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The soil characteristics and morpho-physiological traits of two violet (*Viola* sp.) species as influenced by gibberellic acid and nitrogen

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ABSTRACT

Violet, with its pleasant scent and attractive flower color, is also important for its medicinal and edible uses, in addition to ornamental uses. Proper nutrition of the plant (violet) with essential nutrients and growth hormones improves the quantitative and qualitative characteristics and can increase the economic value and popularity of these flowers. In this regard, the current research aims to investigate the combined effect of nitrogen (N) and gibberellic acid (GA) on the morphophysiological traits of two native Iranian violet species (*Viola tricolor* and *Viola odorata*), as a factorial experiment with three factors including two species of violets, three levels of GA (0, 150 and 300 mg/L) and three levels of N (0, 100 and 200 mg/L) in a completely randomized design with 18 treatments, 3 replications and a total of 216 plants. Some characteristics of planting medium, morphophysiological traits and activity of antioxidant enzymes were evaluated. The results of ANOVA showed that the three-way effect of experimental treatments on all evaluated traits is significant. According to the results of comparing the averages the N application reduced the soil electrical conductivity (EC) versus its non-application in both violet species and at all GA levels. The highest soil N in both species was obtained from the application of 0 mg/L GA × 100 or 200 mg/L N. In two species of violets, the highest number of leaves, flowers, and stolons was obtained from the treatment of 300 mg/L GA × 200 mg/L N. The highest chlorophyll *a* and total chlorophyll among the treatments were related to *V. odorata* × 150 mg/L GA × 200 mg/L N. The application of GA and N in both species increased peroxidase activity versus the control. The highest level of this enzyme activity was related to 300 mg/L GA × 200 mg/L N. *V. odorata* had higher peroxidase activity than *V. tricolor*. The lowest and highest levels of catalase activity were recorded by control × *V. tricolor* (0.13 nM/g FW/min) and *V. odorata* × 300 mg/L GA × 200 mg/L N (0.676 nM/g FW/min), respectively. According to the results, with the application of 300 or 150 mg/L GA × 200 mg/L N, the ornamental and edible properties of these two types of violets are improved, and their economic value and marketability are increased, and there will be a change in the sales market of these flowers.

Keywords: *Viola tricolor*, *Viola odorata*, foliar application, growth stimulator, ornamental-edible plants, seedbed acidity.

RESUMO

Características do solo e morfofisiológicas de duas espécies de violeta (*Viola* sp.) influenciadas por ácido giberélico e nitrogênio

A violeta, com seu aroma agradável e cor atraente das flores, também é importante para usos medicinais, comestíveis e ornamentais. A nutrição adequada dessa planta com nutrientes essenciais e hormônios de crescimento melhora as características quantitativas e qualitativas e pode aumentar o valor econômico e a popularidade dessas flores. Assim, a presente pesquisa visa investigar o efeito combinado do nitrogênio (N) e do ácido giberélico (GA) nas características morfofisiológicas de duas espécies nativas de violeta iraniana (Viola tricolor e Viola odorata), em experimento fatorial com três fatores (2 espécies de violetas), três níveis de GA (0, 150 e 300 mg/L) e três níveis de N (0, 100 e 200 mg/L), em delineamento inteiramente casualizado com 18 tratamentos, 3 repetições e um total de 216 plantas. Neste estudo foram avaliadas algumas características do meio de plantio, características morfofisiológicas e atividade de enzimas antioxidantes. Os resultados da ANOVA mostraram que o efeito triplo dos tratamentos experimentais em todas as características avaliadas é significativo. De acordo com os resultados da comparação das médias, a aplicação de N reduziu a condutividade elétrica (CE) do solo versus sua não aplicação em ambas as espécies de violeta e em todos os níveis de GA. O maior N no solo em ambas as espécies foi obtido a partir da aplicação de 0 mg/L GA × 100 ou 200 mg/L N. Em duas espécies de violetas, o maior número de folhas, flores e estolões foi obtido no tratamento 300 mg/L GA × 200 mg/L N. Os maiores valores de clorofila a e clorofila total entre os tratamentos foram relacionados a V. odorata × 150 mg/L GA × 200 mg/L N. A aplicação de GA e N em ambas as espécies aumentou a atividade da peroxidase em relação ao controle. O maior nível de atividade desta enzima foi relacionado a 300 mg/L GA × 200 mg/L N. V. odorata apresentou maior atividade de peroxidase que V. tricolor. Os níveis mais baixos e mais altos de atividade de catalase foram registrados por controle × V. tricolor (0,13 nM/g FW/min) e V. odorata × 300 mg/L GA × 200 mg/L N (0,676 nM/g FW/min), respectivamente. De acordo com os resultados, com a aplicação de 300 ou 150 mg/L GA × 200 mg/L N, as propriedades ornamentais e comestíveis destes dois tipos de violetas são melhoradas, e o seu valor econômico e comercialização são aumentados, e é possível haver uma mudança no mercado de vendas dessas flores.

Palavras-chave: *Viola tricolor*, *Viola odorata*, aplicação foliar, estimulador de crescimento, plantas ornamentais comestíveis, acidez do solo

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Nitrogen (N) is an essential macronutrient for plant growth and development. This nutrient is a structural component of proteins, photosynthesizing pigments, and cell walls and is involved in all physiological and biochemical processes of plants, as well as in root development, vegetative growth, and the improvement of plants' quantitative and qualitative yields (Patni *et al*., 2020; Mussarat *et al*., 2021). N is, on the other hand, the most limiting nutrient of plant growth, but N availability contributes to preserving the vital processes of plant tissues by increasing the uptake of nutrients and water (Patni *et al*., 2020; Ye *et al*., 2022). Increasing plant resistance to environmental stresses, improving vegetative growth, preserving and increasing photosynthesizing pigments, escalating the activity of antioxidant enzymes, and improving quantitative and qualitative yields are some effects of N application on plants (Agami *et al*., 2018). The positive effect of N has been reported on plant adaptation to nutrient deficiency and over-availability and resistance to environmental stresses (Ye *et al*., 2022), increasing plant height, root length, chlorophyll content, and nutrient contents in *Ficus deltoidea* (Sheikh & Ishak, 2016), and maintaining pigments, proteins, and nutrients in coffee (Reis *et al*., 2015), which reflect the desired effect of N at optimal rates on plant growth and development and the need for its application to gain maximum yields.

Gibberellin is a stimulator of cell division and elongation. This plant growth regulator is involved in all physiological processes of plant growth and development from germination to senescence. Gibberellic acid (GA) enhances plant growth and development by increasing the activity of photosynthesis enzymes, improving the photosynthesis process, and increasing the efficiency by which photosynthates are distributed and consumed (Khan *et al*., 2002). GA slows down senescence by inhibiting the degradation of photosynthesizing pigments. GA application has reportedly increased the vegetative and reproductive growth and improved photosynthesizing pigments in two snapdragon cultivars (Chehrazi *et al*., 2017), increased leaf number, plant height, corm weight, and floret number and preserved pigments in gladiolus (Hassanpur Asil *et al*., 2017), improved growth, flowering, and bulb characteristics, extended longevity, and intensified flower color in tulips (Hojjati *et al*., 2019), increased nutrient concentrations and improved quantitative and qualitative yield of lettuce and *Eruca sativa* (Miceli *et al*., 2019), and increased nutrient uptake, enhanced the activity of antioxidant enzymes, and alleviated oxidative stress (Alharby *et al*., 2021).

GA improves N use efficiency by influencing N metabolism, mobilization, and re-distribution in plants. On the other hand, GA-induced growth enhancement increases the N requirement of the plants and supplies the N requirement of 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 within plant organs so that gibberellin content is severely decreased in the shoots of N-deficient plants (Mozafari & Khaleghi, 2016). It can, therefore, be said that plants are benefited from GA via the facilitation of N mobilization and uptake and from N via the acceleration of GA translocation within their organs.

Mozafari & Khaleghi (2016) studied the combined effect of GA and N on the quantitative and qualitative traits of pistachio. The results showed that the combined application of GA and N improved plant resistance to salinity stress, preserved and increased photosynthesizing pigments, and increased nutrient concentrations in the leaves. Khan *et al*. (2002) state that GA application is beneficial when adequate N is available to the plant. These researchers found that the foliar application of GA along with 80 kg/ha N was the most effective treatment in improving the quantitative and qualitative yield of mustard.

The violet (*Viola* sp.) is an ornamental, medicinal, and edible flower from the Violaceae family. There are over 500 species in the genus of *Viola* around the world (Mousavi *et al*., 2016), about 30 species of which have been identified in the north and northwest of Iran. *V. tricolor* and *V. odorata* are two important violet species that are native to Iran (Abolghasemi *et al*., 2020).

V. odorata has antioxidant, anti-inflammatory, anti-asthmatic, anti-cough and anti-bacterial effects, and it is used in traditional medicine to treat respiratory infections. It has been reported that *V. odorata* syrup is effective in reducing lung inflammation and reducing the symptoms of Covid-19 (Mehraban *et al*., 2023). *V. tricolor* is a good source of flavonoids and antioxidant compounds and is used as a medicinal plant. In addition, *V. tricolor* is one of the most popular edible flowers due to its unique texture and taste as well as attractive flower color, and it is used in preparing all kinds of food, including salad, soup, sweets and drinks. Also, the petals of this plant are used to extract blue and yellow food colors (Koike *et al*., 2015). There is no information about the production and commercial supply of edible violet flowers, and only the research of Fernandes *et al*. (2020) stated that the price of 20 edible violet flowers produced by Flores Frescas company is 6.80 euros. It is expected that by increasing the production and performance of fragrant and commercial violets, in addition to increasing their supply to the ornamental markets and the pharmaceutical and health industries, an effective step will be taken in the direction of the prosperity of the market of edible flowers, especially violets.

One of the most important prerequisites for the commercial production of various plants is to pay attention to the nutrition of plants in different stages of growth. However, there is not much information about growing and feeding violets.

In this regard, the present study aimed to shed light on the effect of N and GA on the seedbed characteristics and some morpho-physiological traits of aromatic and commercial violet.

MATERIAL AND METHODS

The effects of gibberellic acid (GA) and nitrogen (N) were investigated on the morpho-physiological traits of two native Iranian violet cultivars in a factorial experiment based on a completely randomized design with 3 replications, 18 treatments, 54 plots, and 4 pots/plot. The experimental treatments included two violet cultivars (*V. odorata* cv. Queen Charlotte and *V. tricolor* cv. Hortensis), three GA rates (0, 150, and 300 mg/L), and three N rates (from the source of liquid nitrogen fertilizer Crystaliq 40-0-0 company SUPER MAX, Iran) (0, 100, and 200 mg/L). The rhizomes of *V. odorata* and *V. tricolor* were collected from the green spaces of Rasht County and were immediately transferred in proper packages to Kishestan Greenhouse Town of Guilan (37°33'N, 49°48'E, 18 m altitude). The research was conducted in an open space as a pot experiment for 8 months.

Plastic pots, 14 cm mouth diameter or the volume of 830 cc were prepared for the research and were filled with seedbed which included a mixture of leaf mold, rotten manure, sand, and peanut shell at an equal volumetric ratio. Then, the rhizomes were sown in the pots. The irrigation, weeding, and nutrition operations were performed as per the plants' needs and the experimental design. It should be noted that N used in the research was supplied from the liquid nitrogen fertilizer Crystaliq (40-0-0 N-P-K) made in the company Super Max, Iran, and GA made by Merck Group, Germany. The plants were treated with the Crystaliq (40-0-0 N-P-K) fertilizer by foliar application one month after their establishment (totaling three times of use every once 15 days) and with GA by foliar application 45 days after their establishment once 15 days (totaling three times of application).

Assessment of traits

Morphological traits

The number of flowers per plant (fully open flowers) was counted from the emergence of the first flower (135 days after planting) until the end of the flowering period, and the number of stolons and leaves per plant was counted at the end of the flowering period.

Chemical characteristics of seedbed

To measure the pH and EC of the seedbeds, a 1:2.5 mixture of the soil extract was first prepared for which 20 g of the seedbed was mixed with 20 mL distilled water. The result was shaken with a shaker for 1 h. Then, they were kept at room temperature for some hours for their suspended particles to precipitate. After they all precipitated, the supernatant was infiltrated through Whatman filter paper, and it was used to determine pH and EC with an MI150 pH-meter (Martini, Milwaukee, Italy) and an MI170 digital electric EC-meter (Milwaukee, Hungary), respectively (Mohammadi Torkashvand, 2009). The total N content of the seedbeds was measured by Kjeldahl's method.

Measurement of petal nutrients (P and K)

To measure the potassium (K) and phosphorus (P) content of the violet petals, some dried petals were converted into ash by putting them in an electrical furnace at 480°C for 24 h. Then, 1.25 g of the petal ash was mixed with 10 mL of an acid mixture (300 mL thick HCL + 100 mL thick HNO_3 + 600 mL distilled water). The sample was placed in a hot bath at 100°C for 30 min. After cooling, it was infiltrated through Whatman filter paper and adjusted to 50 mL by adding distilled water. The extract was used to measure K and P contents. The petal K content was determined by flamephotometery and its P content by spectrophotometry at 470 nm (Rengel & Romheld, 2000).

Chlorophyll a, b, and total

After the first flower has bloomed (140 days after planting), the intact healthy leaves were sampled to measure their chlorophyll contents. Then, 0.5 g of the leaves was extracted with 80% acetone in a china mortar. The extract was infiltrated through Whatman grade-2 filter paper. The absorbance of the samples was read at 643 and 660 nm with a spectrophotometer (Shimadzu UV-120-02, Japan), and the results were put in the following equations to determine the chlorophyll content in mg/g fresh weight (FW) (Mazumdar & Majumder, 2003):

Chlorophyll *a* = 9.93 (A_{660}) – 0.777 (A_{643})

Chlorophyll *b* = 17.6 (A_{643}) – 2.81 (A_{660})

Total chlorophyll = 7.12 (A_{643}) + 6.8 (A_{660})

Activity of antioxidant enzymes (catalase and peroxidase)

To determine the activity of antioxidant enzymes, after the first flower has bloomed, the petals were sampled and kept in liquid nitrogen at -4°C until the extraction of enzymatic extract. To derive the enzymatic extract, 0.5 g of the petal was extracted with 50 mM potassium phosphate buffer containing polyvinylpyrrolidone 1% and EDTA 1 mM. The resulting extract was centrifuged at 10500 rpm at 4°C for 25 min. The centrifugation was repeated three times and at each stage, the clear supernatant was taken from the sample surface with a sampler and used as the enzymatic extract (Gapinska *et al*., 2008).

To measure peroxidase (POD) activity, 100 µL of the enzymatic extract was mixed with 450 µL of H_2O_2 and 450 µL of guaiacol. The absorbance of the sample was read at 470 nm with a spectrophotometer (Jasco V 530, Japan). Finally, the POD activity was reported in nM/g FW (In *et al*., 2007).

For catalase (CAT) activity measurement, 40 µL of the enzymatic extract, 400 µL of 15 mM H_2O_2, and 2.6 mM of 50 mM potassium phosphate buffer (pH = 7) were mixed and the reaction mix was developed. The absorbance of the solution was, then, read at 240 nm with a spectrophotometer (Jasco V 530, Japan). The CAT activity was calculated using an extinction coefficient of 39.4 mM/cm and reported in nM/g FW (Beers & Sizer, 1952).

Data analysis

In this research, the effect of three levels of gibberellic acid and three levels of nitrogen on two species of violets was investigated as a factorial experiment in the form of a completely randomized design in three replications, 54 plots and 216 experimental units. Morphological traits were measured during the experiment and in the field,

and biochemical traits were measured in the laboratory. After the measurements were completed, the data obtained from field visits and laboratory investigations were analyzed for uniformity of variance and normal distribution and then analyzed with SPSS 19 software. To compare the means, the LSD test was used at the probability level of 1 and 5% and finally graphs were drawn using Excel software.

RESULTS AND DISCUSSION

The simple effect of cultivar on all traits except soil EC, petal phosphorus and potassium, chlorophyll b and peroxidase enzyme and the simple effect of GA on all traits except soil EC and chlorophyll b had a significant effect. Also, the simple effect of N on all traits is significant. The interaction of "species × GA × N" was significant ($P<0.01$) for all traits.

Seedbed characteristics

Seedbed pH

The comparison of means showed that in *V. odorata*, the application of 100 and 200 mg/L N at all three GA levels increased the seedbed pH versus N non-application. In *V. tricolor*, the seedbed pH was higher at the N rate of 100 mg/L than at the N rates of 0 and 200 mg/L at all three GA levels. Among all treatments, the highest seedbed pH was related to "*V. tricolor* × 300 mg/L GA × 100 mg/L N" (8.29) and "*V. tricolor* × 300 mg/L GA × 0 mg/L N" (8.27). These two treatments did not differ significantly. The lowest seedbed pH was 6.85 exhibited by "*V. odorata* × 0 mg/L GA × 200 mg/L N" (Table 1).

Soil pH is one of the most important soil variables that affects the physical, chemical and biological characteristics of soil as well as processes related to plant growth and performance. Soil pH is one of the most important factors for the mobility of nutrients and the availability or absorbability of cations and anions in the root environment (Kabata-Pendias, 2010; Neina, 2019).

Elements are most absorbable at certain pHs. The best pH range for agricultural and horticultural crops is 6.5-7.5 (Mohammadi Torkashvand, 2009). In the present research, the seedbed pH was within this range in most treatments. Nutrients are still absorbed in slightly alkaline pHs so that no shortage is detected in plants, but plants face nutrient deficiencies at high pHs (Mohammadi Torkashvand, 2009). In the present study, the two seedbeds of *V. tricolor* treated with "300 mg/L GA × 100 mg/L N" and "300 mg/L GA × 0 mg/L N" had high pHs (8.29 and 8.27, respectively), so they did not limit nutrient (P and K) uptake by the two violet species.

It has been found that the pH of the soil solution is strongly influenced by plant nutrition with different sources of nitrogen fertilizer due to differences in the ratio of cation/anion absorption, nitrogen absorption, and cell pH stabilization (Kabata-Pendias, 2010).

Some researchers have stated that the application of nitrogenous fertilizers affects soil pH. Research shows that N supply from ammonium sources reduced rhizosphere pH, but its supply for nitrate sources increased it (Hamlin & Barker, 2006). The fertilizer used in the

Table 1. Means comparison for the effect of different treatments on soil pH, soil EC, soil nitrogen, leaf, flower and stolon number. Tehran, Iran, Islamic Azad University/Tehran University, 2020.

Species	Treatments	Soil pH	Soil EC (dS/m)	Soil nitrogen (%)	Leaf number	Flower number	Stolon number
V. odorata	GA_0N_0	7.50 cd	1.33 b	0.392 fgh	6.00 g	4.00 ef	3.00 g
	GA_0N_{100}	7.33 de	1.22 bc	0.66 ab	6.08 g	4.00 ef	3.50 g
	GA_0N_{200}	6.85 f	1.26 bc	0.673 a	6.66 g	3.00 f	4.00 g
	$GA_{150}N_0$	7.62 bcd	1.23 bc	0.242 ij	7.33 fg	4.00 ef	5.00 fg
	$GA_{150}N_{100}$	7.60 bcd	1.10 cd	0.403 e-h	14.33 b-e	6.00 cde	9.00 cd
	$GA_{150}N_{200}$	7.42 cde	1.10 cd	0.455 d-h	14.33 b-e	8.00 abc	9.20 cd
	$GA_{300}N_0$	7.57 cd	1.36 b	0.49 c-g	7.66 fg	5.00 def	5.00 fg
	$GA_{300}N_{100}$	7.47 cd	1.10 cd	0.536 b-e	14.66 b-e	7.06 b	10.00 bcd
	$GA_{300}N_{200}$	7.13 ef	1.12 cd	0.548 a-d	16.00 abc	9.02 ab	11.00 abc
V. tricolor	GA_0N_0	7.72 bc	1.20 b-d	0.432 d-h	11.00 ef	5.00 def	6.00 ef
	GA_0N_{100}	7.89 b	1.13 cd	0.511 c-f	11.66 e	5.00 def	6.00 ef
	GA_0N_{200}	7.47 cd	1.04 d	0.6 abc	12.33 cde	6.00 cde	8.00 de
	$GA_{150}N_0$	7.56 cd	1.44 b	0.206 j	12.00 de	7.13 bcd	8.2 de
	$GA_{150}N_{100}$	7.69 bc	1.30 bc	0.353 hi	15.66 a-d	8.06 abc	10.00 bcd
	$GA_{150}N_{200}$	7.61 bcd	1.00 d	0.361 ghi	18.66 a	9.00 ab	12.00 ab
	$GA_{300}N_0$	8.27 a	1.73 a	0.431 d-h	13.00 b-e	8.00 abc	8.00 de
	$GA_{300}N_{100}$	8.29 a	1.36 b	0.432 d-h	16.66 ab	10.00 a	11.00 abc
	$GA_{300}N_{200}$	7.88 b	1.01 d	0.44 d-h	19.50 a	10.60 a	13.00 a

*In each column, means with similar letters are not significantly different ($P<0.05$) using the LSD test

present study (Crystaliq (40-0-0 N-P-K), Super Max, Iran) contained urea and ammonium sulfate, which decreased the pH of the soil solution compared to the control. Ammonium fertilizer after nitrification and during preferential absorption of $NH4^+$ (preferential $NH4^+$) decreases the pH of the soil mass and rhizosphere, respectively (Thomson *et al*., 1993).

Seedbed EC

In both species, increasing the N rate resulted in decreasing the seedbed EC versus N non-application at all three GA levels. So, there was a difference of 0.26 and 0.73 dS/m between the highest and lowest EC recorded in the soil of *V. odorata* and *V. tricolor*, respectively.

The highest seedbed EC (1.73 dS/m) was obtained from the treatment of "*V. tricolor* × 300 mg/L GA × 0 mg/L N". The lowest was related to the treatments of "V. tricolor × 0, 150, or 300 mg/L GA × 200 mg/L N", differing insignificantly. Regarding V. odorata, the application of "150 or 300 mg/L GA × 200 or 100 mg/L N" reduced seedbed EC versus the other treatments significantly (Table 1).

In the present study, the EC of the seedbeds was in the range of 1.00-1.73 dS/m, which is appropriate for most agricultural and horticultural crops. Researchers argue that seedbed EC changes during the plant growing season just like pH, so soil EC may be higher after plant cultivation or even during the growth period than its initial value (Najafi & Towfighi, 2008). Eigenberg *et al*. (2002) studied the interactive effect of manures and composts, along with the NPK fertilizer, on corn. They recorded the highest EC for beds that were treated with compost and N fertilizer. The lowest EC variations during the growing season were related to beds that were fertilized with NPK. These researchers suggest that water and nutrients are the most important factors limiting soil EC. Mirzakhaninafchi *et al*. (2017) stated that soil type, temperature, moisture level, fertilizer amount and salinity have an effect on soil EC value. In the research of these researchers, the amount of EC increased with the increase of nitrogen fertilizer level from 50 to 200 kg/ha with a gentle slope.

Seedbed total N

Table 1 shows that in both violet species and at all GA levels, the N application increased the seedbed N content versus its non-application. However, in both species, the highest seedbed N content was obtained from the application of "100 or 200 mg/L N × 0 mg/L GA" and the lowest from the application of "150 mg/L GA × 0 mg/L N". The highest seedbed N content (0.673%) was related to "*V. odorata* × 0 mg/L GA × 200 mg/L N", not differing from "*V. odorata* × 0 mg/L GA × 100 mg/L N", "*V. tricolor* × 0 mg/L GA × 200 mg/L N", and "*V. odorata* × 300 mg/L GA × 200 mg/L N" significantly (Table 1).

Increasing the N level in both species increased the seedbed N content, but it was decreased with the combined application of GA and N, which can be related to the effect of GA in increasing the plants' N requirements and subsequently, the increase in N uptake from the seedbed.

Morphological traits

Leaf number

The comparison of means revealed that *V. tricolor* had more leaves than *V. odorata*. Among the treatments, "*V. tricolor* × 300 mg/L GA × 200 mg/L N" recorded the highest number of leaves, insignificantly differing from that of "*V. tricolor* × 150 mg/L GA × 100 or 200 mg/L N", "*V. tricolor* × 300 mg/L GA × 100 mg/L N", and "*V. odorata* × 300 mg/L GA × 200 mg/L N". The lowest number of leaves was observed in "*V. odorata* × 0 mg/L GA × 0, 100 or 200 mg/L N", not differing from one another significantly (Table 1).

Flower number

Based on the comparison of means for the interactive effect of the experimental treatments, the highest number of flowers was related to "*V. tricolor* × 300 mg/L GA × 200 or 100 200 mg/L N" (10.6 and 10 flowers, respectively), which did not show statistically significant differences from the treatments of "*V. tricolor* × 150 mg/L GA × 100 or 200 mg/L N", "*V. tricolor* × 300 mg/L GA × 0 mg/L N", and "*V. odorata* × 300 or 150 mg/L GA × 200 mg/L N". Overall, *V. tricolor* produced more flowers than *V. odorata* when the plants were treated with N and GA (Table 1).

Stolon number

According to the results, increasing the GA and N rates increased the number of stolons in both violet species so that in both species, the highest number of stolons was produced by the treatment of "300 mg/L GA × 200 mg/L N". This treatment did not show any statistically significant differences from the two treatments of "*V. tricolor* × 300 mg/L GA × 100 mg/L N" and "*V. tricolor* × 150 mg/L GA × 200 mg/L N". The lowest number of stolons was recorded by the treatment of "*V. odorata* × 0 mg/L GA × 0, 100 or 200 mg/L N" (3, 3.5, and 4 stolons, respectively). These three treatments did not differ significantly (Table 1).

The combined application of N and GA improved the number of leaves, flowers, and stolons in both species. N is an essential element for the vegetative growth and development of plants so that growth cessation is a symptom of N deficiency (Patni *et al*., 2020; Rehman *et al*., 2020). Rehman *et al*. (2020) found that N increased the vegetative growth and leaf number of ramie by enhancing photosynthesis, preserving chlorophylls and proteins, and maintaining the natural functioning of the plants. In Ghasemi *et al*. (2012) research, the application of N fertilizers increased strawberry leaves. These researchers argue that N fertilizers increases N availability for plants to meet their N demand for photosynthesis. The resulting increase in photosynthates improves vegetative growth including leaf number. Increases have been reported in the leaf number of peppermint (Golchin *et al*., 2020) and aloe vera (Hazrati Yadekori & Tahmasebi Sarvestani, 2012) with N application, which agrees with our findings.

GA is a growth stimulator that plays a key role in many physiological processes, e.g., cell division and elongation, and its application generally increases vegetative growth including leaf number and plant yield (Hajisamadi Asl *et al*.,

2011). Consistent with our findings, leaf number was also increased in *Polianthes tuberosa* (Mortezaeinezhad & Etemadi, 2010) with GA application. Akbari Chermahini & Moallemi (2011) state that in addition to stimulating shoot growth, GA increases root growth via stimulating sucrose synthesis and mobilization to underground parts. In our research too, the number of stolons was increased with the combined application of GA and N.

Accelerating flowering, inhibiting flower bud abortion, and increasing the length of flowering stem and the number of flowers is some effective consequences of GA for plants (Giannakoula *et al*., 2012). In the present study, GA positively influenced the flower number of both violet species. Mortezaeinezhad & Etemadi (2010) reported that GA was effective, especially at the rate of 300 mg/L, in advancing flowering and increasing flower and bulb production of *Polianthes tuberosa*. Chehrazi *et al*. (2017) investigated the effect of GA (0, 100, 200, and 400 mg/L) on the morphological traits of white and yellow snapdragon cultivars. The highest number of florets and leaves of the yellow and white cultivars were obtained from the GA rates of 200 and 400 mg/L GA, respectively.

Chlorophyll a, b, and total

V. tricolor at all treatments and *V. odorata* at all treatments except "150 mg/L GA × 0 mg/L N" showed significantly higher chlorophyll *a* content than the control. The treatment of "0 mg/L GA × 100 mg/L N" was related to the lowest chlorophyll *b* content in *V. odorata* and the highest in *V. tricolor*. In *V. odorata*, all treatments except "0 mg/L GA × 100 mg/L N" increased chlorophyll *b* versus the control significantly. But *V. tricolor* showed lower chlorophyll *b* versus the control under all treatments except "300 mg/L GA × 200 mg/L N" and "0 mg/L GA × 100 mg/L N" (Table 2).

Means comparison showed that, increasing N and GA in both violet cultivars, total chlorophyll was increased especially in *V. odorata*. In *V. odorata*, the highest total chlorophyll (21.37 mg/g FW) was recorded by the treatment of "150 mg/L GA × 200 mg/L N", which was not significantly different from the treatments of "150 mg/L GA × 100 mg/L N" and "0 mg/L GA × 200 mg/L N". In *V. tricolor*, the application of "150 mg/L GA × 0 mg/L N" resulted in the lowest total chlorophyll content. The highest total chlorophyll content in *V. tricolor* (18.10 mg/g FW) was recorded by the application of "300 mg/L GA × 200 mg/L N" (Table 2).

N is a major component of proteins, amino acids, and chlorophyll. It plays a role in the synthesis and activity of enzymes involved in the formation of chloroplast membranes and its presence is essential for preserving and increasing photosynthesizing pigments (Patni *et*

Table 2. Means comparison for the effect of different treatments on leaf chlorophyll and petals phosphorus and potassium. Tehran, Iran, Islamic Azad University/Tehran University, 2020.

Species	Treatments	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)	Total chlorophyll (mg/g FW)	Petals phosphorus (%)	Petals potassium (%)
V. odorata	GA_0N_0	6.45f g	1.06 i-k	7.51 i	0.57 gh	1.32 d
	GA_0N_{100}	8.05 d-g	0.39 k	8.44 h	0.62 g	1.87 a
	GA_0N_{200}	16.51 a	3.36 c-e	19.87 ab	1.14 ef	1.73 a
	$GA_{150}N_0$	6.45 fg	1.64 g-j	8.09 h	1.19 ef	1.42 cd
	$GA_{150}N_{100}$	14.76 a	4.32 bc	19.08 ab	1.63 bc	1.76 a
	$GA_{150}N_{200}$	16.79 a	4.58 ab	21.37 a	1.93 a	1.73 a
	$GA_{300}N_0$	11.10 cd	2.24 f-h	13.34 de	1.30 de	1.49 c
	$GA_{300}N_{100}$	14.29 ab	2.41 f-h	16.70 cd	1.76 abc	1.70 ab
	$GA_{300}N_{200}$	14.41 ab	3.01 def	17.41 c	1.82 ab	1.66 b
V. tricolor	GA_0N_0	5.29 g	3.78 bcd	9.07 g	0.30 h	1.02 e
	GA_0N_{100}	7.81 e-g	5.58 a	13.39 de	0.95 f	1.70 ab
	GA_0N_{200}	11.44 bc	2.57 e-g	14.01 d	0.54 gh	1.46 c
	$GA_{150}N_0$	8.22 d-g	0.63 jk	8.85 h	1.03 ef	1.70 ab
	$GA_{150}N_{100}$	8.60 c-f	1.77 g-i	10.37 f	1.96 a	1.83 a
	$GA_{150}N_{200}$	10.67 c-e	2.13 f-h	12.80 e	1.63 bc	1.76 a
	$GA_{300}N_0$	8.85 c-f	1.38 h-k	10.23 f	1.49 cd	1.73 a
	$GA_{300}N_{100}$	14.64 a	2.11 f-i	16.75 cd	1.84 ab	1.87 a
	$GA_{300}N_{200}$	14.22 ab	3.88 bcd	18.10 b	1.63 bc	1.73 a

*In each column, means with similar letters are not significantly different (P<0.05) using the LSD test.

al., 2020; Rehman *et al*., 2020). Since chlorophyll has a nitrogenous structure, N availability increases chlorophyll pigments in leaves. This result was recorded for both violet species in the present study. Reis *et al*. (2015) reported that the application of N fertilizers directly influenced the N content of the plant tissues, chlorophyll synthesis and accumulation, and the improvement of the photosynthesis process. Rehman *et al*. (2020) also revealed that the nutrition of ramie plants with adequate N could hinder the degradation of photosynthesizing pigments and the disruption of the photosynthesis process. It has been reported that the escalation of chlorophyll content following N application was effective in improving the growth of *Ficus deltoidea* (Sheikh & Ishak, 2016) which corroborates with our results.

GA is an anti-aging hormone that prevents chlorophyll degradation. Hejazi & Kafashi Sedghi (2018) reported that GA contributed to preserving pigments by increasing Rubisco activity and stimulating and accelerating the photosynthesis process. Researchers have reported that N is a structural component of chlorophylls, and GA helps maintain chloroplast structure and chlorophyll pigments by increasing leaf N content (Ramteke *et al*., 2016). GA application has reportedly increased chlorophyll synthesis in the leaves of narcissus (Mashahiri & Hassanpour Asil, 2018), wheat (Alharby *et al*., 2021), and two snapdragon cultivars (Chehrazi *et al*., 2017). As well, Mozafari & Khaleghi (2016) reported that the combined application of GA and N increased chlorophyll in pistachio, which agrees with our findings.

Petal elements (P and K)

The comparison of means showed that the application of N increased petal P content of *V. odorata* at all GA levels; the higher the N rate was, the greater the increase in the P content would be. The application of N increased the petal P content of *V. tricolor*, too (0.3% versus the control), but the increase in N rate from 100 to 200 mg/L had no effect on increasing the petal P content of this cultivar. In general, the most effective treatment in increasing petal P was "150 mg/L GA × 100 mg/L N" in *V. tricolor* and "150 mg/L GA × 200 mg/L N" in *V. odorata*, which did not significantly differ from the treatments of "*V. odorata* × 300 mg/L GA × 100 or 200 mg/L N" and "*V. tricolor* × 300 mg/L GA × 100 mg/L N". The lowest petal P content was observed in the control in both cultivars (Table 2).

Table 2 depicts that the application of GA and N increased the petal K content of both cultivars versus the control. The highest petal K content of *V. odorata* was obtained from the treatments of "0 or 150 mg/L GA × 100 or 200 mg/L N" and "300 mg/L GA × 100 mg/L N" and that of *V. tricolor* was obtained from the application of "0 mg/L GA × 100 mg/L N" and "300 or 150 mg/L GA × 0, 100 or 200 mg/L N". They, which did not differ from one another significantly, increased the petal K content versus the other treatments (Table 2).

Pis a nutrient that facilitates vital functions in skeleton and non-skeleton tissues. It is necessary for body health and energy generation. K is also an essential nutrient for the body. Its uptake through foods and supplements reduces blood pressure, prevents stroke and cardiovascular diseases, and enhances bone health (Weaver, 2013). So, P and K intake from a natural food source can improve human health. The results showed that the edible violet flowers can supply a part of the daily P and K requirements of the human body. Also, the results revealed that N and GA could increase the nutrient contents of the violet flowers.

The application of exogenous growth regulators is an effective way to increase yield, improve quality, and regulate the uptake and accumulation of nutrients in plants (Gilani *et al*., 2021). GA stimulates cell division (Alharby *et al*., 2021). Clearly, the need for nutrients increases during cell division and development, so GA increases nutrient uptake in this period, thereby enhancing plant growth and development. Alharby *et al*. (2021) showed that GA increased wheat growth and development by increasing nutrient uptake.

It has been reported that there is a positive correlation between the uptake and mobilization of K and nitrate in plants (Ye *et al*., 2022). Malhotra *et al*. (2018) found that P and N had a synergic effect and N application increased P uptake. In Ye *et al*. (2022) research, it was found that the N and P uptake processes were in interaction and there should be coordination between these two nutrients for the optimal growth of the plants and their nutritional balance. There are reports regarding the acceleration of nutrient uptake and mobilization in plant organs by N (Mohammadi *et al*., 2016) and GA (Miceli *et al*., 2019). According to Mozafari & Khaleghi (2016), the

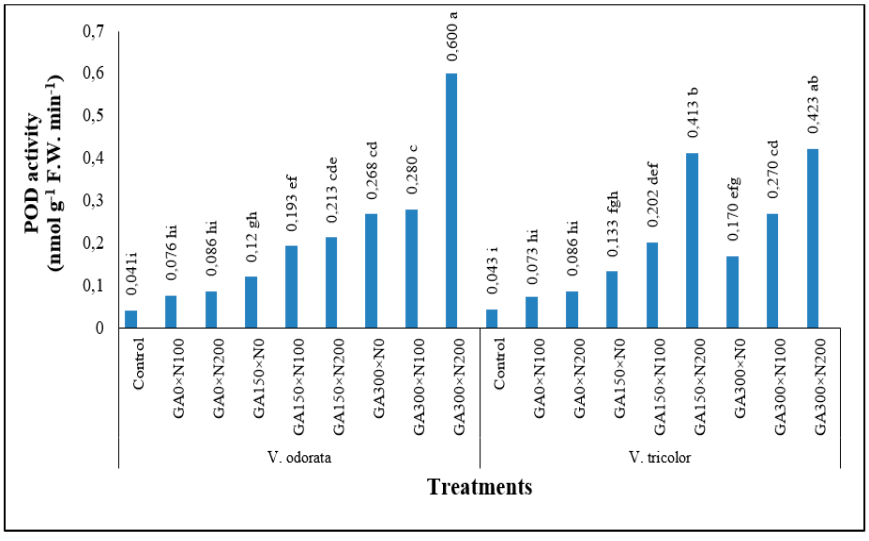

Figure 1. The interactive effect of "species × gibberellic acid × nitrogen" on the peroxidase (POD) activity in two violet species. Tehran, Iran, Islamic Azad University/Tehran University, 2020.

combined application of N and GA increased the nutrient content of pistachio, which is consistent with our findings.

The activity of antioxidant enzymes

Based on the comparison of means for the interaction of "species × GA × N" for POD activity, the activity of this enzyme was increased with the combined application of GA and N, especially by increasing the N rate, in both violet species versus the control. The POD activity was the highest in the treatment of "*V. odorata* × 300 mg/L GA × 200 mg/L N", but this treatment did not show a statistically significant difference from the treatment of "*V. tricolor* × 300 mg/L GA × 200 mg/L N". The lowest POD activity of both species was related to the control, differing from one another insignificantly (Figure 1).

Figure 2 shows that the CAT activity of *V. odorata* was not significantly influenced by applying only 100 or 200 mg/L N, but GA and GA × N increased it versus the control (0.21 nM/g FW) significantly. In *V. tricolor*, increasing the N rate increased the CAT activity versus the control (0.13 nM/g FW) significantly at all three GA levels. The highest CAT activity was related to "*V. odorata* × 300 mg/L GA × 200 or 100 mg/L N". These two treatments did not differ significantly (Figure 2).

Hydrogen peroxidase (H_2O_2) is a sort of reactive oxygen species (ROS) that is produced in a slight quantity during natural reactions and processes and in a very high quantity during stresses and adverse conditions. H_2O_2 accumulation in cells causes the peroxidation of fats, proteins, and nucleic acids, the degradation of enzymes, and the activation of cell apoptosis (Sharma & Ahmad, 2014). POD and CAT enzymes are two important H_2O_2-decomposing enzymes in the antioxidant system of plants. CAT decomposes H_2O_2 into water and oxygen and plays a key role in preserving H_2O_2 balance in cells. Among the antioxidant enzymes, CAT has the highest efficiency in decomposing H_2O_2 so that it reportedly converts H_2O_2 into H_2O and O_2 at a rate of 6 million molecules per minute (Sharma & Ahmad, 2014).

POD is involved in H_2O_2 decomposition into water. As POD activity increases, the invasion of plants by ROS is alleviated. In the present study, the CAT and POD activity was increased in both violet species with the combined application of GA and N. According to Khammari *et al*. (2012), the application of N at the rates of 100 and 150 mg/L significantly increased the activity of antioxidant enzymes in Indian Senna. There are, however, reports about the impact of N application on reducing the activity of antioxidants. For example, Reis *et al*. (2015) stated that with the application of N, the activity of antioxidant enzymes was decreased in coffee, while N non-application resulted in its increase. Rehman *et al*. (2020) found that the application of N at adequate amounts prevented the ramie plants from damages to their tissues, reduced the accumulation of malondialdehyde in their tissues, and reduced the activity of their antioxidant systems. However, they reported that the increase in the N rate from 140 and 280 kg/ha to 420 kg/ha increased SOD and POD activity.

Research on the effect of GA on the activity of antioxidant enzymes shows that GA application reduces POD and CAT activity (Abbas *et al*., 2020). Consistent with our findings, Liang *et al*. (2013) and Alharby *et al*. (2021) recorded an increase in the activity of antioxidant enzymes with GA application in *Salvia miltiorrhiza* and wheat, respectively.

As was expressed in the results section, the cultivation of the violet flowers (*V. tricolor* and *V. odorata*) under different levels of N as an essential element for growth and GA as a growth stimulator had a significant effect on improving their recorded traits. In *V. odorata*, the highest leaf number, flower number, stolon number, and antioxidant activity were obtained from the application of "300 mg/L GA × 200 mg/L N" and the highest P and total chlorophyll content of the shoot from the application of "150 mg/L GA × 200 mg/L N". In *V. tricolor*, the highest flower number, leaf number, stolon number, total chlorophyll content, and antioxidant activity were related to the application of "300 mg/L GA × 200 mg/L N". So, the combined application of 150 or 300 mg/L GA and 200 mg/L N is recommended for the cultivation of *V. odorata* and *V. tricolor* as they produced the best of most studied traits of these two species.

Therefore, it can be said that by using "150 or 300 mg/L GA and 200 mg/L N" it is possible to improve the ornamental and biochemical characteristics of commercial and aromatic violets. With production of violet as an edible source, the harvesting of this flower from nature is decreased. N and GA can help to increase the yield of edible parts of this flower. In the end, it is recommended to

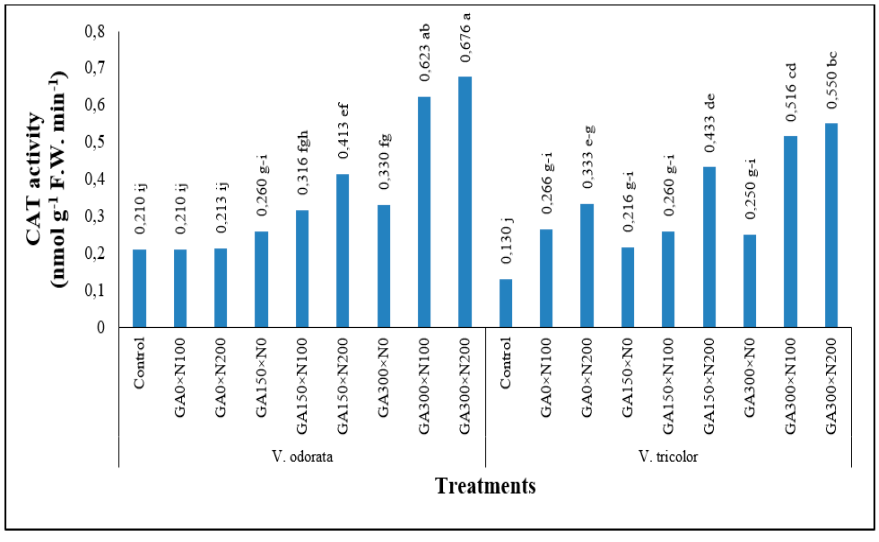

Figure 2. The interactive effect of "species × gibberellic acid × nitrogen" on the catalase (CAT) activity in two violet species. Tehran, Iran, Islamic Azad University/Tehran University, 2020.

improve the ornamental-medicinal and edible properties of different cultivars of *Viola* sp. in order to promote its health benefits for consumers and preserve the health of the environment by organic methods.

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