigor index but statistically on par with untreated aged seed. Seed treated with KH_2PO_4 recorded lowest seedling vigor index (1847) which was lower than that in untreated aged seed (Table 2), which might be due to the minimum germination and seedling length recorded with KH_2PO_4 treatment.

The results are in agreement with the earlier reports of Rao *et al*. (2012) [17] in mungbean where the seedling vigor index was improved significantly by hydration followed by seed treatment with thiram and seed treatment with $CaCl_2$. Significant enhancement in seedling vigor index when primed with polyethylene glycol was earlier reported in wheat by Baque *et al*., (2016) [18].

Seed priming causes enhancement in □-amylase activity that hydrolyses macro starch molecules into smaller and simple sugars which are readily available to the germinating seed making them more vigorous (Rao *et al*., 2012) [17]. The increase in seedling vigor index may be due to the activation of growth promoting substances and translocation of secondary metabolites to the growing seedling (Renganayaki and Ramamoorthy, 2015 [19].

Table 1: Mean squares for seed quality traits in aged seed of chickpea as affected by seed invigoration treatments

Source	d.f	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root / shoot ratio	Seedling vigor index
Treatment	8	80.273**	6.006**	3.361*	16.749**	0.002^{NS}	340395.088**
Error	27	4.209	1.808	1.313	1.862	0.003	16475.187

* Significant difference at 5% probability level

** Significant difference at 1% probability level

NS: Non-significant

Table 2: Influence of seed invigoration treatments on seed quality of aged seed of chickpea

Treatment	Germination (%)		Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root / shoot ratio		Seedling vigor index
T_1 - Aged (*Rabi*, 2015-16 harvested seed – untreated (control)	84.50	(66.82)*^{cde}	11.72^{cd}	12.32^{bc}	24.04^{ef}	0.96	(1.40)**	2030^{ef}
T_2 - Hydration treatment	86.00	$(68.02)^{bcd}$	13.26^{abc}	12.87^{abc}	26.14^{cde}	1.03	(1.43)	2249^{cd}
T_3 - Hydration followed by seed treatment with thiram @ 3 g kg^{-1} seed	89.50	$(71.12)^{b}$	12.56^{bcd}	13.80^{abc}	26.56^{bcd}	0.92	(1.38)	2379^{bc}
T_4 - Osmo-conditioning with PEG 6000 (-0.5 Mpa)	89.50	$(71.12)^{b}$	14.07^{ab}	14.41^{a}	28.48^{ab}	1.01	(1.41)	2550^{b}
T_5 - Seed treatment with 50 ppm GA_3	83.00	$(65.67)^{de}$	13.21^{abc}	12.83^{abc}	26.04^{cde}	1.04	(1.43)	2161^{de}
T_6 - Seed treatment with 2% KH_2P0_4	81.00	$(64.17)^{e}$	10.87^{d}	11.97^{c}	22.84^{f}	0.91	(1.38)	1847^{f}
T_7 - Seed treatment with 2% $CaCl_2$	96.50	$(79.47)^{a}$	14.78^{a}	14.54^{a}	29.32^{a}	1.02	(1.42)	2829^{a}
T_8 - Seed treatment with 2% $KN0_3$	85.50	$(67.62)^{cd}$	11.84^{cd}	13.31^{abc}	25.15^{de}	0.90	(1.38)	2149^{de}
T_9 - Fresh (*Rabi*, 2016-17 harvested seed (Check)	88.00	$(69.84)^{bc}$	13.29^{abc}	14.06^{ab}	27.35^{abc}	0.95	(1.39)	2405^{bc}
Mean	87.06	(69.32)	12.84	13.35	26.21	0.97	(1.40)	2289
CD (5%)	2.98		1.95	1.66	1.98	NS		186.24
S Em ±	1.03		0.67	0.57	0.68	0.03		64.18
CV (%)	2.96		10.47	8.59	5.21	3.92		5.61

*Values in the parenthesis indicate arc-sine transformed values

**Values in the parenthesis indicate square root transformed values

NS: Non-significant

The values in the same column with the same alphabetical letter are not significantly different (P<0.05) as per the Duncan's Multiple Range Test

Conclusion

Aamong the invigoration treatments used in the present study, seed treatment with $CaCl_2$ and osmo-conditioning with PEG showed significantly higher germination, root length, shoot length, seedling length and seedling vigor index over untreated aged seed. The germination and seedling vigor index in $CaCl_2$ treated seed were significantly higher than that in fresh seed also. The remaining treatments except that with KH_2PO_4 also improved the germination and vigour index. These results suggest that seed germination and vigor in aged chickpea seed can be improved to some extent by seed invigoration treatments.

Reference

1. McDonald MB. Seed priming. In Black, M and Bewley, J.D. (Eds.) - Seed Technology and Biological Basis, Sheffield Academic Press, England, 2000, 287-325.
2. Ashraf M, Foolad MR. Pre-sowing seed treatment - a shotgun approach to improve germination, plant growth and crop yield under saline and non-saline conditions. Advances in Agronomy. 2005; 88:223-271.
3. Abdul-Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. Crop Science. 1973; 13:630-633.
4. Kata LP, Bhaskaran M, Umarani R. Influence of priming treatments on stress tolerance during seed germination of rice. International Journal of Agriculture, Environment and Biotechnology. 2014; 7(2):225-232.
5. Christiansen MN, Foy CD. Fate and function of calcium in tissue. Communications in Soil Science and Plant Analysis. 1979; 10:427-442.
6. Faijunnahar M, Baque A, Habib MA, Hossain HMMT. Polyethylene glycol (PEG) induced changes in germination, seedling growth and water relation behavior of wheat (*Triticum aestivum* L.) genotypes. Universal Journal of Plant Science. 2017; 5(4):49-57.
7. Agawane RB, Parhe SD. Effect of seed priming on crop growth and seed yield of soybean [*Glycine max* (L.) Merill]. The Bioscan. 2015; 10(1):265-270.
8. Coolbear P. Studies on the changes in the major nucleic acid components of tomato seeds (*Lycopersicon esculentum* Mill.) resulting from osmotic presowing treatment. Journal of Experimental Botany. 1979; 30:1153-1162.
9. Janmohammadi M, Bashiri A, Asghari-Shirghan R, Sabaghnia N. Impact of pre-sowing seed treatments and fertilizers on growth and yield of chickpea (*Cicer arietinum* L.) under rainfed conditions. Natura Montenegrina. 2013; 12(1):217-229.
10. Bose B, Mishra T. Response of wheat seed to pre-sowing seed treatment with Mg $(NO_3)_2$. Annals of Agricultural Research. 1992; 13:132-136.
11. Farooq M, Basra SMA, Wahid A. Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. Plant Growth Regulation. 2006; 49:285-294.
12. Vishwas S, Chaurasia AK, Bara BM, Debnath A, Parihar NN, Brunda K *et al*. Effect of priming on germination and seedling establishment of chickpea (*Cicer arietinum* L.) seeds. Journal of Pharmacognosy and Phytochemistry. 2017; 6(4):72-74.
13. Sathish S, Sundareswaran S. Standardisation of seed priming technique in maize hybrid COH (M) 5. Madras Agricultural Journal. 2010; 97(10-12):315-318.
14. Farooq M, Basra SMA, Rehman H, Hussain M. Seed priming with polyamines improves the germination and early seedling growth in fine rice. Journal of New Seeds. 2008; 9(2):145-155.
15. Amin R, Khan AZ, Muhammad A, Khalil SK, Gul H, Daraz, G *et al*. Influence of seed hardening techniques on vigor, growth and yield of wheat under drought conditions. Journal of Agricultural Studies. 2016; 4(3):121-131.
16. Farooq M, Basra SMA, Wahid A, Ahmad N. Changes in nutrient homeostasis and reserves metabolism during rice seed priming: Consequences for seedling emergence and growth. Agricultural Sciences in China. 2010; 9(2):191-198.
17. Rao PS, Ankaiah R, Reddy BG. Effect of pre-sowing and invigoration treatment for better crop establishment of mungbean. International Journal of Science and Research. 2012; 3(9):1926-1929.
18. Baque A, Nahar M, Yeasmin M, Quamruzzaman M, Rahman A, Azad MJ *et al*. Germination behavior of wheat (*Triticum aestivum* L.) as influenced by polyethylene glycol (PEG). Universal Journal of Agricultural Research. 2016; 4(3):86-91.
19. Renganayaki PR, Ramamoorthy K. Enhancing planting value of marginal seed lots of blackgram through hydration-dehydration (HD) treatment. Research Journal of Seed Science. 2015; 8(1):22-29.