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Status and role of antioxidant enzymes in sexual and apomictic species of *Boechera*

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Introduction

Enzymatic and non-enzymatic antioxidation systems provide protection against the toxic effects of activated oxygen species including scavenging H₂O₂. It is evident that during stress plants produce different ROS which play a negative role in regulating the antioxidant system and a positive role in signaling events that regulate ion channel activity and gene expression. Microarray data indicate that ovules and pistils from apomictic *Boechera* differ in gene expression from those of sexual *Boechera*. Specifically, these differences involve genes responsible for stress response, RNA processing, ribosome synthesis and function, photosynthesis and cell cycle regulation. Thus, in the present study, activities of three antioxidant enzymes *viz.* guaiacol peroxidase, catalase and ascorbate peroxidase were measured in the young unopened buds of two sexual (*B. stricta* and *B. yellowstonensis*) and two apomictic (*B. retrofracta x stricta* and *B. microphylla*). The activity staining of these enzymes were performed to detect isozymes of these enzymes in a species-specific manner.

Materials and Methods

Unopened buds (150 mg) were homogenized using chilled mortars and pestles, and enzymes were extracted in 1.0 ml sodium phosphate buffer (0.1M, pH 7.0). The homogenate was centrifuged at 14,000 x g for 10 min. at 4 °C. The supernatant was used for enzyme activity and protein content estimations.

Peroxidase was assayed by measuring the oxidation of guaiacol at 470 nm in the presence of H₂O₂ as described by Volk & Feierabend (1989). Catalase was assayed by measuring the degradation of H₂O₂ at 240 nm (Aebi, 1984). Ascorbate peroxidase was assayed by measuring the degradation of ascorbic acid at 290 nm (Nakano and Asada, 1981). The different isozymes were separated on 12% Criterion TGX precast gels (Biorad) under native conditions at 4 °C and were stained for activity of respective enzymes to visualize isozymes and zymograms of each different enzymes was prepared.

Results and Discussion

The highest peroxidase activity was found in *B. microphylla*, and the lowest activity was in *B. stricta* (Fig.1). The activity in *B. microphylla* was about 5x higher more than in *B. stricta* or *B. retrofracta x stricta*. Catalase activity was highest in *B. retrofracta x stricta* and lowest in *B. microphylla* (Fig.2). In this case also, the activity in *B. retrofracta x stricta* was about 5x higher than the activity in *B. microphylla*. For ascorbate peroxidase, *B. microphylla* had the highest activity among the species studied (data not shown). The activity was about 3x higher than the activity in *B. retrofracta x stricta*. Combining the activity profiles of these enzymes reveals that in the group *B. stricta* and *retrofracta x stricta*, catalase is the main enzyme responsible for detoxification of H₂O₂. However, in *B. yellowstonensis* and *B. microphylla*, catalase activity was less and the major antioxidant enzymes were peroxidase and ascorbate peroxidase.

Increasing stress levels resulted in a shift from asexual to a sexual mode of reproduction in bacteria and *Daphnia*. It has been hypothesized that oxidative stress is the trigger for meiosis. Many proteins involved in meiosis are derived from oxidative damage repair proteins of bacteria. During apomixis in flowering plants, there is an alteration or an exclusion of meiosis (apomeiosis) and the development of an unfertilized unreduced egg cell into an embryo (parthenogenesis).

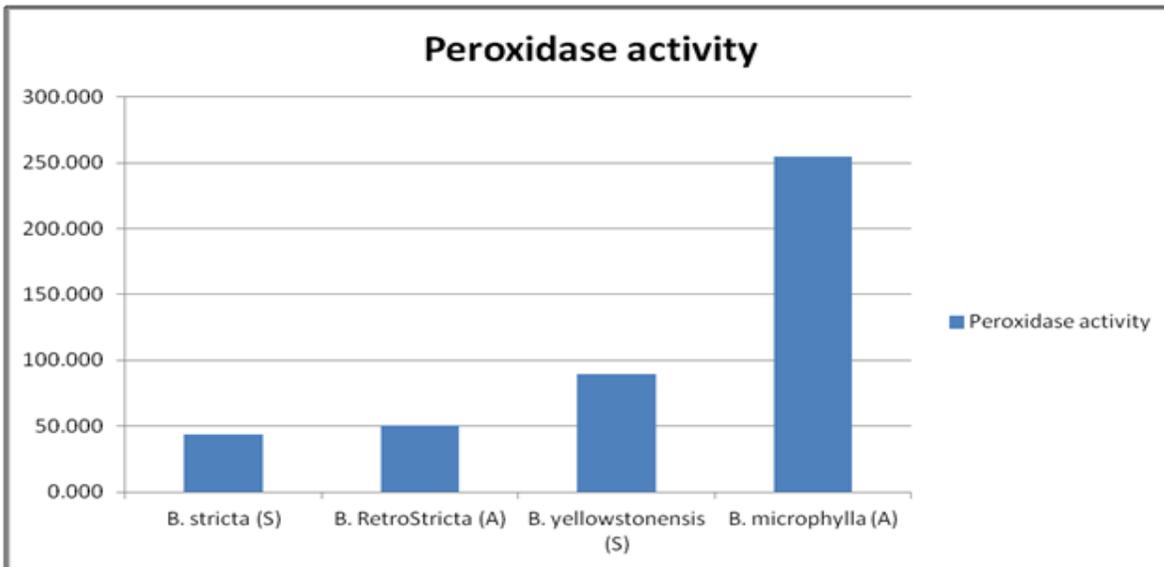


Fig. 1: Profile of peroxidase activity in *Boecheera* species

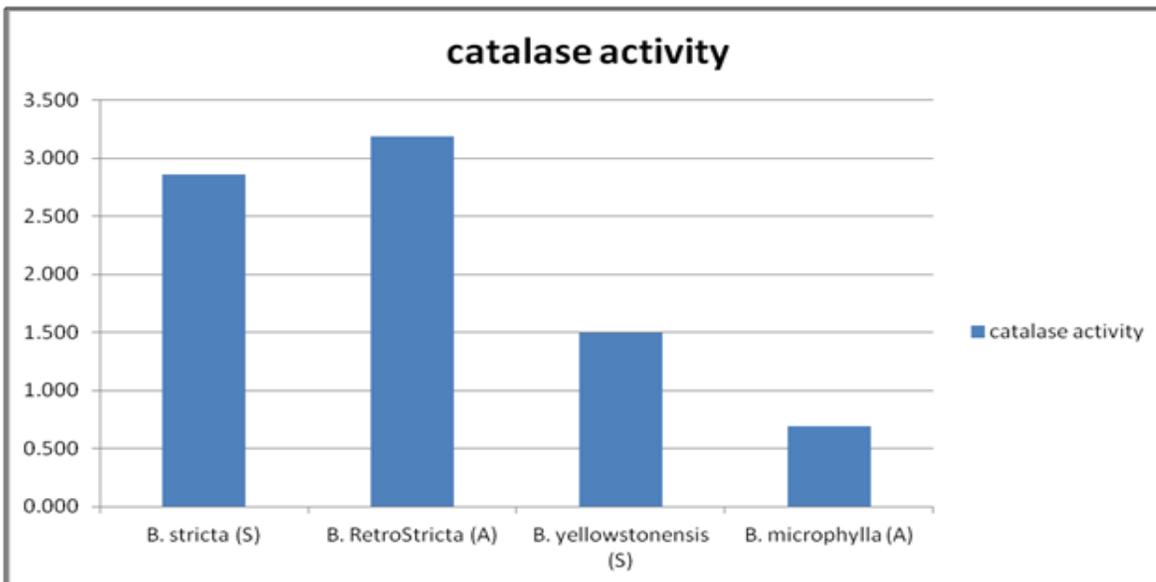


Fig. 2: Profile of catalase activity in *Boecheera* species

Conclusion

Apomixis is a feature of great importance in agriculture owing to its capacity to fix hybrid vigour. Role of ROS and antioxidant enzyme network in determining the mode of reproduction for a particular species will adapt, suggests that stress is a key factor in determining the mode of reproduction in a plant.

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