



Effect of Seed Hardening on Morphological, Growth and Yield Parameters of Chickpea (*Cicer arietinum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted at Main Agriculture Research Station, University of Agricultural sciences, Dharwad during *rabi* 2021-22 to study the impact of seed hardening chemical Aminolevulinic acid (ALA) on morphological, biochemical parameters and yield in Chickpea (var. JG-11). The experiment was laid out in randomized block design with three replications. The treatments consist of seed hardening with Aminolevulinic acid at different concentrations (10, 20, 30, 40, 50 ppm) and CaCl₂ (2%). The results of the investigation revealed that, among the

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treatments the seed soaking with Aminolevulinic acid @ 30 ppm has registered significantly highest plant height, number of primary branches per plant, and total dry matter and SPAD value followed by 2% CaCl₂. Whereas it was lowest in control in all the parameters. The growth parameters viz., leaf area index, absolute growth rate, crop growth rate, relative growth rate, net assimilation rate and leaf area duration increased significantly with seed hardening treatments. yield and yield components increased significantly due to seed hardening with Aminolevulinic acid (30 ppm) followed by 2% CaCl₂ as compared to control. The economics of chickpea was highest with 2 % CaCl₂ compared to aminolevulinic acid @ 30 ppm. As per the results obtained seed hardening with Aminolevulinic acid (30 ppm) and CaCl₂ (2%) are more effective in increasing drought tolerance in chickpea.

Keywords: Seed hardening; morphological parameters; growth parameters; crop performance.

1. INTRODUCTION

India is the world's leading producer of pulses, accounting for 28.8 million hectares and 26.96 million tons of global output Anon., 2023. Pulses are important in Indian agriculture because of their inherent ability to fix atmospheric nitrogen via biological nitrogen fixation (BNF), Pulses enhance soil health with their deep tap roots, improving soil structure and moisture efficiency. They are an essential part of the vegetarian Indian diet, providing high-quality protein. Pulses complement cereal-based diets, offering a balanced source of nutrition.

India's growing population and slow progress in pulse production are leading to serious food security issues. The per capita availability of pulses has declined from 69 g in 1961 to 45 g in 2021, due to the shift toward cereal cultivation after the Green Revolution. To combat protein-energy malnutrition, a minimum of 50 g of pulses per capita per day is necessary, alongside other protein sources. By 2030, India will need around 32.64 million tons of pulses to meet rising demand Kumeera *et al* [1]. This can only be achieved by adopting more productive farming technologies.

Chickpea (*Cicer arietinum* L.) is the third most important food legume, grown in over 45 countries worldwide. It is rich in protein (20-22%), carbohydrates, fiber, and essential minerals such as calcium, magnesium, zinc, potassium, iron, and phosphorus, along with vitamins like thiamine and niacin. Chickpeas improve soil fertility by converting atmospheric nitrogen into plant-available forms through symbiosis, fulfilling 80% of their nitrogen needs. Additionally, they leave residual nitrogen for future crops and contribute organic matter, enhancing soil health and fertility.

Chickpea is the leading legume in India, cultivated on 28.8 million ha with a production of

26.96 million tons. Karnataka ranks fifth in production, contributing 4.18 lakh ha and 2.31 lakh tons [2]. Despite being the largest producer, India's productivity is lower than countries like Italy, Turkey, and Iran, with potential for improvement through better varieties and production technologies.

The low chickpea yield in India is primarily due to reliance on residual soil moisture, leading to increasing moisture stress as the crop matures, especially in peninsular India. This stress negatively impacts pod formation, a critical factor for yield potential. Additionally, physiological issues like flower dropping, poor pod filling, and pod shedding further constrain productivity. These factors collectively limit the crop's growth and yield potential [3,4].

Seed hardening is a low-cost, effective technique to enhance drought resistance in crops by improving seedling vigor, osmotic adjustment, and antioxidant activity. It involves pre-treating seeds with water or chemicals to induce stress tolerance. This approach boosts germination rates and early growth under drought conditions. Though species-specific, seed hardening offers a sustainable, cost-effective solution for improving crop resilience to environmental stresses.

Seed hardening improves chickpea yield, particularly under drought conditions, by inducing physiological, metabolic, and morphological changes. It enhances seed water retention, osmotic pressure, and protein quality, boosting seedling vigour and crop performance. Hardened seeds show increased respiration, higher photosynthesis, and improved starch content, leading to better stress tolerance. Plant growth regulators, such as Aminolevulinic acid (ALA), further improve the source-sink relationship, increasing pod numbers, test weight, and germination. ALA also regulates key processes

like seed germination, nutrient status maintenance, and ROS scavenging, enhancing drought resilience. By increasing photosynthetic assimilation and reducing Na⁺ uptake, ALA improves overall plant health. This offers new opportunities to increase chickpea productivity under moisture stress. Identifying optimal hardening and growth regulator strategies is crucial for improving drought resistance in crops.

2. MATERIALS AND METHODOLOGY

A field experiment was conducted during rabi 2021-22 to study the impact of seed hardening chemicals to induce drought tolerance in chickpea with water, CaCl₂ and Aminolevulinic acid on Morphological, Growth and Yield components in chickpea (*Cicer arietinum* L.) at College of Agriculture Farm, University of Agricultural Sciences, Dharwad. The experiment was carried out on medium black clay soil (Plot no. 126A of 'E' block). Which is situated at 15° 12' N latitude and 75° 07' E longitude with an altitude of 678 m above the mean sea level. The experiment was laid out in randomized block design with three replications. A day before sowing chickpea seeds were soaked separately in water, CaCl₂ (2%), Aminolevulinic acid (10, 20, 30, 40, 50 ppm) for four hours and seeds were dried under shade and used for sowing.

Seeds JG -11 were obtained from Main Research Station, Dharwad. Healthy and bold seeds were dibbled with a spacing of 30 cm x 10 cm to a depth of 5 cm. The crop was provided with protective irrigations as and when required. Recorded morphological parameters like Plant height, no of branches per plant and total dry matter and its portioning and growth parameters like Leaf area index LAI, AGR, CGR and NAR LAI was measured by the formula given by Sestak et al., [5].

Absolute Growth Rate (AGR): It expresses the dry weight increase per unit time and was calculated by using the following formula,

$$AGR = \frac{(W_2 - W_1)}{(t_2 - t_1)}$$

where, W₂ and W₁ are the total dry weights per plant at time t₂ and t₁ respectively.

Crop Growth Rate (CGR): Crop growth rate is the rate of dry matter production per unit ground area per unit time [6]. It was calculated by using

the following formula and expressed as g m⁻² day⁻¹

$$CGR (gm^{-2}day^{-1}) = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{1}{P}$$

Where, W₁ = Dry weight of the plant (gm-2) at time t₁

W₂ = Dry weight of the plant (gm-2) at time t₂

t₁-t₂ = Time interval in days

A = Unit land area (m₂)

Net Assimilation Rate (g dm⁻² day⁻¹): Net assimilation rate (NAR) is the rate of dry weight increased per unit leaf area per unit time. It was calculated by using the following formula [6]

$$NAR (g dm^{-2} day^{-1}) = \frac{(W_2 - W_1) (Log_e L_2 - Log_e L_1)}{(t_2 - t_1) (L_2 - L_1)}$$

where,

L₁ & W₁ = Leaf area (dm²) and dry weight of the plant (g), respectively at time t₁.

L₂ & W₂ = Leaf area (dm²) and dry weight of the plant (g), respectively at time t₂.

Yield parameters: No of pods per plant, Seed yield per plant (grams) and Harvest index

Harvest index: It was calculated by using the following formula.

$$HI = \frac{\text{Economic yield (Pod yield)}}{\text{Total biological yield}} \times 100$$

Statistical analysis: The data were subjected to the analysis of variance by following the method of Panse and Sukhatme (1967). The level of significance used in 'F' and 't' tests was P = 0.05. Critical differences were calculated wherever 'F' test was found significant.

3. RESULTS AND DISCUSSION

Plant height (cm): The data on plant height (Table 1) as influenced by seed hardening chemicals showed significant differences among the treatments at all the stages except at 30DAS. In general, plant height increased from 30DAS to harvest. Aminolevulinic acid at 30 ppm consistently resulted in the tallest plants, reaching 35.79 cm at 45 DAS, 41.87 cm at 60 DAS, and 42.02 cm at harvest. Calcium chloride at 2% also promoted growth, with heights of 34.08 cm, 40.44 cm, and 40.75 cm, respectively. The control group showed the lowest growth at

all stages, with plant heights of 24.62 cm, 32.43 cm, and 32.61 cm. The data on harvest index showed significant differences among the treatments. The treatment with Aminolevulinic acid @ 30ppm (48.93 %) recorded significantly maximum harvest index followed by CaCl_2 @ 2 per cent (47.27 %) and Aminolevulinic acid @ 20 ppm (46.98 %) while, the lowest harvest index was recorded in control (41.16 %).

Number of branches per plant: The data (Table 1) on number of primary branches per plant showed that at 30 DAS, the number of branches per plant differed significantly among the treatments where the number of primary branches per plant was recorded maximum with Aminolevulinic acid @ 30ppm (2.8) followed by 2% CaCl_2 (2.6) and 20ppm Aminolevulinic acid (2.4) while, the lower number of primary branches per plant was recorded in control (2.2) at all the stages. The similar trend was also observed at 45,60DAS and at harvest. Which could be attributed to the plants vigorous growth as a result of seed hardening Chavan et al., [7] observed similar results in soybean, Manjunath and Dhanoji [3] in chickpea.

Total dry matter (g): The data on total dry matter (TDM) accumulation showed that at 30 DAS, Aminolevulinic acid @ 30 ppm had the highest TDM (2.65 g plant⁻¹), followed by CaCl_2 @ 2% (2.49 g plant⁻¹) and Aminolevulinic acid @ 20 ppm (2.38 g plant⁻¹), with the lowest in the control (1.17 g plant⁻¹). At 45 DAS, the trend remained similar, with Aminolevulinic acid @ 30 ppm recording the highest TDM (3.69 g plant⁻¹) and control showing the lowest (1.98 g plant⁻¹). At harvest, Aminolevulinic acid @ 30 ppm had the highest TDM (32.93 g plant⁻¹), followed by CaCl_2 @ 2% (31.24 g plant⁻¹), while the control had the lowest (22.36 g plant⁻¹). TDM levels may rise as crops mature due to an indeterminate growth pattern, a higher rate of CO_2 fixation, and RuBP carboxylase activity. TDM's relationship with grain yield was stronger at all stages of crop development. As a result, TDM is a critical parameter in improving the source-sink relationship and yield potential. Manjunath and Dhanoji [3] made similar observations in chickpea.

Growth parameters:

Leaf area index: Leaf area index (LAI) increased up to 60 DAS and then declined at harvest. Aminolevulinic acid @ 30 ppm consistently recorded the highest LAI at all stages, followed

by CaCl_2 @ 2% and Aminolevulinic acid @ 20 ppm. The control group had the lowest LAI at each stage. The highest LAI was observed at 60 DAS and decreased at harvest. The similar results were reported by Suliman et al. [8] by seed hardening with Aminolevulinic acid in wheat.

Absolute growth rate (AGR) (g plant⁻¹): The absolute growth rate (AGR) increased with crop age, with Aminolevulinic acid @ 30 ppm showing the highest AGR at all stages. From 30-45 DAS, it was 0.069 g plant⁻¹, from 45-60 DAS, 0.424 g plant⁻¹, and from 60 to harvest, 0.762 g plant⁻¹. The control consistently had the lowest AGR at each stage. The similar results were also reported by Prajapati et al. [9] in blackgram.

Crop growth rate (CGR) (g m⁻² day⁻¹): The experimental results on crop growth rate (Table 2) varied significantly among the treatments. At 30-45 DAS crop growth rate was found significantly maximum with Aminolevulinic acid @ 30ppm (0.022 g m⁻² day⁻¹) followed by CaCl_2 @ 2 per cent (0.021 g m⁻² day⁻¹) and Aminolevulinic acid @ 20 ppm (0.021 g m⁻² day⁻¹) while, significantly lowest crop growth rate was recorded in control (0.014 g m⁻² day⁻¹). The similar trend was also followed at 45-60 DAS and 60-harvest. These findings are consistent with the findings of Maitra et al. [10], who reported that seed hardening with 2% CaCl_2 significantly increased CGR over control in finger millet.

Net assimilation rate: At 30-45 DAS, Aminolevulinic acid @ 30 ppm showed the highest NAR (0.337 g dm⁻² day⁻¹), followed by CaCl_2 @ 2% and Aminolevulinic acid @ 20 ppm, with the control having the lowest (0.198 g dm⁻² day⁻¹). A similar trend was observed at 45-60 DAS. At 60-harvest, Aminolevulinic acid @ 30 ppm (0.308 g dm⁻² day⁻¹) still recorded the highest NAR, and the control had the lowest (0.177 g dm⁻² day⁻¹). The net assimilation rate (NAR), also known as the "unit leaf rate," expresses the rate of dry weight increased at any instant on a leaf area basis, with leaf representing an estimate of the size of assimilatory surface [11]. The similar results were also reported by Eshanna and Kulkarni [12] with CaCl_2 (1:3 proportion of seed: chemical) resulted highest NAR over control in maize. Suliman et al. [8] also reported similar results in wheat by seed hardening with Aminolevulinic acid.

Table 1. Influence of seed hardening chemicals on morphological characteristics in chickpea

Treatment	Plant Height (cm)	Number of primary branches	Total dry Matter (gm)
T ₁ : Seed soaking with (5-ALA) @ 10 ppm	35.67 ^{bc}	4.5 ^{a-d}	29.95 ^b
T ₂ : Seed soaking with (5-ALA) @ 20 ppm	36.91 ^b	4.6 ^{abc}	30.52 ^{ab}
T ₃ : Seed soaking with (5-ALA) @ 30 ppm	42.02 ^a	4.9 ^a	32.93 ^a
T ₄ : Seed soaking with (5-ALA) @ 40 ppm	34.76 ^{bc}	4.4 ^{bcd}	28.98 ^b
T ₅ : Seed soaking with (5-ALA) @ 50 ppm	33.89 ^{bc}	4.2 ^{cd}	28.36 ^{bc}
T ₆ : Seed soaking with CaCl ₂ @ 2 %	40.75 ^a	4.7 ^{ab}	31.24 ^{ab}
T ₇ : Seed soaking with water	32.98 ^c	4.2 ^{cd}	25.82 ^c
T ₈ : Control	32.61 ^c	4.1 ^d	22.36 ^d
Mean	36.19	4.45	28.77
S.Em ±	1.12	0.14	0.88
CD (5%)	3.39	0.41	2.66

Table 2. Influence of seed hardening chemicals on growth parameters in chickpea

Treatment	LAI	CGR (g m ⁻² day ⁻¹)		NAR (g dm ⁻² day ⁻¹)		AGR (g plant ⁻¹)	
	60 DAS	45-60	60 DAS	45-60	60 DAS	45-60	60 DAS
T ₁ : Seed soaking with (5-ALA) @ 10 ppm	1.12 ^{abc}	0.135 ^{abc}	0.228 ^b	0.479 ^c	0.261 ^{bc}	0.41 ^b	0.69 ^d
T ₂ : Seed soaking with (5-ALA) @ 20 ppm	1.17 ^{abc}	0.137 ^{ab}	0.231 ^{ab}	0.503 ^{bc}	0.279 ^b	0.41 ^b	0.70 ^c
T ₃ : Seed soaking with (5-ALA) @ 30 ppm	1.30 ^a	0.139 ^a	0.251 ^a	0.565 ^a	0.308 ^a	0.42 ^a	0.76 ^a
T ₄ : Seed soaking with (5-ALA) @ 40 ppm	1.06 ^{bcd}	0.123 ^b	0.223 ^{bc}	0.414 ^{cd}	0.242 ^c	0.37 ^c	0.68 ^d
T ₅ : Seed soaking with (5-ALA) @ 50 ppm	1.02 ^{cd}	0.122 ^{bc}	0.221 ^{bc}	0.362 ^d	0.211 ^{cd}	0.37 ^c	0.67 ^e
T ₆ : Seed soaking with CaCl ₂ @ 2 %	1.24 ^{ab}	0.139 ^a	0.239 ^{ab}	0.527 ^{ab}	0.292 ^{ab}	0.42 ^a	0.72 ^b
T ₇ : Seed soaking with water	0.91 ^{de}	0.11 ^d	0.201 ^c	0.281 ^{de}	0.191 ^{de}	0.33 ^d	0.61 ^f
T ₈ : Control	0.8 ^e	0.089 ^{de}	0.179 ^d	0.267 ^e	0.177 ^e	0.27 ^e	0.54 ^f
Mean	1.07	0.124	0.222	0.424	0.245	0.38	0.67
S.Em ±	0.03	0.05	0.04	0.01	0.03	0.01	0.02
CD (5%)	0.10	0.14	0.12	0.04	0.09	0.03	0.05

Table 3. Influence of seed hardening chemicals on yield parameters in chickpea

Treatment	Pods/plant	Seed yield (q/ha)	HI(%)
T ₁ : Seed soaking with (5-ALA) @ 10 ppm	71 ^{bc}	23.9 ^{abc}	45.88 ^{ab}
T ₂ : Seed soaking with (5-ALA) @ 20 ppm	76 ^b	24.8 ^{abc}	46.98 ^{ab}
T ₃ : Seed soaking with (5-ALA) @ 30 ppm	87 ^a	26.1 ^a	48.93 ^a
T ₄ : Seed soaking with (5-ALA) @ 40 ppm	65 ^{cd}	23.4 ^{bc}	44.95 ^{abc}
T ₅ : Seed soaking with (5-ALA) @ 50 ppm	62 ^d	22.8 ^c	44.26 ^{bc}
T ₆ : Seed soaking with CaCl ₂ @ 2 %	83 ^a	25.2 ^{ab}	47.27 ^{ab}
T ₇ : Seed soaking with water	54 ^e	19.93 ^d	42.78 ^{bc}
T ₈ : Control	48 ^e	19.14 ^d	41.16 ^c
Mean	68.2	23.15	45.27
S.Em ±	2.15	0.71	1.37
CD (5%)	6.52	2.14	4.16

Yield parameters: Among the treatments seed hardening with Aminolevulinic acid @ 30ppm recorded significantly maximum number of pods per plant (87) followed by 2% CaCl₂ (83) and Aminolevulinic acid @ 20 ppm (76) whereas, the minimum number of pods per plant was recorded in control (48) mentioned in (Table 3). Seed hardening with CaCl₂ (2%) recorded significantly higher number of pods per plant and pod yield in blackgram [9].

The data on seed yield shown that Aminolevulinic acid @ 30ppm (16.10 g plant⁻¹) recorded significantly highest seed yield per plant followed by CaCl₂ @ 2 per cent (14.75 g plant⁻¹) and Aminolevulinic acid @ 20 ppm (14.32 g plant⁻¹) while, the lowest seed yield per plant was recorded in control (9.18 g plant⁻¹). The increase in vegetative characters that intern enhanced cell division and quick cell multiplication, whereas the higher yield may be due to better carbon assimilation, and reduced plant respiration. The findings are consistent with Manjunath and Dhanoji [3] in chickpea.

The data on harvest index showed significant differences among the treatments. The treatment with Aminolevulinic acid @ 30ppm (48.93 %) recorded significantly maximum harvest index followed by CaCl₂ @ 2 per cent (47.27 %) and Aminolevulinic acid @ 20 ppm (46.98 %) while, the lowest harvest index was recorded in control (41.16 %).

4. CONCLUSIONS

Seed hardening with 30 ppm ALA significantly increased plant height, number of branches, and dry weight of leaves, stems, and reproductive parts, followed by 2% CaCl₂ and 20 ppm ALA. Leaf area index and leaf area duration also showed significant improvement with 30 ppm ALA treatment. Seed hardening treatments significantly enhanced CGR, AGR, RGR, and NAR during different growth phases. Additionally, 30 ppm ALA resulted in higher relative water content, Proline content, and chlorophyll stability index. Finally, seed yield and yield components were highest with 30 ppm ALA, making it the most effective treatment for increasing chickpea yield. According to the results of various seed hardening treatments, it is concluded that seed hardening with 30 ppm ALA and 2% CaCl₂ is more effective in increasing chickpea yield.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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