Plant Physiology and Biochemistry 49 (2011) 1313–1322

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Possible involvement of polyphenols and polyamines in salt tolerance of almond rootstocks

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ARTICLE INFO

Article history:
Received 27 June 2011
Accepted 24 August 2011
Available online 31 August 2011

Keywords:
Almond rootstocks
Salt tolerance
Anthocyanin
Carotenoids
Polyamines

ABSTRACT

Leaf physiological and biochemical adaptive strategies and more particularly the possible involvement of polyamines and polyphenols in salt stress tolerance were investigated. Three almond rootstocks (GN15, GF677 and bitter almond) were subjected to 0, 25, 50 and 75 mM NaCl for 30 days. The dry mass of leaves, stems and roots decreased with increasing salt concentration in the irrigation solution regardless of genotype. Photosynthetic assimilation rate decreased in the three almond rootstocks, but more so in GF677 and bitter almond. The accumulation of toxic ions was greater in the leaves than in the roots in all genotypes. GN15 accumulated less Na^+ and Cl^- than GF677 and bitter almond. GF677 accumulated polyphenols, but had less anthocyanin and antioxidant activity in its leaves compared to bitter almond. It seems that GN15 was more able to tolerate the excess of toxic ions using anthocyanins which are abundant in its red leaves and free polyamines for a more efficient response to stress. However, most of the antioxidant activity was found in the leaves and was lower in the roots. Given that the upper part of the tree will be of a different cultivar after grafting, this advantage may not be relevant for the tree's survival. GF677 showed a different antioxidant strategy; it maintained a stable carotenoids content and accumulated polyphenols in its leaves. The three rootstocks used different strategies to deal with the excess of salt in the growth medium.

1. Introduction

Increased salinity affects plant growth and development by ion toxicity, induced nutritional deficiencies, water deficit (osmotic effect) and lower rate of photosynthetic assimilation [1]. Plants respond to salt stress by increasing the concentration of compatible osmolyte in their tissues (osmotic adjustment) [2]. Moreover, the inhibition of photosynthesis causes an over-reduction of the photosynthetic electron transport chain and redirects photon energy into processes that favour the production of reactive oxygen species [3], such as superoxide anion radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), which are harmful to plant growth due to their detrimental effects on most sensitive biological macromolecules and membranes. To prevent this oxidative stress from accessing, plants display a multitude of photoprotective processes including leaf positioning and detoxification of chloroplasts via biochemical compounds [4,5]. Most secondary metabolites involved in stress tolerance are synthesized from intermediates of primary carbon metabolism via phenylpropanoid, shikimate, mevalonate or MEP pathways. Among those, chlorophylls, carotenoids, polyphenols, flavonoids and anthocyanins play an important role in scavenging free radicals [5]. Their synthesis is generally stimulated under abiotic stress [6] and they are used during the detoxification process. For instance, Na^+ accumulation in the leaves affects photosynthetic apparatus by decreasing the level of pigments such as chlorophyll and carotenoids, which are the most important defence line in the chloroplast. Under salt stress conditions, the production of polyphenols is related to the leaf carbon economy. Their accumulation is enhanced when carbon production overtakes the metabolic demand for growth [7]. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Among flavonoids, anthocyanins are highly water soluble pigments derived from flavonoid

Abbreviations: A, net photosynthetic rate; ATN, anthocyanin; Car, carotenoids; Chl ($a + b$), total chlorophylls; PA, polyamine; Put, putrescine; TAA, total antioxidant activity; Spd, spermidine.

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doi:10.1016/j.plaphy.2011.08.009

precursors via the shikimate pathway [5]. They protect the chloroplasts of shade adapted [8] and senescing leaves from photooxidative damage during exposure to high solar radiation mainly by absorbing UV–B. Since anthocyanins are osmotically active, their enhanced expression can increase hardiness through increased osmotic control [5]. Kaliamoorthy and Rao [9] reported up to 40% increase in anthocyanin concentration in maize leaves in response to increased salinity. Anthocyanins accumulate also when leaves are exposed to UV–B, drought low temperature, nutrient deficiency or to ozone [8].

Another response that helps plants become more tolerant to unfavourable environmental conditions is the accumulation of low molecular weight osmolytes such as proline [10] and polyamines (PAs) [8]. Many studies suggest that biosynthesis of PAs may be an integral part of the plant's response to stress [8]. A salt-induced increase in endogenous polyamines' content has been reported in several plant species [11]. However, ionic and osmotic stress induced by salinity may influence PA metabolism in different ways, and PAs may have different and specific functions under these stress conditions [11]. The involvement of these compounds in metabolic adjustment remains unclear. It has been suggested that, due to their polycationic nature, PAs could be involved in cellular ionic balance [12].

Almond tree has been described as being sensitive to salinity [13,14]. Different interspecies *Prunus* hybrids are used as rootstocks for almond because of their resistance to pathogens and their tolerance to adverse soil conditions (pH, water logging, and salinity). Among the most widely used are GF677, GN15 and bitter almond.

The aim of this study was to characterize the response of three almond rootstocks (GF677, GN15 and bitter almond) to soil salinity and to determine if polyphenols and polyamines are implicated in this response.

2. Results

2.1. Growth, photosynthetic and ionic characteristics

After 30 days of exposure to NaCl treatment, total plant dry mass of all three rootstocks was reduced considerably compared to control plants but with various degrees (Table 1). The addition of 75 mM NaCl in the culture medium reduced by 33.5%, 48% and 61% the total plant dry mass of bitter almond, GN15 and GF677, respectively. In the case of GF677 genotype, 75 mM NaCl in the culture medium affected considerably (60%) both shoots and roots parts, while in GN15, shoot dry mass (DM) was affected more than root DM (51%, 45%). However, in better almond the roots DM was affected more than shoot DM (46%, 27%). Still, NaCl treatment increased root/shoot ratio in GN15 and GF677 rootstocks, but it decreased in bitter almond (Table 1).

Table 1
Salt effect on growth parameters and photosynthetic assimilation rate (A) of three almond rootstocks. Each point represents the mean (±SE) of 4 replicates. Differences were considered significant at probability level of $P \leq 0.05$ (results of Duncan's test). Different letters indicate significant differences between treatments for a given rootstock.

NaCl (mM)	Total plant dry mass (g)	Total Leaf dry mass (g)	Shoot dry mass (g)	Total root dry mass (g)	Root/Shoot ratio	A (mol m^{-2} s^{-1})
Bitter almond						
0	4.68a	1.65a	3.29a	1.38a	0.56ab	20.34a
25	3.39ab	1.34a	2.79ab	1.10a	0.36b	19.44a
50	3.51ab	1.18ab	2.56b	0.94ab	0.33b	15.22b
75	3.11b	1.01b	2.37b	0.74b	0.32b	14.23b
GF677						
0	14.26a	4.78a	9.45a	4.81a	0.57b	12.64a
25	10.41b	2.73b	6.87b	3.63a	1.14a	12.51a
50	6.83c	1.85bc	4.53c	2.30b	1.17a	11.79a
75	5.65c	1.64c	3.69c	1.95b	0.75b	7.90b
GN15						
0	12.7a	4.25a	8.36a	3.80a	0.52b	10.08a
25	9.48b	2.29b	6.04b	3.41a	0.81ab	8.87ab
50	7.89b	2.23b	5.42b	2.40a	1.17a	8.10ab
75	6.32c	1.81b	4.08c	2.01a	0.82ab	7.52b

In control plants, the net photosynthetic assimilation rate (A) was greater in the rootstocks with green leaves (bitter almond and GF677) than in GN15 which has reddish leaves (Table 1). However, A of GN15 leaves was the last affected by salt addition. For instance, the addition of 75 mM NaCl in the culture medium decreased A by 25%, 30% and 37% for GN15, bitter almond and GF677 respectively.

Our results suggest that the effect of salt addition to the growth medium on Na^+ and Cl^- absorption and partitioning depended on genotype, and salt concentration (Fig. 1). In all three genotypes, the leaves accumulated considerably more Na^+ and Cl^- than roots. At the highest NaCl concentration, GN15's leaves and roots contained less Na^+ levels in their leaves (317 and 188 μeq g^{-1}DW respectively) compared to other genotype. However, the pattern of accumulation of Na^+ was comparable for the three rootstocks. Na^+ was partitioned preferentially to the leaves not the roots. The accumulation started at 25 mM for GN15 and GF677 and only at 50 mM for bitter almond, a maximum concentration which depended on genotype was rapidly reached. This maximum concentration was about four times higher in GF677 and bitter almond compared to GN15. The addition of salt to growth medium increased Cl^- concentration in the leaves but not the roots. The accumulation of Cl^- in the leaves was gradual. After one month of treatment with 75 mM NaCl, the highest increase of Cl^- was recorded in the leaves of bitter almond (60%) and GF677 (50%) as compared to GN15 (31%).

In all three rootstocks, adding NaCl salt to the growth medium significantly reduced K^+ concentration and K^+/Na^+ ratio in the leaves (Table 2). The addition of 75 mM NaCl reduced leaf K^+ concentration by 38%, 34% and 30% in bitter almond and GF677 and GN15 respectively. GN15 leaves maintained the highest K^+/Na^+ ratio at all salinity levels. Root K^+ content changed little because of salinity.

2.2. Leaf pigments

The effect of growth medium salinity on leaf pigments varied between cultivars. In bitter almond, Chl*a*, total chlorophyll and carotenoids' content were depressed by salinity but Chl*b* content and Chl*a*/Chl*b* and carotenoids/Chl ($a + b$) ratio were not affected.

In GF677, chlorophyll's contents were reduced by salinity whereas carotenoids were not affected, Chl*a*/Chl*b* and carotenoids/Chl ($a + b$) ratios increased.

In GN15, chlorophylls were not or little affected by growth medium salinity; Chl*a*/Chl*b* ratio increased, carotenoids' content and carotenoids to chlorophylls ratio decreased (Fig. 2).

The leaves of GN15 which have a reddest appearance had the highest anthocyanin (ANT) concentration (Fig. 3). These pigments decreased progressively with increasing salt level in GN15 rootstock. However, the concentration of these compounds remained unchanged in bitter almond and GF677 leaves. The individual ANT, cyanidin-3.5-glucoside and petunidin-3-glcoside showed the same variation pattern in response to salt stress treatments in bitter almond. However, petunidin-3-glucoside decreased in GF677 mainly with 50 and 75 mM NaCl. Regardless of salt level, GN15 had the highest ANT content, especially cyaniding-3.5-glucoside, compared with GF677 and bitter almond. The ANT/Chl ($a + b$) ratio decreased considerably under salt stress conditions in GN15, but it increased in bitter almond with 75 mM NaCl.