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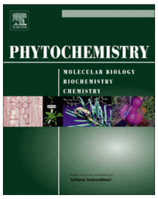

Effect of long-term salinity on cellular antioxidants, compatible solute and fatty acid profile of Sweet Annie (*Artemisia annua* L.)

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ABSTRACT

Impact of long-term salinity and subsequent oxidative stress was studied on cellular antioxidants, proline accumulation and lipid profile of *Artemisia annua* L. (Sweet Annie or Qinghao) which yields artemisinin (Qinghaosu), effective against cerebral malaria-causing strains of *Plasmodium falciparum*. Under salinity (0.0–160 mM NaCl), in *A. annua*, proline accumulation, contents of ascorbate and glutathione and activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) increased, but the contents of reduced forms of glutathione (GSH) and ascorbate declined. The fatty-acid profiling revealed a major salinity-induced shift towards long-chain and mono-saturated fatty acids. Myristic acid (14:0), palmitoleic acid (16:1), linoleic acid (18:2) and erucic acid (22:1) increased by 141%, 186%, 34% and 908%, respectively, in comparison with the control. Contents of oleic acid (18:1), linolenic acid (18:3), arachidonic acid (22:0) and lignoceric acid (24:0) decreased by 50%, 17%, 44% and 78%, respectively. Thus, in *A. annua*, salinity declines ascorbate and GSH contents. However, increased levels of proline and total glutathione (GSH + GSSG), and activities of antioxidant enzymes might provide a certain level of tolerance. Modification in fatty-acid composition might be a membrane adaptation to long-term salinity and oxidative stress.

1. Introduction

Artemisinin is the most effective drug against *Plasmodium* species that causes malaria (Klyman, 1985), affecting some 500 million people besides causing 1–2 million deaths annually (Lindahl et al., 2006), particularly in developing countries (Snow et al., 2005). Moreover, artemisinin has shown potential activity against a number of viruses including hepatitis B, C and others (Efferth et al., 2008). Recent studies have demonstrated that even artemisinin derivatives are equally potent and efficacious against several other human parasites that cause toxoplasmosis, trypanosomiasis, schistosomiasis, cryptosporidiosis, amoebiasis, giardiasis, clonorchiasis and leishmaniasis (Brisibe et al., 2009). *Artemisia annua* still remains the only commercial source of artemisinin, which is present in very low concentrations (0.01–0.8% DW), thus seriously limiting its commercialization and compelling for innovative methods to improve artemisinin production (Van Agtmael et al., 1999; Nafis et al., 2011). The efforts of increasing the artemisinin production might suffer from environmental stress, including soil salinity.

Salinity stress has a negative impact on agricultural yield throughout the world. Of late, the problem has been aggravated by agricultural practices such as irrigation. Approximately, 20% of the world's cultivated lands and more than half of all the irrigated lands are affected by salinity (Rhoades and Loveday, 1990; Arzani, 2008).

High concentrations of salt cause ion imbalance and hyperosmotic stress in plants. As a consequence of these primary effects, secondary stresses, such as oxidative stress (Qureshi et al., 2005) due to production of activated oxygen species (AOS), often emerge and cause damage to DNA, proteins, chlorophylls and membrane functions (Vanderauwera et al., 2011). Oxidative stress also alters the components of plant antioxidant system(s) (Hernandez and Almansa, 2002; Mittova et al., 2002). Plants respond to salinity stress through various mechanisms including ion detoxification, osmolyte accumulation, ion homeostasis and upregulation of non-enzymatic and enzymatic antioxidants (Bartels and Phillips, 2010).

Under the effect of oxidative stress, antioxidants increase in all the components of the ascorbate–glutathione (GSH) cycle, including SOD (Pang and Wang, 2010), APX (Koussevitzky et al., 2008) and GR (Meloni et al., 2003), and thus the antioxidant defence mechanisms provide a strong basis for stress tolerance (Miller

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et al., 2010). Catalase is another antioxidant enzyme important to counter H_2O_2. Processes underlying the antioxidant responses to oxidative stress need to be understood clearly in order to assess the tolerance threshold of the given plant. The magnitude of oxidative stress is subject to several factors. Metabolisms of chloroplasts and mitochondria under stress favor the $O_2^{\bullet -}$ and H_2O_2 production (Pang and Wang, 2010). Transcript levels for mitochondrial Mn–SOD, chloroplastic CuZn–SOD and phospholipid hydroperoxide GSH peroxidase, and cytosolic GSH reductase (GR) and ascorbate peroxidase (APX) are strongly induced in the stress-tolerant varieties, and not in the stress-sensitive ones, suggesting that the induction of antioxidant defense is one of the components of tolerance mechanism against oxidative stress. Evidence suggests that plants adapt to environmental stresses by strengthening their antioxidant defense mechanism (Miller et al., 2010). Salinity induces oxidative stress (Pérez-López et al., 2009) and alters lipid composition besides altering expression of aquaporins and controlling water permeability as an acclimation mechanism against salinity (López-Pérez et al., 2009). However, responses of medicinal plants to oxidative stress are still little studied. To the best of our knowledge, there is no report till date on the impact of long-term salinity on fatty-acid composition of leaf. Since fatty acids are the building blocks of lipids, including membrane lipids, any change in lipid composition would be crucial for plant adaptation to stress (Elenkov et al., 1996; Zheng et al., 2011). Any change in the cell-lipid composition is indicative of the changed composition of protein or multiprotein complexes (Qureshi et al., 2010) under stress such as in thylakoidal and mitochondrial membranes. The present study investigates the impact of salt-induced stress on levels of non-enzymatic and enzymatic antioxidants, proline accumulation and fatty-acid profile of leaf lipid in *A. annua* L., the source of a potent antimalarial compound.

2. Results

The ascorbate content in control was recorded to be the maximum at 130 days after sowing (DAS). NaCl-treated plants showed a dose-dependent decrease up to 85% over the control (160 DAS) with T_3 (Fig. 1A). On the other hand, dehydroascorbate content in the control was the maximum at 160 DAS. The treated plants showed a rapid dose-dependent increase, which was nearly 400% with T_3 at 100 DAS. Later it declined gradually to remain 67% (T_3, 160 DAS) above the control (Fig. 1A). The total ascorbate content in controls was the maximum at 130 DAS. The NaCl-treated plants suffered a decline in a dose-dependent manner up to 59% (T_3, 130 DAS), which later was 45% (T_3, 160 DAS), as compared with the control (Fig. 1A).

The glutathione (GSH) content in the control was the maximum at 130 DAS. In the NaCl-treated plants, it showed a dose-dependent decrease, which went up to 52% (T_3, 160 DAS) with reference to the control (Fig. 1B). The GSSG content in the control was the maximum at 130 DAS. It increased in a dose-dependent manner in the treated plants to become 8.4-fold (T_3, 160 DAS) to 5-fold (T_1, 100 DAS) higher over the control (Fig. 1B). The maximum total glutathione content in the control was recorded at 130 DAS. The treated plants showed a relatively higher level, increasing in a dose-dependent manner and reaching nearly 79% at 130 DAS with T_3 (Fig. 1B).

Salinity modulated the activities of cellular antioxidant enzymes (Fig. 2A–C). The maximum activity of superoxide dismutase (SOD) in control plants was recorded at 130 DAS. The treated plants showed a dose-dependent increase in SOD activity, which varied from the control up to 161% (T_3, 100 DAS). It declined later to 61% (T_3, 160 DAS), as compared with the control (Fig. 2A).

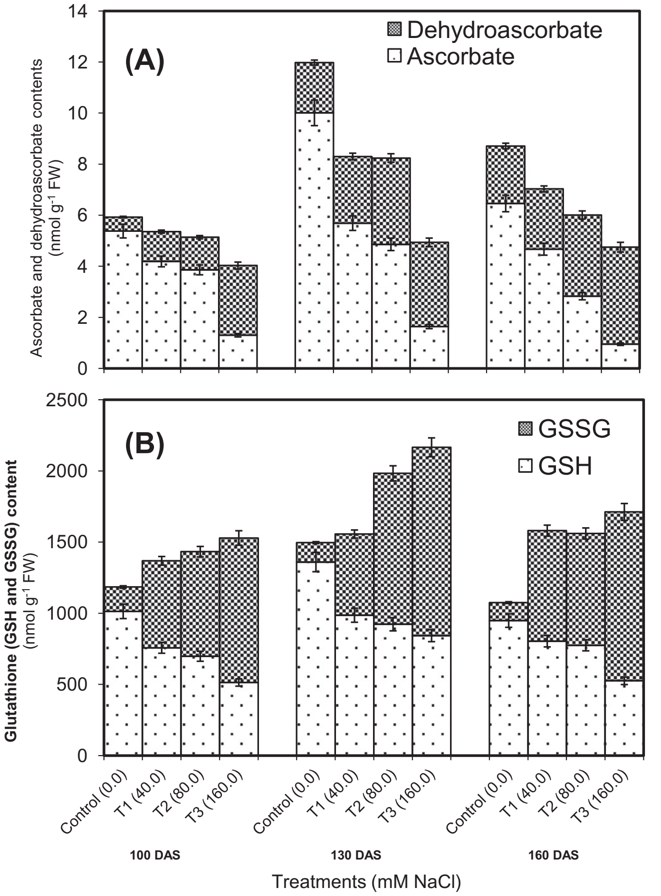

Fig. 1. Impact of different levels of salinity (NaCl) on (A) contents of ascorbate, dehydroascorbate and total ascorbate (nmol g^{-1} FW) and (B). GSH, GSSG and total glutathione (GSH + GSSG) (nmo g^{-1} FW) at 100, 130 and 160 days after sawing (DAS) in leaf of *Artemisia annua*. Five plants from each set of triplicate were considered.

In control plants, the maximum ascorbate peroxidase (APX) activity was noted at 130 DAS. The NaCl-treated plants showed a dose-dependent increase in activity, 19% (T_1, 100 DAS) to 170% (T_3, 100 DAS), over the control. However, at the end of study (160 DAS), APX activity was only 50% higher than in the control (Fig. 2B).

The glutathione reductase (GR) activity in control plants was the maximum at 130 DAS. It showed a dose-dependent increase in treated plants, varying from the control approximately by 14% (T_1, 100 DAS) to 114% (T_3, 160 DAS) (Fig. 2C).

The catalase (CAT) activity in control plants was the maximum at 130 DAS and the least at 160 DAS. It showed a dose-dependent increase in treated plants, varying from the control approximately by 10% (T_1, 100 DAS) to 46% (T_3, 160 DAS) (Fig. 2D).

The maximum proline level in control plants was detected at 130 DAS. The level was higher in the NaCl-treated plants, attaining the maximum with T_1 (40 mM NaCl) at 100 DAS. Proline content became 5.1-fold at 130 DAS with T_1. Treatments beyond 80 mM NaCl resulted in a lesser degree of proline accumulation (Fig. 3).

In control plants, the fatty acid profile of leaf consisted of 14:0, myristic acid (2%); 16:0, palmitic acid (24%); 16:1, palmitoleic acid (2%); 18:1, oliec acid (9%); 18:2, linoleic acid (28%); 18:3, linolenic acid (25%); 22:0, arachidonic acid (2%); 22:1, erucic acid (2%); 24:0, lignoceric acid (6%) (Fig. 4A). Under salinity (160 mM NaCl) at 160 DAS, the leaf showed altered levels of all the fatty acids, shifting the compositions of 14:0 (from 2% to 6%), 16:1 (from 2% to 6%), 18:1 (from 9% to 5%), 18:2 (from 28% to 18%), 18:3 (from 25% to

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