

Article

Effect of Varied Salinity on Marigold Flowers: Reduced Size and Quantity Despite Enhanced Antioxidant Activity

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Abstract: Salinity is an increasing problem worldwide that limits crop production. The cultivation of salt-tolerant flowers is a potential sustainable strategy for the exploitation of saline soils while avoiding the use of freshwater resources. However, our understanding of how flowers can adapt to salinity is still limited. In this study, we investigated the effects of salinity on a widely-used ornamental plant (*Tagetes patula*) by submitting three cultivars (cv. Aurora Orange, Fireball, Safari Scarlet) to different salinity levels (0, 50, 100, 300 mM). The plants were grown under controlled conditions and followed over two weeks. We tested the effect of salinity on plant growth and flower production, as well as on the levels of total carotenoids, polyphenols, and flavonoids, and the activities of antioxidant enzymes (catalase, peroxidase activity, ascorbate peroxidase). The findings indicate a substantial decline in both plant growth and flower production under saline conditions. Overall, plant height was ¼ lower, and there were 1/3 less flowers under salinity. Additionally, there was a consistent rise in antioxidant compounds, highlighting the swift response of defense mechanisms, both enzymatic and non-enzymatic, to combat stress. The high levels of antioxidant compounds sustain the use of marigold flowers as a new source of nutritional compounds, with enriched nutritional contents. Yet, cultivation of these plants in saline conditions should carefully consider the pronounced adverse effects observed at high salinity levels (e.g., 100 and 300 mM) on both plant and flower production.

Keywords: antioxidant; edible flowers; nutritional compound; salinity; tolerance

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1. Introduction

Soil salinity stands out as one of the most severe abiotic stresses, since it causes major reductions in cultivated land area, as well as in crop yield and quality [1]. High soil salinity causes ion toxicity, osmotic stress, nutrient deficiency, and oxidative stress in most plants. This stress condition also leads to oxidative stress, resulting in the overproduction of reactive oxygen species (ROS) [2,3]. To counterbalance these effects, plants activate a complex detoxification system through the actions of enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), and non-enzymatic antioxidants such as vitamins, flavonoids, and carotenoids, to reduce the excess ROS [4–7]. At the same time, plant antioxidants play a potential role in human diets, and can be used for many purposes, including serving as new sources of bioactive and nutritional compounds [8]. Thus, exploring the role of these antioxidants not only provides important information on plant adaptation mechanisms to harsh environmental conditions but also identifies functional compounds with benefits for human health.

In response to high salinity, salt-tolerant species elevate antioxidant levels to balance ROS, coordinating cellular processes such as water uptake and ion regulation [6,9–11]. However, most plants are not tolerant to salinity, and a high degree of responses can even

be found among sensitive and tolerant species. These responses also vary within the same species, being also dependent on the duration and the level of the stress [10,12–14]. For instance, salinity delays flowering in some species such as rice [15] and *Arabidopsis* [16], while in others, namely salt-tolerant species, flowering occurs earlier and faster [17]. But even in salt-tolerant plants, there can be a reduction in pollen fertility, seed set, and seed viability in the presence of salinity [18]. Ultimately, the combined effects of osmotic stress and ion toxicity triggered by salinity generate a secondary stress that affects germination, growth, and overall plant development [6,19].

Understanding salt tolerance in ornamental plants also holds global significance as it could contribute to the conservation of freshwater resources in floriculture [20]. Although some research has advanced our understanding of salt tolerance of ornamental plants, the underlying mechanisms are complex as they vary widely between plants. For instance, some ornamental plants, such as snapdragon (*Antirrhinum majus*), petunia (*Petunia × hybrida*), verbena (*Verbena × hybrida*), coleus (*Solenostemon scutellarioides*), and begonia (*Begonia hiemalis*) can tolerate moderate levels of salinity (7.0–14.2 dS m⁻¹ NaCl for 5 weeks) without a significant reduction in plant growth [21]. Other ornamental plants, such as *Zinnia*, are highly affected by salinity, even at low concentrations [22], while others, such as periwinkle (*Catharanthus roseus*), may show some tolerance, but only under low salinity levels (up to 3 dS m⁻¹ [23]). Thus, to breed new salt-tolerant plants, it is important to understand the tolerance threshold where salinity does not compromise physiological processes, resulting in decreased quality or reduced yield.

Marigold species (*Tagetes* L.) demonstrate potential for cultivation under specific levels of salinity and are highly demanded in the flavor, fragrance, food, medicinal, and floricultural industries [24]. Marigolds contain a variety of bioactive components, including carotenoids, flavonoids, and phenolic acids, making their flowers valuable in the search for antioxidant compounds [25]. Pigments such as zeaxanthin and lutein from marigold flowers have also been used as food coloring agents [26,27]. Nevertheless, most marigold plants are highly affected by abiotic factors such as drought and temperature [28–30]. However, some marigold species and varieties are viewed as promising cash crops that can grow in saline environments, although with significant challenges. For instance, exposure to short-duration salinity levels (10 days at 50, and 100 nM NaCl) of *T. patula* L. (French marigold) plants triggered an accumulation of antioxidants and minerals in its flowers, but also led to a decrease in plant weight and the marketability of post-harvested flowers [24]. The growth of *T. erecta* L. (Aztec or Mexican marigold) cv. Sumo orange plants under 0, 50, 100, 150, and 200 mM NaCl for 25 days was also affected by increased salinity [31]. As salinity stress increased, Ca²⁺, Mg²⁺, and Na⁺ concentrations also increased, while the K⁺ concentration decreased in the roots and shoots. Additionally, the K⁺/Na⁺ and Ca²⁺/Na⁺ ratios of the roots and shoots were significantly lower in all salinity treatments, even though plants received a salt pre-treatment (except controls) to avoid a salt stress shock [31]. Seed germination of *T. minuta* L. (Mint marigold) cv. Him Swarnima grown under 0, 50, 100, 150, and 200 mM of soluble salts (CaCl₂, KCl, MgCl₂, Na₂SO₄, and NaCl) decreased significantly with increased levels of salinity under all salts, especially after 100 mM [32].

In this study, we investigated the salt-tolerance mechanisms of three French marigold cultivars (cv. Aurora Orange, Fireball, Safari Scarlet) under varying NaCl concentrations (0, 50, 100, and 300 mM). Our aim was to understand the extent to which flowers can tolerate salinity. We studied the effects of salinity on overall plant growth and flower production, examining how enzymatic and non-enzymatic components reacted to salinity. Despite the positive impacts from an edible point of view, salinity may impose constraints on plant development. While marigolds have been reported to tolerate salinity, being a source of edible flowers and nutritional compounds, the level of salinity and the cultivars used play a crucial role [24]. Thus, we specifically aim to (1) unravel the impacts of different salinity levels on French marigold flowers, (2) understand the antioxidant response mechanisms of flowers to salt stress, (3) detect the occurrence of intraspecific tolerance variability to

salinity, and (4) test the salt-tolerance trade-off between the level of beneficial antioxidant compounds versus plant growth and flower production.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

Seeds from three *Tagetes patula* widely sold cultivars (cv. Aurora Orange, Fireball, Safari Scarlet) were chosen for this study since they are recommended for use in gardens, including city and courtyard gardens, and coastal sites as they are thought to be resistant to abiotic stresses. Yet, the effects of stresses have not been tested in these varieties. Thus, initially seeds were grown in 2 L capacity pots (50% peat, 25% perlite, and 25% vermiculite following [30]) in a nursery ($T = 20.5\text{--}25.3\text{ }^{\circ}\text{C}$; $\text{RH} = 72\text{--}76\%$, light and dark period = 16:8 h) for 3 weeks to ensure plant uniformity at the stage of two-true leaves. Plants were watered (50 mL per pot) twice a week using Hoagland nutritive solution ($\text{Na}_2\text{Fe}\cdot\text{EDTA}$ 20 mg/L, H_3BO_3 2.86 mg/L, $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$ 2.13 mg/L, $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ 0.22 mg/L, $\text{CuSO}_4\cdot 7\text{H}_2\text{O}$ 0.08 mg/L, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 0.02 mg/L).

Seedlings were then transplanted to 200 L capacity tanks and transferred to a controlled environmental chamber with the following conditions: long-day photoperiod (16 h light), temperature of 23 and 19 $^{\circ}\text{C}$ during the light and dark periods, respectively, and relative humidity of 72–76%, with an irradiance of 700–800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Tenney WIT-983; New Columbia, PN, USA). Each drained pot contained only one plant being supported by a polyethylene tray. Plants were irrigated with sterile water (200 mL per pot) twice a week and allowed to acclimatize for one month, before the imposition of stress. The optimal pH and EC of the NS were 5.8 and 2.1 mS cm^{-1} , respectively.

After one month, plants started to flower and were exposed to the following salinity treatments: 0, 50, 100, and 300 mM NaCl levels for two weeks following previous stress experiments [24]. Each treatment consisted of 10 biological replications, totaling 40 plants (biological replicates) per cultivar. The regular electrical conductivity (EC) of the nutrient solution was 2.0 mS cm^{-1} for the control treatment (0 mM NaCl), 6.0 mS cm^{-1} for the salinity of 50 mM NaCl, 10.0 mS cm^{-1} for the salinity of 100 mM NaCl, and 28.0 mS cm^{-1} for the salinity of 300 mM NaCl, being constant during salinity stress treatments. At the end of the experiment, the following traits were measured: plant height (cm), number of flowers produced, and size of flowers, e.g., its diameter (cm). Flowers were then harvested and dried using a thermo-ventilated oven at 65 $^{\circ}\text{C}$.

2.2. Determination of Total Carotenoid, Polyphenol, and Flavonoid Content

The total carotenoid content was based on 500 mg of fresh flowers, which were extracted with 30 mL of 80% ice-cold acetone before being vortexed and centrifuged. The supernatant was separated, and its absorbance was measured at 480 nm, as described by [24]. The following equation was used for quantification: Carotenoids (μg) = $4 \times A_{480} \times \text{volume (mL)}$. Results were converted and expressed as mg of carotenoids per gram of DW.

The total polyphenol content of flowers was determined based on extracts prepared from 500 mg of fresh flowers following repeated (four-fold) addition of 2.5 mL 50% (*v/v*) methanol under ultrasonication (MSE Sonicprep 150 ultrasonic disintegrator; Sanyo, Loughborough, UK). The aliquot was collected and centrifugated for 5 min at 4 $^{\circ}\text{C}$ at $3000 \times g$ (Harrier 18/80, Sanyo Scientific, Torrance, CA, USA). From the supernatant, we collected and transferred to a new Eppendorf tube 125 μL , adding 1.5 mL of Milli-Q water, 125 μL of FolinCiocalteu reagent (Sigma Aldrich, Poole, UK), and 1.25 mL of 7% (*w/v*) sodium carbonate. The reaction mix (3 mL) was incubated in the dark for 90 min. The absorbance was then measured at 755 nm (Genesys 10 Vis, ThermoSpectronic, Rochester, NY, USA) according to [24]. Results were expressed in terms of μmol of gallic acid equivalents (GAE; Sigma Aldrich, Poole, UK) per gram of DW.

The total flavonoid content was extracted from 500 mg of fresh flowers, following [30], using 4 mL of Milli-Q water and 0.3 mL of sodium nitrite solution (5% concentration). The

reaction mix was incubated for 5 min, after which we added 0.3 mL of aluminium chloride (10% concentration). The sample was left to incubate for 6 min, after which we added 2 mL of sodium hydroxide (1 M). Immediately after that, the final volume was completed and mixed with water (Milli-Q) until 10 mL. The absorbance was measured at 510 nm. Results were expressed as mg of catechin equivalents (CE) per gram of DW.

2.3. Antioxidative Enzyme Activities

Maximal cellular enzyme activities were assayed using 500 mg fresh weight (FW) of flower tissues, pooled from 3 flowers per plant and treatment. After that, samples were homogenized under 1 mL of buffer containing 200 mM Tris-HCl (pH 8), 10 mM MgCl₂ 6H₂O, 30 mM β-mercaptoethanol, 4 mM DTT, 2% Triton X-100, "Complete cocktail EDTA" (2 pills), and 10% glycerol, adding 1% (1 mL) of polyvinylpolypyrrolidone (PVPP) to each sample in the homogenization phase. The samples were centrifuged (13,000 × g, 20 min, 4 °C), and the supernatant was used to quantify enzymatic activities.

The activity of catalase (CAT) was determined in a 1.5 mL reaction mixture containing 10 mM H₂O₂, 50 mM phosphate buffer (pH 7.0), and the enzyme extract. The activity was evaluated following [24] through the rate of H₂O₂ consumption at 240 nm. Results were expressed in CAT units mg⁻¹ of protein DW (1 unit = 1 mM of H₂O₂ reduction per min).

Peroxidase activity (POX) was determined as described by [33], by measuring the absorbance at 430 nm, and using an extinction coefficient of 2.47 mM⁻¹ cm⁻¹ for calculations. Results were expressed in POX units/mg of protein. One POX unit was defined as the amount of enzyme to decompose 1 μmol of H₂O₂ per minute. Results were expressed in units mg⁻¹ of protein DW.

Ascorbate peroxidase (APX) was determined according to [34]. Each reaction mixture contained 20 mM ascorbate and 0.1 mM H₂O₂ in 50 mM phosphate buffer (pH 7.8) and 10 μL of the enzyme extract in a total volume of 1 mL. The sample reaction was assessed through the H₂O₂-dependent oxidation of ascorbate at 290 nm, using an extinction coefficient of 2.8 mM⁻¹ cm⁻¹ for calculations. Results were expressed in APX units mg⁻¹ of protein DW.

2.4. Statistical Analysis

Mean values (±SE) were calculated from the 10 replicates per cultivar using IBM SPSS v.22. To analyze the effects of salinity within each cultivar, we used a multivariate ANOVA at the 5% significance level after checking the homogeneity of variance using Levene's Test for Equality of Variances. Additionally, a second ANOVA was performed to test for significant differences between cultivars within the same salinity level. Significant differences between means were also followed by Tukey's test for post-hoc comparisons at the 5% significance level. The Pearson correlation coefficient was used to test the relationship between plant height, the number of flowers produced, size, and the production of antioxidant compounds (carotenoids, polyphenols, and flavonoids) and the activity of antioxidative enzymes (CAT, POX, APX).

3. Results

3.1. Influence of Salinity on Plant Growth and Flower Production

Salinity significantly decreased the plant height of *T. patula* cv. Aurora Orange ($F_{3,14} = 10.035$; $p = 0.001$), cv. Fireball ($F_{3,14} = 52.518$; $p = 0.001$), and cv. Safari Scarlet ($F_{3,14} = 5.788$; $p = 0.011$). Overall, salinity reduced plant height by 1/4 in comparison to non-saline conditions. In cv. Aurora Orange, plant height was significantly reduced under 100 mM and 300 mM NaCl, while no significant differences were found between control and 50 mM NaCl conditions (Figure 1A). In contrast, plant height already decreased in the other two cultivars under the lowest salinity level (Figure 1A).

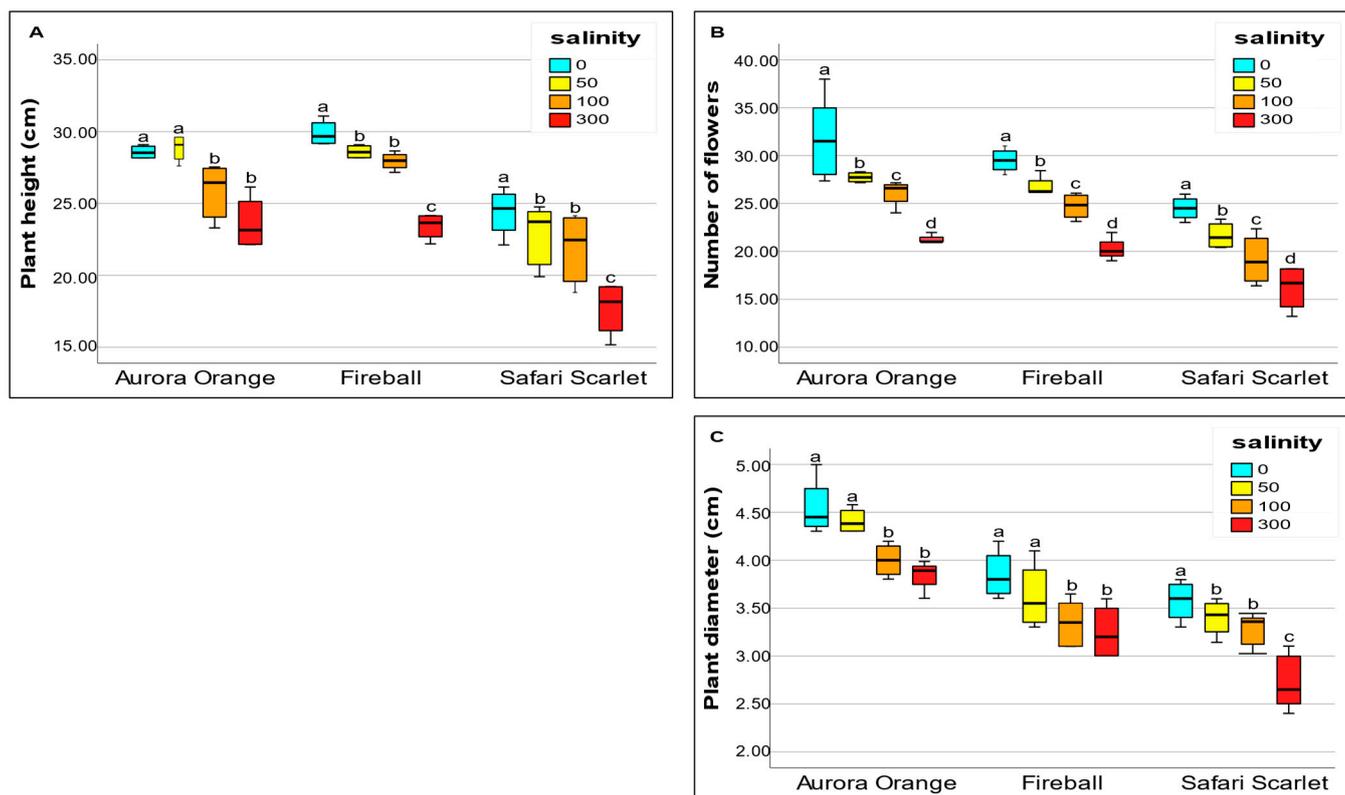


Figure 1. Impact of salinity levels (0, 50, 100, and 300 mM NaCl) on plant height, (A) the number of flowers produced, (B) and the size of flowers, (C) considering *Tagetes patula* cultivars cv. Aurora Orange, Fireball, and Safari Scarlet. Each box plot represents the minimum, lower quartile, median, upper quartile, and maximum values. Different superscripts indicate significant differences between salinity levels for the same cultivar (ANOVA followed by a Tukey test at $p < 0.05$).

Salinity also affected the number of flowers produced (Figure 1B), since a significant decrease of almost 1/3 was reported with increased salinity levels, either in cv. Aurora Orange ($F_{3,14} = 9.331$; $p = 0.002$), cv. Fireball ($F_{3,14} = 37.500$; $p = 0.001$), or cv. Safari Scarlet ($F_{3,14} = 10.008$; $p = 0.001$). The average number of flowers decreased from 32 and 21 in cv. Aurora Orange, 30 and 20 in cv. Fireball, and from 24 and 18 in cv. Safari Scarlet, as salinity increased. This negative effect was especially felt under 300 mM NaCl in all cultivars, where the lowest number of flowers was recorded due to the abortion of several flower buds (Figure 1B).

Likewise, salinity also affected the size of flowers, becoming smaller with the increase in salinity (Figure 1C). The average flower size decreased from 4.46 to 3.58 in cv. Aurora Orange, 3.56 to 3.20 in cv. Fireball, and from 3.54 to 2.52 in cv. Safari Scarlet as salinity increased. Significant differences were recorded either in cv. Aurora Orange ($F_{3,14} = 8.461$; $p = 0.003$), cv. Fireball ($F_{3,14} = 2.716$; $p = 0.032$), or cv. Safari Scarlet ($F_{3,14} = 7.310$; $p = 0.005$). Some intraspecific variability was found since the size of flowers in cvs. Aurora Orange and Fireball was only significantly affected by the highest salinity levels (100 and 300 mM NaCl) while cv. Safari Scarlet showed the lowest size only under 300 mM NaCl (Figure 1C).

3.2. Determination of Total Carotenoid, Polyphenol, and Flavonoid Content

Carotenoids varied between 1.23 and 3.44 in cv. Aurora Orange, 1.28 and 3.19 in cv. Fireball, and between 1.27 and 3.74 in cv. Safari Scarlet (Table 1). They significantly increased with salinity, almost triplicating the values under the highest concentration ($F_{3,14} = 12.370$; $p = 0.0002$). The levels of polyphenols also increased as salinity increased ($F_{3,16} = 9.561$; $p = 0.0002$). Polyphenols varied between 23.21 and 55.45 in cv. Aurora Orange, 22.25 and 55.66 in cv. Fireball, and 24.21 and 58.11 in cv. Safari Scarlet, and

doubled under 300 mM NaCl (Table 1). Likewise, salinity increased the levels of flavonoids, more than doubling under the highest salinity concentration ($F_{3,11} = 11.221$; $p = 0.0003$): 4.31 and 10.41 in cv. Aurora Orange, 4.23 and 11.23 in cv. Fireball, and 6.29 and 14.74 in cv. Safari Scarlet (Table 1). The highest levels were reported under 300 mM NaCl in all cultivars (Table 1). Significant differences were found between cultivars, being the levels of carotenoids, polyphenols, and flavonoids higher in cv. Safari Scarlet than in the other cultivars ($F_{2,11} = 4.291$; $p = 0.0001$, $F_{2,10} = 5.506$; $p = 0.0001$ and $F_{2,8} = 3.101$; $p = 0.0001$, respectively). Also, in the case of cv. Aurora Orange, the levels of carotenoids only increased under 100 and 300 mM NaCl in comparison to control conditions (Table 1).

Table 1. Impact of salinity levels (0, 50, 100, and 300 mM NaCl) on total carotenoids, polyphenols, and flavonoids on the flowers of three *Tagetes patula* cultivars (cv. Aurora Orange, Fireball, and Safari Scarlet). Results are expressed as mean values \pm SE ($n = 10$). Different superscripts indicate significant differences between salinity levels for the same cultivar (ANOVA followed by a Tukey test at $p < 0.05$).

		0 mM NaCl	50 mM NaCl	100 mM NaCl	300 mM NaCl
Carotenoids	Aurora Orange	1.23 \pm 0.10 ^c	1.29 \pm 0.15 ^c	2.64 \pm 0.71 ^b	3.44 \pm 0.91 ^a
	Fireball	1.28 \pm 0.12 ^d	1.42 \pm 0.19 ^c	2.59 \pm 0.81 ^b	3.19 \pm 0.95 ^a
	Safari Scarlet	1.27 \pm 0.14 ^d	1.57 \pm 0.21 ^c	3.11 \pm 0.90 ^b	3.74 \pm 0.92 ^a
Polyphenols	Aurora Orange	23.21 \pm 0.66 ^d	33.99 \pm 0.55 ^c	44.61 \pm 0.51 ^b	55.45 \pm 1.01 ^a
	Fireball	22.25 \pm 0.55 ^d	33.98 \pm 0.66 ^c	44.50 \pm 0.44 ^b	55.66 \pm 0.99 ^a
	Safari Scarlet	24.21 \pm 0.77 ^d	34.99 \pm 0.88 ^c	45.66 \pm 0.90 ^b	58.11 \pm 1.75 ^a
Flavonoids	Aurora Orange	4.31 \pm 0.34 ^d	6.32 \pm 0.44 ^c	8.11 \pm 0.61 ^b	10.41 \pm 1.92 ^a
	Fireball	4.23 \pm 0.31 ^d	6.23 \pm 0.55 ^c	8.59 \pm 0.55 ^b	11.23 \pm 1.95 ^a
	Safari Scarlet	6.29 \pm 0.55 ^d	7.44 \pm 0.61 ^c	9.11 \pm 1.78 ^b	14.74 \pm 2.11 ^a

3.3. Antioxidative Enzyme Activities

Enzyme activities increased significantly with enhanced levels of salinity, considering CAT ($F_{3,14} = 8.221$; $p = 0.0002$), POX ($F_{3,11} = 6.734$; $p = 0.002$), or APX ($F_{3,16} = 6.356$; $p = 0.001$) (Table 2). The activity of CAT more than doubled with salinity, varying from 1.23 to 3.44 in cv. Aurora Orange, 1.28 to 3.19 in cv. Fireball, and 1.37 to 3.74 in cv. Safari Scarlet (Table 2). In cv. Aurora Orange, CAT activity only increased at the highest salinity levels (100 and 300 mM NaCl), while in the other cultivars, the activity of this enzyme already increased at 50 mM NaCl. The activity of POX also more than doubled with the increase in salinity levels. POX activity varied between 23.21 and 55.45 in cv. Aurora Orange, 22.25 to 55.66 in cv. Fireball, and 24.21 to 58.11 in cv. Safari Scarlet (Table 2). A significant variation in the activity of enzymes was found between cultivars, with enzymatic levels usually higher in cv. Safari Scarlet than in the other two cultivars (Table 2). Nevertheless, the highest level of activity was recorded at 300 mM NaCl in all cultivars, and considering all enzymes tested.

Overall, considering all cultivars together, a significant negative correlation was found between plant traits (plant height, number of flowers, and their sizes) and the activity of antioxidant compounds and enzymes, as salinity increased (Figure 2; $p < 0.05$).

Table 2. Impact of salinity levels (0, 50, 100, and 300 mM NaCl) on enzyme activities of catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX), expressed as units/mg of protein DW on the flowers of three *Tagetes patula* cultivars (cv. Aurora Orange, Fireball, and Safari Scarlet). Results are expressed as mean values \pm SE (n = 10). Different superscripts indicate significant differences between salinity levels for the same cultivar (ANOVA followed by a Tukey test at $p < 0.05$).

		0 mM NaCl	50 mM NaCl	100 mM NaCl	300 mM NaCl
CAT	Aurora Orange	1.23 \pm 0.10 ^c	1.29 \pm 0.25 ^c	2.64 \pm 0.71 ^b	3.44 \pm 0.91 ^a
	Fireball	1.28 \pm 0.12 ^d	1.42 \pm 0.19 ^c	2.59 \pm 0.81 ^b	3.19 \pm 0.95 ^a
	Safari Scarlet	1.37 \pm 0.14 ^d	1.57 \pm 0.21 ^c	3.11 \pm 0.90 ^b	3.74 \pm 0.92 ^a
POX	Aurora Orange	23.21 \pm 0.66 ^d	33.99 \pm 0.55 ^c	44.61 \pm 0.51 ^b	55.45 \pm 1.01 ^a
	Fireball	22.25 \pm 0.55 ^d	33.98 \pm 0.66 ^c	44.50 \pm 0.44 ^b	55.66 \pm 0.99 ^a
	Safari Scarlet	24.21 \pm 0.77 ^d	34.99 \pm 0.88 ^c	45.66 \pm 0.90 ^b	58.11 \pm 1.75 ^a
APX	Aurora Orange	4.31 \pm 0.34 ^d	6.32 \pm 0.44 ^c	8.11 \pm 0.61 ^b	10.41 \pm 1.92 ^a
	Fireball	4.23 \pm 0.31 ^d	6.23 \pm 0.55 ^c	8.59 \pm 0.55 ^b	11.23 \pm 1.95 ^a
	Safari Scarlet	6.29 \pm 0.55 ^d	7.44 \pm 0.61 ^c	9.11 \pm 1.78 ^b	14.74 \pm 2.11 ^a

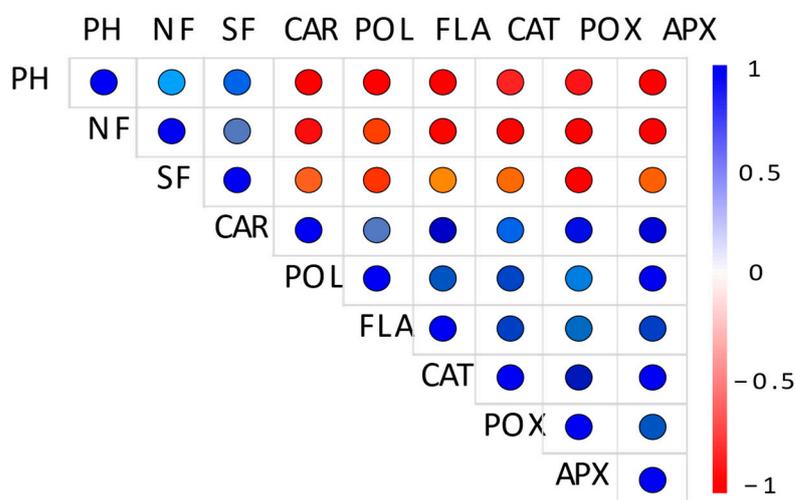


Figure 2. Pearson's correlation among the different morphological and physiological parameters considering *Tagetes patula* cultivars exposed to different salt stress levels (0, 50, 100, and 300 mM NaCl). Each circle indicates the Pearson's correlation coefficient between a pair of parameters. PH; plant height, NF; number of flowers, SF; size of flowers, CAR; carotenoids, POL; total polyphenols, FLA; flavonoids, CAT; catalase, POX; peroxidase, and APX; ascorbate peroxidase. In all cases, the correlation was significant at 0.05.

4. Discussion

4.1. Effects of Salinity on Plant Height and Flower Production

Salinity significantly reduced plant height, the number of flowers, and their size in all cultivars studied (Figure 1), likely due to the stress triggered by salinity [35]. This oxidative, osmotic, and toxic stress affects the direction of cell division, the formation of phragmoplasts, and the structural organization of cells, often leading to significant damage in the cytoskeleton [36–38]. Consequently, these processes may lead to the death of cells and/or changes in plant morphology and growth [36,37], as reported here.

Flowering and the underlying processes of the development of flower reproductive structures are also disrupted by adverse conditions. For instance, the number of inflorescences in the halophyte *Crithmum maritimum* decreased significantly as salinity increased, at least in some genotypes [39]. Here, we also found a decrease in the number of flowers with the increase in salinity levels. Likewise, flower abortion, reduction of pollen number, and viability have often been reported to be high in other species [40], being limiting factors in

the floriculture industry [35]. In addition, some morphological changes were already seen in some flowers, especially under 300 mM NaCl, together with a high number of aborted buds as reported here. The expression of some MADS-box transcription factors, which regulate flower development suffer changes under salinity [41,42] and may be responsible for the levels of aborted flowers, although this needs to be further studied.

4.2. High Levels of Antioxidant Molecules on Flowers

To balance the levels of ROS produced by saline stress, plants trigger several antioxidant compounds, such as the ones reported here. As such, carotenoids increased as salinity increased in all cultivars, except in Aurora Orange, where no differences were found between control conditions and the lowest saline level (Table 1). This differs from a previous study involving other *T. patula* cultivars, where an increase in carotenoid content was reported under saline conditions, although with higher levels under 50 mM NaCl and decreasing under 100 mM NaCl [24]. Carotenoids are involved in quenching $^1\text{O}_2$ and peroxy radicals generated during excess excitation of the chlorophyll, helping to protect the photosynthetic apparatus and avoiding plant damage [43]. In salt-tolerant plants, salinity triggers carotenoid biosynthesis and increases carotenoid concentration, as reported in the taproots of carrots [44] or the leaves of *Thellungiella* [45], being key for achieving tolerance under saline conditions [46]. Thus, the increased levels of carotenoids under saline conditions seem important to maintain the quality of marigold flowers. Carotenoids are an important contributor to the petal colors of marigolds, and during flower development, carotenoid biosynthesis genes are upregulated [26].

As reported here, total polyphenol and flavonoid content also plays a role in *Tagetes* responses, since their levels increase with increased salinity levels in all cultivars. Increases in total phenols have also been reported in the flowers of other *T. patula* cultivars, while the levels of anthocyanins decrease under salinity stress [24]. Water-soluble polyphenols are a major group of antioxidants in many species, showing a rapid ability to scavenge O_2^- , OH, H_2O_2 , and $^1\text{O}_2$ compared with the lipid-soluble carotenoids [47]. Among phenolic compounds, flavonoids are the largest and best-studied group, playing a major role under abiotic stresses [47]. Increased flavonoid levels are crucial for balancing Na^+/K^+ ions, reducing the effects of stress through the regulation of transcriptional and hormonal mechanisms involving ABA hormone signaling [48]. Thus, altogether, the three components—carotenoids, polyphenols, and flavonoids—were highly responsive to saline stress in French marigold flowers.

4.3. The Role of Antioxidant Enzymatic Machinery on Flowers

Together with non-enzymatic components, the high activity of the enzymes CAT, POX, and APX suggests a fast response of marigold flowers to balance the oxidative stress imposed by salinity in all studied cultivars. CATs are primary scavenging enzymes that directly dismutate H_2O_2 and reduce the levels of ROS, especially under drought and salt stress because of their critical involvement in photorespiration [49]. POXs are a family of isoenzymes, also capable of scavenging H_2O_2 , mainly in the apoplastic space [50]. Another major hydrogen peroxide detoxifying system under abiotic stresses is the ascorbate-glutathione cycle, where APX enzymes catalyze the conversion of H_2O_2 into H_2O , using ascorbate as a specific electron donor [51]. Therefore, the increased activity of these enzymes in the stressed French marigold plants studied here allows for the removal of the H_2O_2 produced by salinity. The activity of CAT, SOD, APX, and POD has been found to increase in the flowers of other *T. patula* cultivars, albeit only under 50 mM NaCl and decreasing under 100 mM NaCl [24], contrary to our results. This highlights the need to study more cultivars, since responses to stresses are very dependent on the cultivar used, as reported here and in other studies [24,28,30,31,52,53].

4.4. Usefulness of Saline Conditions for French Marigold Plants: Implications for the Floriculture Industry

Ornamental plants are usually irrigated with high-quality water. Yet, as freshwater resources become scarcer and soil salinity becomes higher, the cultivation of plants that can tolerate (at least some) degree of salinity becomes a helpful solution. Ornamental plants that can grow and survive under saline levels at or over 200 mM NaCl are considered salt-tolerant, providing a beneficial resource for use in saline or degraded soils [54]. However, few plants can tolerate high levels of salinity [21]. It is, thus, interesting to find tolerant *T. patula* cultivars, at least to some levels of salinity, as reported in this study. In fact, after the end of the experiment, the plants returned to non-saline conditions and no cases of senescence or plant mortality were recorded, suggesting that these plants could recover from short-term salinity exposures. Nonetheless, since reproductive stages are usually highly sensitive to stresses [55], further evaluation is needed to understand the effects of salinity on flower development and reproductive processes, including under higher periods of exposure. The significant negative correlations already found in this study between plant height, the number and sizes of flowers, and the activity of antioxidant compounds and enzymes might change under different salinity exposures. *Tagetes patula* flowers are also viewed as nutritional or functional foods, with potential economic value [24]. There is a growing interest in the antioxidant compounds, carotenoids, flavonoids, and polyphenols produced by plants since they may contribute to human health. An ongoing study suggests that these cultivars have high levels of total phenolic content and proteins, while the level of fatty acids is relatively low, supporting the presence of nutritional compounds in these *Tagetes* cultivars (*unpub. results*).

Based on our results, farmers who are considering the use of saline conditions to produce flowers with nutritional compounds could start to employ low levels of salinity. However, due to the negative effects of salinity on French marigold flowers, we recommend the production of *T. patula* flowers under short exposure to salinity (of up to 50 mM NaCl) to achieve high production of nutritional compounds without compromising flower production. Higher values may be beneficial to produce antioxidants and minerals, but they significantly compromise the quantity of flowers produced.

5. Conclusions

Salinity significantly reduced plant height, the number of flowers, and their size in all cultivars studied, despite the increase in antioxidant compounds. These compounds are beneficial for human consumption and increased at the lowest salinity level (50 mM). This stress also had minimal effects on plant growth and flower production, suggesting the possibility of using saline irrigation water to enhance useful antioxidant compounds. Thus, these cultivars can be used in saline soils, but only under small levels of salinity (up to 50 mM NaCl). This contrasts sharply with the adverse impacts observed at higher salinity levels (100 and 300 mM NaCl). Future studies should investigate the effects of varying salt levels over different durations on flower production to provide a more comprehensive understanding. Exploring additional methodological approaches, such as exogenous foliar application, may prove beneficial for enhancing salt tolerance in these plants, as suggested by previous studies [56,57]. Using saline water holds significance for sustainable agricultural practices by: (1) providing an alternative to farmers beyond traditional glycophyte crops, (2) helping diversify the agricultural market, and (3) contributing to the sustainable use of scarce freshwater resources. Thus, based on our results, salinity triggered an increase in important health antioxidant compounds that can be used as a new source of nutritional food, despite the negative impacts on plant height and flower production.

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