Effect of Seed Priming with Ascorbic Acid on Seed Germination and Seedling Growth of Cowpea (*Vigna unguiculata* L. Walp)

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ABSTRACT

Seed priming enhances seedling growth in cowpeas, a crop of nutritional and economic importance. However, its physiological effects, particularly its interaction with antioxidants like ascorbic acid, remain underexplored. We hypothesized that priming cowpea seeds with ascorbic acid would enhance germination percentage, root and shoot length, and seedling biomass. The objective of the study was to investigate the effects of ascorbic acid seed priming on cowpea (*Vigna unguiculata* L. Walp) germination and seedling growth. An experiment was conducted using a Randomized Complete Block Design (RCBD) with three treatments and three replications. Treatments included three levels of ascorbic acid (0 as control, 0.5, and 1 mM), where seeds were soaked for 12 hours for priming and air-dried for an hour. Each dish contained ten seeds subjected to the respective treatments. The results indicated a significant effect of ascorbic acid priming on germination and seedling growth. The highest germination percentage (100%), shoot length (10.84 cm), root length (9.97 cm), and fresh weight of seedlings (5.60 g) were observed in seeds primed with 1 mM of ascorbic acid. However, the control (0 ascorbic acid) exhibited lower values with 80% germination, shoot length of 6.84 cm, root length of 5.54 cm, and seedling fresh weight of 3.77 g. These findings suggest that ascorbic acid enhances early-stage cowpea growth and improves metabolic activation during germination. Further research is recommended to explore the interaction of ascorbic acid priming with mixed-seed systems and other vegetable crops to determine its broader agricultural applications.

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1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a vital legume crop known for its high nutritional value and adaptability to diverse growing conditions (Abebe & Alemayehu, 2022; Osipitan et al., 2021; Simion, 2018). Cowpea, with its adaptability to a variety of agroecological zones (e.g., climate, soil, topography and land use/land cover), plays a positive role in providing food security, particularly in areas where environmental stresses such as drought and extreme heat challenge its growth and development (Boukar et al., 2019; Kebede & Bekeko, 2020). Improving the germination and early growth of cowpea seeds is critical for generating maximum crop yields and guaranteeing sustainable agricultural practices (Dorvlo et al., 2022; Omomowo & Babalola, 2021). Poor seed germination and suboptimal early growth often limit its yield potential. This might be due to water deficit during the initial stage of cowpea seed imbibition which results in delayed and erratic seedling emergence and stand establishment, and in severe cases, complete inhibition of seedling emergence (Agbicodo et al., 2009; Carvalho et al., 2019).

Water deficit during the imbibition phase of germination is the primary reason for decline in seedling growth and establishment (Wafa'a, 2010). Researchers have found that water deficit is one of the major problems in cowpea seedling emergence and establishment in arid regions (Melo et al., 2022; Nkomo et al., 2021). Seed priming, a pre-sowing treatment that enhances seed metabolism, has been widely used to improve germination and seedling vigor. Among various agents, ascorbic acid is known for its role in enhancing seedling development, yet its effect on cowpeas remains inadequately studied. The need for this study arose from the limited understanding of how ascorbic acid influences cowpea seed germination and early growth. While previous studies have explored other priming agents, the potential of ascorbic acid in improving seed performance is not well established.

Seed priming involves controlled hydration and dehydration of seeds and has gained popularity as a promising strategy for improving seed performance and crop output. Ascorbic acid, a well-known antioxidant, has

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been recognized for its potential function in seed priming, as it participates in multiple physiological processes, including the regulation of reactive oxygen species (ROS) and the activation of enzymatic antioxidants (Adetunji et al., 2021). Ascorbic acid reduced the adverse impacts of drought stress on seedling development, catalase, and peroxidase activity in cowpeas, implying that ascorbic acid could improve the plant's tolerance to drought stress. Seed priming is a double technology to enhance rapid and uniform emergence, and to achieve high vigor and better yields in vegetables and floriculture (Thakur et al., 2022; Corbineau et al., 2023).

Seed priming with ascorbic acid is one of the most important techniques and is highly responsive to existing environmental conditions (Terzaghi et al., 2023; Shah et al., 2019). Ascorbic acid plays multiple roles in seed germination, plant growth, functioning cell division, cell wall expansion, and other developmental processes (Akram et al., 2017). It is a good medium that can counteract the adverse effects of abiotic stress (Hosseinifard et al., 2022; Khan and Ashraf, 2008). The viable seeds do not germinate due to limited water uptake and a subsequent loss of metabolic activity (Long et al., 2015). Through a series of controlled environment experiments, we want to uncover the biochemical and molecular mechanisms connected with ascorbic acid priming, shedding light on its potential for a sustainable approach to improving cowpea crop establishment. We hypothesized that cowpea seed priming with ascorbic would improve its germination, root and shoot growth, and seedling biomass. The objective of the study was to evaluate the effects of ascorbic acid on cowpea seed germination and seedling growth and to determine the optimal rate for improved early-stage development of cowpea.

2. Materials and Methods

An experiment of cowpea seed priming with ascorbic acid was conducted at the Agronomy laboratory of the Institute of Agriculture and Animal Science (IAAS), Lamjung campus, Nepal using cowpea variety 324. Cowpeas were kept on moist filter paper in growth trays on May 1st, and the study continued until May 14th. The experiment consisted of 3 treatments with 15 replications and was laid out as a complete randomized block design. Seeds of cowpea variety 324 were collected from a local agrovet. The seeds were surface sterilized by immersion in 0.5% sodium hypochlorite (NaOCl) solution for 5 minutes to prevent fungal infections and then washed three times with sterile, distilled water to remove any residual NaOCl. After washing, seeds were subjected to priming with ascorbic acid solutions. Three concentrations of ascorbic acids, i.e., control (distilled water priming or no ascorbic acid), 0.05mM, and 1mM were used for seed priming. Seeds were primed for 12 hours and air-dried for an hour. Ten seeds per treatment were placed in petri dishes and kept in a germinator. Seed germination was observed daily and the final germination percentage was calculated after a week. Average shoot length and root length were calculated after a week of germination. The fresh weight of seedlings from each treatment was measured after a week.

2.1. Experiment Layout

The layout was done in three steel trays by keeping 15 glass petri dishes in each tray. Tray one was considered the first replication; trays two and three served as the second and third replications, respectively (Fig. 1). We used the following combination of seed and treatment numbers: 1 tray = 15 petri dishes; 3 trays = 45 petri dishes; 1 petri dish = 10 cowpea seeds; 45 petri dishes = 450 cowpea seeds.

2.2 Observations and Data Analysis

Germination was observed daily for up to 1 week (Fig. 1). A seed was classified as germinated when the radicle had emerged and reached a length greater than 2 mm. The number of germinated seeds was counted from each petri dish and the germination percentage was calculated as:

$$Germination\ \% = \frac{Actual\ Germinated\ Seeds}{Total\ Seeds} * 100$$

The root length was measured by using a centimeter (cm) scale. The radicle was measured from five randomly chosen seedlings from each petri dish. Similarly, the shoot length (plumule) was measured by a cm-scale from five randomly chosen cowpea seedlings. From each treatment, seedlings were packed in separate paper bags, and fresh weight was measured using a digital balance (A & E lab company limited, London, UK). This was done one week after germination. Data were tested to meet the assumption for normal distribution. We did a log transformation to achieve normality before running ANOVA. Data analysis was done by using SPSS version 16 and means separation was done using Tukey's HSD test at a 5% level of significance.

Figure 1. *Laid out experimental setup for seed germination, root length, shoot length, and fresh weight of seedlings with 3 treatments, 15 replications, and 45 experimental units.*

3. Results

The effect of ascorbic acid treatments on the seed germination percentage of cowpea was not significant ($p > 0.05$). However, the treatment that received 1 mM of ascorbic acid gave the highest germination percentage (100%), while the control gave the lowest germination percentage (80%) (Table 1). A significant difference ($p < 0.01$) was observed in shoot length among ascorbic acid treatments. The treatment that received 1 mM of ascorbic acid produced longer shoots (10.84 cm), and 0.5 mM treatment was at par, but lower than 1 mM treatment. The treatment that did not receive any ascorbic acid produced shorter shoot lengths of 6.84 cm (Table 1). Root length was significantly affected by ascorbic acid priming ($p < 0.05$). The treatment that received a higher dose of ascorbic acid produced longer roots (9.97 cm), while the control produced shorter roots (5.54 cm). However, the treatments that received 0.5 mM of ascorbic acid showed an intermediate effect, indicating a dose-dependent response to ascorbic acid priming (Table 1).

There was a significant effect of ascorbic acid treatments on the fresh weight of seedlings ($p < 0.05$). The treatment that received a higher dose of ascorbic acid produced a higher fresh weight of seedlings (5.6 g), while the control produced a lower fresh weight (3.77 g). However, the treatments that received 0.5 mM of ascorbic acid were at par with the control and treatment that received 1 mM of ascorbic acid solution (Table 1).

Table 1. *Effects of different levels of ascorbic acid on germination, shoot length, root length, and fresh weight of seedlings of cowpea.*

Ascorbic acid treatments	Germination (%)	Confidence limits		Shoot length	Confidence limits		Root length	Confidence limits		Fresh weight of seedlings	Confidence limits	
		Lower	Upper	(cm)	Lower	Upper	(cm)	Lower	Upper	(g)	Lower	Upper
Control (0mM)	80	67.60	92.40	6.84^{b}	6.20	7.40	5.54^{b}	4.80	6.20	3.77^{b}	3.20	4.40
0.5mM	90	81.00	99.00	10.48^{a**}	10.00	11.00	7.88^{ab*}	7.30	8.50	4.62^{ab*}	4.00	5.20
1mM	100	78.20	100.00	10.84^{a**}	10.20	11.40	9.97^{a*}	9.40	10.60	5.60^{a*}	5.10	6.10

^{ab}Values in the same column with different superscripts differ ($^{*}p < 0.05$, $^{**}p < 0.01$).

4. Discussion

The study hypothesis that priming cowpea seeds with ascorbic acid would enhance germination percentage, root and shoot length, and seedling biomass is partially accepted as the treatment did not improve seed germination but enhanced the seedling growth. In contrast to the seed-germination results found in the current study, previous study suggests that seed priming substantially improved germination under drought conditions owing to the early completion of pre-germination metabolic activities during priming (George et al., 2024). For instance, ascorbic acid has been shown to help build germination metabolites, resulting in earlier and uniform establishment in most crops worldwide (Farooq et al., 2013). Although the statistical insignificance suggests that ascorbic acid does not fundamentally alter germination rates, its potential to optimize germination under stress conditions warrants further investigation.

Shoot length was influenced by ascorbic acid treatment, with the longest shoots recorded for the 1 mM treatment, followed closely by the 0.5 mM treatment. These results support our hypothesis. Similarly, the control resulted in the shortest shoot length, which suggests that ascorbic acid enhances shoot elongation, likely by promoting cell expansion and division through its role in antioxidant defense and hormonal signaling (Celi et al., 2023; Ejaz et al., 2019). Similar results have been reported in other legumes where ascorbic acid priming improved shoot growth by enhancing nutrient mobilization and enzymatic activity during early seedling development. For instance, Beltagi (2008) reported that exogenous application of ascorbic acid improved the growth and physiological activities of chickpeas (*Cicer arietinum*).

The root length of the cowpea was also affected by ascorbic acid treatment, and we accept our hypothesis. The longest root in the 1 mM ascorbic acid treatment suggests that the observed improvement in cowpea roots may be attributed to enhancing root meristem activity in developing tissues (Bilska et al., 2019; Moustafa-Farag et al., 2016). The 0.5 mM treatment showed an intermediate effect, suggesting a dose-dependent response to ascorbic acid. This tells us that the potential application of ascorbic acid can improve root growth, particularly in resource-limited environments. For instance, Dolatabadian et al. (2008) reported that seed priming increased the cellular activity in canola. This was primarily due to an adequate supply of ascorbic acid, which increased stomatal conductance under lower water status by enhancing root performance (Loutfy et al., 2020; Naz et al., 2016).

Our study also found that the fresh weight of cowpea seedlings was increased by ascorbic acid treatments, and we accept our hypothesis. The highest fresh weight of seedlings obtained in the 1 mM treatment indicates improved water uptake, enhanced enzymatic activity, and better stress tolerance facilitated by ascorbic acid (Kanwal et al., 2024; Sharma et al., 2024). A study by Behairy et al. (2012) and Gaafar et al. (2020) documented that seed priming with ascorbic acid improved the role of antioxidants in promoting early seedling vigor and biomass accumulation in various plants.

5. Conclusion

Overall, higher dose of ascorbic acid enhanced early seedling growth in cowpeas, supporting its potential as a seed priming agent for improved crop establishment. This study demonstrated that ascorbic acid seed priming positively influences early growth and seedling vigor in cowpeas. Although ascorbic acid did not influence germination under the conditions of current study, its potential benefit under stress demands further investigation. The results, which highlight the role of ascorbic acid in enhancing early cowpea establishment, may be valuable in resource-limited or stress-prone environments. Future research should explore the long-term effects of ascorbic acid priming on field performance and yield, as well as its interaction with abiotic stress factors such as drought and salinity. Moreover, investigating the molecular mechanisms governing ascorbic acid-induced growth enhancement could provide deeper insights into its physiological benefits and further optimize its application in sustainable agriculture.

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