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Effect of salt stress on the growth and survivability of different cultivars of strawberry (*Fragaria* spp.) under protected cultivation

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Abstract

Strawberry is known for its flavour in the world. Its cultivation is being popular due to its pleasant aroma. The major challenge of the crop is withstanding in stress condition. The salt stress acts as negative impact on plant growth and productivity. In current study, four concentrations of sodium chloride salt (0, 25, 50 and 75 µmol) were given to five most adopted low chilling cultivars of strawberry, *viz*., Camarosa, Ventana, Winter Dawn, Nabila and Raina. The experiment was laid out in factorial randomized blocked design with three replications. The results showed that in all the cultivars and salt stress combinations, Ventana with 50 µmol NaCl had the highest proline content (1.38 mmol/gFW). Winter Dawn cv. without NaCl had the highest chlorophyll content (56.26 SPAD index), and Ventana with 25 µmol had the maximum number of leaves (31.66) and highest survivable percentage (100 %). However, Camarosa with 25 µmol had the highest lipid peroxidation content (81.93 µmol MDA/gFW). Overall, the results indicate that cv. Ventana performed the best among the different cultivars under varying salt stress conditions. These findings can be useful in developing strategies to enhance strawberry cultivation and productivity under stress conditions.

1. Introduction

The strawberry (*Fragaria* × *ananassa* Duch.) is a popular fruit crop that may be cultivated economically in both subtropical and temperate regions of the globe. The strawberry fruit is technically not a berry but rather an "accessory fruit" (Shulaev *et al*., 2008). There are 23 different species of strawberry in the genus *Fragaria*, which is part of the Rosaceae family. Octoploid fruits are the most common kind consumed by humans (Davis *et al*., 2007). People are paying more attention to fruit eating because of the possible health benefits. The strawberry, like other berries, is rich in bioactive compounds that have been linked to health benefits and illness prevention. Many studies have examined the positive effects of strawberries on health, and they have been shown to reduce the risk of heart disease and obesity-related disorders and to even prevent cancer (Afrin *et al*., 2016).

Among different cultivars, Ventana strawberry, one of the most popular varieties worldwide, has a medium-sized, conical fruit with a pointed tip. It has large, consistent berries throughout the growing season and having a long shelf life, which makes it a desirable option for shipping and storage. Camarosa strawberry known for their sweetness and firmness, with a conical shape and blunt tip that ranges in size from medium to giant. Additionally, it is resistant to common diseases like verticillium wilt and powdery mildew (Castro *et al*., 2002). Winter Dawn strawberries are a cold-tolerant variety suitable for winter fruit production in areas with mild winters. Its conical, medium-sized fruit is deep blood red, and the plant produces abundant fruit in the late fall and early winter. Its resilience to freezing temperatures, withstanding as low as 10°F (–12°C), and the ability to blossom and bear fruit with less freezing than other strawberry varieties are its distinctive qualities (Khound *et al*., 2021). The Nabila strawberry, developed in Spain, has a high level of disease resistance and is highly productive. Its medium to large, conical-shaped fruit is a vibrant red colour with a subtle aroma and juicy and sweet flesh with firm, glossy skin. The Nabila strawberry noted as disease resistance to powdery mildew, verticillium wilt, and red stele. The Raina strawberry, developed by the Indian Institute of Horticultural Research, is a relatively new high-yielding variety that thrives in subtropical and tropical climates. Its conical, medium-sized fruit is bright red and has a juicy, sweet flesh with a rich fruity flavour and firm, glossy skin. The Raina strawberry's distinctive quality is its ability to produce abundant fruit despite hot and sunny weather.

Two important concerns for social, environmental, and economic sustainability are water scarcity and soil salinity (Ibrahim *et al.,* 2022). In many agricultural regions across the world, salinity is a significant environmental source of stress that inhibits plant development and yield (Zahedi *et al*., 2020). Salinity is a major environmental stress that negatively impacts the productivity of various crops worldwide. High levels of salt in the soil can lead to significant reductions in agricultural yields. This stress affects critical physiological processes in plants, resulting in delayed cell division, increased cell size, or both in the developing zone. Furthermore, salt stress limits the uptake of essential mineral nutrients, causing a nutritional imbalance in the plant (Mohammad *et al*., 2003).

Salinity stress adversely affect biochemical and physiological traits, such as reducing chlorophyll levels and moisture content, decreasing the intake of essential nutrients, and enhancing the rate of electrolyte leakage. Increased MDA levels led to oxidative damage during salt stress (Alluqmani and Alabdallah, 2022). Under conditions of salt

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stress, osmotic adjustment is accomplished by the production and accumulation of appropriate organic solutes, such as proline and sugars. Hence, NaCl's harmful effects can be reduced by proline by stabilising the cell's proteins and enzymes (Koc *et al.*, 2016). Strawberry cultivars' shoot and root Na^+ accumulation increased while K^+ concentration reduced under salt stress conditions. Improved nutrient absorption and efficiency, increased tolerance to biotic and abiotic stressors, and heightened rhizospheric activity are only a few of the ways in which biostimulants increase crop output (Chitra *et al*., 2022). Strawberry plants, on the other hand, are salt-sensitive and can suffer substantial harm when grown in salty soils. In this regard, it is vital to introduce new, cutting-edge cultural methods as a means of mitigating the detrimental effects of salinity stress on plants.

2. Materials and Methods

Lovely Professional University provided the necessary facilities for conducting the research. The research was done during November 2022 to April 2023 under the polyhouse conditions, where the temperature and humidity was observed 21 ± 8°C and 80-85%, respectively. The experiment was done with five strawberry cultivars well suited under Punjab agro-climatic conditions, *i.e.*, Camarosa, Ventana, Winter Dawn, Nabila and Raina. As a stress factor, four different concentrations (0, 25, 50, 75 µmol) of sodium chloride salt stress were given to all the cultivars. The experiment was laid under three replications of each twenty treatments combination and ten plants were studied under each replication. The experiment was designed under factorial randomized block design. The R and SPSS software were used for data analysis. In November 2022, a group of uniform runners were chosen for a planting experiment. The soil, farm yard manure, vermicompost and cocopeat (2:1:1:1) growth medium was created for the strawberry plants. The mixture was filled in black polybag of 6/8 inch. The salt solutions were given at weekly intervals from 1st January 2023 to March 14th 2023. While irrigations were given manually at two days interval. The observations were recorded as;

2.1 Leaf proline content

The technique provided by Ábrahám *et al.* (2010) was used to calculate the proline content of leaves. The leaves were weighed and 0.2 g was collected for the sample. After adding a 3% sulfonic acid solution and placing the vial in a boiling water bath for 30 min, the vial was allowed to cool. The extract was then combined with ninhydrin and glacial acetic acid before being heated and chilled once more in the vial. Chromophores formed when the mixture was mixed with toluene and left to sit in the dark for 5 h. UV-Visible spectrophotometer readings at 520 nm were taken of the resultant chromophore. Using a standard calibration curve, we calculated the quantity of proline in the sample, which was then reported as mg of proline per g of fresh leaf weight:

$$\text{mmoles per gram tissue} = \frac{(\text{mg proline / ml}) \times \text{ml toluene}) \times 115.5 \text{ mg / mmole}}{(\text{g sample}) \times 5}$$

2.2 Chlorophyll content

A portable chlorophyll meter was utilized to determine the greenness of the leaves of various plant species. This device is highly precise and can accurately quantify the total concentration of chlorophyll in leaves. The measurement process involved taking three readings on each treatment for all fully grown leaves. The resulting data for each plant were then averaged.

2.3 No. of leaves/plant

During the growing season of November to March, the number of leaves on each plant was monitored every two weeks, from the point of transfer to the end of the season. The average number of leaves per plant was calculated for each observation. Additionally, the number of leaves per plant was recorded on four separate intervals at 40, 80, 120, and 150 days after transplanting and the average number of leaves per plant was determined.

2.4 Plant survivability

This can be calculated by multiply the total number of plants of each species that have survived by the total number of planted plants:

$$\text{Plant survivability} = \frac{\text{No. of plants survives}}{\text{Total number of plants}} \times 100$$

2.5 Lipid peroxidation

Malondialdehyde (MDA) content was measured using the Heath and Packer (1968) technique to assess lipid peroxidation in leaf samples. We homogenised 0.5 g samples of fresh leaves in 5 millilitres of 20% trichloroacetic acid (TCA) and centrifuged them at 10,000 revolutions per minute for 5 min. The resultant supernatant (1ml) was mixed with 0.6% (w/v) thiobarbituric acid solution (10% TCA) to form a new solution. After 30 min, the mixture was heated in a boiling water bath before being chilled rapidly in an ice bath. Three wavelengths (450, 532 and 600 nm) were used to test the mixture's absorbance. The following formula, based on absorbance, may be used to determine the degree of lipid peroxidation:

$$\text{MDA } (\mu\text{mol g}^{-1}\text{ FW}) = 6.45(A_{532} - A_{600}) - 0.56 \times A_{450}$$

3. Results

3.1 Proline content (mmol/gFW)

Table 1 shows the results obtained for proline content in different strawberry cultivars and salt concentration interactions. As per the analysis of the data, cv. Ventana was found to have the highest (1.38 mmol/gFW) proline under the concentration of 50 µmol salt. Further, it was followed by cv. Nabila having proline content of 1.28 mmol/gFW under control conditions as compared to Nabila and Rania which showed least proline content of 0.45 mmol/gFW at 75 µmol of salt concentration. Amongst the cultivars (Figure 1), Ventana showed highest proline of 1.38 mmol/gFW, however the least proline content was observed in Rania and Nabila (0.45 mmol/gFW). Although, least proline content was observed to be 0.45 mmol/gFW in 75 µmol concentration of salt as compared to control (1.28 mmol/gFW).

Table 1: Effect of salt stress on proline (mmol/gFW) of different varieties of strawberry under protected structure

Cultivars (C)	NaCl salt concentration			
	0 µmol (S1)	25 µmol (S2)	50 µmol (S3)	75 µmol (S4)
Camarosa (C1)	1.05^{cde}	1.05^{cde}	1.09^{cde}	0.55^{h}
Ventana (C2)	1.05^{cde}	1.16^{bcd}	1.38^{a}	1.21^{bc}
Winter Dawn (C3)	1.22^{bc}	0.93^{ef}	0.42^{h}	0.56^{h}
Nabila (C4)	1.28^{ab}	1de	0.77^{g}	0.45^{h}
Raina (C5)	1.19^{bc}	0.97^{ef}	0.82^{fg}	0.45^{h}

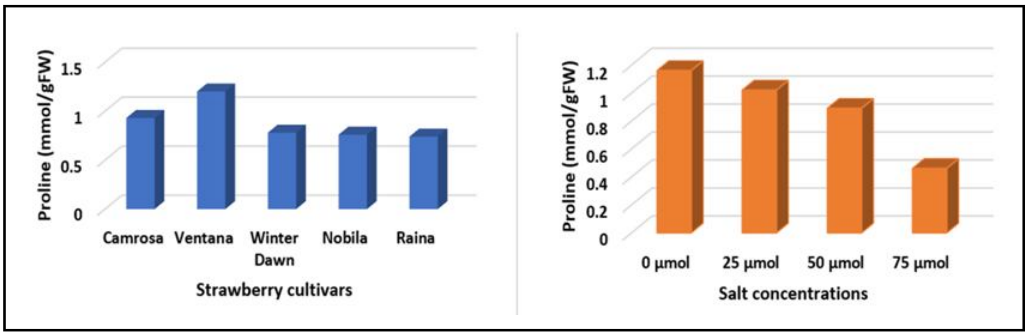

Figure 1: Effect of different salt concentrations on proline of strawberry cultivars.

3.2 Chlorophyll content

Interaction of different cultivars and salt concentrations representing the significant difference in chlorophyll content was observed in Table 2. Conferring the data analysis, highest chlorophyll content (56.26) was observed in cv. Winter Dawn with 0 µmol of salt concentration. It was followed by Ventana showing chlorophyll content of 51.36 with salt concentration of 25 µmol as compared to least (42.16), which was observed in cv. Rania at 25 µmol of salt (Figure 2). Within the cultivars (Figure 3), highest chlorophyll content of 56.26 was observed in Winter Dawn, whereas the least content of 42.16 was shown by Rania. However, 42.16, the least of chlorophyll content was observed in 75 µmol of NaCl salt as compared to control showing chlorophyll content of 56.26.

Table 2: Effect of salt stress on chlorophyll content (SPAD Index) of different varieties of strawberry under protected structure

Cultivars (C)	NaCl salt concentration			
	0 µmol (S1)	25 µmol (S2)	50 µmol (S3)	75 µmol (S4)
Camarosa (C1)	50.73^{bcd}	49.03^{cd}	48.73^{cd}	49.06^{cd}
Ventana (C2)	50.63^{bcd}	51.36^{bcd}	50.43^{bcd}	49.43^{cd}
Winter Dawn (C3)	56.26^{a}	52.16^{bc}	53.7^{ab}	50.16^{bcd}
Nabila (C4)	45.26^{ef}	49.06^{cd}	49.06^{cd}	42.76^{f}
Raina (C5)	43.00^{f}	42.16^{f}	47.7^{de}	43.7^{f}

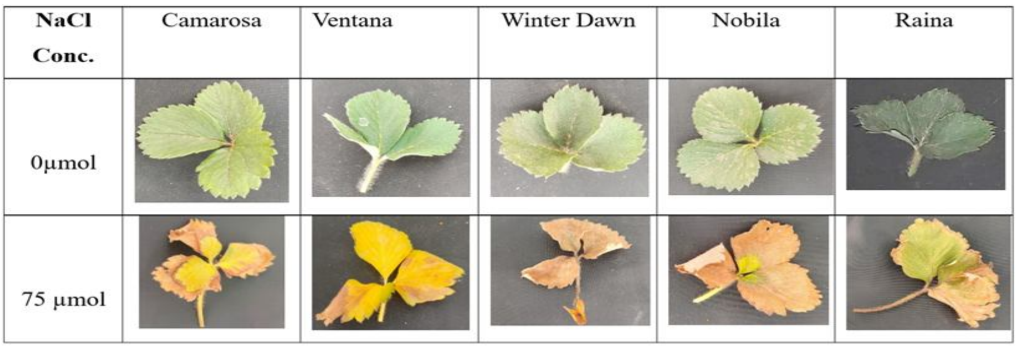

Figure 2: Effect of salt stress on different cultivars of strawberry.

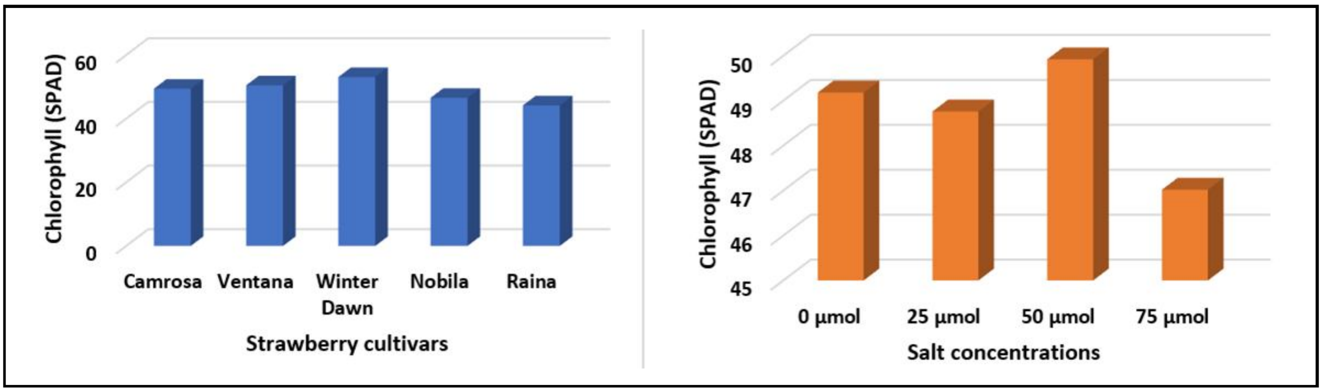

Figure 3: Effect of different salt concentrations on chlorophyll of strawberry cultivars.

3.3 Number of leaves

Table 3 showed significant differences in number of leaves amongst different cultivars and their interactions with salt concentrations. As per the analysis of the data, maximum number of leaves was found in cv. Ventana (31.66) with a concentration of 25 µmol of salt followed by cv. Camarosa (24.33) in control (without salt) as compare to minimum leaf number in Nabila (12.33) at 75 µmol salt. Amongst the cultivars (Figure 4), Ventana got the maximum number of leaves (31.66), while the minimum leaf number was observed in Nabila (12.33). However, number of leaves was found to be least up to 12.33 in 75 µmol NaCl salt as compare to 25 µmol of salt showing 31.66 of number of leaves.

Table 3: Effect of salt stress on number of leaves of different varieties of strawberry under protected structure

Cultivars (C)	NaCl salt concentration			
	0 µmol (S1)	25 µmol (S2)	50 µmol (S3)	75 µmol (S4)
Camarosa (C1)	24.33^{b}	19.33^{cdef}	21.33^{bcde}	17.00^{efghi}
Ventana (C2)	19.00^{cdefg}	31.66^{a}	18.33^{defg}	18.00^{defgh}
Winter Dawn (C3)	19.00^{cdefg}	18.00^{defgh}	24.0^{b}	23.33^{bc}
Nabila (C4)	16.33^{fghi}	22.0^{bcd}	15.33^{fghi}	12.33^{i}
Raina (C5)	13.33^{hi}	15.00^{fghi}	15.33^{fghi}	14.33^{ghi}

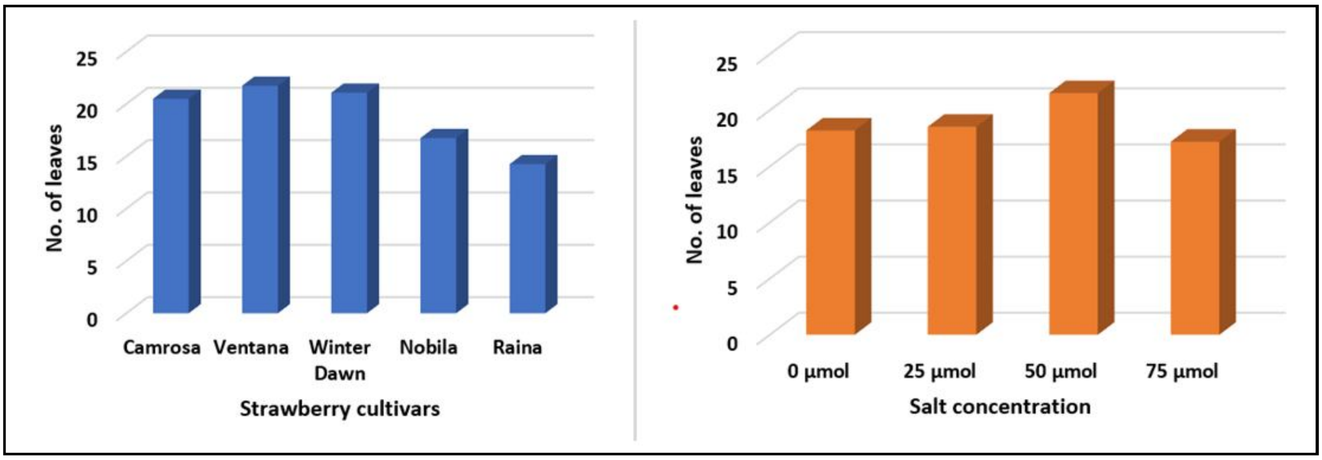

Figure 4: Effect of different salt concentrations on number of leaves of strawberry cultivars.

3.4 Survivability (%)

Table 4 representing the data showed significant difference in survivability among the interactions of different cultivars and salt concentrations. The analysis of data indicated that cv. Ventana has the highest survivability of 100% with 25 µmol concentration of salt followed by cv. Camarosa which exhibited survivability of 91.66% in salt concentration of 25 µmol as compared to cv. Rania which showed least survivability of 33.33% at 75 µmol salt concentration. Ventana showed maximum survivability of 100%, amongst the cultivars (Figure 5), while the minimum survivability was observed in Rania (33.33%). However, 33.33% of minimum survivability was observed in 75 µmol as compared to 25 µmol which showed 100% of survivability.

Table 4: Effect of salt stress on survivability (%) of different varieties of strawberry under protected structure

Cultivars (C)	NaCl salt concentration			
	0 µmol (S1)	25 µmol (S2)	50 µmol (S3)	75 µmol (S4)
Camarosa (C1)	83.33[abc]	91.66[ab]	83.33[abc]	75.00[abc]
Ventana (C2)	91.66[ab]	100.00[a]	83.33[abc]	66.66[abcd]
Winter Dawn (C3)	83.33[abc]	91.66[ab]	91.66[ab]	58.33[bcd]
Nabila (C4)	50.00[cd]	75.00[abc]	50.00[cd]	50.00[cd]
Raina (C5)	83.33[abc]	75.00[abc]	66.66[abcd]	33.33[d]

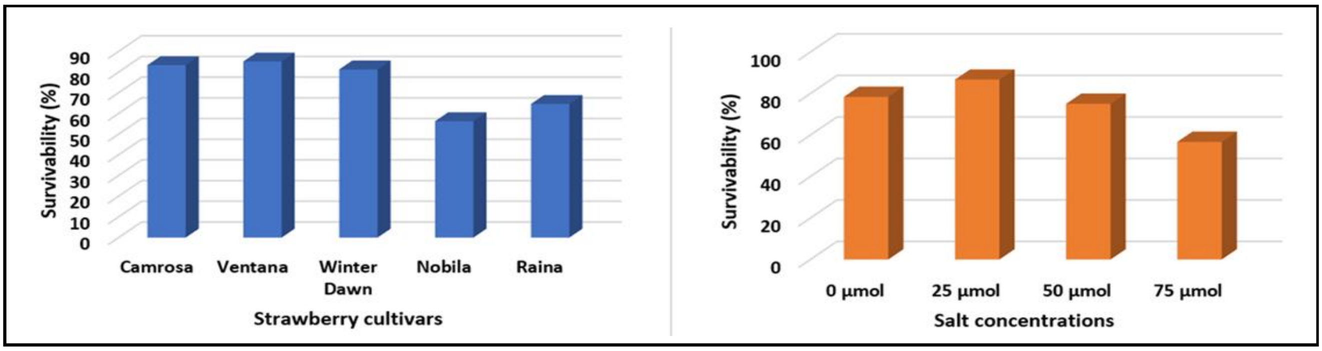

Figure 5: Effect of different salt concentrations on survivability of strawberry cultivars.

3.5 Lipid peroxidation (µmol MDA/gFW)

A significant difference was observed in Table 5 for lipid peroxidation content among different cultivars and salt concentrations interactions. As per the analysis of the data, enhanced lipid content of 81.93 µmol MDA/gFW was observed in cv. Camarosa under the salt concentration of 25 µmol. Further, it was followed by Winter Dawn which showed lipid content of 75.26 µmol MDA/gFW in control as compared to the decreased lipid content (32 µmol MDA/gFW) in Rania at 75 µmol salt concentration. Amongst the cultivars (Figure 6), Camarosa got highest lipid content of 81.93 µmol MDA/gFW, whereas least was observed in Raina with a lipid content of 32.35 µmol MDA/gFW. However, least content of lipid (32.35 µmol MDA/gFW) was observed in 75 µmol as compared to the 25 µmol which resulted in the lipid content of 81.93 µmol MDA/gFW.

Table 5: Effect of salt stress on lipid peroxidation (µmol MDA/gFW) of different varieties of strawberry under protected structure

Cultivars (C)	NaCl salt concentration			
	0 µmol (S1)	25 µmol (S2)	50 µmol (S3)	75 µmol (S4)
Camarosa (C1)	48[h]	81.93[a]	49.66[g]	54.28[f]
Ventana (C2)	46.06[j]	46.8[i]	64.89[d]	58.9[e]
Winter Dawn (C3)	75.26[b]	43.44[k]	65.44[d]	48.02[h]
Nabila (C4)	33.75[m]	43.33[k]	69.2[c]	33.64[n]
Raina (C5)	32.84[n]	36.98[l]	36.69[l]	32.35[n]

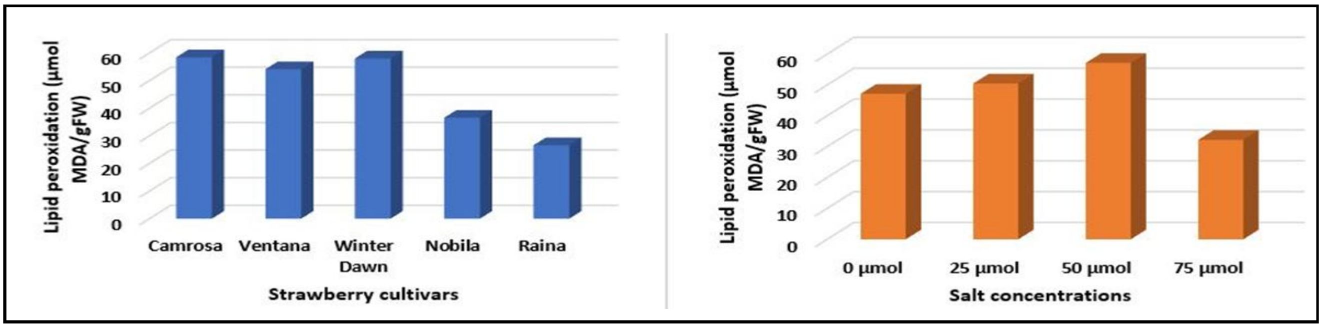

Figure 6: Effect of different salt concentrations on lipid peroxidation of strawberry cultivars.

4. Discussion

In the case of the cultivars mentioned earlier, cv. Ventana displayed the highest proline content, indicating its better adaptation to salt stress conditions. On the other hand, cv. Winter Dawn, cv. Nabila, and cv. Raina showed a decreasing trend in proline content, suggesting their lower tolerance to salt stress. However, cv. Camarosa demonstrated consistent proline content at all salt concentrations, except for 75µmol, which indicates its moderate adaptation to salt stress (Al-Shorafa *et al*., 2014). In order to survive under salt stress conditions, plants increase their cellular osmotic pressure by producing various secondary metabolites, chemicals, and stress proteins such as proline. The higher accumulation of proline has been associated with better osmotic adjustment, and thus, higher adaptation to salt conditions. This is why proline content in plants is often used as an indicator of their tolerance to salt stress. According to Koc *et al*. (2016), proline accumulation is an important adaptive mechanism in plants to cope with salt stress. Proline acts as an osmoprotectant, which helps to maintain water balance and protect cellular structures and functions under salt stress conditions. In addition, proline also plays a role in scavenging reactive oxygen species (ROS) generated under salt stress, thereby preventing oxidative damage to plant cells. Overall, the study of proline content in plants under salt stress provides important insights into their adaptation mechanisms and can be used to identify salt-tolerant cultivars for cultivation in saline environments.

The study found that cv. Camarosa had the highest SPAD value in untreated plants, and equal SPAD values in plants treated with 25 µmol and 75 µmol of salt. cv. Ventana had the highest chlorophyll content when treated with 25 µmol of salt, and equal amounts of chlorophyll content in plants treated with 50 µmol and no salt. cv. Nabila had the same amount of chlorophyll content in untreated plants and plants treated with 25 µmol of salt. In cv. Raina, the highest chlorophyll content was observed in plants treated with 25 µmol of salt. However, in all cultivars, chlorophyll content decreased when exposed to 75 µmol of salt stress. It is worth noting that alkaline stress can lead to a decline in both chlorophyll SPAD value and photosynthesis rates. Nonetheless, the analysis of chlorophyll fluorescence can provide valuable insights into a plant's ability to tolerate environmental stress and the extent of damage caused to its photosynthetic system. In general, it appears that moderate levels of salt can be beneficial to plant growth, while high levels can be detrimental. It was found that plants grown in soil with a salt concentration of 25 µmol produced the most leaves. This suggests that a certain level of salt is necessary for the plant to thrive, but too much can be harmful. While the plants grown in soil with a salt concentration of 75 µmol produced fewer plants. A recent study by Bahmanbiglo and Eshghi (2021) suggested that the soil was saturated with sodium and chloride ions, which interfered with the plant's growth and the development of new shoots. The excess salt essentially poisoned the plant, preventing it from reaching its full potential.

The rising levels of salt stress have led to a decrease in plant survivability, particularly in strawberry plants that exhibit low resistance to salinity. Out of the strawberry cultivars tested, cv. Camarosa demonstrated the highest level of survivability, even in the presence of varying levels of salt concentration. However, cv. Nabila showed poor results at all levels of salt concentration. The survivability rate of cv. Raina decreased significantly in comparison to the other cultivars. The results showed that cv. Ventana exhibited the best survivability rate at a salt concentration of 25 µmol, whereas the cultivar did not show such results at other concentrations. Overall, the findings suggest that cv. Camarosa is the most tolerant cultivar to salt stress, while cv. Nabila is the least tolerant. The results highlight the importance of identifying salt-tolerant cultivars to ensure the sustainability of agriculture in regions experiencing salt stress. Hasanuzzaman and Fujita (2022) conducted a study that to implement strategies to improve plant resistance to salt stress to ensure global food security. Along with a 25% salt stress condition, plant survival is normal and healthy in normal conditions. However, treating the plant with a salinity concentration of 75% influenced it. Because fewer nutrients are being taken up by plants from the soil due to an increase in soil salinity, plant survival rates have decreased.

The lipid peroxidation levels in the five strawberry cultivars were found to be highest at 25 µmol concentration of salt, except for cv. Winter Dawn, which showed the best results in untreated plants. However, the values were found to be the lowest at 50 and 75 µmol concentrations in all cultivars. The overproduction of reactive oxygen species and lipid peroxidation occurred in strawberry leaves when exposed to alkalinity stress, but treatment with H_2S alleviated this oxidative damage. The H_2S treatment decreased the levels of reactive oxygen species and lipid peroxidation induced by alkalinity stress. Bahmanbiglo and Eshghi (2021) have also reported similar findings.

5. Conclusion

When assessing various strawberry cultivars, it was found that cv. Ventana performed the best at a salt concentration of 25 µmol, while cv. Winter Dawn showed the best results in untreated soil. Ventana is highly resistant in salt concentration upto 75 µmol followed by Camarosa which show decline after 50 µmol. This suggests that cv. Winter Dawn is suited to slightly saline conditions, while Ventana thrives well in saline conditions. As a result, farmers could potentially benefit from utilizing both cultivars for strawberry cultivation. Ventana cv. is the superior choice when soil salinity is present, whereas cv. Winter Dawn performs best in untreated soil. This information is beneficial for farmers looking to optimize their crop yield and choose the most appropriate cultivars for their specific growing conditions. Ultimately, by considering these factors, farmers can make informed decisions that can lead to successful strawberry cultivation.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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