

Research Article:**QUALITY RESPONSES OF STRAWBERRY (*Fragaria ananassa* DUCH.) FRUITS TO EXOGENOUS GIBBERELIC ACID APPLICATIONS UNDER PROTECTED CONDITION****Bishal Shrestha^{a*}, Kalyani Mishra Tripathi^a, Arjun Kumar Shrestha^a and Rajendra Gautam^b**^aFaculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal^bSamriddhi Agriculture Research and Development Pvt. Ltd., Kathmandu, Nepal

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DOI: <https://doi.org/10.3126/jafu.v6i1.78151>**ABSTRACT**

A greenhouse experiment was conducted to evaluate the qualitative traits of strawberry varieties as influenced by GA₃ applications in Bharatpur-11, Chitwan Nepal. The experiment was laid out in two factorial RCBD comprising 3 strawberry varieties as Factor A i.e. Winter Dawn, Sweet Sensation, and Florida Beauty, and five concentrations of gibberellic acid (GA₃) i.e. Control, 50 ppm, 75 ppm, 100 ppm, and 125 ppm as Factor B replicated 4 times. The physicochemical analyses revealed that the maximum TSS (8.65 °Brix) was observed in the variety Florida Beauty and the minimum TSS (7.03 °Brix) was recorded in the variety Winter Dawn. Similarly, the maximum TSS (8.62 °Brix) was observed in plants treated with 100 ppm GA₃ and the minimum TSS (7.34 °Brix) was recorded in plants treated with 125 ppm GA₃. A higher amount of antioxidants (71.10%) was observed in the variety Sweet Sensation and a minimum in the variety Winter Dawn (67.02%) while the maximum antioxidants (74.45%) were recorded in fruits obtained from plants treated with 100 ppm and the minimum amount of antioxidants (64.32%) was observed in fruits obtained from control. This study revealed the application of 100 ppm GA₃ was found to be better in all nutritional characteristics of strawberry varieties in protected conditions.

Key words: Antioxidants, strawberry varieties, vitamin C**INTRODUCTION**

The natural hybrid strawberry (*Fragaria ananassa* Duch.) is cultivated in cooler climates across the world for its tasty fruits that are abundant in vitamins, minerals, and other bioactive substances. (Oszmianski & Wojdylo 2009). It belongs to the Rosaceae family, and all cultivated varieties are octoploid with a chromosome count of 2n=56. Strawberries are highly popular globally and are considered an important soft fruit second to grapes. Among the many fruits grown, it gives the quickest return in the shortest possible time, hence it is an important high-value horticultural crop (Bakshi et al., 2014). The fruits are quite popular for their distinct aroma, red color, juicy pulp, sweet and acidic blend, and higher content of phenolics, flavonoids, aromas, and vitamins (Hakkinen & Torronen, 2000). A wide range of agroecological conditions ranging from the Terai in the south to the Hills in the north provide climate conditions that are suitable for an array of crops. Farmers in the temperate hills cultivate strawberries as an important fruit crop, especially in the Nuwakot district. There are early indications that Okharpauwa and Kakani have benefited greatly from strawberry farming. Strawberry is a newly identified export product of Nepal, with improved marketing; this crop will be the major livelihood option of the farmers in Nuwakot along with adjoining potential districts (Dhakal et al., 2022). However,

issues related to fruit size, uniformity, and overall quality often arise in strawberry cultivation, especially under varying environmental conditions. There are different constraints regarding the blooming period and the size of the flower. Depending upon its market cycle and perishable nature, there is a need for efficient flowering and fruiting mechanisms to balance the production and demand scenario. Besides this, strawberries in Nepal are of small size and their quality is also low. In this context, the exogenous application of plant growth regulators could be a viable option to sustain the production and quality of strawberries in Nepal.

Plant growth regulators (PGRs) have a direct impact on both the quantitative and qualitative elements of fruit growth and are useful instruments for increasing fruit yield. Together with the appropriate growing climate, they are essential in regulating the different growth and developmental stages of plants (Ingle et al. 2001). It was discovered that the synthetic cytokinin CPPU [N-(2-chloro-4-pyridyl)-N phenyl-urea], GA₃, NAA, and ethephon were highly effective in promoting fruit growth in cranberries, strawberries, apples, and grapes. (Hamano et al., 2006). The use of GA₃ in strawberries has been reported to influence early flowering, prolonging the duration of flowering, fruit set, fruit size, harvesting, and yield (Palei et al., 2016; Vishal et al., 2016). It promotes vegetative development, speeds up maturity, aids in cell elongation and expansion, and boosts fruit output and quality.

As strawberries have been cultivated for around 25 years but are not a fully utilized crop in Nepal, there is no standardization or optimization of PGR for commercial production, and a study on their use efficiency for different varieties has not been conducted yet. Gibberellic acid (GA₃) is a plant growth regulator influencing fruit development, including size, shape, and ripening processes. Despite its potential, the optimal application of exogenous GA₃ for enhancing strawberry fruit quality, particularly under protected or controlled environmental conditions (such as greenhouses or tunnels), remains insufficiently explored. Different interventions are required to manage the flowering and fruiting period as well as the yield and quality of strawberry fruits. As it is a crop of export potential and industrial purpose, intervention in its production package is also a must. Therefore, proper varietal selection and other managerial interventions need to be studied.

MATERIALS AND METHODS

Location of the research:

The experiment was conducted in the green greenhouse of Nepal Polytechnic Institute, Bharatpur-11, Chitwan, and the postharvest study was conducted at the laboratory of the same institute.

Duration of research

The potted greenhouse experiment was carried out for two years during the winter – summer season of the planting years 2021 and 2022. The first-year experiment was conducted from October 2021 to March 2022 and the postharvest study took place from January 2022 to April 2022. Similarly, the second-year experiment was conducted from October 2022 to March 2023 and the postharvest study was conducted from January 2023 to April 2023.

Experimental design

The research was conducted on two factorial Randomized Complete Block Design (RCBD). Factor A comprised three varieties of strawberry, and Factor B comprised five concentrations of gibberellic acid (GA₃). There were altogether 15 treatment combinations which were replicated four times. Each experimental unit comprised 25 plants and 5 plants were selected as samples and tagged from each unit. GA₃ was sprayed two times during the total growth period, i.e., 30 days and 40 days after transplantation. The fruit from sample plants was collected, and juice

was extracted for chemical studies,

Treatment details

Factor A: Varieties

V₁ = Winter Dawn

V₂ = Sweet Sensation

V₃ = Florida Beauty

Factor B: Concentrations of gibberellic acid (GA₃)

G₁: Control

G₂: 50 ppm

G₃: 75 ppm

G₄: 100 ppm

G₅: 125 ppm

Treatment combinations

Table 1. Details of treatment combinations used

SN	Variety (V)	Concentrations of GA ₃ (G)	Treatment combinations (V*G)
1	Winter Dawn	Control	Winter Dawn × Control
2	Winter Dawn	50 ppm	Winter Dawn × 50 ppm
3	Winter Dawn	75 ppm	Winter Dawn × 75 ppm
4	Winter Dawn	100 ppm	Winter Dawn × 100 ppm
5	Winter Dawn	125 ppm	Winter Dawn × 125 ppm
6	Sweet Sensation	Control	Sweet Sensation × Control
7	Sweet Sensation	50 ppm	Sweet Sensation × 50 ppm
8	Sweet Sensation	75 ppm	Sweet Sensation × 75 ppm
9	Sweet Sensation	100 ppm	Sweet Sensation × 100 ppm
10	Sweet Sensation	125 ppm	Sweet Sensation × 125 ppm
11	Florida Beauty	Control	Florida Beauty × Control
12	Florida Beauty	50 ppm	Florida Beauty × 50 ppm
13	Florida Beauty	75 ppm	Florida Beauty × 75 ppm
14	Florida Beauty	100 ppm	Florida Beauty × 100 ppm
15	Florida Beauty	125 ppm	Florida Beauty × 125 ppm

Planting:

For planting, bare-root healthy strawberry transplants were planted in a plastic pot with 30cm diameter and 40 cm height in the first week of October. The potting media used were cocopeat, peat moss, and obifert at the ratio of 1:1:1.

Nutrient management

The nutrition was managed as early growth to 1st fruit set= 3:5:4 NPK 4kg/ha/wk. and 1st fruit set to harvest = 2:1:4 NPK 4kg/ha/wk. The nutrients were supplied by urea, Pramukh, and NP plus. Nutrient solutions were made and used 150 mL at a time by drenching.

Harvesting

Harvesting took place when the fruits reached 70% red in color indicating physiological maturity.

Parameters studied:

Various quality parameters were recorded from the fruits of sample plants. Total soluble solids (TSS) were measured by a hand-held refractometer (ERMA Inc., Tokyo, Japan) using juice extracted from the strawberry fruit and expressed as °Brix, pH was measured by using an electric pH meter (Hanna), juice content (%) was measured by percentage volume method, titratable acidity (TA) (%) and ascorbic acid content (mg/100 g) were measured by following Rangana (1986). The antioxidant activity (%) was measured by following the DPPH assay method as described by Williams et al. (1995), and total phenolic content was determined using Folin-Ciocalteu's colorimetric assay method (Singleton & Rossi, 1965).

Data analysis:

All data recorded was processed using Microsoft Excel. Statistical analysis and relation among treatments were established for the selected parameters (Gomez & Gomez, 1984). The statistical tool R-stat was used for the analysis of variance, DMRT test, and other data analysis as per the requirement.

RESULTS AND DISCUSSION

Table 2 presents pooled data concerning total soluble solids, titratable acidity, and TSS/TA of different varieties of strawberries as influenced by the different GA₃ concentrations. The statistical analysis result showed a significant difference in the TSS of different strawberry varieties. The maximum TSS (8.65 °Brix) was observed in the variety Florida Beauty and the minimum TSS (7.03 °Brix) was recorded in the variety Winter Dawn. The result of statistical analysis showed a significant difference in TSS because of the application of different GA₃ concentrations in strawberries. The maximum TSS (8.62 °Brix) was observed in plants treated with 100 ppm GA₃ followed by plants treated with 75 ppm GA₃ (8.49 °Brix) and the minimum TSS (7.34 °Brix) was recorded in plants treated with 125 ppm GA₃.

Differences in the chemical characteristics between the varieties were noted in the experiment. The fluctuation in different parameters may be caused by the varying levels of expression of the genes that affect flowering and fruiting because of tropical agroclimatic conditions. The TSS concentration of the fruits was shown to be influenced by the temperature and humidity levels during the fruit's growth and ripening phases. The increase in TSS is due to the lower number of fruits and higher accumulation of photosynthates. Similarly, minimum days to flowering and more days to fruit maturity may have resulted in increased TSS values. The above results are consistent with the findings of Menzel and Smith (2014). According to Rajbhar et al. (2015), strawberry varieties with GA₃ 100ppm + vermicompost @100q/ha have higher TSS (10.68 °Brix).

Table 2. Mean values for qualitative properties of strawberry varieties as influenced by the different GA₃ concentrations at Bharatpur, Chitwan, Nepal during the planting years 2021 and 2022.

Treatments	Total soluble solids (⁰ Brix)	Titrateable acidity (%)	TSS/TA
Varieties (Factor A)			
Winter Dawn	7.03 ^c	0.72 ^a	9.82 ^b
Sweet Sensation	8.13 ^b	0.69 ^b	11.81 ^a
Florida Beauty	8.65 ^a	0.74 ^a	11.85 ^a
SEM	0.48	0.01	0.67
LSD _{0.05}	0.36 ^{***}	0.02 ^{***}	0.73 ^{***}
GA ₃ Doses (Factor B)			
Control	7.37 ^c	0.62 ^d	11.91 ^a
50 ppm	7.86 ^b	0.68 ^c	11.57 ^{ab}
75 ppm	8.49 ^a	0.79 ^b	10.83 ^{bc}
100 ppm	8.62 ^a	0.82 ^a	10.51 ^c
125 ppm	7.34 ^c	0.67 ^c	10.96 ^{abc}
SEM	0.27	0.04	0.26
LSD _{0.05}	0.47 ^{***}	0.028 ^{***}	0.94 [*]
Interaction effect			
SEM	0.24	0.02	0.30
LSD _{0.05}	Ns	Ns	Ns
Grand mean	7.94	0.72	11.16
CV %	7.19	4.78	10.24

Means followed by the same letter(s) in a column do not differ significantly at 5% ($p \leq 0.05$) in DMRT. Level of significance in DMRT * = Significant at 5% ($p \leq 0.01$), ** = Significant at 1% ($p \leq 0.001$), ns = Not significant at 5% ($p \leq 0.05$). LSD = Least Significant Difference, SEM = Standard error of the mean, CV = Coefficient of variation.

A highly significant difference was observed in the titrateable acidity of strawberry varieties at a 5% level of significance. Variety Florida Beauty showed significantly higher (0.74%) titrateable acidity compared to variety Sweet Sensation (0.69%). The result of statistical analysis showed a significant difference in titrateable acidity because of GA₃ application in strawberries. Maximum titrateable acidity (0.82%) was observed in plants treated with 100 ppm GA₃ followed by plants treated with 75 ppm GA₃ (0.79%) and the minimum titrateable acidity (0.62%) was recorded in the control. The higher TA might have been observed due to the lower TSS content and late fruiting stage which initiated early ripening. The result follows the findings of Kurian (2015), who obtained higher TA in late varieties.

The result of statistical analysis showed a significant difference in the TSS/TA ratio of strawberry varieties. Variety Florida Beauty showed a significantly higher (11.85) TSS/TA ratio which was at par with variety Sweet Sensation (11.81) while the minimum TSS/TA ratio was recorded in the variety Winter Dawn (9.82). The result of statistical analysis showed a significant difference in the TSS/TA ratio because of GA₃ applications in strawberries. Maximum TSS/TA ratio (11.91) was observed in control plants followed by plants treated with 50 ppm GA₃ (11.57) and the minimum TSS/TA ratio (10.51) was recorded in plants treated with 100 ppm GA₃. No

significant difference in TSS, TA, and TSS/TA ratio was observed in strawberry varieties due to the interaction effect with GA₃ concentrations.

Table 3. Mean values for qualitative properties of strawberry varieties as influenced by the different GA₃ concentrations at Bharatpur, Chitwan, Nepal during the planting years 2021 and 2022.

Treatments	Vitamin C (mg/100 g)	Antioxidant (%)	Total phenols content (mg GAE/g)
Varieties (Factor A)			
Winter Dawn	61.95	67.02 ^b	0.24 ^c
Sweet Sensation	62.55	71.10 ^a	0.41 ^a
Florida Beauty	63.04	67.91 ^b	0.39 ^b
SEM	0.32	1.24	0.05
LSD _{0.05}	Ns	1.45***	0.02***
GA ₃ Doses (Factor B)			
Control	58.29 ^c	64.32 ^d	0.29 ^d
50 ppm	61.55 ^b	66.93 ^c	0.33 ^c
75 ppm	64.87 ^a	72.28 ^b	0.38 ^b
100 ppm	67.30 ^a	74.45 ^a	0.41 ^a
125 ppm	60.56 ^{bc}	65.40 ^{cd}	0.33 ^c
SEM	1.60	1.99	0.02
LSD _{0.05}	2.59***	1.87***	0.02***
Interaction effect			
SEM	0.88	1.16	0.02
LSD _{0.05}	Ns	Ns	Ns
Grand mean	62.51	68.68	0.35
CV %	5.04	3.32	8.04

Means followed by the same letter(s) in a column do not differ significantly at 5% ($p \leq 0.05$) in DMRT. Level of significance in DMRT *=Significant at 5% ($p \leq 0.01$), **=Significant at 1% ($p \leq 0.001$), ns=Not significant at 5% ($p \leq 0.05$). LSD= Least Significant Difference, SEM= Standard error of the mean, CV=Coefficient of variation.

There was no significant difference in the Vitamin C content of different strawberry varieties. A significant difference was observed in the Vitamin C content by application of GA₃ on strawberries as shown in Table no.3. The maximum Vitamin C content (67.30 mg/100g) was recorded in plants treated with 100 ppm GA₃ followed by treatment with 75 ppm GA₃ (64.87 mg/100g) and the minimum Vitamin C content (58.29 mg/100g) was observed in fruits obtained from plants from control. The result of statistical analysis showed no significant difference in Vitamin C content due to the interaction effect of variety and GA₃ concentrations.

The result of statistical analysis at a 5% level of significance showed a significant difference in the antioxidant properties of different strawberry varieties. A higher amount of antioxidants (71.10%) was observed in the variety Sweet Sensation and a minimum in the variety Winter Dawn (67.02%).

Sharma and Singh (2009) found that the strawberry variety Chandler had the highest juice content (74.8%) and ascorbic acid content (50.4 mg/100 g) when plants were sprayed twice with GA₃ (75 ppm) in mid-November and mid-February. The higher TA might have been observed due to the lower TSS content and late fruiting stage which initiated early ripening. The result follows the findings of Kurian (2015), who obtained higher TA in late varieties. Higher acidity levels and lower total soluble solids may have led to higher Vitamin C content. The higher ascorbic acid content in the early period could be due to its precocity. According to Singh et al. (2008), fruit with a greater ascorbic acid content may have grown more vegetatively because there was more photosynthate available for the buildup of sugar and acid.

There was a significant difference in the amount of antioxidants because of the application of different concentrations of GA₃ in strawberries. Maximum antioxidants (74.45%) were recorded in fruits obtained from plants treated with 100 ppm GA₃ followed by treatment with 75 ppm GA₃ (72.28%) while the minimum amount of antioxidants (64.32%) was observed in fruits obtained from control.

The antioxidant capacity, flavonoids, phenolic acids, and vitamins may further enhance protective properties against oxidative cellular damage. The determination of the total antioxidant activity allows a more realistic evaluation of the potential protective effect of a strawberry. In similar kinds of studies, three varieties of strawberry, Dover, Campineiro, and Oso Grande presented similar antioxidant activities (Cordenunsi et al., 2005). Phenolic compounds found in fruits are secondary metabolites whose major portion is represented by flavonoids and phenolic acids. The higher phenols are one of the important varietal attributes.

The statistical analysis result showed a significant difference in the total phenols content of different strawberry varieties. The maximum total phenols content (0.41 mg GAE/g) was recorded in the variety Sweet Sensation followed by Florida Beauty (0.39 mg GAE/g) and the minimum total phenols content (0.24 mg GAE/g) was recorded in the variety Winter Dawn. The result of statistical analysis showed a significant difference in total phenols content in strawberries because of different GA₃ concentrations. The maximum total phenols content (0.41 mg GAE/g) was observed in plants treated with 100 ppm GA₃ followed by plants treated with 75 ppm GA₃ (0.38 mg GAE/g) and the minimum total phenols content (0.29 mg GAE/g) was recorded in control. No significant difference in total phenols content was observed in strawberry varieties due to the interaction effect with GA₃ concentrations.

Furthermore, the endogenous balance between promoters and inhibitors was changed in favor of the metabolic pathway that forms fruit by the exogenous application of GA₃ (Sharma & Sharma, 2006). According to Kumar et al. (2012), applying 80 ppm of GA₃ to strawberries enhanced the TSS, Acidity, and other bioactive compounds in strawberries. The fact that gibberellin hormones regulate the mobilization of metabolites within the plant and that developing fruit are known to be highly active metabolite sinks that mobilize metabolites and their flow vegetative parts may also account for the increase in berry weight. Kumar et al. (2014) reported that high-concentration GA₃ hastens the days to initiate flowering. Stuart and Cathey (1961) observed that Gibberellins have a huge impact on flowering and inflorescence production which affects the quality parameters of the fruits. Adams et al. (1975) reported that the gibberellins are known to influence both cell division and cell enlargement. Moreover, the successful fertilization of the ovule is followed by cell division and cell expansion resulting in the growth of the fruit. Davis (2004) reported that the application of gibberellic acid increased cell size and/or cell numbers. The contribution of GA₃ in fruit quality, taste, and aroma is due to the role of these hormones in transporting metabolites to the developing fruit which is considered an extremely active

metabolic “sink”, and it is perhaps enough to contribute to fruit growth. The increase in fruit nutritional quality may also be explained by the fact that hormones play a regulatory role in the mobilization of metabolites within the plant, and it is a well-established fact that developing fruits are extremely active metabolite sinks that mobilize metabolites and their flow vegetative parts.

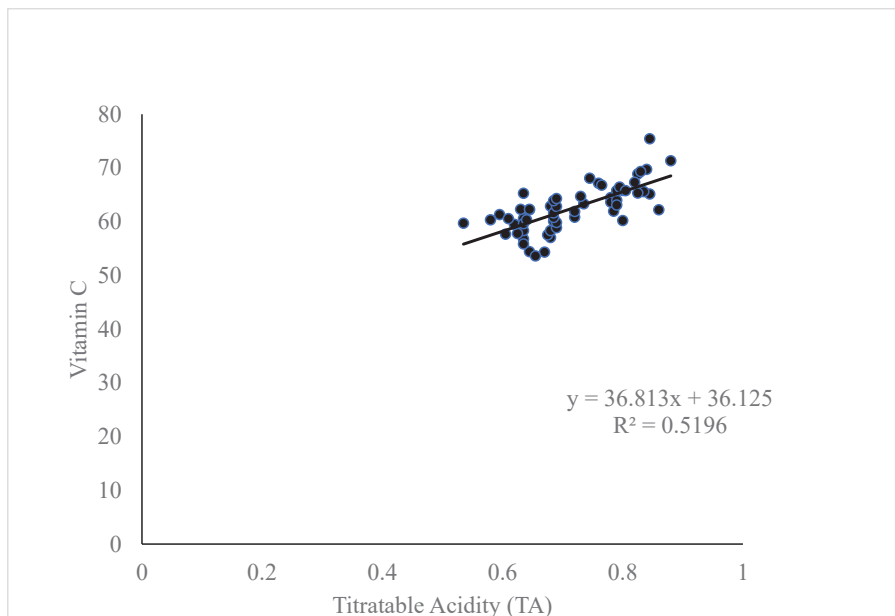


Fig. 1. Simple linear regression between Titratable Acidity (TA) and vitamin C

The graph illustrates the relation between titratable acidity (TA) and vitamin C concentrations. There was a positive linear trend, as TA rises the vitamin C concentration also tends to rise. Similarly, the regression equation, $y = 36.813x + 36.125$, indicates that for each unit increase in TA, vitamin C increases by 36.813 units, starting from a baseline value of 36.125 when TA is zero. The coefficient of determination ($R^2 = 0.5196$) reveals that about 52% of the variation in vitamin C can be attributed to changes in TA, suggesting a moderate correlation.

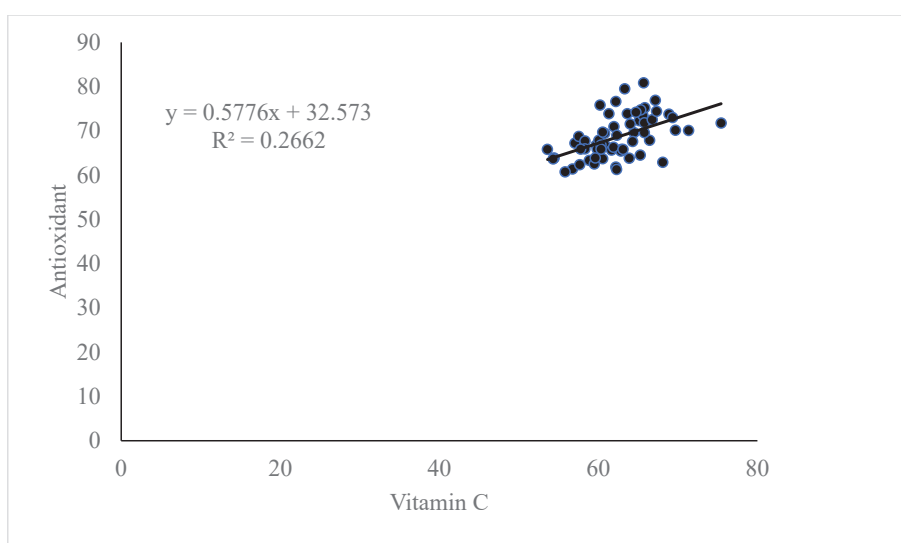


Fig. 2. Simple linear regression between vitamin C and antioxidant content

This graph illustrates a positive linear trend between vitamin C and antioxidant content, suggesting that as vitamin C levels increase, antioxidant levels also tend to rise. The regression

equation, $y = 0.5776x + 32.573$, indicates that for each unit an increase in vitamin C, antioxidant levels rise by 0.5776 units, starting from a baseline antioxidant value of 32.573 when vitamin C is at zero. The R^2 value of 0.2662 implies that about 27% of the variation in antioxidant levels can be explained by vitamin C, indicating a weaker correlation compared to the relationship between TA and vitamin C.

Table 4. Correlation between Titratable acidity (TA), Vitamin C, and Antioxidants.

	Titratable acidity (TA)	Vitamin C	Antioxidants
Titratable acidity (TA)	1		
Vitamin C	.721**	1	
Antioxidant	.646**	.516**	1

** Correlation is significant at the 0.01 level (2-tailed)

The correlation table reveals notable positive relationships between Titratable acidity (TA), Vit C, and Antioxidant levels, all significant at the 0.01 level. There is a strong correlation between TA and Vit C ($r = 0.721$), indicating that higher levels of Titratable acidity (TA) are linked to increased levels of Vitamin C. Additionally, Titratable acidity (TA) and Antioxidants show a moderately strong correlation ($r = 0.646$), suggesting a positive connection between these two variables. Lastly, Vitamin C and Antioxidants exhibit a moderate correlation ($r = 0.516$), emphasizing their positive relationship. These findings imply that the three variables are interconnected with the strongest associations involving Titratable acidity (TA).

CONCLUSION

The present study concluded that there is a presence of significant variability in chemical attributes among the tested varieties of strawberries and the application of different concentrations of GA_3 . Considering desired and selective attributes, Florida Beauty was found superior among tested varieties in terms of sweetness, acidity, and vitamin C while Sweet Sensation was better for antioxidant properties and phenols. The application of 100 ppm GA_3 was found to be better in all nutritional characteristics of strawberry varieties. This study widens the prospect of the selection of low-chilling strawberry varieties and the use of GA_3 for commercial cultivation in the tropical region of Nepal based on grower and consumer demand. However, selected varieties and their response to hormones should be tested in farmer's fields at multiple representative locations before recommendations are made considering the yield, market, and consumer preference.

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