# The relationship between citrulline accumulation and salt tolerance during the vegetative growth of melon (*Cucumis melo* L.)

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**ABSTRACT**

Citrulline has been recently shown to behave as a novel compatible solute in the *Citrullus lanatus* (*Cucurbitaceae)* growing under desert conditions. In the present study we have investigated some aspects of the relationship which might occur in leaves of melon seedlings, also known to produce citrulline, between the capacity to accumulate this ureido amino acid and salt tolerance. With this end in view, salt-induced changes at the citrulline level have been compared in two melon genotypes exhibiting contrasted abilities to withstand the damaging effects of high salinity. Progressive salinization of the growing solution occurred at 23 days after sowing. The final 250 mmol/l external NaCl concentration was reached within 5 days and further maintained for 16 days. In response to this treatment, it was found that the citrulline amount increased in fully expanded leaves of both genotypes according to different kinetics. The salt tolerant genotype Midyat was induced to accumulate citrulline 4 days before the salt sensitive Yuva and as a consequence the final amount of this amino acid was twice higher in the former than in the latter. Compared with citrulline, the free proline level was found to be relatively low and the changes induced in response to the salt treatment exhibited different trends according to the genotypes under study. Thus at the end of the treatment mature leaves of the salt sensitive Yuva contained higher amount of proline than those of Midyat. The changes in the calculated molar ratio between citrulline and free proline suggested that salt tolerance might be associated with high values for this ratio and vice et versa for sensitivity. The interest of citrulline as a biochemical marker for salt tolerance of melon genotypes is discussed.

**Keywords:** salt tolerance; free amino acids; proline; biochemical markers; plant abiotic stress

World population is increasing at an alarming rate and is expected to reach about six billions by the end of year 2050. On the other hand food productivity is decreasing due to the effect of various abiotic stresses on crop plants (Mahajan and Tuteja 2005). Drought and salinity are becoming particularly widespread in many regions, and may cause serious salinization of more than 50% of all arable lands by the year 2050. Drought, salinity, extreme temperatures and oxidative stress are often interconnected, and may induce similar cellular damages (Wang et al. 2003). Plants respond and adapt to some extent to these stresses in order to survive. Drought and salinity tolerant plants accumulate various organic osmolytes, especially organic compatible solutes in response to osmotic stress (Rhodes et al. 2002). The primary function of compatible solutes is to maintain cell turgor and to take up more water from the soil (Wang et al. 2003). Compatible solutes fall into three major groups: amino acids (e.g. proline), quaternary and tertiary onium compounds (e.g. glycine betaine, dimethylsulfoniopropionate) and polyol/small sugars (e.g. mannitol, trehalose), all substances which are highly soluble in water (Rhodes et al. 2002). The compatible solutes can also act as free-radical scavengers and directly stabilize membranes and/or proteins (Wang et al. 2003).

Citrulline is a non-essential amino acid first identified from the juice of watermelon, *Citrullus lanatus* (Wada 1930) and then found to occur in other cucurbitaceous fruits, especially in the

rind, including bitter melon, cucumber, muskmelon, pumpkin, bottle gourd, dishrag gourd, and wax gourd (Rimando and Perkins-Veazie 2005). Kawasaki et al. (2000) reported that in the Bostwana desert, under the conditions of severe water deficit associated with strong sunlight and high temperatures, C4 plants turned to yellow, whereas wild watermelon, which is a C3 plant, still remained green and survived for a longer period. Survival under so harsh conditions is temporally associated with accumulation of the ureido amino acid citrulline in non-damaged leaves. The accumulation of citrulline in response to drought stress in wild watermelon is a unique phenomenon in C3 plants (Kawasaki et al. 2000). Akashi et al. (2001) and Yokota et al. (2002) reported that the accumulated citrulline could contribute to protect green tissues from the secondary oxidative stress induced under drought conditions because *in vitro* it behaves as a more potent hydroxyl radical scavenger than compatible solutes like mannitol, proline and glycinebetaine. Those last substances were not accumulated by leaves of watermelon growing under desert conditions. In spite of the fact that wild watermelon was not induced to accumulate citrulline in response to salinity (Kawasaki et al. 2000) we have hypothesized that citrulline itself and/or its metabolism could be involved as metabolic determinant(s) involved in salt tolerance of melon plants whose growth and agronomic yield are known to be drastically restricted even at low salinity (Navarro et al. 1999, Sagot 2005). It was reported that salt tolerance in melons is dependent on cultivar traits and there are sensitive and tolerant ones (Shannon and Francois 1978, Yasar et al. 2006, Kusvuran et al. 2007).

As a preliminary investigation of the citrulline function in salt tolerance of melon plants we have used local varieties of melon from Turkey known to exhibit, under experimental conditions, contrasted levels of salt tolerance: the genotype Midyat is salt tolerant while the genotype Yuva is salt sensitive. Young seedlings of those cultivars growing under experimental conditions have been submitted to a progressive salinization through incremental increase of external NaCl concentration from 0 to 250 mmol/l within 5 days and further treated under such high salinity for 16 days. Fully expanded leaves collected either on salinized seedlings or on those maintained under non-saline conditions were then analyzed to determine the changes induced at the citrulline level. The level of proline, which was assumed to be regulated as that of a true osmolyte (Yoshiba et al. 1997) and to be a relevant marker in predicting either salt sensitivity or salt tolerance, has also been measured. This preliminary approach allowed characterizing some aspects of the relationships between the salt-induced changes occurring in the level of these amino acids and the respective salt tolerance of the genotypes.

## MATERIAL AND METHODS

**Plant material and growth conditions.** Two melon (*Cucumis melo* L.) genotypes were used. Midyat is a native melon genotype originating from hot and dry region of the south-east part of Turkey, and is tolerant to both salt and drought stress. Yuva is a local cultivar from the central part of Turkey which is known to be susceptible to environmental stress. Seeds were germinated and the young seedlings were grown in vermiculite at 26°C/22°C ± 2°C day/night temperatures with a 16/8 h light/dark regime. They received a photosynthetic photon flux density of 350 µmol/m$^2$/s. Relative humidity during daytime was maintained at 65 to 70%. The plants were irrigated with the Hoagland's nutrient solution. The composition of the nutrient solution used was as follows (M): $Ca(NO_3)_2 \cdot 4H_2O$, $3.0 \times 10^{-3}$; $K_2SO_4$, $0.90 \times 10^{-3}$; $MgSO_4 \cdot 7H_2O$, $1.0 \times 10^{3}$; $KH_2PO_4$, $0.2 \times 10^{-3}$; $H_3BO_3$, $1.0 \times 10^{-5}$; $10^{-4}$M FeEDTA, $MnSO_4 \cdot H_2O$, $1.0 \times 10^{-6}$; $CuSO_4 \cdot 5H_2O$, $1.0 \times 10^{-7}$; $(NH)_6Mo_7O_{24} \cdot 4H_2O$, $1.0 \times 10^{-8}$; $ZnSO_4 \cdot 7H_2O$, $1 \times 10^{-6}$. The melon plants were 23 days old at the onset of the salt treatment with 50 mmol/l NaCl. This salt level was enhanced daily by 50 mmol/l NaCl up to a final concentration of 250 mmol/l within 5 days. Seedlings were then grown under the saline conditions for 16 days. Control plants were grown under non-saline conditions for the same period of time. Fully expanded melon leaves were sampled for measurement of citrulline and proline starting from 32 days after sowing (DAS) and then every 4 days i.e. at 36, 40, 44 DAS. At the end of the saline treatment, the total plant dry weights of 44-day-old salinized or control seedlings were recorded after desiccation of the fresh matter in oven (48 h, 60°C).

### Citrulline determination

**Citrulline extraction and crude extracts fractionation.** Five hundred milligrams of fresh melon leaves were homogenized in 1.5 ml of EtOH (96%). Extracts were heated at 100°C until complete

evaporation of EtOH. The residues were then dissolved in 1.5 ml of cold water and vigorously mixed. After centrifugation of the homogenates (10 min, 5000 g, 24°C) the supernatants (the crude extracts) were removed and stored at –20°C until purification by chromatography on ion exchanger. Two-millilitre columns of Dowex 50W-X8(H+) resin (16–40 mesh) were first successively washed with 2 ml of $H_2O$, 2 ml of 2N HCl and 3 ml of $H_2O$. Then aliquots from crude extracts (300 µl) were loaded on the columns which were firstly washed with 5 ml $H_2O$ to discard sucrose and other neutral and acidic substances. Sucrose is expected to be abundant in melon leaves and it reacts with the color-developing reagent for citrulline by giving brown color, which makes citrulline determination impossible (Prescott and Mangnall 1976). On this ion exchanger citrulline, other amino acids and basic substances were adsorbed and further eluted with 2 ml 4M $NH_4OH$ and 2 ml $H_2O$. Aliquots from eluates were then directly used for the measurement of citrulline. Under our chromatographic conditions, fractionation of crude extracts enriched with known concentrations of citrulline led to current yields of 80% for citrulline.

**Photometric method.** The photometric method used has been adapted from the colorimetric well microtiter plate assay described by Knipp and Vasak (2000). This procedure is based on the reaction of citrulline with diacetylmonoxime in strong acidic conditions which lead to an imidazolino intermediate that reacts secondarily with the ureido group of citrulline and form an unstable glycourile. This chromogen is protected from the side product hydroxylamine by thiosemicarbazide used as a reducing agent and is then further converted at high temperature and in the presence of catalytic amount of $Fe^{3+}$ into a dye of unknown structure exhibiting a maximum absorbance at 540 nm. Preparation of solutions as well as setting up the calibration curve were done according to these authors, all the assays being performed in test tubes containing a final volume of 2.6 ml corresponding to 0.6 ml of citrulline solutions or eluates and 2 ml of the color developing reagent freshly prepared from stock solutions. The mixture was heated for 15 min at 95°C and then cooled to room temperature (10 min). Since the developed light-sensitive dye is stable for at least 20 min, the absorbance measurement (at 540 nm) should be done during this time.

Table 1. Changes in shoot dry weight of two varieties of *Cucumis melo* exhibiting different tolerance to high salinity at vegetative growth stage. The 28-day-old seedlings were treated for 16 days with 250 mmol/l NaCl. Data are means for shoots collected on three different seedlings ± SD

| Varieties | Treatments | Total shoot dw at 44 DAS (g/plant) | Inhibition rate of shoot biomass production (%) |
|---|---|---|---|
| Midyat | control | 0.886 ± 0.092[a] | – |
| | salinized | 0.829 ± 0.034[a] | 6.4 |
| Yuva | control | 0.759 ± 0.059[a] | – |
| | salinized | 0.629 ± 0.054[b] | 17.6 |

Data followed by different letters showed at the least significant difference at $P = 0.05$

**Proline determination**

The proline amounts were measured directly on aliquots from the crude extracts obtained as indicated for citrulline according to the method of Troll and Lindsley (1955) improved by Magné and Larher (1992).

## RESULTS AND DISCUSSION

The melon plants under study were 28 days old at the onset of the 16-day salt treatment with 250 mmol/l NaCl. At this stage of vegetative development, the salt sensitive genotype Yuva was moderately damaged by salinization while the salt tolerant Midyat exhibited less severe symptoms of salt toxicity. The plant dry weight of 44 days old melon plants treated for 16 days with 250 mmol/l NaCl are given in Table 1. The dry weight of the sensitive genotype Yuva under salt stress was decreased by 17.7% in comparison with that of the non-salinized control plants while that of the salt tolerant genotype Midyat was only decreased by 6.4%.

When collected at C1 (32 DAS) leaves from Yuva and Midyat genotypes already contained important amounts of citrulline. In response to the salt treatment, the citrulline level was found to be enhanced in both genotypes reaching 22.6 and 57.7 µmol/g dw in Yuva and Midyat, respectively (Figure 1). For each of the cultivars, citrulline amount actually doubled within 4 days; this increase was observed more precociously in Midyat than in Yuva. In parallel the citrulline contents remained quite

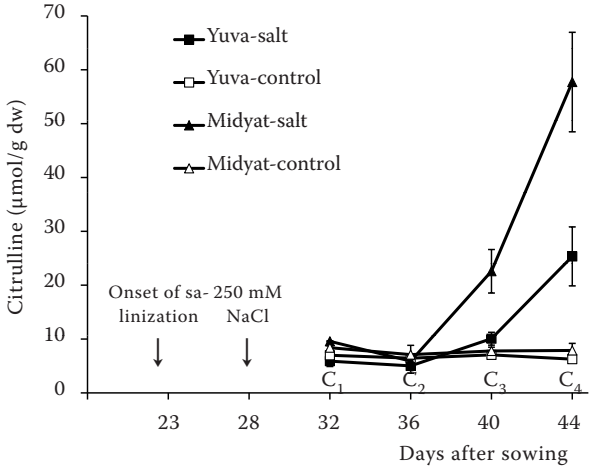



Figure 1. Changes in the citrulline level in leaves of two melon genotypes treated for 16 days with 250 mmol/l NaCl. Twenty three-day-old melon plants were subjected to incremental increases of NaCl concentration up to 250 mmol/l within 5 days and were further kept to grow under such conditions for 16 days. Control plants were maintained for the same period of time on the non-salinized reference medium. Mature leaves were collected (C1, C2, C3 and C4) for analyses at 32, 36, 40 and 44 DAS. Data shown were means of three replicates ± SD

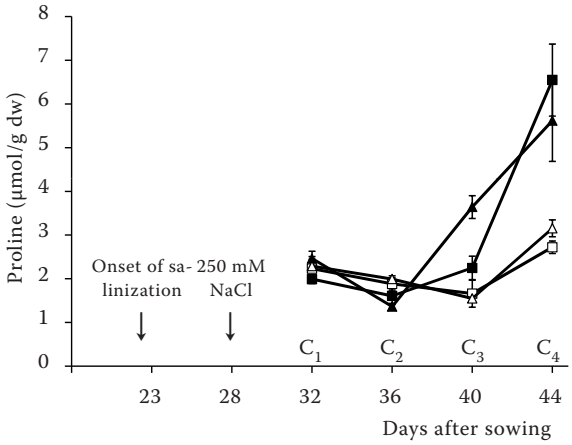



Figure 2. Changes in the proline level in leaves of two melon genotypes treated for 16 days with 250 mmol/l NaCl. Twenty three-day-old melon plants were subjected to incremental increases of NaCl concentration up to 250 mmol/l within 5 days and were further kept to grow under such conditions for 16 days. Control plants were maintained for the same period of time on the non-salinized reference medium. Mature leaves were collected (C1, C2, C3 and C4) for analyses at 32, 36, 40 and 44 DAS. Data shown were means of three replicates ± SD

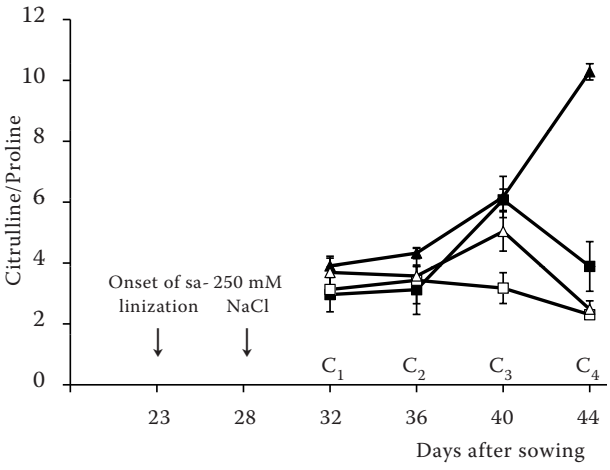



Figure 3. Changes in the ratio between the citrulline and the proline levels in leaves of two melon genotypes treated for 16 days with 250 mmol/l NaCl. Twenty three-day-old melon plants were subjected to incremental increases of NaCl concentration up to 250 mmol/l within 5 days and were further kept to grow under such conditions for 16 days. Control plants were maintained for the same period of time on the non-salinized reference medium. Mature leaves were collected (C1, C2, C3 and C4) for analyses at 32, 36, 40 and 44 DAS

similar and stable in both genotypes growing under control conditions. Finally under our experimental saline conditions, the citrulline level was found to be enhanced by 8 in the leaves of Midyat and by 3 in those of Yuva; this suggested that the salt tolerant genotype is more responsive in terms of salt-induced citrulline accumulation.

In comparison with citrulline, free proline (Figure 2) was found to be very low in both genotypes at the first period of saline treatment. However, a slight increase occurred in both genotypes starting from 36 DAS for the salt sensitive genotype and 40 DAS for the salt tolerant one. Clearly, when subjected to saline conditions melon plants under investigation did not behave as proline accumulators; the salt sensitive genotype was found to induce the typical proline stress response before the salt tolerant one.

The calculated molar ratios between the citrulline level and that of proline in the leaves of both melon plants are shown in Figure 3. They strongly suggested that at the end of the saline treatment the salt tolerance of Midyat could be associated with a cellular environment where citrulline is 10 times more abundant than proline.

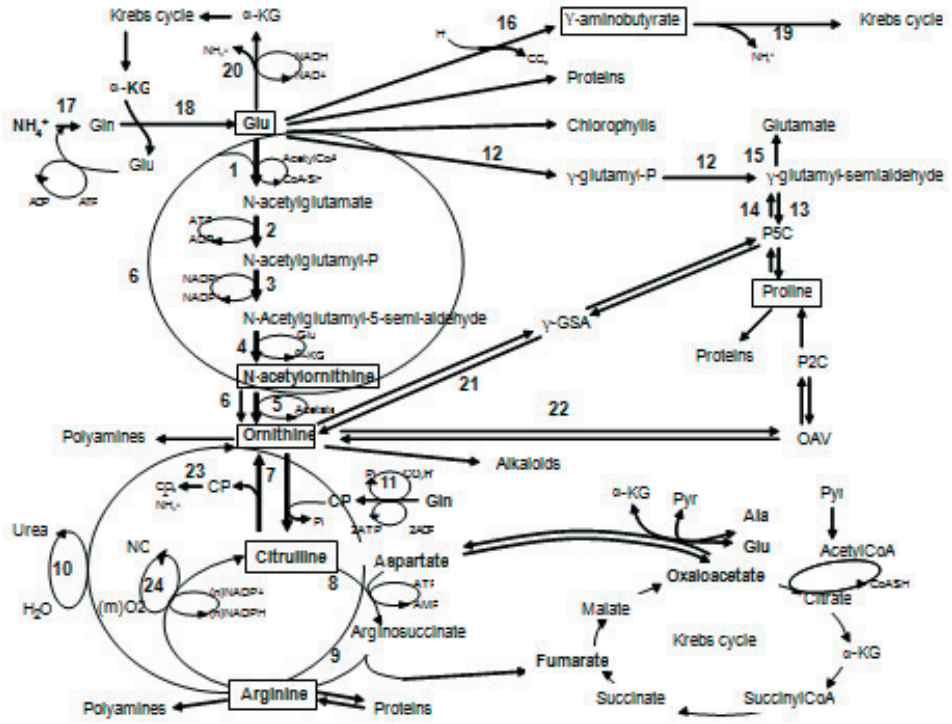
Previous reports devoted to characterization of biochemical indicators associated with different levels of salt tolerance in genotypes of *Cucumis melo* (Yasar et al. 2006, Kusvuran et al. 2007) showed that anti-oxidant defence systems of leaves consisting in constitutive and salt-induced changes of activities of ascorbate peroxidase, glutathione reductase and catalase and ascorbic acid content were significantly higher in the salt tolerant Midyat than in the salt sensitive Yuva. In accordance with such observations the amounts of $Na^+$ and $Cl^-$ were lower, while those of $K^+$ and $Ca^{2+}$ were higher in the tolerant Midyat than those found in the salt-sensitive Yuva (Yasar et al. 2006, Kusvuran et al. 2007). In the present study, we have compared the magnitude of the changes induced by salinity in the amounts of citrulline and proline occurring in the leaves of the varieties, assuming that they could behave as biochemical indicators of the ability to withstand saline conditions through their possible involvement as compatible solutes or scavengers of ROS (reactive oxygen species).

As postulated, it first appears that the ureido amino acid citrulline, which is present at low but significant concentration in leaves of both melon varieties under control conditions, accumulated in the plant parts in response to the treatment with high concentration of NaCl. It will be interesting to further investigate the cause and effect relationships between salt stress intensity and the amount of citrulline accumulated. Here the "citrulline response" appeared after a 4-day lag phase in the salt tolerant genotype and an 8-day lag phase in the salt sensitive one. As a consequence the final amount of citrulline in Midyat, after a 16-day treatment, was found to be twice higher than in Yuva. This strongly suggests a greater responsiveness of citrulline metabolism to NaCl in excess in the salt tolerant variety. The relationship between citrulline accumulation and salt tolerance remains to be ascertained because citrulline overproduction might merely be one of the metabolic consequences of adaptation to high saline conditions.

Still, it has to be said that citrulline accumulation in the watermelon plant growing in the Botswana desert was shown to be involved in osmotic adjustment owing to its very high concentration, its compatibility and its putative protecting properties against oxidative stress through its high potency in scavenging hydroxyl radicals (Akashi et al. 2001). According to these authors, wild watermelon subjected to saline conditions overproduced gamma-aminobutyric acid, proline and glutamine, not citrulline. The reasons for such discrepancy between the metabolic salt responses of *C. lanatus* and that of *C. melo* remain to be elucidated.

In the present study the changes induced in the amount of free proline present in leaf tissues of both melon varieties have also been determined because a wide range of plant species are known to accumulate this amino acid when subjected to saline conditions (Delauney and Verma 1993, Hare and Cress 1997) providing beneficial effects in osmotic adjustment and in control of cellular redox homeostasis. We have found that a very weak "proline response" of melon leaves had the same magnitude in both genotypes, irrespective of their salt tolerance. In comparison with the "citrulline response" detected in plants treated for 16 days with 250 mmol/l NaCl, it was up to 10 times lower, which is quite unusual in higher plants. This leads to a suggestion that salt-induced changes in proline metabolism, of minor importance in melon plants, might be restricted due to the counteracting effects exerted by unknown regulator(s) of proline metabolism. In contrast, the metabolic adjustments that take place at the level of citrulline metabolism and which are responsible for enhancement of its amount strongly suggests that this substance does not behave as a mere intermediate of arginine metabolism (Figure 4). Therefore, in leaf tissues of salt treated melon plants it could play a role in cellular osmotic adjustment. At this level of organization, compartmentation of citrulline in the cytosol might significantly participate in adjusting the osmotic potential of this compartment to that of the vacuole and the apoplastic domains.

Giving the fact that biosyntheses of both citrulline and proline are thought to rely on glutamate as a sole precursor (Figure 4), the question arises of the raison d'être of the differences occurring in the regulatory processes involved in the proline producers on the one hand and the citrulline producers on the other hand. Glutamate channelling towards ornithine rather to proline (Figure 4) in *C. lanatus* (Kawasaki et al. 2000) was previously shown to partly rely on overexpression and activation of N-acetylornithine deacetylase which catalyses the last step of conversion of glutamate into ornithine (Figure 4) (Yokota et al. 2002). Conversely, the reason of the apparent stability of the metabolic pathway responsible for the conversion of glutamate to proline owing the activities of P5CS and P5CR (Figure 4) remains a matter of speculation in the *Cucurbitaceae* investigated today i.e. wild watermelon and melon. We are prone to suggest that the plasticity of proline metabolism (Yoshiba et




1. N-acetylglutamate synthase
2. N-acetylglutamate kinase
3. N-acetylglutamyl phosphate reductase
4. N-acetylornithine aminotransferase
5. N-acetylornithine aminotransferase
6. N-acetylornithine deacetylase = DRIP1
7. Ornithine transcarbamoylase
8. Argininosuccinate synthase
9. Argininosuccinate lyase
10. Arginase
11. Carbamoyl phosphate synthetase
12. P5C synthetase
13. P5C reductase
14. Proline dehydrogenase
15. P5C dehydrogenase
16. Glutamic acid decarboxylase
17. Glutamine synthetase
18. Glutamate synthase
19. GABA-transaminase
20. Glutamate dehydrogenase
21. delta-ornithine transaminase
22. alpha-ornithine transaminase
23. Carbamate kinase
24. No synthase

Figure 4. Outline of the interrelationships between the metabolic pathways involved in proline and arginine synthesis and degradation in higher plants

al. 1997), which is surprisingly restricted in stressed leaf tissues of *Cucurbitaceae*, could result from the suppressing effect exerted by citrulline itself on the osmo-indiced "proline response" (Larher et al. 2008). A similar situation was described in the *Chenopodiaceae* which accumulate glycine betaine rather than proline in response to saline conditions (Briens and Larher 1982, Tipirdamaz et al. 2006) and it was also shown that this betaine could behave as a negative regulator of the proline accumulating system when provided to plant species that do not produce it spontaneously (Larher et al. 1996).

The results presented in this study show that the salt-tolerant melon genotype Midyat accumulated more citrulline in its leaves than the sensitive Yuva. This result, taken together with that reported on the drought-tolerant watermelon (Kawasaki et al. 2000), led us to suggest that the citrulline accumulated in leaves as well as the salt-induced metabolic plasticity which is needed might help in protecting plants from salt stress especially in Midyat.

For assessing and screening melon genotypes for tolerance to salinity during their vegetative development, the amount of citrulline accumulated in response to a given treatment at high salinity might be considered in the future as a novel biochemical indicator of interest for plant breeders.

## REFERENCES

Akashi K., Miyake C. Yokota A. (2001): Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. FEBS Lett., *508*: 438–442.

Briens M., Larher F. (1982): Osmoregulation in halophytic higher plants: a comparative study of soluble

carbohydrates, polyols, betaines and free proline. Plant Cell Environ., *5*: 287–292.

Delauney A., Verma D.P.S. (1993): Proline biosynthesis and osmoregulation in plants. Plant J., *4*: 215-223.

Hare P.D., Cress W.A. (1997): Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul., *21*: 79–102.

Kawasaki S., Miyake C., Kohchi T., Fuji S., Uchida M., Yokota A. (2000): Responses of wild watermelon to drought stress: Accumulation of an ArgE homologue and citrulline in leaves during water deficits. Plant Cell Physiol., *41*: 864–873.

Knipp M., Vasak M. (2000): A colorimetric 96-well microtiter plate assay for the determination of enzymatically formed citrulline. Anal. Biochem., *286*: 257–264.

Kusvuran S., Ellialtioglu S., Yasar F., Abak K. (2007): Effects of salt stress on ion accumulation and activity of some antioxidant enzymes in melon. J. Food Agr. Environ., *5*: 351–354.

Larher F., Dasgan H.Y., Moulin M., Le Vot A., Monnier C., Le Port L., Bouchereau A. (2008): The novel compatible solute citrulline could act as a suppressor of the osmo-induced proline response. In: XVI$^{th}$ Congress of the FESPB, Tampere, Finland.

Larher F., Rotival-Garnier N., Lemesle P., Plasman P., Bouchereau A. (1996): The glycine betaine inhibitory effect on the osmo-induced proline response of rape leaf discs. Plant Sci., *113*: 21–31.

Magné C., Larher F. (1992): High sugar content of extract interferes with colorimetric determination of amino acids and free proline. Anal. Biochem., *200*: 115–118.

Mahajan S., Tuteja N. (2005): Cold, salinity and drought stresses: an overview. Aust. J. Plant Physiol., *13*: 1–13.

Navarro J.M., Botella M.A., Martinez V. (1999): Yield and fruit quality of melon plants grown under saline conditions in relation to phosphate and calcium nutrition. J. Hortic. Sci. Biotechnol., *74*: 573–578.

Prescott A., Mangnall D. (1976): Interference by sucrose in the chemical determination of citrulline with diacetylmonoxime. Anal. Biochem., *76*: 551–555.

Rhodes D., Nadolska-Orczyk A., Rich P.J. (2002): Salinity, osmolytes and compatible solutes. In: Laüchli A., Lüttge U. (eds.): Salinity: Environment, Plant, Molecules. Kluwer Academic Publishers, the Netherlands: 181–204.

Rimando A.M., Perkins-Veazie P.M. (2005): Determination of citrulline in watermelon rind. J. Chromatogr. A, *1078*: 196–200.

Sagot E. (2005): Etude physiologique, biochimique, moléculaire et agronomique du stress salin chez le melon *Cucumis melo* L. [Ph.D. Thesis.] University of Poitiers, France.

Shannon M.C., Francois L.E. (1978): Salt tolerance of three muskmelon cultivars. J. Am. Soc. Hortic. Sci., *103*: 127–130.

Tipirdamaz R., Gagneul D., Duhazé C., Aïnouche A., Monnier C., Özkum D., Larher F.R. (2006): Clustering of halophytes from an inland salt marsh in Turkey according to their ability to accumulate sodium and nitrogenous osmolytes. Environ. Exp. Bot., *57*: 139–153.

Troll W., Lindsley A. (1955): A photometric method for determination of proline. J. Biol. Chem., *215*: 655–660.

Wada M. (1930): Ueber Citrulline, eine neue Aminosäurein Pressaft der Wassermelone, *Citrullus vulgaris* Schrad. Biochem. Z., *224*: 420–429.

Wang W., Vinocur B., Altman A. (2003): Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, *218*: 1–14.

Yasar F., Kusvuran S., Ellialtioglu S. (2006): Determination of anti-oxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress. J. Hortic. Sci. Biotechnol., *81*: 627–630.

Yokota A., Kawasaki S., Iwano M., Nakamura C., Miyake C., Akashi K. (2002): Citrulline and DRIP-1 protein (ArgE homologue) in drought tolerance of wild watermelon. Ann. Bot., *89*: 825–832.

Yoshiba Y., Kiyosue T., Nakashima K., Yamaguchi-Shinozaki K., Shinozaki K. (1997): Regulation of levels of proline as an osmolyte in plants under stress. Plant Cell Physiol., *38*: 1095–1102.

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