



RESEARCH ARTICLE

Biochemical characterization of *Phaseolus vulgaris* L. seeds primed with calliterpenone and gibberellic acid

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Abstract

Calliterpenone, a phyllocladane diterpenoid extracted from Priyangu (*Callicarpa macrophylla*) has been reported to enhance the population of desirable microorganisms along with the sprouting of seeds, overall root, shoot biomass and blooming in Angiosperms. This paper highlights the effect of calliterpenone on protein and enzymatic assay (α -amylase, catalase, ascorbate peroxidase & protease) in *P. vulgaris* seeds, compared to similar concentration of gibberellic acid. Maximum protein content and α -amylase activity was observed in 0.01 mM calliterpenone treated seeds. However as for the catalase and ascorbate peroxidase activity is concerned it was maximum in 0.001 mM calliterpenone supplemented treatments. According to this study, calliterpenone has the potential to significantly increase the output of a variety of crops by acting as a natural plant growth stimulator. It may be a useful agricultural input because of its growth-stimulatory effects and bioactive qualities, especially in organic and sustainable farming systems.

Keywords: Calliterpenone, *Callicarpa macrophylla*, Catalase, Gibberellic acid.

Introduction

The Global population is projected to increase around 2.3 billion people by the year 2050. The major fraction of this escalation is contributed mainly by the developing countries. It leads to increase in food demand up by an estimated 3 billion tones. Systemic breeding programmes for improved agriculture output under the green revolution undoubtedly have led to spectacular improvements in agriculture and in

eradicating world hunger, but excessive fertilizers use have led to generate disturbance in environment, particularly nutrient imbalance. So researchers have invented another way for increasing agriculture crop production by applying plant growth regulators. Plant growth boosters have become particularly appealing in the agro-business of high-value crops for organic production and have commercial significance in extensive farming. Out of all noted plant growth regulators (Auxins, Cytokinins, Absciscic Acid, Gibberellic Acid, and Ethylene) used to increase the productivity of cash crops, Gibberellins, especially GA₃, are the most significant category (Srivastava 2007). *Callicarpa macrophylla* Vahl is the source of phyllocladane diterpenoids, which have recently been shown to enhance plant development and ameliorate the effects of growth-retardant allelo-chemicals (Singh *et al.* 2007). *Callicarpa macrophylla*, commonly known as the beautyberry, is a deciduous shrub belonging to family Verbenaceae (Paprikar *et al.* 2021). Native to tropical and subtropical region of Asia, including the Himalayas, India and Southeast Asia, it thrives in forests, open grasslands and riverbanks. The plant is characterized by its broad, ovate leaves and clusters of small, purplish pink flowers that bloom in summer, followed by vibrant, glossy purple berries in autumn. These berries are known for their ornamental appeal and are often used in traditional medicine for their purported anti-inflammatory and astringent properties (Rahim *et al.* 2025). Adaptable and hardy, *C. macrophylla* plays a role in soil conservation and

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is valued in ecological restoration projects. Many chemicals from the genus *Callicarpa* have been identified, including amino acids, benzinoids, carbohydrates, lipids, numerous diterpenes, flavanoids, phenylpropanoids, phytosterols, sesquiterpenes, and triterpenes (Arya *et al.* 2023; Panday *et al.* 2014; Pandey *et al.* 2022). Thus the aim of our study is to determine the effect of GA and Calliterpenone on protein and enzymatic assay (Protease, α -amylase, Catalase and Ascorbate peroxidase) in *P. vulgaris*, a valuable food crop.

Phaseolus vulgaris, commonly known as the common bean, holds significant economic importance as a major food crop worldwide (Gudeta Dida *et al.* 2018). It is a versatile legume grown for its edible seeds and pods, which are consumed as dry beans or fresh vegetables. Rich in protein, fiber, vitamins and minerals (Gudeta Dida *et al.* 2018), it serve as a staple food and a key source of nutrition, especially in developing countries. Economically, it supports farmers and agricultural economies due to its adaptability to diverse climates and its role in crop rotation, improving soil fertility through nitrogen fixation. Additionally, *P. vulgaris* contributes to the food processing industry, generating income through the production of canned, frozen and packaged bean products. Its high demand in local and International markets underpins its value in global trade.

Present study explored the usefulness of growth promoter, calliterpenone (CA) extracted from the plant *Callicarpa macrophylla* augmented with different doses (0.1mM, 0.01mM & 0.001mM) over Gibberellic acid for protein content and enzymatic activity.

Material and Methods

Calliterpenone was obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP) Lucknow and was used as received (Singh *et al.*, 2007). To prepare stock solution of 0.1 mM, 3.2 mg of Calliterpenone (320 g/mol) and 3.46 mg of gibberellic acid (346 g/mol) were dissolved separately in 1 milliliter of absolute alcohol, followed by pouring this solution into 20 ml distilled water in a volumetric flask (100 ml). The solutions were mixed and prepare final volume of 100 mL with distilled water. Thereafter 0.01 mM and 0.001 mM solution were prepared by taking 10 mL of this stock solution (0.1mM) to a final volume of 100 mL and 1000 mL by adding distilled water for both the compounds. *P. vulgaris* seeds were initially soaked (8 hours) in varying concentrations of plant growth promoters (GA₃ & CA), subsequently seeds were maintained under moist condition with respective treatments upto 72 hours. One set of seeds first imbibed followed by moistened with distilled water upto same hours, used as standard. After 72 hours Protein (Lowry *et al.* 1951), Protease activity (Kembhavi *et al.* 1993), α -amylase (Filner and Warner. 1967), Catalase (Aebi. 1984) and Ascorbate peroxidase act (Nakano and Asada. 1981) activity of all the treated seeds were tested out. All the experiments were made in triplicate and results are expressed in terms of mean $\pm$ SD.

Results

Effect on protein and enzymatic activity in *P. vulgaris* seeds treated with varying concentrations of Calliterpenone (CA) and Gibberellic acid (GA₃)

Effect on Protein

Maximum gain in protein content (18.6 %) was observed in 0.01mM calliterpenone treated sets followed by 0.001mM gibberellic acid, while the lowest protein content was observed in 0.01 mM GA₃ treated sets (Fig. 1). Increase in protein content in calliterpenone treated sets may be attributed to enhanced synthesis of enzymatic protein. The effects on protein content vary between the treatments and concentrations. Both GA₃ and CA show concentration –dependent effects on protein levels. The direction of the changes (increase or decrease) differs between the two treatments at each concentrations.

Protease activity

Gibberellic acid (GA₃) appears to have a complex effect on protease activity. At different concentrations, it either increase or decrease protease activity. Calliterpenone (CA) seems to generally lead to a decrease in protease activity, and the effect is more pronounced at higher concentration. Protease activity was found to be highest (47.2%) at 0.1mM gibberellic acid supplementation (Fig. 2A)

Alpha-amylase activity

Similar α -amylase activity was observed in 0.1mM GA₃ and 0.01mM CA treated sets. Calliterpenone treated sets generally have a higher α amylase activity as compared to GA₃ and distilled water. Alpha amylase plays a crucial role in mobilizing carbohydrate reserves hydrolysis during seed germination. The resulting sugar serves as an energy source for the developing seedling. The concentration of GA₃ at 0.1 mM seems to have a stimulatory effect on α -amylase activity. For both GA₃ and CA, there is a decrease

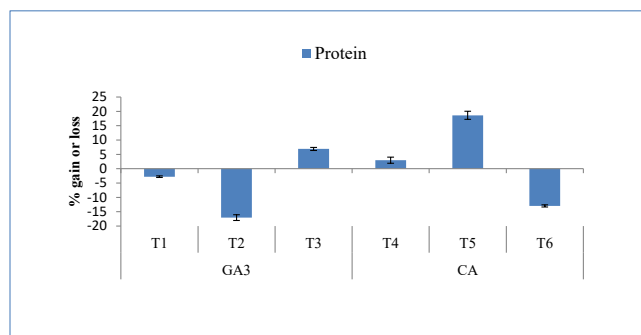


Figure 1: Gain or loss (%) in protein content in GA₃ and CA treated *P. vulgaris* seeds as compared to control (imbibed in distilled water). Each bar represents the mean value and Error bars represents the standard deviation $\pm$ SD (n=3). **T1**- Seed primed in 0.1 mM GA₃, **T2**- Seed primed in 0.01 mM GA₃, **T3**- Seed primed in 0.001 mM GA₃, **T4**- Seed primed in 0.1 mM CA, **T5**- Seed primed in 0.01 mM CA, **T6**- Seed primed in 0.001 mM CA.

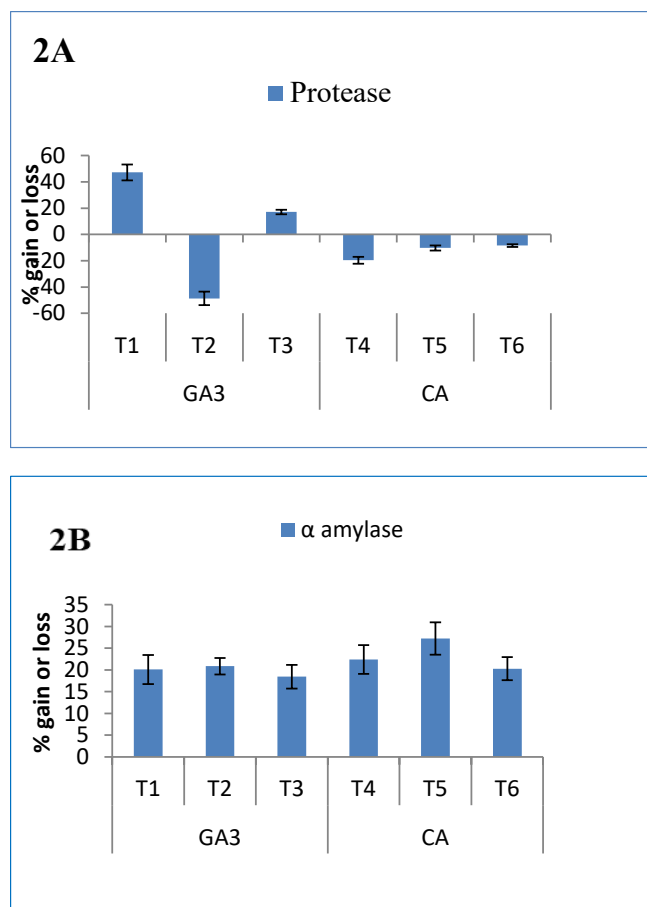


Figure 2: Gain or loss (%) in protease activity (2A) and α -amylase activity (2B) in GA₃ and CA treated *P. vulgaris* seeds as compared to control (imbibed in distilled water). Each bar represents the mean value and Error bars represents the standard deviation $\pm$ SD (n=3). **T1**- Seed primed in 0.1 mM GA₃, **T2**- Seed primed in 0.01 mM GA₃, **T3**- Seed primed in 0.001 mM GA₃, **T4**- Seed primed in 0.1 mM CA, **T5**- Seed primed in 0.01 mM CA, **T6**- Seed primed in 0.001 mM CA.

in α -amylase activity at lower concentrations (0.001mM). It's interesting to note that at 0.01mM the α -amylase activity is the highest for CA but the lowest for GA₃ (Fig. 2B). This suggests that calliterpenone may be an effective alternative to GA₃ in promoting the energy supply needed for seedling development.

Ascorbate peroxidase activity

Ascorbate peroxidase activity was found to be higher at 0.1mM and 0.001mM GA₃ concentration, similar results also available in CA treated sets (Fig. 3A). Previous studies have explained that application of GA₃ can enhanced antioxidant activity of plants by inducing synthesis of phenolic compounds, decreasing lipid peroxidation and upregulating proline and glycine betaine synthesis. Studied also concluded that PGRs both individually and in combination, plays a pivotal role in regulating the transcription of certain genes along with mRNA that ultimately in turn ameliorate the synthesis of a specific hormone to make proteins.

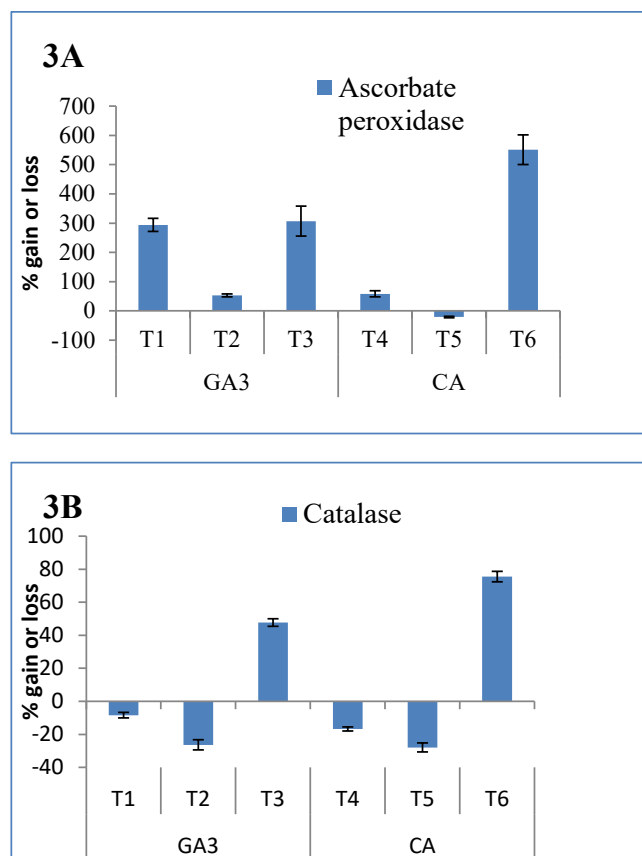


Figure 3: Gain or loss (%) ascorbate peroxidase activity (3A) and in catalase activity (3B) in GA₃ and CA treated *P. vulgaris* seeds as compared to control (imbibed in distilled water). Each bar represents the mean value and Error bars represents the standard deviation $\pm$ SD (n=3). **T1**- Seed primed in 0.1 mM GA₃, **T2**- Seed primed in 0.01 mM GA₃, **T3**- Seed primed in 0.001 mM GA₃, **T4**- Seed primed in 0.1 mM CA, **T5**- Seed primed in 0.01 mM CA, **T6**- Seed primed in 0.001 mM CA.

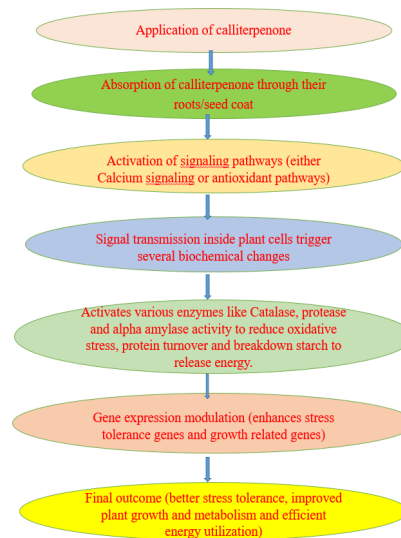


Figure 4: A Proposed flowchart illustrating mechanism of action of calliterpenone in Plants.

Catalase activity

In the case of GA₃ at 0.1mM the catalase activity decreased by 8.35% (Fig. 3B). In case of calliterpenone, catalase activity was found to be highest at 1.0 micro-molar concentration in both the treatments. This demonstrates that small concentrations of calliterpenone are more effective in enhancing the seed ability to manage oxidative stress during germination.

Discussion

This study conclusively demonstrates that calliterpenone (CA), a di diterpenoid compound extracted from *Callicarpa macrophylla*, act as effective plant growth regulator as compare to gibberellic acid in case of *P. vulgaris* seeds (Kumar *et al.* 2011, Bose *et al.* 2013, Haider *et al.* 2009). The application of calliterpenone during seed imbibition has significantly enhanced the germination and stimulated key biochemical process tied to early seedling establishment. Similar to other PGRs, calliterpenone treatment also provides adaptability to plant against stress (Stylar *et al.* 2019).

During the germination of seed, calliterpenone treated seeds show enhanced proteases activity. These enzymes catalyze the hydrolysis of peptide bonds in proteins, leading to protein processing or degradation. Based on their catalytic mechanisms, proteases are classified into serine, cysteine, aspartic, and metalloproteases. They are essential for protein turnover, metabolic regulation, apoptosis, and stress responses in plants and microorganisms. Enhanced protease activity reflects active protein remodeling, which is necessary for proper seedling development (Taiz *et al.* 2015). Furthermore, the increased total protein content observed in treated seeds suggests enhanced metabolic activity and biosynthesis, contributing to improved growth performance.

Alpha amylase is one of the key enzyme behind seed germination. It's a calcium dependent enzyme, which breaks down the α -1, 4 glycosidic linkage in starch, glycogen and similar polysaccharides. This hydrolysis releases maltose and other smaller carbohydrate, which serves as immediate energy source for the growing embryo. Alpha amylase has a wide distribution range from microbe to plants and animals. In human, it aids in digestion through its salivary and pancreatic form, whereas in plants it is essential for breaking down stored starch reserve during the process of germination. Enhanced alpha amylase activity observed in CA treated seeds at all concentration, suggests improved carbohydrate mobilization, thereby supplying sufficient energy to support faster seedling growth (Nelson and Cox, 2008).

The present findings suggest the significance of ascorbate–glutathione (AsA–GSH) cycle in minimizing damage to plant cells caused by abiotic stress, through production of large amount of Reactive Oxygen Species (Hasanuzzaman *et al.* 2020). This pathway act as major

detoxification pathway mechanism in plant cell. Ascorbate serves as foremost electron donor, while ascorbate peroxidase and glutathione reductase simultaneously facilitate H₂O₂ scavenging and regenerate reduced form of glutathione (Gill and Tuteja, 2010; Barwal *et al.* 2023). Notably, APX activity was highest at lower CA supplementation, suggesting the calliterpenone modulates antioxidant defense system in optimal quantity.

Similar to APX, catalase also plays a key role detoxification of ROS. Catalase is an enzyme, which catalyze the breakdown of hydrogen peroxide into H₂O and O₂, therefore helps in diminishing oxidative damage to cellular components. By rapidly eliminating H₂O₂, catalase maintains cellular redox homeostasis and enhances stress tolerance in plants, microorganisms, and animals (Li *et al.* 2016; Gill and Tuteja, 2010; Berg *et al.* 2023). Increased catalase activity in calliterpenone-treated seeds justifying its role in maintaining the redox balance under stress conditions.

Overall, calliterpenone, particularly at concentrations of 0.01 mM and 0.001 mM, significantly enhanced the activity of enzymes involved in carbohydrate metabolism and oxidative stress management. Its effects were comparable to those of GA₃-treated seeds and superior to distilled water controls. Similar improvements in enzymatic activity have been reported in mung bean (Tiwari *et al.* 2015) and *Passiflora* species (Domingues *et al.* 2014). These findings collectively support the potential of calliterpenone as a promising natural plant growth regulator. A proposed flowchart illustrating its mechanism of action in plants is presented in Fig. 4.

Conclusion

In conclusion the result clearly explained that seed imbibed in varying concentration of calliterpenone have increased alpha amylase activity, Ascorbate peroxidase activity and catalase activity. Total protein content also increased in seed primed with Calliterpenone. Application of varying Gibberellic acid, also promote alpha amylase activity and protein level. In CA treated seeds increased catalase and ascorbate peroxidase activity helps in reducing oxidative damage and stress tolerance. This results highlights the role of calliterpenone as an alternative or complementary growth promotor similar to GA₃. It stimulates both growth and protective mechanism, makes it a promising alternative for agricultural applications. Further research could explored its extensive use across diverse crop species. Its persistent impact on plant growth and integration into agriculture practiced targeted to enhance crop yield and resilience.

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