Spermine and putrescine enhance oxidative stress tolerance in maize leaves

Nuran Durmuş* and Asım Kadıoğlu

Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080, Trabzon, Turkey
*Author to whom all correspondence should be addressed, e-mail: durmusn@hotmail.com

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Abstract

The protective effects of spermine (SPM) and putrescine (PUT) against paraquat (PQ), a herbicide in agriculture and oxidative stress inducer, were investigated in the leaves of maize. Maize leaves were pretreated to SPM and PUT at concentrations of 0.2 and 1 mM and treated with PQ afterwards. Pretreatment with 1 mM of SPM and PUT significantly prevented the losses in chlorophyll and carotenoid levels induced by PQ. Ascorbic acid content in the leaves pretreated with both polyamines was found to be higher than those of the leaves pretreated with water. Also, pretreatment with SPM and PUT was determined to have some effects on the activities of superoxide dismutase (SOD) and peroxidase (POD). 1 mM of SPM increased SOD activity, but PUT has no significant effect on SOD activity. On the other hand, POD activity was recorded to increase slightly in response to both concentrations of SPM and 1 mM of PUT. The results showed that such polyamine pretreated plants may become more tolerant to oxidative stress due to increases in the antioxidative enzymes and antioxidants.


Introduction

Crop loss due to environmental stresses is the primary source of decrease in agricultural productivity. The reason is partly due to oxidative stress that is the overproduction of reactive oxygen species (ROS) in plant cells under these environmental conditions. Abiotic and biotic stresses (pollutants, herbicides, extremes of temperature and high light, high O2 pressures, salinity and pathogen invasion) all cause increases in toxic ROS in plant cells (Sakaki et al. 1983, Kenylon and Duke 1985). The containment of ROS has proved to be important for problems as diverse as aging and cancer in human health and crop loss in agriculture. Therefore, understanding of oxidative stress and antioxidant defense mechanisms and alleviation of oxidative damage are important for plant productivity. It has been proposed that polyamines could take part in cellular defense mechanism against oxidative damage through the inhibition of lipid peroxidation (Tadolini 1988). In addition, polyamines are well known for their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties, as well as to their membrane and cell wall stabilizing abilities (Velikova et al. 2000). Free radical scavenging properties of polyamines have also been documented (Drolet et al. 1986). Despite extensive studies on polyamine metabo-
lism, the exact role that these compounds play in plant physiology remains unclear (Tiburcio et al. 1997).

Paraquat herbicidal activity in higher plants is thought to be the result of increased amount of superoxide radical. Paraquat accepts an electron from the primary electron acceptor of photosystem I to become a reduced free radical which rapidly reacts with oxygen to form the superoxide radical (Dodge 1994). Superoxide serves as a source of hydrogen peroxide and the highly active hydroxyl radical. Thus, toxicity of paraquat stems from the generation and activity of oxygen species that lead to oxidative stress in biological systems. Reactive oxygen species can react with numerous cell components causing inactivation of enzymes, pigment bleaching, lipid peroxidation, and proteolysis. Thus, they need to be scavenged for maintenance of normal plant growth. Plant cells contain substances such as glutathione, ascorbic acid and carotenoids, and also enzymes like superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), glutathione reductase (GR; EC 1.6.4.2) which participate in scavenging ROS (Halliwell 1982). The primary scavenger is the enzyme superoxide dismutase which converts superoxide to hydrogen peroxide (Asada and Kiso 1973). This toxic product of SOD is removed by POD. Enhanced production of oxygen free radicals is responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells was controlled by the potency of the antioxidative POD enzyme system (Sreenivasulu et al. 1999). In several plant species paraquat tolerance was correlated with increased capacity of enzymes detoxifying activated oxygen species (Shaaltiel et al. 1988; Furusawa et al. 1984). Correspondingly, paraquat tolerance in _Conyza, Lolium_ or _Nicotiana_ was also accompanied by a cross-tolerance to other environmental factors involving oxidative stress, such as SO2 or ozone (Shaaltiel et al. 1988; Tanaka et al. 1988).

In this study we investigated if prior exposure to SPM and PUT may protect plants against exposure to paraquat and if this protection may be related to antioxidative enzyme activities and antioxidant levels.

Materials and Methods

Plant material and treatments

Maize (_Zea mays_ L. cv RX 947) seeds which were obtained from Agriculture Research Center in Trabzon were sown in plastic pots (11 cm high, 23 cm top and 13 cm bottom diameter) filled with soil and sand (5:1). They were maintained in a growth chamber under a 16-h light/8 h dark regime with a light intensity of 350 μE m−2 s−1, 75 % relative humidity, and day/night temperatures of 25/22 °C. Ten day-old maize plants were sprayed until run off with SPM and PUT at 0.2 and 1 mM concentrations, each containing 0.05 % Tween 20 as a surfactant once daily for 4 days. Then, for paraquat treatment, leaves of 14-d-old plants were exposed in a surface application to 10−4 M paraquat (methyl viologen) in a 0.05 % solution of the Tween 20 for 24 h into the light period. Control plants were sprayed with 0.05 % Tween 20 in distilled water. Foliar samples were collected for analyses at 8, 12 and 24 h following PQ application, immediately frozen in liquid nitrogen and stored at −20 °C for determination of enzyme activities. All treatments were repeated at least three times on different days.

Determination of chlorophyll and carotenoids

For chlorophyll and carotenoid determinations, the leaves were homogenized in 5 ml of 80 % acetone and centrifuged at 3000 rpm for 5 min. The optical density of the supernatant was read at 450, 645 and 663 nm with a spectrophotometer. The amounts of total chlorophyll and carotenoids were estimated according to Arnon (1949) and Jaspars (1965), respectively.

Determination of ascorbic acid

The determination of ascorbic acid was performed using the procedure of Shieh and Sweet (1979) with pure ascorbic acid as the standard. Two g samples were homogenized with 0.01 M phosphate-citric acid buffer, pH 3.0, filtered and centrifuged at 5000 rpm, for 5 min at 25 °C. The supernatant was used to determine the ascorbic acid content. The assay mixture consisted of 0.5 ml of 0.01 M phosphate-citric acid, pH 3.0, 2.4 ml of 2,2′-Cu-biquinoline solution (1.0 mM 2,2′-biquinoline and 0.38 mM...