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Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress

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ABSTRACT

Selenium (Se) is considered an essential micronutrient for humans, animals and plants due to its physiological and antioxidative properties. The positive role of Se in attenuation of drastic effects of various environmental stresses in plants is, however, still unclear and need to be explored. The present study aimed at investigating the physiological and biochemical changes induced by Se foliar spray to improve the drought tolerance potential of wheat. Additionally, we also examined the effect of supplemental Se on uptake of nutrients using detection by ICP-OES. Foliar Se application significantly lowered osmotic potential (13%) that markedly improved turgor by 63%, enhanced transpiration rate (60%), improved accumulation of total soluble sugars (33%) and free amino acids (118%) and activity of antioxidant system which ultimately increased the grain yield by 24%. Supplemental Se also significantly increased Se contents (5.77 $\mu g\ g^{-1}$ DW) and improved Fe (91%) and Na (16%) uptake, whereas it reduced Zn accumulation by 54% and did not affect Ca contents. The results supported our hypothesis that supplemental Se influences nutrients uptake and wheat yield through maintenance of turgor and gas exchange characteristics and enhancement in antioxidant system activity.

1. Introduction

Drought stress is one of the major limitations to agricultural productivity around the globe (Waraich et al., 2011). The identification of strategies to improve plant productivity under limited water conditions is a big challenge for plant scientists. The arid and semi-arid regions of the world, especially in the developing countries, are at greater risk because these regions are already facing acute shortage of water. Furthermore, an increasing frequency of droughts in days ahead will make natural and cultivated vegetation more vulnerable to severe and acute shortage of water (Nawaz et al., 2012). Wheat is a major food grain crop of the world. Hence it is necessary to develop better drought mitigation strategies, with the ultimate goal of increasing drought tolerance potential and wheat yield to meet the demands of ever growing world's population.

The physiological and antioxidative properties of selenium (Se) have raised the curiosity of biologists in recent past. It plays beneficial role in plants by enhancing growth of plants (Cartes et al., 2010), reducing damage caused by UV-induced oxidative stress (Yao et al., 2013), increasing chlorophyll and carotenoids in plant leaves (Dong et al., 2013), stimulating enzymatic and non-enzymatic antioxidant system against Cd-toxicity (Kumar et al., 2012; Lin et al., 2012) and improving plant tolerance to drought stress by regulating water status (Yao et al., 2009). Low levels of Se stimulate the antioxidant machinery in plants but it acts as a prooxidant at high levels (Feng et al., 2013).

The increase in acidity, iron oxides/hydroxides, organic matter and high clay content of soil (Mikkelsen et al., 1989; Kabata-Pendias, 2001) decreases the effectiveness and bioavailability of Se to plants. The soil moisture also affects the availability of Se as it is more available under low precipitation conditions (Zhao et al., 2007). The foliar application of Se can be used as an alternative for enrichment of agricultural products with Se (Smrkolj et al., 2006) because it minimizes the impact of soil chemistry and microbiology on Se uptake and accumulation, hence improving efficacy even with low volumes of foliar applied Se solution (Kapolna et al., 2012). Furthermore, the actively growing tissues usually contain large amounts of Se (Kahakachchi et al., 2004) and accumulation is higher in shoot than in root tissues (Zayed et al., 1998). Hence Se foliar spray is much more viable and effective approach than soil application to improve Se content in plants (Kapolna et al., 2009). Foliar Se application facilitates Se transport through xylem and

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phloem (Boldrin et al., 2013). Poggi et al. (2000) observed great mobility of Se in the phloem of potato plants by Se foliar spray. The foliar Se application results in direct transfer and accumulation of Se in plants. Diffusion of Se ions to leaf epidermal cells increases by foliar application (Wójcik, 2004) but its high concentrations may lead to toxicity and cause damage to leaf surface (Marschner, 1995). The foliar spray of Se has been reported to significantly promote growth in vegetables such as onion bulbs and leaves (Kapolna et al., 2012), carrot roots and leaves (Kapolna et al., 2009), radish flowers and leaves (Hladun et al., 2013) as well as garlic bulbs (Põldma et al., 2011) and in cereals like rice (Boldrin et al., 2013) and wheat (Yao et al., 2013).

Literature indicated positive effect of Se in improving drought tolerance through accumulation of compatible solutes and activation of enzymes in barley (Habibi, 2013), rapeseed (Hasanuzzaman and Fujita, 2011) and wheat seedlings (Yao et al., 2009; Nawaz et al., 2013). However, these results involved short-term studies and reports regarding Se mediated physiological and biochemical mechanisms responsible for enhancing yield of food crops particularly wheat under environmental stresses like drought are scant. This study provides useful information concerning the effects of Se foliar application on physiological and biochemical mechanisms in wheat under normal and water deficit conditions. We presumed that supplemental Se influences wheat yield and nutrients content through maintenance of turgor, accumulation of osmoprotectants and activation of antioxidant machinery.

2. Materials and methods

2.1. Experimental conditions

Lysimeters (cemented tanks) measuring 9 m^2 (3×3 m^2) and 1 m in depth were used for the present study. A buffer zone consisting of 15 cm thick cemented wall on each side was used to prevent seepage between tanks. The tanks were precision levelled before planting to ensure even distribution of water. The physiochemical characteristics of the soil were determined by following the method of Jackson (1962), whereas the method published by Ramos et al. (2010) was used to determine total Se content in soil with results as follows: sand=46.54%; silt=28.71%; clay=24.75%; saturation percentage=41.24%; pH=7.12; electrical conductivity=1.19 dS m^{-1}; soil organic matter=0.27%; available nitrogen (N)=31 mg kg^{-1}; available phosphorous (P)= 7.44 mg kg^{-1}, available potassium (K)=84 mg kg^{-1} and total Se content=0.114 mg kg^{-1}. A basic dose of N, P and K fertilizers in the form of urea (110 kg N ha^{-1}), diammonium phosphate (80 kg P_2O_5 ha^{-1}) and potassium sulfate (60 kg K_2SO_4 ha^{-1}) was broadcasted and mixed with the surface layer (0–15 cm) one week before sowing. A pre-planting irrigation of 75 mm was applied in both tanks before the experiment.

The randomly selected healthy seeds of wheat (*Triticum aestivum* L. cv Pasban-90) provided by Ayyub Agricultural Research Institute (AARI), Faisalabad (Pakistan) were sterilized with 5% sodium hypochlorite solution for five minutes prior to sowing. The seeds were hand drilled in rows of 0.80 m length keeping $R \times R$ distance of 0.30 m when soil was at field capacity condition. The tanks were manually weeded and hoed whenever found necessary. The experimental design was a 2×2 factorial scheme as follows: normal water supply without supplemental Se (W), Se supply under normal conditions (Se), drought stress without supplemental Se (D) and Se supply under water deficit conditions (D$\pm$Se) with three replications. The plants were sampled at flowering stage (90 days after sowing) and flag leaves were used for the investigation of physiological and biochemical changes in wheat.

2.2. Drought stress and Se foliar application

Each tank in the lysimeter was allocated water stress treatment i.e., normal (100% field capacity) and drought stress (60% field capacity) conditions. The normal plants were irrigated with 75 mm tap water approximately twice per week, whereas water deficit conditions were imposed by the application of 45 mm irrigation water till maturity. The quantity of applied irrigations was measured using a water meter. A manually operated shelter equipped with movable sheet of transparent flexible polythene was used for protection from rain.

The foliar application of Se was carried out using sodium selenate (Na_2SeO_4, Sigma-Aldrich, USA) solution of 40 mg Se L^{-1}, verified analytically using atomic absorption spectrometry technique (Krishnaiah et al., 2003), containing 0.1% Tween-20 as a surfactant for foliar spray during flowering. A compression sprayer was used for this purpose to ensure even distribution of Se on all leaves. The spraying was performed in the morning (between 08:00 and 10:00) with a compression sprayer of 1 L capacity on a dry, sunny day and was repeated after three weeks. The same amount of distilled water was used for treatments without Se supply. The plants were harvested at maturity and randomly selected shoots (stem plus leaves) were used for the estimation of nutrients contents.

2.3. Leaf water relations determination

The flag leaves of plants from each treatment were used to determine leaf water potential (Ψ_w). The measurements were made early in the morning between 08:00 and 10:00 with Scholander type pressure chamber (ARIMAD-2, ELE-International). The same leaves as used for Ψ_w measurements, were frozen at -20 °C for osmotic potential (Ψ_s) measurements. The frozen leaf material was then thawed and cell sap was extracted while crushing the leaves with a glass rod. The sap so extracted was directly used for the determination of Ψ_s using an osmometer (Wescor, 5520). The leaf turgor potential (Ψ_p) was calculated as the difference between Ψ_w and Ψ_s values.

$$(\Psi_p) = (\Psi_w) - (\Psi_s)$$

For relative water content's (RWC) measurements, five leaves (flag leaf) from each treatment were taken. Fresh weight (FW) of each sample was recorded using a digital electrical balance (Chyo, MK-500 C) and leaves were dipped in test tubes containing distilled water. After 24 h, the leaves were taken out, wiped with the tissue paper and the turgid weight (TW) was recorded. The samples were dried at 65 °C for 72 h and dry weight (DW) of each sample was determined. Relative water contents were calculated using the formula given by Cornic (1994).

$$RWC=[(FW - DW)/(TW - DW)] \times 100$$

2.4. Determination of gas exchange characteristics

The net photosynthetic rate (P_n), transpiration rate (E) and stomatal conductance (g_s) were recorded using photosynthesis measuring-system, CI-340 portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were taken between 9.00 and 11.00 a.m. with the following adjustments: molar flow of air per unit leaf area 403.3 mmol m^{-2} s^{-1}, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 mbar, PAR at leaf surface was maximum up to 1711 mol m^{-2} s^{-1}, temperature of leaf ranged from 28.4 to 32.4 °C, ambient temperature ranged from 22.4 to 27.9 °C and ambient CO_2 concentration was 352 mol mol^{-1}.