Effect of Exogenous Selenium Intake on Yield and Quality of Tomatoes Grown Under Salt Stress

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Abstract. The aim of the work undertaken is to study the impact of the exogenous supply of a biostimulant, which is selenium (sodium selenate Na2SO4), on tomatoes fruits quality under salt stress conditions. To this end, an experimental trial has been conducted on a seasonal tomato crop. Within the agricultural domain of the Regional Centre for Agricultural Research (CRRA), Sidi Bouzid, Tunisia (9°430 E, 35° 010 N; altitude 354 m above sea level). The variety used is Firenze. The latter is irrigated with saline water with two different concentrations of NaCl (3 and 6 g/l of NaCl). For each type of salinity, three levels of treatment with the biostimulant were used which correspond to the control plants (without foliar spraying) and to the two doses of application of sodium selenate C1 and C2 which are respectively in the order of 0.5 mM and 1 mM Na2SeO4. The results show that salt stress has a depressive effect on the morphometric and physicochemical characteristics of fruits such as weight, size, color, Brix degree, and lycopene content. However, the application of selenium further improved these parameters. This improvement was more significant in plants that were grown at moderate salinity (3g/l) and were also sprayed with a high dose of selenium (1mM Na2SeO4). For example, yield and fruit weight increased by 228.45 percent and 73.07 percent, respectively, when compared to non-treated plants. Also, fruit width and length increased by 42.31% and 35.57%. In addition, for the physicochemical parameters the Brix degree and the lycopene content increased respectively by 18.45% and 237.38%.

1. Introduction

Tomato (Solanum Lycopersicum) is one of the most important vegetable plants in the world. Global production is estimated at 180.76 million tons in 2019 [1] . Soil and water salinity is a major problem in many countries of the world. It is considered the main abiotic factor limiting plant productivity and agricultural yield. Salinity reduces the world's irrigated area by 1 to 2% each year [2]. Its action is particularly pronounced in arid and semi-arid regions where 25% of irrigated land is contaminated [3].

In Tunisia, salinity affects about 1.5 million hectares, about 10% of the country's total area. The aridity of the climate and the use of non-conventional water resources are slowly worsening the situation. Indeed, in most parts of the country, water resources are becoming increasingly scarce and the gap between good quality water supplies and irrigation is growing so it's inevitable to address the use of relatively saline water. This salt stress essentially results in the toxic accumulation of ions in the cells and / or a nutritional imbalance due to an excess of certain ions [4]. In fact, depending on the
degree of stress in the environment, plants are subject to modifications in their morpho-physiological, anatomical and biochemical behavior [5].

Under these circumstances, tolerance of plants to the presence of salts is, therefore, a widely sought-after quality. For overcoming the saline problem different adaptive strategies are used. In recent decades, regulators of growth also called biostimulants applied exogenously such as osmoprotectants, polyamines, plant hormones, antioxidants, signaling molecules, and trace elements have been used to provide significant protection to plants under environmental stress.

The present study was conducted to investigate the effect of the exogenous application of different doses of selenium on growth, yield, and quality attributes of tomato plants subjected to different NaCl concentrations.

2. Materials and Methods

2.1. Experimental design

The experimental plot is located within the agricultural domain of the Regional Centre for Agricultural Research (CRRA), Sidi Bouzid, Tunisia. A single variety of tomato for seasonal cultivation, the Firenze variety, was used as the plant support for this experiment. The experiment was carried out in a two-factor test design, which was the saline treatment and the treatment with the selenium biostimulant (sodium selenate: Na2SeO4).

The first factor (salt stress) includes 2 levels for which irrigation was carried out with saline water with two different concentrations of NaCl: 3g/l and 6g/l. The second factor, which concerns the treatment with the selenium biostimulant, includes 3 levels corresponding to the control plants (without foliar spray) and the two application doses of sodium selenate C1 and C2 which are respectively of the order of 0.5 mM and 1mM Na2SeO4.

The experimental plot is thus cultivated with 360 tomato plants of the Firenze variety on the 4th of March 2019. It is then divided into two parts. One part is irrigated with saline water at 3g/l NaCl and the other part is irrigated with 6g/l NaCl. For each type of irrigation, the plants are grown in 10 rows with a spacing of 80 cm between plants.

Each part is divided in turn into three sub-units, each with 60 plants. The first sub-unit corresponds to the control plants that have not been sprayed with selenium. The second and third sub-unit correspond to the plants that were sprayed with selenium at C1 and C2 doses respectively.

2.2. Yield and morphometric quality of fruits

The fruit yield was determined at the harvest stage. It was estimated through the number of fruits and the average weight of fruits per plant.

The description of the fruits is carried out according to the worldwide description standards of the International Union for the Protection of New Varieties of Plant (UPOV). The samples taken must be free from defects such as sunburn and damage caused by insects or disease, which may have affected the normal ripening process. Fruit dimensions (size and length) were studied on the fruits of 6 plants at the level of each sub-unit. They were measured with a digital caliper 150 mm type (T304BW-1215).

The colour density was studied for all samples using a CR10 colorimeter of the Konica Minolta type (Japan). This instrument uses the L*a*b* colour space system, also known as CIELAB, defined by the International Commission on Illumination in 1976. Readings were taken on the sidewall of the fruit. The expression of the colour is done according to 3 values: L: determines the brightness; a: indicates the variation from red to green and b: indicates the variation from blue to yellow. The ratio a/b shows the darkening of the products (the lower the values, the less dark the products). Products are less obscure. This ratio presents the quality of the colour and should be as low as possible [6].

2.3. Physicochemical parameters

The studied parameters in this section are the water and juice content of the fruit. Also, pH, electrical conductivity, Brix degree, and Nitrate content were measured in juice.
Titratable acidity (TA) in juice was determined by a titration method (to pH 8.1 with 0.1 M NaOH).
The TA is given in g of citric acid (AC) / 10 ml of juice it is calculated according to the following
formula: $TA = k \times a \times f$. With: $TA =$ titratable acidity expressed in [g AC / 10 ml of juice]; $K =$
conversion factor for the passage into citric acid = 0.64; $f =$ factor of 0.1 M sodium hydroxide solution
$= 1$ and $a =$ amount of 0.1 M sodium hydroxide solution used in ml.

For the extraction of lycopene, samples of fresh tomato fruits were finely processed into a 5 gram
puree using an electric blender. The tomato purees were placed in 125 ml vials, which were wrapped
in aluminium to prevent exposure to light. To each vial, 100 ml of a mixture of hexane/acetone/ethanol
(2:1:1, v/v/v) is added to solubilise the carotenoids. The samples are shaken for 30 min, 10 ml of
distilled water is then added. The solution is allowed to stand for 5 minutes to allow separation of the
two phases, the top layer (containing lycopene) is recovered for spectrometric determination. The
absorbance is measured in a quartz cuvette at 503 nm against an hexane blank. The lycopene content
of the tissue is then estimated by the equation proposed by [7].

2.4. Statistical analysis
All parameters were measured for at least six plants ($n = 6$). Statistical analysis was performed using
the General Linear Models (GLM) procedures of SPSS software for Windows: version 20. The study
of experiments was performed using standard analysis of variance (ANOVA) with interactions. The
comparison of the means of each factor was made by the Duncan test at the probability threshold of
5% ($p < 0.05$) is considered significantly different).

3. Results and Discussion

3.1. Productivity parameters
The Table 1 describes the variation of the number of fruits per plant and the total weight of fruits per
plant as a function of the Selenium-NaCl interaction. These two parameters reflect the productivity
of the tomato crop stressed and sprayed with selenium. Overall, the productivity of tomato plants grown
under $3g/l$ salinity is higher than in plants grown under $6g/l$ salinity. Selenium supply leads to a
significant ($p<0.05$) increase in the number of fruits as well as their total weight per plant.

Our results are in affinity with the results found by [8, 9], which show beneficial effects of
selenium and salicylic acid on increasing the number of tomato fruits exposed to salt stress. The
highest productivity for all plants is recorded at the $1 mM$ sodium selenate dose. This behaviour
suggests that selenium is involved in enhancing the growth process. Furthermore, the number and
yield of fruits are also closely related to the number of flowers.

In this context, many studies have shown a positive correlation between selenium and the two
studied parameters such as [10] who showed that the use of $0.51 mg / l$ selenium increases the number
of flowers of Raphanus sativus more than two times. [11] Also showed that selenium could positively
and directly affect pollination in olive under water stress by improving pollen tube formation leading
to increased crop productivity. Another study found that applying selenium to mungbean plants grown
under salt stress ($100mM$ NaCl) increased the development of pollen tubes, resulting in increased
production [12].

Table 1. Effect salt stress and treated with different concentrations of Na2SeO4 in productivity parameters of
tomato plants of the Firenze variety

| Treatments | Parameters | Yield (g) per plant |
|------------|------------|---------------------|
|            | Number of fruits per plant |                   |
| [0 mM] Se | 28 ± 2 c | 731 ± 29,30 a |
| 3g/l NaCl + [0.5 mM] Se | 38 ± 2 b | 1213 ± 105,29 b |
| [1 mM] Se | 53 ± 4 a | 2401 ± 506,83 c |
| [0 mM] Se | 22 ± 2 c’ | 503 ± 29,98 a’ |
| 6g/l NaCl + [0.5 mM] Se | 36 ± 2 b’ | 1081 ± 85,36 b’ |
3.2. Morphometric parameters

Morphometric parameters are represented by average fruit weight, size, length, and colour. Table 2 shows the variation of these parameters according to salt stress and selenium spraying. The results concerning the variation of the average fruit weight show that salinity causes its decrease and on the other hand selenium induces a good fruit development. The most notable weight development was marked in plants treated with 1mM sodium selenite and under 3g/l of NaCl. These results are in agreement with the study of [8].

By causal effect, the increase in the average weight of the fruit is the result of the increase in its volume. The latter is characterised by size and length. Due to the increase in salt stress, the size of the fruit has been reduced. However, selenium supply leads to a significant increase in size. To understand this expansion of fruit size and weight, many suggestions have been made. Some authors have attributed this expansion to the ability of selenium to increase cell wall plasticity, but recently the metabolic profile of fruit treated with exogenous selenium supply has revealed the accumulation of dihydrozeatin, an intermediate in zeatin biosynthesis. Zeatin is a cytokinin known to be involved in the control of cell division. The upward accumulation of this intermediate in the presence of selenium could be the reason that improves biometric parameters for example in the case of strawberry treated with 10 µM of selenium [13].

The variation of the different colour criteria is described by the CIELAB spatial colour system. (L) describes the luminance intensity or integrity from white to black, (a) describes the colour integrity from green to red and (b) describes the colour integrity from blue to yellow. The (a/b) parameter is one of the most important parameters in the study of colours. This ratio describes the quality of colours and it also describes the percentage of presence of the red colour [14]. In fact, as this ratio increases, the proportion of red colour increases. The statistical analysis of this parameter gives a significant result (p<0.05). The ratio (a/b) tells us about the rate of chlorophyll degradation and the production of lycopene and carotenoids in the fruit, which controls the intensity of red colour. Increasing the dose of salinity causes an increase in the ratio (a/b), this increase can be explained by the effect of salinity which stimulates respiration and ethylene production in climacteric fruits like tomato [15]. The application of Selenium still leads to an increase in the (a/b) ratio. Indeed, the increase of this ratio is explained by the effect of Selenium which stimulates the production of lycopene as an antioxidant enzyme. Also, [16] showed that the application of silicon improves the development of red colour in tomato under NaCl stress (150 mM). The values presented in this table correlate with the results of lycopene analysis.
Table 2. Effect salt stress and treated with different concentrations of Na2SeO4 in morphometric parameters of tomato plants of the Firenze variety. (L): determines the brightness or variation from black to white; (a): indicates the variation from red to green; (b): indicates the variation from blue to yellow and (a / b) indicates the quality of the colors and it also describes the percentage of presence of red color.

| Treatments             | Parameters                                      |
|------------------------|-------------------------------------------------|
|                        | Average weight of fruit(g) | fruit width (mm) | Fruit length (mm) | L     | a     | b     | a/b   |
| [0 mM] Se              | 26 ± 1 a                                      | 38,14 ± 4,37 a  | 44,64 ± 1,52 a    | 32,47 ± 1,76 a | 35,37 ± 1,62 a | 41,74 ± 2,17 a | 0,85 ± 0,063 a |
| 3g/l NaCl + [0.5 mM] Se | 32 ± 4 b                                      | 48,16 ± 3,75 b  | 55,40 ± 1,54 b    | 34,33 ± 1,84 b | 37,21 ± 1,62 b | 37,27 ± 1,41 b | 1,00 ± 0,05 b  |
| [1 mM] Se              | 45 ± 6 c                                      | 54,28 ± 3,57 c  | 60,52 ± 1,53 c    | 41,68 ± 1,43 c | 40,73 ± 1,98 c | 32,47 ± 1,98 c | 1,26 ± 0,08 c  |
| [0 mM] Se              | 23 ± 2 a’                                     | 35,35 ± 2,57 a’ | 40,89 ± 1,26 a’   | 33,65 ± 1,51 a’| 42,69 ± 2,18 a’| 32,77 ± 1,75 a’| 1,31 ± 0,08 a’ |
| 6g/l NaCl + [0.5 mM] Se | 31 ± 4 b’                                     | 43,55 ± 2,57 b’ | 50,12 ± 1,52 b’   | 35,19 ± 1,91 b’| 40,02 ± 1,28 b’| 30,33 ± 1,72 b’| 1,34 ± 0,09 a’  |
| [1 mM] Se              | 33 ± 2 b’                                     | 50,46 ± 3,35 c’ | 54,16 ± 3,32 c’   | 37,04 ± 3,36 c’| 37,89 ± 2,31 c’| 24,94 ± 4,14 c’| 1,52 ± 0,24 b’  |

Note: in each column, the different letters (a, b, c, a’, b’, and c’) indicate statistically significant differences among treatments according to Duncan test at the significance level (P ≤ 0.05).
3.3. Physico-chemical parameters

Table 3 shows the variation of the different physicochemical parameters studied according to the different selenium and NaCl treatments. First of all, we will start with the water content and the juice yield, which show a decrease under the influence of increased salinity. On the other hand, selenium intake leads to an increase in both parameters.

The most remarkable increase was recorded in terms of juice content with the intake of selenium in 1mM. It was about 8% and 7% respectively for plants grown under 3g/l and 6g/l of NaCl. The water content of the fruit and the juice yield are in fact positively correlated for fruits such as tomatoes, it is very important to maintain a stable water level in fruit to maintain the functioning of metabolic processes and also to maintain fruit quality. For this, plants tend to concentrate a lot of mineral elements in fruits in order to maintain their watery state compared to other plant components. This behavior has been shown by studying the application’s effect of selenium on the formation of strawberry fruits in which Na+ levels have increased in the fruits to maintain their high fresh weight. Fresh fruit weight could be maintained directly by selenium itself, which accumulates directly in fruit [17].

The degree Brix describes the soluble content of juice specifically sugar. The increase in salinity is associated with the increase in degree Brix by 29.52%. The addition of Selenium is accompanied by its increase, the largest of which was recorded with the dose of 1 mM of selenium. It was of the order of 6 and 7-degrees Brix respectively for plants treated with 3g/l and 6g/l NaCl. These results are correlated with the study of [9] which shows that selenium stimulates the increase in the degree Brix of tomato as a strategy to combat saline stress.

The Titratable acidity is sensitive to salinity in the sense that its severity favours its increase. On the other hand, selenium intake leads to a decrease in this parameter. This decrease is significant for fruit of plants grown under 3g/NaCl but not significant for fruit of plants grown under 6g/NaCl. A similar result was obtained by [18], who reported that firmness was higher in peach fruits from plants treated with selenium than those from plants not treated with selenium. These results suggest that the ability of selenium to maintain fruit quality may be related to the inhibition of ethylene production. Concerning the variation of the PH and the electrical conductivity, it is noted that the largest increase of these two parameters is marked in plants treated with 3g/l of NaCl and 1 mM of selenium. In fact, PH and electrical conductivity play a very important role in determining fruit quality. Several studies show that the application of selenium ensures the maintenance of a balanced pH and electrical conductivity to ensure the best quality of the fruit. This can be explained by the ability of selenium to interact in the process of transporting H+ ions and other minerals from the plant to the fruit [17].

Concerning the nitrate content, it is noted that under the influence of the increase in salinity this parameter decreases by 11.49%. On the other hand, the effect of selenium on nitrate accumulation is negligible and does not exceed 7%. This behaviour is considered one of the beneficial effects of selenium. In fact, the accumulation of nitrate at significant levels threatens human health. For this, the application of selenium presents a solution that maintains a balanced nitrate level. This has been proven by [19].

Salinity leads to a 47.66% increase in lycopene content, which stimulates early maturation. The application of Selenium is accompanied by a positive and significant effect on lycopene content in fruit. Moreover, for plants irrigated by 3g/l of NaCl, the exogenous intake of selenium in 1mM and 0.5mM leads respectively to the increase in lycopene content by 237.38% and 195.32% compared to the control. For plants irrigated by 6g/l of salinity the rates of increase were respectively around 222% and 62% the intake of 1mM and 0.5mM of selenium compared to the control. Several authors find similar results, for example,[9, 20] showed that the application of selenium may interfere with lycopene biosynthesis in tomatoes but with delayed maturation.
Table 3. Effect salt stress and treated with different concentrations of Na2SeO4 in physicochemical parameters of tomato plants of the Firenze variety

| Treatments                  | Parameters                              | Water content (%) | Juice yield (%) | EC (mS/cm) | Brix degree | pH | Titratable acidity (g/10ml juice) | Nitrate content (mg/kg) | Lycopene content (mg/kg of tissue) |
|-----------------------------|-----------------------------------------|-------------------|----------------|------------|-------------|----|-----------------------------------|------------------------|-------------------------------|
| [0 mM] Se                   |                                         | 86.06 ± 1.85 b    | 45.08 ± 3.65 b  | 3.81 ± 0.22 a | 5.42 ± 0.45 a | 4.31 ± 0.07 b | 5.6 ± 0.17 a | 145.00 ± 10.49 a | 1.07 ± 0.11 b |
| 3g/l NaCl + [0.5 mM] Se     |                                         | 90.92 ± 0.42 b    | 51.03 ± 2.86 ab | 3.81 ± 0.27 a | 5.82 ± 0.43 a | 4.27 ± 0.07 a | 3.3 ± 0.13 b | 143.33 ± 8.16 a | 3.16 ± 0.09 a |
| [1 mM] Se                   |                                         | 92.81 ± 0.84 a    | 53.52 ± 8.43 a  | 4.51 ± 0.30 b | 6.42 ± 0.49 b | 4.72 ± 0.11 a | 1.9 ± 0.09 c | 148.33 ± 23.17 a | 3.61 ± 0.38 a |
| [0 mM] Se                   |                                         | 85.79 ± 1.61 b’   | 43.05 ± 2.40 b’ | 3.99 ± 0.32 a’| 7.02 ± 0.87 a’| 4.33 ± 0.04 a’| 6.5 ± 0.47 a’| 128.33 ± 7.53 a’| 1.58 ± 0.24 b’|
| 6g/l NaCl + [0.5 mM] Se     |                                         | 87.80 ± 1.30 b’   | 48.70 ± 4.68 b’ | 4.05 ± 0.30 a’| 6.98 ± 0.45 a’| 4.56 ± 0.42 a’| 4.5 ± 0.91 b’| 126.67 ± 10.33 a’| 2.56 ± 1.11 b’|
| [1 mM] Se                   |                                         | 90.79 ± 0.49 a’   | 50.10 ± 4.30 b’ | 3.61 ± 0.82 a’| 7.38 ± 0.41 a’| 4.16 ± 0.39 a’| 3.5 ± 0.10 b’| 120.00 ± 8.94 a’| 5.09 ± 0.44 a’|

Note: in each column, the different letters (a, b, c, a’, b’, and c’) indicate statistically significant differences among treatments according to Duncan test at the significance level (P ≤ 0.05).
4. Conclusion
The analysis of parameters related to the biological quality of tomato fruits showed the beneficial effect of selenium application under salt stress conditions on their pomological and physicochemical characteristics. Indeed, whatever the saline concentration in the tomato culture medium, the application of selenium at high dose [1Mm] resulted in a clear improvement of the morphometry of the fruits and their organoleptic quality. This improvement was mainly manifested by a significant increase in fresh weight, colour and size of the fruit. Also there was an increase in sugar and lycopene levels in the tomato juice.

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