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Influence of macro nutrients and plant growth regulators on biochemical traits and antioxidant production in strawberry

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Abstract
A field experiment was conducted during 2010-12 to study the effect of different levels of macro-nutrients viz. nitrogen (N) and potassium (K) in combination with plant growth regulators viz. gibberellic acid (GA_3) and paclobutrazol (PP_{333}) on bio-chemical attributes of strawberry cv. Chandler. The experiment was laid out in the factorial randomized block design with three replications and three plants per replication. The treatments were formulated with 3 levels of N (40, 60 and 100 Kg ha^{-1}) and K (20, 40 and 60 kg ha^{-1}) applied in the form of urea and MOP, respectively along with foliar spray of 50 ppm GA_3 and 300 ppm PP_{333}. Among the treatment combinations, application of 60 kg of N and 40 kg of K along with 300 ppm PP_{333} was found to be superior in terms of total soluble solids (9.39 and 9.46 °Brix), total sugars (6.94 and 7.09%) and total anthocyanin content (46.67 and 48.77 mg $100g^{-1}$ edible portion) during 2010-11 and 2011-12, respectively, while total soluble proteins and total free amino acids were significantly more in plants supplied with 60:40 kg ha-1 N:K combined with 50 ppm GA_3 and plants treated with 100:60 kg ha-1 N:K in combination with 50 ppm GA_3 in both the years, respectively.

Keywords: Antioxidant, macro nutrient, plant growth regulator, strawberry.

Introduction
In recent years, there has been a great deal of attention toward the effect of free radicals on human health. Free radicals are produced within our body from exposure to various factors such as smoking, chewing tobacco, excessive exposure to sunlight and exposure to heavy metals for a long duration (Van Wijk *et al.*,) [40]. Antioxidants are thought to be highly effective in scavenging free radicals, suppressing their formation or opposing their action (Kattappagari *et al.*) [20]. Strawberry (*Fragaria x ananassa* Duch.), the herbaceous perennial plant of family Rosaceae, possesses good source of natural antioxidants like vitamin C, total phenols, anthocyanins etc. which provide protection against harmful free radicals (Tulipani *et al.*, Panico *et al.*,) [39, 32] and thus antioxidant activity of strawberry contributes to the prevention of cancer, cardiovascular and other chronic diseases (Hannum; Wang,) [13, 41]. It is well known as "super fruit" for its potential in the nutraceutical and functional food markets (Tulipani *et al.*,) [39]. It is one of the most important soft fruits acclaimed for its refreshing characteristics, aroma, lucrative appearance and good nutritive value (Singh *et al.*,) [38]. Delicacy in flavour and richness in vitamins and minerals, makes strawberry a highly favoured food in the diet of millions of people around the globe (Bhat *et al.*,) [5].

Strawberry is a short day plant having fast growth (two to three months) and is highly influenced by environmental conditions, such as light, salinity, water quality, temperature and nutrients (Li *et al.*,) [26]. Due to its development speed, the crop needs sufficient absorption of macronutrients in order to meet the photosynthetic demand and adequate fruit growth (Li *et al.*,) [26]. Harvesting strawberry fruit removes a significant amount of nitrogen (N) from plants (Archbold and Mac Kown, 2). Potassium (K) is also highly demanded by the crop for directly favoring fruit quality and increasing the contents of total soluble solids and ascorbic acid (Pettigrew,) [33]. Rodas *et al.* [36] indicated that physical and chemical properties of strawberry were influenced by combined doses of N and K fertilizers. The growth regulators are known to have effect on improvement of fruit quality like increase in total soluble solids, reducing sugars, total sugars and vitamin-C and decrease in fruit acidity (Singh *et al.*, Reddy *et al.*,) [37, 34]. The combined application of mineral nutrients and growth regulators are the new practices nowadays adopted for higher fruit production and improved fruit quality (Dutta and Banik,) [10]. Therefore, this study aimed to evaluate different biochemical attributes of the strawberry cultivar 'Chandler' in response to different doses of N and K and foliar spray of gibberellic acid (GA_3) or paclobutrazol (PP_{333}).

Material and Methods

The present investigation was conducted at Experimental Orchard, Dept. of Horticulture, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) during 2010-12. The experimental site was situated between a latitude of 24.47°-26.56° N and longitude of 81.12°- 85.89° E on an elevation of about 113.00 metres above mean sea level of gangetic alluvium in eastern Uttar Pradesh. The climate of this area was subtropical and semi-arid having hot summer and cold winter with average annual rainfall of around 1200 mm. The soil of the experimental plot was silt loam, alkaline in reaction with soil pH 8.10; the available N, P and K were 182.33, 13.22 and 202.50 kg ha^{-1} with 0.33% organic carbon. The experiment was laid out in randomized block design (RBD) with 19 treatments having three replications and three plants per replication. The runners of strawberry cv. Chandler were collected from Dr. Y.S. Parmar University of Horticulture & Forestry, Solan, Himachal Pradesh for planting. About nine runners were planted at a distance of 30 cm x 30 cm in raised bed of 1 m x 1 m in the first week of October during 2010 and 2011. The graded doses of N (40, 60 and 100 Kg ha^{-1}) and K (20, 40 and 60 kg ha^{-1}) in the form of urea and MOP were applied before planting (i.e. at the time of preparation of raised experimental beds), while recommended quantity of P_2O_5 (40 kg ha^{-1}) through SSP and FYM (50 t ha^{-1}) were applied as a basal dose in all the treatment combinations. The stock solution of PP_{333} was directly made in distilled water. The required amount of GA_3 was first dissolved in alcohol and then desired volume was made with distilled water. Spraying was done on clear and calm day during the morning hours in third week of December. The various treatments followed for the investigation were as follows: T_1 = Control, $T_2 = N_1 K_1$ + 50 ppm GA_3, $T_3 = N_1 K_2$ + 50 ppm GA_3, $T_4 = N_1 K_3$ + 50 ppm GA_3, $T_5 = N_2 K_1$ + 50 ppm GA_3, $T_6 = N_2 K_2$ + 50 ppm GA_3, $T_7 = N_2 K_3$ + 50 ppm GA_3, $T_8 = N_3 K_1$ + 50 ppm GA_3, $T_9 = N_3 K_2$ + 50 ppm GA_3, $T_{10} = N_3 K_3$ + 50 ppm GA_3, $T_{11} = N_1 K_1$ + 300 ppm PP_{333}, $T_{12} = N_1 K_2$ + 300 ppm PP_{333}, $T_{13} = N_1 K_3$ + 300 ppm PP_{333}, $T_{14} = N_2 K_1$ + 300 ppm PP_{333}, $T_{15} = N_2 K_2$ + 300 ppm PP_{333}, $T_{16} = N_2 K_3$ + 300 ppm PP_{333}, $T_{17} = N_3 K_1$ + 300 ppm PP_{333}, $T_{18} = N_3 K_2$ + 300 ppm PP_{333}, $T_{19} = N_3 K_3$ + 300 ppm PP_{333}.

Observations on various bio-chemical attributes were recorded by using standard methods. Nine plants per treatment were selected randomly and eighteen berries from each treatment were randomly selected to record the data on bio-chemical characters. The total soluble solids (TSS) of the berry juice were determined with the help of Erma Hand Refract meter (0-32ºB). The titratable acidity, sugars and ascorbic acid were determined by method as suggested in A.O.A.C. [1]. Soluble proteins were estimated by the method of Lowry *et al*., [27]. Total phenols were calculated against a standard curve tannic acid (Bray and Thorpe,) [6]. Total free amino acids were estimated according to the method described by Lee and Takahashi [24]. Anthocyanin, content was expressed in absorption units at 530 nm per cent gram fresh berry (Harborne,) [14]. The two years experimental data were subjected to ANOVA (Gomez and Gomez, 12), where appropriate means were separated with least significant difference analysis.

Result and Discussion

Soil application of nutrients (N and K) prior to planting followed by foliar spray of plant growth regulators (GA_3/ PP_{333}) in present study significantly influenced bio-chemical attributes in strawberry cv. Chandler. Significantly highest total soluble solids (9.39 and 9.46 ºBrix) were recorded in T_{15} during both 2010-11 and 2011-12, respectively (Table 1).

Table 1. Effect of macro nutrients and plant growth regulators on bio-chemical attributes of strawberry.

Treatment	TSS (ºBrix)		Acidity (%)		Total sugars (%)		Reducing sugar (%)		Non-reducing sugar (%)	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
T_1	7.23	7.45	0.68	0.77	6.17	6.34	4.15	4.08	2.02	2.26
T_2	7.70	8.33	0.77	0.76	6.23	6.52	4.38	4.23	1.84	2.29
T_3	7.88	8.85	0.77	0.79	6.46	6.59	4.45	4.20	2.00	2.38
T_4	8.17	8.62	0.74	0.80	6.34	6.46	4.32	4.25	2.02	2.21
T_5	8.41	8.83	0.76	0.73	6.49	6.66	4.31	4.27	2.17	2.38
T_6	8.74	9.08	0.76	0.82	6.68	6.74	4.55	4.38	2.13	2.36
T_7	9.09	9.12	0.73	0.83	6.50	6.56	4.43	4.33	2.07	2.22
T_8	9.21	9.26	0.66	0.81	6.52	6.59	4.35	4.37	2.16	2.22
T_9	8.96	9.10	0.71	0.83	6.61	6.77	4.54	4.38	2.06	2.39
T_{10}	8.78	8.91	0.71	0.80	6.47	6.59	4.57	4.32	1.89	2.26
T_{11}	8.15	8.43	0.73	0.75	6.39	6.49	4.18	4.25	2.21	2.24
T_{12}	8.29	8.52	0.65	0.81	6.45	6.58	4.39	4.28	2.06	2.30
T_{13}	8.41	8.39	0.82	0.79	6.55	6.45	4.39	4.34	2.16	2.11
T_{14}	9.20	9.34	0.67	0.80	6.70	6.74	4.33	4.36	2.37	2.38
T_{15}	9.39	9.46	0.65	0.74	6.94	7.09	4.43	4.51	2.51	2.58
T_{16}	8.77	9.07	0.76	0.85	6.83	6.86	4.46	4.52	2.37	2.34
T_{17}	9.13	9.35	0.74	0.81	6.74	6.80	4.39	4.42	2.35	2.37
T_{18}	9.24	9.26	0.72	0.73	6.83	6.56	4.19	4.27	2.64	2.28
T_{19}	9.05	9.28	0.76	0.73	6.74	6.78	4.29	4.20	2.44	2.57
SE±	0.02	0.02	-	-	0.02	0.01	-	-	-	-
$CD_{0.05}$	0.07	0.09	NS	NS	0.06	0.08	NS	NS	NS	NS

With the onset of fruit ripening, carbohydrates reserves of roots and stem were drawn upon heavily into the fruits and transfer of assimilates from leaves to developing fruits increased due to metabolic sink of fruits. The application of major nutrients (N and K) followed by spray of PP_{333} might have played regulatory role on the absorption and translocation of various metabolites like carbohydrares which affected the quality of fruits. Similar observations have been made by Pandey *et al*. [31] in guava fruits. Bussi and Amicot [7] also reported that nitrogen fertilizer application has positive effect on TSS content. Omotoso and Akinrinde [30] reported significant increase in TSS of pineapple plants with increase in nitrogen rates. They stated highest TSS in crop plants that received 50 kg kg ha^{-1} than those received 100 kg ha^{-1}. Asma *et al.* [4] emphasized that minimum and maximum nitrogen applications had no effect on TSS content but, potassium applications had increased TSS content of fruit. The increase in TSS content with application of K could be related with role of potassium in translocation of sugars from leaves to fruits (Havlin *et al.*,) [15]. The increase in fruit TSS with the application of PP_{333} was confirmed by Reynolds [35] who suggested that the paclobutrazol-induced suppression of lateral-shoot development reduced the photo assimilate demands of these superfluous sinks, resulting in an increase in fruit soluble solids (ºBrix). Similarly, T_{15} recorded maximum total sugars content in fruits (6.94 and 7.09%) during 2010-11 and 2011-12, respectively (Table 1). As sugar content in plant organs is directly related to the size of the cells in the respective plants (Kano *et al.*,) [18] and nitrogen is the major component of chlorophyll and amino acids, thus nitrogen has positive effect on increase in total sugars. Since K is involved in photophosphorylation and transportation of photo assimilates from source tissues via the phloem to sink tissues (Pettigrew,) [33], thus adequate K nutrition has also been associated with increased soluble solids (Lester *et al*.,) [25]. Increase in total sugar content by paclobutrazol application is presumed to be the result of favourable mobilization of photo-assimilates to the developing sink created by maturing fruits (Reddy *et al*.) [34]. Thus increase in fruit TSS and total sugars could be attributed to the cumulative effect of both the fertilizers and PP_{333} application.

Our study exhibited significant influence of nutrients and PGR on free amino acids and total soluble proteins of strawberry (Table 2). Among different treatments, T_{10} recorded significantly highest total free amino acids in fruits (74.29 and 74.51 mg/100g) in both the years, respectively. Total soluble proteins in fruits were found significantly more in T_6 (2.55 and 2.63 mg/g) in 2010-11 and 2011-12, respectively, which were statistically at par with those of T_{17} (2.54 mg/g) in 2010-11. It could be justified by the fact that the inorganic N taken up by the plant is known to be converted into organic N compounds which contain the nitrogen primarily as NH_2 groups. Good K nutrition favours the rapid turnover of Inorganic nitrogen into amino acids and proteins and consequently, potassium improves the effect of nitrogen fertilizer (Kumar *et al.*,) [22]. The crucial importance of potassium in quality formation stems from its role in promoting synthesis of photosynthates and their transport to fruits and to" enhance their conversion into starch, proteins etc. (Mengel and Kirkby,) [28]. Our results also signified the contribution of GA_3 for improvement in total free amino acids and soluble proteins in fruits. Our finding is in close conformity with those of Kaur *et al*., [21] in kinnow mandarin, which could be attributed to involvement of PGR in increasing the formation of rough endoplasmic reticulum that provides the appropriate medium for increasing polyribosome and mRNA (Kaber,) [17]. Thus synergistic effects of fertilisers (N and K) and GA_3 resulted in increased photosynthetic efficiency and translocation of nutrients and other metabolites to the developing fruits thereby leading to the improvement of total free amino acids and soluble proteins (Childers,) [8].

Fruit ascorbic acid content was significantly highest in T_{10} (53.66 mg/100g) during 2010-11 and T_{11} (53.99 mg/100g) during 2011-12 (Table 2). Our study showed that nitrogen application had positive effect on ascorbic acid content in fruits, indicating sufficient N was required to maintain ascorbic acid synthesis, as also stated by Miceli and Miceli [29]. Plausible mechanisms for K-induced increase in fruit ascorbic acid contents include increased synthesis through enzyme activation as well as increased substrate(carbon skeletons) availability, resulting from K-induced improvements in fruit sugar content (Jifon and Lester,) [16]. Our study recorded stimulative effect of PP_{333} on fruit ascorbic acid, which was confirmed by Reddy *et al* [34] in mango. The increase in ascorbic acid of fruits with the application of GA3 was also reported in Cape gooseberry (Kumar *et al.*,) [23].

Table 2. Effect of macro nutrients and plant growth regulators on free amino acids, soluble proteins and antioxidant property traits of strawberry.

Treatment	Total free amino acids (mg/100g)		Total soluble Proteins (mg/g)		Ascorbic acid (mg/100g)		Total phenols (mg/g)		Total anthocyanin (mg/100g)	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
T_1	65.88	64.89	2.31	2.29	45.66	46.26	20.88	22.15	39.43	36.77
T_2	67.56	69.55	2.41	2.49	49.65	51.62	23.25	25.12	44.98	45.88
T_3	70.22	72.38	2.38	2.46	48.22	49.59	25.55	26.33	44.77	42.67
T_4	70.41	72.21	2.32	2.42	48.69	49.66	21.88	22.32	43.88	45.09
T_5	67.08	74.18	2.37	2.47	49.18	49.56	20.77	22.92	44.08	45.09
T_6	70.77	72.24	2.55	2.63	51.26	53.31	20.55	23.55	45.66	45.39
T_7	66.93	73.17	2.42	2.52	52.77	53.78	25.44	25.66	44.05	46.33
T_8	69.55	72.46	2.42	2.47	52.65	53.42	23.94	27.24	42.54	46.33
T_9	72.34	72.35	2.38	2.40	43.15	53.19	23.78	25.49	42.65	46.56
T_{10}	74.29	74.51	2.36	2.38	53.66	53.92	23.73	25.63	44.36	47.34
T_{11}	71.08	66.88	2.35	2.38	53.46	53.99	26.17	23.22	45.18	46.21
T_{12}	70.29	66.98	2.44	2.48	48.52	51.43	21.09	16.09	43.86	41.44
T_{13}	69.05	66.74	2.44	2.44	48.75	51.33	24.93	25.86	43.55	44.49
T_{14}	68.41	69.61	2.45	2.53	48.27	51.23	23.67	21.22	44.46	44.56
T_{15}	68.34	69.77	2.44	2.56	50.28	52.83	24.77	26.92	46.67	48.77
T_{16}	67.87	69.57	2.44	2.54	50.20	52.24	27.09	23.33	44.67	48.43
T_{17}	69.42	68.51	2.54	2.56	50.12	50.86	24.98	23.11	45.88	48.21
T_{18}	68.41	67.88	2.46	2.52	49.88	53.71	24.42	25.88	45.23	46.56
T_{19}	69.33	67.78	2.45	2.48	49.77	52.66	24.93	24.22	44.15	46.51
SE±	0.07	0.08	0.03	0.05	0.01	0.07	-	-	0.28	0.04
$CD_{0.05}$	0.15	0.17	0.06	0.09	0.05	0.22	NS	NS	0.80	0.13

Total anthocyanin content in fruit was significantly more in T_{15} (46.67 and 48.77 mg/100g) during both 2010-11 and 2011-12, respectively (Table 2). The present results exhibited synergistic effect of nutrients like N and K and growth regulator PP333 on fruit anthocyanin content. It could be attributed to the fact that adequate nitrogen levels stimulate the activity of phenylalanine ammonia-lyase, which is involved in anthocyanin synthesis (Kataoka *et al*.,) [19]. Further, as potassium supplies improve the assimilation of nitrogen, both the nutrients might synergistically interact to stimulate anthocyanin synthesis in fruits (Delgado *et al*.,) [9]. Besides, the application of PP_{333} also had effect on reduction in vegetative growth, thereby exposing the fruits to direct sunlight which might have significantly increased total anthocyanin of the fruits (Ashraf *et al*., 3). The increase in total anthocyanin content in fruits with the application of PP_{333} was also reported by Eliwa and Ashour [11] in peach.

Data presented in Table 3 revealed existence of significant positive corrlation between TSS was total sugars (r = 0.827). Total sugars content was positively correlated with total anthocyanin (r = 0.691) and total soluble proteins (r = 0.696). Further, total anthocyanin exhibited significant positive correlation with ascorbic acid (r = 0.667), total phenols (r = 0.507) and total soluble proteins (r = 0.606). Thus the application of nutrients in combination with PGR might have played regulatory role on increased photosynthetic efficiency and translocation of nutrients and other metabolites to the developing fruits which affected the quality of fruits.

Table 3. Correlation coefficient values of bio-chemical characters and antioxidant property traits of strawberry.

Trait	TSS	TA	TS	RS	NRS	A	AA	TP	TSP	TFA
TSS	1.000									
TA	-0.072	1.000								
TS	0.827*	-0.076	1.000							
RS	0.577*	0.398	0.618*	1.000						
NRS	0.647*	-0.363	0.842*	0.098	1.000					
A	0.735*	0.145	0.691*	0.605*	0.462*	1.000				
AA	0.510*	0.010	0.256	0.331	0.099	0.667*	1.000			
TP	0.392	0.220	0.314	0.263	0.206	0.507*	0.416	1.000		
TSP	0.658*	0.195	0.696*	0.530*	0.522*	0.606*	0.369	0.086	1.000	
TFA	0.346	0.213	0.094	0.551*	-0.268	0.373	0.356	0.164	-0.010	1.000

Value with '*' indicates significant correlation between the variables at $t_{0.05}$ (Students T test, Web Agri Stat Package version WASP 2.0, ICAR Research Complex for Goa, India); TA = Titrable acidity, TS = Total sugars, RS = Reducing sugar, NRS = Non-reducing sugar, A = Total anthocyanin, AA = Ascorbic acid, TP = Total phenols, TSP = Total soluble proteins, TFA = Total free amino acids.

From the results of the present investigation, it can be concluded that the combined application of nutrients (60 kg N and 40 kg K ha^{-1}) prior to planting followed by foliar spray of 300 ppm PP_{333} prior to flowering improved bio-chemical attributes of strawberry cv. Chandler. Hence, concerning about nutritional aspects, the particular treatment combination can be recommended for quality fruit production.

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