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Original Article

Higher Biomass and Biochemical Compounds of *Stevia rebaudiana* Through Exogenous Gibberellic Acid (GA) and Kinetin (KN) hormones Treatments

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ABSTRACT

Hormones are chemical messengers that regulate various physiological processes in plants. Stevia growth is affected by them, so this study investigated how gibberellic acid (GA) and kinetin (KN) affect it. Two RCBD experiments were conducted in 2016 in Shirvan, Iran, to study gibberellic acid (GA) and kinetin (KN) hormones. First experiment included the following GA treatments: 1. control (no GA) 2. 100 ppm 3. 200 ppm 4. 300 ppm (PPM). In the second experiment, KN was included in the treatments: 1. control (no KN) 2. 20 ppm 3. 40 ppm 4. 60 ppm. At all concentrations of KN and GA, plant height, leaf number, leaf dry matter, and biomass significantly enhanced while yield index decreased. Additionally, although GA application decreased non-reducing sugars (TSSN) and total soluble sugars (TSS), KN at 40 and 60 ppm increased TSS. In contrast to KN treatments, GA treatments improved total stevia glycosides (TSGs). Furthermore, FLV was not affected by GA, but plants treated with KN 60(KN at 60) ppm had the highest FLV. All GA concentrations induced more CHL b than control. Additionally, it boosted N, P, and K, however, only KN at 40 and 60 ppm increased N and K. Overall, the positive effects of both hormones are dose-dependent; while KN treatment was ineffective in terms of TSG productivity, GA treatment was beneficial.

INTRODUCTION

healthcare programs may require a sugar substitute that falls between plant-based and synthetic sweeteners to promote natural alternatives, support calorie control and blood sugar management [1]. Stevia [*Stevia rebaudiana* (Bertoni) Bertoni] is a perennial herbaceous plant of the Asteraceae family that can be a suitable option for healthcare programs aiming to promote healthier dietary choices. The importance of stevia lies in its potential as a natural alternative to artificial sweeteners [2]. Stevia contains compounds called steviol glycosides (SGs), which are 100-300 times sweeter than sucrose [3]. Furthermore, the cultivation and use of stevia can have positive environmental impacts. Stevia is a perennial plant that requires less water and land compared to traditional sugar crops like sugarcane or sugar beet. Its cultivation can help reduce the demand for these resource-intensive crops, leading to a more sustainable agricultural system [3]. Stevia also has potential medicinal properties. Research suggests that (SGs) may have anti-inflammatory, antioxidant, and antimicrobial effects. These properties make stevia a potential candidate for use in the development of new drugs or natural remedies [1]. Although stevia is a preferable alternative for artificial and natural sweeteners, but the market value of Stevia has already risen in recent years. So, finding a suitable technique of for increasing yield production is essential.

hormones, also known as plant regulators, are chemical messengers that regulate various physiological processes in plants. Hormones play crucial roles in plant growth, development, and responses to environmental stimuli [4] They regulate processes such as seed germination, stem elongation, flowering, fruit development, and stress responses. Hormones are essential for coordinating and

controlling the growth and development of plants in response to internal and external signals [5]. Gibberellic acids (GAs) and cytokinin (CKs)are key hormones involved in maintaining development.

Gibberellins are a group of plant hormones that play important roles in plant growth and development. One of the main functions of gibberellins is to promote stem elongation. They stimulate cell division and elongation in the stem, leading to increased plant height. This is particularly important in the early stages of plant growth when rapid elongation is needed [6, 7]. Furthermore, gibberellins are involved in regulating flowering. They promote the transition from vegetative growth to reproductive growth, leading to the formation of flowers. Gibberellins can also influence flower development and fruit set, contributing to overall plant reproduction [4]. In addition to their effects on growth and development, gibberellins can also influence plant responses to environmental stimuli. They can enhance tolerance to abiotic stresses such as drought and high temperatures, as well as biotic stresses such as insect attacks and pathogen infections [6].

Kinetin (KN) is a synthesized cytokinin (CKs) that plays several important roles in plants. One of the main functions of kinetin is to promote cell division and differentiation. It stimulates the growth of lateral buds, leading to branching in plants. This branching increases the overall biomass of the plant by providing more sites for photosynthesis and nutrient uptake. Kinetin also has a role in delaying senescence or aging in plants by restoring CKs signaling which reduces ABA contents. It helps to maintain the greenness and vitality of leaves by preventing chlorophyll degradation and promoting protein synthesis. This prolongs the photosynthetic capacity of the plant, contributing to increased biomass production. Furthermore, kinetin is involved in regulating plant growth and development. It promotes root formation and elongation, leading to increased nutrient uptake and biomass accumulation in the roots [8,9].

It has been reported that since Steviol glycosides (SGs) and GAs have a common precursor in their biosynthesis pathway in the plant, there may be an effect on the production of SGs after the application of GAS due to their biosynthetic relationship. The multiplication of carbohydrates in response to GA has also been reported in literature [10].

Application of exogenous KN enhances the contents of major hormones including SA, JA, and GAs. Moreover, KN enhances photosynthesis and reduces ABA content by restoring CKs signaling. In a greenhouse experiment, Hajihashemi studied [10] the effects of paclobutrazol (PBZ) and gibberellin (GA) on the *S. rebaudiana* herb. Photosynthesis pigments were not affected by PBZ or GA treatments, but carbohydrates, amino acids, and proteins were increased. Yoneda [11] reported that the GA biosynthesis pathway influences the number of *SGs.* In addition, hormonal crosstalk between GA and auxin also affects the number of SGs in S. *rebaudiana*. The effects of GA, kinetin, and benzyl aminopurine (BAP) on propagation, growth, morphological properties, and SG content of stevia leaf samples were investigated by Pazuki [3]. They found that treatments containing 0.5 GA produced the highest biomass and largest leaf areas. There was little difference between the three hormonal treatments in terms of the number of leaves, the fresh to dry weight ratio, and the length of the leaves. There was no difference between GA and the control group in terms of rebaudioside-A (Reb-A) production (16.2 and 18.04 mg/g, respectively). Although different hormones cause a wide range of plant responses, only a few studies have examined the effects of GA and KN hormones on stevia [12-14], As a result, in this study, stevia's biochemical and physiological responses were assessed to exogenous GA and KN.

MATERIALS AND METHODS

An experimental field trial was conducted on Shirvan's experimental farm in 2016 (longitude 57 93', latitude 37 10', altitude 1097 meters). It had typical arid and semiarid climatic characteristics. The weather conditions included average annual precipitation of 251.8 mm, average annual temperature of 18 °C, average minimum annual temperature of 7 °C, and total annual evaporation of 2046.34 mm). Before the experiment, soil was sampled for its physicochemical characteristics (Table 1). gibberellic acid (GA) and kinetin (KN) were foliar applied before and after flowering. Both experiments used the same sampling method and cultivation operation. A complete randomized design with three replications was used in both experiments. Treatments in the first experiment included GA at different levels: 1. control (no GA) 2. 100 ppm (GA) 3. 200 ppm (GA) 4.300 ppm (GA).

Table 1 Physicochemical properties of farm soils

Soil texture	EC (dS/m)	Nitrogen %	Bulk density (g/m^3)	pH	Organic matter(%)	Phosphorus (%)	Fe (mg/kg)	Cu (mg/kg)	Potassium (ppm)
Loam clay	0.98	0.080	1.5	7.79	0.091	2.9	7.46	1.14	290

The second experiment involved KN treatments 1. control (no KN) 2. 20 ppm (KN) 3. 40 ppm (KN) 4. 60 ppm (KN). In this study, plot sizes were 3.5 × 2 m^2, and plants were planted at 25 × 45 cm spacing. Each plot contained 8-10 plants. Afterward, the plots were uniformly flooded one day before transplanting, and seedlings were transferred at 3-4 leaves on 15 May.

Growth Parameters Measurement

The plants were harvested and transferred to the laboratory at the end of the season. In order to determine the fresh weight of the leaves and stems, the leaves and stems were weighed separately. Following this, the plant height was measured, and the dry weights of the stems and leaves were calculated separately.

Biochemical Measurement

Total Phenolic and Flavonoids

Folin-Ciocalteu was used to determine total phenolic content with slight modifications [15]. A colorimetric method was used to determine total flavonoid content [16].

Carbohydrates

A modified Omokolo [17] method was employed for extraction and measurement of soluble sugars. The total soluble sugars (TSS) were calculated using a glucose curve standard [18]. TSSN were determined using the anthron. TSSRs were calculated by subtracting non-reducing sugars from total soluble sugars [19].

Chlorophyll

Leaf chlorophyll concentration was determined by a spectrophotometer and the adsorption rate of the samples containing chlorophyll measured *(chlorophyll samples measured)* at 663, 645 and 470 nm. Then the amount of chlorophyll a, b was calculated by the fallowing equation [20].

Chl a (µg/ml) = (12.25 A 663) – (2.55 A 645) s(Eq.1)
Chlb (µg/ml) = (20.31 A 645) – (4.91 A 663) (Eq.2)

Mineral Nutrients Measurement

Nitrogen was obtained by the Kjeldahl procedure [21] and potassium content was recorded by flame photometer using a standard curve [22]. The amount of phosphorus was determined using a spectrophotometric device at 420 nm wavelength [23].

Total Stevia Glycosides (TSGs)

0.10 g of leaves were extracted from ethanol (80%) then centrifuged at 12,000×g for 10 min. Then the supernatant was evaporated by rotary, and the SGs were determined by HPLC [24].

Statistical Analysis

Data analysis was performed by Statistical variance analysis (SAS 9.4) (SAS Institute, Cary, USA), and treatments' means were compared using the least significant difference (LSD) test ($p < 0.05$).

RESULTS

Growth and Yield Parameters

The Plant Height

According to the variance analysis, different levels of GA significantly influenced plant height (Tables 2 and 3). The Stevia's height was increased by 18.72, 32.96, and 55.13%, respectively, when GA levels were 100, 200, and 300 ppm, respectively, in comparison to control. In addition, the highest and lowest heights were related to 300 ppm treatment (91.66 cm) and control (58.66 cm), respectively (Table 4). The Stevia height was similarly affected by KN (Table 3). The largest and smallest heights were associated with the 60ppm KN treatment (77 cm) and the control (58.66 cm). Additionally, there was no significant difference between the 60 and 40 ppm KN treatments. Compared to the control, three levels of KN (60, 40, and 20 ppm) increased plant height by 31.24, 24.43, and 11%, respectively. Using the analysis of variance, both GA and KN hormones significantly affected the number of stevia leaves (Tables 2 and 3). A 200 ppm GA treatment produced the greatest number (799) of stevia leaves, and a control treatment produced the lowest number (697). In addition, the application of 100, 200, and 300 ppm of GA increased the number of leaves by 4.8, 14.63, and 11.76%, respectively, compared with the control (Table 4). As compared to the control, 60 and 40 ppm KN respectively increased the number of leaves by 8.27% and 15.11%.

Table 2 Result of analysis variance (ANOVA) of the effects of GA hormone on the growth and yield of stevia

S.O. V	D.F	Plant height	Leaf numbers	Leaf dry matter	Shoot dry matter	biomass	Harvest index
Block (B)	2	22.75 ^{ns}	207.25 ^{ns}	0.83 ^{ns}	1.80 ^{ns}	13592.5 ^{ns}	0.0006 ^{ns}
GA	3	581 **	6403 **	31.49 **	260.06 ^{ns}	4504695.5**	0.0070 **
Error	6	8.75	92.91	0.51	6.07	73934.8	0.0003
CV (%)	-	3.97	11.28	14.87	9.72	6.77	4.93

ns, (*) and (**) non-significant and significant at $p<0.05$ and $p<0.01$, respectively

Table 3 Result of analysis variance (ANOVA) of the effects of KN hormone on growth and yield of stevia

S.O. V	Df	Plant height	Leaf numbers	Leaf dry matter	Shoot dry matter	biomass	Harvest index
Block (B)	2	0.08 ^{ns}	121.33 ^{ns}	0.64 ^{ns}	7.24 ^{ns}	107064.2 ^{ns}	0.00040 ^{ns}
KN	3	201.41 **	7592.22	29.77 **	136.9 **	2935318.9 **	0.00170 **
Error	6	8.75	112.55	0.56	0.45	18233.13	0.00006
CV (%)	-	4.32	11.43	5.14	12.89	13.57	12.04

ns, (*) and (**) non-significant and significant at $p<0.05$ and $p<0.01$, respectively

Table 4 Mean comparison of the effects of GA and KN hormones on growth and yield traits of stevia

Treatment (ppm)	Plant height (cm)	Leaf numbers (number)	Leaf dry matter (g per plant)	Shoot dry matter (g per plant)	Dry Biomass (kg/h)	Harvest index
Control	58.6 d	697 d	10.5 d	14.2 c	2477 c	0.4 a
GA100	69.6 c	731 c	14.3 c	21.3 b	3573.3 b	0.4 ab
GA200	78 b	799 a	18.3 a	31.2 a	4956.7 a	0.3 b
GA300	91.6 a	779 b	15.8 b	34.5 a	5043.3 a	0.3 c
Control	58.6 c	697 c	10.5 c	47 b	2477 d	0.4 a
KN20	65 b	699.3 c	14.3 b	66 c	3680 c	0.3 b
KN40	73 a	754.6 b	15.4 b	78 b	4143.3 b	0.3 b
KN60	77 a	802.3a	18.1a	30.1 a	4826.7 a	0.3 b

Means followed by similar letters have not significantly different at α= 5% probability level, LSD test.

Leaf Dry Matter

The leaf dry matter was highest at 200 ppm (18.3 gr) and lowest in the control (10.566 gr). Leaves dry matter increased by 35.98, 13.82, and 50.18 in comparison to the control treatment at 100, 200, and 300 ppm, respectively (Table 4). The highest leaf dry matter (18.166 gr) was obtained with 60 ppm KN and the lowest leaf dry matter (10.566 gr) was obtained with the control treatment. Furthermore, no significant difference was observed between treatments at 40 and 20 ppm KN. Moreover, 60 ppm KN increased leaf dry matter by 71.96% compared to the control treatment (Table 4).

Shoot Dry Matter

The effect of different concentrations of GA and KN on shoot dry matter was significant (Table 2 and 3). The highest shoot dry matter was obtained in the treatment of 300 ppm GA (34.56 gr), which was not significantly different from the treatment of 200 ppm (31.267 gr). The lowest dry matter level was obtained in the control treatment (14.203 gr). (Table 4). Also, the application of 60 ppm of KN resulted in the highest shoot dry matter (30.1), while the lowest shoot dry matter (14.203 gr) was observed in the control treatment (Table 4).

Biomass

Application of different levels of GA and KN had significant effects on stevia biomass (Table 2 and 3). The result of the mean comparison showed that the highest biomass belonged to the treatment of 300 ppm GA with an average of 5043.3 kg/h. which was not significantly different from the treatment of 200 ppm GA. The lowest biomass (2477 kg/h) also found in the control treatment. (Table 4). The application of 20, 40, and 60 ppm KN also caused a significant increase in biomass compared to the control treatment (2477 kg/ha). The rate of this increase varied from at least 48.5% in the treatment of 20 ppm KN to 95% in the treatment of 60 ppm KN (Table 4).

Harvest Index (HI)

According to the statistical results, the control treatment had the highest HI (0.426), which did not

show a significant difference with the 100 ppm GA treatment. As GA concentration increased from 0 (control) to 300 ppm, the HI decreased, resulting in the lowest harvest index of (0.313). The results of KN application were similar to those of GA (Table 4). The highest harvest index was associated with the control treatment (0.426) and the lowest harvest index was observed in the treatment with 40 ppm KN (Table 4).

Biochemical Traits

Total Soluble Sugars (TSS)

The results showed that the effect of different levels of GA and KN on TSS was significant (Table 5 and 6). The highest TSS was obtained from control treatment with an average of 93.76 mg/g of dry leaf. there was no significant difference between concentrations of 100, 200, and 300 ppm GA in TSS (Fig 1). The highest TTS in different KN levels was related to the 60 ppm KN treatment between an average of 111.16 mg/g dry leaf. However, there was no significant difference with the 40 and 20 ppm KN treatments. The control treatment had the lowest TSS (93.76 mg/g) (Fig 2).

Reducing Sugars (TSSR)

The analysis of variance showed that the effect of GA on the amount of TSSR was not significant, whereas KN had a significant effect on this trait (Table 5 and 6). Comparisons of the mean related to the KN hormone revealed that the highest TSSR was related to the use of the KN hormone at 60 ppm. Also, the results showed that with increasing KN concentrations, the amount of TSSR increase. The least amount of TSSR was observed in the control treatment. Furthermore, treatment of 40 and 20 ppm KN had a similar effect on TSSR (Fig 2).

Non-reducing Sugars (TSSN)

Application of both GA and KN hormones decreased TSSN. Results showed that the highest TSSN obtained in the control treatment in both hormones, while there were no significant differences in different levels of both hormones in the amount of TSSN (Fig 1 and Fig 2).

Total phenolic content (TPC)

The results showed that GA and KN treatments significantly affected TPC (Table 5 and 6). The highest amount of TPC was obtained by application of 200 ppm GA, which increased TPC by 50% compared to the control treatment. However, different levels of GA had the same effects on TPC. Among all the KN treatments, 40 ppm KN had the highest amount of TPC, but the lowest amount of TPC, with an average of 40.5%, belonged to the control treatment (Table 7).

Total Stevia Glycosides (TSGs)

The analysis of variance revealed that different KN treatments did not significantly affect total stevia glycosides (TSGs), while GA had a significant effect (Tables 5 and 6). According to mean comparisons, compared to control, GA application at concentrations of 100 ppm, 200 ppm and 300 ppm increased TSGs by 10%, 33%, and 21%, respectively (Table 7).

Flavonoid (FLV)

The application of GA hormone had no significant effect on the amount of stevia flavonoids. (Table 5 and 6). But the KN increased flavonoids. The application of 20, 40 and 60 ppm KN treatments reduced flavonoids by 46.57, 18.1 and 12.8% respectively compared to the control (Table 7).

Content (CHL)

The results showed that chlorophyll a and b were affected by different amounts of KN and GA (Table 8 and 9). Application of 200 and 300 ppm of GA caused the highest amount of chlorophyll a. The lowest amount of chlorophyll a found in to the control treatment and 100 ppm (0.36 mg/g). The amount of chlorophyll b in different concentrations of GA was higher than in the control treatment; also, no significant difference was observed between the concentrations of GA (Fig 3). An increasing trend was observed in the amount of chlorophyll a ((Fig *3)*. Thus, the highest amount of chlorophyll a was found in the 60 ppm KN treatment. By increasing *KN* concentrations the amount of chlorophyll b increased (Fig 4). This was while no significant difference was observed between 40 and 20 ppm concentrations. The highest amount of chlorophyll b was present in the treatment of 60 ppm, which had an increase of about 74% compared to the control (Fig 4).

Plant Nitrogen (N)

Both GA and KN hormones affected the amount of N in the plant. Among the different levels of GA, the highest amount of N observed at 200 ppm and the lowest amount was observed in the control treatment. Also, the application of 60 ppm KN increased N amounts by 23% compared to the control treatment (Table 10).

Plant Phosphorous (P)

GA and KN hormones had positive effects on the amount of P in the plant (*plant P levels)* (tables 8 and 9). The highest amount of P was observed in the treatment of 200 ppm GA and the lowest in the control treatment. The application of KN hormone also showed a significant increase in this trait compared to treatment. Among the different levels of KN hormone, the highest amount of phosphorus was observed in the (at) 40 ppm (*ppm level*). Applying 20 and 60 ppm KN also increased P by 50% and 55% compare to control (Table 10).

Plant Potassium (K)

Among the different amounts of GA, the application of 300 ppm GA caused a significant increase in the amount of K in *(in K levels in)* the plant. Among the different amounts of KN, the treatment of 40 ppm KN obtained the highest amount of K, and the use of levels of 60 and 20 ppm KN increased the potassium level by the same amount (Table 10).

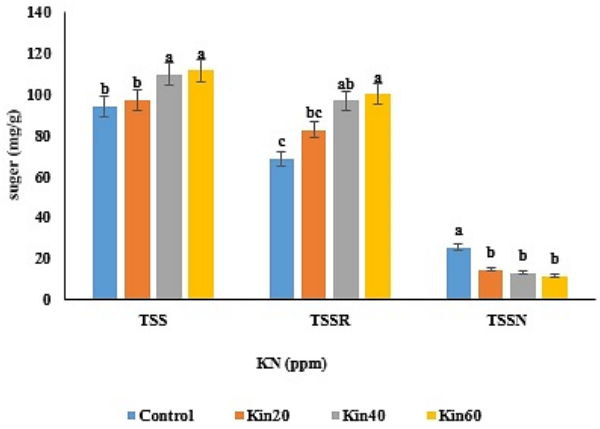

Fig. 1 Effects of different levels of gibberellic acid (GA) on Total soluble sugars (TSS), Reducing sugars (TSSR), Non-reducing sugars (TSSN) in stevia

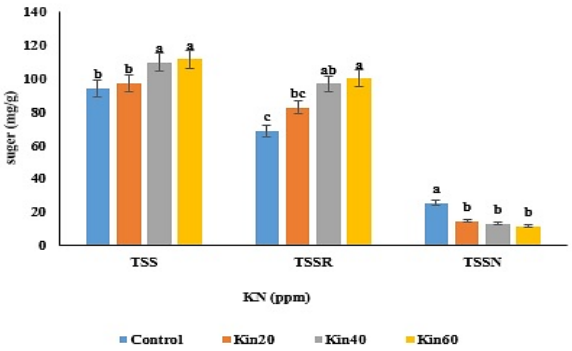

Fig. 2 Effects of different levels of Kinetin (KN) on Total soluble sugars (TSS), Reducing sugars (TSSR), Non-reducing sugars (TSSN) in stevia

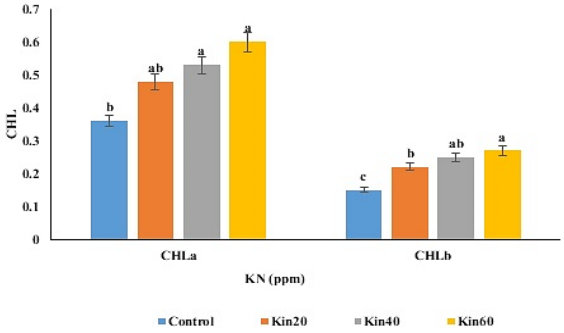

Fig. 3 Effects of different levels of gibberellic acid (GA) on Chlorophyll a (CHLa) and Chlorophyll b (CHLb) content in stevia

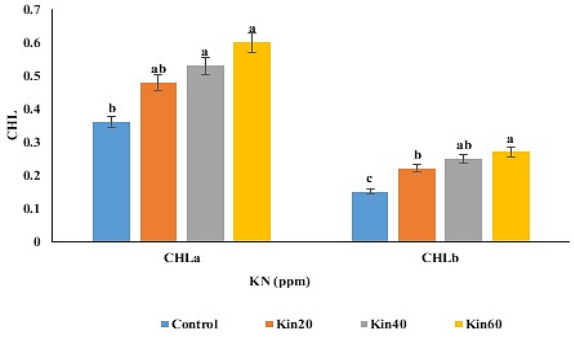

Fig. 4 Effects of different levels of kinetin (KN) on Chlorophyll a (CHLa) and Chlorophyll b (CHLb) content in stevia

Table 5 Analysis of variance of the effects of GA hormones on biochemical traits of stevia

S.O. V	Df	TSS	TSSN	TSSR	TPC	TSGs	FLV
Block	2	22.15 ^{ns}	6.02 ^{ns}	47.3 ^{ns}	2.43 ^{ns}	0.51 ^{ns}	0.18 ^{ns}
GA	3	315.70 **	7.01 **	25.4 ^{ns}	269.25 **	5.50 *	4.77 **
Error	6	16.26	100.08	34.16	5.54	0.95	0.50
CV (%)	-	5.13	18.39	7.04	14.31	8.80	5.46

ns, (*) and (**) non-significant and significant at $p<0.05$ and $p<0.01$, respectively (Total soluble sugars (TSS), Reducing sugars (TSSR), Non-reducing sugars (TSSN), Total phenolic content (TPC), Total stevia glycosides (TSGs), flavonoid (FLV))

Table 6 Analysis of variance of the effects of KN hormone on biochemical traits of stevia

S.O. V	Df	TSS	TSSN	TSSR	TPC	TSGs	FLV
Block	2	45.54 ^{ns}	17.73 ^{ns}	119.75 ^{ns}	15.20 ^{ns}	o.38 ^{ns}	0.89 ^{ns}
KN	3	230 *	120.47 *	619.51 **	262.75 **	0.39 ^{ns}	11.16 *
Error	6	27.10	6.16	55.89	13.40	0.26	0.47
CV (%)	-	5.06	15.46	8.61	13.40	5.28	6.87

ns, (*) and (**) non-significant and significant at $p<0.05$ and $p<0.01$, respectively
(Total soluble sugars (TSS), Reducing sugars (TSSR), Non-reducing sugars (TSSN), Total phenolic content (TPC), Total stevia glycosides (TSGs), flavonoid (FLV))

Table 7 Mean comparisons of GA and KN hormones on biochemical traits of stevia

S.O. V	Df	CHLa	CHLb	N	P	K
Block	2	0.001 ^{ns}	0.001 ^{ns}	0.010 ^{ns}	0.002 ^{ns}	0.010 ^{ns}
GA	3	0.037 *	0.010 *	0.140 **	0.020 **	0.310 *
Error	6	0.004	0.001	0.004	0.002	0.070
CV (%)	-	13.64	18.19	3.84	12.55	11.81

Means followed by similar letters have not significantly different at α= 5% probability level, LSD test.
ns, (*) and (**) non-significant and significant at $p<0.05$ and $p<0.01$, respectively

Table 8 Analysis of variance of the effects of GA hormones on biochemical traits of stevia

Treatment ppm	TPC (mg/g)	TSGs (%)	FLV (mg/g)	TSGs (%)
Control	40.55 a	9.56 c	11.10 a	9.56 c
GA100	59.37 a	10.50 bc	13.47 a	10.50 bc
GA200	61.00 a	12.70 a	13.67 a	12.70 a
GA300	57.46 a	11.56 ab	13.69 a	11.56 ab
Control	40.55 c	9.54 a	11.10 c	9.54 a
KN20	52.22 b	9.48 a	12.53 b	9.48 a
KN40	61.79 a	10.04	13.11 b	10.04
KN60	58.50 ab	10.21 a	16.87 a	10.21 a

(Chlorophyll a (CHLa) and Chlorophyll b (CHLb), Nitrogen (N), Phosporous (P), potassium (K))

Table 9 Analysis of variance of the effects of KN hormone on biochemical traits of stevia

S.O. V	Df	CHLa	CHLb	N	P	K
Block	2	0.0007 ^{ns}	0.0004 ^{ns}	0.0006 ^{ns}	0.0010 ^{ns}	0.1500 ^{ns}
KN	3	0.0300 *	0.0070 **	0.0800 *	0.0500 *	0.1700 *
Error	6	0.0040	0.0002	0.0090	0.0010	0.0700
CV (%)	-	14.24	7.55	5.93	9.92	11.52

ns, (*) and (**) non-significant and significant at $p<0.05$ and $p<0.01$, respectively
(Chlorophyll a (CHLa) and Chlorophyll b (CHLb), Nitrogen (N), Phosporous (P), potassium (K))

Table 10 Mean comparisons of GA and KN hormones on biochemical traits of stevia

Treatment ppm	N (%)	P (%)	K (%)
Control	1.53 c	0.27 b	2.05 b
GA100	1.75 b	0.45 a	2.23 b
GA200	2.03 a	0.48 a	2.23 b
GA300	1.93 a	0.47 a	2.80 a
Control	1.53 c	0.27 c	2.05 b
KN20	1.56 bc	0.43 b	2.44 ab
KN40	1.73 ab	0.60 a	2.60 a
KN60	1.89 a	0.42 b	2.5 ab

Means followed by similar letters have not significantly different at α= 5% probability level, LSD test.

DISCUSSION

Based on our results, it can be outlined that the growth induction in stevia causing by external application of GA and KN hormones may be created through accelerate Chl biosynthesis and their crucial roles in promoting cell division because an increment was observed in the number of stevia leaves, plant height and concentrations of Chla and Chlb. it also

has been clearly observed GA and KN hormones stimulated the dry matter allocation in its stem and induce photo assimilates flow into the leaves which could be a useful trait in leafy plant, such as stevia, confirming previous findings observed in stevia in both well-watered and drought stress conditions [25]. In the present study, the low value of HI in hormones-treated plants, in comparison with the control might be because of relatively more increase in total biomass of plant as compare to economic yield. The increased biomass production also coupled with moderate concentration of N, P and K in leaf and stem [26]. There was also an increase in the nutrient content of grain (N, P, and K) in hormonal treatments. The results of this study are in agreement with those reported by Tariq Aftab [26] who reported that foliar spray of GA increased shoot and root lengths, plant height, and leaf lengths in *Artemisia annua* L. Gurmani also reported similar results for cucumbers treated with KN [27]. Similarly, Rafique found that GA enhanced N, P, and K content of chickpea seeds [28]. Foliar GA treatment improved nitrate reductase activity as well as stomatal leaf function. In mustard, GA application increased nitrate reductase activity by 24.68% and nitrogen content in the leaves by 21.45%. CK application can alleviate the enzymatic activity of nitrate reductase (NR)under water deficit stress, which contributed to less reduction in total N content in plant. Enhancing stomatal leaf function may contribute to the enhancement of photosynthetic rate [28]. There is evidence that hormone concentrations are positively correlated with phenolic compound biosynthesis and accumulation [6]. Moreover, recent studies have confirmed previous findings that GA and KN hormones significantly increased flavonoid content in Taraxacum officinale [29]. For KN application on stevia, similar relationships were observed [6]. The concentration of hormone influences soluble sugar content in Magnolia shoots [30], stevia [31]. The same results were reported by Simlat in stevia plants treated with KN [6]. According to the results of KN experiments, there were no differences between the control and other treatments in terms of TSGs. In contrast, GA treatment resulted in significant changes. Previous studies have shown that GA biosynthesis can modulate SG production. A number of previous studies have indicated that GA had a synergistic effect on SGs because SG-related genes were upregulated by GA treatment compared with controls [10]. However, some research suggests GA may not have a repulsion effect on SG contents [12].

CONCLUSION

The results of the present study show that GA and KN at different concentrations significantly improved the contents of CHL, N, P, and K. These findings suggest that GA and KN are dose-dependent, and higher GA levels may result in higher TSG levels. Furthermore, 100 ppm of GA is recommended for sugars. Furthermore, our findings indicated that KN has a negligible effect on TSGs. Accordingly, it is concluded from this study that exogenous GA and KN application of a particular hormone concentration improved growth and yield in stevia; however, more research is required to determine the effects of hormonal interactions.

DECLARATION OF COMPETING INTEREST

The authors approved the manuscript and declared no conflict of interest

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