

## Effect of Seed Priming on Germination Properties and Seedling Establishment of Cowpea (*Vigna sinensis*)

Hamdollah ESKANDARI\*, Kamyar KAZEMI

Payame Noor University, Agriculture Department, 19395-4697, Tebran, Iran; [ehamdollah@gmail.com](mailto:ehamdollah@gmail.com) (\*corresponding author)

### Abstract

Early emergence and stand establishment of cowpea are considered to be the most important yield-contributing factors in rainfed areas. Laboratory tests and afield experiment were conducted in RCB design in 2011 at a research farm in Ramhormoz, Iran, to evaluate the effects of hydropriming (8, 12 and 16 hours duration) and halo priming (solutions of 1.5% KNO<sub>3</sub> and 0.8% NaCl) on seedling vigor and field establishment of cowpea. Analysis of variance of laboratory data showed that hydropriming significantly improved germination rate, seed vigor index, and seedling dry weights. However, germination percentage for seeds primed with KNO<sub>3</sub> and non-primed seeds were statistically similar, but higher than those for NaCl priming. Overall, hydropriming treatment was comparatively superior in the laboratory tests. Invigoration of cowpea seeds by hydropriming and NaCl priming resulted in higher seedling emergence and establishment in the field, compared to control and seed priming with KNO<sub>3</sub>. Seedling emergence rate was also enhanced by priming seeds with water, suggesting that hydropriming is a simple, low cost and environmentally friendly technique for improving seed and seedling vigor of cowpea.

**Keywords:** cowpea, hydro priming, salt priming, seed germination

### Introduction

Germination and seedling establishment are critical stages in the plant life cycle. In crop production, stand establishment determines plant density, uniformity and management options (Cheng and Bradford, 1999). One of the major contributing factors to low crop yield in many developing countries is poor establishment, especially as a result of water shortage at crucial time during crop growth period. High speed and uniform germination of seed and its ability to germinate under water deficiency affect crop establishment (Fischer and Turner, 1978). However, if the stress effect can be alleviated at the germination stage, chances for attaining a good crop establishment would be high (Ashraf and Rauf, 2001). Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance. Seed priming is soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radical emergence (McDonald, 2000).

There are reports that seed priming permits early DNA replication, increase RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites. Seed priming is seen as a viable technology to enhance rapid and uniform emergence, high vigor and better yields in some field crops (Basra *et al.*, 2002; Chiu *et al.*, 2002; Harris *et al.*, 1999; Murungu *et al.*, 2004). Common priming techniques include os-

mopriming (soaking seeds in osmotic solutions such as polyethylene glycol), halo priming (soaking seeds in salt solutions) and hydropriming (soaking seeds in water). Previous studies on pepper (Amjad *et al.*, 2007), sugarcane (Patade *et al.*, 2009) and melon showed that halo priming improves seed germination, seedling emergence and growth under saline and drought conditions. Harris *et al.* (1999) demonstrated that on-farm seed priming (soaking seeds overnight in water) markedly improved establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. Hydropriming value has already been shown for many crops, for example wheat (Harris *et al.*, 2001); chickpea (Musa *et al.*, 2001), maize (Ashraf and Rauf, 2001), sunflower (Kaya *et al.*, 2006) and Barley (Abdulahmani *et al.*, 2007).

Since there is not much evidence about seed priming of cowpea, this research was aimed to investigate the effects of halo priming and hydropriming on seed invigoration and seedling emergence of this crop in the field.

### Materials and methods

Seed moisture content of cowpea seeds was determined by the high-temperature oven method at 130±2°C for 4 hours (ISTA, 2003). Mean moisture content of the seed sample was about 9.5%. Seeds were pre-treated with mancozeb fungicide at a rate of 3.3 g/kg, in order to control possible fungal contamination during priming. Seed sample was divided into six sub-samples. One of the sub-sam-

ples was considered as control (unprimed) and the other five sub-samples were prepared for priming treatments.

#### Seed priming

The effects of seed priming was evaluated by soaking seeds in solutions of  $KNO_3$  (1.5%), NaCl (0.8 %) (Ghassemi-Golezani *et al.*, 2011) and distilled water for 8, 12 and 16 hours. All priming treatments were performed in an incubator adjusted on  $20 \pm 1^\circ C$  under dark conditions. After priming, samples of seeds were removed and the seeds of salt priming treatments were rinsed three times in distilled water and then dried to the original moisture level.

#### Laboratory germination and seedling vigor

Four replicates of 25 seeds were germinated between double layered rolled germination papers. The rolled paper with seeds was put into plastic bags to avoid moisture loss. Seeds were allowed to germinate at  $10 \pm 1^\circ C$  (Ghassemi-Golezani *et al.*, 2008) in the dark for 14 days. Germination was considered to have occurred when the radicles were 2 mm long. Germinated seeds were recorded every 24 h for 14 days. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980):

$$MGT = \sum(Dn) / \sum n$$

where  $n$  is the number of seeds germinated on day  $D$ ,  $D$  is the number of days counted from the beginning of the test and is mean germination rate.

Seed vigor index (SVI) was calculated as:

$$SVI = SDW / MGT$$

where MGT is mean germination time and SDW is seedling dry weight.

The seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated (ISTA, 2003). At the end of germination test (14 days), radicles and shoots were cut from the cotyledons and then dried in an oven at  $75 \pm 2^\circ C$  for 24 hours. The dried radicles and shoots were weighed to the nearest milligram and the mean radicle and shoot dry weight and consequently mean seedling dry weight were determined.

#### Field emergence

Field experiment was conducted in 2011 at a Research Farm in Ramhormoz, Iran ( $46^\circ 36' N$ ,  $31^\circ 16' E$ , altitude 150 m above sea level). The plots were  $10 m^2$  with six sowing rows of 2 m long. Seeds were treated with Mankozeb at a rate of 3 g/kg before sowing. The plots size was  $12 m^2$  consist of six rows of 2 m long. The rows located 50 cm apart. The seeds were then sown in a silt-loam soil at a depth of about 3 cm during the last week of July. Number of emerged seedlings in an area of  $1 m^2$  within each plot was counted in daily intervals until seedling establishment became stable. Seedling emergence rate was calculated in accordance with the equation introduced by Ellis and Roberts (1980).

#### Experimental design

Laboratory tests were carried out at the Seed Technology Laboratory of Payame-Noor university of Ahwaz, Iran, using randomized complete block (RCB) design with 3 replicates. Field experiment was conducted with 3 replicates on the basis of RCB design in 2011. Analysis of variance (ANOVA) of the laboratory and field emergence data were carried out, using MSTATC software. Excel software was used to draw figures. Means were compared by applying Duncan's multiple range test (DMRT) at 5% probability.

#### Results and discussion

The effect of seed priming on germination percentage of cowpea seeds was significant (Tab. 1). The highest and the lowest germination percentages were obtained for seeds primed with 16 hours of hydropriming and NaCl, respectively (Fig. 1). However, differences between seeds primed with different hydropriming treatments and  $KNO_3$  were not significant (Fig. 1). Seed germination rate was significantly affected by seed priming (Tab. 1). The highest germination rate obtained for seeds primed with water with 16 and 12 hours duration. Germination rate of seed primed with water (8 hours) was significantly higher than that of salt priming (Fig. 1). The highest value of seed vigor index was observed for hydropriming. However, this trait was statistically similar for salt primed and unprimed seeds (Fig. 1). Seeds primed with water were significantly superior in seedling dry weight, compared to other seed treatments.

There were significant differences in percentage and rate of seedling emergence among seed treatments (Tab. 2). Seedling emergence percentage of hydro primed seeds

Tab. 1. Analysis of variance (ANOVA) for seed priming effects on cowpea seed germination and vigor in laboratory

Source of variance	df	Mean of squares			
		Germination (%)	Germination rate (day)	SVI	SDW
Replication	2	17.1	0.003	0.168	0.000031
Treatment	5	53.14**	0.1456**	0.499**	0.0000241**
Error	10	5.4	0.005	0.0301	5.441E-6
CV (%)		5.6	11.2	10.5	13.4

\*\* significant at  $P \leq 0.01$ , SVI: Seed vigor index; SWD: Seedling dry weight

Tab. 2. Analysis of variance (ANOVA) for seed priming effect on cowpea fo field emergence and establishment

Source of variance	df	Mean of squares	
		Seedling emergence (%)	Seedling emergence rate (day)
Replication	2	16.6	0.005
Treatment	5	49.18**	6.218**
Error	10	4.4	0.009
CV (%)		8.3	9.1

\*\* significant at  $P \leq 0.01$

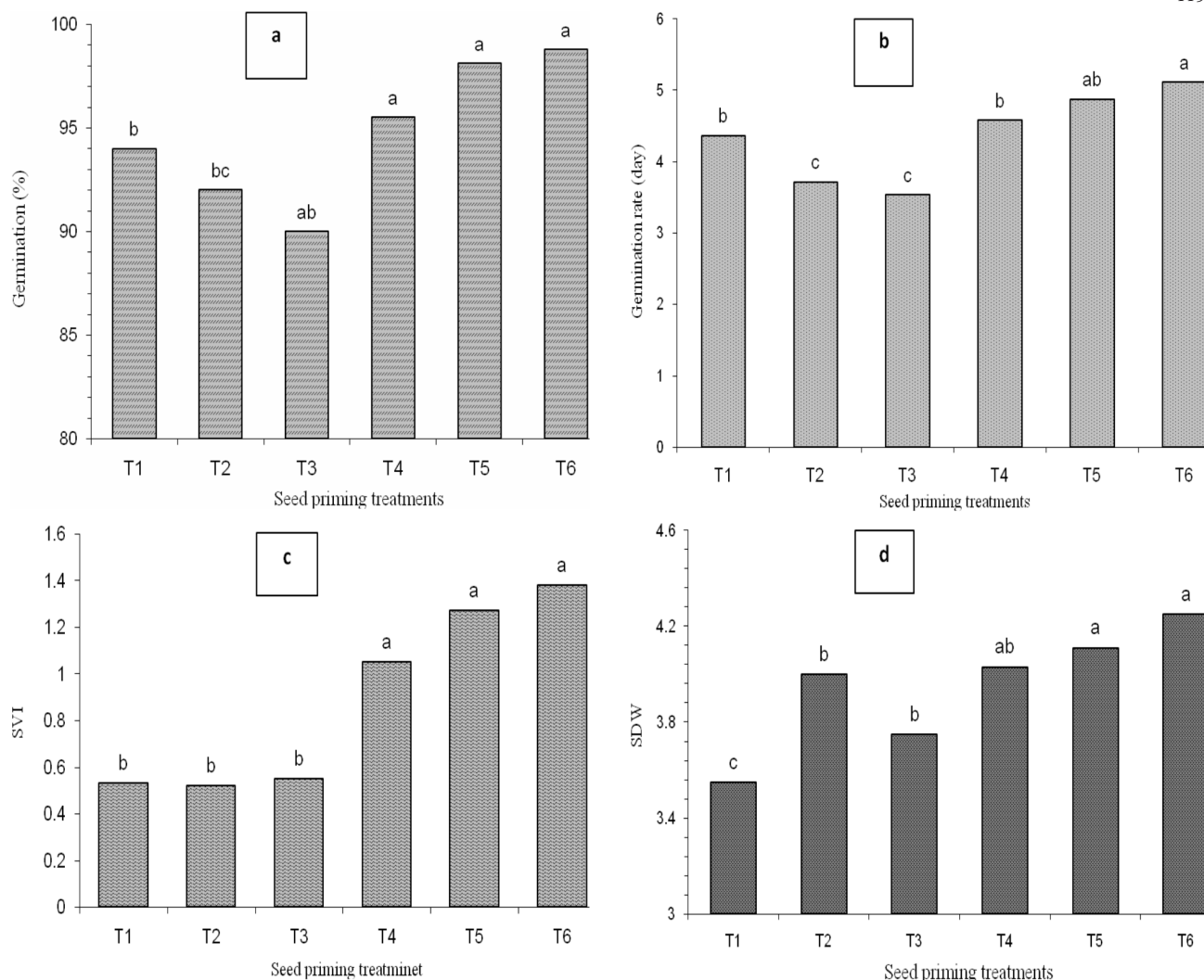


Fig. 1. Effect of priming on germination properties of cowpea seeds in laboratory; (a) Germination percentage; (b) Germination rate; (c) Seed vigor index (SVI); (d) Seedling dry weight (SDW). T<sub>1</sub>: non-primed; T<sub>2</sub>, T<sub>3</sub>: Salt priming of NaCl and KNO<sub>3</sub>, respectively; T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>: Hydropriming of 8, 12 and 16 hours

and seeds primed with NaCl was higher than those with KNO<sub>3</sub> primed and unprimed seeds (Tab. 3). Seed priming with water enhanced seedling emergence rate in the field. The highest rate of seedling emergence was observed in primed seeds with water with 16 hours duration (Tab. 3).

Tab. 3. Comparison of means for seed priming effects on cowpea for field emergence and establishment

Treatment	Seedling emergence (%)	Seedling emergence rate (day)
T <sub>1</sub>	51.16 a	0.081 b
T <sub>2</sub>	60.18 a	0.079 b
T <sub>3</sub>	53.1 b	0.075 c
T <sub>4</sub>	66.6 a	0.091 b
T <sub>5</sub>	65.4 a	0.090 b
T <sub>6</sub>	67.4 a	0.095 a

T<sub>1</sub>: non-primed; T<sub>2</sub>, T<sub>3</sub>: Salt priming of NaCl and KNO<sub>3</sub>, respectively; T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>: Hydropriming of 8, 12 and 16 hours

According to Sadeghi *et al.* (2011) completion of pre-germination metabolic activities during seed priming, making the seed ready for soon germination after planting compared with unprimed seeds. During priming, the embryo expands and compresses the endosperm (Liptay and Zaiffa, 1993). The compression force of the embryo and hydrolytic activities on the endosperm cell walls may deform the tissues that have lost their flexibility upon dehydration (Lin *et al.*, 1993), producing free space and facilitating root protrusion after rehydration. The fastest rate of germination was obtained by soaking seeds in water, probably due to faster water uptake and earlier initiation of metabolism processes. Hydro primed seeds produced the seedlings, compared to the other seed treatments (Fig.1). This means that during priming, seeds would be simultaneously subjected to processes of repair and deterioration and force between the two determined the success or failure of the treatment (McDonald, 2000).



Hydro priming and halo priming with NaCl improved seedling emergence rate and percentage and consequently seedling establishment in the field (Tab. 3). Good germination and emergence are the key to controlling stand establishment. Kibite and Harker (1991) reported that seed hydration of wheat, barley and oats seeds improved the uniformity of seedling emergence. Harris *et al.* (1999) found that hydropriming enhanced seedling establishment and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. Similarly, vigorous early growth is often associated with better yields (Carter *et al.*, 1992). The resulting improved stand establishment can reportedly increase drought tolerance, reduce pest damage and increase crop yield (Harris *et al.*, 1999). These results suggest that hydropriming and priming with NaCl are useful techniques for improving seedling vigor and establishment of cow pea in the field.

### Conclusions

Priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. Our findings revealed that hydropriming is a simple and useful technique for enhancing seedling emergence rate and percentage of cowpea. These effects can improve seedling establishment and field performance of this important food legume.

### References

- Abdulrahmani B, Ghassemi-Golezani K, Valizadeh M, Feizi-Asl V (2007). Seed priming and seedling establishment of barley (*Hordeum vulgare* L.). J Food Agri Environ 5:179-184.
- Amjad M, Ziaf K, Iqbal Q, Ahmad I, Riaz MA (2007). Effect of seed priming on seed vigor and salt tolerance in hot pepper. Pak J Agri Sci 44(3):408-419.
- Ashraf M, Rauf H (2001). Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts, growth and ion transport at early growth stages. Acta Physiol Plant 23:407-414.
- Basra SMA, Zia MN, Mehmood T, Afzal I, Khaliq A (2002). Comparison of different invigoration techniques in wheat seeds. Pak J Arid Agric 5:11-16.
- Carter DC, Harris D, Youngquist JB, Persaud N (1992). Soil properties, crop water use and cereal yields in Botswana after additions of mulch and manure. Field Crops Res 30:97-109.
- Cheng Z, Bradford KJ (1999). Hydrothermal time analysis of tomato seed germination responses to priming treatments. Exp Bot 33:89-99.
- Chiu KY, Chen CL, Sung JM (2002). Effect of priming temperature on storability of primed sh-2 sweet corn seed. Crop Sci 42:1996-2003.
- Ellis RH, Roberts EH (1980). Towards rational basis for testing seed quality, p. 605-635. In: Hebblethwaite PD (Ed.). Seed Production. Butterworths, London.
- Fischer RA, Turner NC (1978). Plant productivity in the arid and semiarid zones. Ann Rev Plant Physiol 29:277-317.
- Ghassemi-Golezani K, Aliloo AA, Valizadeh M, Moghaddam M (2008). Effects of hydro and osmo priming on seed germination and field performance of lentil. Not Bot Horti Agrobo 36(1):29-33.
- Ghassemi-Golezani K, Jabbarpour S, Zehtab-Salmasi S, Mohammadi A (2011). Response of winter rapeseed cultivars to salt priming of seeds. African J Agri Res 5(10):1089-1094.
- Ghassemi-Golezani K, Khazaei F, Yari L, Sheidaei S (2011). Effect of seed osmopriming on seed germination behavior and vigor of soybean. J Agri Biol Sci 6(1):39-43.
- Harris D, Joshi A, Khan PA, Gothkar P, Sodhi PS (1999). On-farm seed priming in semi-arid agriculture development and evaluation in maize, rice and chickpea in India using participatory methods. Exp Agric 35:15-29.
- Harris D, Raghuwanshi BS, Gangwar JS, Singh SC, Joshi KD, Rashid A, Hollington, PA (2001). Participatory evaluation by farmers of 'on-farm' seed priming in wheat in India. Nepal and Pakistan Exp Agric 37:403-415.
- ISTA (2003). International Seed Testing Association, ISTA Handbook on Seedling Evaluation, 3<sup>rd</sup> Ed.
- Kaya MD, Okcu G, Atak M, İkili Y, Kolsarıcı O (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Europ J Agr 24:291-295.
- Kibite S, Harker KN (1991). Effects of seed hydration on agronomic performances of wheat, barley and oats in central Alberta. Can J Plant Sci:71:515-518.
- Lin Y, Van Der Burg WJ, Aartse JW, van Zwol RA, Jalink H, Bino RJ (1993). X-ray studies on changes in embryo and endosperm morphology during priming and inhibition of tomato seeds. Seed Sci Res 3:171-178.
- Liptay A, Zariffa N (1993). Testing the morphological aspects of polyethylene glycol-primed tomato seeds with proportional odds analysis. Hort Sci 28:881-883.
- McDonald MB (2000). Seed priming, Black, Seed Technology and Its Biological Basis, p. 287-325. In: Bewley MJD (Ed.). Sheffield Academic Press, Sheffield, UK.
- Murungu FS, Chiduzo C, Nyamugafata P, Clark LJ, Whalley WR, Finch-Savage WE (2004) Effects of 'on-farm seed priming' on consecutive daily sowing occasions on the emergence and growth of maize in semi-arid Zimbabwe. Field Crops Res 89:49-57.
- Musa AM, Harris D, Johansen C, Kumar J (2001). Short duration chickpea to replace fallow after aman rice: the role of on-farm seed priming in the High Barind Tract of Bangladesh. Exp Agric 37:509-521.
- Patade VK, Bhargava S, Suprasanna P (2009). Halopriming impacts tolerance to salt and PEG induced drought stress in sugarcane. Agri Ecosys Environ 134(1-2):24-28.
- Sadeghi H, Khazaei F, Yari L, Sheidaei S (2011). Effect of seed osmopriming on seed germination behavior and vigor of soybean (*Glycine max* L.). ARPJ Agri Biol Sci 6(1):39-43.