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INTERACTIVE EFFECT OF FOLIARLY APPLIED ASCORBIC ACID AND SALT STRESS ON WHEAT (*TRITICUM AESTIVUM* L.) AT THE SEEDLING STAGE

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Abstract

A hypdoponic experiment was conducted to assess the role of ascorbic acid in alleviation of the adverse effects of salt stress on growth of two wheat cultivars, S-24 (salt tolerant) and MH-97 (moderately salt sensitive). Seven-day old plants were subjected to normal or saline conditions (0 and 150 mM) for four weeks. Ascorbic acid was exogenously applied as foliar spray with varying levels (0, 50, 100 mg L^{-1}) in hydroponics. Salt stress severely reduced growth of both wheat cultivars. Foliar spray with AsA improved the growth of non-stressed plants of both cultivars, but did not alleviate the adverse effects of salt stress on plants. However, salt-induced reduction in leaf chlorophyll 'a' was improved with AsA application. Salt-induced enhanced activities of all antioxidant enzymes were not substantially changed with AsA application. However, AsA application enhanced the Na^+ accumulation in the leaves of salt stressed plants of both cultivars, but it did not change the K^+ accumulation in the leaves and roots of the salt stressed plants of both cultivars. Furthermore, AsA applied as a foliar spray did not induce substantial changes and hence salt tolerance of both wheat cultivars. Thus, it can be concluded that though foliar spray with applied ascorbic acid protected the photosynthetic machinery from the damaging effects of salt stress, it did not improve growth of the two wheat cultivars under saline conditions.
Keywords: chlorophyll a, foliar spray, antioxidant, salt tolerance

Introduction

Salinity stress is a major threat to crop productivity in the arid and semi-arid regions of the world (Shannon, 1998). Although all soils contain some amount of soluble salts of multifarious nature, when soil and environmental conditions allow the concentrations in soil profiles to a high level, soil salinity becomes severe threat to land degradation (Wiebe *et al*., 2005) and crop productivity.

There is a strong evidence that in many crop plants natural accumulation of osmoprotectants and other organic compounds is very low and this deficiency can be overcome by their exogenous application (Makela *et al*., 1996, 1998). Exogenous applications of osmoprotectants, plant growth regulators, fertilizers, and antioxidants have been reported to successfully mitigate the adverse effects of salinity on plants. Of these, exogenous application of antioxidants has recently gained a ground as a very promising means of mitigating the adverse effects of salt on plant growth and metabolism (Kefeli, 1981; Janda *et al*., 1999; Shalata & Neumann, 2001).

Of the various non-enzymatic antioxidants, such as tocopherols, carotenoids, and phenols, ascorbic acid occurs ubiquitously in plants and has been reported to play a vital

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role in alleviating the adverse effects of salt on plant growth and metabolism in many crop plants (Hamada, 1998). In general, effects of ascorbic acid in mitigating the adverse effects of salt stress have been ascribed to activation of some of the enzymatic reactions (Kefeli, 1981). Furthermore, such positive effects of ascorbic acid in overcoming the adverse effects of salt stress were attributed to the stabilization and protection of photosynthetic pigments and the photosynthetic apparatus from oxidative damage (Neubauer & Yamamoto, 1992; Choudhury *et al*., 1993; Hamada, 1998).

From the earlier mentioned reports it is evident that ascorbic acid plays a key role in the regulation of a number of metabolic processes in plants exposed to salt stress. However, information on how ascorbic acid regulates physiological/biochemical processes in wheat plants subjected to salt stress is not much available in the literature. Thus, the main objective of the present study was to examine whether the adverse effects of salt stress on wheat plants could be mitigated by exogenous application of ascorbic acid as a foliar spray and how far it regulates the plant antioxidant enzyme system.

Materials and Methods

The study regarding the role of exogenously applied ascorbic acid in alleviating the adverse effects of salt stress on growth of wheat was carried out in the growth room of Department of Botany, University of Agriculture, Faisalabad. Seeds of two genotypes, S-24 (salt tolerant) and MH-97 (moderately salt sensitive) were surface sterilized with 5% sodium hypochlorite solution prior to experimentation. Surface sterilized seeds were placed in Petri plates double lined with filter paper and moistened with 10 ml of half strength Hoagland's nutrient solution containing 0 or 150 mM NaCl and were allowed to germinate for 7 days. Thereafter, seven-day old wheat seedlings were transferred to plastic pots of 15 cm diameter containing with 300 ml of half strength Hoagland's nutrient solution along with 0 or 150 mM NaCl. Seedlings that were grown under normal or saline conditions sprayed with different concentrations of ascorbic acid (mol. wt =156.1) [0, and 100 mg L^{-1}] prepared in distilled water containing tween-20 as a surfactant. Control (0 mg L^{-1} AsA) plants were sprayed with distilled water only. All pots were placed in a growth room under white florescent light (*PAR* 300 μmol m^{-2} s^{-1}) at 25 ºC ± 2 ºC and relative humidity ranged from 45.9 to 58.6%. The experiment was arranged in a completely randomized design with four replicates. The seedlings were harvested two weeks after AsA applied as a foliar spray, and different growth parameters and chlorophyll contents were measured. Shoots and roots were separated and their fresh biomass was measured. These plants were then oven-dried at 65°C for 72 hours and dry biomass was recorded.

The chlorophyll *a* was determined with the method as described by Arnon (1949). Fresh leaves (0.5 g) were chopped in fine pieces and extracted overnight with 80% acetone at -10 °C. The extract was centrifuged at 14000 x *g* for 5 min. and the absorbance of the supernatant was read at 480, 645 and 663 nm using a spectrophotometer (IRMECO U2020) and total amount of chlorophyll *a*, was calculated.

Analyses of variance of data for all the parameters were computed using the MSTAT-C computer package. The least significant differences between the mean values were calculated following Snedecor & Cochran (1980).

Results

Salt stress caused a significant reduction in the shoot fresh and dry weights of both cultivars. Cultivar S-24 produced greater shoot fresh and dry biomass than MH-97 under both saline and non-saline conditions. Application of ascorbic acid as a foliar spray caused a significant increase in shoot fresh and dry weights of both cultivars under non-saline conditions. This increasing effect was more in S-24 than that in MH-97 (Fig. 1).

Root fresh and dry weights of both wheat cultivars were reduced significantly due to salt stress. However, cv. S-24 generally exhibited higher root fresh and dry weights than those of MH-97 under saline conditions. Exogenous application of ascorbic acid as a foliar spray was found to be effective in increasing the root fresh and dry weights of both cultivars under saline conditions (Fig. 1.).

Imposition of salt stress caused a significant reduction in chlorophyll *a* of the seedlings of both wheat cultivars. Application of 100 mg L^{-1} ascorbic acid as a foliar spray enhanced chlorophyll *a* content in both wheat cultivars under saline conditions (Fig 2).

Activities of all antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)] increased in both wheat cultivars due to the imposition of salt stress. However, foliarly applied AsA caused a further increase in SOD activity of salt stressed plants of MH-97, whereas it remained unchanged in the salt stressed plants of S-24.

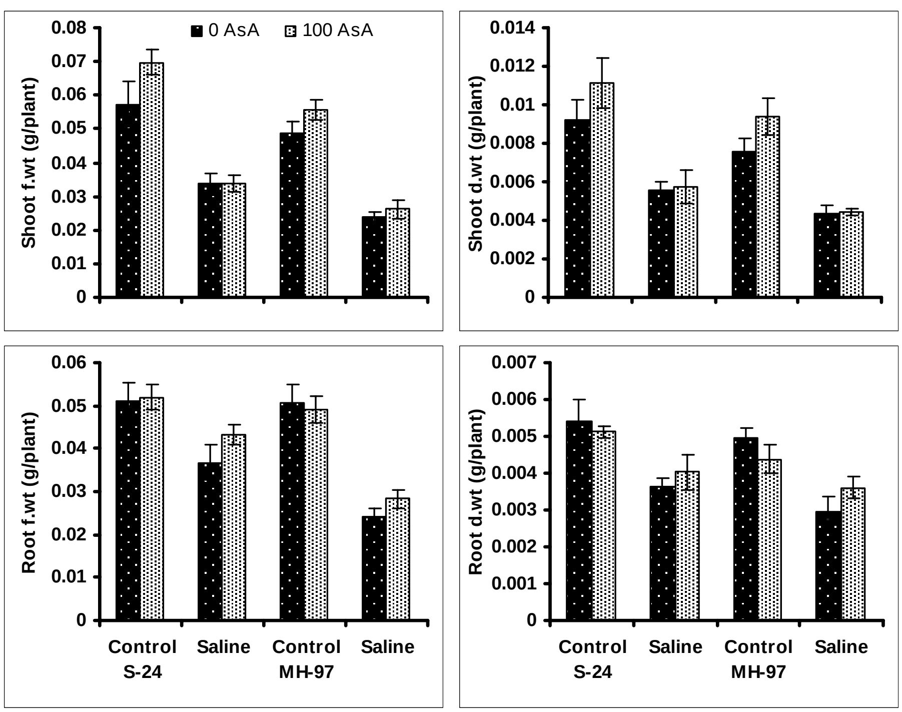

Fig. 1. Fresh and dry weights of shoots and roots of three-week-old seedlings of two spring wheat cultivars when 0 or 100 mg L^{-1} ascorbic acid (AsA) were applied as a foliar spray.

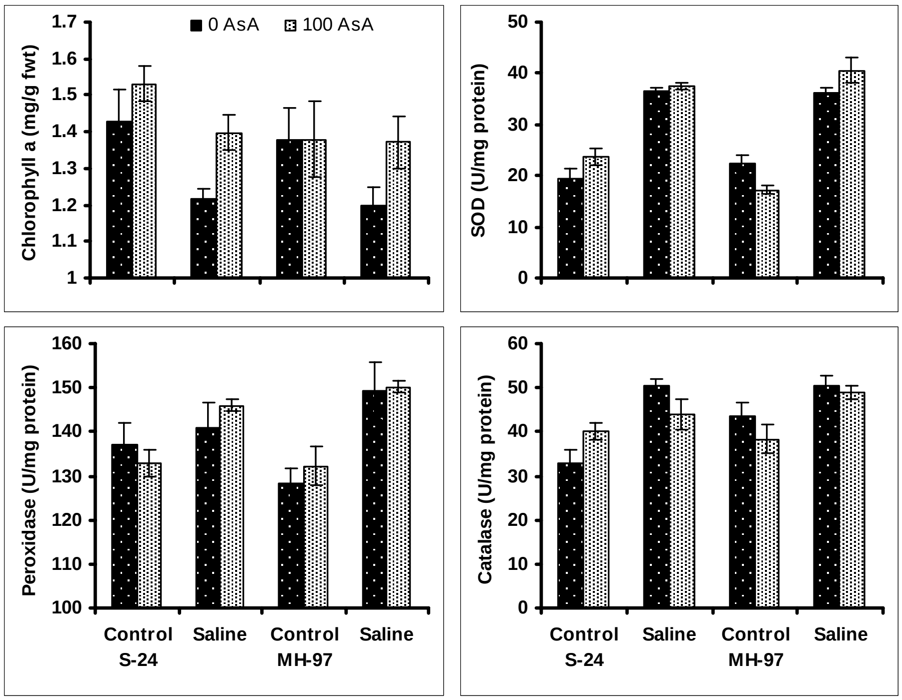

Fig. 2. Chlorophyll *a*, and activities of SOD, POD and CAT of three-week-old seedlings of two spring wheat cultivars when 0 or 100 mg L^{-1} ascorbic acid (AsA) were applied as a foliar spray.

However, foliarly applied AsA did not change the activity of POD in both wheat cultivars under both saline and non-saline conditions. In contrast, the activity of CAT increased only in non-stressed plants of S-24 due to AsA application. Foliar application of AsA did not affect CAT activity of MH-97 under both non-saline and saline conditions (Fig. 2).

Imposition of salt stress increased the leaf and root Na^+ in both wheat cultivars. However, exogenous application of AsA also increased the accumulation of Na^+ in the leaves of both wheat cultivars. In contrast, root Na^+ increased only in salt stressed plants of MH-97 (Fig. 3). Potassium content both in leaf and root was decreased in the salt stressed plants of both wheat cultivars. Foliar application of AsA did not enhance the accumulation K^+ both in the leaves and roots of both cultivars, except in the salt stressed plants of S-24 where it increased significantly (Fig. 3).

Discussion

A number of effective ways to overcome salt-induced reduction in growth has been proposed by different researchers (Ashraf, 1994; 2004; Munns *et al*., 2002; Ashraf & Harris, 2004; Flowers, 2004; Ashraf & Foolad; 2005; 2007). However, exogenous application of osmoprotectants, antioxidants, or plant growth regulators is considered to be an alternative short-term solution to induce salt tolerance in some of the important crop cultivars (Gadallah, 1999; Khan *et al.*, 2003; Raza *et al*., 2006; Waseem *et al*., 2006).

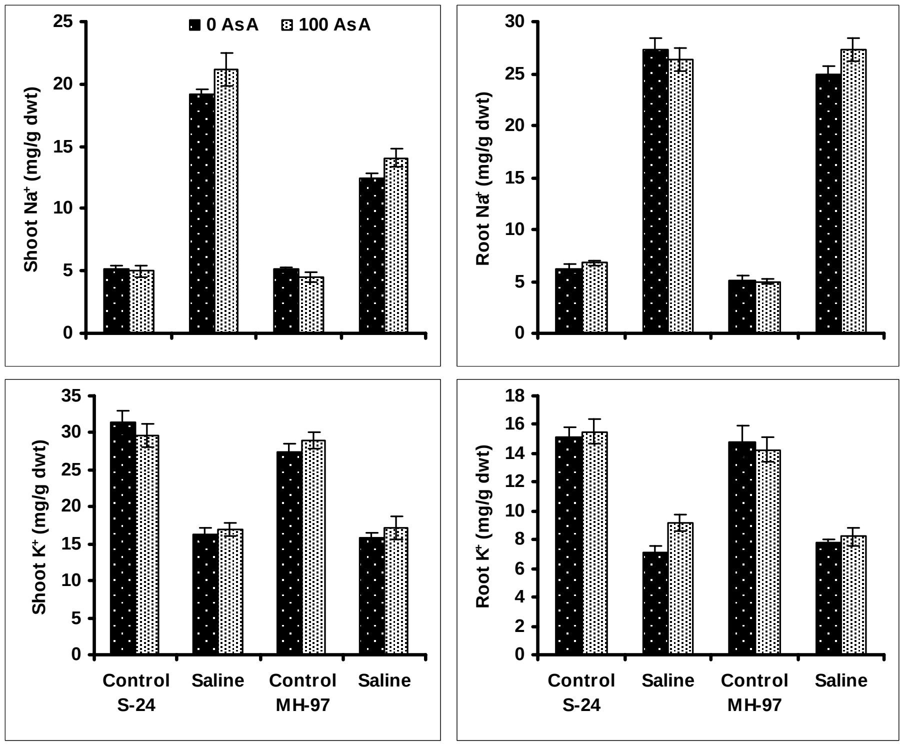

Fig. 3. Accumulation of Na^+ and K^+ in shoots and roots of three-week-old seedlings of two spring wheat cultivars when 0 or 100 mg L^{-1} ascorbic acid (AsA) were applied as a foliar spray.

Antioxidants, enzymes or non-enzymes, have the ability to scavenge reactive oxygen species (ROS). Of non-enzymatic antioxidants, salicylic acid and ascorbic acid are more important due to having their promotive role in plant growth and development in addition to their antioxidant capacity (Raskin *et al*., 1992; Barth *et al.*, 2004; 2006; Pavet *et al*., 2005).

In view of the results obtained from this study, it is obvious that salt stress caused a reduction in the growth of both wheat cultivars at the seedling stage. However, exogenously applied ascorbic acid (AsA) as a foliar spray promoted the growth of both wheat cultivars under non-saline conditions. This growth promoting effect of AsA was more pronounced in S-24. These findings can be related to some earlier studies in which it has been observed that exogenous application of AsA promoted growth in wheat (Hamada & Al-Hakimi, 2001; Al-Hakimi, 2001), and tomato (Shalata & Neumann, 2001). AsA-induced increase in growth under non-saline may have been due to accelerated cell division and/or cell enlargement (Arrigoni, 1994). However, from the results of the present study it is obvious that foliar spray with AsA was less effective in improving the growth of salt stressed plants of both cultivars, which is in contrast to the findings of Shalata & Neumann (2001) who found that AsA applied through the rooting medium counteracted the salt induced reduction in growth. However, in the present study, AsA was applied as a foliar spray. Thus, effectiveness of exogenously applied AsA depends on the mode of application too, which may enhance endogenous level of AsA.

It is generally known that photosynthetic efficiency depends on photosynthetic pigments such as chlorophylls '*a*' and '*b*', which play an important role in photochemical reactions of photosynthesis (Taiz and Zieger, 2006). In the present study, adverse effect of salt stress on chlorophyll '*a*' was counteracted by AsA application. The changes in leaf chlorophyll content may have been due to reduced biosynthesis or increased degradation of chlorophyll under saline conditions. Furthermore, in salt stressed plants, breakdown of ultrastructure of chloroplasts including plastid envelop, thylakoids (Santos, 1998), and photosynthetic apparatus may result due to direct Na^+ toxicity or salt-induced oxidative damage (Mittler, 2002). In the present study, salt stress increased Na^+ accumulation but decreased that of K^+ in the leaves and roots. Thus salt induced reduction in chlorophyll *a* of both wheat cultivars might have been due to Na^+ toxicity. However, from the results of AsA-induced enhancement in chlorophyll '*a*', it is suggested that foliar applied AsA protected photosynthetic apparatus from salt induced oxidative stress. This view is further supported by the fact that chloroplast is a major source of production of reactive oxygen species (ROS) in plants (Ormaetxe *et al*., 1998), but it lacks catalase to scavenge ROS, therefore AsA acts as a substrate for ascorbate peroxidase (APX) to scavenge ROS produced in the thylakoid membranes (Davey *et al*., 2000). Furthermore, foliar applied AsA did not cause substantial changes in antioxidant enzymes, which may affect chlorophyll '*a*'. Thus, it seems that enhanced activities of SOD-CAT-POD in salt stressed plants did not completely scavenge ROS or toxic effects of Na^+ on chlorophyll 'a'.

Although AsA promoted the growth of non-stressed plants of both wheat cultivars, salt induced reduction in growth was not ameliorated by the foliar application of AsA. In addition, exogenous application of AsA enhanced chlorophyll *a* contents, which suggests a protective role of AsA on photosynthetic pigments against salt-induced oxidative damage to photosynthetic pigments.

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