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Phytohormones Promote the Growth, Pigment Biosynthesis and Productivity of Green Gram [*Vigna radiata* (L.) R. Wilczek]

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Abstract: Globally, optimized doses of exogenously applied growth regulators hold the potential to sustainably boost the growth and productivity of leguminous crops, including green gram. A field investigation was undertaken at the Agronomy Farm of the University of Agriculture Faisalabad, Pakistan in 2021–2022 to determine the highest-performing doses of foliar-applied salicylic acid (S_1 = 0 and S_2 = 75 ppm) and gibberellic acid (G_1 = 0, G_2 = 30, G_3 = 60, G_4 = 90 and G_5 = 120 ppm) for green gram (cv. NIAB-MUNG 2011) sown under irrigated conditions in a semiarid climate. The response variables included physiological growth traits (CGR and net assimilation rate (NAR)), yield attributes (plant height (PH), PBs and the number of pods per plant^{-1} (NP), pod length (PL) and SW, grain (GY) and biological yields (BY), the biosynthesis of pigments (chlorophyll a, chlorophyll b and total chlorophyll along with carotenoids) and protein (P) contents. The results revealed that S_2G_5 remained unmatched in that it exhibited the highest crop growth rate, while it remained on par with S_2G_4 and S_2G_3 in terms of its net assimilation rate. Additionally, S_2G_5 maximized plant height, the number of pod-bearing branches and pods per plant, pod length, seed number per pod^{-1} and 1000-seed weight, which led to the highest grain yield and biological yield (104% and 69% greater than those of the control, respectively). Moreover, the same treatment combination also surpassed the rest of the treatments because it recorded the largest amounts of chlorophyll and carotenoid contents, and the P content was increased to 24% greater than that observed for the control treatment. Thus, the exogenous application of salicylic acid (75 ppm) and gibberellic acid (120 ppm) might be recommended to green gram growers to sustainably increase the plant's yield and nutritional value, and these findings may serve as a baseline for conducting more studies to test higher doses of these growth regulators.

Keywords: biological nitrogen fixation; crop growth rate; growth regulators; green gram; grain protein

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1. Introduction

Globally, the attainment of sustainable development goals (SDGs), especially zero hunger and poverty alleviation, is directly linked with the provision of nutritional food at affordable prices. Green gram [*Vigna radiata* (L.) R. Wilczek], also known as mung bean, constitutes a vital source of dietary protein and thus strategically contributes to ensuring the nutritional security of the rapidly increasing population in South Asia [1–3]. Besides being a vital source of food and feed, it plays a crucial role in restoring soil fertility through the fixation of atmospheric nitrogen (N), along with improving the physicochemical properties (bulk density, soil porosity, organic matter percentage, macronutrient availability, etc.) of the soil [4]. Additionally, it contains a remarkably high content of easily digestible protein and many essential amino acids [5]. Among the South Asian countries, Pakistan is one the chief producers of green gram, but its average production has remained stagnant in the past decade, which has reduced farmers' income [6–9]. Suboptimal plant nutritional management has been reported to be the leading cause of lower yields [7,10]. Therefore, increasing the yield of green gram per unit of land area through effective plant nutrition management has been identified as imperative to assuring self-sufficiency and reducing import bills [2,6,11].

Among plant nutrition management strategies, the application of growth regulators such as salicylic acid (SA) has the potential to trigger the growth of crop plants, leading to higher grain yield [12–17]. SA is a natural plant hormone which belongs to the group of plant phenolics that possess an aromatic ring bearing a hydroxyl group [13,14,18–20]. Along with promoting growth, it also serves as a signal molecule that increases plant tolerance to various biotic and abiotic stresses [12,21–24]. Exogenously applied SA regulates plant growth by optimizing glycolysis, ion uptake, stomatal conductance, photosynthesis and transpiration and biosynthesizing numerous enzymatic and nonenzymatic compounds [25–28]. It has been inferred previously that the effectiveness of foliar-applied SA remained dependent on its dose and plant species. Exogenously applied SA in a concentration of 1 mM was more effective in increasing plant growth in comparison with higher concentrations, while the underlying mechanism of the growth-restricting impact of higher doses of SA still awaits further research [14,16]. Moreover, foliar-applied SA resulted in significant increments in Ca^+ and K^+ content within plants, which improved cell walls permeability for nutrients present in the cell sap [29–32]. Furthermore, it boosted plant growth and development by reducing the biosynthesis of ethylene and preventing micronutrient toxicity, and through lipid peroxidation, which protected the crop plants from membrane damage [33–36]. SA's roles in regulating the physiological and biochemical activities of plants, such as the germination of seeds, nodulation in legumes, the regulation of stomatal conductance, respiration, delaying fruit ripening, preventing leaf senescence and enhancing fruit yield, have been previously reported [16,23,36]. Efficient cell divisions and the rapid conduction of photoassimilates due to SA application favored the enhancement of the mean crop growth rate and net assimilation rate [37,38]. Analogous findings were also reported by Nawalagatti et al. [39] on the growth of French beans as well. Likewise, the growth-promoting effects of SA stem from its role in the activation of osmotic regulation and antioxidant systems which prevent structural cell damage, the disruption of chlorophyll, lipids and proteins, and the disordering of metabolic activity by maintaining ionic homeostasis under normal and stressful environments. However, there are currently no validated studies available pertaining to the impact of exogenously applied SA on green gram under semiarid conditions.

Along with SA, gibberellic acid (GA) is a naturally produced growth regulator which has a critical role in promoting cell divisions and ultimately results in higher plant growth and yield [40–43]. Additionally, GA triggers cell elongation and increases in leaf area, flowering and other morphological traits of the crop plants [44]. GA is isolated from the fungus Gibberella fujikuroi and it fosters vegetative growth through the elongation of internodes, increases the production of pods, flowers and leaves, promotes early flowering and pod development, breaks seed dormancy, hastens maturity and also controls fruit cracking in horticultural crops [45–47]. Exogenously applied GA has enhanced mung bean growth by mitigating the deleterious impacts of salt toxicity through improvements in the ionic balance [42,46,48]. Likewise, GA application stimulates cell division, which has led to stem elongation and accelerated fruit development in legume crops [44,49–52]. Moreover, GA improved the yield attributes of crops under both normal and unfavorable growth conditions [53] by increasing grapevine fruit production along with improving the productivity and quality of ornamental plants [54,55]. Interestingly, it also proved its effectiveness by increasing cotton lint productivity [56], field pea protein content and the chemical composition of crotons, and suppressed the biosynthesis of numerous undesirable chemical compounds in pulse grains [57]. GA application promoted the reproductive stage of crop plants, especially the flowering process (morphogenesis), along with triggering generative developments such as pod setting, anther dehiscence, pollination and fertilization, which led to greater numbers of pods per plants and seeds per pods and a greater pod area [45]. Likewise, GA foliar application effectively promoted vegetative growth traits and seed development in black gram and horse gram [58], the soybean, the common bean, the cowpea and the pigeon pea [59] by improving the supply of assimilates to pods, which resulted in a greater number of seeds that had significantly higher seed weights. GA application tended to increase the pigment content in *Vicia faba* [60], along with boosting the water use efficiency of winter wheat [61], which led to higher yield attributes. However, it has been inferred that it was actually the promotion of the photosynthetic rate through the improved activity of carboxylase in broad beans and soybeans that increased the number of pods and the seed weight [62]. GA induced significant increments in water uptake, causing cell expansion due to more dilution of the sugars, which resulted in the production of taller plants and a greater number of branches per plant [63]. It was also suggested that GA promoted starch hydrolysis, which led to sugar accumulation that improved the water balance, and ultimately, growth was triggered. Moreover, exogenously applied GA decreased transpiration and promoted reproductive growth in plants [64].

Although numerous research findings have portrayed the effectiveness of GA and SA in terms of mitigating the deleterious effects of abiotic stress in crop plants, a lack of sufficient research, as well as contrasting findings pertaining to the dose optimization of these phytohormones for green gram grown under normal irrigated conditions in a semiarid climate, necessitated conducting fresh studies. Therefore, the research hypothesis of this field study was that green gram might respond differently in terms of morphological traits, pigment biosynthesis and yield to varying doses of salicylic acid and gibberellic acid. Thus, the prime aim of this research was to evaluate the effects of exogenously applied salicylic acid and gibberellic acid on green gram in order to determine the highest-performing dose of these exogenously applied phytohormones.

2. Materials and Methods

2.1. Experimental Site's Meteorological Features and Physicochemical Description

A field experiment was performed for two consecutive growth seasons in 2021 and 2022 at the Agronomy research area of University of Agriculture Faisalabad, Pakistan. The experimental site has an altitude of 186 m, and its geographical coordinates are 31.4504° N, 73.1350° E. The study locality has plain topography, as it is situated in central Punjab region and has sufficient canal water for crop production. The meteorological characteristics (temperature and precipitation) of the experimental location during the crop-growing months (mean values of both years) are illustrated in Figure 1. The study location allows for irrigated farming systems that ensure economical production of a variety of crops including wheat, cotton, maize and different vegetables.

Prior to the sowing of green gram, soil samples from the experimental block were taken from depths of 0–15 and 15–30 cm using soil auger. The soil samples were collected from the middle of experimental block and four corners and thereafter mixed thoroughly and stored in zip-lockable polythene bags for further analysis.

For pH estimation, soil mixing with water in a ratio of 1:2.5 was performed in order to prepare the paste, which was thereafter subjected to analysis with a glass electrode [65]. The estimation of electrical conductivity (EC) was performed with the help of a conductivity meter [29–33]. In addition, organic carbon (OC) content assessment was performed by putting the wet oxidation method into practice, and the Walkley–Black protocol was used to determine the organic matter (OM) content [66]. Moreover, total nitrogen (N) was evaluated using Kjeldahl apparatus to perform distillation with H_2SO_4 (concentrated acid) titration [67]. Likewise, phosphorous (P) of soil samples was analyzed by following Olsen's method, which encompasses 0.5 N $NaHNO_3$ reaction with a soil: extractant paste prepared in a 1:10 ratio at 8.5 pH, and subsequently, spectrophotometer (882 nm) was used in a system containing H_2SO_4 [68]. Moreover, potassium (K) content was calculated by performing ammonium acetate extraction, which involved shaking of soil samples in the solution of ammonium acetate for 30 min, which led to displacement of K^+ ions, and flame photometer was used for the detection of displaced K^+ ions.

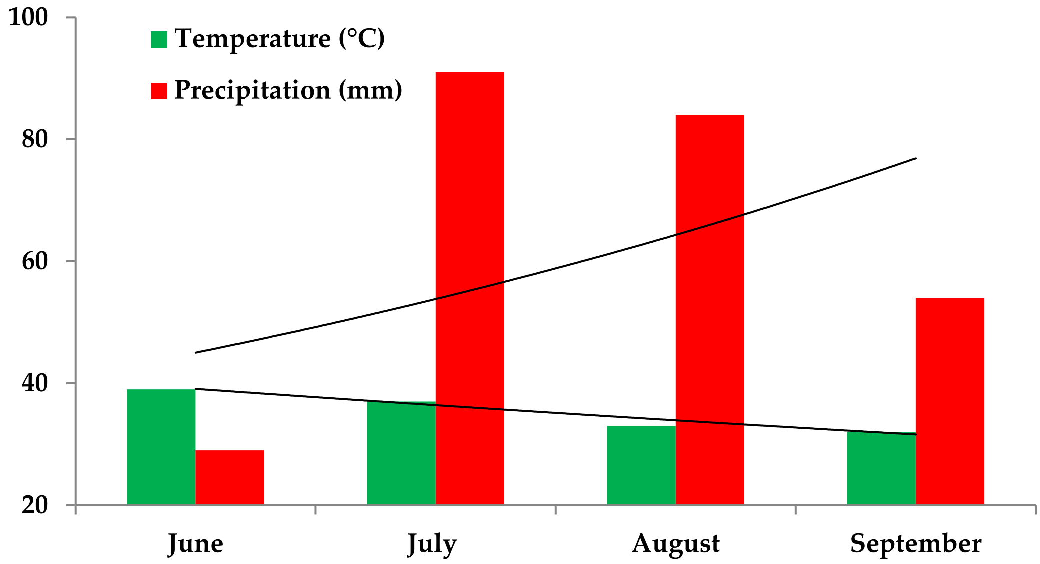

Figure 1. The study area's (Faisalabad, Punjab province, Pakistan) meteorological characteristics (having minimum to maximum values of temperature and precipitation in the ranges of 32–39 °C and 29–91 mm, respectively) during the course of field trial (black lines are exponential trend lines).

For recording micronutrient contents of soil samples, the extraction method involving ammonium acetate solution (CH_3COONH_4) was subject to reaction with soil paste while maintaining a pH of 3.0 for estimation of iron (Fe) content. Thereafter, colorimetric method using spectrophotometer (510 nm wavelength) was performed for precise estimation of Fe content. Moreover, other micronutrient contents, such as boron (B), zinc (Zn), copper (Cu) and manganese (Mn) contents, were also determined by following an extraction method entailing the use of diethylenetriaminepentaacetic acid [65–72]. The experimental soil's texture was sandy loam, while its pH and OM were 7.9 and 0.63%, respectively, indicating the need to give due consideration to optimizing the fertilizer dose to achieve the potential yield of green gram. Likewise, the bulk density and EC of the experimental soil were 1.15 cm^{-3} and 0.42 dS m^{-1}, respectively (indicating the soil was not salt-affected). Interestingly, the NPK concentrations were 89, 5.6 and 179 mg kg^{-1}, respectively. However, all micronutrients were present in appropriate concentrations, including B (1.03 mg kg^{-1}), Mn (14.4 mg kg^{-1}), Fe (13.6 mg kg^{-1}), Cu (1.59 mg kg^{-1}) and Zn (1.17 mg kg^{-1}).

2.2. Details of Treatments and Experiment's Execution

The planting material for this trial was green gram cultivar NIAB-MUNG 2011 (a short-duration cultivar suitable for cultivation as a catch crop in rice–wheat cropping system, having large seed size and being quite fit for mechanical harvesting) that was procured from the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. In this two-year field trial, two factors were studied, including exogenously applied doses of salicylic acid (S_1 = 0 and S_2 = 75 ppm) and gibberellic acid (G_1 = 0, G_2 = 30, G_3 = 60, G_4 = 90 and G_5 = 120 ppm). The foliar sprays of SA and GA were applied as per treatment after 35 days of sowing using manual backpack sprayer with flat fan nozzle. The experiment was executed as per randomized complete block design (RCBD) with factorial arrangement and was replicated thrice. The experimental plots receiving both SA and GA foliar applications had intervals of three days between foliar sprays. The net plot size was 3.6 m × 10 m after excluding walking paths, water channels, field bunds, etc. There were twelve rows in the crop in each experimental unit, and manual thinning was performed after two weeks of sowing in order to maintain plant-to-plant spacing.

In order to prepare a fine seedbed, ploughing was performed using a tractor-driven common cultivator thrice, and each ploughing followed planking (with wooden plank) to break the clods and pulverize the soil. The crop was sowed using a seed rate of 35 kg ha^{-1} and sowing was performed using a single-row hand drill, maintaining R × R and P × P spacings of 30 cm and 10 cm, respectively. The sowing was performed on 4 June and 9 June during 2021 and 2022, respectively, while harvesting was performed on 28 September and 30 September, respectively. Plant protection measures were practiced to avoid weed and pathogen attacks.

Areas of 1.5 m and 4 m were kept among the experimental units and replications, respectively. Regarding plant nutrition management in the experimental units, well-composted poultry manure (5 tons ha^{-1}) was applied in conjunction with mineral fertilizers (urea and DAP at rates of 50 and 60 kg ha^{-1}, respectively). Prior to sowing, green gram seeds were subjected to hydropriming involving seed soaking in sterilized water for 12 h to promote seed germination. Thereafter, shade drying of hydroprimed seeds on muslin cloth sheets was performed, and they were subsequently stored at 10 °C.

2.3. Response Variable Recordings

The response variables were recorded through random selection of ten plants from the central rows of every experimental plot, and their means were computed so that they could be used in further analyses. The plant height of green gram plants was measured from the base of plant to the tip of the uppermost leaf using tailor's measuring tape. The grain and biological yields of green gram were determined by harvesting all plants in experimental units; the plants were bundled and weighed separately using a spring balance. Thereafter, the recorded yields were converted into a hectare basis with the help of Equation (1). The crop growth rate (CGR) and net assimilation rate (NAR) of green gram were calculated using Equations (2) and (3), respectively. The harvest index (HI) was estimated using Equation (4). The chlorophyll a, b and total amounts were estimated with the help of Equations (5)–(7), respectively. Finally, carotenoid content was determined using Equation (8).

$$\text{Yield of green gram} = \frac{\text{Yield per plot} \times 10{,}000\ \text{m}^2}{\text{Plot area}\ (\text{m}^2)} \quad (1)$$

Mean CGR and mean NAR were computed using the following formula:

$$\text{CGR} = (W_2 - W_1)/(t_2 - t_1) \quad (2)$$

$$\text{NAR} = \text{TDM}/\text{LAD} \quad (3)$$

Agronomic data on plant height, number of pod-bearing branches and pods per plant, pod length, number of seeds per pod, 1000-seed weight, seed yield and biological yield were recorded after harvesting. Harvest index was recorded using the following formula:

$$\text{H.I} = (\text{Seed yield})/(\text{Biological yield}) \times 100 \quad (4)$$

The amounts of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were evaluated. For this purpose, 100 mg leaf tissue was suspended in 80% acetone solution at 4 °C overnight. The amount of chlorophyll was estimated using formulas given by [73,74] (1979), respectively, as follows:

$$\text{Chl. a}\ (\text{mg g}^{-1}) = [12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645)] \times \text{V}/1000 \times \text{W} \quad (5)$$

$$\text{Chl. b (mg g}^{-1}) = [22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663)] \times \text{V}/1000 \times \text{W} \quad (6)$$

$$\text{Total Chl. (mg g}^{-1}) = [20.2\ (\text{OD } 645) + 8.02\ (\text{OD } 663)] \times \text{V}/1000 \times \text{W} \quad (7)$$

$$\text{Carotenoid (mg g}^{-1}) = [(\text{OD } 480) + 0.114\ (\text{OD } 663) - 0.638\ (\text{OD } 645)] \times \text{V}/1000 \times \text{W} \quad (8)$$

2.4. Statistical Analyses

The data pertaining to all response variables under investigation were recorded, thoroughly arranged and subjected to statistical analyses using Bartlett's test, which revealed the effects of the year to be nonsignificant, and thus, data were transformed into mean values for determining statistical significance among treatments. Thereafter, Fisher's analysis of variance (ANOVA) was employed to determine overall significance, and comparison among treatment means was performed by subjecting data to Tukey's honest significant difference (HSD) test at 5% probability level using the SAS statistical package (9.2 Version, SAS Institute, Cary, NC, USA) [75].

3. Results

3.1. Crop Growth and Net Assimilation Rates

The results showed that the individual and interactive effects of SA and GA applied exogenously in varying doses were statistically significant in terms of the physiological growth traits of green gram (Table 1). The SA application (S_2 = 75 ppm) remained effective in boosting the mean crop growth rate (CGR) of green gram by 28.8% compared with the control treatment. Likewise, the GA foliar spray in a dose of 120 ppm (G_5) outperformed the rest of the doses by exhibiting a 40% higher CGR than the control treatment. Regarding the interaction effect of foliar-applied SA and GA, the maximum CGR (0.63 gm^{-2} d^{-1}) was obtained with the level of S_2G_5, which was 0.29 gm^{-2} d^{-1} higher than that of the control treatment. It was followed by S_2G_4, which recorded a 73% higher CGR in comparison with the control treatment. Regarding SA's impact on the net assimilation rate (NAR), S_2 remained superior, giving a 13 gm^{-2} d^{-1} higher NAR compared with the control treatment, while the G_5 and G_4 treatments remained statistically on par with each other in terms of the NAR of green gram. However, in contrast to CGR, statistically nonsignificant values of NAR were recorded for S_2G_5, S_2G_4 and S_2G_3. These treatments were followed by S_2G_2, which exhibited a 23% higher NAR than the control treatment. Overall, the S_2G_5 treatment remained unmatched, giving 85% and 25% higher CGR and NAR, respectively, compared with the control treatment.

Table 1. Impact of varying doses of foliar-applied salicylic acid and gibberellic acid on crop growth rate and net assimilation rate of green gram grown under irrigated conditions in semiarid climate.

Treatments	Crop Growth Rate (gm^{-2} d^{-1})	Net Assimilation Rate (gm^{-2} d^{-1})
	Salicylic acid levels	
S_1 (0 ppm)	0.45 b	113.83 b
S_2 (75 ppm)	0.58 a	126.98 a
CV (%)	8.247	11.378
	Gibberellic acid levels (G)	
G_1 (0 ppm)	0.42 e	111.50 e
G_2 (30 ppm)	0.48 d	117.47 d
G_3 (60 ppm)	0.53 c	119.08 c
G_4 (90 ppm)	0.57 b	126.75 a
G_5 (120 ppm)	0.59 a	127.23 a
CV (%)	6.574	17.235

Table 1. *Cont.*

Treatments	Crop Growth Rate (gm^{-2} d^{-1})	Net Assimilation Rate (gm^{-2} d^{-1})
	S × G interaction effects	
S_1G_1	0.34 j	103.93 f
S_1G_2	0.39 i	108.70 e
S_1G_3	0.47 h	107.90 e
S_1G_4	0.55 f	124.00 c
S_1G_5	0.55 e	124.00 c
S_2G_1	0.51 g	119.07 d
S_2G_2	0.57 d	127.05 b
S_2G_3	0.59 c	129.16 a
S_2G_4	0.61 b	129.60 a
S_2G_5	0.63 a	130.02 a
CV (%)	10.287	14.973

Values with atypical letters within same column indicate significant variation at $p = 0.05$.

3.2. Yield Attributes

The research results showed that foliar-applied SA and GA in varying doses remained effective in boosting the yield attributes of green gram grown under semiarid conditions (Table 2). In terms of plant height (PH) and the number of pod-bearing branches (PBs), the SA application (S_2 = 75 ppm) remained effective, producing plants that were 17% taller and PBs that were 50% higher compared with the control treatment. In addition, the GA foliar spray (120 ppm) outperformed the rest of the doses by exhibiting 23% and 80% higher PH and PB values, respectively, compared with the control, which received no exogenous application of GA. Regarding the interaction effects, S_2G_5 produced the tallest plants (8% and 7% greater than S_2 and G_5, respectively) and the highest number of PBs; however, S_2G_5 and S_2G_4 performed statistically on par with each other in terms of the number of PBs for green gram.

Regarding the number of pods per plant (NP), the pod length (PL) and the number of seeds per pod (SP) of green gram, the individual and interactive effects of exogenously applied SA and GA remained remarkably significant with respect to the control treatment (the experimental units that received no foliar spray) (Table 2). The S_2 and G_5 treatments resulted in unmatched findings in terms of NP, PL and SP, and their interactive effect (S_2G_5) also remained superior to the rest of the treatment combinations, exhibiting 52%, 57% and 50% greater NP, PL and SP values, respectively, compared with S_1G_1. This treatment combination was followed by S_2G_4, which in turn was followed by S_2G_3. Overall, all treatment combinations performed better than the experimental units where no foliar application was performed.

The foliar-applied SA and GA also boosted the 1000-seed weight; the S_2 and G_5 treatments were the highest-performing treatments, recording 9% and 14% greater 1000-seed weights for green gram compared with S_1 and G_1, respectively (Table 2).

Table 2. Impact of varying doses of foliar-applied salicylic acid and gibberellic acid on plant height, number of pod-bearing branches and pods per plant along with pod length and seed number per pod of green gram grown under irrigated conditions in semiarid climate.

Treatments	Plant Height (cm)	Number of Pod-Bearing Branches	Number of Pods per Plant-1	Pod Length (cm)	Number of Seeds per Pod
Salicylic acid levels					
S_1 (0 ppm)	74.05 b	6.45 b	27.10 b	8.62 b	8.23 b
S_2 (75 ppm)	87.18 a	9.38 a	32.15 a	10.21 a	10.98 a
CV (%)	12.548	10.981	8.417	6.954	11.684
Gibberellic acid levels (G)					
G_1 (0 ppm)	71.02 e	5.64 e	25.47 e	8.29 e	7.81 e
G_2 (30 ppm)	76.99 d	7.30 d	28.62 d	8.96 d	8.90 d
G_3 (60 ppm)	80.43 c	7.95 c	29.82 c	9.52 c	9.52 c
G_4 (90 ppm)	86.32 b	9.14 b	31.71 b	9.96 b	10.65 b
G_5 (120 ppm)	88.32 a	9.55 a	32.52 a	10.35 a	11.16 a
CV (%)	11.249	16.584	9.640	12.729	7.624
S × G interaction effects					
S_1G_1	62.67 i	3.37 h	23.42 i	7.67 h	6.47 g
S_1G_2	70.43 h	5.53 g	26.67 h	8.21 g	7.33 f
S_1G_3	73.42 g	6.57 f	27.17 gh	8.52 f	7.82 f
S_1G_4	81.23 e	8.12 de	28.86 f	9.23 d	9.57 de
S_1G_5	82.51 d	8.67 cd	29.51 e	9.51 cd	10.00 cd
S_2G_1	79.37 f	7.91 e	27.53 g	8.92 e	9.15 e
S_2G_2	83.54 d	9.08 bc	30.67 d	9.71 c	10.47 c
S_2G_3	87.45 c	9.33 b	32.47 c	10.50 b	11.21 b
S_2G_4	91.42 b	10.17 a	34.56 b	10.70 b	11.74 b
S_2G_5	94.13 a	10.43 a	35.53 a	11.20 a	12.33 a
CV (%)	8.861	11.337	17.249	10.684	15.294

Atypical letters within same column indicate significant variation at $p = 0.05$.

3.3. Grain and Biological Yields and Harvest Index

Likewise, S_2G_5 surpassed the rest of the treatment combinations in terms of demonstrating the highest 1000-seed weight of green gram, which was 2% and 25% higher than the following treatment (S_2G_4) and the control treatment (S_1G_1), respectively. Interestingly, S_2G_4 gave a statistically nonsignificant difference in 1000-seed weight compared with S_2G_3, while S_2G_2 and S_1G_5 remained on par with each other as far as the 1000-seed weight of green gram was concerned. Moreover, it was revealed that the highest-performing treatment combination (S_2G_5) recorded 9% and 14% greater 1000-seed weights compared with the S_2 and G_5 treatments, respectively (Table 3). Like all the yield attributes, the grain (GY) and biological yields (BY) of green gram were significantly influenced by the individual and interactive effects of varying doses of foliar-applied SA and GA (Table 3). Following this trend, the highest seed yields were observed for the S_2 and G_5 treatments; these yields were 40% and 53% greater than the control treatments of S_1 and G_1, respectively. The same trend was also revealed for the BY of green gram sown under semiarid conditions. However, the S_2G_5 treatment combination remained unmatched, recording 104% and 69% greater GY and BY values, respectively, than the control treatment (S_1G_1). This treatment combination was followed by S_2G_4, which in turn was followed by S_2G_3 for BY, while

S_2G_4 and S_2G_3 remained on par with each other for the GY of the green gram. Similarly, as far as the harvest index (HI) of the green gram was concerned, S_2 exhibited a 2% higher HI than S_1, while on the other hand, G_5 recorded a 4% higher HI compared with the control treatment of G_1 (Table 3). Moreover, the highest-performing interaction effect in terms of the HI was noted for S_2G_5, for which the effect was 7% higher than that of S_1G_1. This was followed by S_2G_4 which remained on par with S_2G_3; however, both of these treatment combinations gave significantly higher HI values compared with the experimental plots that received no foliar spray at all.

Table 3. Impact of varying doses of foliar-applied salicylic acid and gibberellic acid on 1000-seed weight, grain and biological yields and harvest index of green gram grown under irrigated conditions in semiarid climate.

Treatments	1000-Seed Weight (g)	Grain Yield (t ha^{-1})	Biological Yield (t ha^{-1})	Harvest Index (%)
Salicylic acid levels				
S_1 (0 ppm)	44.13 b	1.38 b	3.83 b	35.87 b
S_2 (75 ppm)	48.19 a	1.94 a	4.94 a	39.20 a
CV (5%)	6.289	9.105	10.824	12.774
Gibberellic acid levels (G)				
G_1 (0 ppm)	42.78 e	1.28 e	3.60 e	35.41 d
G_2 (30 ppm)	45.07 d	1.53 d	4.12 d	36.83 c
G_3 (60 ppm)	46.72 c	1.68 c	4.39 c	37.77 b
G_4 (90 ppm)	47.56 b	1.85 b	4.82 b	38.35 b
G_5 (120 ppm)	48.67 a	1.97 a	5.00 a	39.32 a
CV (%)	12.665	10.579	14.371	12.690
S × G interaction effects				
S_1G_1	40.44 g	1.10 h	3.19 j	34.51 g
S_1G_2	42.85 f	1.22 g	3.46 i	35.24 fg
S_1G_3	44.59 e	1.28 g	3.58 f	35.97 ef
S_1G_4	45.85 d	1.59 e	4.35 h	36.67 de
S_1G_5	46.92 c	1.70 d	4.60 e	37.12 d
S_2G_1	45.12 e	1.45 f	4.02 g	36.15 de
S_2G_2	47.29 c	1.85 c	4.8 d	38.59 c
S_2G_3	48.85 b	2.08 b	5.22 c	39.86 b
S_2G_4	49.28 b	2.12 b	5.31 b	40.05 b
S_2G_5	50.42 a	2.25 a	5.4 a	41.63 a
CV (%)	12.658	10.420	14.982	11.128

Atypical letters within same column indicate significant variation at $p = 0.05$.

3.4. Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carotenoid and Protein Contents

The individual and interaction effects of exogenously applied SA and GA in varying concentrations remained significant for the chlorophyll a (chl-a), chlorophyll b (chl-b), total chlorophyll (chl-t), carotenoid (Ct) and protein (P) contents of the green gram (Table 4). The results revealed that the S_2 treatment recorded 45%, 34% and 25% higher chl-a, chl-b and chl-t levels, respectively, compared with S_1, and the same treatment also exhibited the highest Ct and P contents for the green gram. In addition, the G_5 treatment surpassed the rest of the GA doses in terms of the chl-a, chl-b and chl-t contents along with giving the highest Ct and P content levels, which were 39% and 14% greater than those of G_1, respectively. Following this trend, the interaction effects pertaining to chl-a, chl-b and chl-t were significantly higher for S_2G_5 compared with S_1G_1. Furthermore, the same treatment combination also recorded 92% and 24% higher Ct and P content levels in comparison with the control treatment. This treatment was followed by S_2G_4, which was followed in turn by S_2G_3 as far as the Ct and P contents of the green gram were concerned. Overall, lower doses of both SA and GA remained significantly less effective in boosting the chlorophyll contents along with the Ct and P concentrations in green gram sown under irrigated conditions.

Table 4. Impact of varying doses of foliar-applied salicylic acid and gibberellic acid on pigment biosynthesis and protein content in green gram grown under irrigated conditions in semiarid climate.

Treatments	Chlorophyll a (mg g^{-1} F. Wt.)	Chlorophyll b (mg g^{-1} F. Wt.)	Total Chlorophyll (mg g^{-1} F. Wt.)	Carotenoids (mg g^{-1} F. Wt.)	Protein (%)
Salicylic acid levels					
S_1 (ppm)	1.81 b	0.86 b	2.17 b	2.04 b	21.61 b
S_2 (ppm)	2.43 a	0.56 a	2.72 a	2.66 a	24.06 a
CV (%)	9.854	12.501	14.879	13.248	10.251
Gibberellic acid levels					
G_1 (ppm)	1.66 e	0.41 e	1.85 e	1.95 e	21.28 e
G_2 (ppm)	1.99 d	0.66 d	2.34 d	2.20 d	22.08 d
G_3 (ppm)	2.14 c	0.76 c	2.60 c	2.28 c	22.87 c
G_4 (ppm)	2.37 b	0.83 b	2.69 b	2.64 b	23.75 b
G_5 (ppm)	2.46 a	0.87 a	2.75 a	2.71 a	24.19 a
CV (%)	8.615	9.348	12.440	12.054	13.570
S × G interaction effects					
S_1G_1	1.11 i	0.09 j	1.14 i	1.51 j	20.06 j
S_1G_2	1.61 h	0.48 i	1.98 h	1.84 i	20.53 i
S_1G_3	1.84 g	0.66 h	2.44 g	1.91 h	21.68 h
S_1G_4	2.23 f	0.76 f	2.61 e	2.45 f	22.79 f
S_1G_5	2.29 e	0.79 e	2.66 d	2.51 e	22.99 e
S_2G_1	2.22 f	0.73 g	2.56 f	2.38 g	22.50 g
S_2G_2	2.37 d	0.83 d	2.69 c	2.56 d	23.64 d
S_2G_3	2.45 c	0.86 c	2.75 b	2.65 c	24.06 c
S_2G_4	2.51 b	0.91 b	2.78 b	2.83 b	24.70 b
S_2G_5	2.63 a	0.95 a	2.84 a	2.92 a	25.39 a
CV (%)	8.641	11.224	10.993	12.504	13.856

Values with atypical letters within same column indicate significant variation at $p = 0.05$.

4. Discussion

The results of this trial are in concurrence with the postulated hypothesis, because green gram responded differently to varying doses of exogenously applied SA and GA in terms of its physiological traits, yield attributes and seed and biological yields, along with its harvest index. In this field study, higher doses of both SA and GA remained superior, in that the green gram treated with these doses exhibited a significantly higher CGR and NAR, which might be attributed to accelerated growth caused by SA and GA. These findings are in concurrence with those of Huang et al. [76], who opined that exogenous application of 2.00–4.00 mg L^{-1} SA triggers vegetative growth in plants through the effective modulation of antioxidant activities. It was suggested that foliar-applied SA maintained an optimized osmotic environment which boosted the photosynthetic rate, and ultimately, the crop growth rate was remarkably increased. Similar results have also been reported previously [77], whereby foliar-applied SA in a 5.0 mM concentration improved 2-phenylethyl-glucosinolate levels in the leaves of cabbage, which led to a significantly higher growth rate and the partitioning of assimilates from source to sink. Contrastingly, it was affirmed that no significant influence of SA on *Vigna mungo* occurred when it was applied as a foliar spray in 10.0 µM [78]. These contrasting findings probably reflect that there could be numerous factors (for instance, experimental materials, crop varieties, SA concentrations, the time of the foliar spray with respect to the crop growth stage, etc.) responsible for triggering the crop growth and net assimilation rates. Additionally, GA also remained effective in increasing the CGR, NAR, leaf area index and plant dry weight in mung bean [79,80]. These results are in line with those of Feng et al. [81], who reported a systemic effect of SA on leaf growth; however, it was suggested that SA's impacts varied per SA concentration and CGR measurement time after the exogenous application of SA. It was also inferred that SA (0.5 mM) improved the total amounts of phenolics by 28% 12 h after the foliar application of SA, and also boosted the amounts of polyphenol oxidase and superoxide dismutase, which assisted crop plants in off-setting the deleterious effects of stresses and boosted the CGR and NAR in crop plants. Contrastingly, it was reported that foliar application of SA in low doses (1.0–2.5 mM) remained more effective in producing strong chemical defense responses which improved the growth rate of maize.

The yield attributes of green gram determine productivity in terms of seed yield and biological biomass production. The higher doses of SA and GA remained unmatched in terms of triggering vegetative growth (plant height, the number of pod-bearing branches, etc.) and reproductive traits (the number of pods and seeds per pod, pod length and 1000-seed weight) in green gram grown under irrigated conditions in a semiarid climate. Taller green gram plants with a greater number of branches per plant and foliar-applied phytohormones might be attributed to accelerated cell division, which improves internodal distance in mung beans, cowpeas, okra, chickpeas and field peas [82–85]. Plants being taller and having a greater number of pod-bearing branches and a greater pod length could be due to the enhanced transverse divisions of cells and the translocation of nutrients by the interactive spray of salicylic acid and gibberellic acid [86]. Gad et al. [87] confirmed that SA and GA were involved in increasing the number of pod-bearing branches in Ixora coccinea. Concerning pod length, the given results are related to the results of Kumar et al. [88] in green gram. GA and SA were very crucial in regulating compounds that helped in the formation of floral buds and developed a good source–sink relationship for the uptake of minerals and nutrients [89]. These results are also supported by a previous study in which foliar application of GA promoted stem elongation by boosting the biosynthesis of auxin, which led to a significant increment in plant height along with a greater number of branches per plant for field peas [90]. Similar to our findings, it was reported that GA (100 ppm) remained superior by producing an 8% higher number of pods per plant in mung bean and okra [82,91]. Moreover, SA application remained effective in boosting the yield attributes of *Brassica napus* by triggering its antioxidant capacity and by lowering oxidative stress. It was also observed that SA improved carbohydrate and proline metabolism, enhanced cell viability in roots and triggered the activity of ribulose 1, 5-bisphosphate carboxylase (Rubisco). Furthermore, it was opined that SA up-regulated the assimilation of sulfur, which triggered the synthesis of cysteine, methionine, heavy metal chelators such as metallothioneins, nonprotein thiols, phytochelatins and various coenzymes and vitamins, and ultimately, reproductive growth in the crop plants was triggered [92].

The findings of this study pertaining to greater pod length being recorded for GA and SA application are consistent with those of [93–95], who suggested that pod enlargement occurred by virtue of growth-regulator-triggered cell division. Similar results are also reported for *Brassica* species [94]. However, in contradiction to our findings, there was no significant impact of exogenously applied GA on pod length or the number of seeds per pod [96,97], and El-Shraiy and Hegazi [98] noted a significant reduction in the reproductive yield attributes of field peas treated with foliar-applied GA. These contrasting results could be attributed to atypical crop–growth-regulator–environmental factor interactions, which resulted in varying impacts of foliar-applied growth regulators [99]. It has been previously reported that significant increments in pod number, grains per pod^{-1} and weed weight caused by phytohormone application and an improvement in yield attributes were further exaggerated with the use of dual spraying in comparison with a single spraying. Likewise, the highest number of seeds per pod for mung bean was recorded after GA (200 ppm) application [100–102], and these results also confirm our findings pertaining to growth regulators' effectiveness in boosting reproductive yield attributes. The greater number of seeds per pod and the higher 1000-seed weight were probably caused by the stimulation of thermogenesis under the influence of applied chemicals which accelerated the regulation of photoassimilates, embryogenesis and nucleic acids, which in turn promoted the creation of flowers, seeds and proteins [103]; Basuchaudhuri [104] mentioned parallel results for soybean plants.

These findings also corroborate earlier reported results in which foliar-applied growth regulators significantly promoted seed setting, seed number and seed weight, which led to a significantly higher seed yield in mung beans [105]. Similarly, exogenously applied GA boosted black gram, pea, cowpea and linseed grain yields [106–108]. The underlying mechanism behind the higher seed yield produced by growth regulators has been attributed to improved biosynthesis in the assimilates which are directly related to yield, as GA foliar application on 30-day-old seedlings produced higher yields in mustard, while GA (100 ppm) also produced the highest soybean seed yield [109]. Similar to our findings, it was reported that GA triggered vegetative growth in crop plants by increasing plant height, stem diameter, weight and leaf numbers, and the plants' fresh and dry weights [110–113], which led to higher biomass productivity. Seed and biological yield are vital indicators for determining the effectiveness of foliar-applied phytohormones. The significantly higher biomass yielded by SA and GA might be attributed to the amplification of photosynthetic and translocating processes [114]. Externally applied GA was reported to boost the production of DNA, RNA and enzymes such as phosphatases, which play a role in the development of floral buds and seeds. All the growth stages of the plants contribute towards the biological yield of the crop [109], and the harvest index was notably improved due to interactions between growth promoters. Khatun et al. [115] also found identical conclusions in the case of soybeans. Our findings are also consistent with previously reported results in which foliar-applied phytohormones improved the biological and stover yields, along with the harvest index, of mung beans [116,117]. The present findings are in conformity with those of Verma et al. [118], who noted a 12% increment in HI caused by GA application in a concentration of 150 ppm. However, these findings are not consistent with the results of Nagar et al. [119], who inferred that GA could not increase the HI of wheat.

In the present study, the experimental plots receiving both SA and GA in higher doses produced the highest amounts of chlorophyll a, chlorophyll b and total chlorophyll content in green gram. The same treatment combination remained instrumental in recording the highest carotenoids and protein content. These results agree with the findings of Talaat et al. [120], in which SA foliar application in an optimized dose (3 μM) significantly improved wheat growth by inducing increased biosynthesis of photosynthetic pigments such as chlorophyll a, chlorophyll b and carotenoids. Another underlying mechanism behind higher levels of growth and development was that SA induced a greater acquisition of nutrients, ionic homeostasis of K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+), and osmolyte accumulation, along with reducing Na+ accumulation as well as the chlorophyll a/b ratio. Likewise, GA boosted protein content by triggering nitrate reductase activity in cowpea [121] and wheat plants [61]. These findings correlate with those of Chaves et al. [122], who recorded that exogenous SA application improved the antioxidant system in crop plants as well activating the phenylpropanoid pathway, which resulted in secondary metabolite biosynthesis. Moreover, SA effectively improved the net CO_2 assimilation rate, transpiration rate, stomatal conductance and water use efficiency, which led to higher protein content. Likewise, Wang et al. [10] opined that SA application (1.5 mmol L^{-1}) remained effective in increasing the soluble protein and soluble sugar contents, along with improving cell membrane structural stability and osmoregulation in crop plants. Similar findings were previously reported by Gacnik et al. [123], who opined that exogenously applied SA accelerated plant growth and development along with enhancing the quality of the traits of Mentha piperita. Xu et al. [124] reported that foliar-applied SA in a 0.6 mmol L^{-1} dose increased the growth of crop plants by preventing electrolyte leakage, and increased the photosynthesis rate in addition to promoting the biosynthesis of chlorophyll, soluble sugar and soluble protein. Additionally, it increased the accumulation of K^+, Mg^{2+} and Ca^{2+} content and the K^+/Na^+ ratio, and maintained ionic homeostasis. However, it was observed that SA's effectiveness was not linearly related to its concentration. Likewise, foliar-applied SA improved various physiological and biochemical characteristics of crop plants; however, it was opined that SA's efficacy in terms of pigment synthesis was dependent on the application dose, the rate of SA absorption and utilization by the plant species, the genotypes and the crop's developmental stage [125]. Furthermore, GA and SA application might have escalated the biochemical reactions, light intensification and absorbance, stomatal conductance and chloroplast activity that might be involved in the improvement of photosynthetic pigments [126]. Exogenously applied SA assisted plants in withstanding the deleterious impacts of different diseases through robust vegetative growth and development, and resultantly, nutritional quality in terms of factors such as protein content was improved significantly [127]. Likewise, it remained unmatched in terms of increasing the nutritive contents and antioxidant profile of mung beans [128]. Moreover, it increased pigment synthesis, which triggered the photosynthesis process, nitrogen metabolism and membrane permeability of the crop plants [129,130]. Furthermore, the external application of GA resulted in improved protein content in mung beans, as it reduced the accumulation of reactive oxygen species under stressful environments, and nutritional quality was improved owing to the protection offered by GA of plasma membrane integrity [131]. In a similar respect, Rashad and Hussien [132] inspected the effect of growth hormones on the quality of the contents in maize and inferred that growth hormones were effective in increasing amino acid content, owing to improved rates of transpiration and nitrogen metabolism, which led to significantly higher protein content.

5. Conclusions

In the India-Pakistan subcontinent, farmers are consistently switching to other cash crops owing to the declining productivity of green gram, which has necessitated testing farmer-friendly and pro-environment strategies such as the exogenous application of phytohormones to boost its yield. From the recorded data, it was revealed that the research findings were in complete conformity with the research hypothesis, as green gram responded differently to varying doses of foliar-applied SA and GA. The interaction effect of SA and GA applied in the higher doses remained unmatched, recording the maximum crop growth, yield attributes, grain and biological yield, along with pigment synthesis and protein content. Overall, the individual effects of SA and GA were also effective, but the highest doses' interaction effects surpassed the rest of the treatments, indicating their greater efficacy compared with solo application. Thus, SA and GA foliar application in doses of 75 and 120 ppm, respectively, 35 days after sowing might be recommended to green gram growers in order to obtain significantly higher grain and biological yields along with better nutritional quality, especially protein content. Moreover, these encouraging research findings might serve as a baseline to test more doses of SA and GA, as the higher doses of both growth regulators remained superior.

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Abbreviations

CGR	crop growth rate
NAR	net assimilation rate
PH	plant height
PBs	pod-bearing branches
NP	number of pods
PL	pod length
SP	seeds per pod
GY	grain yield
BY	biological yield
SA	salicylic acid
GA	gibberellic acid
P	Protein

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