

DOSE-DEPENDENT EFFECTS OF AMINO-ACID APPLICATION ON AGRONOMIC GROWTH AND FLOWERING PERFORMANCE OF CELOSIA ARGENTEA L.

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Abstract

Amino-acid-based biostimulants are increasingly recognized as promising inputs for improving plant growth and flowering in ornamental crops. However, their effects on the agronomic and floral performance of *Celosia argentea* L. remain insufficiently documented. Therefore, the present study evaluated the effects of different amino-acid application levels on germination, vegetative growth, flowering, and floral characteristics of *C. argentea*. Plants were subjected to four treatments: T1 (control), T2 (amino acid 6 mL), T3 (amino acid 12 mL), and T4 (amino acid 18 mL). Germination percentage, days to flower initiation, number of leaves, side branches, plant height, stem diameter, number of flowers per plant, flower diameter, flower stalk length, and flower fresh and dry weight were recorded. Germination was high, reaching 98% under the experimental conditions. Amino-acid application significantly improved most vegetative and flowering traits compared with the control. Treatment T4 produced the highest number of leaves (15.75 plant⁻¹), side branches (10.00 plant⁻¹), plant height (16.60 cm), and stem diameter (5.62 cm). It also promoted earlier flower initiation (7.00 days) and produced the highest number of flowers (2.75 plant⁻¹), flower diameter (8.45 cm), flower stalk length (5.90 cm), and fresh flower weight (15.92 g). Correlation analysis showed strong positive relationships among vegetative growth and floral characteristics, indicating that improved vegetative development was associated with enhanced flowering performance and flower biomass. Overall, amino-acid application, particularly T4, substantially improved the growth and flowering performance of *C. argentea* under the conditions of this study. These findings demonstrate the potential of amino-acid biostimulants for enhancing ornamental quality and productive performance.

1. INTRODUCTION

Along with their aesthetic, economic, environmental and social values, ornamental plants are an important part of horticultural production. The demand for flowers and ornamental plants for gardens, landscapes, public areas, floral arrangements, ceremonies and interior decoration has stimulated the development of better production technologies to improve plant growth and flower quality. In ornamental horticulture, in addition to biomass production, plant performance is determined by plant structure, flowering time, flower number and size, stem quality, flower color, and overall visual quality. Therefore, efficient nutrient management and application of environmentally friendly technologies for growth of ornamental plants have received growing attention in order to enhance the productivity and value of the crop in the market (Calvo et al., 2014; du Jardin, 2015). Cockscomb (*Celosia argentea* L.) is a lovely annual ornamental plant, which is much appreciated for its unique and striking flower spikes. Its attractive floral structures and ease of production make it a popular species for cultivation as a bedding plant, garden ornamental, container plant and cut-flower crop. Its inflorescences are extremely variable in ornamentality, in terms of colour, form, size and arrangement, and are suitable for landscape and commercial horticulture use. For cockscomb, the production of a vigorous vegetative framework and profuse and even flowering are essential. Plant height, number of leaves, number of branches, stem diameter, flowering time, number of inflorescences and the characteristics of the inflorescence are thus important attributes that determine its ornamental quality. More recently, *C. argentea* culture has been shown to be sensitive to environmental conditions and irrigation regimes, which affect emergence and early growth, highlighting the need for proper crop management in order to produce a successful crop (Freitas et al., 2024).

Nutrition is one of the most significant factors controlling vegetative growth, physiological activity, reproductive development and flowering performance. Traditional crop nutrition is mostly

based on macronutrients and micronutrients, but modern horticultural research increasingly focuses on the use of plant biostimulants as a complement to achieve better nutrient-use efficiency, physiological performance, stress tolerance and crop quality. Plant biostimulants generally include substances that serve as natural stimulants of plant processes to promote the efficiency of mineral nutrition, abiotic stress tolerance and/or crop-quality traits, and are not just mineral nutrients (du Jardin, 2015). Protein hydrolysates and amino-acid based products are an interesting class of biostimulants; due to their potential activity on plant growth, metabolism, nutrient assimilation and product quality, they have gained increasing interest in the last years (Colla et al., 2015; Calvo et al., 2014).

Amino acids are basic organic compounds which are essential for protein, enzyme, hormones, pigments and for the production of many primary and secondary metabolites. Amino acids are also involved in signaling, nitrogen metabolism, osmotic adjustment, acting as antioxidants, and controlling plant response to environmental stress, as well as being structural components of proteins. Therefore, exogenous amino acid application can have other positive and negative effects on plant physiology than as a nitrogen source. Research on protein hydrolysates containing amino acids and peptides has shown that these compounds can affect plant growth and nitrogen metabolism, such as stimulation of nitrate reductase and glutamine synthetase activities, which would have an impact on N assimilation and plant metabolic activity (Ertani et al., 2009). Likewise, protein hydrolysates isolated from plants has been linked to improved root and shoot growth, better nitrogen assimilation and hormone-like activity in a few plant systems (Colla et al., 2014).

The physiological activity of amino acids is closely related to their concentration, chemical structure, plant species, developmental stage and application method. The use of foliar application is especially appealing as it allows for the application of biologically active compounds directly to the aerial parts of the plant and may circumvent some of the

limitations found with soil application. Foliage can absorb amino acids from the solution and then utilize or transport them within the plant. Experimental results confirm the presence of an important foliar availability of organic nitrogen sources such as amino acids (glycine, glutamic acid and proline) for foliar fertilization, which would allow the use of amino-acid compounds as effective tools to introduce N into plants (Stiegler et al., 2013). More recently, a comprehensive review of the individual amino acids revealed that foliar applied amino acids can alter photosynthesis, chlorophyll biosynthesis, electron transport, antioxidant metabolism and plant response to oxidative stress (Henderson et al., 2025).

Amino acids and protein hydrolysates can stimulate the growth of plants via multiple physiological processes that work together. These effects have been linked with an improvement of the metabolism in carbon and nitrogen, nutritive assimilation, modification of root architecture and modulation of hormone-related processes (Ertani et al., 2009; Colla et al., 2014). Peptides can also have physiological effects that are elicited apart from their nutritional value, and are found in protein hydrolysates. In horticultural crops, these materials are known to enhance production, quality traits, nutrient uptake and resilience to adverse environmental factors (Colla et al., 2015). In addition, application of plant-based biostimulants can have an impact on plant-microorganism relationship, and may lead to better nutrient availability and plant's physiological functioning (Nardi et al., 2016). Amino-acid based biostimulants are especially interesting in intensive ornamental crop production where quick and good development of plants is desired.

The benefits of amino-acid supplementation may become particularly important during periods of rapid vegetative development. Metabolic activity in this stage is crucial for the production of leaves, stem development, branching, plant height and the ability to foster reproductive structures later in the plant's life. Previous research has shown that protein hydrolysate and amino acids containing

biostimulants are able to promote shoot and root growth and increase plant biomass in various horticultural crops (Ertani et al., 2009; Ertani et al., 2013). Amino-acid based biostimulants can also under stressful situations help to enhance the physiological stability via assisting antioxidant mechanisms and osmotic adjustment. Other examples are the beneficial effect of alfalfa based biostimulants on the early growth of maize plants under saline stress, where amino acids were observed to play a role in plant resistance to stress (Ertani et al., 2013). Significant metabolic activity and assimilate and nutrient reserves are needed for flower initiation, bud development, anthesis and development of the inflorescence. Amino acids may be involved in nitrogen metabolism and may be precursors or regulators of various compounds which are related to plant development and stress. Thus, the improvement of the physiological efficiency after application of amino-acids can lead to an indirect effect on the development of the reproduction and flowering performance. Experimental evidence for ornamental crops agrees with this. In potted snapdragon (*Antirrhinum majus* L.), application of an animal derived protein hydrolysate biostimulant resulted in higher plant height, greater number of shoots, production of leaves, total leaf area, and number of flowers, indicating that application of biostimulant had a positive effect on both vegetative and reproductive characteristics of an ornamental species (Cristiano et al., 2018).

The potential value of amino-acid based biostimulants was further reinforced by a study conducted with cut flowers and other ornamental species. Protein hydrolysates from animal and plant origin have been proven to influence morphological and physiological traits, ornamental value, mineral contents and post-harvest traits of chrysanthemum. The intensity and direction of the responses, however, varied depending on the source and composition of the hydrolysate, suggesting that biostimulant effects are not general and might be formulation and genotype dependent (Carillo et al., 2022). The significance of this observation is that for cockscomb, the results experienced in vegetables

cannot be directly extrapolated to the other ornamental species. Therefore, for each species, it is essential to evaluate the species-specific suitability of these concentrations and application schedules.

Another important plant response-determining factor is the concentration of amino acids applied to foliage. If a concentration at which the plants responded positively, then the concentration is not necessarily further increased to achieve a better response. High levels of some amino acids can give adverse physiological effects or disrupt metabolic balance. There is also new evidence that individual amino acids can have concentration dependent effects; beneficial at the right concentration but suppressive of growth or physiological disruption at high concentrations (Henderson et al., 2025). Hence, setting an optimum nutrient application rate is crucial to ensure proper nutrient uptake and maximize benefits of amino-acid foliar applications without application or purchase of nutrients that are not needed or may be harmful.

Amino acid treatments may also be further affected by environmental factors. Leaf absorption, plant metabolism, and the resulting physiological reaction to foliar applied compounds can be affected by the factors of temperature, water availability, humidity, irradiance and nutritional status. This is particularly important for ornamental crops grown in warm and semi-arid climates where heat stress and water stress can adversely impact crop vegetative growth and flowering. Additionally, amino acids like proline are linked to stress-response mechanisms and protein hydrolysates can induce antioxidant activity and osmotic adjustment in adverse environments (Colla et al., 2015; Ertani et al., 2013). Amino-acid biostimulants could thus be considered as a possible approach to enhance plant biotic responses and the ability to keep flowering throughout fluctuating environmental conditions.

There is a more available research on amino acids and protein hydrolysates in agricultural/horticultural crops, but less information available on the impact of amino

acids and protein hydrolysates on cockscomb. Previous research has shown bio-stimulants can enhance the growth of ornamental plants, physiological performance, and flowering traits in several species, with species-specific effects depending on the plant species, formulation, concentration, application method, and environmental conditions (Cristiano et al., 2018; Carillo et al., 2022). Furthermore, most of the literature published has emphasized the use of protein hydrolysates as broad-spectrum products, and not studied the specific effects of foliar application of amino acids to the growth and flowering attributes of *Celosia argentea*. It is an important knowledge gap for growers to find simple and efficient ways to enhance the quality of ornamental plants.

Amino-acid application to foliage is of commercial ornamental value and understanding the response of cockscomb to foliar amino-acid application is of particular importance as the commercial value is dependent on both vegetative and reproductive properties. Growth height cannot be used as a criterion of ornamental quality when coupled with low stems, poor branching, delayed flowering or decreased inflorescence development. Likewise, more vegetative growth does not necessarily lead to better flowering if it is not used commercially. Hence, the evaluation of several growth and flowering parameters is needed to determine an amino acid treatment that yields a balanced improvement of the overall plant performance.

Using amino acids as foliar biostimulants could therefore be an effective strategy to optimize the production of ornamental plants and to promote optimal use of agricultural resources. They have the potential to positively influence nutrient assimilation, photosynthetic activity, antioxidant defense, plant architecture and flowering, and therefore make promising components of integrated nutrient-management programs (Calvo et al., 2014; Colla et al., 2015; Henderson et al., 2025). Nevertheless, the application concentration needs to be optimized as plant response is highly concentration and formulation dependent. Evaluation in the field or in pots under specific environmental conditions can then be valuable in

assessing what might be the best treatment for a specific ornamental species.

So the present study was conducted to assess the effect of foliar application of various concentrations of amino acids on growth and flowering parameters of cockscomb (*Celosia argentea* L.). The specific objective of this study was to find out the effect of amino-acid application on vegetative growth characteristics and important flowering traits; and to find out which treatment gave the best overall plant performance. The results will be used to design an effective and practical foliar biostimulator approach for enhancing the growth, flowering ability, ornamental value and market value of cockscomb.

2. Materials and Methods

2.1 Study Site

Amino acids was tested on growth and flowering responses of cockscomb (*Celosia argentea*) in a greenhouse experiment of the Department of Horticulture, Faculty of Agriculture, Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS), Uthal, Balochistan, Pakistan in the growing season of 2025. The seeds of *Celosia argentea* were collected from an online store (Sunny View Store, Lahore).

The uniform seeds were first started in plastic nursery trays with 128 cells. The growing medium used consisted of peat moss, and the seeds were sown in the greenhouse. The seeds were grown on an even basis by providing regular irrigation and standard nursery management practices to ensure proper seeding and seedling establishment. After attaining a suitable growth stage and adequate size, healthy and uniform seedlings were carefully transplanted into pots of the same size. This experiment was then continued under the greenhouse throughout experimental period.

The experiment treatments were designed in Completely Randomized Design (CRD) with three replications. Environmental and management conditions were kept similar among the plants to minimize variation other than that due to the amino-acid application.

2.2 Treatments and Experimental Design

In the experiment, 12 pots were used—of which 3 pots for each amino-acid treatment along with control treatment. The experiments were conducted in a Completely Randomized Design in 3 replications (Table 1).

Table 1. Experimental treatments and amino-acid application levels

Treatment	Treatment description	Amino-acid application level
T1	Control (only water Spray)	0 mL
T2	Amino acid	6 mL
T3	Amino acid	12 mL
T4	Amino acid	18 mL

In the control treatment, the plants were watered while in the treatment groups, the plants were treated with the various concentrations of the amino acids. The same greenhouse, irrigation and general plant management conditions were used for all plants during the experiment.

2.3 Foliar Application of Amino Acid

Amino acid were prepared according to the different treatments required for foliar application. Uniform application of prepared

solutions was accomplished on the plants with a hand-operated sprayer. Spraying was done by spraying over the foliage to cover the leaves. In order to keep uniformity in experimental operation, water spray was done on the control plants too as done in the experimental plants. The foliar application was made when the crop was in the proper growth stage, making sure the application conditions were similar from one treatment to the next. The plants were then grown

under identical greenhouse conditions until the end of the experiment.

2.4 Parameters Recorded

Foliar-absorption of amino acid effects on growth and flowering performance of *Celosia argentea* was assessed by measuring some growth and flowering parameters at proper stages of plant development. The percentage of germination was obtained by dividing the number of germinated and emerged seeds by the number of seeds sown and multiplying by 100 and was calculated as: Germination (%) = (Number of germinated seeds / Total number of seeds sown) × 100. The number of leaves per plant was measured by hand-counting all fully expanded leaves on each plant sampled. The height of the plants (cm) was determined from the soil surface to the highest fully extended leaf or growing point on a graduated measuring scale. The number of side branches was counted by hand, counting the lateral branches that came from the main stem of each of the selected plants, and the stem diameter (cm) measured at a standard point on the stem with a digital Vernier caliper.

Flowering traits were evaluated based on the number of days to flower initiation, flower numbers per plant, flower diameter and flower stalk length. The days to flower initiation were calculated as the number of days from sowing to the appearance of the first flower bud. Fully grown flowers were counted from each selected plant to determine the number of flowers per plant, by manually counting all the flowers produced by each selected plant during the flowering period. The diameter of flowers (cm) was determined across the widest part of fully open flowers with a ruler/vernier caliper and the length of the flower stalk (cm) was measured from the base of the flower stalk to the point of attachment with the flower with a measuring scale.

The biomass of flowers was measured in terms of fresh and dry flower weight. The fresh weight of flowers (g) was immediately measured after they were harvested from the selected plants on a digital electronic balance after they reached full growth. Fully developed flowers were picked for the dry

flower weight and dried till constant weight and weighed on digital balance. All parameters were recorded on a representative plant in each experimental unit and the means were statistically analysed.

2.5 Statistical Analysis

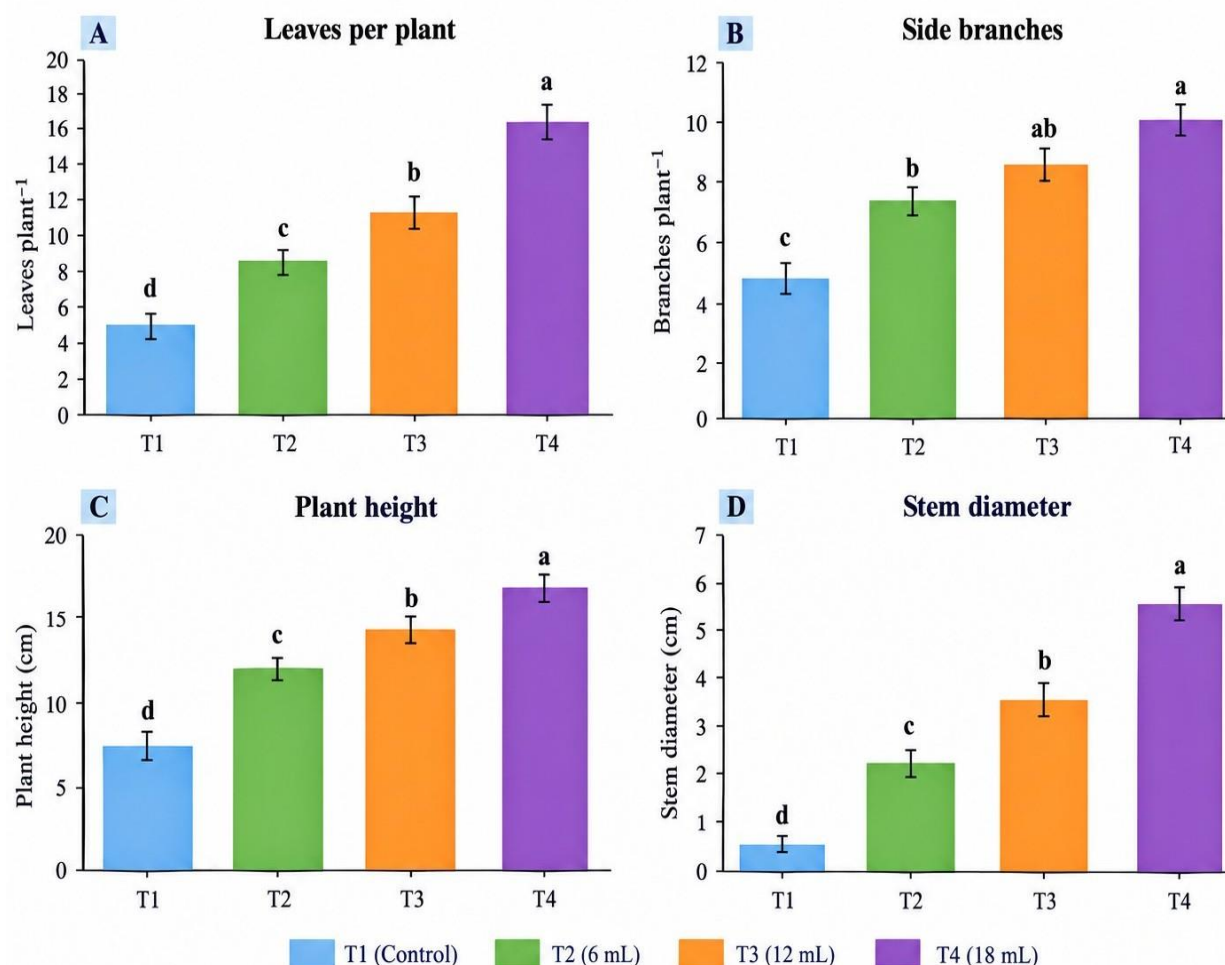
Analysis of variance (ANOVA) was used to analyze the recorded data, and Statistix 8.1 (Statistix, 2006) statistical software was used to determine significance of treatment effects. The Least Significant Difference (LSD) test at 5% probability level ($P \leq 0.05$) was used to compare treatment means. Pearson's correlation analyses were used to identify relationships between the growth and flowering parameters measured. In addition, regression analysis was conducted to identify important relationships between the measured traits and plant performance. Data were checked for normality and homogeneity of variance before statistical analysis and were found to meet these requirements so that the data could be compared statistically.

3. Results

3.1 Agronomic and Phenotypic Responses

The major agronomic and phenotypic traits of *Celosia Argentea* were affected by the treatments to a significant extent. Germination percentage in the experimental condition was 98%, which showed that the seeds germinated successfully and establishment of experimental plants were relatively even.

The vegetative growth was found to have increased significantly with treatment. The control treatment (T1) produced the lowest number of leaves (4.75 ± 0.52 leaves plant⁻¹), whereas leaf production increased to 8.25 ± 0.52 , 10.75 ± 0.52 , and 15.75 ± 0.52 leaves plant⁻¹ under T2, T3, and T4, respectively. Similarly, the number of side branches increased from 4.75 ± 0.54 under T1 to 7.25 ± 0.54 , 8.50 ± 0.54 , and 10.00 ± 0.54 branches plant⁻¹ under T2, T3, and T4, respectively. The ANOVA and LSD test showed that the differences between the treatments were significant at $P < 0.05$.



The same trend was seen in plant height. Plants in the control treatment had the lowest height (7.27 ± 0.53 cm), while T2, T3, and T4 produced progressively taller plants, with mean heights of 11.87 ± 0.53 , 14.35 ± 0.53 , and 16.60 ± 0.53 cm, respectively. Stem diameter also increased markedly with amino-acid application, from 0.53 ± 0.22 cm under T1 to 2.22 ± 0.22 cm under T2, 3.65 ± 0.22 cm under T3, and 5.62 ± 0.22 cm under T4.

Overall, the results show a steady increase in vegetative growth as the amount of amino acids applied increases. Treatment T4 (S4) gave the highest value of leaves, side branches, and plant height and stem diameter while treatment T1 consistently gave the lowest value.

Figure 1. Effect of different amino-acid application treatments on major agronomic and phenotypic traits of *Celosia argentea* L. Panels show (A) number of leaves per plant, (B) number of side branches per plant, (C) plant height, and (D) stem diameter. Treatments consisted of T1 (control), T2 (amino acid 6 mL), T3 (amino acid 12 mL), and T4 (amino acid 18 mL). Values are presented as mean $\pm$ SE (n = 3). Different lowercase letters indicate significant differences among treatments according to the LSD test at P < 0.05.

4.2 Flowering and Floral Characteristics

Application of amino acids had significantly influence flowering and floral traits of *C. argentea*.

The number of days to flower initiation was gradually decreased after the application of amino-acid. The control treatment required 17.00 ± 1.17 days for flower initiation, whereas T2, T3, and T4 required 10.00 ± 1.17 , 8.75 ± 1.17 , and 7.00 ± 1.17 days, respectively. The ANOVA showed that the treatment effect was significant ($F = 13.8$, $P = 0.0003$).

Flower production and flower size were enhanced by the application of the amino-acids, as compared to the decrease in flowering time. The number of flowers per plant increased from 1.00 ± 0.22 under T1 to 1.75 ± 0.22 , 2.50 ± 0.22 , and 2.75 ± 0.22 under T2, T3, and T4, respectively. The diameter of the flowers also increased significantly; the smallest were observed in the control (3.27 ± 0.20 cm) and the largest in T4 (8.45 ± 0.20 cm). The intermediate flower diameters for T2 and T3 plants were 4.92 ± 0.20 cm and 6.25 ± 0.20 cm, respectively.

A similar treatment response was observed for flower stalk length. The control treatment

produced the shortest flower stalks (1.32 ± 0.26 cm), while T2, T3, and T4 increased stalk length to 3.12 ± 0.26 , 4.57 ± 0.26 , and 5.90 ± 0.26 cm, respectively.

The fresh weight of flowers also significantly increased as the application of amino-acids increased. Flowers from T1 had the lowest fresh weight (4.92 ± 0.49 g), followed by T2 (7.92 ± 0.49 g) and T3 (11.17 ± 0.49 g), while T4 produced the heaviest flowers (15.92 ± 0.49 g). The treatment effect was highly significant ($F = 89.9$, $P < 0.001$).

The overall pattern of flower longevity was similar to the observations. Flowers under the control treatment remained fresh for approximately 1.03 ± 0.21 days, compared with 2.35 ± 0.21 , 4.55 ± 0.21 , and 5.92 ± 0.21 days under T2, T3, and T4, respectively. Therefore, the flowering parameters of the plants grown by T4 were the most beneficial: earlier flower initiation, higher flower numbers, higher flower diameter, longer stalks, higher flower weight and longer flower longevity.

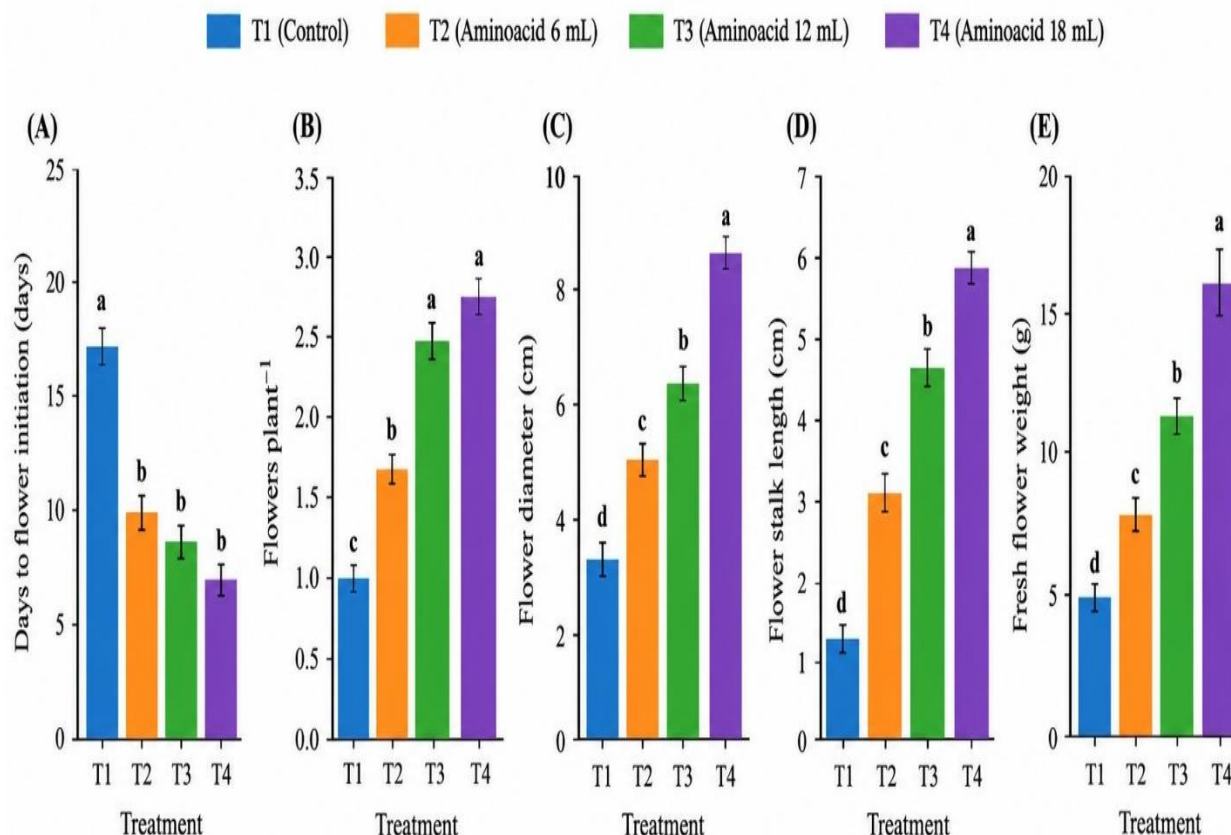


Figure 2. Effect of different amino-acid application treatments on flowering and floral characteristics of *Celosia argentea* L. Panels show (A) days to flower initiation, (B) number of flowers per plant, (C) flower diameter, (D) flower stalk length, and (E) fresh flower weight. Treatments were T1 (control), T2 (amino acid 6 mL), T3 (amino acid 12 mL), and T4 (amino acid 18 mL). Values are presented as mean $\pm$ SE ($n = 3$). Different lowercase letters indicate significant differences among treatments according to the LSD test at $P < 0.05$.

4.3 Relationships among Growth and Flowering Traits

A significant positive correlation was found between all the vegetative and flowering characteristics with the exception of plant height and the number of branches per plant, as shown in Table 4.14. Days to flower initiation were positively correlated with dry weight/flower

longevity ($r = 0.6709$) and various floral and vegetative characteristics. High correlations between flower diameter, flower stalk length, flower weight, flower number, leaf number, side branch number, plant height and stem diameter were found.

The diameter of the flowers had particularly high positive correlation with flower stalk length ($r = 0.9826$), flower weight ($r = 0.9897$), number of flowers per plant ($r = 0.9810$), number of leaves per plant ($r = 0.9899$), number of side branches ($r = 0.9867$), plant height ($r = 0.9917$), and stem diameter ($r = 0.9628$). In the same manner, flower weight correlated highly with flower stalk length ($r = 0.9920$), number of leaves ($r = 0.9931$), plant height ($r = 0.9842$) and number of side branches ($r = 0.9777$).

The number of leaves per plant was also strongly associated with flower stalk length ($r = 0.9898$), flower weight ($r = 0.9931$), number of flowers ($r = 0.9728$), side branches ($r = 0.9842$), and plant

height ($r = 0.9829$). Likewise, plant height was strongly correlated with flower diameter ($r = 0.9917$), flower weight ($r = 0.9842$), flower number ($r = 0.9800$), and side branches ($r = 0.9865$).

Overall, it can be concluded that the correlation analysis shows that an improvement in vegetative development was generally correlated with an

improvement in flowering performance. The plants that developed more leaves, branches, stems and height usually flowered more, larger, and heavier than those that developed less. Note that these relationships are considered associations, not causal relationships as they are only associated with one another and do not cause one another.

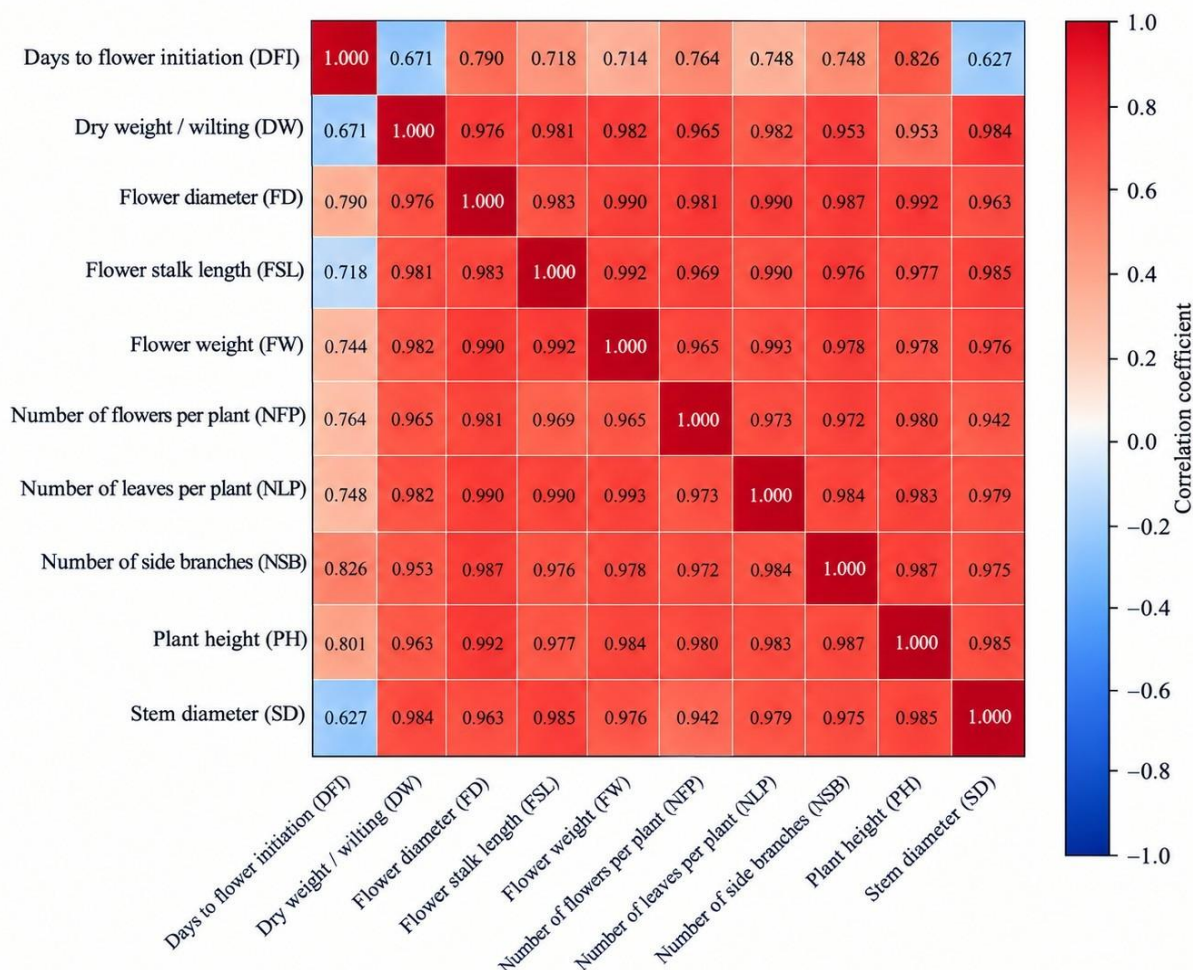


Figure 3. Pearson correlation heatmap illustrating the relationships among flowering, floral, agronomic, and phenotypic traits of *Celosia argentea* L. Traits include days to flower initiation (DFI), dry weight of flowers (DW), flower diameter (FD), flower stalk length (FSL), fresh flower weight (FW), number of flowers per plant (NFP), number of leaves per plant (NLP), number of side branches (NSB), plant height (PH), and stem diameter (SD). Color intensity represents the strength and

direction of the correlation coefficients, with warmer tones indicating stronger positive relationships and contrasting tones indicating weaker or negative associations. Pearson correlation coefficients are shown within the corresponding cells.

5. Discussion

5.1 Agronomic and Phenotypic Responses to Amino-Acid Application

In the present study, foliar application of amino acids resulted in a clear positive effect in the agronomic and phenotypic development of *Celosia argentea*. The number of leaves and side branches, plant height and stem diameter were progressive increases with amino-acid treatments relative to the untreated (T1) treatment and the largest values were observed for the highest treatment (T4; 18 mL amino acid). This fact that several vegetative characteristics showed consistent improvement suggests that the application of amino-acid did not have a direct effect on any one morphological characteristic, but rather the general plant growth was stimulated. The same results have been obtained in the case of amino-acid- and protein-hydrolysate-based biostimulants, which can improve plant growth, nutrient uptake, photosynthetic activity and general plant strength. The present experiment has shown an increase in leaf production, which is significant because the leaf development is the determinant of the photosynthetically active surface area for assimilate production. T4 gave 15.75 leaves plant⁻¹ while T1 gave 4.75 leaves plant⁻¹. The large increase indicated that the amino-acid application might have stimulated cell division, cell enlargement, nitrogen assimilation and metabolism. Amino acids are not only building blocks of protein, they also serve as intermediates in the metabolic pathways and as signal molecules. They are closely related to the balance of carbon and nitrogen in their metabolism and the regulation of the developmental process. Glutamate, for instance, plays a key role in nitrogen metabolism, and is a crucial metabolic intermediate between the assimilation of ammonium and the production of other amino acids.

The continued growth in plant height and stem diameter also reinforces the idea that amino-acid application was beneficial for vegetative growth. Similar increases in plant height, shoot biomass, root system and nutrient uptake have been reported after application of amino-acid based

fertilisers and protein hydrolysates to various horticultural crops. Souri et al. (2017), for instance, have reported that amino-chelate application has resulted in better growth, lateral shoot formation, chlorophyll status, dry weight of shoots, fruit number, yield and leaf N.

This response may also be linked to better nitrogen assimilation. Protein hydrolysates and amino acid-based products can also be used to stimulate endogenous nitrogen metabolism and supply readily available organic nitrogen. Ertani et al. (2009) showed that protein hydrolysates promoted greater activity of both nitrate reductase and glutamine synthetase, which implied that inorganic nitrogen compounds were converted to organic nitrogen compounds. Likewise, Colla et al. (2014) found that nitrogen uptake and growth of tomato increased with plant-derived protein hydrolysate.

Amino acids could also have developmental effects as hormones. Protein hydrolysates have been correlated with the auxin and gibberellin-like activity and certain amino acids can be involved in the metabolic pathways associated with hormonal regulation. The more extended growth of branching seen in *C. argentea* may be due to a combination of better nitrogen metabolism, greater carbon assimilation, cell development and hormone related signalling and not just a fertilizer effect.

The dose-dependent effect reported in the present work is also in line with the general findings that the efficacy of amino-acid bio-stimulants is dependent on dose, composition, crop species, physiological stage and application mode. Recent reviews have highlighted that amino-acid products should not be considered as universally interchangeable as different amino acids and protein hydrolysates may elicit different physiological effects.

The optimum concentration for this experiment was 18mL as this produced the highest response rate for T4. This is not to be taken as an indication that increases in concentration would result in further increases in growth. The reactions to biostimulants often have a concentration-dependent response and excessive application may

result in decreased reactions or even detrimental effects. More experiments to assess the optimum economic and physiological dose for *C. argentea* would be beneficial for concentrations lower and higher than those tested here.

5.2 Flowering and Floral Development

The effects of amino acids on reproductive performance and floral traits of *C. argentea* were also found to be significant. Amino-acid-treated plants flowered earlier than the untreated plants that needed the longest to start flowering. The days to flowers initiation in T1 were 17.00 days, while in T4 were 7.00 days. The tendency toward earlier flowering as the amino-acid concentration increased was evident with the numbers, but the LSD grouping revealed that T2, T3 and T4 were not significantly different. Therefore, the main treatment effect was the flowering advancement of the treatments compared to the control and not the differences among the three amino-acid concentrations.

It may be linked to a nutritional and/or physiological improvement due to the application of amino-acids, which may led to a shift to flowering earlier than usual. Amino acids are involved in nitrogen metabolism, protein synthesis, enzyme function and in signalling pathways that regulate vegetative and reproductive growth. Functional amino acids also have the ability to affect specific developmental processes, as well as to be involved in metabolic and signalling pathways regulating plant growth.

The present results are in accordance with the data obtained in other crops where amino-acid based biostimulants have been shown to have a stimulating effect on reproductive development. For instance, Salmani et al. (2023) demonstrated that foliar application of amino acids enhanced the number of flowers produced in sweet pepper grown in a greenhouse, especially in water limited conditions. Recently Khan et al. (2026) showed that foliar application of amino acids based biostimulant at flower initiation improved plant growth and reproductive efficiency of chickpea.

The application of amino-acids resulted in a greater effect for flower size. Flower diameter was

observed to grow with increasing treatment i.e. T1 (3.27 cm) to T4 (8.45 cm), showing a significant improvement in the floral development. A similar reaction has been reported for ornamental plants. In chrysanthemum, foliar application of plant protein hydrolysates resulted in an increase in the length of the stems and particularly the size of the apex in some cultivars. Similarly, Barsoom et al. (2024) found that application of amino acids to *Hymenocallis speciosa* resulted in better vegetative and reproductive characteristics such as flower diameter and flower weight.

The longer growth of the flower stalks in the present experiments is another indication that amino acids were beneficial in reproductive growth. The length of the flower stalks increased from 1.32 cm in T1 to 5.90 cm in T4. This response is linked with higher availability of assimilates and better cell expansion in the reproductive development. Protein hydrolysates can activate photosynthetic activity, nitrogen assimilation process, and carbon metabolism which lead to more assimilates to form and develop reproductive organs.

The biomass of the flowers was also highly influenced. The weight of the fresh flowers was increased from 4.92 g in T1 to 15.92 g in T4. This is in line with the flowers being larger and vegetative parts being better developed in the same treatment. The same increase in flower biomass is reported in ornamental crops treated with amino-acid- and protein-hydrolysate based biostimulants. When an animal-derived protein hydrolysate was applied to the foliage of petunia, flower production, above-ground biomass, leaf biomass and ornamental quality were all improved.

Another significant discovery was the increased life of the flowers. Flowers of T4 lasted 5.92 days compared to only 1.03 days in the control treatment. This parameter has been described in the section heading as dry flower weight, but the accompanying measurements are obviously not dry flower weight, rather they are days to wilting/flower longevity and the discussion should be made with this terminology as well. The biologic longevity may be related to better nutrition, cell integrity, water relations and

antioxidant protection. The antioxidant capacity has been noted to increase due to protein hydrolysates and the ability of amino acids like proline to regulate osmosis and structural protection.

Interestingly, the literature also shows that the effects of protein hydrolysates on flower longevity may vary depending on the product origin and composition. A plant-based source of protein hydrolysate enhanced the ornamental quality and inhibited flower-stem senescence in some chrysanthemum treatments while an animal-based source of protein hydrolysate had a different effect (Carillo et al. 2022). This indicates that the positive effect of the *C. argentea* should be evaluated only under the present experimental conditions involving the amino-acid formulation. The higher number of flowers per plant from 1.00 flower plant⁻¹ in T1 to 2.75 in T4 further indicates the improved reproductive productivity. The reproductive signalling, nutrient assimilation and photosynthesis may also contribute to increased flower production, as well as increased vegetative source capacity. In pepper, application of amino-acid foliar spray resulted in more flowers, indicating that amino acids can affect reproduction outside of their nutritional effect.

5.3 Interrelationships among Vegetative Growth and Flowering Traits

The correlation analysis further confirms the tight coupling of vegetative growth and floristic response in *C. argentea*. There were mostly high positive correlations between most of the measured traits. The diameter of the flowers was highly correlated with the fresh flower weight, number of leaves, plant height and length of flower stalk. Likewise the weight of fresh flowers was significantly related to flower stalk length ($r = 0.9920$), leaf number ($r = 0.9931$), plant height ($r = 0.9842$) and side branches ($r = 0.9777$).

The relationships indicated that larger vegetative plants tended to have larger floral plants. A larger leaf canopy may offer more photosynthetic surface and more branching may result in more reproductive opportunity sites. The indirectly increased flower size and biomass may have been

due to enhanced vegetative growth caused by the amino acids. This is in line with the overall physiological perspective of how carbon assimilation and nitrogen metabolism both regulate biomass production and reproductive allocation.

The very strong correlation between flower diameter and flower weight is biologically intuitive, as larger flowers are likely to have higher levels of structural biomass or reproductive biomass. Similarly, the strong correlation between the plant height, stem diameter, leaf number, and flower characteristics suggest that better plant architecture was linked to better performance of the flowers. Increase in biomass, leaf growth, chlorophyll status, nutrient uptake and reproductive characteristics were observed in the same direction in horticultural crops treated with amino-acid based biostimulant, as reported.

Correlation should not be taken as a proof of causality, however. The high correlations presented here are statistical association of traits and do not prove that a higher number of leaves led to a greater flower diameter or a greater plant height led to a greater number of flowers produced. Instead, these characteristics could be linked to a shared physiological mechanism, such as increased nutrient assimilation, photosynthesis, allocation of carbon or hormonal signaling.

One of the highlights of the correlation matrix is the positive correlation of days to flower initiation with majority of the growth and flowering parameters. This is a somewhat different treatment level pattern since the application of amino-acids led to a decrease in the number of days to flowering. This apparent discrepancy is to be interpreted with care since correlation is a measure of covariation of the individual observations while treatment means are a measure of responses to the experimental treatments. Hence, no causal inference can be made between days to flowering and increased flower yield or flower production as a result of delayed flowering. In general, the correlation results are in accordance with the idea of an integrated growth-flowering response in *C. argentea*. Application of amino-acids seemed to increase the vegetative basis

of the plants, which was followed by better flower formation and higher flower mass. This type of integrated response has also been observed to amino-acid and protein-hydrolysate treatments in horticultural species.

5.4 Overall Implications for Amino-Acid Application

The results collectively show that the application of amino-acids can be a promising biostimulant approach to enhance the vegetative and reproductive response of *C. argentea*. The response was not limited to one parameter, but rather there was an improvement in leaf production, branching, plant height, stem diameter, flowering time, the number of flowers, flower diameter, length of flower stalk, flower weight and flower longevity after treatment with amino-acids. This led to the conclusion that the highest concentration tested gave T4 the best response under the experimental conditions as it was consistently superior over most of the measured traits.

The magnitude and uniformity of the response is significant because ornamental production is a function of biomass accumulation as well as the ability to flower, flower size, appearance, number of flowers, and post harvest performance. Increased research with protein hydrolysate and amino-acids has shown that they can have a multi-faceted effect on physiological processes such as nutrient uptake, nitrogen metabolism, photosynthesis, hormone signaling, antioxidant defense and stress response.

The results are also applicable to sustainable floriculture. The use of amino-acid-based biostimulants can be used in conjunction with conventional fertilization to benefit from the nutrient-use process instead of just providing nutrients. Navarro-León et al. (2022) reported that the biomass production, photosynthetic activity, nitrogen accumulation, and nitrogen-use efficiency of lettuce were improved by the use of L- α -amino-acid based biostimulants. The same evidence has been found in other crops, including vegetables and ornamentals, and may justify the

use of these amino-acid products as part of an overall more effective crop-management system.

The present experiment, however, should not be construed to be the optimum concentration for *C. argentea* for all cases. Amino-acid response may be affected by such factors as formulation, amino-acid composition, source material, concentration, crop genotype, application timing, environmental factors, and method of application. Thus, the 18 mL treatment should be labeled as the "best" treatment of those investigated, and not as the "optimum" treatment.

Further investigations involving measurement of chlorophyll content, photosynthesis, leaf nitrogen, nutrient uptake, antioxidant activity, endogenous hormones and amino-acid profiles should be explored to determine physiological basis for the response. A dose response experiment running at rates higher and lower than 18 mL will also aid in determining the economically and physiologically optimum application rate. This would shift the interpretation from a predominantly phenotypic to a mechanistic interpretation of the effect of amino acids on growth and flowering.

6. Conclusion

The present study showed that the growth, flowering and floral quality of *Celosia argentea* was significantly enhanced through foliar application of amino acids. Amino-acid treatments resulted in an increase in leaf production, side branching, plant height and stem diameter, as well as earlier initiation of flowers, number of flowers produced, flower diameter, flower stalk length, weight of flower, and longer flower life. Under the experimental conditions, the treatment T4 (18 mL amino acid) was the best in terms of overall performance in the different treatments tested.

The correlation between vegetative and floral traits and their positive relationship are also important and show that better plant architecture was linked with better reproductive performance. The results corroborate the possible use of AA-based biostimulants as complementary management measures to enhance ornamental plant growth and flower quality. The answer however, will probably vary as a function of formulation and

dose, and the results should be confirmed in other environmental conditions and with other doses. Based on the overall results, it is concluded that the application of amino-acids is a promising method for the improvement of the productivity and ornamental value of *C. argentea*, especially when the aim is to produce vigorous plants, earlier flowering, larger flower size, higher floral biomass and a longer flower life span.

Author Contributions:

Afsa Bibi conducted field sampling, data collection, and laboratory analyses; Nimra assisted with data collection, laboratory experimentation, and literature review; Shahmir Ali Kalhor & **Muhammad Ramzan** performed statistical analysis, organized and interpreted the data, prepared the figures, and contributed to manuscript preparation; Aadil Hameed contributed to the conceptualization and design of the study and provided supervision; Tasneem contributed to data interpretation, literature review, and manuscript revision; **Ghulam Khaliq** contributed to the research methodology, data analysis, and critical review of the manuscript; and Muhammad Abuzar Jaffar supervised the overall research, contributed to the interpretation of results, and critically revised the manuscript. All authors reviewed and approved the final manuscript.

Data and material availability

This article contains all the soil and plant analysis data generated during the course of this research study. The analysis and the resulting data presented herein may be obtained at the reasonable request of the corresponding author.

Conflicts of interest

Authors declare no potential conflicts of interests

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