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Relationships between aquaporins gene expression and nutrient concentrations in melon plants (*Cucumis melo* L.) during typical abiotic stresses

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ABSTRACT

Melon (*Cucumis melo* L.), a member of the Cucurbitaceae, in Mediterranean regions is usually affected by abiotic stresses like salinity, nutrients deficiency or high temperature. These abiotic stresses have been shown to produce the modulation of gene expression as a response to the altered conditions. Among these genes, aquaporins (transmembrane proteins) stand out due to their vital function as transporters of water and different solutes. For this reason, the aim of this work was to study the expression levels of all (31) aquaporins of melon plants (CmAQPs) after exposure to salinity (50 mM NaCl), nutrient deficiency (10% Hoagland solution) or high temperature (40 ºC for 1 h/day) and relate them with nutrient content, water relations and hydraulic conductance. There were general decreases in plant nutrient concentrations, especially in the root (Fe, K, Mn or Zn), while the concentrations of some elements for each stress (B, Ca, Mg, Mo or Si) increased. Physiological parameters were regulated depending on the treatment, showing the important role of hydric physiology regulation in the whole melon plant response to the different stresses. For most of the aquaporins, their expression decreased in the root (PIP2;1, PIP2;5 and PIP2;6 within the PIPs; most of the TIPs; NIP6;1, NIP7;1; SIP1;1) in all three treatments, while other aquaporins were over-expressed, such as the PIP1s (high temperature treatment), PIP2;2 (nutrient deficiency and high temperature treatments) and TIP1;1 (salinity and high temperature treatments) and NIP5;1 (nutrients deficiency treatment). The leaf aquaporin expression levels were less affected. This study shows that CmAQPs expression is modified differently in response to distinct abiotic stresses and that this is related to plant water relations and nutrients levels.

1. Introduction

Melon (*Cucumis melo* L.) is the most important member of the Cucurbitaceae family, a consequence of its high economic value, being the fourth most important fruit in the world market (De Campos et al., 2017). This crop has a high demand for water and a correct balance of nutrients is vital for the correct development of the plants and fruits (Lopez-Zaplana et al., 2020a; Preciado et al., 2018). The main proteins responsible for water transport are the aquaporins, transmembrane proteins that generate pores in cell membranes and allow the exchange of water and other solutes (Maurel et al., 2015) like CO_2 (Kaldenhoff, 2012), H_2O_2 (G.P. Bienert et al., 2014; G. Bienert et al., 2014), ammonium (Bertl and Kaldenhoff, 2007; Hwang et al., 2010), urea (Gerbeau et al., 1999), glycerol (Dean et al., 1999) or metalloids (Bienert et al., 2008). They have been characterized recently in *C. melo* (Lopez-Zaplana et al., 2020b).

In higher plants, there are 5 major aquaporins subfamilies: the PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), NIPs (nodulin 26-like intrinsic proteins) (Maurel et al., 2015), SIPs (small basic intrinsic proteins) (Johanson and Gustavsson, 2002) and XIPs (X-intrinsic proteins) (Danielson and Johanson, 2008). In general, most PIPs are responsible for the transport of water, H_2O_2 and CO_2 (G. Bienert and Chaumont, 2014; G.P. Bienert and Chaumont, 2014; Kaldenhoff, 2012). The TIPs are able to transport the same solutes as PIPs and also some others such as N compounds (urea or ammonia) (Dynowski et al., 2008). Some NIPs are able to transport water and other solutes, principally metalloids such as boron (B), silicon (Si), selenium (Se), arsenic (As) or antimony (Sb) (Bienert et al., 2008; Pommerrenig

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et al., 2015; Sabir et al., 2020). SIPs have only been characterised as water transport channels (Johanson and Gustavsson, 2002; Noronha et al., 2014). In contrast, XIPs are known as multifunctional permeable channels for water, metalloids and ROS (Bienert et al., 2011; Lopez et al., 2012).

The regulation of the genes expression of aquaporins is subject to the conditions of the environment in which the plants are found, including abiotic stresses, which affect directly the transport of water and solutes and indirectly the relationships with other elements (Barzana et al., 2020; Boursiac et al., 2008; Kumar et al., 2020; Martínez-Ballesta et al., 2009).

Salinity stress is one of the most widespread abiotic stresses affecting plants. The damaging effect of this stress occurs in two phases: first, an osmotic stress decreases water uptake (Sharma et al., 2012), and then a nutritional stress results from an ionic dysregulation caused by competition between salt ions and nutrients, principally potassium (K) and calcium (Ca) (Acosta-Motos et al., 2017; Munns and Tester, 2008). At a general level, the expression of the genes of PIPs and TIPs is decreased more than 50% after a salinity treatment. In addition, in *Arabidopsis thaliana* L. it has been shown that NIPs also have an important role in salinity tolerance (Afzal et al., 2016; Sutka et al., 2011).

Stress due to nutrient deficiency is highly variable, depending on the nutrient in short supply, but a deficiency usually affects the normal growth and physiology of the plant, disturbing photosynthesis, enzyme activity, signalling or construction of structures (Dell and Huang, 1997; Gong et al., 2010; Guo et al., 2016). Among the most common nutrient deficiencies are those of Mg, often due to decreases in its bioavailability in acidic soils or to high rainfall in some tropical regions (Gransee and Führs, 2013), and iron (Fe), which is an abundant element but usually present as insoluble forms, not available to plants (Ricachenevsky et al., 2013). Further, we find the deficits associated with the competition between nutrients, as occurs in saline soils with K, Ca or Mg (Acosta--Motos et al., 2017; Fageria et al., 2011). In general, some deficiencies - like Ca (Maathuis et al., 2003) or Fe (Hopff et al., 2013) - decrease the activity of aquaporins, while others - like B (Takano et al., 2006) or zinc (Zn) (Ariani et al., 2019) - increase their expression, but this is highly variable, depending on the crop and the nutrient.

Exposure of plants to high temperature can cause deregulation in terms of water transport and affects the expression of aquaporins genes, depending on the intensity and duration of the stress and on the crop (Obaid et al., 2016). For example, in soybean (*Glycine max* L.) root, it has been seen that several PIPs and TIPs genes showed an increase in their expression level after six hours of high temperature treatment, while the expression level of the TIP4;1 and SIP1;3 genes was lower than the control (Feng et al., 2019). On the other hand, the expression of all these aquaporins genes was decreased after twelve hours of treatment (Feng et al., 2019), showing the enormous variability in the behaviour of aquaporins depending on the intensity and duration of the stress to which the plants are subjected.

Despite the fact that it is well known that abiotic stresses like salinity, nutrient deficiency or high temperature modify the expression of aquaporins genes, in melon plants, information is lacking on this process and how it interacts with the mineral content. Thus, the present work studies the connection between aquaporins expression patterns and the physiological status and nutrient concentrations of melon plants under three stresses typical of the summer in Mediterranean areas (salinity, nutrient deficit due to leaching caused by torrential rains and high temperature) (López-Ortega et al., 2016), for determining the different subfamilies or isoforms involvement in each stress or determining a common response.

In this way, as salinity is the stress that mostly affect the osmotic balance of the plant, our hypothesis was based on that the majority of aquaporins should be changed in relation to nutrient for osmotic compensation. In other way, as the nutrient deficiency treatment should decreased mineral uptake, the response should be related to NIPs modification. However, in the high temperature treatment, the observed increase of water transport should be related to aquaporins that only transport water. To investigate the hypothesis, the expression of all aquaporins was studied in leaves and roots for stablish the relationship with mineral concentration, water relations and gas exchange in melon plants.

2. Material and methods

2.1. Plant growth conditions and abiotic stress treatments

Seeds of melon were pre-hydrated with de-ionised water and aerated continuously for 24 h. After this, the seeds were germinated in vermiculite, in the dark at 28ºC, for three days. Then, the seedlings were transferred to a controlled-environment chamber with a light-dark cycle of 16–8 h, a temperature of 25ºC-20ºC and relative humidities of 60%–80%. Photosynthetically-active radiation (PAR) of 400 μmol m^{-2} s^{-1} was provided by LEDs. After two days, the seedlings were placed in 15-L containers with continuously-aerated Hoagland nutrient solution (Hoagland and Arnon, 1950).

After two weeks of growth in control conditions, the plants were divided into four groups, one for each treatment. The experimental design was a completely randomised design (CRD). Eight plants per treatment were distributed randomly in the four abiotic stress treatments. One group of eight plants was used as the control. Another group of eight plants received a salinity treatment (50 mM NaCl in Hoagland solution, providing a final EC 4 dS m^{-1}). The third group was grown under nutrient deficiency (10% Hoagland solution). The last group was grown in Hoagland solution and exposed to high temperature by placing each plant in a cylinder that was submerged in 40 °C bath once per day for one hour, heating the Hoagland solution. Each treatment was carried out for two weeks and each solution was replaced completely every week. The whole experiment was performed twice.

2.2. Fresh and dry weight

After these two-week treatments, four plants from each one were weighed to obtain the fresh weight, separating the root from the aerial part, and then left in an oven (60ºC) for five days until they were completely dry, weighing them again to obtain the dry weight (DW).

2.3. Transpiration rate

The transpiration rate (mmol m^{-2} s^{-1}) was determined using a gravimetric method (Aroca et al., 2007, 2001). Each plant was placed individually in a pot with growth medium. The surface of each pot was covered with a waterproof plastic sheet. The pot-plant system was weighed immediately (W_0) and again after 2 h, 4 h and 6 h (W_f). The leaf transpiration rate was calculated as: $(W_0 - W_f)/(t \times A)$, where t is the time (s) and A is the leaf area (m^2). To calculate the leaf area, all the leaves of each plant were drawn on paper, scanned and analysed using ImageJ 1.52 software bundled with Java 1.8.0 (National Institutes of Health, USA) (Abràmoff et al., 2004).

2.4. Root and leaf water potential (Ψω), osmotic potential (Ψμ) and turgor potential (Ψp)

The osmotic potential (Ψμ) of three leaves and roots was measured using a freezing-point depression osmometer (Digital Osmometer, Roebling, Berlin) at 25 ± 1 ºC (Navarro et al., 2003). The leaf water potential (Ψω) was measured in the fourth fully-expanded leaf of four plants of each treatment using the pressure chamber technique (Turner, 1988). Turgor potential (Ψp) was calculated as the difference between leaf water potential and osmotic potential (Nonami and Schulze, 1989).

2.5. Root hydraulic conductivity (L_o)

The root hydraulic conductivity (L_o) was measured on detached roots exuding under atmospheric pressure for 15 min. It was calculated as $L_o=J_v/\Delta\Psi$, where J_v is the exuded sap flow rate and $\Delta\Psi$ the osmotic potential difference between the exuded sap and the nutrient solution into which the plants were imbibed (Aroca et al., 2007). The measurements were carried out 6 h after the onset of light. The L_o value was expressed in mg H_2O g root DW^{-1} MPa^{-1} h^{-1}.

2.6. Mineral content

The concentrations of macronutrients (Ca, K, Mg, P and S), micronutrients (B, Fe, Mn, Mo, Si and Zn) and Na were measured in four plants, distinguishing between the roots and aerial part. All the samples were finely ground in a mill grinder (model A10, IKA, Staufen, Germany) and digested with HNO_3–$HClO_4$ (2:1) in a microwave oven (CEM Mars Xpress, Mattheus, NC, USA). The analysis of the elements was carried out using a Perkin–Elmer (Waltham, MA, USA) 5500 model ICP emission spectrophotometer (Iris Intrepid II, Thermo Electron Corporation, Franklin, TN, USA), at 589 nm. The concentration was expressed as mg g^{-1} DW (Ca, K, Mg, P, S and Na) or as mg kg^{-1} DW (B, Fe, Mn, Mo, Si and Zn). All the elements were measured, but only those that showed important differences are reported.

2.7. RNA extraction and cDNA synthesis

After grinding the liquid nitrogen frozen roots and leaves with a mortar, total RNA of 50 mg of each sample was extracted using the NZY Total RNA Isolation kit (Nzytech, Lisbon, Portugal), according to the manufacturer's protocol. The quantity and purity of the RNA were measured with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). The integrity of the RNA was measured by electrophoresis in agarose gel. Contaminating DNA was removed using the RNase-free DNase solution provided in the RNA Isolation kit (Nzytech, Lisbon, Portugal), according to the manufacturer's instructions. The RNA extracted was stored at −80 °C until use. The High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) was used to synthesise cDNA from 2 μg of total RNA, according to the manufacturer's protocol.

2.8. Quantitative real-time PCR (RT-qPCR) analyses

The RT-qPCR of all the melon aquaporin genes was carried out using the gene-specific primers designed by Lopez-Zaplana et al. (2020b). It was performed using 2 μL of 1:2 (for *PIP2.1, PIP2.4, PIP2.7, PIP2.8, PIP2.9, TIP1.2, TIP1.3, TIP2.1, TIP2.2, TIP4.1, TIP5.1, NIP1.1, NIP4.1, NIP6.1, NIP7.1, SIP1.1, SIP2.1* and *XIP1.1*) or 1:5 (for *PIP1.1, PIP1.2, PIP2.2, PIP2.3, PIP2.6, PIP2.10, TIP3.1, NIP2.1, NIP2.2, NIP5.1* and *NIP5.2*) diluted cDNA samples, 500 nM of specific primers and 5 μL of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in a total reaction volume of 10 μL. The equipment used was a QuantStudio™ 5 Flex Real-Time qPCR system (Applied Biosystems, Foster City, CA) and the qPCR program consisted of 10 min initial denaturation at 95ºC and then amplification in a two-step procedure: 15 s of denaturation at 95ºC and 60 s of annealing and extension at a primer-specific temperature for 40 cycles, followed by a dissociation stage. Data collection was carried out at the end of each round in step two. These conditions were used for both target and reference genes and the absence of primer-dimers was checked in controls lacking templates. The RT-qPCR analysis was performed on three independent samples for each treatment (biological replicates) and each sample reaction was carried out in triplicate (technical replicates) in 96-well plates. The transcript levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.9. Data analysis

Statistical analyses were performed using the SPSS 25.0.0.1 software package. The weights, physiological parameters, element concentrations and RT-qPCR results were analysed using a one-way ANOVA, followed by the *post hoc* Tukey multiple comparison test. Significant differences among the values of all the parameters were determined at $p \leq 0.05$, according to Tukey's test. The values presented are the means ± SE. To detect outliers in the qPCRs performed, the SPSS 25.0.0.1 software package was used.

3. Results

3.1. Weights and physiological parameters

The fresh weight and the DW (Fig. 1a) of melon roots did not show any significant differences due to the treatments. By contrast, the fresh weight and the DW (Fig. 1b) of the aerial parts were significantly lower in the nutrient deficiency treatment with respect to the other three treatments (control, salinity and high temperature).

Physiological measurements (Fig. 2) were performed to determine the changes in the water relations and the physiology of the plants in response to the treatments. The transpiration rate (Fig. 2a) was significantly higher in the high temperature treatment with respect to the salinity and deficient nutrition treatments. Leaf water potential (Fig. 2b) was more negative in the salinity treatment than in the other three

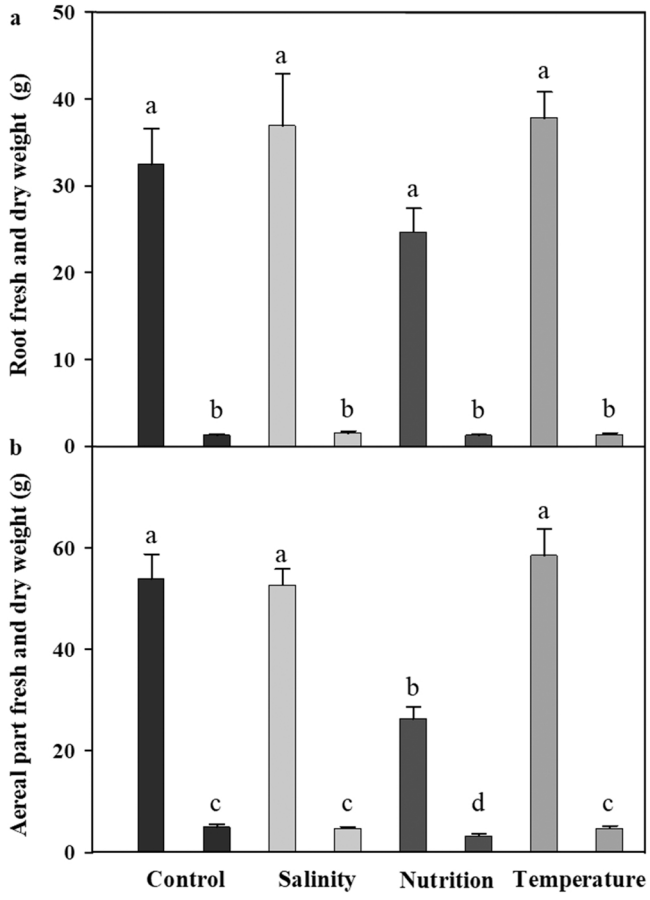

Fig. 1. Fresh and dry weights of the root (a) and aerial part (b) of melon plants for the control and the salinity, deficient nutrition and high temperature treatments. Each value represents the mean of 4 plants with the same treatment. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

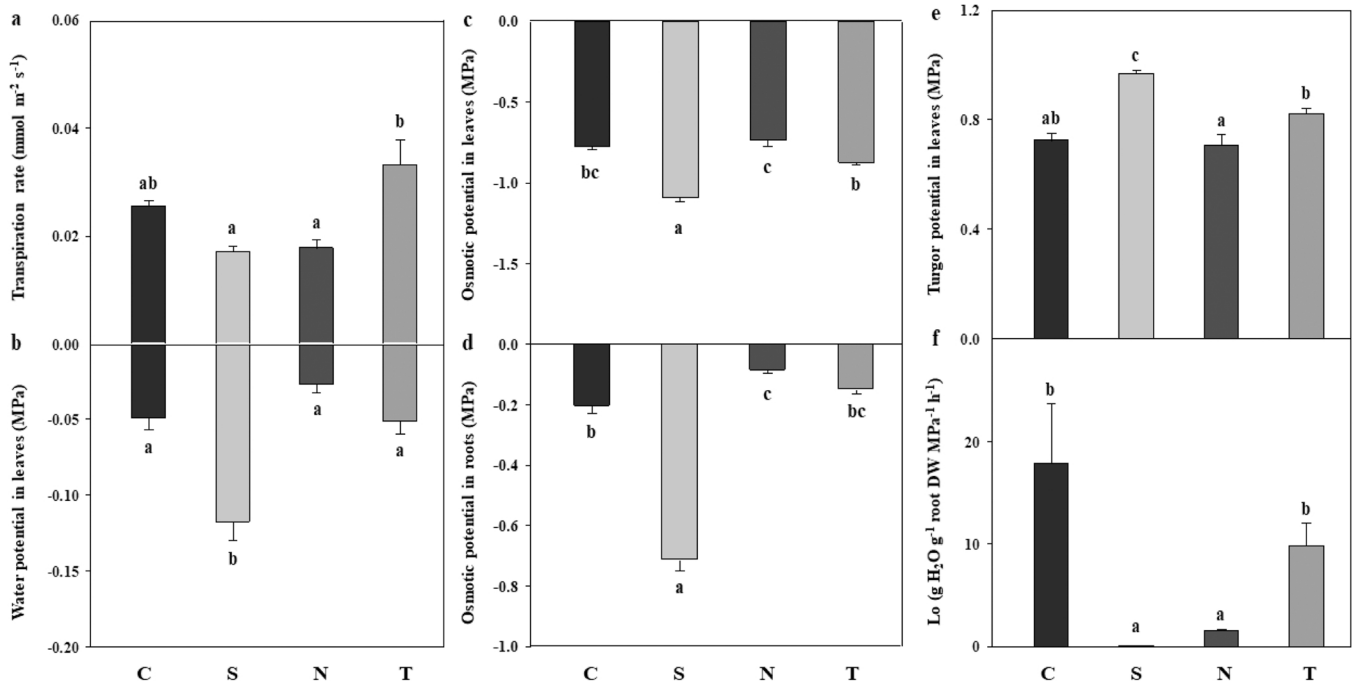

Fig. 2. Physiological parameters: transpiration rate (a), water potential in leaves (b), osmotic potential in leaves (c), osmotic potential in roots (d), turgor potential in leaves (e) and L_o (f) for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 4 samples, each one being an independent plant with the same treatment. Different letters indicate significant differences ($p < 0.05$) between the control and treatments according to a *post hoc* Tukey's multiple comparison test.

treatments. The osmotic potential in leaves (Fig. 2c) was also decreased in the salinity treatment with respect to the other treatments, this diminution is related to an increment in solutes accumulation in the cells. The osmotic potential in roots (Fig. 2d) was more negative in the salinity treatment than in the other treatments and was enhanced in the deficient nutrition treatment with respect to the control. The turgor potential in leaves (Fig. 2e) was highest (significantly so) in the salinity treatment. The osmotic root hydraulic conductance (L_o) (Fig. 2f) was significantly decreased in the salinity and deficient nutrition treatments.

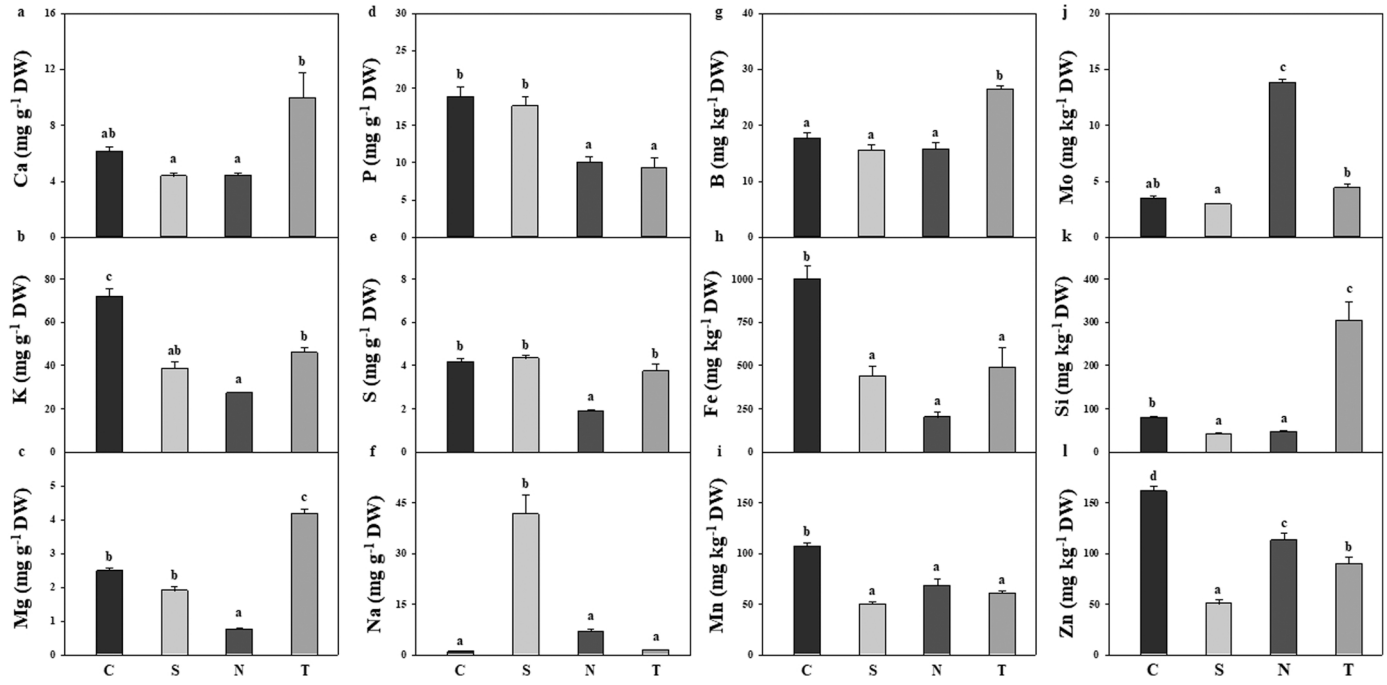

Fig. 3. Mineral concentrations in the root for the control (C) and the salinity (S), deficient nutrition (N) and temperature (T) treatments: macronutrients (Ca, K, Mg, P and S) (a-e), Na (f) and micronutrients (B, Fe, Mn, Mo, Si and Zn) (g-l). Each value represents the mean of 4 total roots, each one being an independent plant with the same treatment. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

3.2. Mineral concentrations

The mineral concentrations, on a dry weight basis, in the melon roots are shown in Fig. 3. Calcium (Ca) (Fig. 3a) showed no significant differences from the control with any stress applied. Potassium (K) (Fig. 3b) was significantly lower in all the treatments (salinity, deficient nutrition and high temperature) with respect to the control. Magnesium (Mg) (Fig. 3c) was significantly higher at high temperature, and significantly lower in the deficient nutrition treatment, than in the other treatments. Phosphorus (P) (Fig. 3d) showed a significant decrease in its concentration in the deficient nutrition and high temperature treatments with respect to the control and salinity treatments. The sulphur (S) concentration (Fig. 3e) in the deficient nutrition treatment was significantly lower than in the other treatments. Sodium (Na) (Fig. 3f) was significantly higher in the salinity treatment with respect to the other treatments. Boron (B) (Fig. 3g) was significantly higher in the high temperature treatment with respect to the other treatments. The iron (Fe) (Fig. 3h) and manganese (Mn) (Fig. 3i) concentrations were significantly lower in all treatments with respect to the control. The molybdenum (Mo) (Fig. 3j) concentration was highest in the deficient nutrition treatment. Silicon (Si) (Fig. 3k) showed a significant decrease in concentration in the salinity and deficient nutrition treatments and a significant increase in the high temperature treatment, relative to the control. The zinc (Zn) (Fig. 3l) concentration was significantly lower in all three treatments with respect to the control.

The mineral concentrations, on a dry weight basis, in the melon leaves are shown in Fig. 4. Ca (Fig. 4a) showed a significant decrease in concentration in the salinity treatment, while K (Fig. 4b) showed a significant decrease in the deficient nutrition treatment, with respect to the control. Mg (Fig. 4c) was highest in the high temperature treatment, and significantly lower in the salinity treatment with respect to the control and high temperature treatments. Root P (Fig. 4d) was highest in the control plants, while S (Fig. 4e) was lowest, and Na (Fig. 4f) was highest, in the salinity treatment. B (Fig. 4 g) showed a significant increase in concentration in the deficient nutrition and high temperature treatments in comparison to the control and salinity treatments, while leaf Fe (Fig. 4h) did not show any significant differences among the treatments. The Mn (Fig. 4i) concentration was significantly lower in the salinity treatment with respect to the control, whereas Mo (Fig. 4j) was significantly increased by the deficient nutrition treatment with respect to the others. The Si (Fig. 4k) concentration was highest in the high temperature treatment, while the Zn (Fig. 4l) concentrations in the salinity and deficient nutrition treatments were significantly lower than in the control.

3.3. Analysis of PIPs

The expression levels of the 12 known PIPs in melon were quantified in roots (Fig. 5) and leaves (Fig. 6). In roots, *PIP1.1* (Fig. 5a) and *PIP1.2* (Fig. 5b) showed a significant increase in expression in the high temperature treatment, with respect to the others. A common pattern found was a significant decrease in expression in all the treatments, with respect to the control, for *PIP2.1* (Fig. 5c), *PIP2.5* (Fig. 5g) and *PIP2.6* (Fig. 5h). *PIP2.2* (Fig. 5d) had a significant increase in expression in the deficient nutrition and high temperature treatments with respect to the control. *PIP2.3* (Fig. 5e), *PIP2.4* (Fig. 5f) and *PIP2.7* (Fig. 5i) expression did not differ significantly among the treatments, while *PIP2.8* (Fig. 5j) showed a significant reduction in the deficient nutrition treatment with respect to the control and salinity treatments. In the deficient nutrition and high temperature treatments *PIP2.9* (Fig. 5k) and *PIP2.10* (Fig. 5l) expression was significantly lower than in the control and salinity treatments.

In the leaf, fewer significant differences between the aquaporins expression levels were found. Most aquaporins showed no changes in expression, as was the case of *PIP1.2* (Fig. 6b), *PIP 2.1* (Fig. 6c), *PIP2.2* (Fig. 6d), *PIP2.3* (Fig. 6e), *PIP2.4* (Fig. 6f), *PIP2.5* (Fig. 6g), *PIP2.8* (Fig. 6j) and *PIP2.10* (Fig. 6l). The most notable results were for *PIP1.1* (Fig. 6a), which showed a significant increase in expression under salinity with respect to the other treatments, *PIP2.6* (Fig. 6h), which had a significant increase in expression at high temperature relative to the

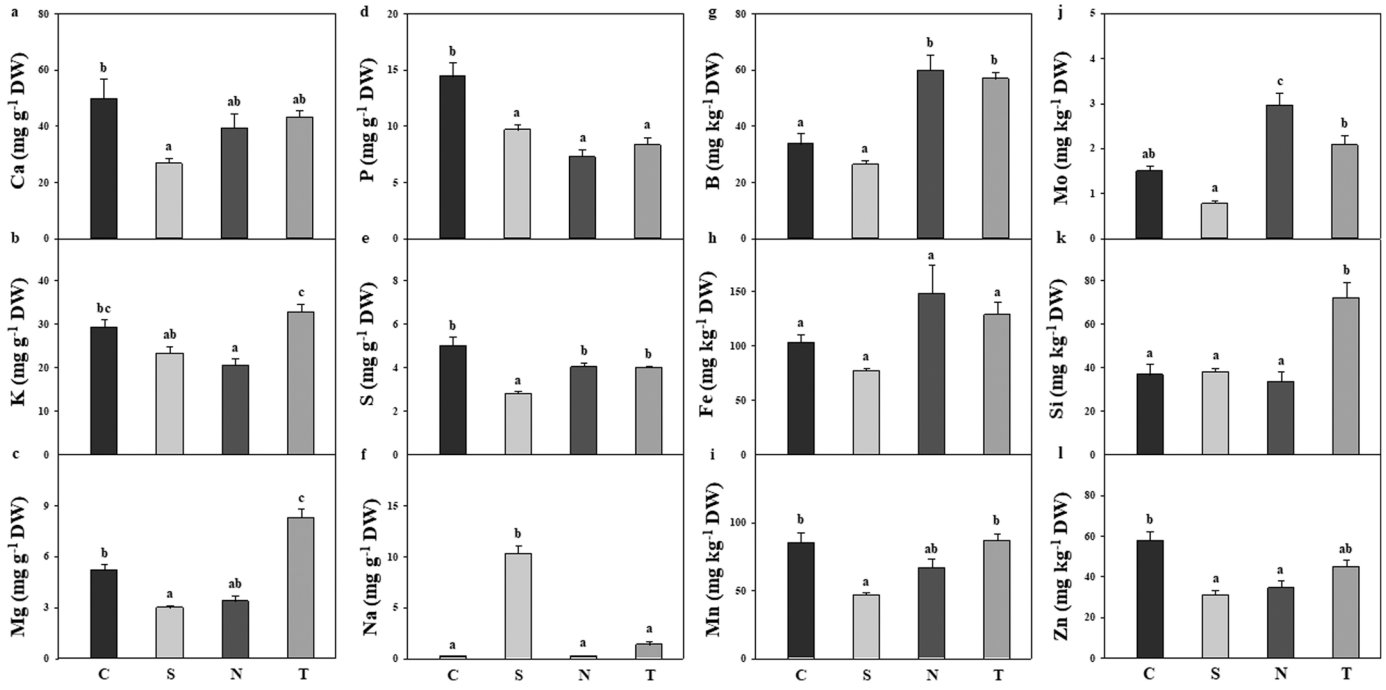

Fig. 4. Mineral concentrations in the leaf for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments: macronutrients (Ca, K, Mg, P and S) (a-e), Na (f) and micronutrients (B, Fe, Mn, Mo, Si and Zn) (g-l). Each value represents the mean of 4 samples, each one being the third and fourth leaves of each plant with the same treatment. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

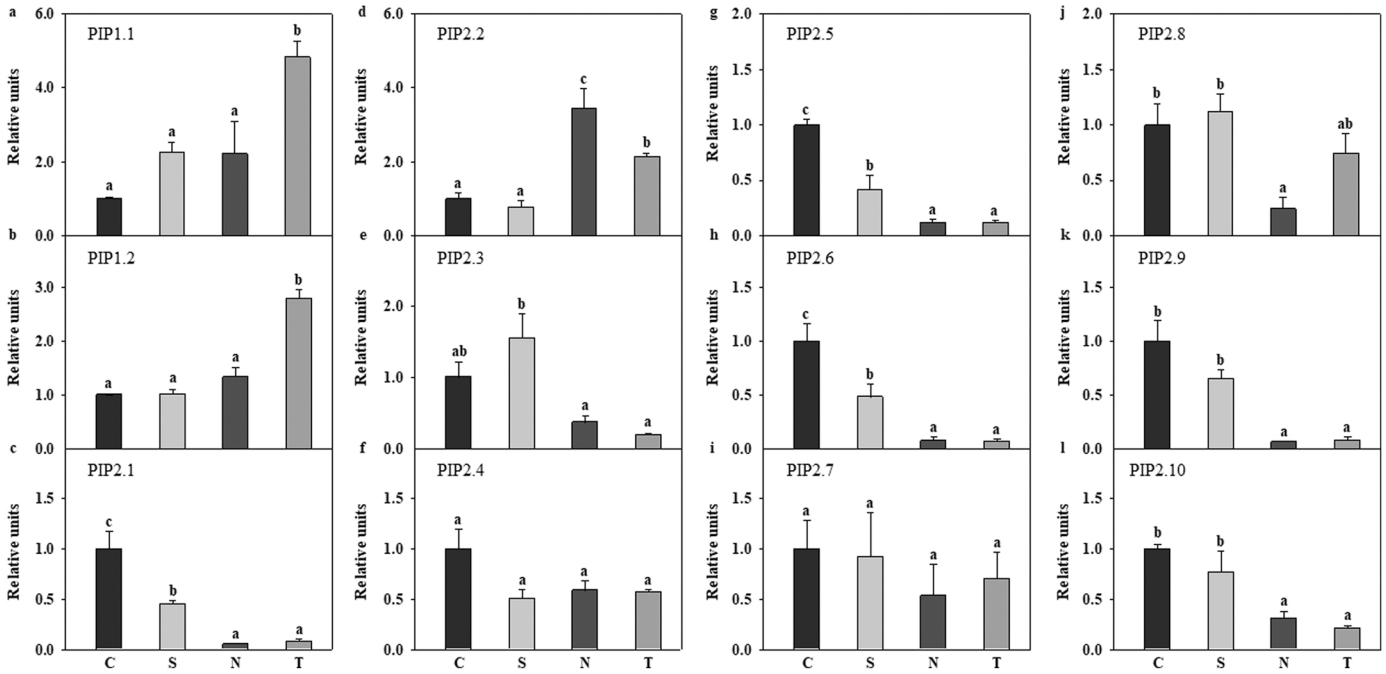

Fig. 5. Relative expression of PIPs in the root for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 roots from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

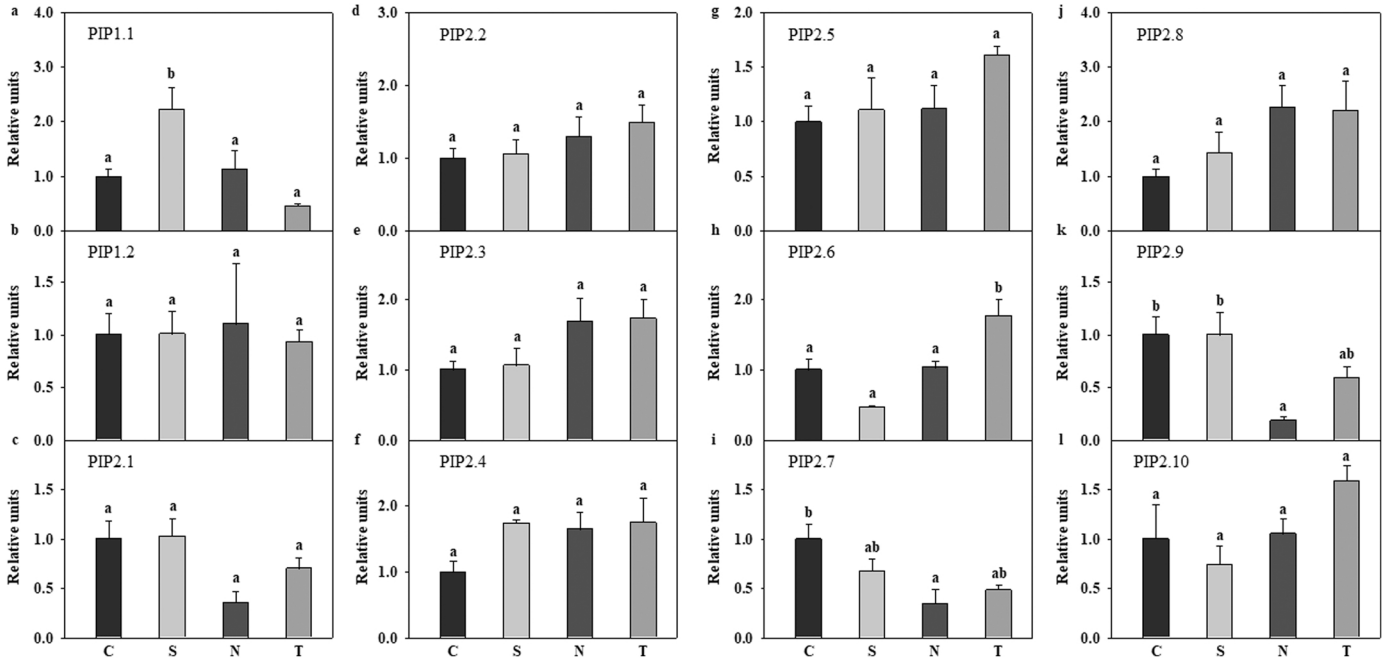

Fig. 6. Relative expression of PIPs in the leaf for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 third and fourth leaves mix from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

other treatments, and *PIP2.7* (Fig. 6h) and *PIP2.9* (Fig. 6k), which had a significant decrease under nutrient deficit with respect to the control.

3.4. Analysis of TIPs

The expression of the eight known TIPs in melon plants was detected and quantified in roots (Fig. 7) and leaves (Fig. 8). In roots, the expression of most of the TIPs was reduced by the treatments. The only exceptions were: TIP1.1 (Fig. 7a), which showed a significant increase in expression in the salinity and high temperature treatments with respect to the control and deficient nutrition treatments, and *TIP2.2* (Fig. 7e) and *TIP4.1* (Fig. 7g), which were unaffected by salinity but showed decreased expression under nutrient deficiency and at high temperature with respect to the control. The expression of the other genes [TIP1.*2* (Fig. 7b), TIP1.*3* (Fig. 7c), TIP2.1 (Fig. 7d), TIP3.*1* (Fig. 7f) and TIP5.*1* (Fig. 7h)] was highest in the roots of the control plants.

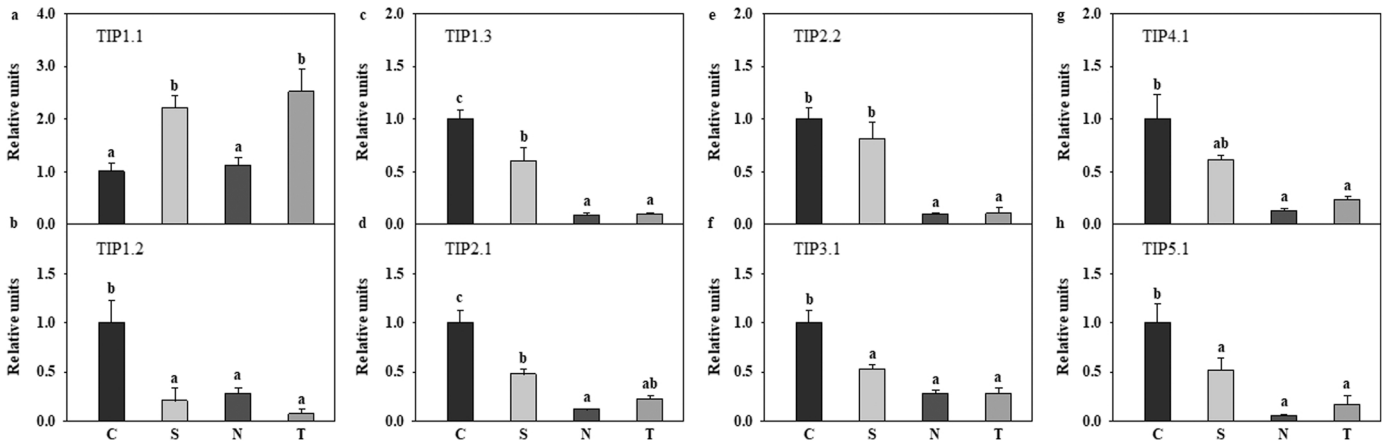

Fig. 7. Relative expression of TIPs in the root for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 roots from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

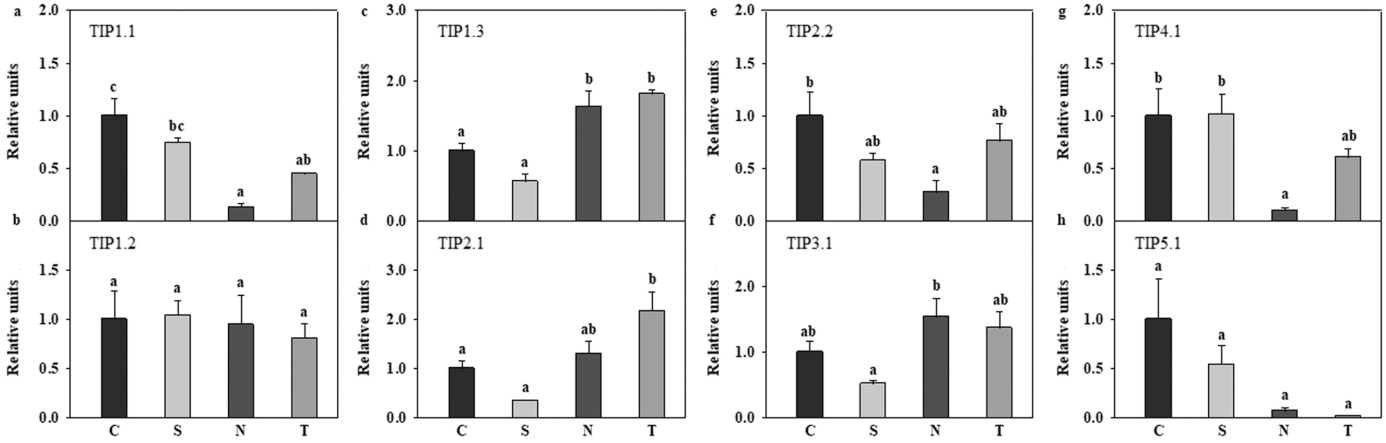

Fig. 8. Relative expression of TIPs in the leaf for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 third and fourth leaves mix from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

In leaves, TIP1.1 (Fig. 8a) showed significant decreases in expression in the deficient nutrition and high temperature treatments, relative to the control, while TIP1.2 (Fig. 8b), TIP3.1 (Fig. 8f) and TIP5.1 (Fig. 8h) did not vary significantly among the treatments. TIP1.3 (Fig. 8c) had a significant increase in expression in the deficient nutrition and high temperature treatments with respect to the control and salinity

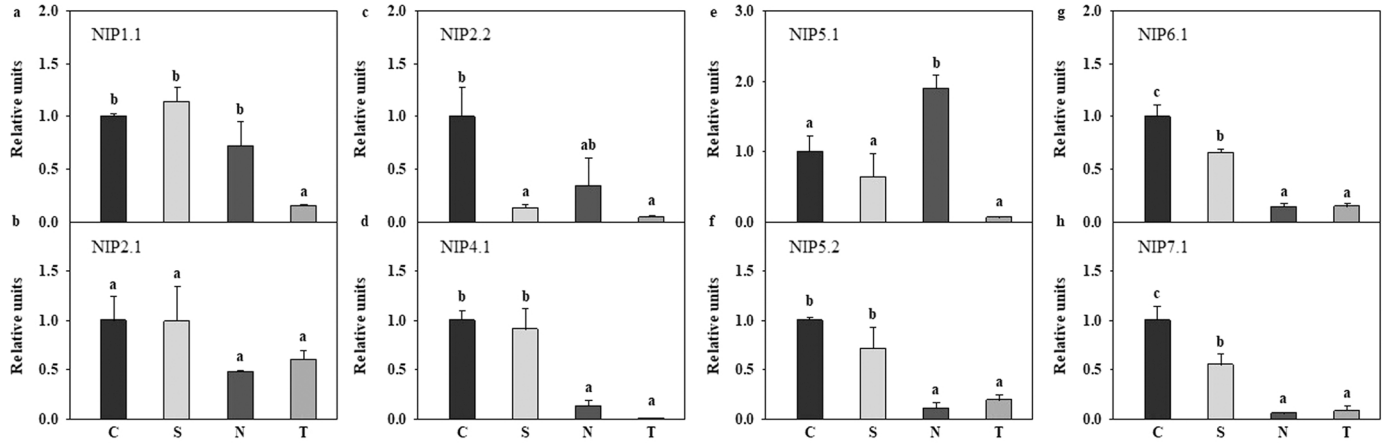

Fig. 9. Relative expression of NIPs in the root for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 roots from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

treatments. Relative to the control, TIP2.1 (Fig. 8d) had an increment in the high temperature treatment, while TIP2.2 (Fig. 8e) and TIP4.1 (Fig. 8g) showed a decrease in expression under nutrient deficiency.

3.5. Analysis of NIPs

The expression of the eight known melon NIPs was detected and quantified in roots (Fig. 9) and leaves (Fig. 10). In roots, *NIP1.1* (Fig. 9a) expression was lowest in the high temperature treatment, while *NIP2.1* (Fig. 9b) expression did not vary significantly among the treatments. *NIP2.2* (Fig. 9c) showed a significant decrease in expression in the salinity and high temperature treatments with respect to the control, while *NIP4.1* (Fig. 9d) and *NIP5.2* (Fig. 9f) were significantly decreased in the deficient nutrition and high temperature treatments with respect to the control and salinity treatments. *NIP5.1* (Fig. 9e) was the only aquaporin gene that showed increased expression under nutrient deficiency, relative to the control, salinity and high temperature treatments, while *NIP6.1* (Fig. 9g) and *NIP7.1* (Fig. 9h) expression were highest in the control.

In leaves, *NIP1.1* (Fig. 10a) showed a significant decrease in expression in the salinity treatment with respect to the other three treatments, and a significant increase in the deficient nutrition and high temperature treatments relative to the control. The expression of *NIP2.1* (Fig. 10b), *NIP4.1* (Fig. 10d) and *NIP5.2* (Fig. 10f) in leaves was not significantly affected by any treatment. With respect to the control, *NIP2.2* expression (Fig. 10c) showed a significant decrease in the salinity treatment, *NIP5.1* (Fig. 10e) had a significant increase in the high temperature treatment, *NIP6.1* (Fig. 10g) showed a significant increase in the salinity treatment and *NIP7.1* (Fig. 10h) had a significant decrease in the deficient nutrition and salinity treatments.

3.6. Analysis of SIPs and XIP

The expression of the two SIPs known in melon was detected and quantified in roots (Fig. 11a, b) and leaves (Fig. 11c, d). The expression of XIP1.1 was not detected (data not shown). In roots, *SIP1.1* (Fig. 11a) had a significant decrease in expression in all treatments with respect to the control, while *SIP2.1* (Fig. 11b) showed a significant decrease in expression in the deficient nutrition and high temperature treatments relative to the control and salinity treatments. In leaves, *SIP1.1* (Fig. 11c) and *SIP2.1* (Fig. 11d) had significantly lower expression under nutrient deficiency with respect to the control.

4. Discussion

The modifications of the measured parameters are consistent with the different types of stress applied, so we will analyse the different responses of the melon plants to the different stresses one by one. Despite this, some generalities in the responses of the melon plants to the environmental stresses were apparent.

As biomass production is the most important marker of stress (Poorter et al., 2009), we expected a significant reduction with our stress treatments. However, we only observed a decrease in weight of the aerial parts in the deficient nutrition treatment. This could be due to the fact that melon is a moderately salinity-tolerant crop species (Akrami and Arzani, 2019; Franco et al., 1997) and seems to be very well adapted to high temperature conditions, with no change in plant weight. In fact, there are some similar species like water melon that show better growth at higher temperatures (35ºC, with respect to 25 ºC) (Rivero et al., 2001). This tolerance of moderate levels of salinity and high temperatures is due to various adaptations of the plant, as reflected in the variations in physiological parameters and the expression of aquaporins genes, as described below.

Regarding the physiological parameters, the transpiration rates in the different treatments did not show a significant difference from the control. The regulation of leaf transpiration is sometimes less crucial than root water uptake regulation in the prevention of stress injury (Aroca et al., 2012). The other physiological parameters were modified according to the specific stress conditions imposed.

In this study, it is shown how the expression of aquaporins is modulated in roots and leaves as a consequence of the abiotic stresses applied. The large number of isoforms and their different functions make it difficult to find general patterns of behaviour beyond a general decrease in expression in the roots of stressed melon plants, with a few exceptions as *TIP1;1* in salinity treatment, *PIP2.2*, *TIP1;3*, *NIP1;1* in nutrient deficiency treatment and *PIPs1*, *PIP2;2, TIP1;1, TIP1;3 and TIP2;1* in high temperature treatment. The treatments mainly affected the aquaporins of the roots, where we found 58 significant changes with respect to the control for the stresses studied, 51 significant decreases *versus* 7 increases. Leaf aquaporin expression was less affected: there were 21 significant changes with respect to the control, 12 decreases and 9 increases.

Root aquaporins are closely related to the root hydraulic conductivity (Aroca et al., 2012; Porcel et al., 2018) and PIPs are the main water transporting aquaporins (Yaneff et al., 2015). In line with this, we can see a relationship between the decrease in expression of some PIP isoforms in roots in all treatments (*PIP2;1, PIP2;5* and *PIP2;6*) and the L_0 parameter in the salinity and nutritional treatments, while in the high

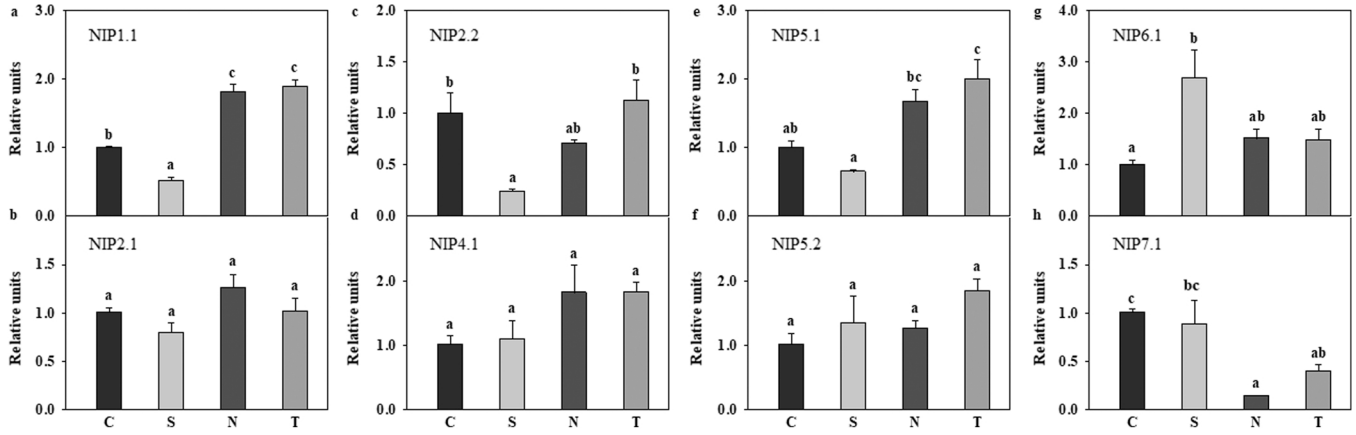

Fig. 10. Relative expression of NIPs in the leaf for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 third and fourth leaves mix from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

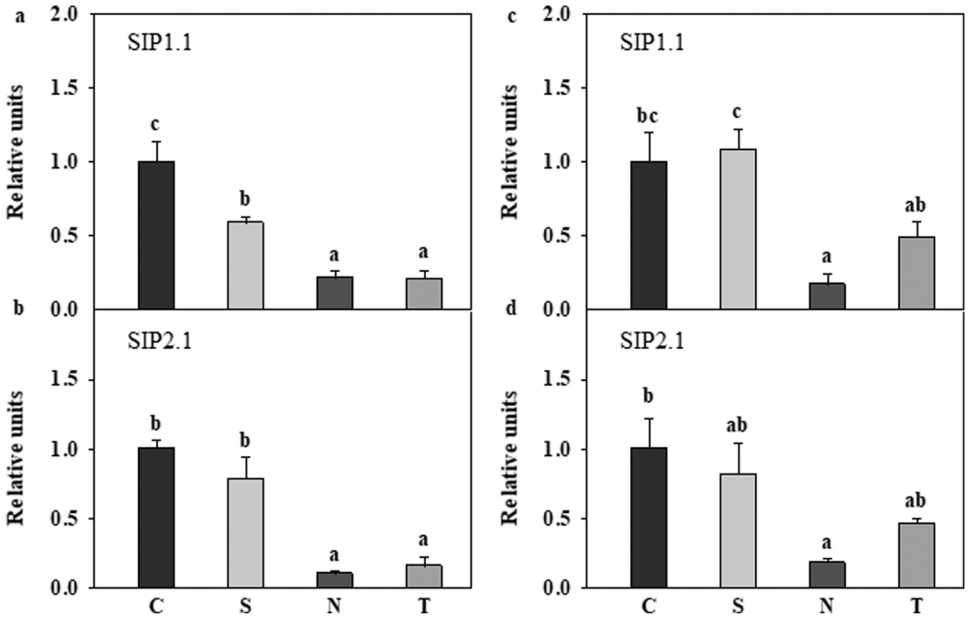

Fig. 11. Relative expression of SIPs in the root (a, b) and leaf (c, d) for the control (C) and the salinity (S), deficient nutrition (N) and high temperature (T) treatments. Each value represents the mean of 3 roots or 3 third and fourth leaves mix from 3 different plants with 3 technical replicates of each measurement. Different letters indicate significant differences ($p < 0.05$) between the control and the treatments according to a *post hoc* Tukey's multiple comparison test.

temperature treatment, despite decreased expression of these three isoforms, the maintenance of hydraulic conductivity could be related to the overexpression of PIP1s and its hetero-tetramerization (Zelazny et al., 2007; Zhu et al., 2019). Despite this, the implication of these three isoforms in the response to environmental stress seems obvious in melon plants. Also, it should be noted that most of the PIPs were unaffected in leaf tissues, which clearly indicates the important role of root water transport control in the abiotic stress response in melon plants.

Within the other groups of aquaporins, only three TIPs isoforms and one NIP isoform had the same pattern within all treatments. All other aquaporins presented differences in behaviour among the treatments, indicating their implications in each abiotic stress, as will be discussed later in the corresponding subsections. As these groups of aquaporins play their most important roles in the maintenance of osmotic pressure, turgor and nutrient exchange, these results point to the importance of the nutrient balance when coping with environmental stresses.

The mineral content was affected by all the treatments applied. All the treatments mainly affected the roots, modifying their mineral content, with 23 significant changes in the elements studied, 18 significant decreases compared to 5 increases. In general, some elements were notably deficient in the roots of the treated plants, such as K, Mn and Zn in all treatments, while others, like B, Mo and Ca, were mostly unaffected or even increased (B at high temperature and Mo under nutrient deficiency). The behaviour of Mg, P, S and Si differed depending on the stress treatment. Notably, in our three treatments, the Fe concentration only declined in the roots, pointing to the redirection of the available Fe from the roots to the leaves, where there were no significant changes in the Fe concentration, probably due to the high importance of Fe in photosynthesis (Terry, 1980). Interestingly, only P was diminished in the leaves by all the treatments, while the concentrations of all the other nutrients were modified according to the specific stress applied.

The interrelations between the concentrations of elements and the expression of aquaporins are very complex. In our experiment we were able to find some relationships already reported by other authors. Regarding B, some studies highlight the interaction of B uptake by some aquaporins with the enhancement of tolerance of different abiotic stresses (Porcel et al., 2018). In our work all the treatments maintained or even increased the concentration of B in both roots and leaves. In all cases we can observe an increase in expression of some aquaporins which have been directly related with B transport, such as *NIP1;1, NIP5;1* or *NIP6;1* (Lopez-Zaplana et al., 2020b). Their implication in the maintenance of B levels has been proved in other species (Kato et al., 2009; Noronha et al., 2020; Zhang et al., 2019). Furthermore, *TIP1;3* had the same pattern as the B concentration in leaves and its capacity to transport B has been suggested (Lopez-Zaplana et al., 2020b). Si is another important element for Cucurbitaceae family that could be transported by some NIPs (Kumawat et al., 2021). In a previous study, *C. melo NIP2;1* and *NIP2;2* were characterised as possible Si transporters based on the comparison of their residues with those of other aquaporins that have been proven to be capable of transporting Si (Lopez-Zaplana et al., 2020b). In our study, few coincidences were found between the Si-transporting aquaporins expression and the level of this micronutrient in the tissues, suggesting that activity, more than regulation of expression or an interaction with other nutrients and transporters, should be taking place. Aquaporins play a significant role in N uptake and mobilisation, mainly in the form of ammonia (NH_3) and urea (Loqué et al., 2005; Wang et al., 2008), and it is well known that N stress produces the greatest impacts on the continuity of plant growth (Drenovsky et al., 2012). Hence, the aquaporins involved in the mobilisation of N throughout the whole plant should have important roles in plant growth maintenance under abiotic stress, as will be described below.

For the rest of the aquaporins, their capacity to transport directly the nutrients studied here is unknown, but there are other links between the expression of their genes and some mineral nutrient concentrations. As an example, low levels of P have been shown to modulate the expression of several PIP aquaporin genes in *Leymus chinesis* L. (Li et al., 2020). All the stresses we applied yielded lower tissue levels of P, and the decrease in expression of some PIPs could be implicated in this, principally in root tissue. Similarly, in K deficiency, a decrease in expression of root PIP2s has been seen in *Hordeum vulgare* L. (Coffey et al., 2018). In our case, all the stresses applied generated a diminution in K in roots and, in parallel, a strong downregulation of different PIPs: *PIP2;1*, *PIP2;*5 and *PIP2;6* in all treatments, and *PIP2;8*, *PIP2;9* and *PIP2;10* in the deficient nutrition and high temperature treatments. Thus, the lower levels of K could be implicated in the decrease in expression of some of our PIPs genes. Zinc is a very important microelement for cucurbits since they are great accumulators of it (Gyulai et al., 2012). Zinc can modify the activity of aquaporins (Ariani et al., 2019; Gitto and Fricke, 2018; Németh-Cahalan

et al., 2007; Yukutake et al., 2009) and the transcription of aquaporins genes (Fatemi et al., 2020). Also, a correlation between *ZmPIP2;1* and a Zn-dependent metalloendopeptidase has been described (Yue et al., 2012) and in our study there was a significant decrease in the expression of *PIP2;1* in the roots in all treatments. In addition, a fall in the expression of several PIPs in *H. vulgare* L. has been described as a response to Zn deficiency (Gitto and Fricke, 2018). The possible role of Zn in the regulation of aquaporins expression in melon is a target which is currently under study (Lopez-Zaplana, unpublished data). Finally, a possible link between the decrease in Ca concentration and the decrease in *NIP1;1* and *NIP2;2* expressions has been described in *A. thaliana* (Maathuis et al., 2003). This could have been the case in the leaves under the salinity treatment, the only one which diminished the Ca concentration and the expression of both these aquaporins. In addition, Ca can directly affect the activity of aquaporins, regulating the gating of the pores directly or by activating phosphorylation cascades (Ji et al., 2017). The maintenance of Ca at high levels in all root tissues could modify the aquaporins functionality, affecting melon stress tolerance.

4.1. Salinity stress response

Saline stress is clearly reflected in the increased Na levels in both roots and leaves of the melon plants. Indeed, the osmotic potential in leaves and roots was significantly more negative in the salinity treatment than in the control. Following the same pattern, the hydric potential in the leaves was also significantly more negative in the saline treatment, all these results being in line with previous studies in *C. sativus* L. (Zhu et al., 2019), *Zea mays* L. (Hajlaoui et al., 2010) and *Olea europea* L. (Mousavi et al., 2008), which show a clear relationship between the potentials, the accumulation of solutes and saline stress (Hajlaoui et al., 2010; Mousavi et al., 2008; Zhu et al., 2019). This increase in solutes in the leaf cells and the increased flow of water towards them jointly produce an increase in cellular turgor, consistent with the growth maintenance of the melon plants under such conditions. Interestingly, the expressions of the water-transporting aquaporins in leaves were not modified under these conditions. The only exception was *PIP1;1*, whose expression nearly doubled in leaves under the saline treatment. This is similar to previous studies where the expression of this aquaporin also doubled under similar conditions (Jang et al., 2004). This increased expression of *PIP1.1* in leaves could be related to the maintenance of the transpiration rate under salinity stress in our plants, as described previously (Aroca et al., 2006; Sade et al., 2009), showing the importance of this aquaporin in salinity stress.

In roots, a diminution of the osmotic potential (increased solutes accumulation) and a significantly lower L_o were found in the salinity treatment, with respect to the control, pointing to a decrease in water transport by roots according to previous studies (López-Berenguer et al., 2006; Zekri and Parsons, 1989). In the literature, this reduction in L_o has commonly been related with a diminution of aquaporins expression and activity, and has been interpreted as a defense mechanism based on the "closure" of the cells to protect them from the water loss due to the osmotic stress (Aroca et al., 2012). In our study, despite the fact that we did not find significant changes with respect to biomass in the saline treatment, we did find a lot of changes in aquaporin expression in roots, demonstrating the good adaptation and the implication of aquaporins of melon under this type of stress. With our treatment with 50 mM NaCl, a reduction in the expression of most aquaporins (*PIP2;1, PIP2;5, PIP2;6, TIP1;2, TIP1;3, TIP2;1, TIP3;1, TIP4;1, TIP5;1, NIP2;2, NIP6;1, NIP7;1, SIP1;1*) was observed. This general decrease in expression correlated with a decrease in water transport by roots (López-Berenguer et al., 2006). Similar results have been described in *C. sativus* L. (a species closely related to melon), where most of the root aquaporins showed decreased expression in the root tissue when applying long-term salinity treatments of 50 and 75 mM NaCl (Zhu et al., 2019). As PIPs are mainly responsible for water uptake in roots, the fall in *PIP2;1*, *PIP2;5* and *PIP2;6* expression seems to be crucial in the L_o reduction under such conditions.

In a long-term salinity treatment (100 mM NaCl for 10 days), in *A. thaliana*, the PIP1s expression levels were similar to the control, but those of the PIP2s were, in general, lower (Martínez-Ballesta et al., 2015), as occurred in our study. However, in another study with *A. thaliana*, a general decrease in aquaporin expression with 2-h and 5-h salinity treatments (80 mM NaCl) was seen, but for longer treatments the expression of most of the genes was increased (Maathuis et al., 2003). The recovery of the activity of aquaporins under long-term high-salinity stress has been described in some species as an adaptation to a reduced apoplastic pathway, which is compensated by an increment in the cell to cell pathway (Aroca et al., 2012). In future studies, it would be interesting to use higher concentrations of NaCl or different durations of treatments and test the changes in expression.

However, despite this general decrease in expression, a significant increase in the expression of *TIP1;1* in the roots was observed, which is consistent with the implication of this aquaporin in the adaptation to salinity stresses in the root tissue (Zhu et al., 2019). *TIP1;1* is the most expressed TIP in *C. melo* (Lopez-Zaplana et al., 2020b), and it could be implicated in the maintenance of root cell turgor, the accumulation of Na in the vacuole or the uptake of water (Shabala et al., 2020). Interestingly, also in roots, there were only two TIPs that were not down-regulated, *TIP2;2* and *TIP4;1*, both of them characterised as ammonia transporters based on their sequences (Lopez-Zaplana et al., 2020b). This points to the mobilisation of N compounds, which could have maintained the normal growth of the plants under the salinity stress applied in our study. Furthermore, *ZmTIP2;2* and *ZmTIP4;1* were related to some Na exchangers in previous work in *Z. mays* (Yue et al., 2012), indicating a role in the salinity adjustment of the plants.

The general reduction in most other TIPs (tonoplast transporters) points again to the preservation of nutrients inside the root cells as a response to salinity stress, which is consistent with the diminished osmotic pressure in root cells and coincides with the reduced transport of nutrients to shoots, where the levels of most of them (Ca, Mg, P, S, Mn and Zn) were diminished. Salinity stress is due principally to excessive concentrations of Na inside the plants, and is related to nutritional stress that is a consequence of decreased transport of nutrients like K and Ca as a result of competition for transport (Acosta-Motos et al., 2017). Salinity also affects the uptake of other elements such as B, Ca, Mg or K (Fageria et al., 2011). In our study, the levels of Ca and Mg were significantly lower in the leaf with respect to the control, while K was significantly lower in the roots, pointing to its competition with Na. Also, a diminution of almost all the micronutrients (Fe, Mn, Si and Zn) was found in the roots, while B and Mo were unaffected in both the roots and leaves. Regarding the NIPs (related to metalloids transport), expression of the *NIP2;2* Si transporter was decreased in the roots, which explains the decline in Si uptake. Also in leaves, *NIP2;2* expression diminished. In this case, a relationship between ZmNIP2;2 and an S transmembrane transporter (SULTR1;3) has been found and suggests the involvement of NIP2;2 in sulphate transmembrane transport in developing leaves (Yue et al., 2012). A possible link between S and this aquaporin can be seen also in our study, where both S and *NIP2;2* were only diminished in the leaves of plants exposed to salinity.

Also, NIP6;1, related to B transport (Lopez-Zaplana et al., 2020b), was also diminished in the roots under salt stress, while the main B transporters in melon roots (NIP5s) were unaffected and, indeed, *NIP6;1* was more abundant in leaf tissue. It has been described in *Z. mays* that the diminution of Lsi6 (ZmNIP6;1) activity did not affect the uptake of B but did alter its distribution to the shoots, with Lsi1 (ZmNIP5;1) being mostly responsible for the entrance of B into the plant (Mitani et al., 2009). Our data correlate with the presence of this nutrient in both tissues. Finally, the presence of Mo is closely related to the assimilation of N and that is probably why it was not affected by the stress, as the N metabolism seemed to continue, according to the plant weight and aquaporin gene expression data.

Despite having found numerous changes in the expression of

aquaporins, it should be noted that these changes are not always correlated with the levels of proteins. In previous articles, it has been shown how, despite no changes in *PIP2;1* expression, the protein levels increased during a short term salinity stress (Suga et al., 2002). Furthermore, in barley in long term salinity stress it can be found the completely opposite response, a decrease of transcript levels of *PIP2;1*, but not changes in protein levels (Katsuhara and Shibasaka, 2007).

4.2. Nutrients deficiency response

The main effect of the nutrients deficiency treatment was the diminution of the weight of melon plants. Physiological parameters were not affected in the leaves in these conditions, but were strongly affected in the roots.

In the roots of the nutrient deficient plants, a significantly lower L_o and enhanced osmotic pressure were found. This indicates that the accumulation of solutes in the cells and water transport were diminished in roots. Some studies show that a deficiency of certain elements - P (Li et al., 2009), S or N (Clarkson et al., 2000) - could induce a reduction in root L_o. In our study, the nutrient deficiency decreased the concentrations of most of the nutrients in the roots (Fe, K, Mg, Mn, P, S, Si and Zn).

In consonance with those results, the expression of most of the aquaporins genes was diminished in the roots. Most of the water-transporting aquaporins were downregulated, including *PIP2;1*, *PIP2;5* and *PIP2;6* (as in the other stress treatments), *PIP2;8*, *PIP2;9* and *PIP2;10* (described as the main water transporter in melon roots (Lopez-Zaplana et al., 2020b)). This diminution explains the reduction in L_o. There is an exception, *PIP2;2*, whose expression in roots was increased; this has been described in other species such as *H. vulgare*, as a response to K deficiency (Coffey et al., 2018) and points to an important role in melon plants under stress.

Most of the TIPs, NIPs and SIPs were also downregulated under nutrient deficiency. The exception was *NIP5;1*, mainly responsible for B uptake by roots, since its expression was enhanced. Increased expression of *NIP5;1* has been shown in other studies in which the expression of AtNIP5;1 increased in response to B deficiency (Gómez-Soto et al., 2019; Takano et al., 2006). Indeed, in our work the B level was maintained in the roots and even increased in the leaves. Regarding this increment, there are two aquaporins that, based on their selectivity filters and homologous analysis, are related in some manner to B transport (Lopez--Zaplana et al., 2020b): *TIP1;3* and *NIP1;1*. Both were upregulated in the leaf tissues, being the only aquaporins upregulated in the leaves under such conditions. These aquaporins are currently being tested to corroborate this function (Lopez-Zaplana unpublished data).

By contrast, in the leaves, the expression levels of the main water-transporting PIPs were not modified. Only some PIPs with very low expression in melon leaves (Lopez-Zaplana et al., 2020b) had their levels reduced, while some TIPs and NIPs isoforms were downregulated, namely *TIP1;1* (the main water transporter in the tonoplast) and the N compounds transporters *TIP2;2*, *TIP4;1* (related to ammonia transport) and *NIP7;1* (related to urea transport) (Lopez-Zaplana et al., 2020b). As mentioned above, TIP1;1 is implicated in the maintenance of root cell turgor and, therefore, its effects could influence plant growth. Also, a decrease in N transport directly affects plant growth, paralyzing it, which is consistent with the halving of the weight of the aerial parts in the nutrient deficiency treatment.

Most of the other nutrients (Ca, Mg, S, Na, Fe, Mn and Si) were unaffected in leaves, while K and P decreased in both roots and leaves, K being lower in the leaves compared to the other stress treatments. Furthermore, the Na and Ca levels were similar to those of the control, in both tissues, while Mo had a significantly higher concentration in both roots and leaves.

In this sense, a decline in K could strongly affect the physiology of the plant as it is involved in many physiological processes (Pettigrew, 2008), while Ca, Na and K are closely related in plant physiology. In K starvation, Ca stimulates the transporters involved in K uptake and mobilisation and it also plays a role in stress signaling events (Bárzana and Carvajal, 2020). Also, it has been shown that Na, when K is limited, compensates for the lost functions due to its chemical similarity (Adams and Shin, 2014). Given that the physiological parameters were maintained in the melon leaf tissues, the maintenance of these two elements, Ca and Na, could work to conserve these functions under K deprivation in the leaves.

For its part, Mo is known to protect against abiotic stresses and the deficit of nutrients (Shoaib Rana et al., 2020) and it increased in the roots and leaves in the deficient nutrition treatment, demonstrating that it could play an important role against this abiotic stress in melon plants also. Furthermore, Mo is closely related to ABA biosynthesis (fundamental in stress conditions) and to the activity of N metabolism enzymes such as nitrate reductase (NR) (Kaiser et al., 2005). The need for N, which directly affects growth, prompts the plant to keep its metabolism active and this could force the increment in Mo in an attempt to compensate the effects of the N deprivation, increasing the N-assimilation through nitrogenase or NR activity (Imran et al., 2019; Muhammad Shoaib et al., 2020).

4.3. High temperature response

High temperature was the stress that produced the fewest changes, with no effects on the physiological parameters measured, while a strong effect could be seen at the level of aquaporins gene expression and for some specific nutrients, pointing to important roles in the adaptation to high temperature in melon plants.

The expression of the genes of the water-transporting aquaporins *PIP2;1*, *PIP2;5*, *PIP2;6*, *PIP2;9* and *PIP2;10* was reduced in the roots but water conductance was not. Interestingly, *PIP2;2* increased, as under nutrition deficiency, pointing again to its role in coping with environmental stresses in melon plants. However, L_o was not reduced, as happened with nutrition deficiency, and the main difference is that in this case *PIP1;1* and *PIP1;2* expressions was improved. The hetero-tetramerization of PIP2s and PIP1s together is essential for the transport of PIP1s to the plasma membrane and the water transport capacity of PIP2s increases in proportion to the amount of PIP1s (Zelazny et al., 2007). Thus, it seems that this co-expression may be relevant in maintaining water flow under high temperature stress. Furthermore, *TIP1;1*, the *TIP* gene that shows the highest expression in melon roots (Lopez--Zaplana et al., 2020b) and that encodes the main water transporter in the tonoplast, is also enhanced, favoring the flow of water within the cell. Thus, all these aquaporins together (PIP1s, PIP2;2 and TIP1;1) seem to have been able to maintain the water flow in the roots.

We can observe some expression patterns similar to those in other studies with *C. sativus*. First, there was an increase in the expression of *PIP1;2* in roots, as occurred with *CsPIP1;2* after 3 and 6 days of heat treatment (Zhu et al., 2019). In *Rhazya stricta* L., the response to heat treatment included an increase in the expression of the aquaporin genes *PIP1;2* and *PIP2;1* in the hours of high temperatures (Obaid et al., 2016). A temperature shock (between 38 °C and 46 °C for 15 days) similar to the one we applied was found to modify the expression of aquaporins genes in Brazilian sour orange and savage citrange, mainly in the first hours after the shock (Shafqat et al., 2021). In this case, the expression of *CsPIP2;4*, *CsTIP1* and *CsTIP2* increased (Shafqat et al., 2021). Other studies in *R. stricta* highlighted an increase in the expression of the aquaporins genes *PIP1;2* and *PIP2;1* in leaves in the hours of high temperatures (Obaid et al., 2016).

In leaf tissues, most of the PIPs were unaffected, according to the maintenance of the hydric potential, osmotic pressure, transpiration and turgor. The only exception was *PIP2;6*, an aquaporin that is predominant in leaf tissues in melon and has been suggested to have some unknown fundamental function (Lopez-Zaplana et al., 2020b). The results suggest its relevance to the tolerance of high temperatures.

The expression of all the *TIPs*, *NIPs* and *SIPs* genes was reduced in the roots, with two interesting exceptions, *NIP2;1* and *NIP5;1*. NIP2;1 has

been characterised in diverse plants as being responsible for Si uptake from the soil and for its involvement in Si translocation within the whole plant (Chiba et al., 2009; Jian et al., 2006; Kumawat et al., 2021; Mitani et al., 2009). The role of NIP5;1 as a B transporter has been seen in *A. thaliana* [75,80]. In parallel, in leaves, there was an increment in the expression of *TIP1;3*, *NIP1;1* and, again, *NIP5;1*, all of them related to some extent with B transport (Lopez-Zaplana et al., 2020b), as previously commented on, while *NIP2;1* was unaffected. Accordingly, B and Si increased significantly in both roots and leaves, indicating a whole-plant high temperature stress defence response. Also, Mg was increased in both roots and leaves, while only P had a significant decrease. An increase in Si could lessen the P deficiency (Zhang et al., 2019) in addition to increasing the resistance to high temperatures (Sivanesan et al., 2014) through stimulation of antioxidant systems, fortification of the cell wall and retention of water, among other mechanisms (Hu et al., 2020). In the same way, B and Mg increases could be related to the adaptation to high temperatures, as described in other studies with rice (Boaretto et al., 2020; Shahid et al., 2018). A regulatory effect of Mg for certain PIPs in *A. thaliana* has been suggested (Kourghi et al., 2017) and it has also been seen that some PIPs are linked to Mg transporters (Yue et al., 2012) but further studies are needed concerning the role of Mg in aquaporins regulation under stress in melon plants. All these results point to the importance of B, Si and Mg in high temperature tolerance in melon plants. The other aquaporin gene that was upregulated in melon leaves was *TIP2;1* (a N compound transporter), suggesting a role in the maintenance of the N metabolism and growth in these plants.

Furthermore, lower levels of K, P, Fe, Mn and Zn were found in the roots, while they were maintained in the leaves, suggesting a translocation effect. As described above, such diminution in roots could downregulate the expression of some aquaporins genes, while in leaves no differences were found - neither in the concentrations of these nutrients, nor in the regulation of most of the aquaporins - and this maintained the physiological parameters at the same levels as in control (non-stressed) plants.

5. Conclusions

Our results indicate that in melon plants aquaporins play an important role in the response to abiotic stress (salinity, deficiency of nutrients and high temperature) and interact with physiological processes and nutrients. In our study, there was only a decrease in biomass production in the nutrient deficit treatment, while the melon plants seemed to tolerate moderate levels of salinity and high temperatures. This could be due to various adaptations of the plants, as reflected in the variations in physiological parameters, nutrient content and gene expression of aquaporins. Of the water-transporting aquaporins, *PIP2;1*, *PIP2;5* and *PIP2;6* were downregulated in the roots by all the stresses studied here, correlating with the decline in water conductance; thus, they seem to be crucial in the response to environmental stress in melon plants. Interestingly, there was a diminution of K and Zn in roots and of P in leaves for all the stresses applied and this has been directly related with the diminution of expression of many PIPs isoforms in other species. The differences in the regulation of TIPs and NIPs depending on the treatment point to the importance of the nutrient balance to the plant's ability to cope with different environmental stresses. Also, the link between the concentration of B and the expression of genes of aquaporins involved in the transport of this element highlights the interaction of B uptake with the enhanced tolerance to abiotic stress in melon plants.

Under salinity stress we observed a generalised decrease in the expression of all root aquaporins, indicating that preservation of water and nutrients inside the root cells was the response. However, in the leaves, the increase in water flow, N mobilisation, accumulation of solutes inside the cells and increased cellular turgor were consistent with the growth maintenance of the melon plants under such conditions. Under nutrient deficit, there was a generalised decrease in the expression of aquaporins genes in the roots and leaves, affecting directly cell turgor and N metabolism and decreasing plant growth. Finally, the melon plants showed great tolerance of the high temperature stress applied with no effects on the physiological parameters measured, while a strong effect could be seen at the expression level of aquaporins and for some specific nutrients, pointing to their important roles in the adaptation to high temperature in melon plants.

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CRediT authorship contribution statement

Alvaro Lopez-Zaplana: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Nicolas Martinez-Garcia:** Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, **Micaela Carvajal:** Conceptualization, Writing – review & editing, Supervision, Validation, Project administration, Funding acquisition. **Gloria Bárzana:** Methodology, Investigation, Writing – review & editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2021.104759.

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