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Evaluation of Drought stress on Antioxidant enzyme activity in Rapeseed cultivars

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ABSTRACT

After cereals, oil seeds are the second source of food, and canola is the third source of oil seeds crop in the world after palm oil and soybean. This study was performed as factorial in RCBD design, First factor included cultivars (Zarfam, Talaye, Okapy) and second factor included drought stress (control, 60% FC, 40%Fc), also some properties were studied such as Superoxide dismutase, Proxidase, Catalase, yield. Totally, results showed that antioxidant enzymes increase as one mechanism in responses to stress and cultivars had different amount of enzyme unit. Between cultivars Zarfam and Talaye had highest antioxidant enzyme, respectively, highest means of SOD (31.6), POD (46) and CAT (23 Enzyme activity unit per mg protein) were obtained by Zarfam cultivar in 40% FC condition. Enzyme unit increased by increasing of drought levels and Zarfam, Talaye and Okapy showed 219, 198 and 112 gr/m2 yield in 40% FC condition.

Introduction

Abiotic stresses, including drought contribute to formation of reactive oxygen species (ROS), superoxide radical (O^{-2}) ,hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), the last one is the most cytotoxic. These ROS cause perturbation of basic metabolic pathways, and damage membranes and organic molecules, mainly proteins, DNA and pigments (Fridovich, 1986; Imlay and Linn, 1988), and sulphur containing amino acids in proteins (Hernandez et al., 2000). However, Plants use different strategies to solve this problem. For instance, one of the strategies is that the plants increase the activity of antioxidant enzymes. On the other hand, the activity of antioxidant enzyme correlates with resistance to salinity (Kayupova and Klyshev, 1984). The peroxidation of membrane lipids and the degree of peroxidative damage of cells happen due to abundant productions of oxygen free radicals, guaiacol peroxidase enzyme system suppresses the increase of oxygen free radicals (Sreeniv et al., 1999), The toxic superoxide radical is rapidly dismutated by superoxide dismutase (SOD) to H2O2, a product which is relatively stable and can be detoxified by catalase (CAT) and guaiacol peroxidases (Grant and Loake, 2000). Increased SOD activity is known to confer oxidative stress tolerance (Bowler et al., 1992). Activities of antioxidant enzymes (SOD, CAT and POX) have been proved to be very effective in discriminating the oilseed rape cultivars to abiotic tolerance (Ashraf and Ali, 2007). There aremany reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stresses in several plant species, such as rice (Guo et al., 2006), foxtail millet (Sreenivasulu et al., 2000), tomato (Mittova et al., 2000), sugar beet (Bor et al., 2003), rapeseed (Zare and Pakniyat, 2012), wheat (Khanna-Chopra and Selote, 2007) and barley (Acar et al., 2001). After cereals, oil seeds are the second source of food, and canola is the third source of oil seeds crop in the world after palm oil and soybean (FAO, 2011).

In Iran, a large amount of vegetable oil for human consumption is imported and, hence, cultivation and appropriate management of oil seeds to enhance yield is very important. Canola (*Brassica napus* L.) species is considered as a relatively moderately drought-sensitive species within which there is certain variability towards drought tolerance (Omidi, 2010). With 240 mm annual average rainfall, Iran is considered as an arid and semi-arid country. Water scarcity, high evapotranspiration, and other factors may cause several limitations in crop production; therefore, research on the effects of drought stress and selection of appropriate cultivars is encouraged (Moradshahi et al., 2004; Tohidi-Moghadam et al., 2009; Youssefi et al., 2011). Drought is considered as one of the most important environmental stresses limiting plant growth and crop productivity (Terzi and Kadioglu, 2006). Up to 45% of the world agricultural lands are subject to continuous or frequent drought stress, wherein 38% of the world human population resides (Ashraf and Foolad, 2007). In addition, water-stressed plants could be more sensitive to other biotic or abiotic stresses such as pathogen attack and weeds, which limit plant productivity (Caruso et al,. 2008). Drought can be defined as the absence of adequate soil moisture necessary for a plant to grow normally and complete its life cycle (Manivannan et al., 2008). The aim of this study was evaluation of drought stress on Antioxidant enzyme activity in Rapeseed cultivars.

Material and Methods

This study was performed as factorial in RCBD design, First factor included cultivars (Zarfam, Talaye, Okapy) and second factor included drought stress (control, 60% FC, 40%Fc), also some properties were studied such as Superoxide dismutase, Proxidase, Catalase, yield. Superoxide dismutase activity, the basis of which is its ability to inhibit the phothochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, 1971), was determined according to the method of Dhindsa et al. (1980).

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For SOD assy, the reaction mixture contained 50mM K-phosphate buffer (pH 7.8), 13mM methionine, 75µM NBT, 0.1µM EDTA, 4µM riboflavin and required amount of enzyme extract. The reaction was started by adding riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal color, served as control. A non-irradiated complete reaction mixture served as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm, which was measured according to the method of Giannopolitis and Ries (1977). Proxidase activity was assayed adopting the method of Polle et al. (1994). According to this method POD activity was determined at 436 nm by its ability to convert guaiacol to tetraguaiacol, The reaction mixture contained 100mM K-phosphate buffer (pH 7.0), 20.1mM guaiacol, 10mM H_2O_2 and enzyme extract. The increase in absorbance was recorded by the addition of H_2O_2 at 436 nm for 5 min. CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm, according to the method of Aebi (1984). The reaction mixture contained 50mM K-phosphate buffer (pH 7.0), 33mM H_2O_2 and enzyme extract. Data were subjected to Duncan's multiple range tests using the SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

Result and Discussion

Superoxide dismutase

According to results it was founded that there were significant differences between cultivars and drought levels treatments (P<0.01), between cultivars, Zarfam had highest means (31.6 Enzyme activity unit per mg protein) of superoxide dismutase. Also results showed that Superoxide dismutase means increased by increasing of drought stress and application of 60 and 40%FC led to 1.72 and 2.13 fold increasing in compare to control. Induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses (Foyer and Noctor, 2003). The activities of these antioxidant enzymes are reported to increase under various environmental stresses (Herinandez et al., 1995; Hernandez et al., 2000). Our results are consistent with other studies reporting the increased SOD activity in response to abiotic stress in Potato (Spychalla, 1990), Pea (Hernandez et al., 2000), Rice (Lee et al., 2001), Wheat (Sairam et al,. 2002) and Brassica (Zare and Pakniyat, 2012).

Peroxidase: According to results it was founded that there were significant differences between cultivars and drought treatments (P<0.05), between cultivars, Zarfam had highest means (46 Enzyme activity unit per mg protein) of peroxidase and lowest mean was obtained by okapy. Also results showed that peroxidase means increased by increasing of drought stress and application of 60 and 40%FC led to 7.8 and 11.2 fold increasing in compare to control. Guaiacol peroxidases play important roles in eliminating these harmful oxygen species by catalyzing reactions where a phenolic substrate is oxidized while the active oxygen species are reduced to a much less harmful form. Another possibility is that guaiacol peroxidase increased with higher levels of stress due to increased lignin or extensin synthesis (Michael et al.. 2006). Evidence has been found for both lignification (Quiroga et al., 2000) and extensin synthesis as a response to salt stress.

Catalase

According to results it was founded that there were significant differences between cultivars and drought treatments (P<0.05), between cultivars, Zarfam had highest means (23 Enzyme activity unit per mg protein) of Catalase. Also results showed that Catalase means increased by increasing of drought stress and application of 60 and 40%FC led to 1.42 and 1.54 fold increasing in compare to control.

Yield

According to results it was founded that there were significant differences between cultivars and drought treatments (P<0.01), between cultivars, Zarfam had highest means (436 gr/m^2) of yield and it founded as resistance cultivar, hence, Okapy showed lowest means and it founded as sensitive cultivar. Also results showed that yield means increased by increasing of drought stress and application of 60 and 40%FC led to 1.42 and 1.54 fold increasing in compare to control. The activity of antioxidant enzymes in normal conditions is an important point because this attribute can be expressed the amount of preparation of plants for against to stress but keep up this amount in stress conditions is more important. Zarfam cultivar had high enzyme activity in stress and in normal conditions. Totally, results showed that antioxidant enzymes increase as one mechanism in responses to stress and cultivars had different amount of enzyme unit. Between cultivars Zarfam and Talaye had highest antioxidant enzyme, respectively, highest means of SOD (31.6), POD (46) and CAT (23 Enzyme activity unit per mg protein) were obtained by Zarfam cultivar in 40% FC condition. Enzyme unit increased by increasing of drought levels and Zarfam, Talaye and Okapy showed 219, 198 and 112 gr/m^2 yield in 40% FC condition.

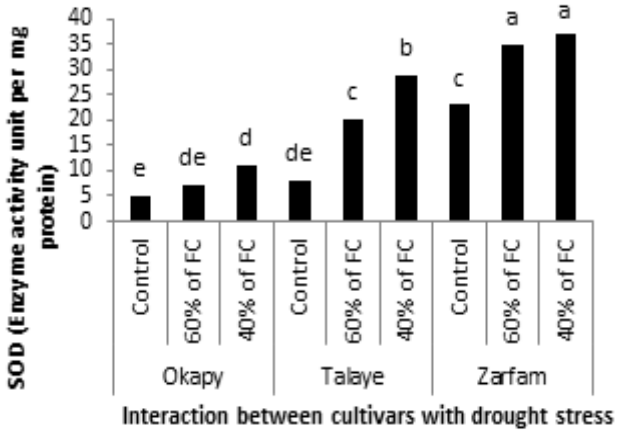

Table 1. Interaction between cultivars and drought stress on SOD.

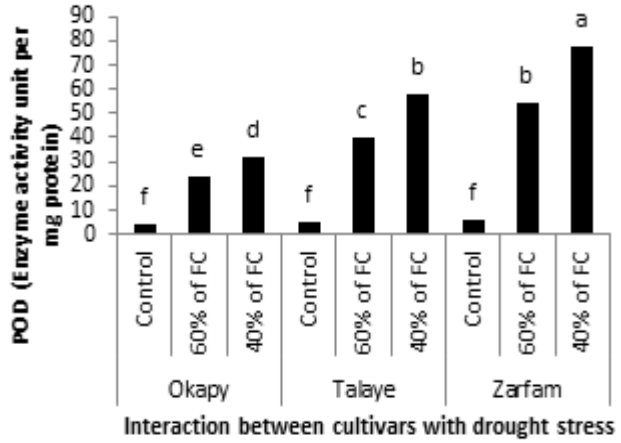

Table 2. Interaction between cultivars and drought stress on POD.

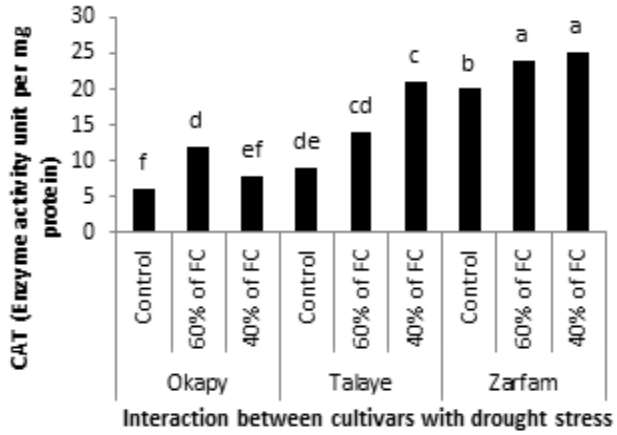

Table 3. Interaction between cultivars and drought stress on CAT.

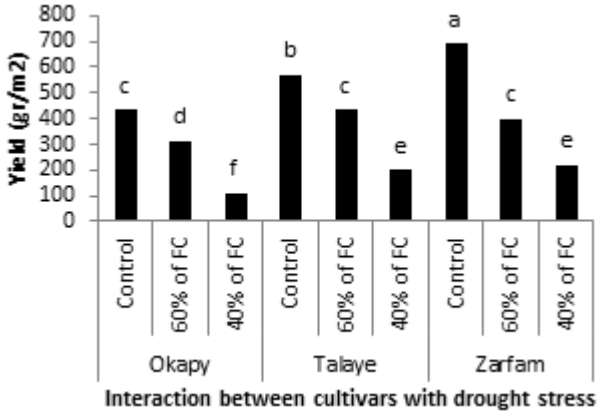

Table 4. Interaction between cultivars and drought stress on Yield.

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