Original article

Calcium effects on changes in chlorophyll contents, dry weight and micronutrients of strawberry (*Fragaria* × *ananassa* Duch.) plants under salt-stress conditions

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Calcium effects on changes in chlorophyll contents, dry weight and micronutrients of strawberry (*Fragaria* × *ananassa* Duch.) plants under salt-stress conditions.

Abstract — **Introduction**. Today, approximately one-third of the world's irrigated lands suffer some degree of salinity. Although there are several reports about the effects of calcium treatments on strawberry growth and development under saline conditions, there are no reports demonstrating the effect on micronutrient concentrations of strawberry due to supplementary calcium applied to the salt-stressed plants. Consequently, we investigated the effects of high salinity levels, with application of supplementary calcium, on chlorophyll contents, dry weight and micronutrients of strawberry. **Materials and methods**. Strawberry (*Fragaria* × *ananassa* Duch.) plants cv. 'Selva' were grown in hydroponic culture in a heated greenhouse to investigate the effectiveness of calcium (Ca) added to nutrient solution applied to plants. Six treatments were applied: nutrient solution alone (= [N]); [N] + NaCl salt (35 mM) (= [NS]); [NS] + $CaCl_2$ (5 mM); [NS] + $CaCl_2$ (10 mM); [NS] + $CaSO_4$ (5 mM); and [NS] + $CaSO_4$ (10 mM). The effect of calcium (different forms and different concentrations) on chlorophyll contents and dry weight of salt-stressed plants was determined. Additionally, micronutrient contents were studied. **Results**. The results indicated that chlorophyll contents and plant dry weight were decreased by salinity. NaCl application increased Cu, Zn, Mn and Fe concentrations of root parts. In shoot parts, Cu accumulation decreased, while other elements increased. Supplementary calcium could ameliorate the negative effects of salinity on chlorophyll and dry mass production. However, micronutrient concentrations of plant parts as influenced by calcium had contradictory results: calcium induced high levels of zinc, manganese and iron concentrations in roots, but high levels of copper and zinc concentrations in shoots. Moreover, there were significant differences between calcium forms in their actions. **Conclusion**. Our results suggest that $CaSO_4$ could be applied to improve the growth of salt-stressed strawberry plants.

Iran Islamic Republic / *Fragaria ananassa* / salt tolerance / chlorophylls / dry matter content / mineral content

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Effets du calcium sur la teneur en chlorophylle, le poids sec et les oligoéléments du fraisier (*Fragaria* × *ananassa* Duch.) en condition de stress salin.

Résumé — **Introduction**. Aujourd'hui, près d'un tiers des terres irriguées du monde souffrent d'un certain degré de salinité. S'il existe plusieurs rapports sur les effets du calcium sur la croissance et le développement du fraisier en conditions salines, il n'y rien sur l'effet du calcium sur les concentrations en oligoélément de fraisiers cultivés en présence de NaCl. En conséquence, nous avons étudié les effets d'un niveau élevé de salinité combiné avec l'application de calcium supplémentaire, sur les teneurs en chlorophylle, en poids secs et en oligoéléments du fraisier. **Matériel et méthodes**. Des plants de fraisiers (*Fragaria* × *ananassa* Duch.) cv. Selva ont été suivis sous culture hydroponique en serre chaude pour étudier l'efficacité de l'ajout de calcium (Ca) dans la solution nutritive apportée aux plantes. Six traitements ont été appliqués : solution nutritive seule (= [N]) ; [N] + sel de NaCl (35 mM) (= [NS]) ; [NS] + $CaCl_2$ (5 mM) ; [NS] + $CaCl_2$ (10 mM) ; [NS] + $CaSO_4$ (5 mM) ; [NS] + $CaSO_4$ (10 mM). L'effet du calcium (sous différentes formes et à différentes concentrations) a été déterminé sur le contenu en chlorophylle et le poids sec des plantes stressées. La teneur en oligoéléments des plantes a également été étudiée. **Résultats**. Nos résultats ont indiqué que la teneur en chlorophylle et le poids sec des plantes étaient diminués par la salinité. L'application de NaCl a augmenté des concentrations en Cu, Zn, Mn et Fe dans les racines. Les teneurs en Cu ont diminué dans la tige, alors que les autres éléments étudiés ont augmenté. L'ajout de calcium a pu compenser les effets négatifs de la salinité sur la teneur en chlorophylle et la production de matière sèche. Cependant, les effets de l'ajout de calcium sur les concentrations en oligoélément dans la plante ont été divers : un niveau élevé a été induit par le calcium pour les concentrations en Zn, en Mn et Fe dans les racines, alors que, dans la tige, ce sont les concentrations en Cu et en Zn qui ont été augmentées. De plus, ces différences ont été significatives. **Conclusion**. Nos résultats suggèrent que le sulfate de calcium ($CaSO_4$) pourrait être appliqué avec succès pour améliorer la croissance des fraisiers soumis à un stress salin.

Iran République islamique / *Fragaria ananassa* / tolérance au sel / chlorophylle / teneur en matière sèche / teneur en éléments minéraux

1. Introduction

Today, approximately one-third of the world's irrigated lands suffer some degree of salinity [1]. The increasing world population and urbanization have forced agricultural producers to utilize marginal lands that are often saline. Moreover, agricultural producers must often use low quality water [2]. Salinity is a factor affecting the whole-plant metabolism processes and its morphology and anatomy [3]. Reduction in growth rate under salinity stress is usually attributed to either ion toxicity or low external osmotic potential [4]. Both effects may influence the plant physiological and biochemical processes [5].

It is unclear how salinity affects the micronutrient composition of plant parts. It is declared that micronutrients are generally less influenced by salinity compared with macronutrients [6]. Hu and Schmidhalter also suggested that micronutrient concentration in plant parts is not much affected by salt stress [7]. However, this stress increased the Zn concentrations in the root and leaf of pepper [8], and the Zn, Cu and Mn concentrations of wheat and rice plants [9]. In the same way, concentrations of Fe, Mn and Zn in leaves of zucchini were increased by intensifying the salt stress; however, the concentration of Cu was reduced [10]. Moreover, Sanchez-Raya and Delgado suggested that salinity generally decreased Fe and Mn transportation from seed to seedling in the growth period in sunflower plants [11]. They also reported that transportation of Fe to aerial parts was distinctly reduced. There are some reports which suggested that calcium would ameliorate sodium stress effects on plant growth and development [12–14]. Furthermore, calcium is well known to have regulatory roles in metabolism [15], and sodium ions may compete with calcium for membrane-binding sites. Therefore, it has been hypothesized that high calcium levels could protect the cell membrane from the adverse effects of salinity [16]. Although gypsum ($CaSO_4$ H_2O) is the most common source of Ca used to ameliorate the effects of Na ions, other sources of calcium such as $CaCl_2$ have been used [2].

There are several reports about the effects of calcium treatments on strawberry growth and development under saline conditions [14, 19, 20]. Nevertheless, to our knowledge, no reports exist which demonstrate the effect of supplementary calcium, applied to the salt-stressed plants, on micronutrient concentrations of strawberry. Consequently, the aim of our study was to investigate the effects of calcium application upon micronutrients, dry weight and chlorophyll contents of strawberry plants submitted to high salinity levels.

2. Materials and methods

A pot experiment was conducted with strawberry cv. 'Selva' plants in hydroponic culture under greenhouse conditions. Cold-stored and bare-rooted plants, each with one well-developed crown of 8–10 mm in diameter, were planted in pots containing perlite. Plant culturing was accomplished on April 1, 2006. Air temperature ranged between 14–16 °C (night) and 21–30 °C (day). Plants were irrigated with a commercial nutrient solution (Melspray, 20 / 20 / 20 NPK). Total concentrations of nutrient solution in the hot and cool seasons throughout the experimental period were 1000–1400 $mg \cdot L^{-1}$ and 550–800 $mg \cdot L^{-1}$, respectively. Nitric acid was used for pH adjustment at 5.5–6.5.

Six treatments were applied after 1 month, on May 2006, when plants were completely established: (a) commercial nutrient solution (20 / 20 / 20 NPK) = [N]; (b) ([N] + NaCl salt at 35 mM) = [NS]; (c) [NS] + $CaCl_2$ at 5 mM; (d) [NS] + $CaCl_2$ at 10 mM; (e) [NS] + $CaSO_4$ at 5 mM; and (f) [NS] + $CaSO_4$ at 10 mM.

Chlorophyll content was measured using a chemical method [17], as well as a chlorophyll meter (Spectronic 20 model) for whole plants.

At the end of the experiment (October 20, 2006) and after plant harvesting, the number of healthy leaves, and shoot and root fresh weight were measured. Dry weight was measured separately after oven-drying the plant parts at 80 °C for 48 h.

Table I.
Effects of salinity and supplementary calcium on chlorophyll contents, and root and shoot dry weights in strawberry plants.

Treatment				Chlorophyll	Root dry weight	Shoot dry weight	[Shoot / root] ratio
Commercial nutrient solution	NaCl	Calcium added	Concentration (mM)	($mg \cdot g^{-1}$ fresh weight)	(g)		
20 / 20 / 20 NPK	None	None	–	1.22 a	37.01 a	60.65 a	1.64 ab
	35 mM	None	35	1.02 bc	10.20 e	10.71 e	1.05 c
		$CaCl_2$	5	0.95 c	13.61 d	16.70 c	1.23 c
			10	1.10 b	19.52 b	13.76 d	0.70 d
		$CaSO_4$	5	1.21 a	15.37 c	28.00 b	1.82 a
			10	1.19 a	12.07 d	18.31 c	1.52 b

Within each column, the same letter indicates no significant difference between treatments at the 5% level.

Chemical analyses were carried out on oven-dried plant materials in both shoots and roots. Ground samples were ashed at 550 °C for 6 h in a porcelain crucible. The white ash was taken up in 2 M-hot HCl, filtered into a 50-mL volumetric flask and made up to 50 mL with distilled water. Fe, Zn, Mn and Cu were determined in these sample solutions. Micronutrients were analyzed by an atomic absorption spectrophotometer [18].

The experiment was set up using a completely randomized design (CRD) with nine replications; there was one plant in each pot. Data were analyzed using MSTAT-C. Differences among means of treatments were compared by Duncan's multiple range tests at the 5% level.

3. Results and discussion

NaCl salinity reduced chlorophyll content in leaves (*table I*), which is in agreement with Kaya *et al.* [19, 20]. However, lower chlorophyll content resulted from $CaCl_2$ (5 mM) treatment. Increment in $CaCl_2$ concentration significantly increased the chlorophyll content from (0.95 to 1.1) $mg \cdot g^{-1}$ fresh weight. Calcium sulfate ($CaSO_4$) was a better form of Ca^{2+} for protecting the chlorophyll content under salt stress. This finding is in agreement with the results of Navarro *et al.* [21] regarding tomato plants. Turhan and Eris [14] observed that total chlorophyll content was unaffected by NaCl applications in both the strawberry varieties 'Camarosa' and 'Tioga'. In fact, different reports about this subject exist in the literature. For instance, chlorophyll content was reduced with NaCl applications in lime [23] and grapevine trees [24].

High NaCl salinity had a negative effect on root dry weight of the strawberry cv. 'Selva' (*table I*). All the treatments with addition of calcium ($CaCl_2$ and $CaSO_4$) induced lower root dry weight when compared with treatment without salt. Nevertheless, Turhan and Eris reported that NaCl salt increased root dry weight in the 'Camarosa' strawberry cultivar [14]. On the other hand, in our experiment, calcium application had a positive effect on root dry weight, and different results were found from the applied calcium forms: root dry weight increased either with raising the $CaCl_2$ concentration in the nutrient solution or with reducing the calcium sulfate concentration.

Shoot dry weight was negatively affected by the high NaCl level (35 mM) applied to plants. Although calcium added to the nutrient solution applied could ameliorate the harmful effects of NaCl salinity, shoot dry weight was decreased by addition of these Ca concentrations (*table I*).

Table II.
Effects of salinity and supplementary calcium on micronutrient content of plants (in $mg \cdot L^{-1}$)

Treatment				Root				Shoot			
Commercial nutrient solution	NaCl	Calcium added	Concentration (mM)	Cu	Zn	Mn	Fe	Cu	Zn	Mn	Fe
20 / 20 / 20 NPK	None	None	–	30.0 b	66.45 e	119.0 f	190.0 d	28.8 b	69.2 d	186.8 c	168.0 f
	35 mM	None	–	31.4 a	77.50 b	158.6 c	192.8 b	26.4 c	79.0 b	198.3 a	198.0 a
		$CaCl_2$	5	25.8 f	77.30 c	168.2 a	173.0 e	24.0 d	71.9 c	192.6 b	188.3 c
			10	26.7 d	70.00 d	164.1 b	167.8 f	22.8 e	83.8 a	152.7 d	194.3 b
		$CaSO_4$	5	28.8 c	53.80 f	132.4 e	195.0 a	29.0 a	64.4 e	127.9 f	179.0 d
			10	26.6 e	88.05 a	138.4 d	191.5 c	22.6 f	68.0 f	140.6 e	169.5 e

Within each column, the same letter indicates no significant difference between treatments at the 5% level.

Considering our data, the [shoot dry weight / root dry weight] ratio was strongly affected by salinity. The highest amounts of this ratio were obtained with the nutrient solution used alone (1.64) and with the nutrient solution used with $CaSO_4$ (5 mM) (1.82). This variable was reduced by an increment in calcium concentration in nutrient solution *(table I)*.

According to our results, $CaSO_4$ treatment was the better form for biomass production by salt-stressed strawberry plants. Several authors have reported contradictory results about shoot dry weight in different plants under saline conditions, among which (10 and 25) mM NaCl in pepper [25] and 25 mM NaCl treatment in eggplant [26] did not affect the shoot dry weight. However, it was determined that leaf dry weight was increased by NaCl application in lettuce and endive plants [27].

Our findings related to micronutrient concentrations of shoot and root parts of the strawberry plants showed that copper concentration of roots was increased by salinity (*table II*), which is in agreement with the results of Alpaslan *et al.* [9] regarding rice and wheat, and with those of Villora *et al.* [10] regarding zucchini plants. NaCl application *via* the nutrient solution significantly increased zinc accumulation by roots in comparison with control plants treated with the nutrient solution alone. Moreover, NaCl application raised manganese and iron concentrations of roots compared with non-saline conditions. These findings are in disagreement with those of Turhan and Eris [14] obtained with other strawberry varieties. Indeed, according to the authors, root Cu was increased by salinity and other elements were unaffected. Supplementary calcium reduced Cu concentration of roots in comparison with non-salinized plants. Nevertheless, this variable increased with $CaCl_2$ increment, while it decreased with $CaSO_4$ increment.

Cornillo and Palloix [8] reported that NaCl salinity increased the concentration of zinc in pepper plant tissues. In our experiment, the highest root Zn accumulation resulted from the $CaSO_4$ (10 mM) treatment, while, with an increase in $CaCl_2$ concentration, zinc accumulation decreased. Likewise, Ca ion application also causes an increment in this variable compared with control.

With $CaCl_2$ reduction [(10 to 5) mM] and $CaSO_4$ increment [(5 to 10) mM], Mn accumulation in root parts increased. Higher and lower Fe concentrations were obtained with the use of $CaCl_2$ (10 mM) and $CaSO_4$ (5 mM) treatments, respectively (*table II*). Our results showed that increasing supplementary calcium concentration caused a reduction in root Fe concentration.

In shoots, supplementary $CaCl_2$ led to a reduction in Cu accumulation in comparison with non-salinized plants. Although high Cu concentrations were obtained with $CaSO_4$ (5mM), an increased level of supplementary calcium strongly reduced this variable in shoots. Similar to the root zinc results already mentioned, NaCl applied to the strawberry plants with the nutrient solution increased shoot zinc accumulation, which is in agreement with the findings of Alpaslan *et al.* [9] regarding pepper plants. Concerning supplementary calcium treatments, root zinc concentration was contrary to the shoot results.The highest manganese accumulation resulted from NaCl treatment. Villora *et al.* [10] also reported that salinity increased the Mn concentration in aerial parts of zucchini plants. Calcium application led to similar results to those for root manganese. NaCl had a positive effect on shoot Fe accumulation in comparison with the control plants, which is in agreement with Turhan and Eris' results [14] obtained with other strawberry cultivars. According to our data, NaCl application caused high Fe accumulation in shoot tissues; $CaCl_2$ treatments increased iron concentration of shoots, while increment in $CaSO_4$ reduced this variable in aerial parts.

Our results showed that there was significant correlation between iron and zinc concentrations in shoot tissue. Moreover, in root tissue, there was significant correlation between iron and manganese concentrations.

4. Conclusions

The response of plants to salt stress is directly related to their stage of growth, duration of exposure and variety [28]. NaCl salt treatments reduced chlorophyll concentrations in leaves of strawberry cv. 'Selva' plants. However, dry mass production was also reduced under stress conditions. Calcium sulfate could restore chlorophyll contents when the plants are exposed to NaCl and, consequently, plant dry weight would be protected, and close to the control plant status. For strawberry plants under salinity conditions, differences in amount of mineral nutrients and reduction in growth, especially at high salinity levels, may depend on ineffective usage of water by plants and interference with ion uptake and ionic imbalance [14]. From our results, we suggest that, in saline conditions, supplementary calcium could be a useful means for improving plant growth and development.

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Efectos del calcio sobre el contenido en clorofila, el peso seco y los oligoelementos de la fresa (*Fragaria* × *ananassa* Duch.) en condición de estrés salino.

Resumen — **Introducción**. Hoy en día, cerca de un tercio de las tierras de riego en el mundo sufren de un cierto grado de salinidad. Si bien existen muchos informes sobre los efectos del calcio sobre el crecimiento y el desarrollo de la fresa en condiciones salinas, no existe nada sobre el efecto del calcio sobre las concentraciones en oligoelementos de fresas cultivadas en presencia de NaCl. Consecuentemente, estudiamos los efectos de un nivel elevado de salinidad combinado con la aplicación adicional de calcio, sobre los contenidos en clorofila, en peso secos y en oligoelementos de la fresa. **Material y métodos**. Se hizo el seguimiento de plantas de fresas (*Fragaria* × *ananassa* Duch.) cv. Selva bajo cultivo hidropónico en invernadero caliente para estudiar la eficacia de la adición de calcio (Ca) en la solución nutritiva aportada a las plantas. Se aplicaron seis tratamientos: solución nutritiva sola (= [N]) ; [N] + sal de NaCl (35 mM) (= [NS]) ; [NS] + $CaCl_2$ (5 mM) ; [NS] + $CaCl_2$ (10 mM) ; [NS] + $CaSO_4$ (5 mM) ; [NS] + $CaSO_4$ (10 mM). Se determinó el efecto del calcio (bajo diferentes formas y en concentraciones diferentes) sobre el contenido en clorofila y el peso seco de las plantas estresadas. Asimismo se estudió el contenido en oligoelementos de plantas. **Resultados**. Nuestros resultados indicaron que el contenido en clorofila y el peso seco de las plantas disminuían por causa de la salinidad. La aplicación de NaCl aumentó las concentraciones en Cu, Zn, Mn y Fe en las raíces. Los contenidos en Cu disminuyeron en el tallo, mientras que los otros elementos estudiados aumentaron. El aporte de calcio compensó los efectos negativos de la salinidad sobre el contenido en clorofila y la producción de materia seca. Sin embargo los efectos del aporte de calcio en las concentraciones en oligoelementos en la planta fueron diversos: se indujo por el calcio un nivel elevado para las concentraciones en Zn, en Mn y Fe en las raíces, mientras que, en el tallo, aumentaron las concentraciones en Cu y en Zn. Asimismo, estas diferencias fueron significantes. **Conclusión**. Nuestros resultados sugieren que el sulfato de calcio ($CaSO_4$) podría aplicarse exitosamente para mejorar el crecimiento de las fresas sometido a un estrés salino.

Iran República Islámica / *Fragaria ananassa* / tolerancia a la sal / clorofilas / contenido de materia seca / contenido mineral