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Selenium Accumulation and Antioxidant Status of Rice Plants Grown on Seleniferous Soil from Northwestern India

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Abstract: Greenhouse experiment was conducted to investigate selenium accumulation and its antioxidant response in two rice varieties (PR116 and Pusa Basmati 1121) grown on normal and seleniferous soils. The plant growth was reduced at early developmental stages and flowering was delayed by a period of 10 d on seleniferous soil. Selenium accumulation increased by 3–20 and 13–14 folds in leaves, 18 and 3 folds in grains from Pusa Basmati 1121 and PR116 varieties, respectively. Selenium accumulation in leaves from rice plants grown on seleniferous soil resulted in significant increase in chlorophyll content, hydrogen peroxide, proline, free amino acids, total phenol and tannin contents. Lipid peroxidation levels and peroxidase activities in leaves increased whereas catalase activity showed a reverse trend. It is concluded that selenium accumulation decreased dry matter content in rice during crop development but these plants were able to combat selenium toxicity by inducing alterations in their defense system.
Key words: antioxidant enzyme; chlorophyll content; rice; selenium

Selenium (Se) is an important trace element in animal and human nutrition but known as a non-essential element in plants, although its beneficial roles in plants capable of accumulating large amount of the element have been reported (Terry et al, 2000; Freeman et al, 2006; White et al, 2007). Plants capable of accumulating high amounts of Se without any toxic symptoms can be used for bioremediation of Se-contaminated soils and water (LeDuc et al, 2006). High levels of Se can cause adverse effects in most of Se-sensitive crop plants (Geoffrey et al, 2007; Yi and Si, 2007), and the metabolic role and regulation of Se toxicity in these plants are poorly understood.

Selenium concentration in soils can vary from 0.01 to 2.00 mg/kg, and in some seleniferous regions, Se content < 1 200 mg/kg has been reported (Fordyce, 2005). Higher Se concentration can be absorbed by plants grown on these Se-rich soils that can be toxic to the grazing livestock. The toxicity of Se is related to its chemical similarity to S, leading to non-specific replacement of S by Se in proteins (Terry et al, 2000) and/or the peroxidant ability to catalyze the oxidation of thiols and simultaneously generate reactive oxygen species (ROS) (Groppa et al, 2007). Active oxygen radicals can directly attack membrane lipids and result in peroxidation of unsaturated fatty acids that leads to formation of products such as malondialdehyde (MDA) (Halliwell and Gutteridge, 1999). Higher plants have active oxygen-scavenging systems comprising of enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), whereas vitamin C, tocopherols, phenolic compounds and glutathione act as nonenzymatic antioxidants within the cells. Activities of antioxidant enzymes are reported to increase in plants to combat oxidative stress induced by various types of stress. High antioxidant activities scavenge the ROS produced during stress in plants, thus increasing their tolerance to environmental stress (Apel and Hirt, 2004).

Pockets of seleniferous soil have been reported in northwestern India with Se in soils ranging from 0.25 to 4.55 mg/kg with a mean value of 3.63 mg/kg (Dhillon and Dhillon, 2003). At the seleniferous sites, it is present in the soil profile up to 2 m depth and the surface layer contains 1.5 to 6.0 times more Se in comparison to the lower layers (Dhillon and Dhillon, 2009). At some of the toxic sites, ground water–the main source of irrigation is contaminated with Se (Dhillon and Dhillon, 2003). Se contaminated water is

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not only used for drinking purposes but also for irrigation of various crops especially rice. Rice is one of the major Kharif crop grown in Punjab, India and an important source of carbohydrates, fibers and vitamins. Rice varieties differ significantly in their Se absorption capacity (Zhang et al, 2006). The objective of the present study was to compare Se accumulation in different plant parts of two rice varieties grown on seleniferous soil from these areas and their antioxidant status at various crop developmental stages.

MATERIALS AND METHODS

Greenhouse experiment

A greenhouse experiment was conducted by growing rice varieties on normal and seleniferous soils. The normal (non-seleniferous) soil was collected from the research farm of Punjab Agricultural University, Ludhiana located between 30.92° N and 75.85° E in northwestern India. The normal soil tested clay loam in texture, alkaline in reaction having a pH of 8.3, electrical conductivity of 0.20 dS/m, organic matter of 0.39% and total Se content of 0.135 mg/kg. Seleniferous sites are located between 31.04° to 31.22° N and 76.14° to 76.41° E in northwestern India. The bulk soil was collected from a seleniferous field located in Barwa village of Nawanshahar district, Punjab, Indian. The soil tested silt loam in texture, alkaline in reaction having a pH of 7.9, electrical conductivity of 0.36 dS/m, organic carbon of 0.48% and total Se content of 2.850 mg/kg.

The soil was air-dried in shade, ground to pass through 2 mm sieve and stored in a clean and dry place. Samples of experimental soil (4 kg) were weighed and placed in series of pots. The treatments consisted of two soil types (normal and seleniferous), two rice varieties (Pusa Basmati 1121 and PR116), and two growth stages with three replications. Three 15-day-old seedlings of each variety were procured from the nursery and transplanted into the respective pots. Irrigation was regularly supplied as distilled water.

Sampling

Leaf samples were collected at 45 d (tillering) from both the treatments and after 80 and 90 d (flowering) from rice plants grown on normal and seleniferous soils, respectively. The grain samples were collected at maturity and separated into husk and grains. Three replicate samples of each rice variety from the normal and seleniferous soils were collected. Leaf samples were brought immediately to the laboratory in an ice box. To remove surface-contaminants, all the samples were washed gently with tap water followed by rinsing with distilled water. Plant tissue samples collected at different growth stages of rice were subjected to analysis of Se and biochemical components. For dry matter determination, a weighed quantity of leaf tissue was oven dried at (55 ± 5) °C to a constant weight. After drying, the tissue was immediately placed in desiccators until final weight was recorded.

Biochemical analysis

For chlorophyll content determination, homogenized fresh leaf material was extracted with dimethyl sulfoxide and the content was determined by measuring the absorbance at 663 and 645 nm (Johnson et al, 1984). Phenolic compounds were extracted by refluxing the seed powder with 80% aqueous methanol at 60 ºC in a water bath with continuous shaking for 2 h. The refluxed material after filtration was used for the estimation of total phenols (Swain and Hillis, 1959). A standard curve of gallic acid (10–100 μg) was simultaneously prepared and the amount of the phenols was calculated and expressed as mg/g seed. Tannins were extracted from the powdered seeds and estimated using Folin-Denis reagent, and the intensity of the colour developed was measured at 700 nm (Sadasivam and Manickam, 1992). A standard curve of tannic acid (10–100 μg) was simultaneously prepared.

Proline content in leaves was determined according to Bates et al (1973). Fresh leaves (1 g) were extracted with 0.1 mol/L sulfosalicylic acid and centrifuged at 5 000 × *g* for 30 min. The proline content of the supernatant was measured at 520 nm, calculated from the standard curve and expressed as μg/g fresh weight. Free amino acids were extracted from fresh leaves with 80% ethanol and determined using a standard ninhydrin assay. Quantitative estimation of free amino acids was made according to Lee and Takahashi (1966). H_2O_2 content was determined at 570 nm using 5% $K_2Cr_2O_7$ and glacial acetic acid and expressed as μmol/g fresh weight (Sinha, 1972).

Lipid peroxidation (LPO) and antioxidant enzymes

Five hundred milligram leaves were hand-homogenized using 5 mL of 5% trichloroacetic acid at 4 °C in pre-chilled mortar and pestle, and the homogenate was centrifuged at 10 000 × *g* for 20 min. To measure the extent of LPO, levels of thiobarbituric acid reactive substances were estimated at 535 nm and MDA