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# Effect of Seed Hardening and Pelleting on Germination and Seedling Attributes of Cowpea under Saline Condition

S. Maamallan, M. Prakash, G. Sathiyanarayanan, S. Rameshkumar[1]

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## ABSTRACT

Cowpea (*Vigna unguiculata*) is an important legume crop. Investigations were undertaken to study the effect of seed hardening, seed pelleting and combined effect of seed hardening with seed pelleting on germination and seedling growth of cowpea under induced saline condition *viz*., $EC_4$, $EC_8$, $EC_{12}$ and $EC_{16}$. Cowpea seeds were hardened with KCl @ 1%, $CaCl_2$ @ 1% and both KCl and $CaCl_2$ @ 1% and hardened seeds were further pelleted with pungam leaf powder @ 100, 150 and 200 g $kg^{-1}$ of seed. Observations on germination % and seedling parameters were recorded. The experimental results revealed that increased salinity reduced germination and seedling growth in terms of germination %, speed of germination, root length, shoot length, dry matter production and vigour index. However, hardening and pelleting treatments significantly increased all these parameters at all the levels of salinity when compared to control. Among the treatments, seeds hardened with KCl @ 1 % + $CaCl_2$ @ 1 % and pelleted with pungam leaf powder @ 200 g per kg recorded higher germination % and all the seedling parameters when compared to control and other treatments.


**Key words:** Cowpea, Germination, Hardening, Seed pelleting.

## INTRODUCTION

Pulses are one of the important food crops globally due to higher protein content. Pulses are the major sources of protein in the diet. Of all categories of people, pulses form an integral part of the Indian diet, providing much needed protein to the carbohydrate rich diet. Pulses are 20 to 25 per cent protein by weight which is double the protein content of wheat and three times that of rice. It is the cheapest and rich source of protein and essential amino acids and thus share a major protein of the vegetarian diet. Food legumes are economically important because they are not only good source of protein but also provide calories, vitamins and minerals in the diet of many individuals in the developed countries.

Among the pulses, cowpea (*Vigna unguiculata*) is an annual herbaceous legume from the genus vigna. Cowpea is mainly cultivated in Africa, Asia, Europe, United States and Central and South America. This is a food and animal feed crop. Generally this is well adapted in drier regions where other food legumes do not perform well. It is cultivated to obtain seeds and pods for human consumption and as a source of green manure and organic material. Due to its tolerance to sandy soil and low rainfall, it is an important crop in the semi-arid regions across Africa and other countries. It requires very few inputs as the plants root nodules are able to fix atmospheric nitrogen making it a valuable crop for resource poor farmers and is also well suited to intercropping with other crops.

Soil salinity is one of the major factors responsible for losses in agricultural production. Salt affected lands cover approximately 10% of the global land surface and nearly 50% of the irrigated cropland worldwide (Iseki *et al.,* 2016). Salinity stress reduced the speed and percentage germination of seed, decreased in shoot length, fresh and dry weights of shoots and roots of plants (Fateme *et al*., 2010). Presence of salt at concentrations higher than 50 mM NaCl affect the germination, seedlings growth and total protein synthesis in cowpea cultivars (Dantas *et al*., 2005).

Dasgan *et al*. (2002) suggested the screening at the seedling stage is not only less laborious, less time consuming and less expensive, but also has a high reliability. Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt. Various strategies can be adopted to cope with salinity stress such as screening and selection, conventional breeding and use of transgenics on the basis of morphological, physiological and biochemical traits (Mozahidul Islam *et al*., 2019).

To increase the crop yield under the adverse climatic conditions like low rainfall and soil salinity which prevents the germination and establishment of seedlings, seed

Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India. [1]Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.

**Corresponding Author:** M. Prakash, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.
Email: geeth_prakash@yahoo.co.in

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hardening and pelleting of seeds has been done which will give an initial boost for germinating seeds and growing seedlings.Hence seedlings can put forth better root and shoot growth and enhance the drought tolerance resulting in increasing yield (Opheliya, 2017). Seed hardening cum seed pelleting are the seed enhancement techniques, which is defined as the post-harvest treatments that improve germination and seedling growth.

Seed hardening is one of the pre-sowing seed management practice recommended for dryland and saline agriculture to resist the seed against the adverse agro ecological conditions the seed faces at the time of sowing until the seeds putforth seedling. Seed hardening imparts tolerance against abiotic stresses like drought and salinity, increase seed germination followed by better and quicker seedling emergence. Bandana Bose *et al.* (2016) observed that the hardening treatments resulted in reduction in membrane leakage, reduced days from sowing to 50% flowering, increased days to plant maturity after sowing and flowering and improved yield attributes. Amin *et al.* (2016) concluded that seed hardening with PEG 8000, $CaCl_2$ and $KNO_3$ for 24 hr showed maximum invigoration for improving vigour, growth and yield in wheat under drought stress.

Seed pelleting is the process of enclosing a seed with small quantity of inert material just large enough to produce globular unit of standard size to facilitate precision planting. The inherent material creates natural water holding media and provide small amount of nutrients to young seedlings. It also reduces the problem of thinning, gap filling and chemicals required in low quantity (Chaya Devi, *et al.* 2017). The cost involved in seed pelleting is also less but the benefit to the farmers especially who depends on monsoon showers is more.

The use of leaf powders in enhancing the crop yield has been reported by Bhattacharya *et al.* (2015). Pushpakaran *et al.* (2018) revealed that 200 g pungam leaf powder pelleted seeds recorded the higher quality parameters. Seeds of cluster bean cv. Pusa mausami were pelleted with pungam, prosopis, notchi, arappu leaf powder @ 50, 100, 150 and 200 g per kg and the results revealed that seed pelleting with pungam leaf powder @ 200 g per kg registered higher values for biometric parameters (Prakash *et al.*, 2018).

In this study, seed hardening chemicals are used to enhance germination under saline conditions and botanical leaf powders are used for seed pelleting to further strengthen the seedling establishment. The present investigation was undertaken with the objective to study the effect of seed hardening and pelleting on growth, yield and seed quality in cowpea under induced saline conditions.

## MATERIALS AND METHODS

Genetically pure seeds of cowpea cv. CO (CP) 7 (*Vigna unguiculata*) were obtained from Palur Research Station, (TNAU) for the study. The experiments were carried out at Seed Technology Laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India (located at 11°24′N, latitude and 79°44′E longitude with an altitude of + 5.79 mts above mean sea level) during 2017-2019.

### Seed hardening

Seeds were hardened with KCl @ 1%, $CaCl_2$ @ 1% and both KCl and $CaCl_2$ @ 1%.

### Seed pelleting

The fresh leaves of pungam (*Pongamia pinnata*) were collected separately and sundried. The dried leaves were powdered using pestle and mortar. A fine leaf powder was obtained by sieving through 0.10 mm wire mesh. To begin with, the required quantities of seeds were smeared with gum arabic as adhesive. The seed pelleting materials were taken in a glass container, gum smeared seeds were added and then container was rotated acentrically so that pelleting mixture was coated on the seed uniformly in required proportion and the pelleted seeds were air dried before use.

### Seed hardening and pelleting

Hardened seeds with KCl and $CaCl_2$ @ 1% concentration were further pelleted with leaf powder of pungam @ 100, 150 and 200 g $kg^{-1}$ of seed and a total of 9 treatments along with a control were taken for this study as per the following details.

### Treatments

$T_0$ - Control.
$T_1$ - Hardening with KCl @ 1% + pelleting with pungam leaf powder @ 100 g/kg.
$T_2$ - Hardening with KCl @ 1% + pelleting with pungam leaf powder @ 150 g/kg.
$T_3$ - Hardening with KCl @ 1% + pelleting with pungam leaf powder @ 200 g/kg.
$T_4$ - Hardening with $CaCl_2$ @ 1% + pelleting with pungam leaf powder @ 100 g/kg.
$T_5$ - Hardening with $CaCl_2$ @ 1% + pelleting with pungam leaf powder @ 150 g/kg.
$T_6$ - Hardening with $CaCl_2$ @ 1% + pelleting with pungam leaf powder @ 200 g/kg.
$T_7$ - Hardening with KCl @ 1% + $CaCl_2$ @ 1% + pelleting with pungam leaf powder @ 100 g/kg.
$T_8$ - Hardening with KCl @ 1% + $CaCl_2$ @1% + pelleting with pungam leaf powder @ 150 g/kg.
$T_9$ - Hardening with KCl @ 1% + $CaCl_2$ @ 1% + pelleting with pungam leaf powder @200 g/kg.

Saline treatment was imposed by using molecular grade Sodium Chloride (NaCl) (Hi-Media) and seedlings were evaluated for seedling parameters *viz*., germination percentage, shoot length, root length, total seedling length, dry matter production, seedling vigour index I and II. Six

different salinity levels used were prepared as per the following details.

**EC levels and concentrations of NaCl used to impose saline treatments**

| EC (dS $m^{-1}$) | Weight of NaCl (g) |
|---|---|
| 0 | Control (Distilled water) |
| 4 | 2.56 gm $l^{-1}$ |
| 8 | 5.12 gm $l^{-1}$ |
| 12 | 7.68 gm $l^{-1}$ |
| 16 | 10.24 gm $l^{-1}$ |

Hardened and pelleted seeds were sown in acid washed sand medium and salt solutions were added at the required quantity to induce 4 different levels (Fig 1) of salinity *viz*., 4, 8, 12 and 16 EC. Then the seeds were allowed to germinate on germination paper in sterile petriplates (60× 15mm), with 5ml of distilled water for control and respective test solutions for inducing salt stress. The experiment was carried out with mean temperature of 28±2°C and relative humidity of 65°C maintained with 16 hours of photoperiod and 8 hours of darkness to minimize the changes of salt concentration and to prevent the evaporation of water, petriplates were sealed tightly with parafilm.

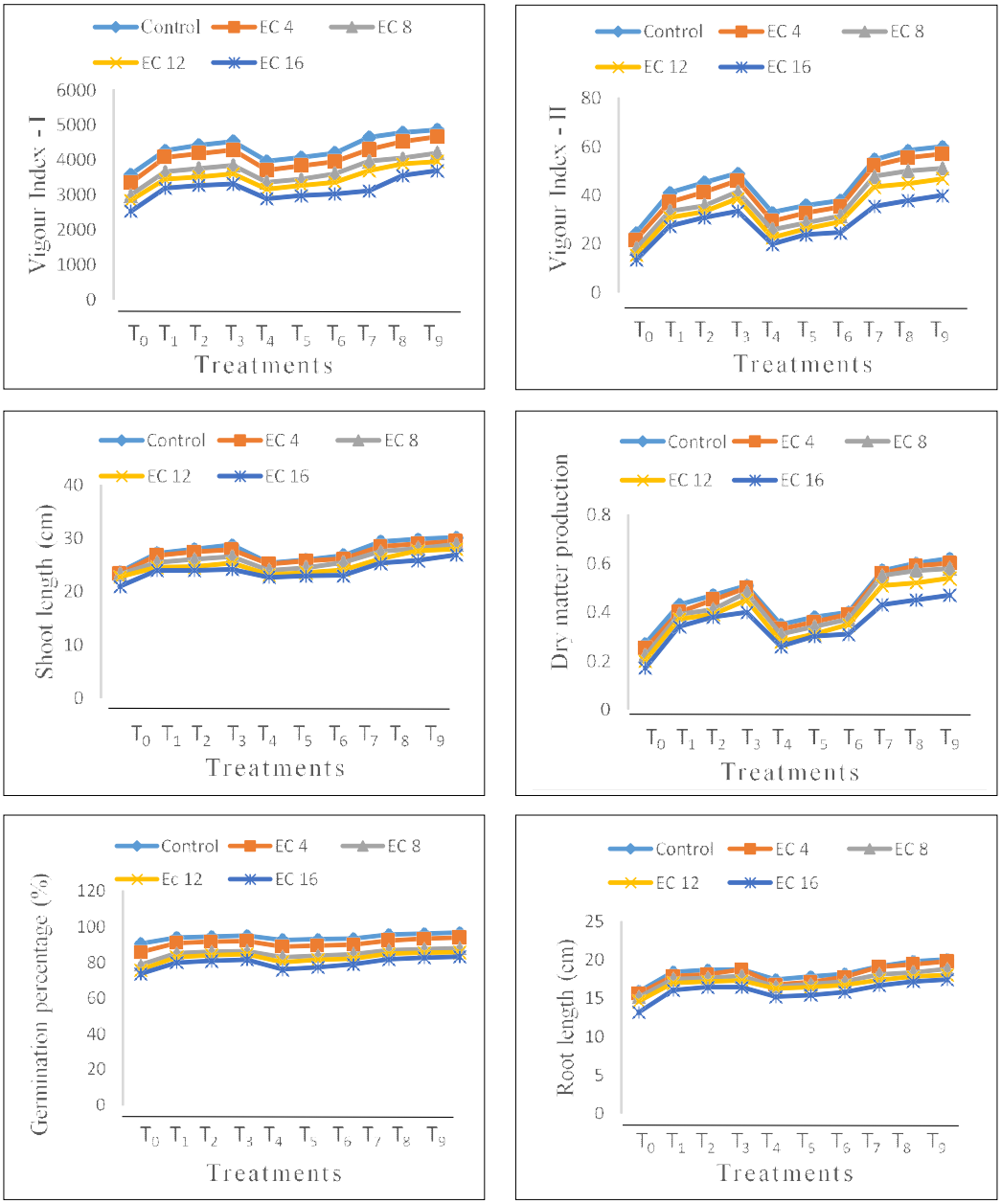


**Fig 1:** Effect of seed hardening and seed pelleting on germination percentage, root length, shoot length, dry matter production, vigour index I and vigour index II parameters of cowpea cv. CO (CP) 7 under saline condition.

All the observations from the seedlings namely germination percentage, shoot length, root length, dry matter production, vigour index I and vigour index II were recorded from each replicate and mean was worked out. Ten normal seedlings were taken randomly at the end of the germination test and the length from the collar region to tip of the primary root was measured and the mean value was expressed in centimeter for root length. The length between the collar region to tip of the primary shoot was measured and the mean value was expressed in centimeter for shoot length.

Germination percentage was calculated using this formula.

$$\text{Germination percentage} = \frac{\text{No. of seed germinated}}{\text{Total no. of seeds taken up for sowing}} \times 100$$

Ten normal seedlings used for growth measurements were placed in a butter paper cover and dried under shade for 24 hrs. Then kept in hot air oven, maintained at 60°C for 24 hrs. The dried seedlings were cooled in a desiccator for 30 minutes and then dry weight was recorded for 10 seedlings and expressed in grams. Vigour index values were computed using the formula suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

Vigour index I =

Germination percentage × Total seedling length in cm

Vigour index II =

Germination percentage × Seedling dry weight in g

The experiment was adopted in a Completely Randomized Design (CRD) with nine hardening and pelleting treatments along with control at 4 EC levels and replicated three times and data were subjected to statistical analysis as per the procedure of Panse and Sukhatme (1985).

## RESULTS AND DISCUSSION

### Effect of salinity on germination and seedling attributes

Salinity is one of the most important factors limiting plant growth and delayed seed germination as well as final germination percentage (Rahman *et al.,* 2000). It is also one of the main factors limiting legume productivity (Iluch *et al.,* 2007). The evaluation of salt tolerance requires a controlled environment. In field screening, seeds are sown directly in soil with high salt concentration. However, because of the variability of salt content in the field and also due to the considerable inûuence of other variable factors such as moisture content in soil, soil fertility, transpiration, and weather, the results may be highly biased and non-conclusive in field (Pathan *et al*., 2007; Yang and Blanchar, 1993). Even though, plant genotypes can be hydroponically screened, such a method is expensive. Therefore, evaluating saline tolerance at seedling stage would be of interest because of less expensive, non-laborious and doing so will also add information to genotypic responses at different levels of salinity.

In the present study in general, germination percentage decreased with increase in salinity levels. The control seeds showed lower values for germination indicating inefficiency of seeds to germinate, whereas, treated seeds showed better germination rate. A similar trend was observed for all the seedling parameters when compared to control and other treatments (Fig 1). Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansourie *et al.,* 2001; Bhattacharjee, 2008). Screening for salt-tolerant genotypes at germination stage is one of the most cost-effective ways to tackle salinity-related issues. Mistura *et al*. (2011) stated that salinity affected germination of legumes and plant vigor.

Dutta and Bera (2014) found a noticeable decrease in seed germination, plant growth, and vigor indices for mung bean supplied with salt treatment. Zahedi *et al*. (2012) reported that salinity decreased germination rate in cowpea, and poor seed germination resulted in a significant reduction in yield. Ashebir *et al*. (2013) studied the effects of salinity at germination in cowpea and revealed that there was significant variability in response to salt stress among cowpea genotypes. Increasing salt concentration reduced the germination rate of cowpea genotypes (Thiam *et al*., 2013; Wests and Francois, 1982; Zahedi *et al*., 2012).

Seed germination varied according to the change in NaCl osmotic potential, *i.e.,* NaCl has direct harmful effects on common bean seed germination (Alihan Cokkizgin, 2012). The increasing concentration of NaCl decreased the growth of lentil plant, expressed as dry weight. A slight reduction of dry weight was observed at lower level of salinity as in comparison to the control (Turan *et al.,* 2007).

Taffouo *et al*. (2009) reported that high sodium chloride concentration significantly affected germination rate in cowpea. It has been reported that salinity delayed germination (Mensah and Ihenyen, 2009). Increased saline concentration caused a harmful effect on seedling vigour. Similar results were observed by Khajeh-Hosseini *et al.* (2003) in soybean seeds. Al-Mutawa (2003) reported that increased salinity leads to decreased root length in chickpea. Bayuelos *et al.* (2002) reported that increase in salinity from 0 to 180 mM of NaCl decreased germination of *Phaseolus* species seeds.

### Effect of seed hardening and seed pelleting on germination and seedling attributes

Among the treatments, seeds hardened with KCl @ 1 % + $CaCl_2$ @ 1 % and pelleted with pungam leaf powder @ 200 g per kg recorded higher values for germination and all the seedling parameters when compared to control and other treatments. The hardening treatment proved to be better for vigour enhancement than the traditional soaking (Manjunath and Dhanoji, 2011). Selvakumari (2010) also reported that pre sowing hardening and designer seeds improved the seed quality characters in maize. Paddy seeds hardened with KCl 1% followed by pelleting with pungam leaf powder @ 200 g/kg also recorded increased growth and biometric characters (Prakash *et al.,* 2013).

The reason for higher germination of KCl 1% + $CaCl_2$ 1% hardened and pungam leaf extract pelleted seeds may

be greater hydration of colloids and higher viscosity of protoplasm and cell membrane that allows the early entrance of moisture that activates the early hydrolysis of reserve food materials in the seed as compared to untreated seeds. It has been reported that pungam leaf powder contains mineral nutrients like nitrogen (5.6%), phosphorus ($P_2O_5$-0.9%), Potassium ($K_2$ 0-3.11%) and calcium (Ca 0-1.0%) and $GA_3$ in traces (Nadeem Binzia, 1992). These nutrients might have helped in enhancing the performance of pelleted seeds when compared with non treated seeds.

The increased shoot length and root length in seeds treated with botanical leaf powder may be attributed to cell wall extension and increased metabolic activities (Afzal *et al*., 2002). The finding of Ramesh Kumar and Muthukrishnan (2015) also corroborated with the results of present study. The pronounced increase in seedling length and dry matter production of pungam pelleted seeds may be due to activation of the growth promoting substances and translocation of secondary metabolites to the growing seedling (Napar *et al*., 2012). It is possible that the bionutrients available in the pelleted seeds might have improved seedling growth resulting in higher growth parameters (Prakash *et al.,* 2013).

Increase in dry matter production with seed pelleting were also reported by Tamilmani (2012) in blackgram, Prakash *et al*. (2013) in rice and Anbarasan *et al*. (2016) in redgram. The increase in dry weight with botanicals treatment may be due to the faster growth and development of seedling and hike in vigour index (Sathiya Narayanan *et al.,* 2016). Seeds coated with *P. flourescens* showed increased germination as well as seedling growth (root and shoot length). Pelleted seeds also showed increased values for dry matter production and vigour index (Ananthi *et al.,* 2015).

The treatment of green gram seeds with albizzia leaf powder (15%) and pongamia leaf powder (15%) recorded increased shoot length, seedling dry weight and vigour index. Increased seedling growth observed due to pungam leaf powder observed is in concomitant with the reports of Harish Babu *et al.* (2005) who observed increased shoot length, seedling dry weight and vigour index

The increase in dry weight was claimed to be due to enhanced lipid utilization and enzyme activity due to the presence of bioactive substances like auxin in pungam leaf extract (Rathinavel and Dharmalingam, 1999) and development of seedling to reach autotropic stage and enabling them to produce relatively more quantity of dry matter with hike in vigour index by pelleting treatment. The physiologically active substances present in the botanical leaves might have stimulated the germination and related processes resulting in more absorption of water due to elasticity of cell wall and development and increased vigour index (Sathish and Bhaskaran, 2015).

Arappu leaf powder pelleted seeds of brinjal @ 250 g per kg recorded significantly higher root length, shoot length, seed vigour index and seedling dry weight during the study (Sathish Kumar *et al.,* 2014). Seeds hardened with 2% $KH_2PO_4$ and film coated with carbendazim @ 2 g $kg^{-1}$ + imidachloprid @ 1 mL in 5 mL of water +30 g DAP + 20 g micronutrient mixture + pink polykote @ 3 g $kg^{-1}$ + *Azospirillum*@ 40 g $kg^{-1}$ of seed recorded higher germination and vigour index under laboratory evaluation (Kavitha *et al.,* 2013).

To conclude, the present study revealed that seed hardening with KCl @ 1 % + $CaCl_2$ @ 1 % and pelleting with pungam leaf powder @ 200 g per kg can be recommended for cultivation of cowpea under saline conditions to get higher yield.

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