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The Effects of Gibberellic Acid and Nitrogen on Edible Traits of Sweet Violet (*Viola odorata*) and Commercial Violet (*Viola tricolor*)

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Abstract

The cultivation of edible flowers, e.g. violet, which is native to Iran, is a good way to increase the availability of these new and nutritionally valuable sources to humans. This research employed a factorial experiment based on a randomized complete block design in three replications to explore the interactive effects of gibberellic acid (GA) at three rates of 0, 150, and 300 mg L^{-1} and nitrogen (N) at three rates of 0, 100, and 200 mg L^{-1} on the traits of two violet species (*Viola tricolor* and *V. odorata*). The results showed that the application of GA and N significantly improved the edible traits of both species so that the highest protein, carotenoid, Fe, and Zn contents in the petals of both species were related to the treatment of '$GA_{300}\times N_{200}$'. The maximum petal anthocyanin content was produced by the treatment of '$GA_0\times N_{100}$' (58.32 mg 100 g^{-1} FW) in the sweet violets and by the treatment of '$GA_{300}\times N_{100}$' (66.84 mg 100 g^{-1} FW) in the commercial violets. The commercial violets were richer in Se than the sweet violets. The highest Se contents in the commercial violets were obtained from the treatments of '$GA_{300}\times N_{200}$', '$GA_{300}\times N_{100}$', and '$GA_{150}\times N_{200}$'. In both violet species, the highest flavonoid contents at the wavelengths of 300 and 330 nm were related to the treatments of '$GA_{300}\times N_{200}$' and '$GA_{150}\times N_{200}$'. However, these treatments were ineffective in flavonoids at 270 nm. The sweet violets had a higher antioxidant capacity than the commercial violets. The highest antioxidant capacity in the sweet violets (84.83 % DPPHsc) and commercial violets (78.17 % DPPHsc) was produced with the application of '$GA_{150}\times N_{200}$'. Based on the results, both species are effective sources of proteins, minerals, and antioxidant compounds. As well, '$GA_{300}\times N_{200}$' and '$GA_{150}\times N_{200}$' are recommended for improving the edible traits of these two species.

Keywords: Antioxidant capacity, Edible flower, Flavonoid, Foliar application, Growth promoter, Selenium.

INTRODUCTION

Over 500 species from the *Viola* genus, which have ornamental, medicinal, and food consumptions, have been detected in the world. Sweet violet (*Viola odorata*) and commercial violet (*Viola tricolor*) are two important species from the Violaceae family that grow in Iran (Lim, 2014; Mousavi *et al*., 2016) and have a special place as edible plants in the world. Buds, flowers, and young leaves are the edible parts of these species consumed both cooked and raw. Violet flowers have a pleasant aroma and sweet taste and their leaves have a mild taste.

Violet flowers and leaves are consumed in salads, desserts, soups, and as flavor and coloring agents in different foods (Lim, 2014).

Although edible flowers like violets are regarded as new and useful food resources for human health, they are mostly collected from nature. This may, however, damage their natural habitats and may even put them at the risk of extinction on the one hand, and the consumption of flowers collected from nature is not available and reliable for all people on the other hand. Therefore, the cultivation of these plant species can prevent the extinction of indigenous species and increase the availability and supply of these species with higher quality and food value (Lim, 2014; Kumari *et al*., 2021).

Nitrogen (N) is the most important nutrient for plant growth and development and its availability is necessary for the economic sustainability of agricultural systems. Nitrogen is the key constituent of proteins, chlorophyll, many organic compounds and plays a vital role in all living processes of plants (Mengel *et al*., 2001; Mussarat *et al*., 2021). It is the element limiting or dictating the rate of plant growth and its shortage reduces or may even stop the growth of different plants. Furthermore, N is the structural constituent of the cell wall, is the osmosis agent, and contributes to the structure of many enzymes. This nutrient improves soil biochemical properties, increases the uptake and retention of water and nutrients by plants, and helps preserve vital processes and improve plant growth and development by preserving cell turgor (Fageria, 2009; Khalaj *et al*., 2012). Golchin *et al*. (2019) state that N is a vegetative growth promoter. They reported that the application of 120 mg kg^{-1} N increased the vegetative growth, photosynthesizing pigments, and essential oil yield of peppermints. Similarly, Vojodi Mehrabani (2017) observed that N application from a urea source and nitroxin increased vegetative and reproductive growth, as well as anthocyanin and flavonoid content, in chrysanthemums versus the control.

Gibberellic acid (GA) is a growth regulator that is involved in many plant processes from germination to aging. GA increases the vegetative and reproductive growth of plants by stimulating cell division and longitudinal growth of cells, influencing photosynthesis, and increasing photosynthates distribution and use efficiency (Iqbal *et al*., 2011). GA is an anti-aging compound that prevents the destruction of photosynthesizing pigments in plants (Ferrante *et al*., 2009) and improves plant growth and development by increasing the activity of photosynthetic enzymes and enhancing the photosynthesis process (Khan *et al*., 2002; Iqbal *et al*., 2011). The increase in minerals is another positive effect of GA application. Al-Rumaih *et al*. (2003) reported that GA application increased minerals in the leaves and roots of cowpeas (*Vigna unguiculata*). Khan *et al*. (2002) revealed that GA application increased N uptake. Researchers argue that GA increases N requirement and uptake from the soil by inducing vegetative growth (Khan *et al*., 2002). There are reports as to the positive effect of GA on the quantitative and qualitative traits of grapes (Zahedi *et al*., 2013), leaf lettuce, *Eruca sativa* (Miceli *et al*., 2019), and snowflakes (Babelian Hendijani *et al*., 2021).

Given the positive effect of N and GA on plant growth and development and the

importance of violet as an edible flower and a new food source, the present research aimed to cast light on the effects of N and GA at different rates on the edible traits of two violet species including *V. tricolor* and *V. odorata*.

MATERIALS AND METHODS

The effects of nitrogen and gibberellic acid at different levels were investigated on the edible traits of two violet species in a factorial experiment based on a completely randomized design with 18 treatments, 3 replications, 54 experimental units, and 216 plants. The experimental treatments included two violet species (*V. odorata* and *V. tricolor*), N (0, 100, and 200 mg L^{-1}), and GA (0, 150, and 300 mg L^{-1}). The leaf-containing rhizomes of sweet violets were collected from the elevations of Guilan (Amlash, Imamzadeh Hashem, and Jafarabad), and those of wild pansy were collected from the green spaces of Tehran. They were then cultivated in a substrate containing leaf mold, cattle manure, sand, and groundnut shell at equal volumes. The nitrogen consumed in the research was from the source of the Yara Kristalon fertilizer and GA was procured from the Merck Group. They were applied as a foliar application at an interval of 15 days after plant establishment. Foliar spraying was done in 3 steps. The irrigation and weed operations were performed as per the plants' requirements.

Assessment of traits

The edible traits of sweet and commercial violets were measured on fully-open flowers. The samples were kept in liquid nitrogen in a freezer (-20 °C) from the flower harvest until the measurement of the traits in a laboratory.

Petal protein

To measure the petal protein content, the nitrogen content was first measured by the indirect Kjeldahl method (Eq. 1). Then, Eq. 2 was used to determine the petal protein content.

(1) $$N(\%)=0.56 \times t \times (a-b) \times \frac{V}{W} \times \frac{100}{DM}$$

in which t is the concentration of the acid used for titration in mol L^{-1}, a is the amount of the acid used for the sample in mL, b is the amount of the acid used for the control in mL, V is the volume of the extract used for digestion in mL, W is the weight of the plant sample used for digestion in g, and DM is the plant's dry matter percentage.

(2) $$\text{Protein}(\%)=N \times 6.25$$

Petal carotenoid

To determine the carotenoid content of the petals, 0.5 g of fresh petals were ground with acetone 80% in a china mortar and its extract was isolated. The filtered extract was read at 440, 645, and 663 nm with an APEL PD-103UV spectrophotometer, and the concentration of the carotenoid pigment was calculated by the following equation in µg g^{-1} FW (Mazumdar and Majumder, 2003):

$$\text{Petal carotenoid}=4.69 \times A_{440}-0.268 \times (20.2) A_{645}+(8.02) A_{663}$$

Petal anthocyanin

The anthocyanin content of the petals was determined by Mazumdar and Majumder's (2003) method. So, 0.5 g of the fresh petal was extracted in a china mortar containing acidic methanol (pure methanol + hydrochloric acid). The extract was then infiltrated with Whatman No. 2 filter paper. Then, the samples' absorption was read at 535 nm with an APEL PD-103UV spectrophotometer, and the anthocyanin content of the petals was measured by the following equation:

$$\text{Anthocyanin (mg /100g FW)} = \frac{e \times b \times c}{d \times a} \times 100$$

in which e is the sample weight, b is the sample volume for measurement, c is the total solution developed, d is the volume of the sample taken, and a is the spectrophotometer reading.

Petal minerals (Fe, Zn, and Se)

To measure the concentration of iron (Fe), zinc (Zn), and selenium (Se) in violet petals, a certain quantity of the petal was first converted to ash in an electric furnace at 550 °C for 7 hours. Then, 1 g of the petal ash was mixed with 3 mL of distilled water and 5 mL of hydrochloric acid 2 M and was put in a hot water bath at 70 °C. After one hour, the samples were taken out of the bath and their volume was adjusted to 50 mL by adding distilled water. Then, the Fe, Zn, and Se concentrations were measured by using an atomic absorption device (Karla, 1998).

Petal flavonoid

The flavonoid content of the petals was measured at 270, 300, and 330 nm using the procedure described by Krizek *et al*. (1998). First, 0.1 g of the fresh petal was extracted by 2.5 mL of acidic ethanol (99 % ethanol + 1 % glacier acetic acid). The extract was then centrifuged at 3600 rpm for 15 minutes. One hour later, the supernatant was separated with a sampler and was put in a hot water bath at 95 °C for 10 minutes. After the samples cooled down, their absorption was read at 270, 300, and 330 nm with an APEL PD-303UV spectrophotometer.

Antioxidant capacity

The antioxidant capacity of the sample was determined by Brand-Williams *et al*.'s (1995) method using the free radical neutralizing property of DPPH. So, 1 g of the fresh petal was extracted with 10 mL of pure methanol and was kept at room temperature for 2 hours. The extract was infiltrated with Whatman No. 2 filter paper. Then, 50 µL of the extract was mixed with 950 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and was kept in darkness at room temperature for 15 minutes. The absorption of the extracts was then read at 515 nm with an APEL PD-303UV spectrophotometer, and the antioxidant capacity of the extracts was calculated as an inhibitory percentage (DPPHsc %) by the following equation:

$$\%\ \text{DPPHsc} = (A_{cont} - A_{samp}) \times \frac{100}{A_{cont}}$$

in which % DPPHsc is the inhibitory percentage, A_{cont} is the sample and DPPH absorption, and A_{samp} is the DPPH absorption.

Data analysis

The data were analyzed in the SPSS19 statistical software package, the means were compared by the LSD test, and the graphs were drawn in the MS-Excel software package.

RESULTS

Petal protein

The interactive effect of 'cultivar × GA × N' was significant ($P < 0.01$) on petal protein content (Table 1). It is observed in Fig. 1 that the treatment of '$GA_{300} \times N_{200}$' was related to the highest petal protein content of the sweet violet (2.08 %) and commercial violet (2.48 %). So, it was the best treatment in increasing petal protein. The lowest of this trait in the sweet violets was related to the control (1.24 %) followed by the treatment of '$GA_0 \times N_{100}$' (1.28 %). Regarding the commercial violets too, the lowest was observed in the control, '$GA_0 \times N_{100 \text{ and } 200}$', and '$GA_{150 \text{ and } 300} \times N_0$', which were not statistically significant (Fig. 1).

Table 1. Analysis of variance for the effect of different treatments on the measured traits.

S.o.V	df	Petals protein	Petals carotenoids	Petals anthocyanin	Iron	Zinc	Selenium	Flavonoid 270 nm	Flavonoid 300 nm	Flavonoid 330 nm	Antioxidant capacity
Gibberellin (GA)	2	0.0498**	82.1**	45.85**	2.72**	0.015**	0.062^{ns}	0.233**	0.127**	0.258**	1446**
Nitrogen (N)	2	0.089**	106.2**	68.1**	0.266*	0.0312**	2.35**	0.247**	0.053**	0.132**	447**
Cultivars (C)	1	0.0075**	73.1**	1.005^{ns}	4.33**	0.0028**	4.98**	0.0073^{ns}	0.038*	0.093**	1992**
GA×N	4	0.015**	18.55**	49.6**	1.25**	0.0123**	0.194**	0.083**	0.0368**	0.126**	2284**
GA×C	2	0.0189**	26.06**	69.95**	0.667**	0.0018*	0.066^{ns}	0.304**	0.105**	0.0283*	198**
N×C	2	0.0097**	17.53**	170.5**	1.59**	0.00406**	1.27**	0.189**	0.096**	0.035*	18.4*
GA×N×C	4	0.0099**	13.95**	71.2**	0.802**	0.0018**	0.183**	0.0103^{ns}	0.0751**	0.0828**	222**
Error	34	0.00062	1.29	7.535	0.0556	0.00036	0.0387	0.0117	0.00702	0.0087	3.58
CV (%)		6.11	12.61	17.1	12.39	11.72	29.51	8.27	7.84	6.97	3.45

*, ** and ^{ns}: significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

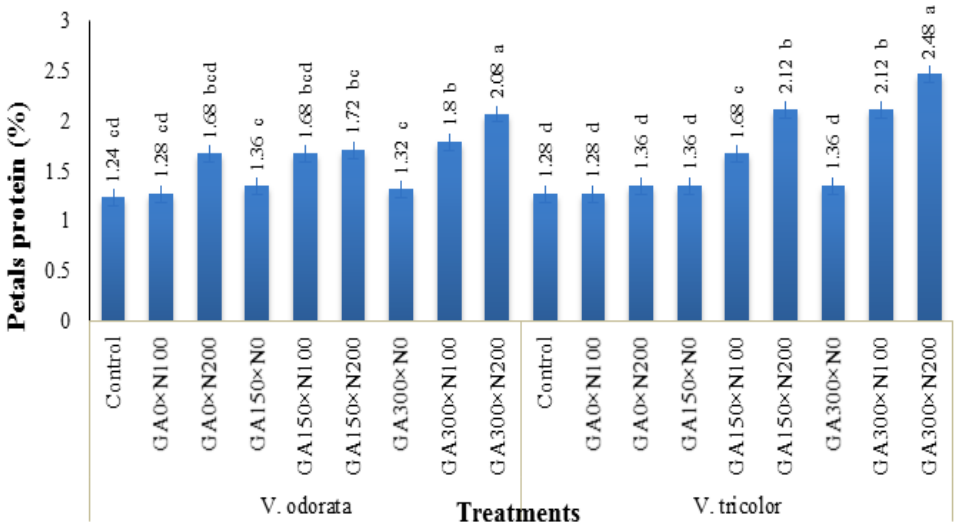

Fig. 1. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on petals protein in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

Pigments (carotenoid and anthocyanin)

Based on the analysis of data variance, the effect of 'cultivar × GA × N' was found to be significant ($P < 0.01$) on petal carotenoid (Table 1). As the comparison of means revealed, the lowest petal carotenoid content in the sweet violets (4.97 µg g^{-1} F.W.) and the commercial violets (4.05 µg g^{-1} F.W.) were related to the control. In the sweet violets, the highest (13.65 µg g^{-1} F.W.) was recorded in the treatment of 'GA_{300}× N_{200}', which did not differ from the treatments of 'GA_{300}×N_{100}' and 'GA_{150}×$N_{100\ and\ 200}$' significantly. In the commercial violets too, the highest (12.57 µg g^{-1} F.W.) was obtained from the application of 'GA_{300} × N_{200}'. This treatment was in the same statistical group as the treatment of 'GA_{300}×N_{100}' (Fig. 2).

The analysis of data variance showed the significant effect of 'cultivar×GA×N' on petal anthocyanin content at the $P < 0.01$ level (Table 1). In both cultivars, the application of 'GA×N' increased petal anthocyanin versus the control significantly. In the sweet violets, the application of 100 mg/L N increased the anthocyanin content significantly at all three GA levels, but when the N rate was further increased to 200 mg L^{-1}, a decline was observed in this trait. The highest petal anthocyanin content in the sweet violets was obtained from the treatments of 'GA_0×N_{100}' (58.32 mg 100 g^{-1} F.W.) and 'GA_{150}×N_{100}' (57.12 mg 100 g^{-1} F.W.). These two treatments did not differ significantly. In the commercial violets, the control had the lowest anthocyanin content (28.56 mg 100 g^{-1} F.W.) and the treatments of 'GA_{300}×N_{100}' and 'GA_{300}× N_{200}' had the highest ones (66.84 and 62.88 mg 100 g^{-1} F.W., respectively) (Fig. 3).

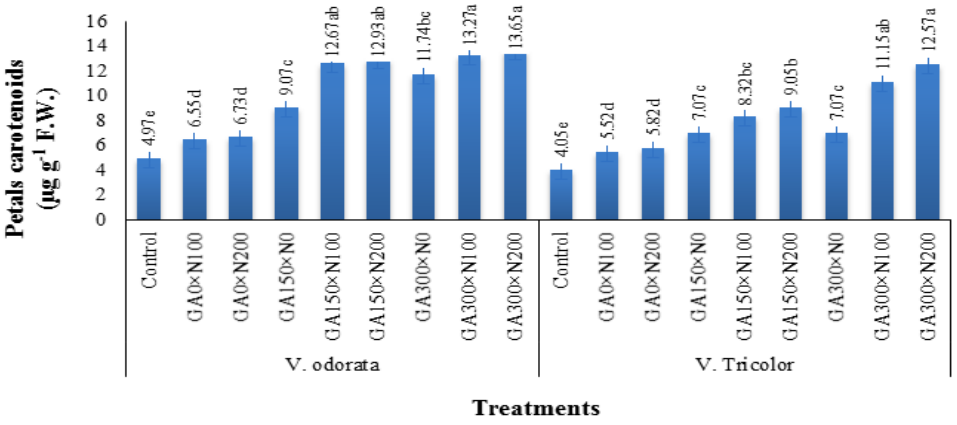

Fig. 2. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on petal carotenoid in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

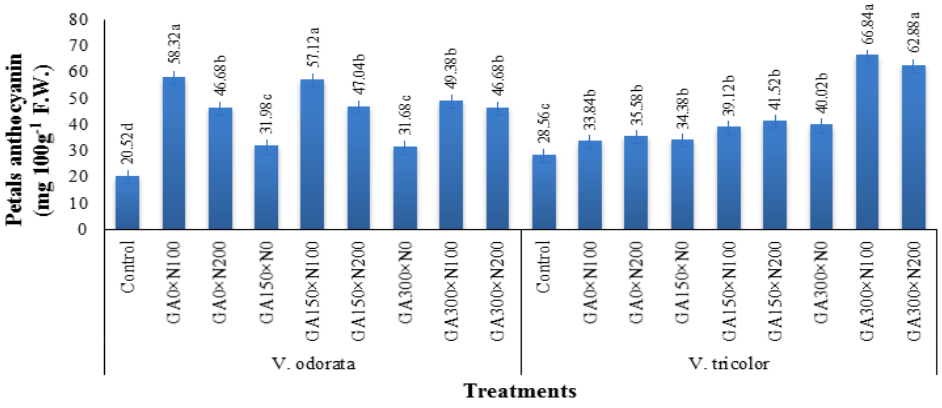

Fig. 3. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on petal anthocyanin in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

Minerals (Fe, Zn, and Se)

The effect of the experimental treatments was significant ($P < 0.01$) on Fe content (Table 1). The sweet violets had higher Fe content than the commercial violets in all treatments. Figure 5 reveals that the highest Fe content of both cultivars was obtained from the treatments of '$GA_{300}\times N_{200}$' and '$GA_{150}\times N_{200}$', which were not significantly different. The lowest Fe content of the sweet violets was related to the control (0.137 mg 100 g^{-1} F.W.) and the treatment of '$GA_0\times N_{100}$' (0.143 mg 100 g^{-1} F.W.). There was not a significant difference between these two treatments. The commercial violets exhibited the lowest Fe content in the control, '$GA_0\times N_{100}$', and '$GA_0\times N_{200}$', which were not significantly different (Fig. 4).

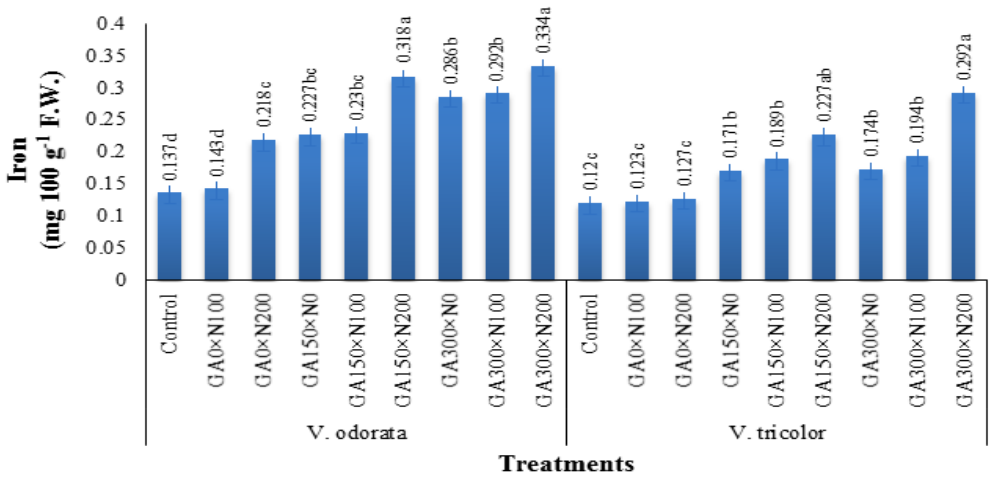

Fig. 4. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on petal Fe content in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

Petal Zn content of the violets was significantly ($P < 0.01$) affected by 'cultivar × GA × N' (Table 1). When GA and N were applied simultaneously, both cultivars accumulated more Zn in their petals than the control although there was no significant difference between the control and '$GA_0 \times N_{100}$' in the sweet violets. In both cultivars, the best treatment for increasing Zn was '$GA_{300} \times N_{200}$', which significantly increased this trait in the petals of the sweet and commercial violets versus the other treatments (Fig. 5).

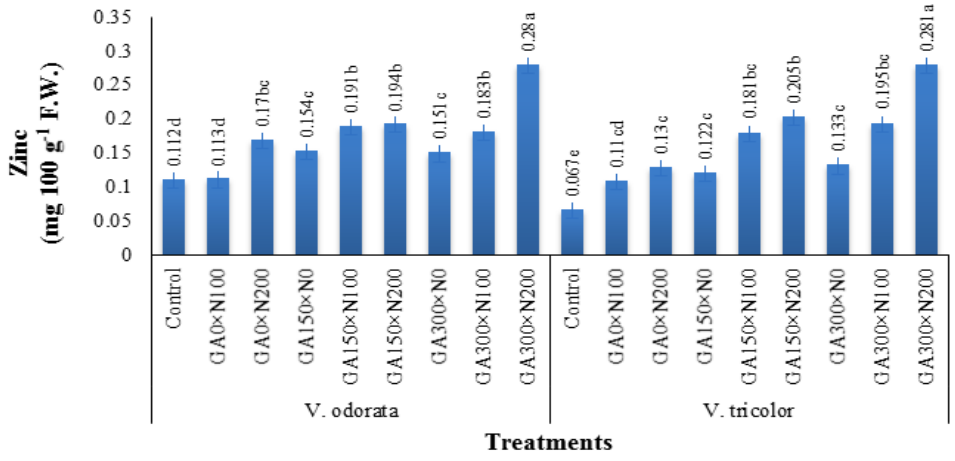

Fig. 5. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on petal Zn content in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

The effect of 'cultivar × GA × N' was significant ($P < 0.01$) on petal Se content (Table 1). It is evident in Fig. 6 that the commercial violets had higher Se content than the sweet violets so that the highest Se content was exhibited by the commercial violets when they were treated with '$GA_{300} \times N_{200}$' (1.62 mg 100 g^{-1} F.W.), which was not different from '$GA_{300} \times N_{100}$' and '$GA_{150} \times N_{200}$' significantly. The sweet violets showed the highest Se content (0.844 mg 100 g^{-1} F.W.) in the treatment of '$GA_{300} \times N_{200}$', which was the best for increasing Se in the petals of the sweet violets (Fig. 6).

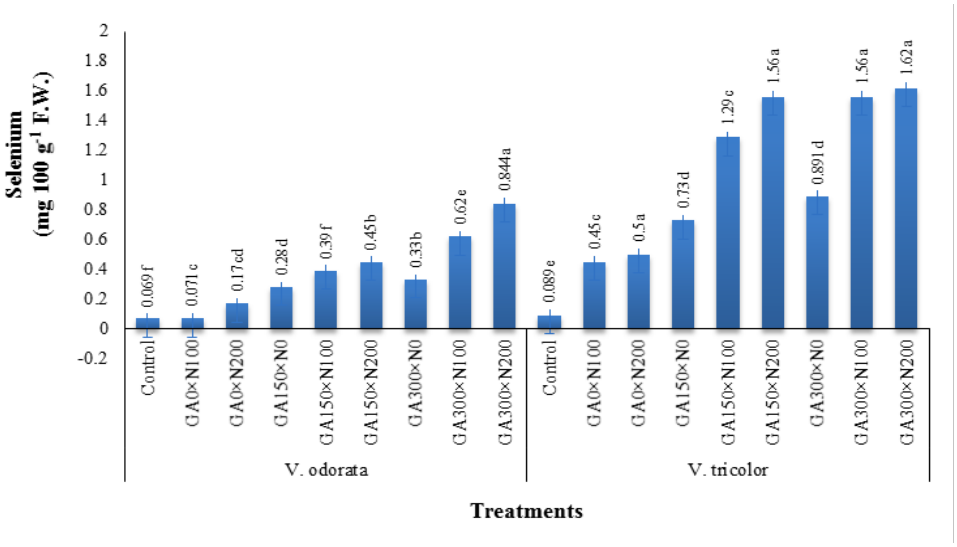

Fig. 6. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on petal Se content in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

Flavonoid content (270, 300, and 330 nm)

The effect of 'cultivar × GA × N' was not significant on flavonoid 270 nm content, but petal flavonoid 300 and 330 nm contents were influenced by the treatments significantly at the $P < 0.01$ level (Table 1). Increasing the N level increased the flavonoid 300 nm content in both cultivars at all three GA levels. The highest flavonoid 300 nm content was recorded by the treatments of '$GA_{300} \times N_{200}$' and '$GA_{150} \times N_{200}$' in the sweet violets, not differing from one another significantly. In the commercial violets, the treatments of '$GA_{300} \times N_{200}$' and '$GA_{150} \times N_{200}$' were the best for the flavonoid 300 nm content, but these two treatments had no significant difference from the treatments of '$GA_{300} \times N_{0 \text{ and } 100}$' and '$GA_{150} \times N_{200}$'. The lowest flavonoid 300 nm was recorded in the control in both cultivars (Fig. 7).

Fig. 8 shows that the highest flavonoid 330 nm content of the sweet violets was obtained from the application of '$GA_{300} \times N_{200}$' (2.141 %) and '$GA_{150} \times N_{200}$' (2.129 %). These two treatments did not differ significantly, but they increased the flavonoid 330 nm content of the sweet violets versus the control (1.361 %) and other treatments significantly. In the commercial violets too, all treatments increased this trait versus the control (1.357 %) and the treatment of '$GA_0 \times N_{100}$' (1.358 %). '$GA_{150} \times N_{200}$' (1.723 %) and '$GA_{300} \times N_{200}$' (1.603 %) were found to be the most successful (Fig. 8).

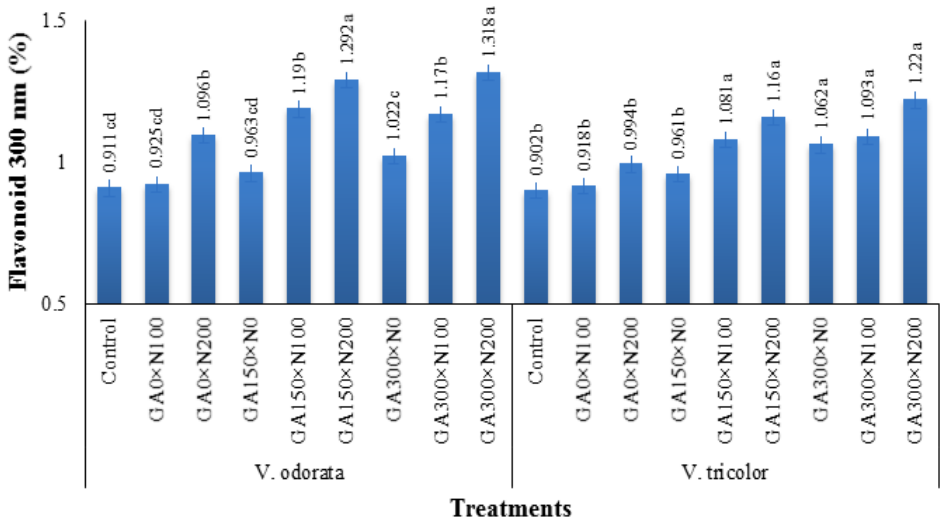

Fig. 7. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on flavonoid 300 nm content in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

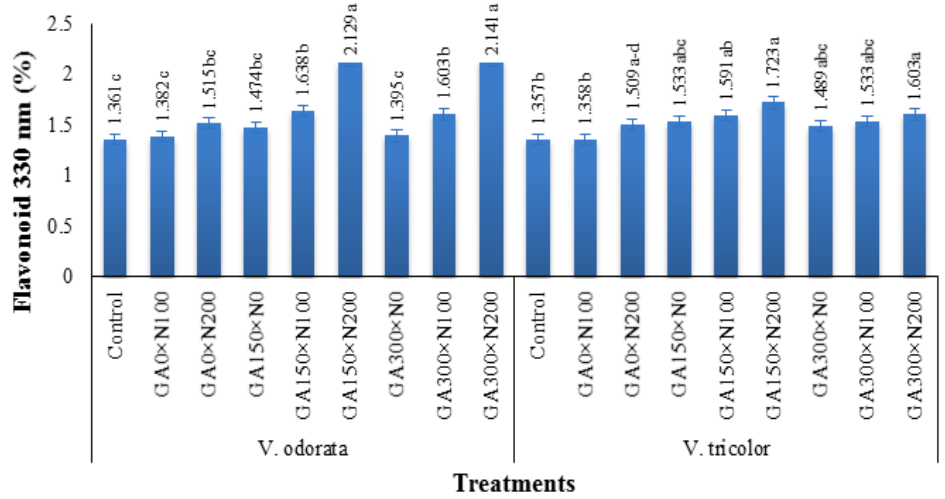

Fig. 8. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on flavonoid 330 nm content in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

Antioxidant capacity

According to the analysis of data variance, the effect of 'cultivar × GA × N' was significant ($P < 0.01$) on the antioxidant capacity of the violets (Table 1). The sweet violets had a higher antioxidant capacity than the commercial violets. The control exhibited the lowest antioxidant capacity in both sweet violets (39.17 % DPPHsc) and commercial violets (20.17 % DPPHsc). The highest antioxidant capacity in the sweet violets was obtained from the application of '$GA_{150} \times N_{200}$', '$GA_{300} \times N_{200}$', and '$GA_{300} \times N_{100}$', not differing from one another significantly. In the commercial violets, the most successful treatments in enhancing antioxidant capacity were '$GA_{150} \times N_{200}$' and '$GA_{150} \times N_{100}$', which did not differ significantly (Fig. 9).

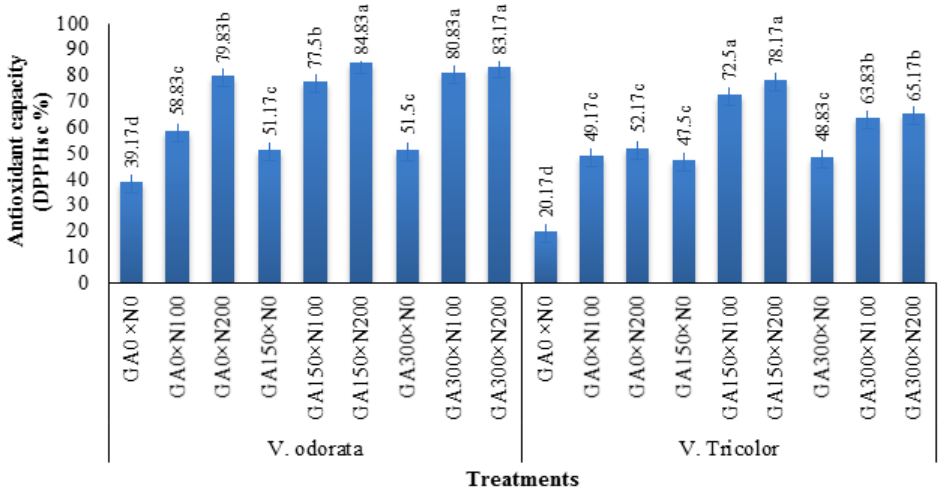

Fig. 9. The interactive effect of 'cultivar × gibberellic acid × nitrogen' on antioxidant capacity in two violet cultivars. GA_0, GA_{150} and GA_{300}: 0, 150 and 300 mg L^{-1} gibberellic acid, N_0, N_{100} and N_{200}: 0, 100 and 200 mg L^{-1} nitrogen.

DISCUSSION

Violet species are famous edible flowering plants in the world. Research on the nutritional value of violets has shown that these species are a good source of phenol and antioxidant compounds, carotenoids, anthocyanins, minerals, and proteins (Gonzales-Barrio *et al*., 2018). Obviously, the cultivation of plants in environmentally and nutritionally ideal conditions can improve their nutritional value. As was reported in the Results section, the cultivation of the sweet and commercial violets at different levels of N, which is a necessary element for growth, and GA, which is a growth promoter, influenced their nutritional traits significantly.

N is the most important nutrient required by plants and is a key constituent of many organic compounds such as amino acids and proteins (Mozafari and Khaleghi, 2016). GA is one of the most important growth regulators involved in many metabolic pathways in plants, such as the production and degradation of photosynthesizing pigments, the uptake and mobilization of nutrients, metabolism, and the redistribution of nitrogen (Miceli *et al*., 2019). GA affects N use efficiency by influencing metabolism, as well as the translocation and redistribution of N in plants. On the other hand, the increased growth induced by GA increases plants' N requirement and provides the N required for different biochemical processes (Miceli *et al*., 2019). The activity of plant hormones, e.g., GA, depends on environmental factors and nutritional conditions. N is the most important element influencing GA translocation in different plant parts so that when there is N deficiency, gibberellin decreases in plant shoots sharply (Mozafari and Khaleghi, 2016). It can, therefore, be said that plants are positively influenced by GA via the facilitation of N uptake and mobilization and by N via the acceleration of GA mobilization in plant parts.

In the present study, the protein content was increased with the application of N and GA so that the highest levels of N and GA were related to the maximum level of protein in both violet cultivars. Dordas and Sioulas (2007) reported that N application increased the protein content in plant tissues, which agrees with the results of the present study. GA reportedly prevents protein degradation by antioxidant effect (Rezaei *et al*., 2011). The effect of GA has also been reported on inducing and increasing the activity of the enzymes involved in protein biosynthesis (Shah, 2007). Bora and Sarma (2006) suggest that plant hormones regulate the rate of protein synthesis by controlling gene transcription and mRNA levels. The increase in

the protein content with the application of GA was also reported by Shahin *et al*. (2010), which is consistent with our findings.

Fe is an essential element for all living organisms and is involved in many metabolic processes, including oxygen translocation, the treatment of anemia and neural disorders, and so on (Lieu *et al*., 2001). Zn is an element with antioxidant and anti-inflammation activities (Prasad, 2014). Se is also an essential element for humans, animals, and microorganisms and plays a role in strengthening the immunity system, improving cardiovascular diseases, and treating thyroid disorders and cancer (Ellis and Salt, 2003). Naturally, the intake of these elements from a natural food source can enhance human health. It was found that the consumption of edible violets can largely satisfy the daily human demand for Fe, Zn, and Se. Also, the results show that the application of N and GA can be effective in increasing the Zn, Fe, and Se content of violets.

The uptake and translocation of minerals in plant parts have been reported to increase by N (Stall *et al*., 1991; Mohammadi *et al*., 2016) and GA (Miceli *et al*., 2019). Mozafari and Khaleghi (2016) reported that the mixed application of N and GA increased the Fe and Zn contents of pistachio. The positive effects of GA (Tuna *et al*., 2008) and N (Drihem and Pilbeam, 2002) on increasing nutrient uptake have been reported in various plants, which is in agreement with our findings.

Flower color partially determines its taste and is one of the main criteria for the selection of edible flowers. Consumers are fonder of edible flowers that are yellow, orange, and blue (Kelly *et al*., 2002, 2002). Carotenoids and anthocyanins are some of the most important plant pigments. Carotenoids are responsible for the red, yellow, and orange colors of flowers (Gamsjaeger *et al*., 2011) and their inclusion in the human diet is particularly important due to their therapeutic and anticancer activities, as well as their role as the precursor of vitamin A and its effect on eyesight and eye health (Bartly and Scolnik, 1995). Gonzales Barrio *et al*. (2018) state that violet species are an appropriate source of carotenoids. As was stated in the results, the petal carotenoid content of both violets was increased with the application of N and GA, which is supported by evidence on the role of GA in accelerating carotenoid biosynthesis and accumulation (Barlley and Scolnik, 1995; Vukics *et al*., 2008). Researchers argue that N application contributes to preserving the structure of photosynthesizing pigments and light interceptors such as carotenoids and increasing these pigments in plants (Mozafari and Khaleghi, 2016; Mardani *et al*., 2019). In the present study too, the increase in N level at all three GA levels increased the petal carotenoid content.

Anthocyanins are the most important flavonoid group and among the largest groups of water-soluble plant pigments, which are used as edible colors in food industries. Anthocyanins are especially important for the human food regime due to their antioxidant activities and their role in strengthening the immunity system (Gould *et al*., 2009). It has been reported that GA accelerates the transcription of the genes that are responsible for the biosynthesis of anthocyanins in some plants (Weiss, 2000; Gould *et al*., 2009). In the present work, N and GA application increased anthocyanins that are responsible for blue and violet colors of violet petals (Gamsjaeger *et al*., 2011). Bartley and Scolnik (1995) reported that GA increased anthocyanin biosynthesis during flower growth, which is consistent with the results of the present study. In Mardani *et al.*'s (2019) study too, the application of 150 kg ha^{-1} N increased the leaf anthocyanins of marshmallows. These researchers attributed the positive effect of N on preserving plant pigments, especially in stressful conditions, to the effect of N on increasing compatible osmolytes and preserving osmotic pressure.

The effect of edible flowers on human health is mostly associated with their antioxidant contents and the role of these antioxidants in suppressing free radicals and preventing cancer (Kucekova *et al*., 2013). Flavonoids are a group of secondary metabolites in plants with medicinal and antioxidant values so that they are used for curing many diseases such as inflammation, diabetes, hypertension, and infections. Research shows that the mean flavonoid intake level is 1-2 g/day for humans (Liu *et al*., 2010). Violets are a rich source of antioxidants and flavonoids (Gonzales-Barrio *et al*., 2018; Vukics *et al*., 2008). In the present study, the sweet violets had the higher antioxidant capacity and flavonoid content than the commercial violets, and they were further increased with the application of N and GA.

Appropriate nutrition of plants plays a significant role in producing secondary metabolites and antioxidants. N regulates the synthesis of secondary metabolites, e.g., flavonoid compounds, in plants (Ibrahim *et al*., 2012). There are various reports as to the impact of N on flavonoid production. Some researchers argue that N reduces flavonoid production in plants (Patil and Alva, 1999; Awad and de Jager, 2002; Ibrahim *et al*., 2012) whereas others have reported the increasing effect of N on flavonoid production (Bongue-Bartelsman and Phillips, 1995). Indeed, it is argued that optimal N level increases flavonoids (Liu *et al*., 2010). Similar results were reported by Vojodi Mehrabani (2017), which is in agreement with our findings. The positive effect of GA on increasing secondary metabolites has been reported in German chamomile (Amiri *et al*., 2014) and periwinkle (Abdul Jaleel *et al*., 2007), which agrees with our findings.

The antioxidant capacity of plants heavily depends on phenol compounds and flavonoids (Ibrahim *et al*., 2012; Zamir *et al*., 2016). Therefore, increasing phenol content or flavonoids increases antioxidant capacity as reported in the present study. Abdul Jaleel *et al*. (2007) showed that the application of GA improved the antioxidant capacity of periwinkle. Similar results were reported by Ahmad *et al*. (2020), which is consistent with the present research.

CONCLUSION

Based on the results, sweet violets and commercial violets are edible flowering plants that have appropriate quantities of proteins, plant pigments, minerals, flavonoids, and antioxidants. Sweet violets performed better in antioxidant capacity, flavonoids, Fe, and carotenoids and commercial violets performed better in anthocyanins and Se. The effect of GA and N was positive on improving these traits. According to the results, it is recommended to apply '300 mg L^{-1} GA × 200 mg L^{-1} N' or '150 mg L^{-1} GA × 200 mg L^{-1} N' for the cultivation of edible violets as they were most influential on most studied traits.

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