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RESEARCH PAPERS

# Genotypic Differences in Photosynthesis and Partitioning of Biomass and Ions in Salinized Faba Bean

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**Abstract**—*Vicia faba* (L.) is a valuable grain legume, rich in nutrients and bioactive constituents with large genotypic variability in resistance to abiotic stress. Growth and performance of two hydroponically-grown *V. faba* cultivars ('Nubaria' 1 and 'Nubaria 2') were investigated under the impact of 0, 20, 50, 100 and 175 mmol/L NaCl. Shoot growth of the two cultivars was moderately reduced by NaCl salinity; but root growth was robust, at the expense of either leaves in the salt-resistant 'Nubaria 1'or stem in 'Nubaria 2'. 'Nubaria 1'showed better vigor and greater leafiness but lesser content of photosynthetic pigments with higher carotenoids content than 'Nubaria 2'. Rates of transpiration and photosynthesis were higher in 'Nubaria 2' than 'Nubaria 1', with more adverse effect of salinity on transpiration than on photosynthesis. The reduced $K^+$ and $Ca^{2+}$ uptake and the enhanced $Na^+$ uptake under salinity were associated with restriction of ion transport to the foliage, particularly the leaves. The role of stem in providing $K^+$ and $Ca^{2+}$ to and retention of $Na^+$ away from the leaves and root under salinity stress was more evident in 'Nubaria 1' than 'Nubaria 2'.

**Keywords:** *Vicia faba*, gas exchange, genotype, minerals, salinity

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## INTRODUCTION

Crop production is hampered by soil problems, such as extreme pH, salinity and water deficit that affect the availability of mineral nutrients and plant performance. Saline soils are more frequent in semiarid and arid regions of the world due to accumulation of soluble salts in the upper soil horizons as a result of limited precipitation. Soil is considered saline when its saturation extract has an EC > 4 dS/m (~40 mmol/L NaCl) and a sodium adsorption ratio <13 [1]. Furthermore, some improper agricultural practices such as irrigation with poor quality water, excessive use of chemical fertilizers and poor drainage systems can create the problem of secondary salinization. The task of improving crop salinity tolerance can be achieved via several approaches, among which is the use of safe amendments either to the soil or to the plant and selection of salt-resistant cultivars which combine both high yield potentiality and good quality.

Salt tolerance is generally low in crop species; where salt damage might vary according to the cultivar, organ and developmental stage. For example, root is more salt-robust than shoot; and most plants are more salt-resistant during germination than at the later stages of growth [1]. Plants exposed to high salt concentrations, undergo osmotic stress at first, which results in tissue dehydration and growth inhibition of young leaves. This is followed by ionic stress with build-up of ions in the shoot up to toxic levels, particularly in mature leaves which accumulate increasing amounts of $Na^+$ and $Cl^-$ because of enhanced transpiration [2, 3]. Therefore, the lowering in transpiration rate of salinized plants might target not only maintenance of water balance within the plant but also restriction of the ascent of salt ions to the foliage; since long-distance transport of $Na^+$ and $Cl^-$ within the shoot is aided by the transpiration stream. The competition between $K^+$ and $Ca^{2+}$ in on hand and $Na^+$ in the other hand can adversely affect membrane functioning, enzyme activity, protein synthesis, photosynthesis and stomatal movement. In addition to the $Na^+$/cation competition, the $Cl^-$/anion competition is equally important [1]. Therefore, salinity tolerance is correlated with the ability of plants to maintain normal $K^+$ concentrations within the cytoplasm under salinity stress; and the lowering in cytosolic K/Na ratio, rather than the $Na^+$ concentration per se, is the most important criterion of salt injury to plant tissues [4].

*Abbreviations:* RWR, StWR and LWR—root, stem and leaf weight ratio, respectively; RKR, StKR and LKR proportion of the plant $K^+$ content allocated to the root, stem and leaves, respectively; RNaR, StNaR and LNaR proportion of the plant $Na^+$ content allocated to the root, stem and leaves, respectively; RCaR, StCaR and LCaR proportion of the plant $Ca^{2+}$ content allocated to the root, stem and leaves, respectively.

The impact of salinity stress on photosynthesis is most severe in the mature leaves because of their high $Na^+$ load [5] in addition to the negative feedback via the decreased demand of sink tissues for assimilates. Salinity might also decrease the concentration of photosynthetic pigments, possibly due to the negative effect of the low K/Na ratios on protein synthesis, particularly in chloroplasts [2]. In contrast to the diminished photosynthesis, salinity, within limits, may enhance respiration, for the plant to meet the energy costs of the compartmentalization of ions or of the repair of cellular damage. However, beyond a certain threshold, respiration may also decrease because of impairment of metabolism as a result of ion toxicity [6].

The plant strategy to tolerate salinity involves a trade-off between alleviation of osmotic stress and ionic stress. Salt inclusion, associated with utilization of $Na^+$ for turgor maintenance and also as a substitute of $K^+$ in protein synthesis and photosynthesis is the strategy adopted by halophytes and natrophilic species [6]. In this group of plants, tolerance necessitates compartmentalization of $Na^+$ and $Cl^-$ ions into specific tissues, cells and subcellular organelles to avoid toxic concentrations within the cytoplasm, especially of the leaf mesophyll. Although salt inclusion affords the solution for osmotic stress, it will at the same time impose ionic stress and vice versa [7]. Glycophytes, on the other hand, adopt an exclusion mechanism, i.e. restriction of $Na^+$ movement to the shoot, with increased reliance on $K^+$ and synthesis of compatible solutes for osmotic adjustment, particularly in the leaves [8]. In addition to restriction of $Na^+$ uptake, retranslocation of $Na^+$ from the shoot to the root and accumulation of ions in mature leaves, which represent strong ion sinks, can protect actively growing and metabolizing cells in the salt-sensitive species [3]. A clear distinction is often made between salt excluders and salt includes; however, in reality there is a continuum spectrum of exclusion and inclusion mechanisms which are employed by either different plant species or even different cultivars of the same species.

Among the grain legumes, faba bean (*Vicia faba* L.) is particularly important because of its high nutritive value and resistance to abiotic stress [9]. In addition to its nutritive value, *V. faba* has outstanding therapeutic potentialities through impacting lipid profiles in human body and providing a good supply of antioxidants and chemo-preventive factors [10]. A large genetic variability has been identified in *V. faba* in terms of tolerance to several biotic and abiotic stresses [11]. The present work investigates the difference in salt tolerance during the vegetative stage of two *V. faba* cultivars, differing in salt tolerance during germination, regarding gas exchange as well as partitioning of biomass, ions and photosynthetic pigments. The differential response of the two *V. faba* cultivars to salinity stress might vary during the germination and vegetative stages as demonstrated for two barnyard grass (*Echinochloa crusgalli* L.) mutants [12]. Furthermore, a wide range of salinity was used to resolve the differential behavior of the two cultivars towards $Na^+$ both at sub-salinity/beneficial levels and at the stressing salinity levels.

## MATERIALS AND METHODS

**Plant material.** Seeds of *Vicia faba* L. 'Nubaria 1' and 'Nubaria 2' were obtained from the Field Crops Research Institute, Agricultural Research Centre at Giza, Egypt. These cultivars were selected because they reflected contrasting behavior towards salinity during germination; where 'Nubaria 2' was more salt-tolerant than 'Nubaria 1' [11].

**Effect of NaCl salinity on plant performance**. Uniform seeds of the two *V. faba* cultivars were surface-sterilized with 1% sodium hypochlorite for 10 min, washed with distilled water and sown on 20 cm-diameter plastic pots filled with water-washed coarse sand (>2 mm) and irrigated with 0.5 mmol/L $CaSO_4$ for 10 days; by that time, the seedlings produced the second vegetative leaf. Plants were then irrigated for 4 days with a nutrient solution of the following composition:

—macronutrients (mmol/L): N 15 (as 2 mmol/L $NH_4^+$ and 13 mmol/L $NO_3^-$), K 6, P 1.5, Ca 5, Mg 1.5 and S 3.5.

—micronutrients (μmol/L): Fe (as Fe-EDTA) 100, Mn 10, Cu 1, Zn 1, B (as boric acid) 50, Mo 0.5 and Co 0.2.

Plants were grown in a controlled growth room at irradiance of 250 μmol/($m^2$ s) supplied from white fluorescent tubes in 12/12 h photoperiod, average temperature of 25–27°C and relative humidity of 70%. After four days from nutrient application, salinity treatment started by irrigation with 0, 20, 50, 100 and 175 mmol/L NaCl superimposed on the nutrient solution for 15 days. Plants were irrigated daily with an excess of the treatment solutions to avoid buildup of salts.

**Plant harvest and analysis.** Plants were harvested 15 days after application of salinity treatment. Plant roots were carefully extracted from sand, washed thoroughly with distilled water and blotted gently between two layers of adsorbent tissue. Plants were then dissected into root, stem and leaves; fresh weights were recorded and dry weights were measured after drying in an air-forced oven at 80°C for 48 hours. The dry matter was grounded into fine powder before analyses.

**Gas exchange measurements.** Just before harvest, photosynthesis rate (A), stomatal conductance ($g_s$), transpiration rate (E), leaf temperature ($T_l$) and substomatal $CO_2$ ($C_i$) were measured in the third youngest leaf at 10:30 a.m. usingan LCA-4 portable gas exchange system (Analytical Development Company, United Kingdom). Measurements were conducted with leaf area of 6.25 $cm^2$, leaf chamber $CO_2$ concen-

tration of 390 ppm at chamber temperature of 37°C and PPFD of 1800 μmol photons/($m^2$ s).

**Determination of photosynthetic pigments.** A known weight of the third youngest leaf was extracted in 80% buffered acetone (pH 7.8) in dim light. The mixture was centrifuged at 9500 rpm for 10 min, and the supernatant was brought up to volume with 80% acetone. Absorbance of the extract was read at 663, 646 and 470 nm using a UNICO 7200 series spectrophotometer. Pigment concentrations were calculated (μg/mL) using the following equations [13]:

$$\text{Chlorophyll } a\,(\text{Chl } a) = 12.21 \times \text{E663} - 2.81 \times \text{E646},$$

$$\text{Chlorophyll } b\,(\text{Chl } b) = 20.13 \times \text{E646} - 5.03 \times \text{E663},$$

$$\text{Carotenoids (Carot)} = \frac{(1000 \times \text{E470} - 3.27 \times \text{Chl } a - 104 \times \text{Chl } b)}{229}.$$

The results were then estimated as mg pigment per gram of fresh weight of leaf.

**Determination of soluble minerals**. The powdered plant material was extracted in 1.5 mL distilled water in Eppendorf tubes at 95°C for 2 hours [14]. The debris was removed by centrifugation at 9500 rpm for 10 min. and the clear extract was used for determination of soluble $K^+$, $Na^+$ and $Ca^{2+}$ using a Jenway PFP7 flame photometer.

**Experimental design and statistical analysis**. The experiment was factorial with two factors and four replications in a completely randomized design. The main factors were: cultivar with two levels ('Nubaria 1' and 'Nubaria 2') and salinity with five levels (0, 20, 50, 100, 175 mmol/L NaCl). Data were analyzed using SPSS version 22. The effects of the main factors and their interaction were assessed by using two-way ANOVA. Mean separation was performed using the Duncan's multiple range test at $P < 0.05$.

## RESULTS

### *Plant Growth and Biomass Partitioning*

ANOVA revealed highly significant effect ($P < 0.01$) of salinity but mild effect of cultivar and the cultivar × salinity interaction on shoot growth of *V. faba*, with non-significant effect of treatments on root growth (Table S1). Leaf dry weight was significantly higher in 'Nubaria 1' than 'Nubaria 2' but dry weights of root and stem were comparable in the two cultivars. 'Nubaria 1' exhibited a beneficial 20 mmol/L NaCl, post which further increase in salinity up to 175 mmol/L NaCl reduced dry weights of leaves, stem and root by 38%, 31% and 25%, respectively. In 'Nubaria 2', increasing salinity from 0 to 175 mmol/L NaCl led to progressive reductions of 33% and 44% in dry weights of leaves and stem with non-significant reduction in root dry weight (Figs. 1a, 1b). The differential effect of treatments on biomass of leaves, stem and root led to alteration of dry matter partitioning. Generally, 'Nubaria 1' had greater LWR at the expense of StWR compared with 'Nubaria 2', with comparable RWR in the two cultivars. Increasing salinity from 0 to 175 mmol/L NaCl increased RWR by 31% in the two cultivars; but whereas this was at the expense of 12% reduction in LWR without effect on StWR of 'Nubaria 1', it occurred at the expense of 19% reduction in StWR without effect on LWR of 'Nubaria 2' (Figs. 1c, 1d).

The number of expanded leaves was appreciably greater in 'Nubaria 1' than 'Nubaria 2' with about 50% reduction due to salinity in both cultivars, either across the whole range of salinity in 'Nubaria 1' or post a 50 mmol/L NaCl threshold in 'Nubaria 2'. By contrast, the number of folded leaves was comparable in the two cultivars and exhibited 55% and 33% increases in 'Nubaria 1' and 'Nubaria 2', respectively as salt level exceeded a threshold of 20 mmol/L NaCl up to 175 mmol/L NaCl. The proportion of folded leaves was comparable in the two cultivars and increased by 100% and 66% in 'Nubaria 1' and 'Nubaria 2', respectively across the whole range of salinity (Table 1). The length and width of blade were comparable in the two cultivars, with a mild effect of salinity which was manifested as a beneficial effect of 20 mmol/L NaCl. The blade width/length ratio was comparable in the two cultivars with an average 15% reduction as salinity exceeded 20 mmol/L up to 175 mmol/L NaCl (Table 1).

### *Photosynthetic Pigments and Pigment Partitioning*

ANOVA revealed a significant ($P < 0.05$) to highly significant ($P < 0.01$) effect of treatments on pigment content of *V. faba* leaves (Table S2). The concentrations of photosynthetic pigments in the leaves were four times (Chl *a*), two times (Chl *b*) or 15% (carotenoids) higher in 'Nubaria 2' than 'Nubaria 1'. In 'Nubaria 1', increasing salinity from 0 to 175 mmol/L NaCl progressively increased Chl *a* and Chl *b* concentrations by 370% and 270% respectively; but in 'Nubaria 2' an average increase of 40% in both pigments was found at 20 mmol/L NaCl, followed by 40% and 20% reductions in Chl *a* and Chl *b*, respectively with further increase in salinity up to 175 mmol/L NaCl. Carotenoids concentration of 'Nubaria 1' leaves was increased by 86% with the increase in salinity from 0 to 100 mmol/L NaCl with steady levels at higher salinity; but in 'Nubaria 2',the effect of salinity was mild (Figs. 2a, 2b). As a consequence of the marked genotype × salinity interaction on leaf pigments, the fractionation of photosynthetic pigments varied considerably. On the average, Chl *a* and Chl *b* contributed each with 30% of the pigment composition of 'Nubaria 1', leaving 40% for carotenoids; but this pattern was radically different in 'Nubaria 2' with 50% for Chl *a*, 30% for Chl *b* and only 20% for carotenoids. In 'Nubaria 1', increasing salinity from 0 to 175 mM

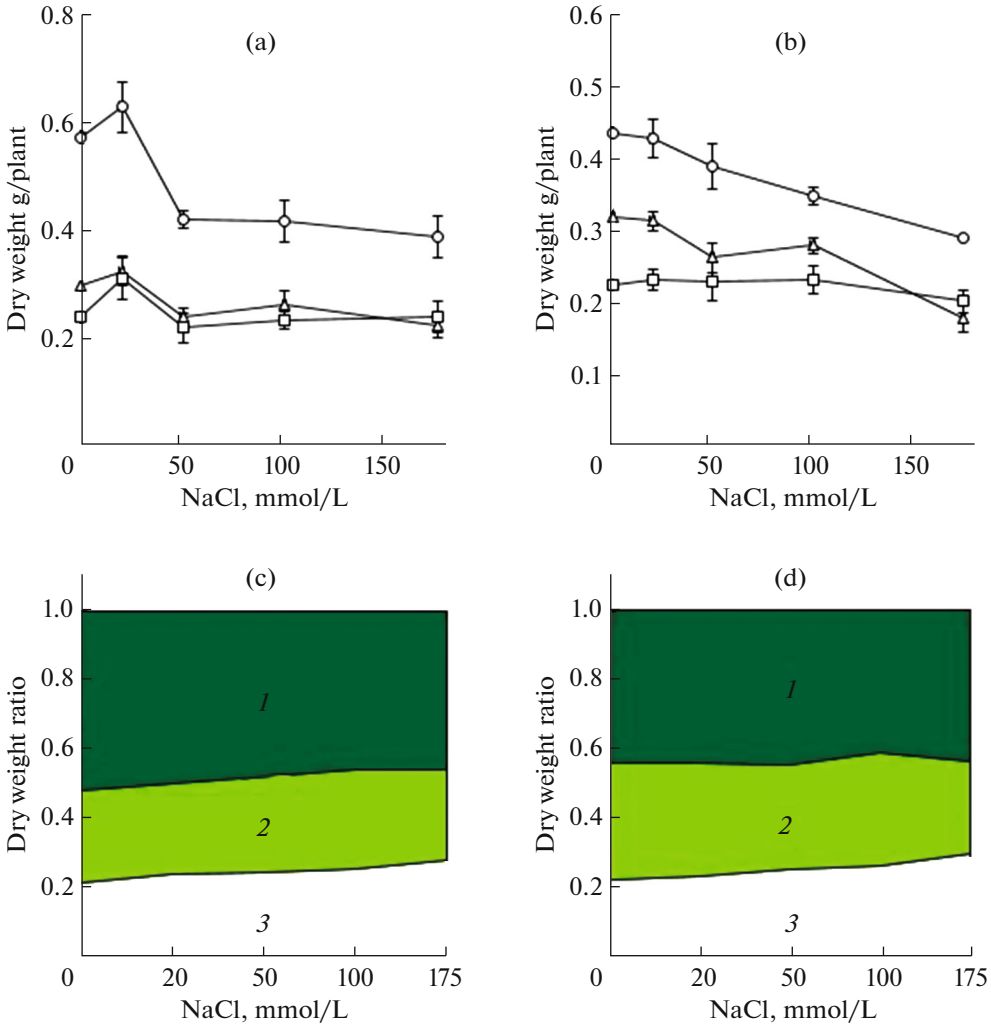


**Fig. 1.** Dry weights of leaves (circles), stem (triangles) and root (squares) of (a) 'Nubaria 1' and (b) 'Nubaria 2'of *V. faba*; and partitioning of the dry matter among leaves (*1*), stem (*2*) and root (*3*) of (c) 'Nubaria 1' and (d) 'Nubaria 2' under the impact of NaCl salinity. For dry weights, each value is the mean of 4 replicates ± SE. RWR, StWR and LWR abbreviate for root weight ratio, stem weight ratio and leaf weight ratio, that is the proportion of plant dry mass allocated to root, stem and leaves, respectively.

**Table 1.** Effect of NaCl salinity on number of leaves and dimensions of the third youngest leaf of 'Nubaria 1' and 'Nubaria 2' cultivars of *V. faba*

| Cultivar and salinity level, mM NaCl | Number of leaves | | Leaf dimensions | |
|---|---|---|---|---|
| | expanded | folded | blade length, cm | blade width, cm |
| 'Nubaria 1' | | | | |
| 0 | $7.75 \pm 0.25^{a}$ | $2.25 \pm 0.25^{a}$ | $6.45 \pm 0.18^{bc}$ | $4.25 \pm 0.02^{bcd}$ |
| 20 | $7.00 \pm 0.41^{ab}$ | $2.25 \pm 0.25^{a}$ | $7.17 \pm 0.14^{a}$ | $5.13 \pm 0.23^{a}$ |
| 50 | $5.50 \pm 0.50^{cd}$ | $3.50 \pm 0.29^{bc}$ | $6.36 \pm 0.22^{bc}$ | $4.08 \pm 0.07^{cd}$ |
| 100 | $4.25 \pm 0.41^{de}$ | $4.25 \pm 0.25^{c}$ | $6.37 \pm 0.05^{bc}$ | $4.08 \pm 0.21^{cd}$ |
| 175 | $4.00 \pm 0.25^{e}$ | $3.50 \pm 0.25^{bc}$ | $6.40 \pm 0.14^{bc}$ | $3.92 \pm 0.24^{cd}$ |
| 'Nubaria 2' | | | | |
| 0 | $6.25 \pm 0.82^{bc}$ | $2.50 \pm 0.48^{a}$ | $6.57 \pm 0.23^{b}$ | $4.03 \pm 0.27^{cd}$ |
| 20 | $6.00 \pm 0.48^{bc}$ | $2.25 \pm 0.00^{a}$ | $6.70 \pm 0.20^{ab}$ | $4.95 \pm 0.14^{a}$ |
| 50 | $6.00 \pm 0.71^{bc}$ | $3.00 \pm 0.41^{ab}$ | $6.60 \pm 0.15^{b}$ | $4.67 \pm 0.12^{ab}$ |
| 100 | $4.25 \pm 0.48^{de}$ | $3.75 \pm 0.48^{bc}$ | $6.77 \pm 0.06^{ab}$ | $4.42 \pm 0.18^{bc}$ |
| 175 | $3.25 \pm 0.25^{e}$ | $3.00 \pm 0.41^{ab}$ | $6.02 \pm 0.12^{c}$ | $3.80 \pm 0.11^{d}$ |

Each value is the mean of 4 replicates ± SE. Means with common letters are not significantly different at $P < 0.05$.

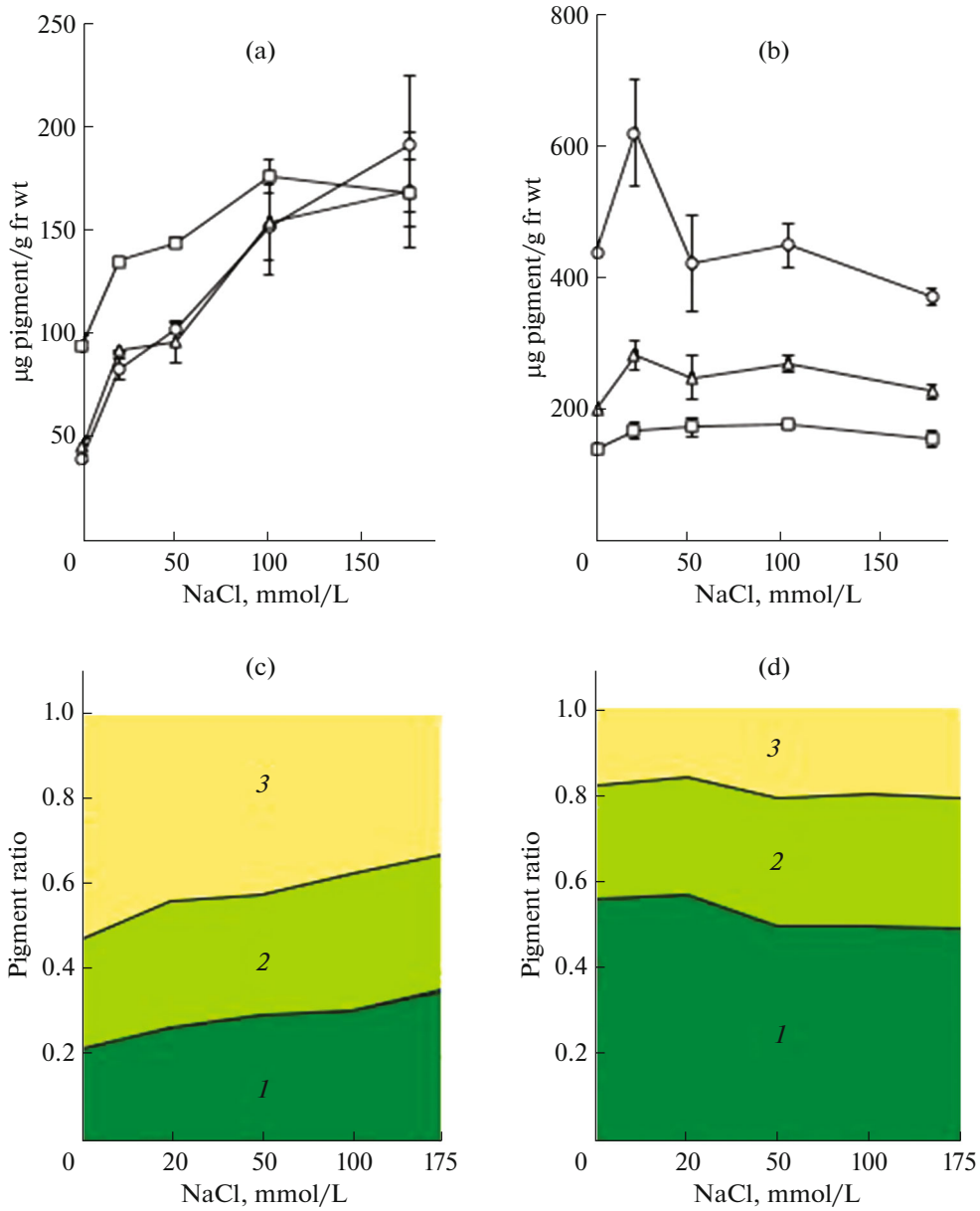


**Fig. 2.** Concentrations of Chl *a* (circles), Chl *b* (triangles) and carotenoids (squares) in the third youngest leaf of (a) 'Nubaria 1' and (b) 'Nubaria 2'of *V. faba*; and partitioning of pigment content among Chl *a* (*1*), Chl *b* (*2*) and carotenoids (*3*) in 'Nubaria 1' (c) and 'Nubaria 2' (d) under the impact of NaCl salinity. For pigment concentrations, each value is the mean of 4 replicates ± SE. Pigment partitioning was expressed as the ratio of Chl *a*, Chl *b* and carotenoids in the total pigment content of the leaf.

NaCl increased the Chl *a* ratio and Chl *b* ratio by 59% and 26%, respectively at the expense of 38% reduction in carotenoids ratio. By contrast, the effect of salinity on pigment fractionation was less marked in 'Nubaria 2' and manifested as mild increases in Chl *b* ratio and carotenoids ratio at the expense of mild reduction in Chl *a* ratio (Figs. 2c, 2d).

### *Gas Exchange*

Photosynthesis rate, transpiration rate and stomatal conductance were very highly significantly ($P < 0.001$) affected by salinity with non-significant effect of genotype; but the reverse was true for leaf surface temperature. The effect of both factors was highly significant on substomatal $CO_2$ concentration (Table S2). 'Nubaria 2' exhibited higher transpiration rate, substomatal $CO_2$ concentration and leaf surface temperature than 'Nubaria 1', but photosynthesis rate and stomatal conductance were comparable in the two cultivars. Photosynthesis rate, transpiration rate and stomatal conductance experienced 53%, 81% and 90% reductions, respectively in response to salinity in the two cultivars. Leaf surface temperature was non-

**Table 2.** Effect of 175 mM NaCl salinity on gas exchange parameters of the third youngest leaf of 'Nubaria 1' and 'Nubaria 2' cultivars of *V. faba*

| Parameter | 'Nubaria 1' | | 'Nubaria 2' | |
|---|---|---|---|---|
| | Control | 175 mM NaCl | Control | 175 mM NaCl |
| Leaf surface temperature, °C | $28.5 \pm 1.17^{a}$ | $29.7 \pm 0.42^{ab}$ | $30.8 \pm 0.04^{b}$ | $31.4 \pm 0.19^{bc}$ |
| Sub-stomatal $CO_2$, μL/L | $299 \pm 10.4^{a}$ | $127 \pm 18.7^{d}$ | $263 \pm 18.1^{abc}$ | $297 \pm 10.7^{ab}$ |
| Transpiration rate, mmol $H_2O$ /($m^2$ s) | $6.30 \pm 0.56^{b}$ | $1.20 \pm 0.11^{cd}$ | $8.27 \pm 0.84^{a}$ | $1.56 \pm 0.37^{c}$ |
| Stomatal conductance, mol $CO_2$/ ($m^2$ s) | $0.46 \pm 0.03^{a}$ | $0.04 \pm 0.00^{cd}$ | $0.41 \pm 0.06^{ab}$ | $0.05 \pm 0.01^{c}$ |
| Photosynthesis rate, μmol $CO_2$/ ($m^2$ s) | $17.32 \pm 2.95^{ab}$ | $7.82 \pm 0.74^{cd}$ | $19.04 \pm 1.63^{a}$ | $9.29 \pm 2.09^{c}$ |

Each value is the mean of 4 replicates ± SE. Means with common letters are not significantly different at $P < 0.05$.

significantly increased in the two cultivars, while sub-stomatal $CO_2$ concentration exhibited 57% reduction in 'Nubaria 1' versus mild increase in 'Nubaria 2' in response to salinity (Table 2).

### *Mineral Content and Partitioning*

The effect of salinity on mineral composition of *V. faba* was highly significant versus either a non-significant or just a significant effect of genotype (Table S3). In absence of salinity, tissue $K^+$ concentration was generally higher in 'Nubaria 1' than 'Nubaria 2', with higher levels in the stem compared with leaves and root in both cultivars. Salinity reduced $K^+$ concentration of plants to different extents according to the cultivar and plant organ. In 'Nubaria 1', increasing salinity from 0 to 175 mmol/L NaCl reduced $K^+$ concentration of stem, leaves and root by 77%, 67% and 35%, respectively; which brought stem $K^+$ from the highest level in control plants down to low level comparable to that of leaves keeping the root with the highest $K^+$ concentration in salinized plants. In 'Nubaria 2', the reduction in stem and leaf $K^+$ concentrations averaged around 54% across the whole range of salinity versus only 30% reduction in root $K^+$ beyond a threshold of 20 mmol/L NaCl, which led to higher $K^+$ concentrations in stem and root above that of leaves in salinized plants (Figs. 3a, 3b).

In absence of salinity, $Na^+$ concentration was higher in the root than the foliage in the two cultivars, but this pattern differed in salinized plants. Increasing salinity from 0 to 175 mmol/L NaCl, increased $Na^+$ concentration of leaf by 11.5 and 20 folds, of stem by 12.5 and 11 folds and of root by 82 and 150% in 'Nubaria 1' and 'Nubaria 2', respectively. This differential effect raised stem $Na^+$ concentration in the two cultivars from low levels comparable to those of leaves in absence of salinity to high levels above those of root and leaves in salinized plants; and this pattern was more evident in 'Nubaria 1' (Figs. 3c, 3d).

The K/Na ratio of plant tissues was comparable in the two cultivars, with higher values in the foliage than the root. Increasing salinity from 0 to 175 mmol/L NaCl reduced K/Na ratio of the leaves and stem by an average of 97% in the two cultivars, and most of the reduction occurred at 20 mmol/L NaCl. The root K/Na ratio was subjected to an average 66% progressive reduction in the two cultivars across the whole range of salinity (Figs. 3e, 3f).

Tissue soluble $Ca^{2+}$ concentration followed the same pattern of $K^+$ in response to treatments, with higher $Ca^{2+}$ levels in the stem than in leaves and root in absence of salinity but with a different pattern in the salinized plants. Increasing salinity from 0 to 175 mmol/L NaCl reduced soluble $Ca^{2+}$ concentration of stem by 70% and 38% in 'Nubaria 1' and 'Nubaria 2', respectively. The soluble $Ca^{2+}$ concentration of leaves and root was progressively reduced by 63% and 36%, respectively across the whole range of salinity in 'Nubaria 1' versus respective reductions of 32% and 19% post a threshold of 20 mmol/L NaCl in 'Nubaria 2'. Similar to $K^+$, the differential impact of salinity on tissue $Ca^{2+}$ concentrations of the three organs brought stem $Ca^{2+}$ concentration of 'Nubaria 1' from the highest level in control plants down to low level comparable to that of leaves and lower than that of root in salinized plants; whereas in 'Nubaria 2', salinity led to comparable $Ca^{2+}$ concentrationsin the stem and root which were higher than that of the leaves (Fig. 4).

Salinity exerted a very highly significant effect ($P < 0.001$) on partitioning of minerals among the plant, with non-significant effect of genotype and the salinity × genotype interaction (Table S4). In absence of salinity, the plant $K^+$ content was partitioned among root, stem and leaves in a ratio of 0.20 : 0.35 : 0.45, respectively in 'Nubaria 1' and 0.20 : 0.45 : 0.35, respectively in 'Nubaria 2'. Salinity increased RKR at the expense of the foliage $K^+$ ratios, with minor variation among the two cultivars (Figs. 5a, 5b). In absence of salinity, plant $Na^+$ was partitioned among root, stem and leaves in a ratio of 0.60 : 0.20 : 0.20, respec-

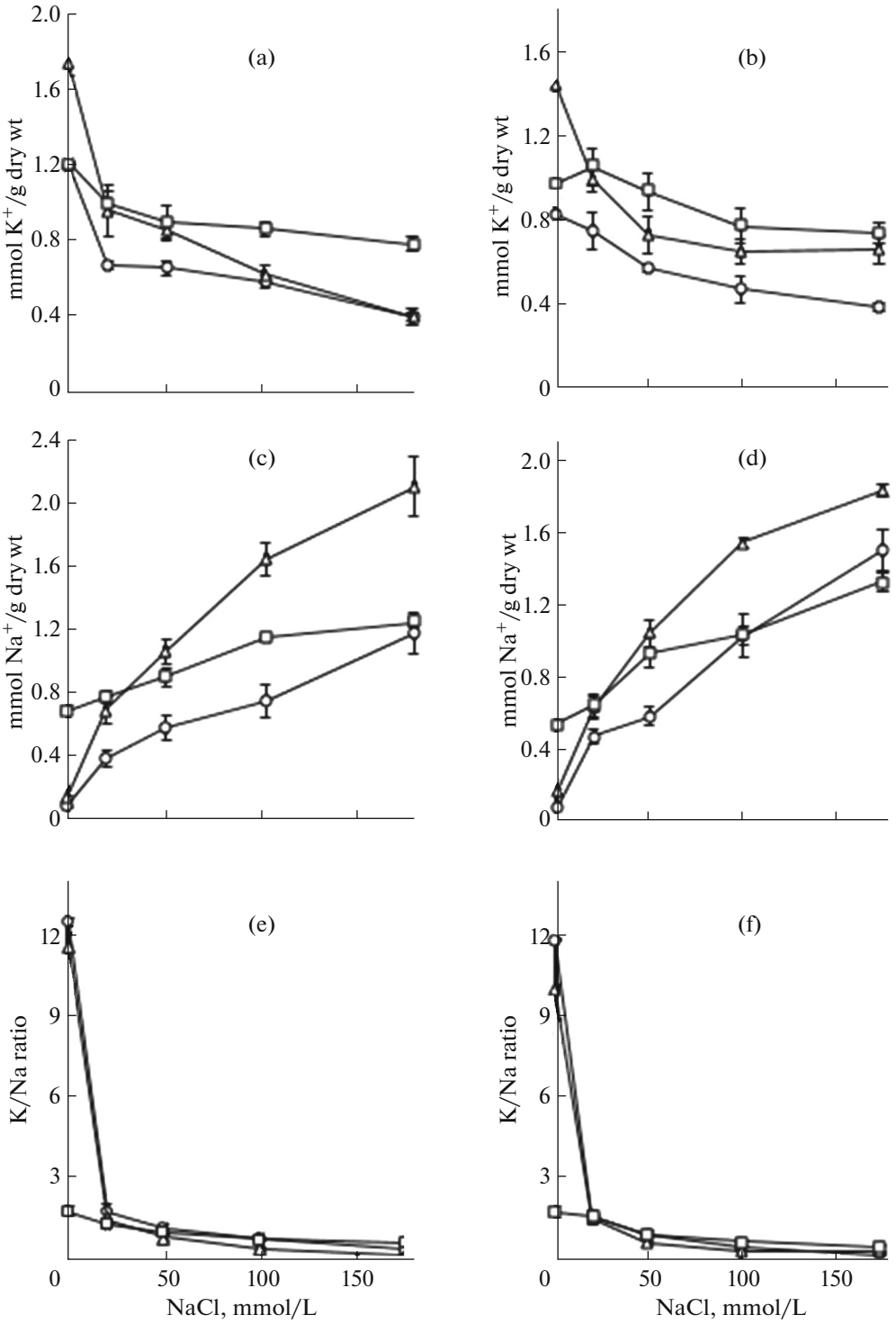


**Fig. 3.** Concentrations of $K^+$ (a, b), $Na^+$ (c, d) and the K/Na molar ratio (e, f) in the leaves (circles), stem (triangles) and root (squares) of 'Nubaria 1' (a, c, e) and 'Nubaria 2' (b, d, f) of *V. faba*, respectively in response to NaCl salinity. Each value is the mean of 4 replicates ± SE.

tively in the two cultivars. In contrast to $K^+$, salinity increased foliage $Na^+$ ratios at the expense of RNaR; and the increase was marked in StNaR of 'Nubaria 1' and LNaR of 'Nubaria 2' (Figs. 5c, 5d). Similar to $K^+$, in absence of salinity, plant $Ca^{2+}$ was partitioned among root, stem and leaves in a ratio of 0.21 : 0.33 : 0.46, respectively in 'Nubaria 1' and 0.21 : 0.42 : 0.37, respectively in 'Nubaria 2'. Salinity increased RCaR at the expense of foliage $Ca^{2+}$ ratios in 'Nubaria 1' and particularly StCaR in 'Nubaria 2' (Figs. 5e, 5f).

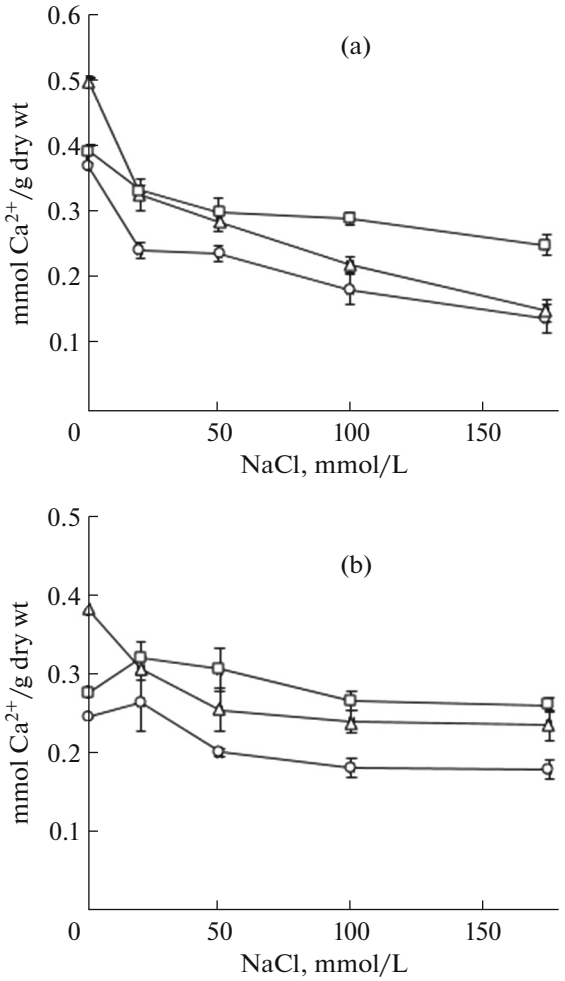


**Fig. 4.** Concentration of soluble $Ca^{2+}$ in of leaves (circles), stem (triangles) and root (squares) of (a) 'Nubaria 1' and (b) 'Nubaria 2' of *V. faba* in response to NaCl salinity. Each value is the mean of 4 replicates ± SE.

## DISCUSSION

The moderate reduction of shoot growth of the two *V. faba* cultivars by 175 mmol/L NaCl, along with the robust root growth suggest that *V. faba* is moderately salt-tolerant during the vegetative stage. Within the foliage, the effect of salinity was more severe on leaves than stem in 'Nubaria 1' but with comparable effect on the two organs of 'Nubaria 2'. However, the beneficial effect of 20 mmol/L NaCl on growth of 'Nubaria 1' suggests that this cultivar exhibits some natrophilic traits versus the natrophobic behavior of 'Nubaria 2'. The beneficial role of $Na^+$ has been documented in natrophilic and $C_4$ species and was attributed to its participation in osmotic adjustment [15, 16]. Although the genotypic difference in salt tolerance among the two *V. faba* cultivars is vague within the used range of salinity, extrapolation of the existing salinity-growth relationship beyond the experimental salinity level (175 mmol/L NaCl) predicts that the natrophilic cultivar ('Nubaria 1') is more salt-tolerant than the natrophobic one ('Nubaria 2'). Considerable differences in salt tolerance among cultivars of the same species can exist, which allows selection of the more salt-tolerant one for cultivation in salt-affected lands provided assuring the quality criteria. Although the present work revealed greater salt tolerance for 'Nubaria 1' than 'Nubaria 2' during the vegetative stage, the reverse behavior was demonstrated by the same cultivars during germination [11]. Such contradiction between the plant response to salinity during germination and vegetative stages has been demonstrated for two barnyard grass (*Echinochloa crusgalli* L.) mutants [12].

The natrophilic cultivar ('Nubaria 1') was characterized with greater leafiness (more investment of plant biomass in production of leaves) at the expense of stem, with almost equal root proportion in the two cultivars. Furthermore, the two cultivars shared a common trait of increasing allocation of plant biomass to the root under salt stress. But, whereas this occurred at the expense of leaves in 'Nubaria 1', it occurred at the expense of stem in 'Nubaria 2'. The greater leafiness of 'Nubaria 1' was also manifested as greater proportion of expanded leaves relative to 'Nubaria 2'. However, the lowering in the proportion of expanded leaves under salinity, which was more evident in 'Nubaria 1' than 'Nubaria 2', represents a way to reduce the transpiring surface. Increasing RWR is an adaptive response to abiotic stress arising from edaphic factors such as low P supply [1], water deficit [17] and salinity [18]; and it arises from rapid inhibition of shoot growth along with delay of leaf appearance and expansion versus maintenance of root growth; processes that are mediated by plant hormones, definitely ABA.

*V. faba* exhibited substantial genotype × salinity interaction on the leaf photosynthetic pigment composition. The natrophilic 'Nubaria 1' was characterized with less foliage greenness (lower chlorophyll content) compared with 'Nubaria 2'. The genotypic effect was most evident on Chl *a*, for which the effect of genotype was greater even than that of salinity. In addition, whereas salinity substantially increased pigment content of 'Nubaria 1', it led either to a reduction post a peak at mild salinity (Chl *a*) or a mild increase (Chl *b* and carotenoids) in 'Nubaria 2'. This genotype × salinity interaction on pigment composition led to different patterns of pigment partitioning in the two cultivars, summarized as increased proportions of the green pigments (particularly Chl *a*) at the expense of carotenoids in 'Nubaria 1', versus an overall mild effect on 'Nubaria 2'. In agreement with our results, salinity, within limits, has been reported to increase Chl *a* concentration, with no effect on Chl *b* and carotenoids in *V. faba* [15], and marked genotype × salinity interaction on pigments concentrations and ratios has been reported for *Cucumis melo* [19]. By contrast, the salinity-induced reduction of chlorophyll in wheat [20] was attributed either to salt-

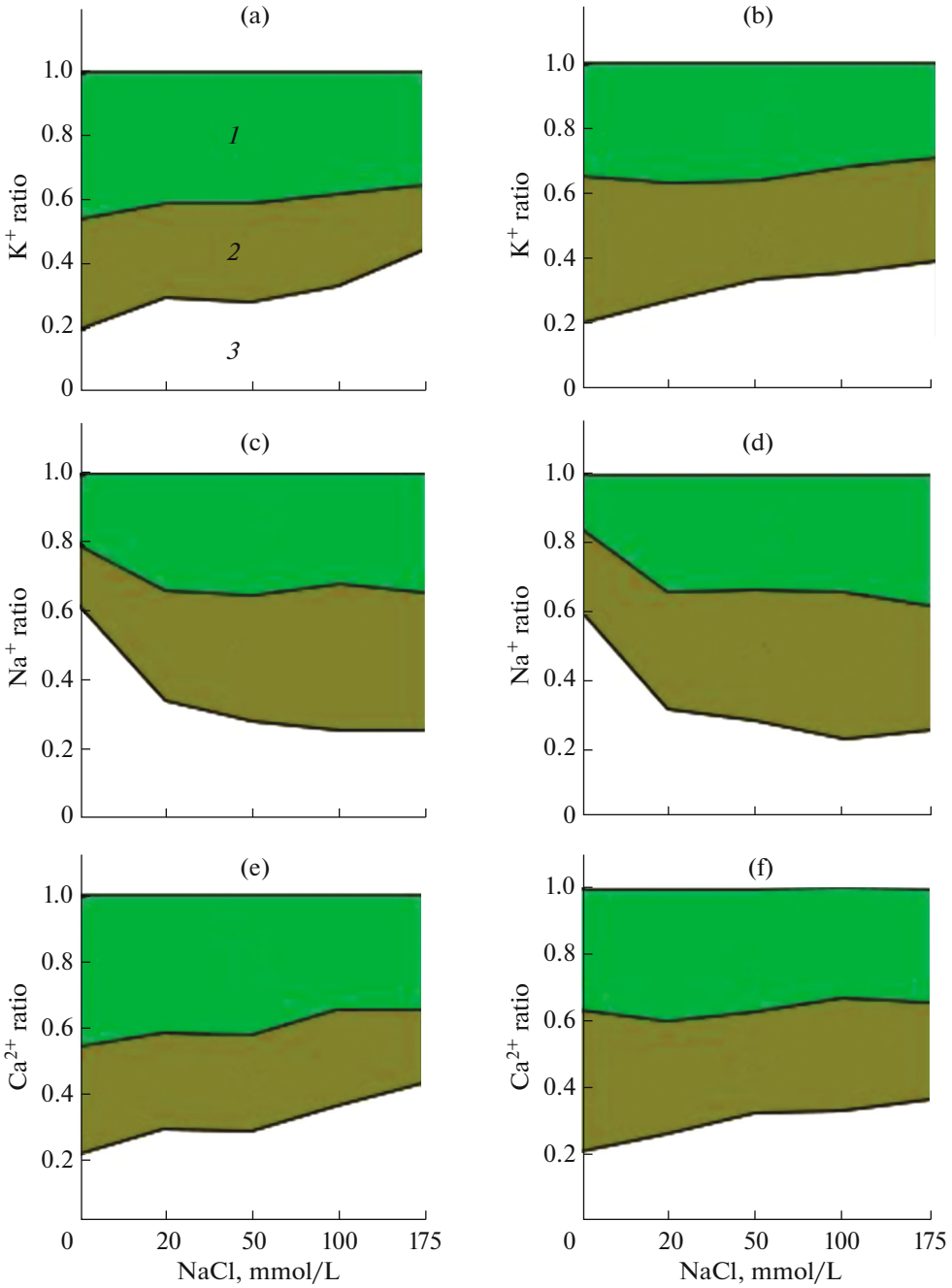


**Fig. 5.** Partitioning of plant content of $K^+$ (a, b), $Na^+$ (c, d) and soluble $Ca^{2+}$ (e, f) among of leaves (*1*), stem (*2*) and root (*3*) of 'Nubaria 1'and 'Nubaria 2' of *V. faba* in response to NaCl salinity.

induced retardation of synthesis or accelerated degradation of the pigment through enhanced chlorophyllase activity [21].

The genotype × salinity interaction was evident also on gas exchange parameters, with higher rates of transpiration (E) and, to a lesser extent, photosynthesis (A) in 'Nubaria 2' than 'Nubaria1'. But, stomatal conductance ($g_s$) was comparable in the two cultivars, with sharp reduction due to salinity. The more adverse effect of salinity on E than on A seems reasonable since E is a mere diffusion process controlled by the water vapor pressure gradient and stomatal aperture whereas A is a multiphasic process with participation of several determinants other than gas exchange through stomata, e.g. the cell metabolic machinery. Thus, in addition to the reduction in leaf biomass and extension under salinity stress in a way to reduce water loss; salinity can also induce stomatal closure which

might lead, as a side effect, to reduction of A. This mechanism was more evident in 'Nubaria 1', with a bit more depression in $g_s$, than in 'Nubaria 2'. The reduction in A of the salt-marsh plants *Atriplex portulacoides* [22] and *Chenopodium quinoa* [23] under salinity stress could be accounted for by lower $g_s$. Although transpiration is an unavoidable drawback of photosynthesis, it has important effects on leaf cooling [24]. In spite of the more salinity-induced reduction in $g_s$ in 'Nubaria 1' relative to 'Nubaria 2', the reduction in E as well as the increase in $T_l$ was comparable in the two cultivars which suggest more contribution of cuticular transpiration in 'Nubaria 2' than 'Nubaria 1'.

Despite the significantly higher $C_i$ in 'Nubaria 1' above 'Nubaria 2' in absence of salinity, the differential effect of salinity on $C_i$ of the two cultivars; that is the sharp reduction in 'Nubaria 1' versus a mild increase in 'Nubaria 2', rendered $C_i$ of salinized plants lower in 'Nubaria 1' than 'Nubaria 2'. These findings suggest a salinity-induced enhancement of respiration only in the natrophobic 'Nubaria 2'. The salinity-induced reductions in A and $g_s$ of sesame were genotype-dependent and associated with increased $C_i$ [24]. Salinity, within limits, can enhance respiration to meet the energy costs of ion compartmentalization and repair of cellular damage [7]; but beyond a certain threshold, respiration may decrease because of impairment of metabolism as a result of ion toxicity [25]. Sodium is a beneficial element for $C_3$ plants but is a nutrient for obligate halophytes, CAM and some$C_4$ plants; the latter group is characterized with low photorespiration rates compared with the $C_3$ plants [16]. Therefore, it seems that the natrophilic traits of 'Nubaria 1' include also low photorespiration rate.

The higher $K^+$ concentration in the stem of *V. faba* above that of the leaves and root in absence of salinity, along with the differential effect of salinity on biomass and $K^+$ concentration of the different organs, suggests that the reduced $K^+$ uptake under salinity stress was associated with accumulation of $K^+$ in the root at the expense of either stem ('Nubaria 1') or stem and leaves ('Nubaria 2'). This behavior was particularly evident in the natrophilic 'Nubaria 1'. The same pattern can be applied, but to a lesser extent, to $Ca^{2+}$. Another aspect of the specific ion effect of salinity, besides $Na^+/K^+$ competition, is the indirect competition between $Na^+$ and $Ca^{2+}$ [6]. The role of stem as a storage pool for $Ca^{2+}$ to minimize salinity-induced $Ca^{2+}$ deficiency in root and leaves is less evident in 'Nubaria 1' than 'Nubaria 2'. Thus, it can be concluded that while salinity restricts upward translocation of $Ca^{2+}$ in the xylem; it favors further mobilization of $Ca^{2+}$ from stem to leaves. The role of $Ca^{2+}$ in maintenance of integrity of cellular membranes and cell wall, and hence in the normal functioning of the cell is well-established. Therefore, avoidance of salt-induced $Ca^{2+}$ deficiency of leaves and root is a necessary prerequisite for efficient salt resistance.

On the other hand, the higher $Na^+$ concentration in the root of *V. faba* than that in the foliage in absence of salinity, along with the differential increase in $Na^+$ concentration and retardation of growth of the three organs, suggests that the enhanced $Na^+$ uptake under salinity stress was associated with restriction of $Na^+$ transport from the root to the foliage and retention of $Na^+$ in the stem at the expense of root and leaf. This mineral distribution within the plant points to appreciable role of stem in providing $K^+$ to and retention of $Na^+$ away from leaves and root under salinity stress, which was more evident in 'Nubaria 1' than 'Nubaria 2' and also indicates limited capacity of the root to sequester $Na^+$. Plant tolerance to salinity is dependent on $Na^+$ exclusion from leaves [2]. Re-translocation of $Na^+$ from the shoot to the root can contribute to low $Na^+$ concentrations in the shoots of salt-sensitive species such as *Phaseolus vulgaris* and salt-tolerant species such as *Phragmites communis* [1].Maintenance of photosynthetic capacity and salt tolerance in wheat was associated with the maintenance of high $K^+$, low $Na^+$ and consequently high K/Na ratio in the foliage, particularly the cytoplasm of mesophyll cells [26].

The overall higher $Na^+$ levels in 'Nubaria 1' above 'Nubaria 2' lends support to the natrophilic behavior of 'Nubaria 1'. But, retention of most of the plant $Na^+$ in the root rather than in the leaves, in absence of salinity, suggests partial expression of the natrophilic trait; that is the beneficial effect of 20 mmol/L NaCl on plant growth along with the generally higher $Na^+$ levels in 'Nubaria 1' is not associated with preferential ascent of $Na^+$ to the leaves as would be expected from a natrophilic plant at low salinity [27]. In addition, the retention of $K^+$ and $Ca^{2+}$ in the root, along with the preferential transport of $Na^+$ to the shoot under salinity stress suggests priority of root over leaves in maintenance of ion hemostasis under salinity stress.

The salinity-induced decrease in plant $K^+$ concentration, along with the rise in that of $Na^+$ led to sharp decline in the K/Na ratio, which was most evident in the leaves but least in the root of the two *V. faba* cultivars. The reduction in K/Na ratio of plant tissue under salinity stress is considered one of the most important aspects of the salinity-induced ion imbalance. The K/Na ratio served as a reliable indicator of salt stress tolerance in rice [28], where low $Na^+$ and high $K^+$ are essential for maintenance of enzymatic reactions in the cytoplasm [26]. The specific ion effect of salt stress on tomato was attributed to the accumulation of the toxic $Na^+$ and $Cl^-$, along with depletion of $K^+$ and $Ca^{2+}$ [1]. The marked salt tolerance of certain cultivars of wheat [29] and barley [30] is related to a more effective restriction of transport of $Na^+$ and/or $Cl^-$ to the

shoot. In wheat, two gene loci confer salinity tolerance (*Nax1* and *Nax2*); which are most likely expressed in the xylem parenchyma and act in retrieval of $Na^+$ from the xylem sap of the root, thus reducing the amount of $Na^+$ entering the shoot and leaf blades [29, 31].

In conclusion, *Vicia faba* is moderately salt-tolerant during the vegetative stage, with salinity-induced increase in the R/Sh ratio at the expense of either leaves in the natrophilic salt-tolerant cultivar or stem in the salt-sensitive cultivar. But, retention of $Na^+$ in the root rather than in the leaves at low salinity suggests partial expression of the natrophilic trait. Salt tolerance was associated with salinity-induced increase in chlorophyll concentration at the expense of carotenoids. In a way to reduce water loss under salt stress, the reduction in leaf growth was associated with stomatal closure and reduction in photosynthesis. The contribution of cuticular transpiration to leaf cooling and also the salinity-induced enhancement of transpiration are marked in the salt sensitive cultivar. The role of stem rather than the root in providing $K^+$ to and retention of $Na^+$ away from leaves under salinity stress contributes to salt resistance in *V. faba*.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests*. The authors declare that they have no conflicts of interest.

*Statement on the welfare of humans or animals*. This article does not contain any studies involving animals performed by any of the authors.

## AUTHOR CONTRIBUTIONS

Taha Mohamed El-Katony designed the experiments, wrote and edited the manuscript, and supported the experiments. Shaimaa Nassim Abd El-Fatah performed the experiments and participated in writing and preparation of the manuscript. The two authors revised the manuscript.

## SUPPLEMENTARY INFORMATION

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