

Reaction of Three Strawberry Cultivars to the Salinity: Vegetative Parameters

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Summary

The study aimed at analysing the reaction of three strawberry *Fragaria x ananassa* Duch. cultivars to the salinity (0-control, 0.5, 1.0, 2.0, 3.0 and 4.0 g of NaCl L⁻¹) in the irrigation water: equivalent of the following EC values: (0.73, 1.65, 2.66, 4.37, 5.93 and 7.81 mS cm⁻¹). Vegetative trial was carried out at the Faculty of Agriculture in Zagreb during 1997 and 1998. Following parameters were tested: degrees of damage of shoots, number of leaves, number of runners (stolons), yield of fresh biomass and dry matter of shoots and roots, and percentage of dry matter of shoots and roots. Tested cultivars showed significant differences of the level of damage (necrosis and decay of plants due to high NaCl concentration). The highest degree of damage was shown by 'Elsanta' in both trial years, followed by 'Marmolada' and 'Miranda' respectively. The analysis of the number of leaves and stolons confirmed the same trend. The yield of fresh biomass and dry matter of shoots and roots was significantly reduced with the application of NaCl. 'Miranda' was characterised by the highest fresh biomass and dry matter yield, followed by 'Marmolada' and 'Elsanta'. Average decrease of fresh biomass yield of shoots in comparison to the control was 45, 33 and 22% in 'Elsanta', 'Marmolada' and 'Miranda'. Dry matter yield of shoots decreased correspondingly for 34, 28 and 18%. Total dry matter yield (shoots + roots) of 'Elsanta' was significantly reduced at 0.5 g NaCl L⁻¹ and of 'Marmolada' and 'Miranda' at 1.0 and 2.0 g L⁻¹ respectively.

Key words

strawberry, NaCl, root, shoot, fresh biomass, dry matter

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Introduction

Excessive salts in soil often are limiting factor in cultivation of agricultural crops in arid and semiarid areas (Marschner, 1995). These areas are significant for high temperatures and evapotranspiration as well as for low precipitation rate, which would rinse excess salts to lower soil layers. One third of world areas that are irrigated have high salt content (Epstein et al., 1980). When it comes to high salt content in soil (growing substrate), three main factors are influencing plants unfavourably: 1) water deficiency, induced lower (or more negative) water potential, 2) ion toxicity – excessive receipt of Cl^- and Na^+ , and 3) disturbance in balance of nutrient receipt and transport (Greenway and Munns, 1980; Ream and Furr, 1976; Zekri and Parsons, 1992; Peres-Alfocea et al., 1993), which impacts negatively on growth and yield of majority of agricultural crops (Maas, 1986).

Few measures can be used for correction and usage of salted soils, although each of them has limited possibilities. Sibbett (1995) quotes *salts leaching* as the only correct measure, but only in cases when proper drainage system is put in place and sufficient level of quality water needed for salt leaching is available. Liu et al. (1997) recommend long-term cultivation of tolerant crops with high capacity for salts removal. Marschner et al. (1981b) recommend cultivation of tolerant genotypes. Soils with high sodium content are 'repaired' best if fertilized profusely with NPK fertilizer before planting (Finck, 1982). Positive impact of calcium on decreasing of adverse salts effect, and on growth and yield were recognized by: Cramer et al. (1986); Rengel (1992) and Lopez and Satti, (1996). Importance of balanced fertilization in management of soil salinity was implied by other authors as well (Garg et al., 1993; Alarcon et al., 1994; Adler et al., 1995; Bar et al., 1997; Lei et al., 1997). Plant species react differently to high salts content (Greenway and Munns, 1980; Bergmann, 1992). Salinity tolerance, also, varies depending on growth stage of plant development (Mass, 1986; Zekri, 1993; Ashraf and McNeily, 1988). Significant differences to salts tolerance were determined within the same species (Heimler et al., 1995; Cornillon and Palloix, 1997; Serraj et al., 1998; Shannon and Noble, 1995; Marschner et al., 1981a). Tolerant genotypes are used for cultivation on salted soils, and as a basis in breeding. Previous research on strawberry has shown its strong susceptibility to salts (Maas, 1986; Maroto and Lopez-Galarza, 1989; Ehlig and Bernstein, 1958; Ehlig, 1961; Martinez Baroso and Alvarez, 1997; Awang et al., 1993, Ondrašek et al., 2006). In addition to the research executed in vegetative or field trial, Badawi et al. (1990) and Biško et al. (1997a) were studying NaCl impact on regeneration and shoot growth of strawberry in *in vitro* conditions.

Regardless of research conditions (*in vitro*, *ex vitro* – field or vegetative trial), significant differences to salinity tolerance of certain genotypes were determined. The main objectives of this research were to study NaCl impact on growth and development of strawberries in vegetative trial, to establish the best parameter as selection criteria, and to establish reactions conditioned by a cultivar.

Material and methods

The study was carried out in a plastic tunnel at the Faculty of Agriculture, University of Zagreb in the course of two veg-

etation years (1997 and 1998). In a study, growing substrate 'FLORATERA' was used, distinguished by suitable nutrient concentration and very low salt content [pH in water (10/100, g/vol) in naturally moist sample=7.05; E.C. mS cm^{-1} (1:2 vol./vol.) in naturally moist sample = 1.40; Na = 43.00 mg L^{-1} , Cl = 78.90 mg L^{-1}]. Prior to filling, entire substrate mass was mixed and in each container (6 litres vol) perforated pvc bag was inserted, filled with 3.6 kg of substrate. Prior to planting, 'frigo plants' were prepared by washing in water; roots were shortened to 8 cm and 60 uniform plants of each cultivar were planted individually in vegetative containers. Strawberry frigo plants (medium early, homogeneous cultivars: 'Elsanta', 'Marmolada' and 'Miranda') were planted in the middle of April. Containers were arranged in completely randomized design (CRD) – a trial of six treatments in 10 repetitions for each cultivar was formed, followed by irrigation of 1.0 litre of water per pot. Afterwards, in each container 400 mL of substrate and 0.5 cm layer of sand (\varnothing 2- 4 mm) were added, previously rinsed in 2.5% of HCl and water for several times. Trial was carried out in partially controlled conditions (quantity and quality of water for irrigation). Irrigation with salted water started week after planting. The trial variants (options) were determined by adding NaCl into irrigation water in concentration of: 0.0 (control); 0.5; 1.0; 2.0; 3.0 and 4.0 g L^{-1} , equivalent to following EC_w : 0.73; 1.65; 2.66; 4.37; 5.93 and 7.81 mS cm^{-1} and pH values: 7.23; 7.26; 7.28; 7.30, 7.30 and 7.32. Plants were irrigated every 5 - 6 days with 500 ml/pot (in April, May, and June – 14 times per year in total). Underneath containers, plastic pads were placed in which strained water was collected. Potable water was used in control and for variants preparation. EC water value during treatment was 0.71 - 0.75 mS cm^{-1} . Top dressing was done four times per year; during irrigation (1% v/v: at irrigation 3 and 7 with Fertina G - composition in %: N=8, K_2O =7, MgO=2, B=0.5, Fe=0.2, and at irrigation 5 and 9 with Fertina C – composition in %: N=4, P_2O_5 =6, K_2O =8, B=0.01, Cu=0.002, Fe=0.02, Zn=0.005). Plants in a trial were observed regularly and biological changes in growth and development were recorded, as well as occurrence of chlorosis and necrosis. Moment of occurrence of runners (stolons) and their number was determined. Immediately before plants were taken to determine the growth parameters, degree of damage caused by salts – 'damage ranking' was determined:

- 1 – completely healthy plants, no sign of chlorosis or necrosis,
- 2 – chlorosis of marginal parts of older leaves (zone $< \frac{1}{2}$ cm),
- 3 – necrosis of marginal parts of older leaves (zone $< \frac{1}{2}$ cm),
- 4 – necrosis of marginal parts of older leaves (zone up to 1 cm),
- 5 – complete necrosis of older leaves,
- 6 – complete necrosis of older and partly of younger leaves,
- 7 – complete necrosis of older and younger leaves (only terminal bud left green),
- 8 – dead plants.

With ending of vegetative trial (1 decade in July), plant material of strawberries was taken to determine the growth parameters. Plants were taken out of the substrate, and with light shaking at the beginning followed by manual cleaning, the substrate was completely removed from roots. Subsequently, plants were washed twice in potable, distilled and redistilled water and dried out on a filter paper (see photo 1). Flower shoots were removed from the runner; plants were divided to roots and shoots.

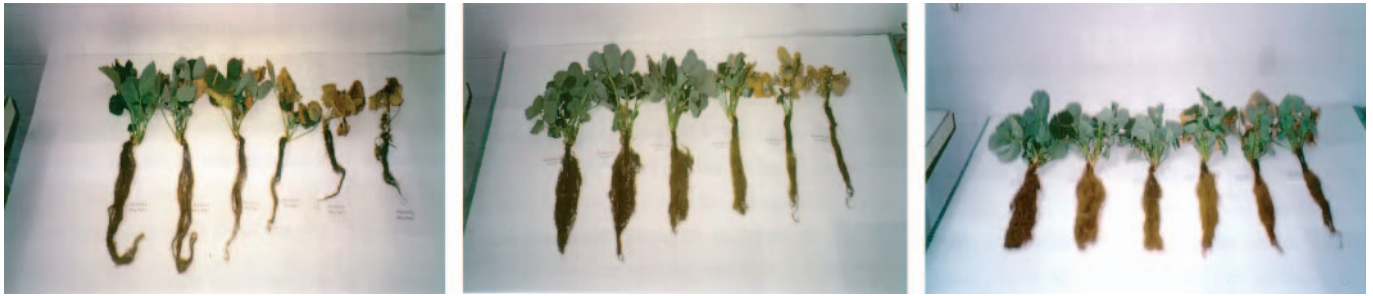


Photo 1. Plant material of strawberries (left - Elsanta, middle - Marmolada, right - Miranda; from left to right: 0; 0.5; 1.0; 2.0; 3.0 and 4.0 g NaCl L⁻¹ water for irrigation)

Yield of fresh biomass (FB), dry matter (DM), and percentage of dry matter (%DM) were determined.

Once plants were divided to roots and shoots, fresh biomass yield was determined by weighing on analytical scale. Dry matter yield was determined (weighing on analytical scale) subsequent to drying of a sample until constant mass at 105°C was reached. Dry matter percentage was calculated: (DM/FB)x100.

Statistical data analysis: Both years, trial was set according to completely randomized design (CRD): three cultivars x six treatments x 10 repetitions. Collected parameters that were measured were analyzed by using variance analysis (ANOVA test), Petz (1985). LSD (last significant different) was calculated in case when F-test was significant at levels $\alpha = 0.05$. Computer program EXCEL 7.0. was used to calculate Linear regression (Serdar, 1977; Bender et al., 1989) and for graphic design.

Results

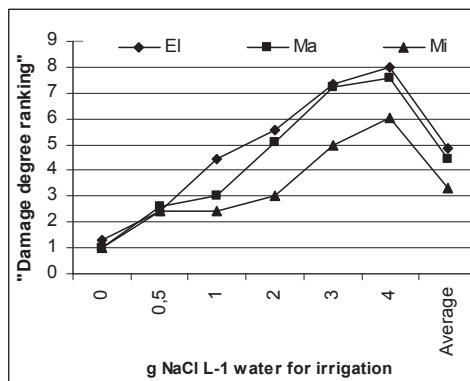
First signs of salts impact appeared in older leaves 15 days after trial started. It manifested as marginal chlorosis that was transferring gradually to marginal necrosis of older leaves, their total decay followed by marginal and total necrosis of younger leaves, and finally decay of plants on substrate with higher salts concentration.

“Damage ranking of shoots” shows that in both trial years, salts application significantly influenced the degree of strawberry damage (graph 1). Marginal leaf chlorosis was caused by concentration of 0.5 g NaCl L⁻¹, and necrosis (different degree)

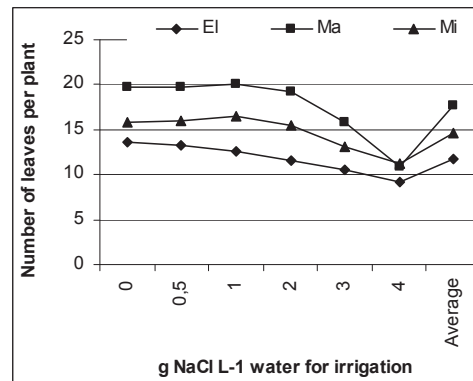
was caused by higher concentration. Every increase of salts concentration in irrigation water above 0.5 g NaCl L⁻¹, was influencing significantly to the degree of strawberry damage (with the exception of damage degree values of ‘Miranda’ in variants with 0.5 and 1.0 g NaCl L⁻¹, where there were no significant differences). The impact of salts on growth and development of shoots of three cultivars, as well as damage degree during salts application is shown in graph 1. In both trial years the highest degree of damage occurred in ‘Elsanta’, followed by ‘Marmolada’, and then ‘Miranda’. Differences established are statistically justified (regression analysis).

Statistical data analysis (graphs 2 and 3) demonstrates the impact of salts on number of strawberry leaves and stolons (runners). For both researched characteristics, significant cultivar characteristic was established, where ‘Marmolada’ had significantly more leaves, while ‘Miranda’ had most of the stolons. The application and increase of salts concentration in a nutritive base had stronger impact on regeneration and number of stolons than on number of leaves. For both characteristics, ‘Elsanta’ demonstrated higher sensitivity than ‘Marmolada’, and ‘Mirnada’ especially. Application of two the highest dosages (3.0 and 4.0 g NaCl L⁻¹) very significantly influenced both observed parameters in all three tested cultivars.

The salts impact on fresh biomass yield of shoots is shown in graph 4 and table 1. Adding of salts in concentration of 0.5 g of NaCl L⁻¹ significantly impacted decrease of fresh biomass yield of ‘Elsanta’ in both trial years. By studying both trial years, re-



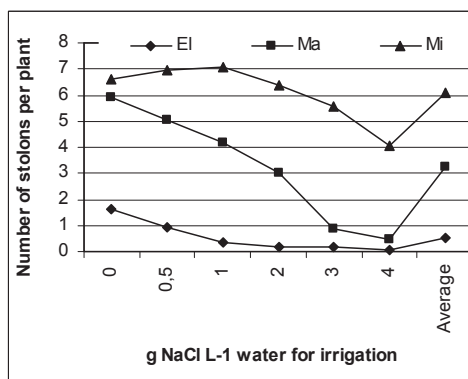
Graph 1. Impact of NaCl in irrigation water on degree of damage: chlorosis, necrosis, decay (two-year average)



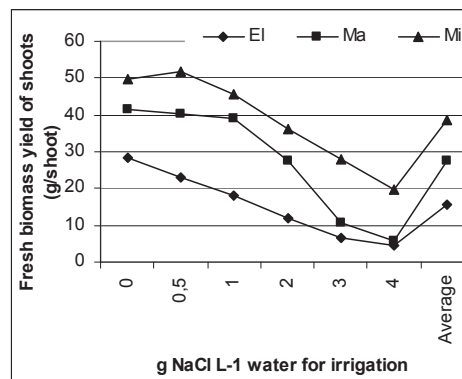
Graph 2. Impact of NaCl in irrigation water on number of leaves per plant (two-year average)

Table 1. Relative yield of: fresh biomass (FB) and dry matter (DM) of shoots, fresh biomass (FB) and dry matter (DM) of roots, total (roots+shoots) fresh biomass (FB) and dry matter (DM), mass ratio of fresh biomass of shoots and roots, and mass ratio of dry matter of shoots and roots (two-year average)

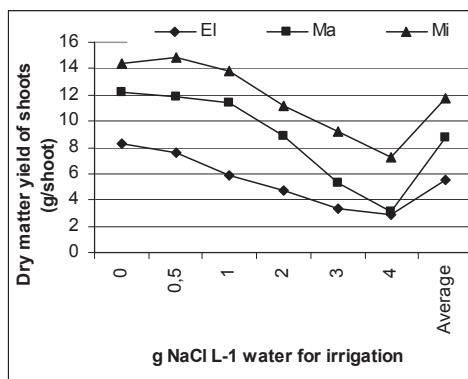
gNaCl L ⁻¹	FB (shoots)	DM (shoots)	FB (roots)	DM (roots)	FB (total (r+s))	DM (total (r+s))	FB (shoot/root)	DM (shoot/root)
‘Elsanta’								
0.0	100.0	100.0	100.0	100.0	100.0	100.0	3.7	3.3
0.5	80.5	91.3	87.2	88.2	84.2	90.5	3.4	3.4
1.0	63.5	70.6	77.5	68.2	67.3	70.0	3.0	3.4
2.0	41.9	56.9	65.9	56.2	47.7	56.7	2.3	3.3
3.0	22.8	40.9	54.9	37.5	29.0	40.1	1.5	3.6
4.0	16.3	34.0	49.8	35.4	22.9	34.4	1.2	3.2
Average	54.2	65.6	72.3	64.4	58.5	65.3	2.8	3.3
‘Marmolada’								
0.0	100.0	100.0	100.0	100.0	100.0	100.0	4.1	3.6
0.5	96.8	96.3	92.2	90.8	94.8	95.1	4.4	3.8
1.0	93.7	92.9	98.2	90.0	93.6	92.3	4.0	3.7
2.0	66.9	72.2	82.5	67.8	69.8	71.2	3.4	3.8
3.0	25.1	43.3	56.8	41.2	30.1	42.8	1.8	3.8
4.0	14.1	25.1	56.4	41.3	22.7	28.6	1.0	2.3
Average	66.1	71.6	81.0	72.0	68.5	71.7	3.4	3.6
‘Miranda’								
0.0	100.0	100.0	100.0	100.0	100.0	100.0	2.3	2.8
0.5	103.7	102.9	99.7	101.8	100.9	102.7	2.4	2.8
1.0	91.1	94.7	79.6	84.4	86.2	91.9	2.7	3.1
2.0	72.6	78.2	62.1	58.4	69.6	72.9	2.7	3.7
3.0	56.7	64.9	48.5	45.1	56.8	59.6	2.7	4.0
4.0	40.1	50.4	39.0	34.5	41.9	46.1	2.5	4.2
Average	77.4	81.9	71.5	70.7	75.9	78.9	2.5	3.2



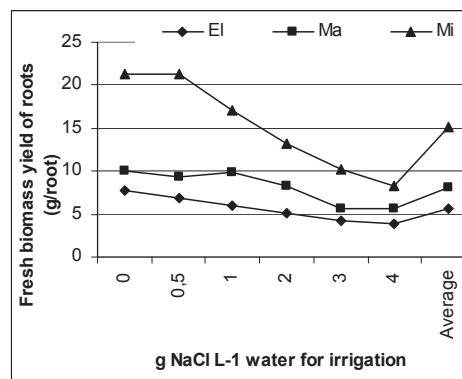
Graph 3. Impact of NaCl in irrigation water on number of stolons per plant (two-year average)



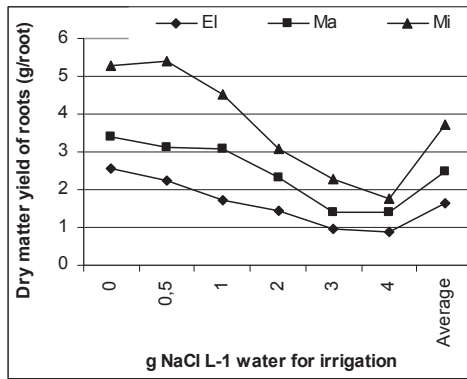
Graph 4. Impact of NaCl in irrigation water on yield of fresh biomass of shoots (two-year average)



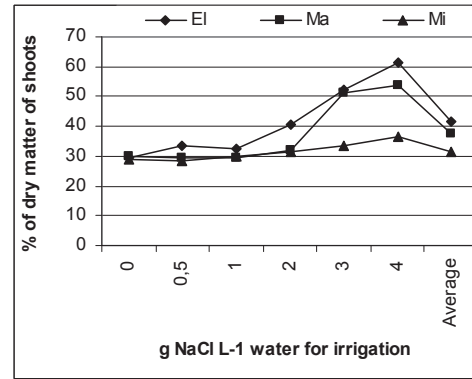
Graph 5. Impact of NaCl in irrigation water to yield of dry matter of shoots (two-year average)



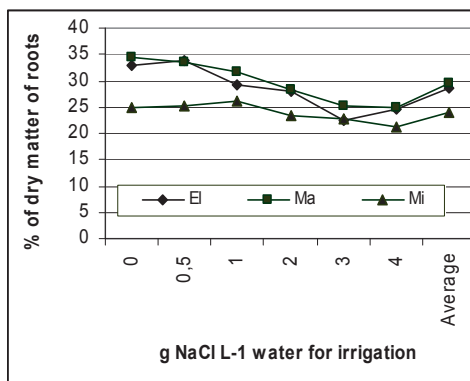
Graph 6. Impact of NaCl in irrigation water on yield of fresh biomass of roots (two-year average)



Graph 7. Impact of NaCl in irrigation water on yield of dry matter of roots (two-year average)



Graph 8. Impact of NaCl in irrigation water on % of dry matter of shoots (two-year average)



Graph 9. Impact of NaCl in irrigation water on % of dry matter of roots (two-year average)

duction of fresh biomass yield was determined in 'Marmolada' and 'Miranda' at 1.0 g NaCl L⁻¹ in irrigation water in comparison to the control. For every further increase in salts concentration in both trial years the fresh biomass yield of shoots in all three strawberry cultivars was reduced significantly.

The highest fresh biomass yield of shoots, in both trial years had 'Miranda', followed by 'Marmolada', and 'Elsanta' respectively. The differences are statistically justified.

As expected, the dry matter yield of shoots was under salts influence, of which results are shown on Graph 5 and Table 1. Upon analyzing two-year results, the dry matter yield of 'Elsanta' shoots was significantly reduced at 1.0 g NaCl L⁻¹ application and of 'Marmolada' and 'Miranda' at 2.0 g NaCl L⁻¹. 'Miranda' had increase of dry matter yield at 0.5 g NaCl L⁻¹, however, this difference was not statistically significant in comparison to the control. In both trial years 'Miranda' had higher dry matter yield of shoots, followed by 'Marmolada', and 'Elsanta'; differences are statistically justified.

The fresh biomass yield of roots was decreasing with the increase of salts (Graph 6 and Table 1). Slightly higher fresh biomass yield of roots was established in 'Marmolada' and 'Miranda' (as well as of shoots) in variants with lower salts concentration (0.5 and 1.0 g NaCl L⁻¹). The highest fresh biomass yield of roots in both trial years had 'Miranda', followed by 'Marmolada', and 'Elsanta' respectively; differences are statistically justified.

The impact of salts on dry matter yield of roots is shown in graph 7 and table 1. In both trial years, the highest dry matter yield of roots had 'Miranda', followed by 'Marmolada', and 'Elsanta' respectively; differences are statistically justified. Two-year average of dry matter yield of roots demonstrates that application of higher salts concentration in irrigation water (2.0, 3.0 and 4.0 g NaCl L⁻¹) strongly influenced reduction of dry matter yield of roots in all three strawberry cultivars.

Salts impact on percentage of dry matter of shoots is shown in graph 8. Increase in salts concentration significantly impacted increase of percentage of dry matter of shoots. The highest percentage of dry matter of shoots in the first trial year had 'Elsanta', followed by 'Marmolada' and 'Miranda', respectively; differences are statistically justified. In the second trial year, the highest percentage of dry matter of shoots was established in 'Elsanta', followed by 'Marmolada' (difference was not statistically justified). Yet again, significantly lower percentage of dry matter of shoots was established in 'Miranda'.

The salts impact on percentage of dry matter of roots demonstrates the reduction in percentage of dry matter as a consequence of salts application (graph 9). The highest percentage of dry matter of roots, in the first trial year had 'Elsanta', followed by 'Marmolada' and 'Miranda' respectively. In the second trial year, the highest percentage of dry matter of roots was established in 'Marmolada', followed by 'Elsanta' and again by 'Miranda'; differences are statistically justified.

Discussion

The impact of NaCl on strawberry growth, in both vegetation trial years, was shown in first sign of necrosis of older leaves; in both years differences between cultivars were established. Necrosis was developing further with time; however, it was the fastest and in higher degree in 'Elsanta'. Established results are consistent with other authors that were studying salts impact on strawberry (Ehlig and Bernstein, 1958; Ehlig, 1961; Bruyn and Voogt, 1989; Martinez Baroso and Alvarez Gonzalez, 1990; Keutgen and Keutgen, 2003; Ondrašek et al., 2006; Saied et al., 2003; Iapichino and D'Anna, 2003; Wahome, 2003; Kurunc and Cekic, 2005) Authors established as well significant differences between genotypes in degree of leaves damage, or decaying at higher concentrations. Most of authors reference that necrosis of leaves was influenced by chlorine and (in lesser degree)

sodium in leaves. Significance of establishing the intensity and degree of necrosis caused by salts is quoted by other authors as well (Shannon, 1979; Ream and Furr, 1976; Huang et al., 1995).

Regression analysis of salts impact on damage degree of strawberry in vegetative first year cycle indicated significant differences between characteristics: 'Elsanta' ($y = 1.67x + 1.98$), 'Marmolada' ($y = 1.67x + 1.42$), and 'Miranda' ($y = 1.14 + 1.22$), in all cases at high and significant determination coefficient (0.95). While there were no significant differences in second year either in values of damage degree in comparison to previous year, it can be said with certainty that cultivars are significantly different when it comes to salinity tolerance. Zaither and Mahfouz (1993) emphasize that specific reaction of genotype, i.e. damage degree of leaves can be used as the best indicator in initial selection of tolerant (or more tolerant) genotypes.

Significant differences in salinity tolerances were determined on vegetative parts of strawberry in vegetation trial in both years. Average decrease of number of leaves in comparison to the control was between 10 and 15% (depending on genotype), with the smallest influence on 'Miranda'. The impact of salts on growth and development, as well as on number of leaves per plant was established by other authors also (Lopez and Satti, 1996, in tomatoes; Huang et al., 1995, in pumpkin), as a result of imbalance in nutrient and water receipt (low water potential), excess sodium and chlorine build up (Greenway and Muns, 1980; Pasternak, 1987), and their unfavourable effect on photosynthesis (Cheeseman, 1988). Yield of fresh biomass and dry matter of shoots and roots was reduced significantly in both trial years. In most plants shoots adversely react on increased salts level in substrate (Greinerberg et al., 1996; Cordovilla et al., 1995; Al-Karaki, 1997; Ehlig and Bernstein, 1958), where weight ratio of shoots/roots was decreasing. However, Bar et al. (1997) quote that high chlorine concentration in irrigation water more influenced reduction of dry matter yield of roots than of shoots. In our case the stronger impact on reduction of dry matter of roots in relation to shoots was determined in 'Miranda', while other two cultivars (more sensitive ones) did not show significant difference between reduction of dry matter of roots and shoots. 'Elsanta' had higher decrease in researched parameters than 'Marmolada' and 'Miranda'. The average decrease of fresh biomass yield of shoots in comparison to the control was: 43 and 48%, 32 and 35% and 21 and 24%, and of dry matter: 34 and 35%, 24 and 33% and 16 and 20% (depending on the trial year), for cultivars 'Elsanta', 'Marmolada' and 'Miranda'; this demonstrates significant specific reactions caused by genotype. Impact of salts to decline of fresh biomass yield of roots was in lesser degree than in shoots. Results are in line with the results of Ehlig and Bernstein (1958), but only for cultivars 'Elsanta' and 'Marmolada' which demonstrated their susceptibility. Alberico and Cramer (1993) considered yield of total dry matter as a realistic indicator when it comes to tolerance assessment of maize to salts. Yield of total dry matter in strawberry for two trial years demonstrates that 'Elsanta' had significant decrease at 0.5 g NaCl L⁻¹, and 'Marmolada' and 'Miranda' at 1.0, or 2.0 g NaCl L⁻¹ respectively (depending on the trial year). The average decrease of total dry matter yield in comparison to the control, in both trial years, was highest in 'Elsanta', followed by 'Marmolada' and 'Miranda', respectively. At 0.5 g NaCl L⁻¹ certain increase

in dry matter yield of cultivars 'Marmolada' and 'Miranda' was established. Mentioned increase was influenced by portions of dry matter of shoots, and Mamo et al. (1996) have established significant increase in dry matter of shoots at 2.0 dS m⁻¹ (1.23 g NaCl L⁻¹) in lentil and chick-pea. Authors quote that it is not known why the application of smaller dosages of NaCl improve the yield of dry matter of shoots, and that other authors have obtained similar results (Marschner, 1995, in maize, Greenway and Rogers, 1963, in barley). According to Marschner (1995) possible cause for growth stimulation rests in potassium and sodium switch in vacuole. One of possibilities for better shoots growth (as well as to lower the degree of leaves damage) in cultivar 'Miranda' is better control of sodium and chlorine intake and transfer and/or excessive build up of essential mineral nutritive elements and adjustments to create their more favourable relations. Mentioned thesis should be methodically verified through chemical analysis (per fractions – plant parts), calculation of content and relation of essential and non-essential cations.

Conclusions

- (i) Adding of NaCl into irrigation water has increased significantly all researched vegetative parameters; reaction threshold depended on researched vegetative parameter and cultivar.
- (ii) Strawberry cultivars were differentiating significantly in reaction to stress (occurrence of stress signs: chlorosis and necrosis of older leaves, damage intensity: number of necrotic leaves and decay of plants at high salt concentration in substrate).
- (iii) The average number of leaves per plant and especially the average number of stolons per plant were under influence of salts in substrate. Threshold reaction that was manifested in decrease of number of leaves emerged later on (at higher concentrations) in comparison to threshold reaction of reduction in number of stolons.
- (iv) The fresh biomass yield and dry matter yield of shoots and roots decreased significantly during NaCl application. The yield of total dry matter of strawberry (shoots + roots) in cultivar Elsanta decreased significantly at 0.5 g NaCl L⁻¹, and in 'Marmolada' and 'Miranda' at 1.0, or 2.0 g NaCl L⁻¹. The average decrease of total dry matter yield in comparison to the control was the highest in 'Elsanta', followed by 'Marmolada' and 'Miranda', respectively.
- (v) The percentage of dry matter of shoots was increasing significantly, and the percentage of dry matter of roots was reducing significantly with the increase in salinity of nutritive base. The most consistent cultivar for both observed characteristics (the smallest increase, i.e. the reduction of dry matter percentage) under the salts influence was Miranda.
- (vi) The sequence of analyzed vegetative parameters according to the degree of selection to salinity is following: "damage degree ranking of shoots" > number of stolons > mass ratio of fresh biomass of shoots and roots > total fresh biomass > total dry matter > yield of fresh biomass of shoots > yield of dry matter of shoots > yield of fresh biomass of roots > yield of dry matter of roots > percentage of dry matter of roots > percentage of dry matter of shoots > number of leaves.

- (vii) According to their salts sensitivity cultivars were ranked: 'Elsanta' > 'Marmolada' > 'Miranda'.

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