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Gibberellic Acid Induced Changes on Growth, Yield, Superoxide Dismutase, Catalase and Peroxidase in Fruits of Bitter Gourd (*Momordica charantia* L.)

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Abstract: Bitter gourd is one of the important cucurbits and highly liked among both farmers and consumers due to its high net return and nutritional value. However, being monoecious, it exhibits substantial variation in flower bearing pattern. Plant growth regulators (PGRs) are known to influence crop phenology while gibberellic acid (GA_3) is one of the most prominent PGRs that influence cucurbits phenology. Therefore, a field trial was conducted at University of Agriculture Faisalabad to evaluate the impact of a commercial product of gibberellic acid (GA_3) on growth, yield and quality attributes of two bitter gourd (*Momordica charantia L.*) cultivars. We used five different concentrations (0.4 g, 0.6 g, 0.8 g, 1.0 g, and 1.2 g per litre) of commercial GA_3 product (Gibberex, 10% Gibberellic acid). Results showed that a higher concentration of gibberex (1.0 and 1.20 g L^{-1} water) enhanced the petiole length, intermodal length, and yield of bitter gourd cultivars over control in Golu hybrid and Faisalabad Long. A significant decrease in the enzyme superoxidase dismutase, peroxidase, and catalase activities were observed with an increasing concentration of gibberex (1.0 and 1.20 gL^{-1} water) as compared to control. These results indicate that the exogenous application of gibberex at a higher concentration (1.2 g L^{-1}) has a dual action in bitter gourd plant: i) it enhances the plant growth and yield, and ii) it also influenced the antioxidant enzyme activities in fruits. These findings may have a meaningful, practical use for farmers involved in agriculture and horticulture.

Keywords: gibberex; *Momordica charantia L*; dismutase; peroxidase; catalase; vegetative growth

1. Introduction

Bitter gourd (*Momordica charantia L.*), well-known as bitter melon, balsam pear, and bitter cucumber [1] exhibits high nutritional value in terms of enriched mineral, vitamins, and macro-nutrient contents [2]. Despite providing essential elements it also possesses antioxidant, antiviral and antimicrobial pressure in addition to lowering blood sugar makes it favourite among consumers [3].

Meanwhile, farmers prefer its cultivation due to shorter growing period, early maturity, incremented yield, and high net return per acre [4,5]. However, bitter gourd belongs to a monoecious family which naturally bears staminate to pistillate flowers and expresses substantial variation [6]. A similar trend was observed in bitter gourd that variates with a ratio of 50:1 [7], but these ratios are quite uncertain and may vary from 9:1 to 48:1 [8]. This variation in sex expression is regulated by certain internal factors, i.e., genetic or hormonal [9], and external factors, i.e., nutrient application, modulating environmental conditions [10], water regime, and the application of plant growth regulators [11].

Among the discussed alternatives, use of plant growth regulators is a promising technique to be adopted as they are known to affect and enhance various physiological and biological functions of plants thus enabling a prompting transformation in plant phenotype [12]. Among plant growth regulators, gibberellins (GAs) are highly valuable as they affect flower initiation and development as in particular they have a crucial role in the differentiation of floral organs such as GA's application are known to induce feminization in maize (*Zea mays L.*) while masculinizing in spinach, cucumber, and asparagus [13]. In this regard, cucumber has been used as a model plant for studying genetic and molecular aspects of sex determination in cucurbits [14]. However, it is revealed that GA can influence sex expression of cucumber by regulating sex differentiating genes i.e., F, M, and A genes via both ethylene dependent and independent pathways [15]. Organic Fertilizer addition to soil increases risk of xenobiotic contamination [16–19]. Moreover, GA signalling pathway also regulates stamen and anther development in Arabidopsis and rice [20,21]. While this hormonal activity is also dependent on certain environmental factors, particularly short days having low night temperatures led to an increment in ethylene concentration, thus regulating the process of sexual differentiation in plant species [22].

Besides sexual differentiation, GA_3 is also known for promoting vegetative growth by inducing hydrolytic enzyme activity [23] and reproductive behaviour by affecting male and female fertility [24] with enhancing quality attributes by regulating antioxidants enzymes [25] of harvested product. Generally, GAs enhance cell division and elongation; promote stem elongation (in particular stalk length) and improving flower and fruit volume [26]. These are also known to be most economical and beneficent in achieving bigger fruit size, heavier fruit weight and sustainable fruit yield in addition to enhancement of produce quality in terms of total soluble salts (TSS), acidity (TA), TSS/TA ratio, and reducing sugar [27]. Plant growth hormones, i.e., auxins, gibberellins, cytokinins, and ethylene have become a center of interest regarding vegetative growth, modulating sex expression, and controlling yield components [28]. Therefore, various gibberellins (GA_3), auxins (IAA and NAA), cytokinins and ethylene are being applied exogenously to enhance the productivity of bitter gourd [29–31]. It was hypothesized that exogenous gibberellic acid application influences the morphological and antioxidative behavior in cucurbits. Thus, the present research trial was conducted with an aim to evaluate the effect of commercial gibberellin, gibberex (10% GA_3), at various concentrations, on the growth, yield, and antioxidative attributes of two bitter gourd varieties.

2. Materials and Methods

2.1. Experimental Design

A field experiment was conducted following randomized complete block design (RCBD) at Vegetable Farm, Institute of Horticultural Sciences (31.4303° N, 73.0672° E), University of Agriculture Faisalabad. The mean temperature, humidity and light duration hours during the experiment were 31.92 °C, 50.95% and 8.75 h respectively. Healthy and diseased free seeds of two bitter gourd cultivars *viz.*, Golu hybrid (G. Hybrid) and open pollinated Faisalabad Long (F. Long) were collected from Bayer Pakistan (Private) Limited and local market, respectively. Raised beds (5.4 × 1.5 m) were prepared on finely pulverized soil (Table 1). Soil properties changes with land use [32–34] were followed by seed sowing by dibbling method at 2 cm depth with 2 feet plant to plant distance on one side of the bed. Seedlings emergence were observed on a daily basis and sowing was done again in case of any dead or week seedlings.

Table 1. Pre-experiment soil characteristics.

Soil	Units	Value
Texture	-	Loam
pH	-	8.02
EC	dS m^{-1}	2.47
Organic matter	%	0.65
Organic N	%	0.024
Available P	mg Kg^{-1}	6.54
Extractable K	mg Kg^{-1}	165

Abbreviations: EC = Soil electrical conductivity, N = Nitrogen, P = Phosphorus, K = Potassium

Moreover, recommended cultural practices, i.e., thinning, weeding, and trellising, were adopted to attain proper growth of bitter gourd plants. Trellising on high-density polyethylene (HDPE) net was done using bamboo poles (six feet high) taut with steel ropes.

2.2. *Collection and Application of GA_3*

Gibberex (10% Gibberellic acid), a product from Evyol group was acquired and applied in different concentrations viz., T_0 = control, T_1: 0.4 g, T_2: 0.6 g, T_3: 0.8 g, T_4: 1.0 g and T_5: 1.2 g per one liter of distilled water, on bitter gourd plants. Three foliar sprays, starting from 30 days after sowing, were done at two weeks intervals till flowering. The volume of spray per plant was kept constant with each plant receiving approximately 20 mL of solution. The spray was done on the abaxial and adaxial surface of leaves to have complete coverage of plant with a commercial hand-held sprayer.

2.3. *Vegetative and Reproductive Parameters*

Vegetative growth expressed as petiole length (cm) and internodal distance (cm) was determined by the method described by Gocmen et al. [35]. Data regarding reproductive parameters on number of fruits [36], fruit weight (g) and yield per plant (kg) [37] were collected.

2.4. *Extraction and Determination of Antioxidant Enzymes*

Fresh fruit (100 mg) from healthy (control and treated) plants was homogenized with 4 mL of 50 mM phosphate buffer (pH 7.0), containing 1 mM EDTA and 1% polyvinyl polypirrolidone (PVP). The homogenate was centrifuged for 25 min at 15,000× *g* and the supernatant was used for enzyme activity assays on an immediate basis.

Superoxide dismutase (SOD) enzyme was assayed following the protocol of Stagner and Ppovic [38] by measuring 50% blocking of photochemical reduction of nitro blue tetrazolium (NBT). Aliquots of supernatant (100 μL) were mixed with 500 μL of phosphate buffer, 200 μL of methionine, 100 μL of riboflavin with 800 μL of distilled water and placed under UV light for 15 min followed by absorbance determination at 560 nm by using a microplate reader. One unit of SOD activity was expressed on the basis of protein contents as U mg^{-1} protein.

Activities of catalase (CAT) and peroxidase (POD) enzyme determined following the protocol of Chance and Maehly [39]. Enzyme extract (100 μL) was mixed in 500 μL solution containing 300 mM of phosphate buffer with 100 μM of 5.9 mM H_2O_2. Changes in reaction solution were observed by absorbance at 240 nm while one unit of catalase activity was expressed as nmol min^{-1} g^{-1} protein. Whereas, for POD activity assay, a solution containing 500 μL of 50 mM phosphate buffer (5.0 pH) with 200 μL of 40 mM H_2O_2, 500 μL of 20mM guaiacol and 100 μL of enzyme extract. Changes in the absorbance of reaction solution were recorded at an interval of 20 s at wavelength of 470 nm. One unit of POD activity was expressed as μmol min^{-1} g^{-1} protein.

2.5. Statistical Analysis

Randomized Complete Block Design (RCBD) was used to evaluate the impact of five treatments and control having three replications with three plants per replication to conduct this experiment. Data were analyzed with the help of two way ANOVA (analysis of variance) and means were compared by applying LSD test at 5% level of significance [40].

3. Results and Discussion

3.1. Petiole and Internodal Length

Results indicated that gibberex application as treatment significantly ($p \leq 0.05$) increased the internodal length (IL) and petiole length (PL) of Golu hybrid and Faisalabad Long (Figure 1A,B). Interaction of different application rates of gibberex and varieties of bitter gourd was ordinal for petiole length (Figure 2A). A positive non-significant correlation was observed between petiole length of Golu hybrid and Faisalabad Long with different application rates of gibberex (Figure 2A). Application of treatment 1.0 gL^{-1} gibberex remained significant as compared to control in Golu hybrid for petiole length. In Faisalabad Long, treatments 1.2 and 0.6 gL^{-1} gibberex showed a significant positive response for improvement in petiole length. Maximum petiole length (9.33 and 7.33 cm) was recorded in Golu hybrid and Faisalabad Long at 1.0 and 1.2 gL^{-1} gibberex respectively.

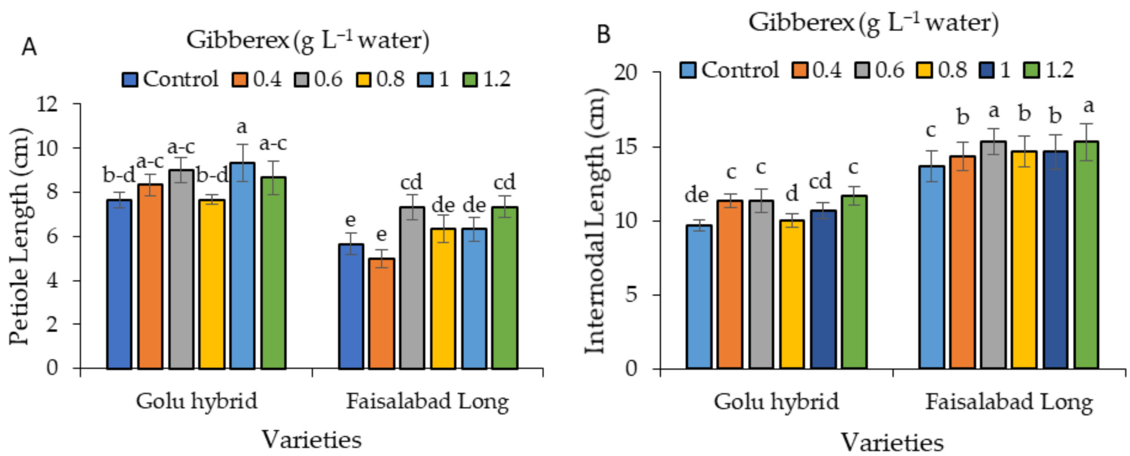

Figure 1. Effect of concentrations of Gibberex on petiole length (**A**) and Internodal length (**B**) of two bitter gourd cultivars. Vertical Bars indicate standard error of means (n = 9). Means sharing different letters are significantly different from each other. * Significant ($p \leq 0.05$), Highly significant ($p \leq 0.01$) while results depicts that for petiole length: Varieties = **, GA = *, Varieties × GA = ^{NS}; for Internodal Length: Varieties = **, GA = *, Varieties × GA = $^{NS.}$

For intermodal length, 1.2 gL^{-1} gibberex differed significantly over control in Golu hybrid and Faisalabad Long. Interaction of different application rates of gibberex and varieties of bitter gourd was disordinal for internodal length (Figure 2B). A positive non-significant correlation was observed between intermodal lengths of Golu hybrid with different application rates of gibberex (Figure 9A). However, intermodal length of Faisalabad Long showed positive significant correlation with different application rates of gibberex (Figure 9B). All application rates of gibberex remained significant over control in Faisalabad Long for internodal length. For Golu hybrid, treatments 0.4 and 0.6 gL^{-1} gibberex also remained equally significant over control for internodal length. Maximum internodal length (15.33 and 11.66 cm) was recorded in Faisalabad Long and Golu hybrid at 1.2 gL^{-1} gibberex respectively. Internodal elongation is based on the ability of plants to perform cell division and cell elongation [41] that varies from genotype to genotype. In contrast, this increment could also be due to the application of gibberex stimulated cell division and cell elongation [42] leading to stem elongation via stretching internodal distance [43]. These findings are comparable with outcomes of Bostrack and

Struckmeyer [44] that observed elongation of pith parenchyma cells leading to elongation of internodal length under GA_3 application. Similar findings were reported by Ros et al. [45] and Mishra et al. [13] that GA_3 application enhanced the internodal distance and petiole length in pea and bitter gourd.

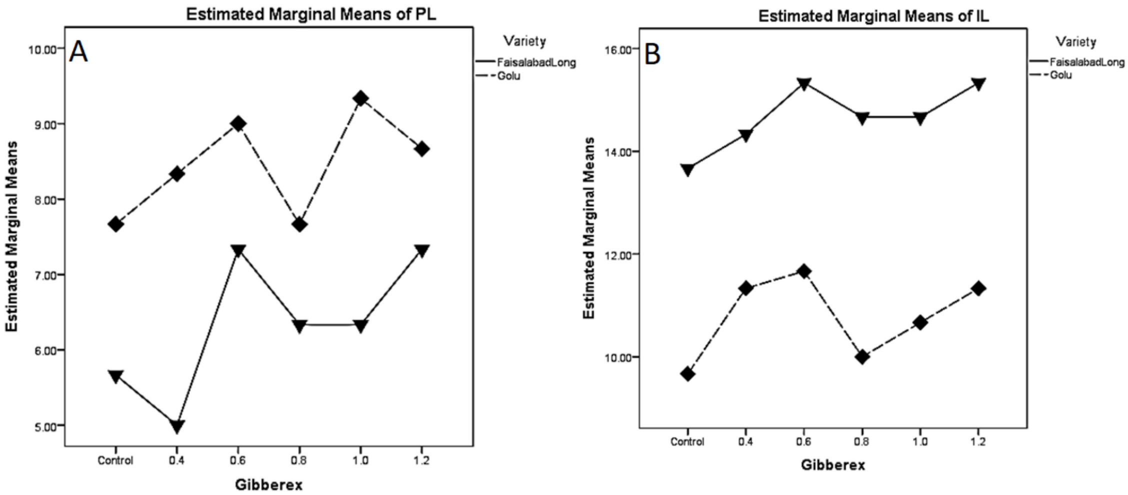

Figure 2. Interaction graph of different application rates of gibberex and bitter gourd varieties for petiole and intermodal length. Figure (**A**) represents petiole length while (**B**) represents the internodal length.

3.2. Fruit Number and Weight

Effect of different application rates of Gibberex was significant ($p \leq 0.05$) on number of fruits (NF) (Figure 3A) and fruit weight (FW) (Figure 3B) of Golu hybrid and Faisalabad Long. Interaction of different application rates of Gibberex and varieties of bitter gourd was disordinal for number of fruits (Figure 4A). A positive significant correlation was observed between Golu hybrid and Faisalabad number of fruits with different application rates of gibberex (Figure 9A,B). It was noted that application of treatment 1.2 gL^{-1}. Gibberex was significantly different as compared to other application rates of Gibberex for improvement in number of fruits of Golu hybrid and Faisalabad Long. However, number of fruits were significantly higher in Golu hybrid over Faisalabad Long. For the increase in fruit weight of Golu hybrid and Faisalabad Long, higher application rate 1.2 gL^{-1} Gibberex as treatment differed significantly from control. Interaction of different application rates of Gibberex and varieties of bitter gourd was significant ordinal for fruit weight (Figure 4B). A positive significant correlation was observed between Golu hybrid and Faisalabad fruit weight with different application rates of gibberex (Figure 9A,B). Maximum increase of 17.8 and 31.4% in number of fruits and 26.4 and 36.1% in fruit weight were noted where 1.2 gL^{-1} gibberex in Golu hybrid and Faisalabad Long respectively. Balance uptake of nutrients by application of Gibberex played an imperative role in the improvement of number of fruits and fruit weight in bitter gourd varieties [43]. Application of GA_3 enhanced the synthesis of chlorophyll which led to enhanced production of proteins [46] whereas varietal genetic potential to produce a low or high number of fruits can also affect fruit number per plant. Moreover, Sadia et al. [47] concluded another factor that GA_3 enhances the initial growth of ovaries along with reducing the abscission layer that leads to a reduction premature flower and fruit drop.

3.3. Yield per Plant

Results indicated a significant difference ($p \leq 0.05$) among the bitter gourd cultivars for yield per plant (YP) (Figure 5A). A significant ordinal interaction was observed among different varieties of bitter gourd and different application rates of gibberex (Figure 5B). A positive significant correlation was observed between Golu hybrid and Faisalabad Long regarding yield per plant with different application rates of gibberex (Figure 9A,B). It was observed that the application of 1.0 and 1.2 gL^{-1} gibberex proved to be significantly best application rate over control for the improvement in the yield per plant in Golu hybrid over Faisalabad Long respectively. Maximum increases of 41% and 88% in yield per plant were noted where 1.0 and 1.2 gL^{-1} gibberex treatments were applied in Golu hybrid and Faisalabad Long respectively. Role of GA_3 had been in focus in various plant species for increasing biomass and yields [48,49] as its exogenous application has proved to promote source and sink relation with an additional increment of dry matter accumulation. However, a high increment of yield in Faisalabad long following gibberex application could be ascribed to both improved genetic traits and GA_3 mediated enhanced nutritional uptake subsequently promoting the leaves photosynthetic capacity [50]. Moreover, GAs had also been set up to facilitate the integration of source to the developing reproductive plant parts followed by enhanced sink strength due to improved photosynthate translocation [51]. Further, GA_3 enhances the transformation of sucrose to glucose, thus improving extracellular invertase [52] that leads to phloem delivering carbohydrates to sink organs, thus ultimately enhancing plant yield.

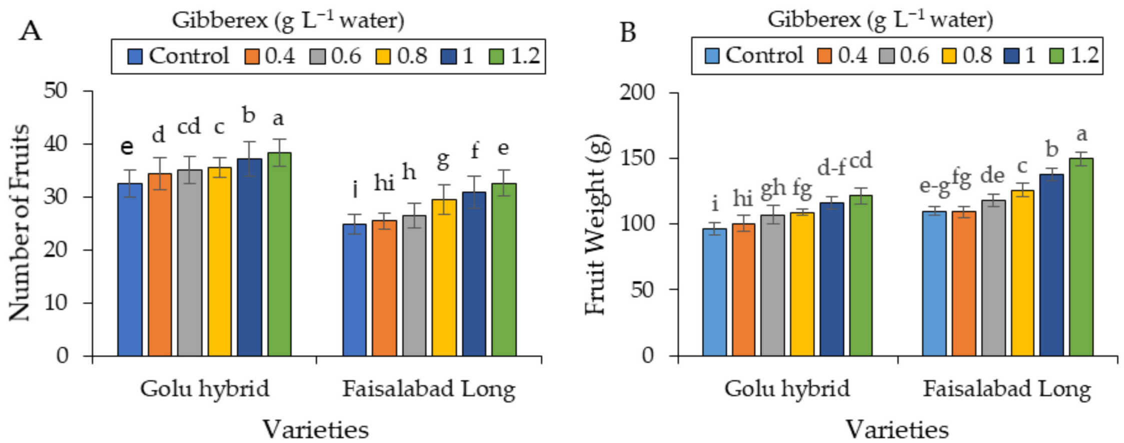

Figure 3. Effect of concentrations of Gibberex on number of fruits (**A**) and fruit weight (**B**) of two bitter gourd cultivars. Vertical Bars indicate standard error of means (n = 9). Means sharing different letters are significantly different from each other. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$). while statistical analysis depicts that results for number of fruits: Varieties = **, GA = **, Varieties × GA = **; for Fruit weight: Varieties = **, GA = **, Varieties × GA = *.

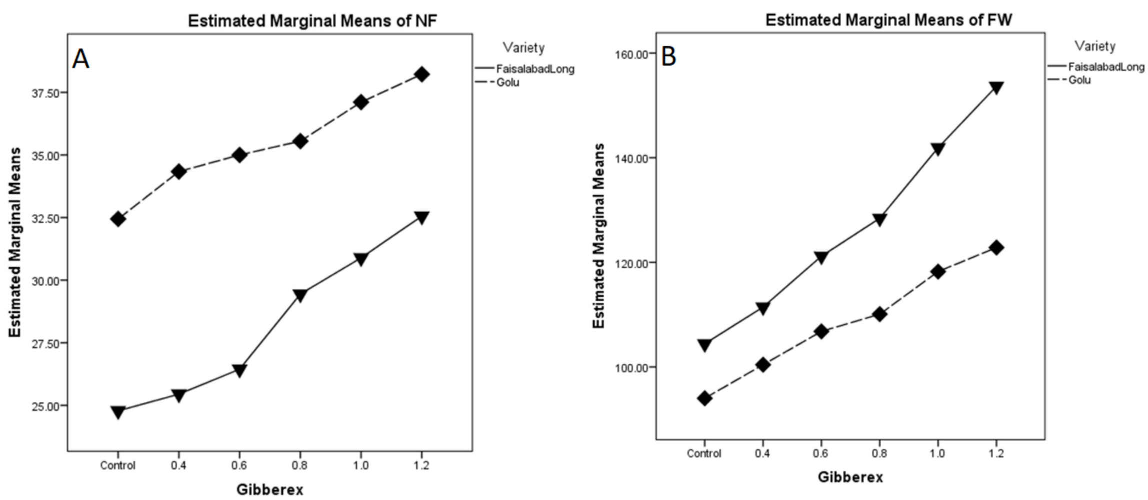

Figure 4. Interaction graph of different application rates of gibberex and bitter gourd varieties for number of fruits and fruit weight. Figure (**A**) represents number of fruit while (**B**) represents fruit weight.

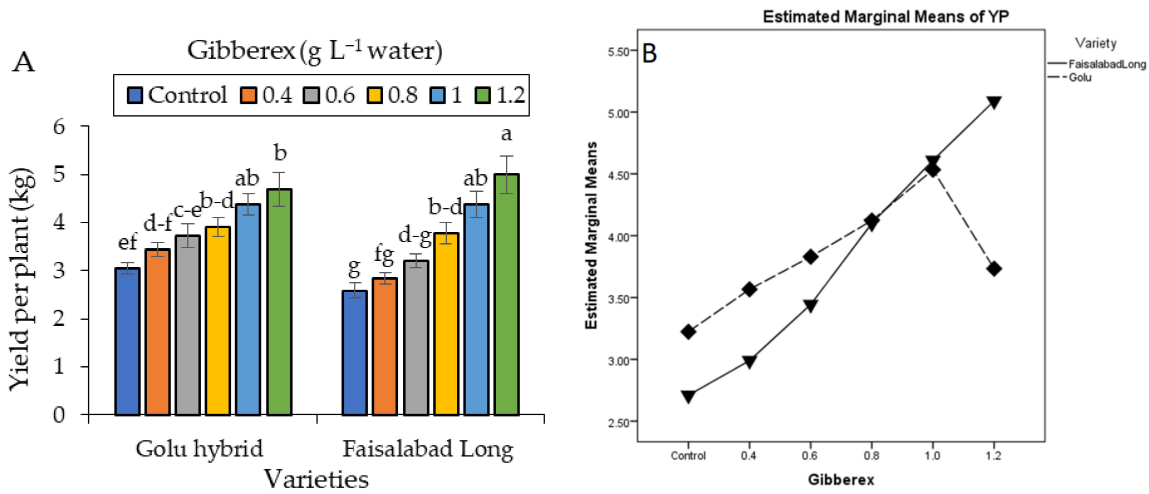

Figure 5. Effect of concentration of Gibberex on yield per plant of two bitter gourd cultivars. Figure (**A**) represents yield per plant while (**B**) represents the interaction graph of different application rates of gibberex and bitter gourd varieties for yield per plant. Vertical Bars indicate standard error of means (n = 9). Means sharing different letters are significantly different from each other. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) while results depicted that for yield per plant: Varieties = ^{NS}, GA = **, Varieties × GA = **.

3.4. SOD, POD and CAT

Effect of different application rates of gibberex significantly ($p \leq 0.05$) affect the SOD (Figure 6A), POD (Figure 7A) and CAT (Figure 8A) in bitter gourd varieties. Interaction between different application rates of gibberex and bitter gourd varieties was significant ordinal for SOD (Figure 6B), POD (Figure 7B) and CAT (Figure 8B). A negative significant correlation was observed between Golu hybrid and Faisalabad SOD, POD and CAT with different application rates of gibberex (Figure 9A,B). Increasing levels of gibberex significantly decreased the SOD, POD and CAT in Golu hybrid and Faisalabad Long. Significant reductions in SOD (79% and 72%), POD (62% and 61%), and CAT (55% and 51%) were noted where treatment 1.2 gL^{-1} gibberex was applied in Golu hybrid and Faisalabad Long respectively. These findings are comparable with the outcomes of Rosenwasser et al. [53]. They also reported that exogenously applied GA_3 reduced the production of reactive oxygen species and thus leads to improvement in the activity of SOD in *Catharanthus roseus*. Previous studies have concluded that GA_3 application can enhance the antioxidant activity of plants by inducing phenolic production [54] reducing lipid peroxidation with increasing proline and glycine-betaine biosynthesis [55]. Previous studies have reported that plant growth regulators alone or in combination are involved in regulating the transcription of genes [56] along with mRNA [57] that ultimately, in turn, improve the synthesis of a specific hormone to make proteins.

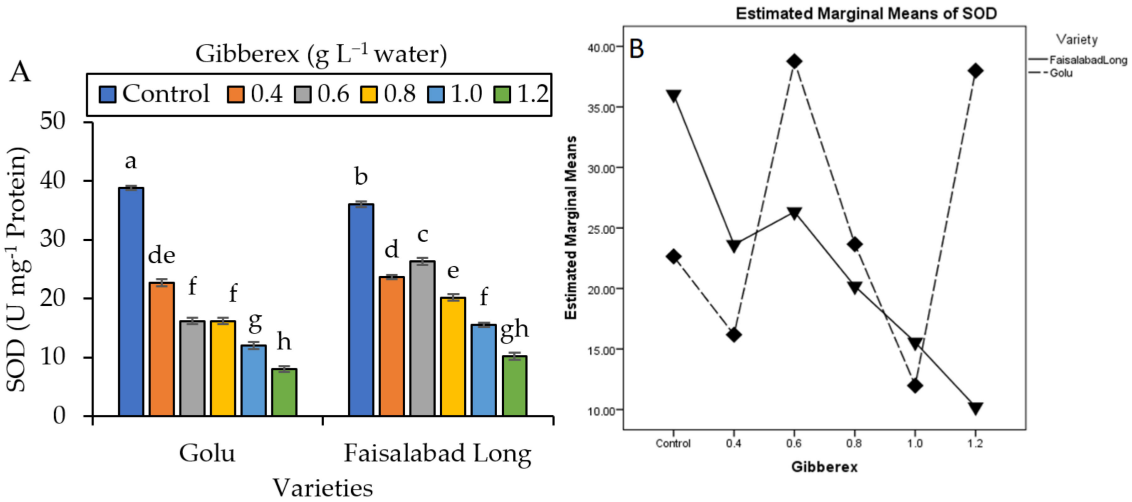

Figure 6. Effect of different concentrations of Gibberex on SOD of two bitter gourd cultivars. Figure (**A**) represents SOD while (**B**) represents the interaction graph of different application rates of gibberex and bitter gourd varieties for SOD. Vertical Bars indicate standard error of means (n = 9). Means sharing different letters are significantly different from each other. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) while results depicted that for SOD activity: Varieties = **, GA = **, Varieties × GA = **.

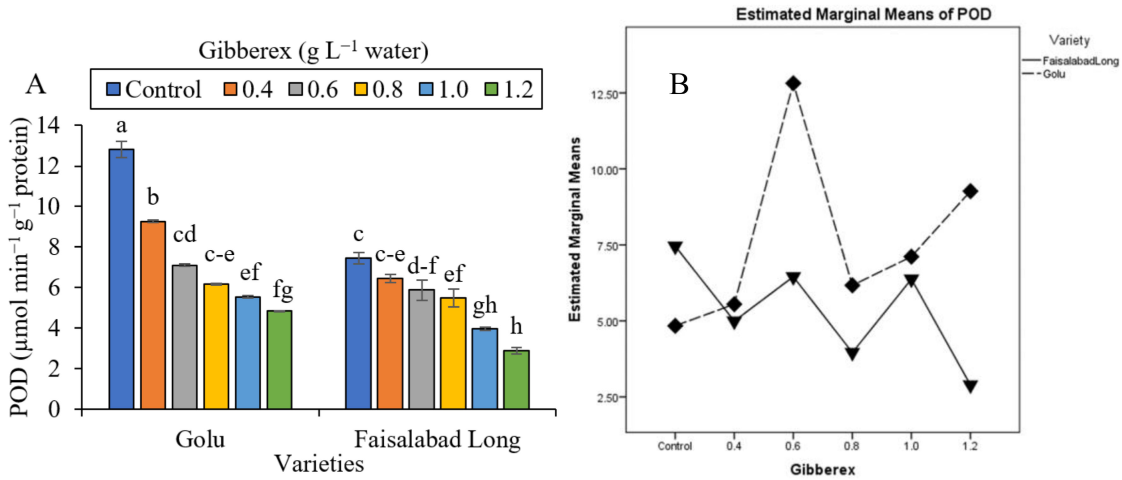

Figure 7. Effect of different concentrations of Gibberex on POD of two bitter gourd cultivars. Figure (**A**) represents POD while (**B**) represents the interaction graph of different application rates of gibberex and bitter gourd varieties for POD. Vertical Bars indicate standard error of means (n = 9). Means sharing different letters are significantly different from each other. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) while results depicted that for POD activity: Varieties = **, GA = **, Varieties × GA = **.

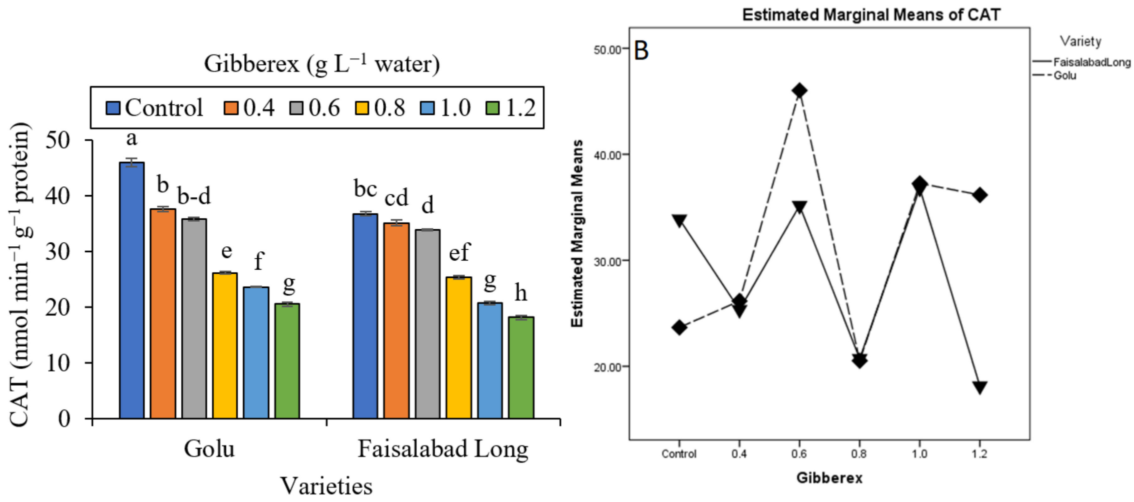

Figure 8. Effect of different concentrations of Gibberex on CAT of two bitter gourd cultivars. Figure (**A**) represents CAT while (**B**) represents the interaction graph of different application rates of gibberex and bitter gourd varieties for CAT. Vertical Bars indicate standard error of means (n = 9). Means sharing different letters are significantly different from each other. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) while results depicted that for yield per plant: Varieties = **, GA = **, Varieties × GA = **.

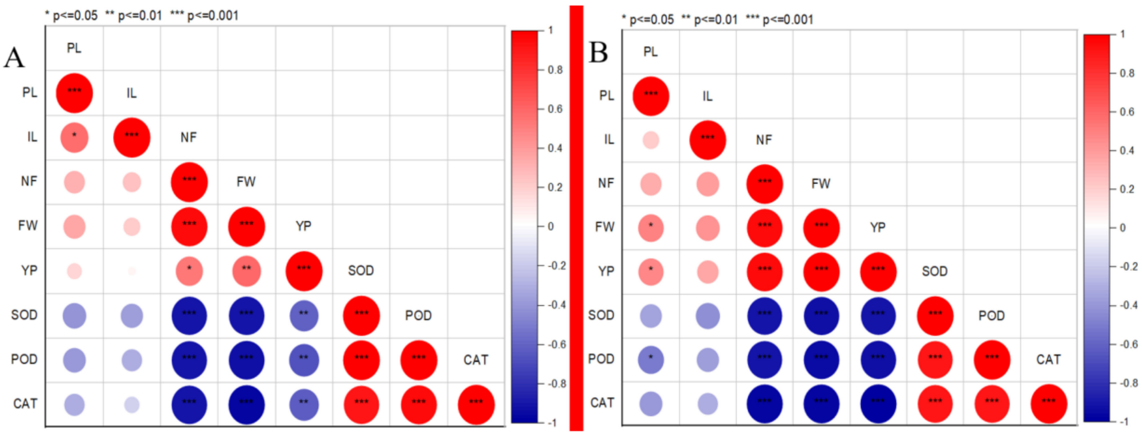

Figure 9. Pearson correlation between different application rates of gibberex and bitter gourd growth, yield and antioxidants. Figure (**A**) represents Golu hybrid and (**B**) represents Faisalabad Long. Red circles showed a positive correlation with attributes. Blue circles showed a negative correlation with attributes. * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.01$) while results depicted that for yield per plant: Varieties = **, GA = **, Varieties × GA = **.

4. Conclusions

It is concluded that 1.0 and 1.2 gL^{-1} gibberex application lead to significant enhancement of petiole and intermodal length in bitter gourd. In particular, it enhances the number of fruits with heavier fruit weight hence increasing yield and giving good returns to the growers. Application of 1.0 and 1.2 gL^{-1} gibberex is also efficacious in decreasing SOD, POD, and CAT in Golu hybrid and Faisalabad Long. However, further research is suggested with molecular analysis to have a detailed insight into gibberellic acid induced changes responsible for the enhancement of growth and yield in Golu hybrid and Faisalabad Long.

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and S.D.; writing—review and editing, M.A.; supervision, M.A. All authors have read and agreed to the published version of the manuscript.

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