Different phenotypic responses in the root of Vigna marina and Vigna luteola under salt stress

Fanmiao Wang (fummyaow754@affrc.go.jp)
National Agriculture and Food Research Organization https://orcid.org/0000-0003-2526-5403
Yurie Iki
National Agriculte and Food Research Organization
Keitaro Tanoi
The University of Tokyo: Tokyo Daigaku
Ken Naito
National Agriculture and Food Researh Organization

Short Report

Keywords: Vigna genus, wild species, salt tolerance, root architecture, plant growth, chlorophyll fluorescence

Posted Date: April 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2834092/v1

Version of Record: A version of this preprint was published at Genetic Resources and Crop Evolution on November 19th, 2023. See the published version at https://doi.org/10.1007/s10722-023-01794-3.

Abstract

Some wild species in *vigna* genus are halophyte that have high potential in salt tolerance. *V. marina* and one accession of *V. luteola* have been previously identified as salt tolerant. However, the mechanisms remain elusive and studies on the phenotypic responses to salt can be a starting point for understanding salt tolerant mechanisms. Additional to salt accumulation and leaf chlorophyll fluorescence, this study evaluated root system architecture, including total root length, root surface area and root tip number, in response to salt. Results demonstrate 1) *V. marina* maintained total root length and surface area in salt stress; 2) *V. marina* grew more but shorter fine roots in salt stress; 3) The leaves of *V. luteola*-beach tolerated high Na concentration which severely damaged those of *V. luteola*-river, and such damage started from older leaf while it happened from younger leaf in *V. luteola*-beach. This is the first report on root architectural change in response to salt in *Vigna* genus. Our results suggest importance of studying relationship between root architecture and salt tolerance.

Introduction

Soil salinity is one of the limiting factors in agriculture in the last half-century (Kopittke et al. 2019). Salinity causes both osmotic and ion toxic stress on plants, which has impacts on the physiological and biochemical functions of plant cells, inhibiting photosynthesis and growth of both aerial part and root system of plants (Santos *et al.* 2022). It has been estimated that over 50% of the arable land would be salinized by the year 2050 (Jamil et al. 2011). Increasing salt tolerance is essential to ensure food security.

Research on salt tolerance has been mainly focused on model plants (Hanin et al. 2016), which are mostly glycophytes and their potential for salt tolerance is limited. Unlike glycophytes, halophytes are plants that can survive at a salt concentration of 200 mM or more without suffering significant impact on their growth or development (Lu et al. 2021). The tolerance mechanisms of halophytic species include development of salt excretory structures that excrete excess salt from plant tissues and accumulation of osmoprotectants for counteracting osmotic pressure accompanied with salt stress (Sun et al. 2013; Wu et al. 2010; Yuan et al. 2016). Despite such promising features of halophytes, the exploration of their intrinsic mechanisms for salt tolerance have been lagged and is far from complete (Pantha and Dassanayake 2020).

As members of halophytic plants, *Vigna marina* and *V. luteola* are wild relatives of cultivated *Vigna* species and good studying materials for salt tolerance. *V. marina* is one of the “pantropical plants with sea-drifted seeds” and grows mainly in sandy beaches. The capability of oceanic dispersal of *V. marina* and the specific habitat makes it robust against adversity stress, especially salt stress. Plants of *V. marina* can survive for at least four weeks in 400 mM of NaCl (Yoshida et al. 2020). Similarly, *V. luteola* (JP233389) grows at seaside thus exhibit outstanding salt tolerance that they can survive for four weeks in 250 mM (Yoshida et al. 2020). Previous studies confirmed their salt tolerance regarding leaf wilting score and chlorophyll fluorescence (Iseki et al. 2016; Yoshida et al. 2020).

In addition to leaf traits, root system architecture including root length and number of lateral roots change notably in response to salt (Khan et al. 2016; Zou *et al.* 2021). In *Arabidopsis*, lateral root length has shown to be reduced by salt stress across studies (Julkowska et al. 2014; Zhao et al. 2011; Zolla et al. 2010). In this study, in addition to photosynthetic activity and ion distribution, we investigated on changes in root architecture under salt stress in two species of *Vigna* genus. The results show that the effect of salt on root growth differed between accessions of *Vigna* genus. Particularly, our results indicated that the super-tolerant species *V. marina* invested on maintaining root surface area and growing fine roots in salt stress. The results suggest importance of studying relationship between root architecture and salt tolerance.

Materials And Methods

Plant materials and growth condition

Three accessions of *Vigna* genus were used in this study: *Vigna marina* (JP247202), *V. luteola* (JP233389) (hereafter *V. luteola*-beach), as salt tolerant accessions and another accession *V. luteola* (JP235855) (hereafter *V. luteola*-river) originally collected from riverside of inland areas and is supposed to be salt sensitive. Seeds were obtained from the Research Center of Genetic Resources, National Agriculture and Food Research Organization (NARO), Japan. Seeds were sterilized with 70 % ethanol and 5 % sodium hypochlorite and rinsed with tap water. Sterilized seeds were scratched on seed coat and germinated on Seramis granule (Effem GmbH, Verden, Germany) for one week. Germinated seedlings were transplanted in hydroponic culture containing 1X Otsuka house No. 1 and 1X Otsuka house No. 2) (Otsuka Chemical Co., Osaka, Japan: N, P, K, Ca, and Mg = 18.6, 5.1, 8.6, 8.2 and 3.0 mEq L^{-1}, respectively). Plants were grown in a growth chamber at 28 ^{o}C with light from 6:00 am–20:00 pm (14 h) and at 24 ^{o}C in a dark from 20:00 pm to 6:00 am (10 h).

Fresh and dry weight measurement

Plants were transferred to hydroponic culture with 200 mM of NaCl at one-week after transplanting. Control plants were in salt-free condition throughout the experiments. Roots and shoots were sampled on day2 and day5 for control and salt-treated plants. Fresh weight was measured immediately after sampling and dry weight was measured after drying plants at 50 °C for one week.

Ion measurement

Methods was same as documented in Noda *et al.* (2022). Briefly, after the 3rd leaf fully expanded, plants were transferred to hydroponic culture with 100 mM of NaCl for two days. Roots, lower and upper stems (stem1 and stem2, respectively) and the 1st, 2nd and 3rd leaves (leaf1, leaf2 and leaf3, respectively) were separately sampled and dried at 50°C for three days. Samples were digested with 69 % HNO_3 for determination of sodium (Na) and potassium (K) by inductively coupled plasma-mass spectrometry (ICP-MS, NexION 350S, PerkinElmer, Waltham, MA, USA).

Root architectural traits analysis

Salt treatment of 200 mM of NaCl started at one-week after transplanting. On day5, control and salt-treated roots were cut at the shoot-root junction and stained overnight in neutral red dye solution (0.08 g L^{-1}, Sigma-Aldrich). Stained roots were rinsed with tap water and scanned using an EPSON Perfection V700/V750 scanner. Scanned images were analyzed for total root length and surface area using WinRHIZO (v. 2008a, Regent Instruments Canada Inc., Arsenault, Pouleur, Messier, & Guay, 1995). Roots were further categorized into three classes by diameter (0–0.15mm, 0.15–0.3mm, 0.3mm–) for analysis of total root tip number, length and surface area.

Quantum yield measurement

Salt treatment of 100 mM, 150 mM and 200 mM of NaCl started at two-weeks after transplanting. Chlorophyll fluorescence was measured by using a portable JUNIOR-PAM fluorometer and the effective quantum yield (Y(II)) of Photosystem II (PSII) was calculated by the WinControl-3 software (Heinz Walz GmbH, Pfullingen, Germany). Measurement was carried out for the 1st, 2nd and 3rd leaves (leaf1, leaf2 and leaf3, respectively) at time-series of day0, 1, 2, 3, 5, 7 and 14 days after salt treatment.

Results

Growth evaluation

To study the effect of salt stress on plant growth, fresh and dry weight were measured on day2 and day5 for plants under control and salt. On day2, there was no significant difference between three accessions in control; on day5, *V. luteola*-beach grew most vigorously among the three accessions (Fig. 1). While significant decreases were observed in *V. luteola*-beach and *V. luteola*-river under salt stress in all conditions, they were not in *V. marina* on day2 for root fresh/dry weight (Fig. 1).

To evaluate the growth response of the three accessions, we calculated relative biomass including salt treated plant/control plant and root/shoot ratio. On day2, as shown in Fig. 2, salt/control ratio of shoot fresh and dry weight reduced to 40–50 % in all the three accessions. *V. marina* in salt stress retained 85 % of root fresh weight and 95 % of root dry weight, while *V. luteola*-beach retained 61 % and 65 %, respectively, and *V. luteola*-river retained 38 % and 38 %, respectively. (Fig. 2). On day5, shoot fresh/dry weight dramatically decreased in the three accessions (16 % - 29 %). The decrease in root fresh/dry weight was the least for *V. marina* (45 % for both root fresh and dry weight), second in *V. luteola*-beach (31 % for root fresh weight and 38 % for dry weight), and the most in *V. luteola*-river (16 % for root fresh weight and 17 % for dry weight) (Fig. 2).

As to root/shoot ratio, there was no difference between three accessions in control condition (Fig. 2). In salt stress, *V. marina* largely increased root/shoot ratio both on day2 and day5, while *V. luteola*-beach showed slight increase in the ratio for dry weight on day2 and *V. luteola*-river showed no significant difference (Fig. 2).-

Patterns of salt allocation in plant body

To understand patterns of salt allocation and accumulation in the three accessions, concentration of Na and K was assessed separately in roots, lower and upper stems (stem1 and stem2, respectively) and the 1st, 2nd and 3rd leaves (leaf1, leaf2 and leaf3, respectively). As shown in Fig. 3, Patterns largely differed between three accessions: *V. marina* accumulated little Na in the stems and leaves and retained some in the roots; *V. luteola*-beach allocated Na in the roots and the leaves but little in the stems; *V. luteola*-river allocated Na in the whole plant body. As for K, *V. marina* accumulated a lot in the whole plant body; *V. luteola*-beach accumulated less to *V. marina*; *V. luteola*-river accumulated high concentration of K in the roots but less in the aerial parts. *V. marina* had the smallest Na/K ratio in aerial parts among the accessions. *V. luteola*-beach maintained low Na/K ratio in the stems but not in the leaves whereas *V. luteola*-river had high Na/K ratio in both stems and leaves.

Root architecture changes in salt stress

To know the effect of salt stress on root architecture, we scanned the roots to evaluate total root length, surface area and tip number (Fig. 4). Total root length was significantly decreased in *V. luteola*-beach and *V. luteola*-river under salt stress compared to control while it was not in *V. marina* (Fig. 5). On average, total root length was reduced by 33 % for *V. luteola*-beach and 62 % for *V. luteola*-river. Although a decrease of 21 % was observed in *V. marina* but such decrease was not significant compared to control (Fig. 5). Similar trend was found for root surface area (Fig. 5). It was decreased by 25 % in *V. marina* in salt stress but still not significantly different from control, whereas it was decreased by 40 % in *V. luteola*-beach and 66 % in *V. luteola*-river. These results suggested that *V. marina* retained global root growth in salt stress compared to control.

Other than global features of root architecture, the scanned image gave us an impression that *V. marina* developed more finer roots in salt stress (Fig. 4). Therefore, we categorized the lateral roots into three classes by diameter (D1 to D3: representing finer to thicker, see methods for the details) and evaluated total root tip number, length and surface area of each category.

As a result, *V. marina* increased the number of D1 roots to more than double in salt stress, with retention of total length and surface area, while the number of D2 and D3 roots was reduced but total length and surface area were fairly maintained (Fig. 6). This indicates *V. marina* produced more but shorter D1 roots, and fewer but longer D2 and D3 roots.

*V. luteol*a-beach showed a similar trend as *V. marina* in increase of D1 root number though not significant, and in decrease of D2 and D3 roots (Fig. 6). Different from *V. marina*, *V. luteol*a-beach maintained total length and surface area only for D1 roots (Fig. 6).

Different from the above two accessions, *V. luteola*-river decreased in all parameters except D3 root number, which was not significantly different between salt and control (Fig. 6).

Spatio-temporal patterns of chlorophyll fluorescence

In addition to the evaluation on root growth, effective quantum yield (Y(II)) was estimated by measuring fluorescence of chlorophyll in the 1st, 2nd and 3rd leaves (leaf1, leaf2 and leaf3, respectively) in 100, 150 and 200 mM of NaCl at seven time points.

Not surprisingly, Y(II) of *V. marina* was stable across time regardless of salt concentrations and position of leaves, except a slight decrease at day14 at 200 mM of NaCl (Fig. 7).

The pattern of Y(II) in *V. luteola*-beach was similar to that of *V. marina* for leaf1 and leaf2, where no decrease was observed. However, Y(II) of leaf3 decreased to zero by day14 under 200 mM of NaCl (Fig. 7).

As for *V. luteola*-river, Y(II) decreased in all the leaves under any conditions of salt stress (Fig. 7). At 100 mM, leaf1 and leaf2 lost fluorescence on day14 while leaf3 still maintained its chlorophyll fluorescence. At 150 mM, Y(II) dropped to zero on day7 in all the three leaves. At 200 mM, Y(II) in leaf1 dramatically decreased at day2. It completely lost fluorescence on day3, and this happened to leaf2 and leaf3 on day5.

In summary, *V. marina* maintained chlorophyll fluorescence even in 200 mM NaCl condition for two weeks. *V. luteola*-beach also retained the chlorophyll fluorescence high but in 200 mM NaCl condition, the leaf3 had completely lost chlorophyll fluorescence before day14. In contrast, *V. luteola*-river lost chlorophyll fluorescence across time under all tested conditions and the lower leaves were damaged earlier than the upper leaves.

Discussion

In this study, we have demonstrated importance of root architecture in salt stress. We should remark that *V. marina* maintained total root length and surface area even under salt stress (Fig. 5), despite its root biomass has been significantly reduced (Fig. 1, 2). This contrasts with the other two accessions (Fig. 5) and those reported in other studies (Ijaz et al. 2019; Mu et al. 2022; Robin et al. 2016). Given root architecture including total root length and surface area are closely related to the capacity of water uptake (Tachibana *et al.* 1983), *V. marina* has a greater ability to keep water uptake in salt-stressed conditions.

What's more interesting is that *V. marina* increased fine roots (D1) in salt stress compared to control, while the number of thicker roots (D2, D3) was reduced (Fig. 6). This was observed in common with the retention of the total root length and surface area. At the beginning, we have considered that finer roots could be a strategy to maximize root surface area and thus to maintain water uptake. However, the root surface area of fine roots accounted for only a tiny proportion of the total root surface area (Fig. S1). Therefore, increase of fine roots did not truly contribute to the total root surface area. Instead, we noticed that while *V. marina* increased in the number of D1 roots, the total length of D1 roots was not different between salt and control (Fig. 6). Therefore, *V. marina* grew more but shorter D1 roots in salt compared to control. Given water uptake occur around root tips and not in basal area (Deng et al. 2022; Gambetta et

al. 2013), it could be another strategy to maximize water uptake with limited root biomass. Thus, those changes in root architecture in response to salt stress could be a unique feature of salt tolerance in *V. marina.*

Securing water uptake under salt stress could contribute to high photosynthetic activity in the leaves of *V. marina*. Though water deficit by osmotic stress leads stomatal closure and negatively affect photosynthesis, *V. marina* kept high chlorophyll fluorescence in all the tested leaves (Fig. 7). Also, Yoshida et al. (2020) reported that the plants of *V. marina* have much higher transpiration rate than *V. luteola* or other species under salt stress. These facts indicate the root of *V. marina* can supply enough water to keep photosynthetic activity under high salt condition.

In addition, *V. marina* had an excellent ability to suppress Na accumulation (Fig. 3) (Noda *et al.* 2022). This is surprising, considering the higher the water uptake and transpiration, the higher the risk of Na uptake. *V. marina* might avoid this risk, at least to some extent, by allocating high amount of K in the plant (Fig. 3). The higher the K concentration in a cell, the lower the chance of Na entrance into a cell across the plasma membrane (Schachtman and Liu 1999). This feature highly contrasts with *V. luteola*-river, where it failed in transporting K, though stocked a lot in the root, to the stem or the leaves. However, it does not explain everything because high K does not always mean low Na (see leaf2 in *V. luteola*-beach in Fig. 3). Thus, *V. marina* could also have other mechanisms such as apoplastic barriers and active exclusion of Na, which should be elucidated in the future studies.

Being salt tolerant as well, *V. luteola*-beach had both similarity to and difference from *V. marina*. *V. luteola*-beach exhibited similar pattern to *V. marina* in that it increased tip number for finer roots but not thicker roots (Fig. 6) and it was as good as *V. marina* in retaining chlorophyll fluorescence in salt stress except leaf3 at day14 under 200 mM (Fig. 7). While *V. marina* invested on root growth more than shoot growth in salt stress (Fig. 2), *V. luteola*-beach did not invest as much on root growth as *V. marina* and was more likely to keep shoot growth in salt stress (Fig. 1, 2). It is possibly because that *V. luteola*-beach did not spend as much energy on suppressing Na accumulation as *V. marina* (Fig. 3).

The leaves of *V. luteola*-beach tolerated high Na concentration which severely damaged those of *V. luteola*-river (Fig. 7). Particularly, having similar level of Na concentration (Fig. 3), leaf1 and leaf2 of *V. luteola*-beach was however in good shape for at least two weeks in salt stress while that of *V. luteola*-river completely lost chlorophyll fluorescence (Fig. 7). Such rapid loss of photosynthetic activity, which may due to stomatal closure to avoid water loss by transpiration, reflects high-sensitivity of *V. luteola*-river under salt stress. Moreover, Na/K ratio was not different between *V. luteola*-beach and *V. luteola*-river. These indicates mechanisms other than Na/K homeostasis enhance salt tolerance in *V. luteola*-beach, like Na compartmentalizing to vacuoles or some unknown mechanism that counters Na toxicity.

What's more, *V. luteola*-beach started to appear salt damage in leaf3 (200 mM) when leaf1 and leaf2 kept chlorophyll fluorescence (Fig. 7). This contrasts to *V. luteola*-river that leaf1 always lost chlorophyll fluorescence earlier than younger leaves. Interestingly, previous study by Noda *et al.* (2022) has revealed that the youngest fully expanded leaves of *V. luteola*-beach take the role of Na loading at 100 mM of NaCl

so that it avoids sacrificing any leaf unless salt stress is too severe to keep growth. As this study demonstrated, the salt treatment of 200 mM for 14 days may exceed the tolerable level for such leaf of *V. luteola*-beach therefore it lost chlorophyll fluorescence. Further study is needed to elucidate the mechanism for this phenomenon.

Declarations

Funding

This work was financially supported by Moonshot Research & Development Program for Agriculture, Forestry and Fisheries by Cabinet Office, Government of Japan (20350204).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgments

This work was financially supported by Moonshot Research & Development Program for Agriculture, Forestry and Fisheries by Cabinet Office, Government of Japan (20350204). We appreciate Dr. Matthias Wissuwa and Dr. Juan Pariasca Tanaka for providing facility for root scan and analysis.

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Figures

Fig. 1

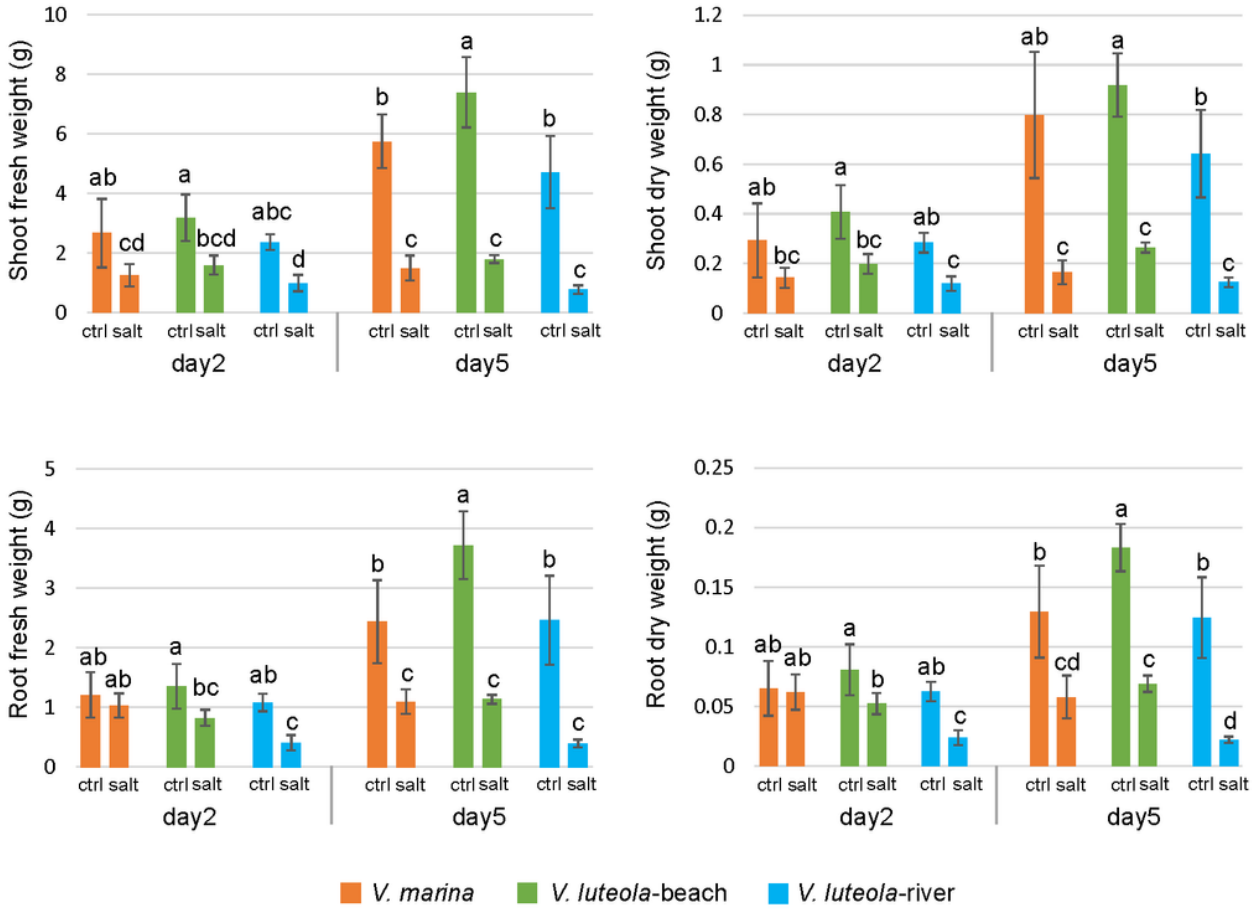

Figure 1

Root/shoot fresh/dry weight of control and salt-treated (200 mM of NaCl) plants on day2 and day5. Orange, green and blue bars represent values for *V. marina*, *V. luteola*-beach and *V. luteola*-river, respectively. Means not sharing the same alphabet are statistically different (Tukey LSD test for day2 and day5 separately, $P < 0.05$)

Fig. 2

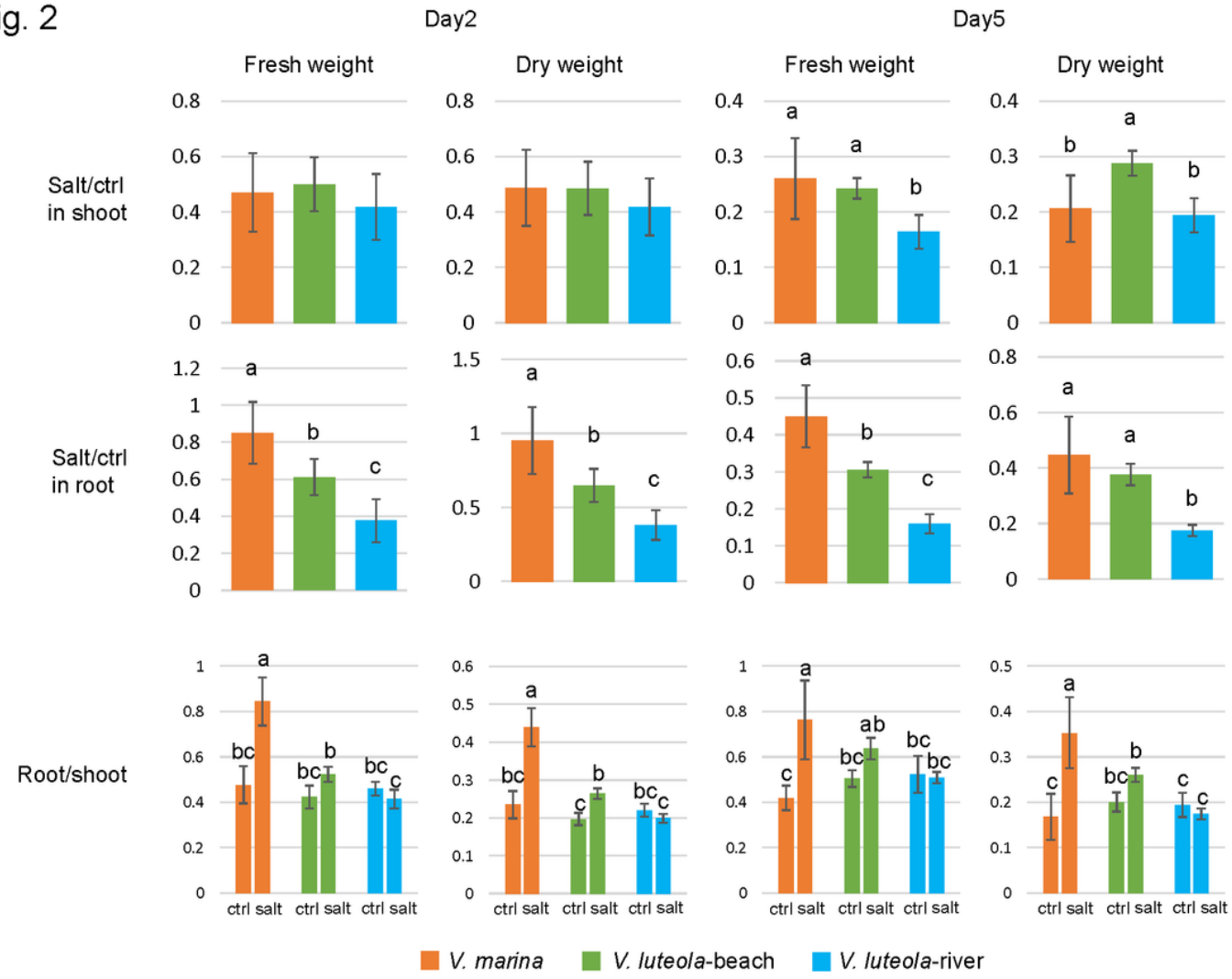

Figure 2

Relative fresh and dry weight calculated as salt-treated (200 mM of NaCl) plant/control plant and root/shoot ratio on day 2 and day5. Orange, green and blue bars represent values for *V. marina*, *V. luteola*-beach and *V. luteola*-river, respectively. Means not sharing the same alphabet are statistically different (Tukey LSD test, $P < 0.05$)

Fig. 3

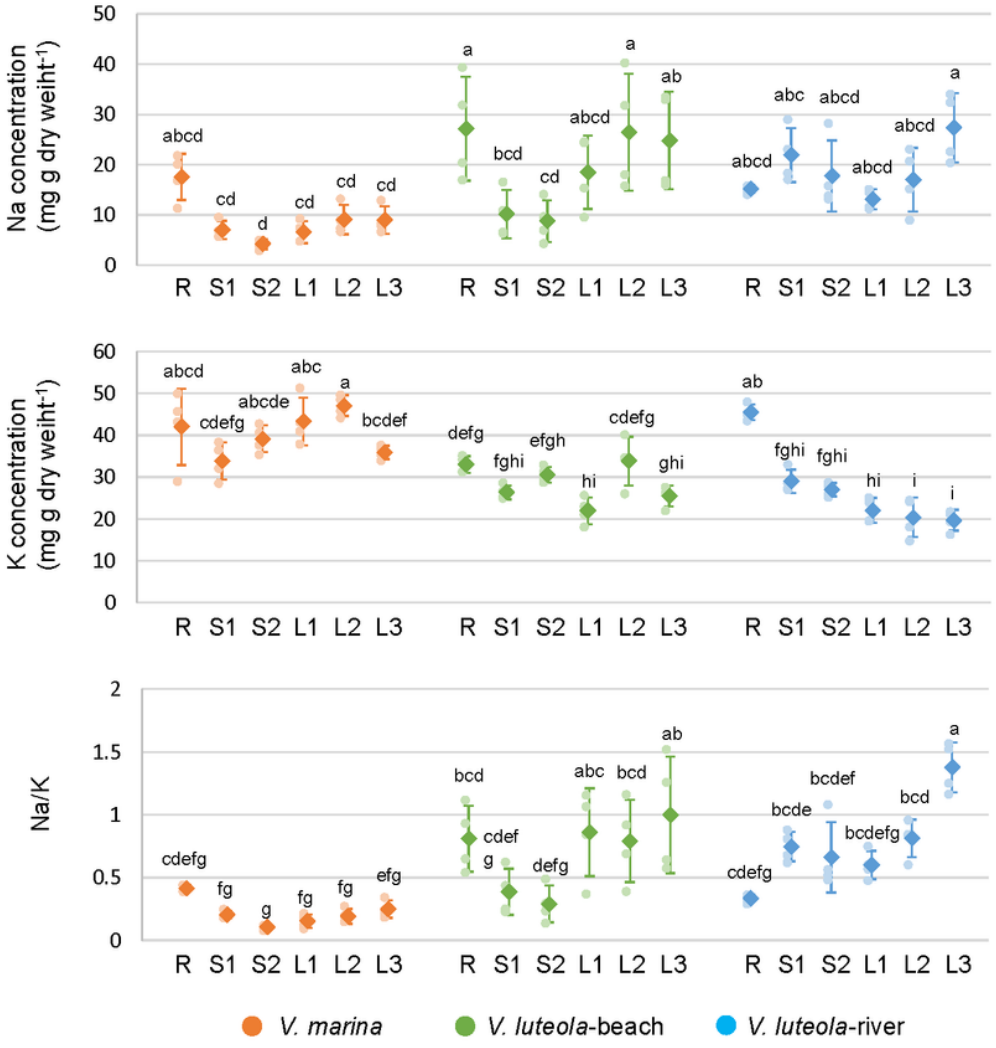

Figure 3

Concentration of Na, K and Na/K ratio in roots (R), stem1 (S1), stem2 (S2) and leaf1 (L1), leaf2 (L2) and leaf3 (L3) of salt-treated (100 mM) plants on day2. Orange, green and blue dots represent values for *V. marina*, *V. luteola*-beach and *V. luteola*-river, respectively. Means not sharing the same alphabet are statistically different (Tukey LSD test, $P < 0.05$)

Fig. 4

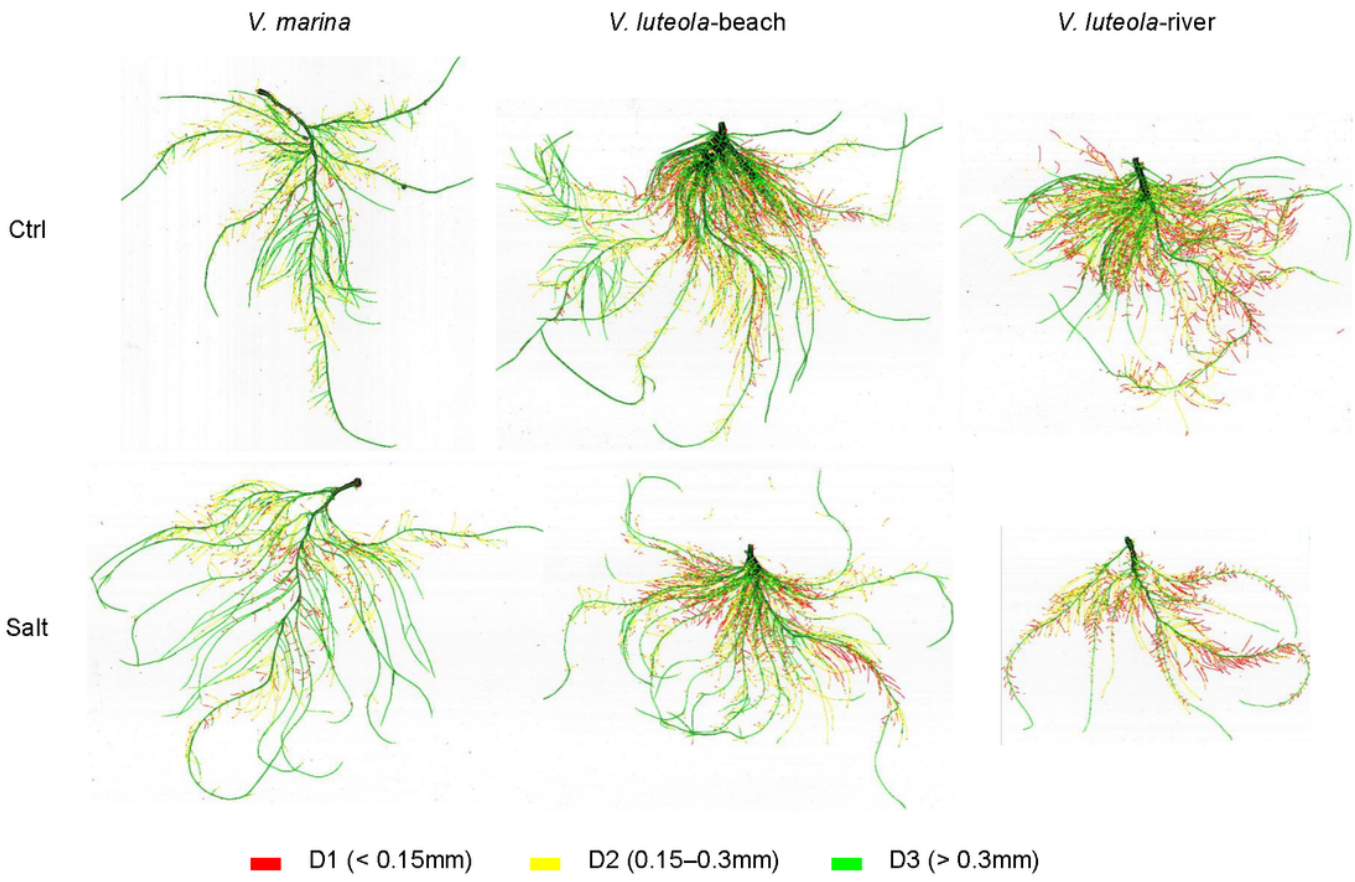

Figure 4

Image analyses of roots of control and salt-treated (200 mM of NaCl) plants on day5. Red, yellow and green represent root with diameter of 0–0.15mm, 0.15–0.3mm, 0.3mm–, respectively

Fig. 5

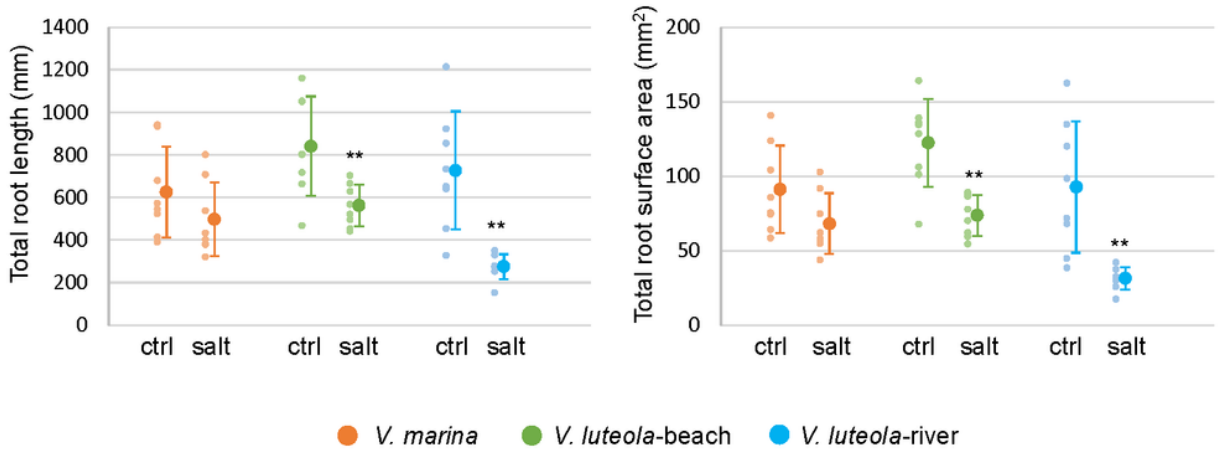

Figure 5

Total root length and surface area of control and salt-treated (200 mM) plants on day5. Orange, green and blue dots represent values for *V. marina*, *V. luteola*-beach and *V. luteola*-river, respectively. ** stands for significant difference of $P < 0.01$ (t-test between control and salt)

Fig. 6

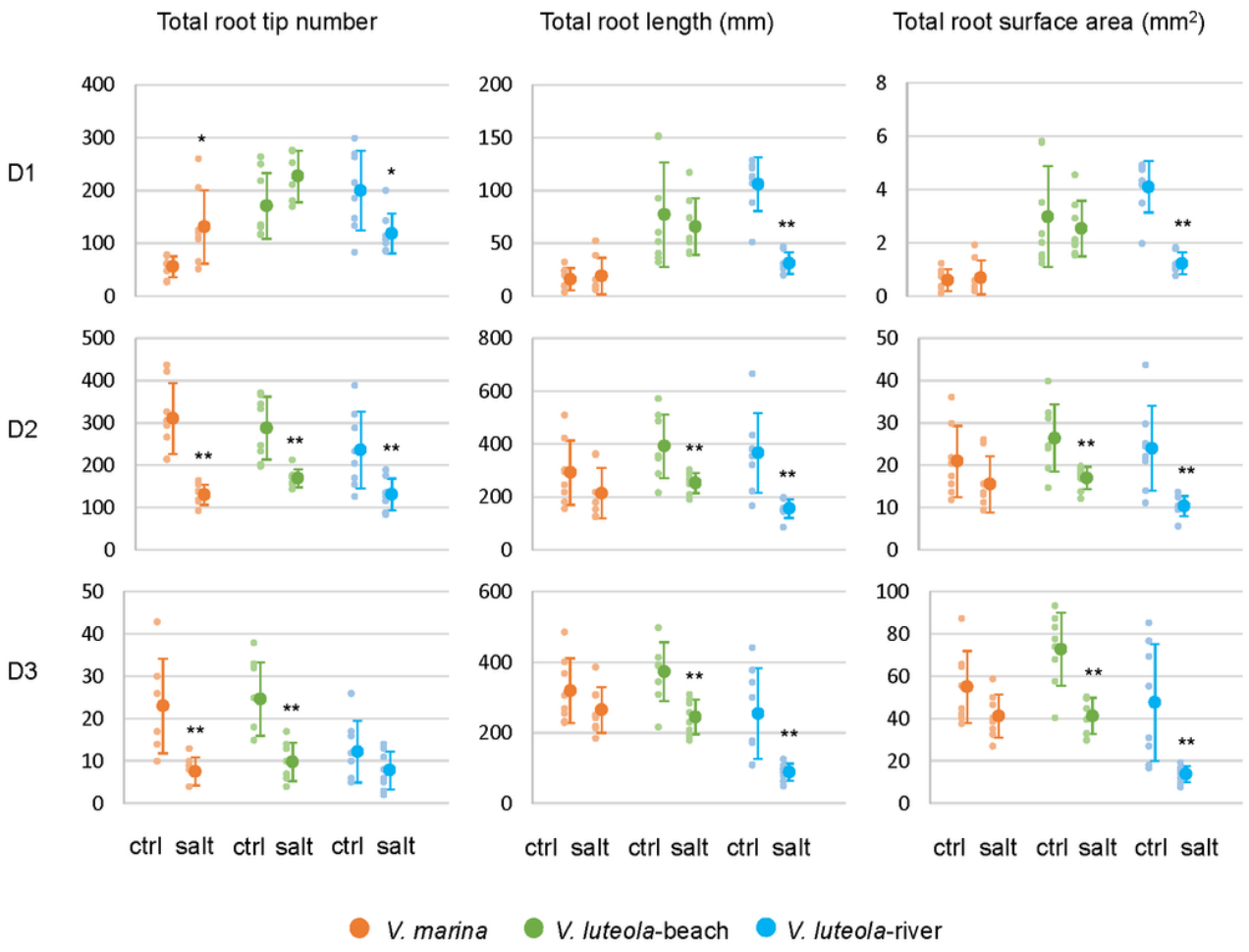

Figure 6

Total root tip number, length and surface area of D1, D2 and D3 roots of control and salt-treated (200 mM) plants. D1, D2 and D3 represent root with diameter of 0–0.15mm, 0.15–0.3mm, 0.3mm–, respectively. Orange, green and blue dots represent values for *V. marina*, *V. luteola*-beach and *V. luteola*-river, respectively. * and ** stands for significant difference of $P < 0.05$ and $P < 0.01$, respectively (t-test between control and salt)

Fig. 7

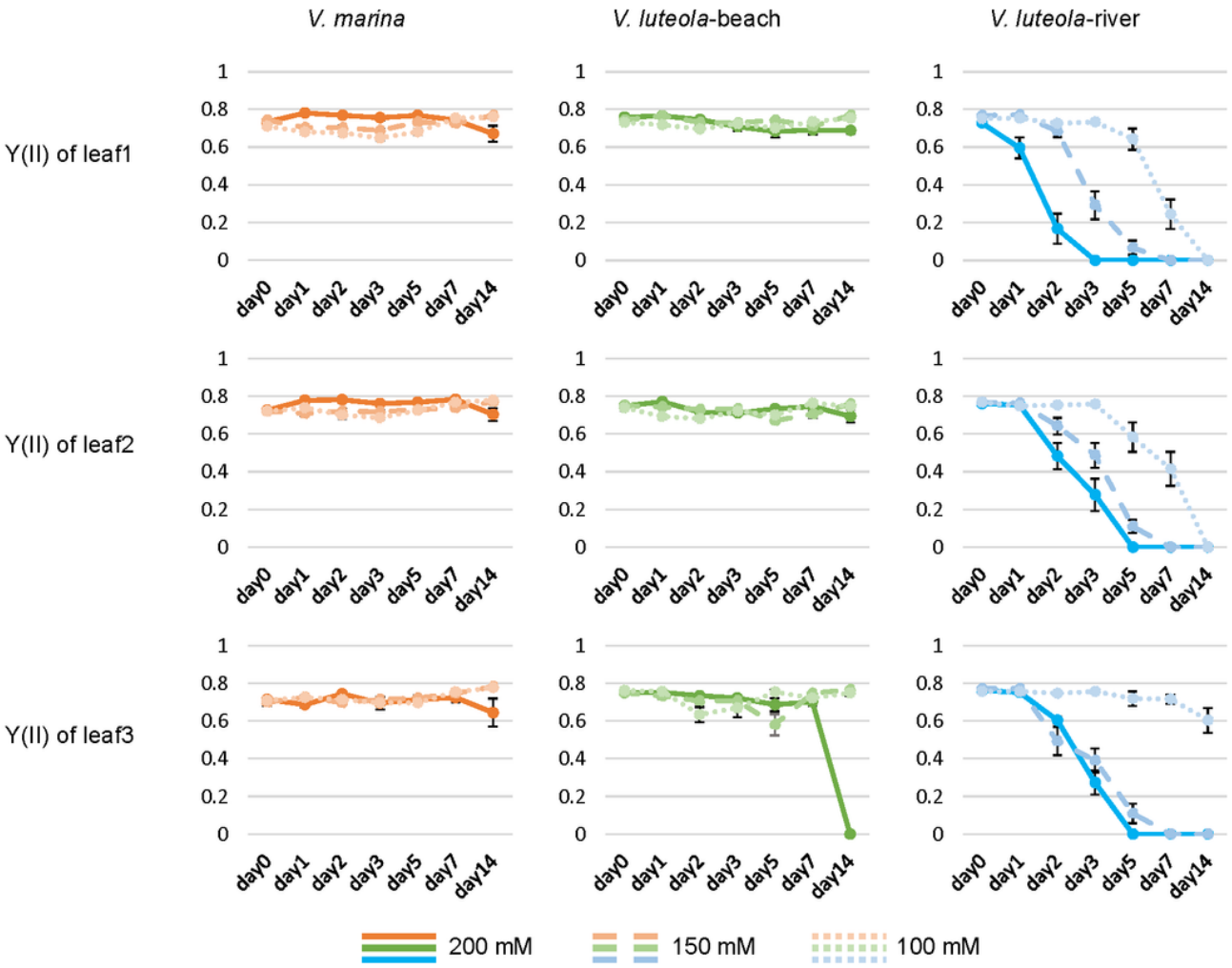

Figure 7

Effective quantum yield (Y(II)) of leaf1, 2 and 3 on day0, 1, 2, 3, 5, 7 and 14 under 100 mM, 150 mM and 200 mM of NaCl treatment

Supplementary Files

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- Fig.S10418.pdf