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Supplementary Material

Root hydraulics in salt-stressed wheat

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PART-A: Details of Calculations

Exudation experiments

The rate of exudate flow was calculated by plotting exudate volume (unit: m^3) against time (unit: min) and calculating for each time interval the slope of line (unit: m^3 min^{-1}) and averaging these values to yield a final exudation rate value for that particular root or plant. Examples for whole-root system and individual root analyses are shown in Fig. S1 and Fig. S2, respectively.

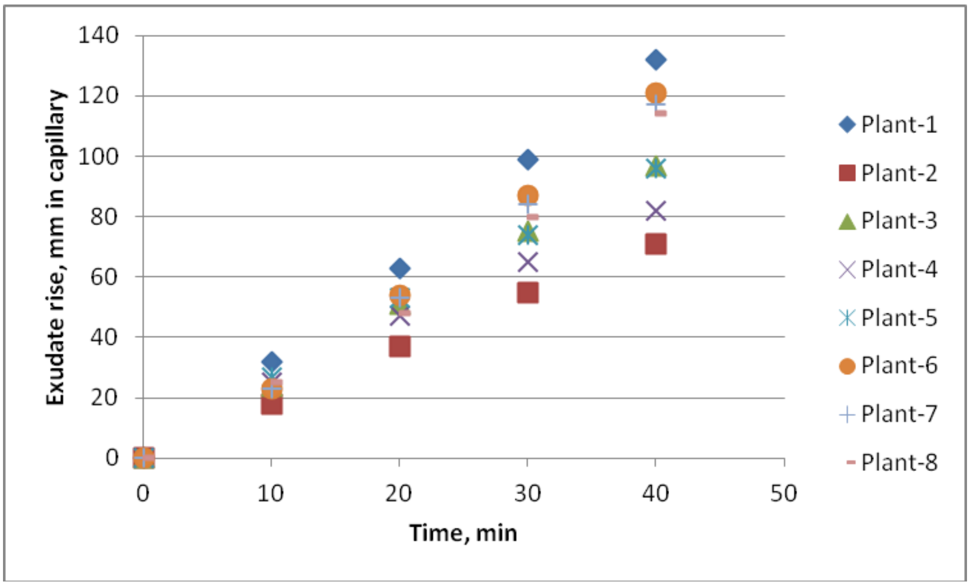


Fig. S1. Example of exudate experiments for whole-root systems of eight plants (Shiraz and Bahar, control, 15–17 days old). The inner diameter of capillary was 0.58 mm, and a 1-mm rise in exudate corresponded to a volume of 0.264 mm^3.

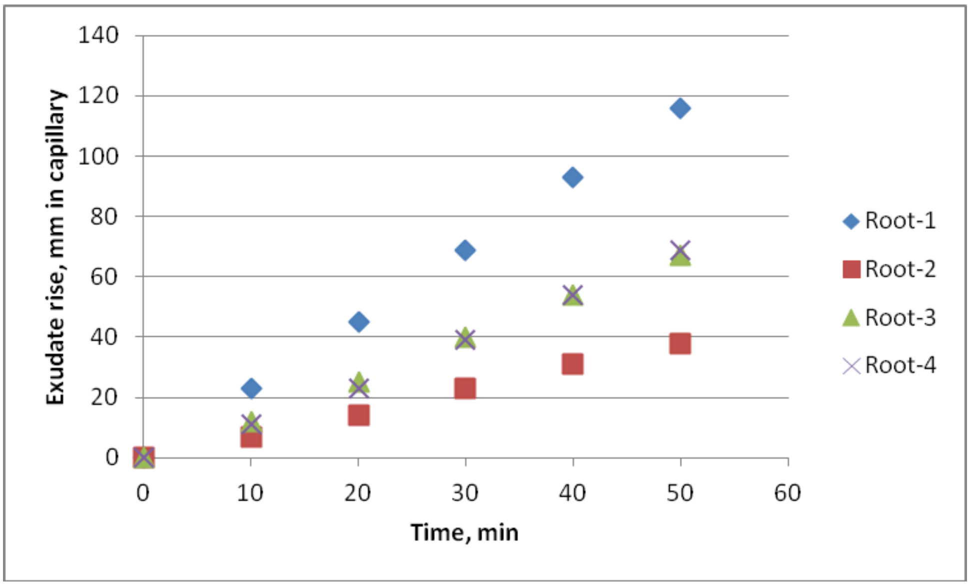


Fig. S2. Example of exudate experiments for individual seminal roots of four plants (Shiraz, NaCl, 24 days old). The inner diameter of capillary was 0.58 mm, and a 1-mm rise in exudate corresponded to a volume of 0.264 mm^3.

The average exudation rate for a particular root system or root was then divided by factor 60 to express the flow rate in 'm^3 s^{-1}'. For the root systems shown in Fig. S1, this resulted in exudation rates (m^3 s^{-1}) listed in Table S1.

Table S1. Values of variables for whole-root systems shown in Fig. S1 to calculate hydraulic conductivity (Lp)

Variable	Plant-1	Plant-2	Plant-3	Plant-4	Plant-5	Plant-6	Plant-7	Plant-8
Exudation rate, m^3 s^{-1}	1.5E-11	7.8E-12	1.1E-11	9.02E-12	1.1E-11	1.3E-11	1.3E-11	1.3E-11
Driving force, MPa	0.10307	0.1227	0.09571	0.122699	0.10552	0.15215	0.12025	0.10061
Root surface area, m^2	0.00286	0.00251	0.00216	0.001909	0.00179	0.00215	0.00172	0.00144
Lp, m s^{-1} MPa^{-1}	4.9E-08	2.5E-08	5.2E-08	3.85E-08	5.6E-08	4.1E-08	6.2E-08	8.6E-08

To obtain the osmotic hydraulic conductivity (Lp; units: m s^{-1} MPa^{-1}), the surface area of the root (A, units: m^2) and the osmotic driving force ($\Delta\Psi$; units: MPa) between root medium and exudate were determined. Osmotic hydraulic conductivity was calculated according to:

$$Lp = (\text{Exudation rate}) / [(\Delta\Psi) \times (A)]$$

The osmotic driving force was determined through analyses of osmotic pressure of exudate and root medium. Experiments on the closely-related barley (Knipfer and Fricke 2011) have shown that the root solute reflection coefficient is close to 1.0 and that, therefore, the measured osmotic gradient approximates $\Delta\Psi$. For details of discussion of root reflection ceofficients in wheat, see Discussion section of main manuscript.

Xylem solute net loading rate

The rate of xylem solute loading (XSL) was calculated from exudation rates (ER; unit, $m^3\ s^{-1}$) and exudate osmolality (EO; unit, mosmol kg^{-1}, approximating mmol l^{-1})) obtained for entire root systems, whereby both variables were determined for the same plants. The xylem solute net loading rate (XSL; unit, mmol s^{-1}) was calculated according to:

$$XSL = ER \times EO$$

PART-B: *Root cross-sections of Shiraz*

Hand-cut cross-sections were prepared from seminal and adventitious roots of 21–24 day-old Shiraz plants, whereby NaCl-plants had been exposed to 100 mM NaCl in the nutrient solution from day 10 onwards. Only adventitious roots showed a difference in root anatomy between control and NaCl-treated plants and are, therefore, shown (Fig. S2A). Salt-treatment caused the formation of an exodermis in adventitious roots; the development of the endodermis was also slightly promoted by salt-treatment. Cross-section were prepared from the region close to the root tip (5–10 mm from tip), representing immature tissue; and cross-sections were prepared from the region 40–60 mm from the tip, representing more mature tissue. Cross-sections were stained with berberine hemisulfate and counterstained with toluidine blue and viewed under fluorescence light (390–420 nm) (Brundrett MC, Enstone DE, Peterson CA. 1988. A berberine-aniline blue staining procedure for suberin, lignin, and callose in plant tissue. *Protoplasma* **146**, 133–142). Tissues are explained in Fig. S2B.

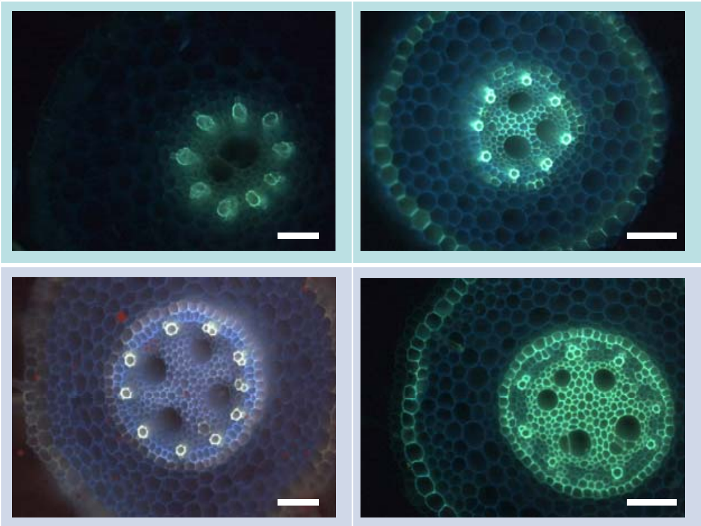
Fig S2A

SHIRAZ, Adventitious root (mm from root tip)

Control NaCl

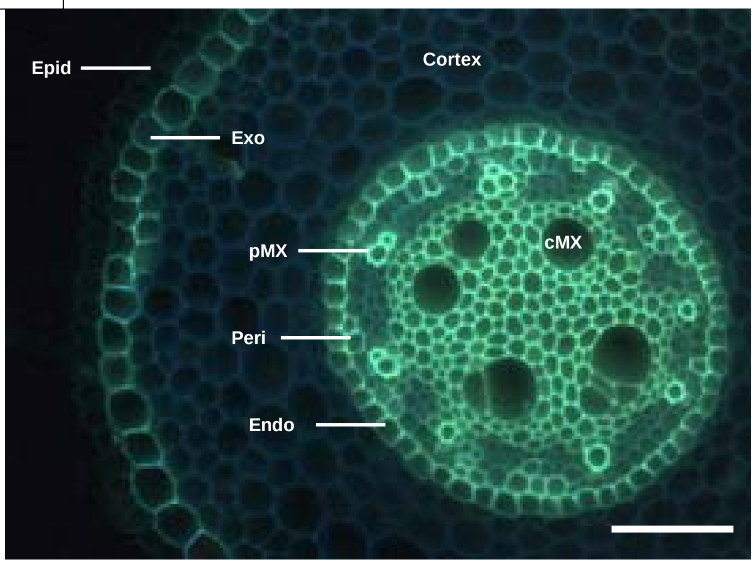
5-10

40-60



Bar, 0.1 mm

Fig S2B

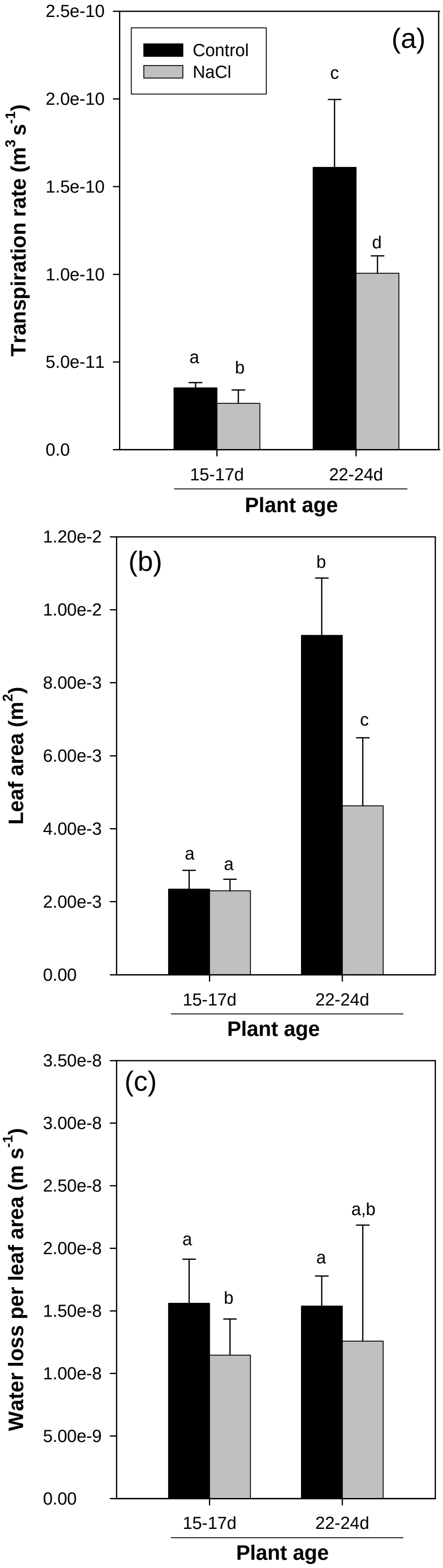


Epid, Epidermis; Exo, Exodermis; pMX, peripheral (early) metaxylem; cMX, central (late) metaxylem; Peri, Pericycle; Endo, Endodermis; bar, 0.1 mm

PART-C: Data for the bread wheat cultivar Bahar (Figs 1–3)

All of the analyses, which did not include use of the cell pressure probe or picolitre osmometry, including determination of root reflection coefficient and leaf cell water potential, were also carried out for another Iranian bread wheat cultivar, Bahar. Previous studies had shown that Bahar and Shiraz differ in drought tolerance (Shiraz, less tolerant; Bahar, more tolerant; Bijanzadeh and Emam, 2010), and this difference may extent to the response to salt stress, considering the common osmotic stress component of drought and salinity. However, the analyses showed that Shiraz and Bahar responded very similar to salt treatment. Exceptions were that transpirational water loss and root surface area in Bahar were reduced significantly already at the younger growth stage, and that Lp of seminal roots did not decrease as control plants grew older. The results for Bahar are shown below.

Fig. 1. (*a*) Day-time plant transpiration rate, (*b*) leaf area and (*c*) water loss per unit leaf area in the bread wheat cultivar Bahar in response to 100 mM NaCl. Plants were 15–17 days and 22–24 days old, respectively, and had been exposed to 100 mM NaCl for 5–7 days and 12–14 days, respectively, prior to analyses. Results are averages and SD (error bars) of 8–10 plant analyses. Different letters denote statistically significant differences between values of control and NaCl-treated plants and developmental stages ($P < 0.05$; Student's t-test).



(a)
Control
NaCl
Transpiration rate (m³ s⁻¹)
2.5e-10
2.0e-10
1.5e-10
1.0e-10
5.0e-11
0.0
a
b
c
d
15-17d
22-24d
Plant age
(b)
Leaf area (m²)
1.20e-2
1.00e-2
8.00e-3
6.00e-3
4.00e-3
2.00e-3
0.00
a
a
b
c
15-17d
22-24d
Plant age
(c)
Water loss per leaf area (m s⁻¹)
3.50e-8
3.00e-8
2.50e-8
2.00e-8
1.50e-8
1.00e-8
5.00e-9
0.00
a
b
a
a,b
15-17d
22-24d
Plant age

Fig. 2. Root surface area, diameter and osmotic hydraulic conductivity (Lp) of the bread wheat cultivar Bahar grown hydroponically under control conditions and in presence of 100 mM NaCl. Plants were 15–17 days and 22–24 days old, respectively, and had been exposed to 100 mM NaCl for 5–7 days and 12–14 days, respectively, prior to analyses. (*a*) The surface area of the entire root system of plants ('Root system total') was provided through the main axis of seminal roots, lateral roots which emerged from seminal roots and adventitious roots; the latter contained few if any lateral roots, even at the more advanced growth stage. (*b*) The diameter of roots was determined through image analysis (see Methods section) for the main axis of roots. Results in (*a*) and (*b*) are averages and SD (error bars) of 14–16 plant analyses, whereby all variables shown were determined for each plant, and between 6 and 14 diameter readings were averaged per type of root to obtain a root diameter value for that particular plant. (*c*) Osmotic hydraulic conductivity (Lp) was determined through exudation analyses for the entire root system of plants ('Root system total'), and for individual seminal and adventitious roots. Whole-root system analyses could only be performed for 15–17 day-old plants, and adventitious roots were sufficiently developed only in 22–24-day-old plants. Results are averages and SD (error bars) of 8–14 plant or individual-root analyses. Different letters denote statistically significant differences for each variable between control and NaCl-plants and developmental stages ($P < 0.05$; Student's t-test).

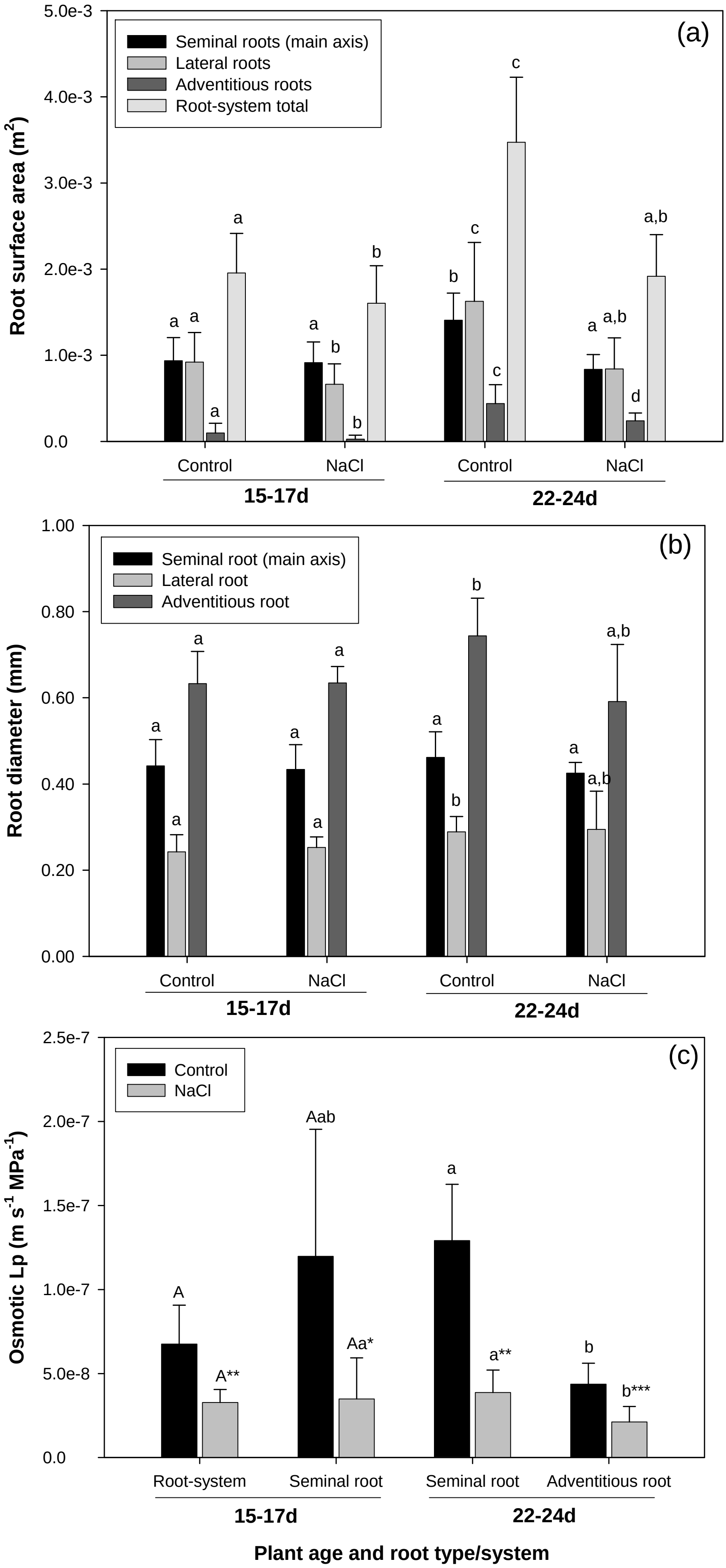
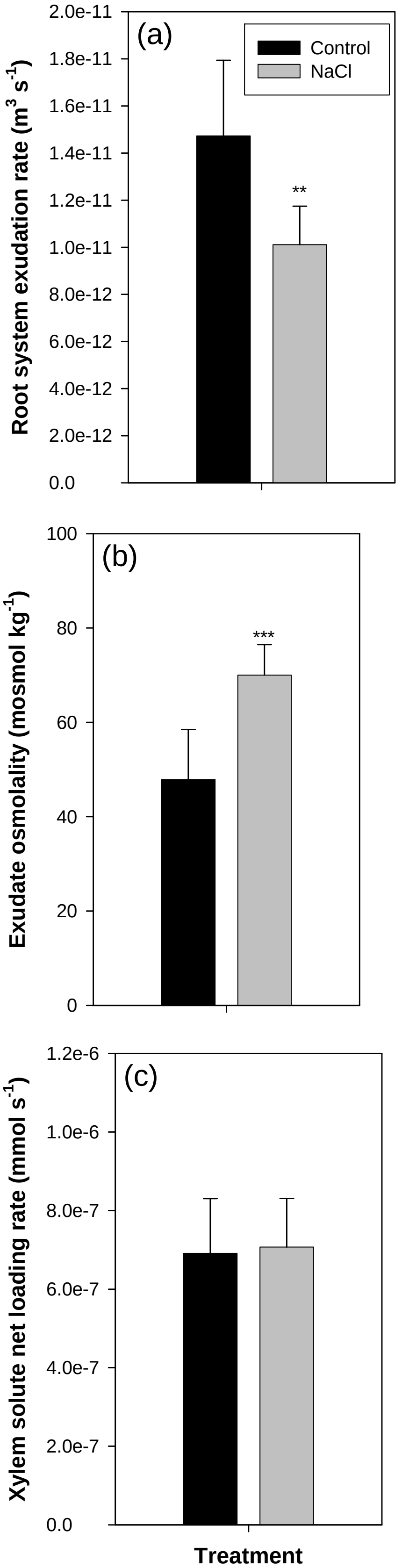

(a)
Seminal roots (main axis)
Lateral roots
Adventitious roots
Root-system total
Root surface area (m2)
5.0e-3
4.0e-3
3.0e-3
2.0e-3
1.0e-3
0.0
Control
NaCl
Control
NaCl
15-17d
22-24d
(b)
Seminal root (main axis)
Lateral root
Adventitious root
Root diameter (mm)
1.00
0.80
0.60
0.40
0.20
0.00
Control
NaCl
Control
NaCl
15-17d
22-24d
(c)
Control
NaCl
Osmotic Lp (m s-1 MPa-1)
2.5e-7
2.0e-7
1.5e-7
1.0e-7
5.0e-8
0.0
Aab
A
A**
Aa*
a
a**
b
b***
Root-system
Seminal root
Seminal root
Adventitious root
15-17d
22-24d
Plant age and root type/system

Fig. 3. Xylem solute loading rate during exudation in the bread wheat cultivar Bahar grown hydroponically under control conditions and in presence of 100 mM NaCl. Plants were 15–17 days old and had been exposed to 100 mM NaCl for 5–7 days prior to analyses. For each treatment, eight plants were first analysed for transpiration (see Fig. 1*a*, values for 15–17 day-old plants) and subsequently used in exudation experiments, in which root system Lp (see values in Fig. 2*c*) were determined. (*a*) Exudation rate, (*b*) exudate osmolality, and (*c*) calculated net rate of xylem solute loading (= [(exudation rate) × (exudate osmolality)], with mosmol kg^{-1} approximating mmol L^{-1}). Results are averages and SD (error bars) of eight plant analyses. Asterisks denote statistically significant differences for each variable between control and NaCl-plants (Student's t-test); *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.


(a)
Control
NaCl
**
Root system exudation rate (m3 s-1)
2.0e-11
1.8e-11
1.6e-11
1.4e-11
1.2e-11
1.0e-11
8.0e-12
6.0e-12
4.0e-12
2.0e-12
0.0
(b)
***
Exudate osmolality (mosmol kg-1)
100
80
60
40
20
0
(c)
Xylem solute net loading rate (mmol s-1)
1.2e-6
1.0e-6
8.0e-7
6.0e-7
4.0e-7
2.0e-7
0.0
Treatment