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THE ROLE OF ANTIOXIDANTS IN DROUGHT TOLERANCE OF TWO NON-SUCCULENT XEROPHYTIC SPECIES , *Launaea spinosa* (FORSSK.) SCH. BIP. EX KUNTZE AND *Leptadenia pyrotechnica* (FORSSK.) DECNE.

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ABSTRACT

Enzymatic and non-enzymatic antioxidants effect were studied in two non-succulent xerophytes, *Launaea spinosa* and *Leptadenia pyrotechinca* growing naturally at wadi Hagul eastern desert of Egypt .The data revealed that catalase, peroxidase, polyphenol oxidase as well as □-tocopherol were higher during dry than wet season. The reverse was true for ascorbic acid.

INTRODUCTION

Drought is one of the most important abiotic stress factors (Dash and Mohanty, 2001), which affects almost every aspect of plant growth. The drought tolerance of plants can be characterized by growth response, changes in water relations of tissue exposed to low water potential, accumulation of ions in tissues and stomatal conductance of leaves (Blum, 1998). Desert plants generally follow two main strategies i.e., they tolerate the drought through phonological and physiological adjustments referred to as tolerance or avoidance mechanisms contribute to the ability of a plant to survive under drought but it also depends on the frequency and sensitivity of the drought periods (Youssef *et al.,* 2003). Under severe water stress conditions caused by salinity or drought, plants stop growing completely and accumulate solutes in cells in order to maintain the cell volume and turgor against dehydration. This phenomenon is known as osmotic adjustment. Osmotic adjustment has been observed in stems, leaves, roots and fruits (Patakas *et al.*, 2002). The plants chosen for this study; *Launaea spinosa* (Compositae) and *Leptadenia pyrotechinca* (Asclepiadaceae). The Asclepiadaceae comprises many medicinal plants with a wide range of therapeutic activities. Many Asclepiadaceous constituents have been intensively investigated as possible antitumor agents and also as potential bioactive chemicals (Gopiesh and Kannabiran, 2007). Many medicinal uses of *Leptadenia pyrotechnica* are reported in traditional medicine, the branches of the plant are diuretic and the bedouins use the infusion of branches for treatment of retention of urine and to help expel uroliths (Panwara and Tarafdarb, 2006). Also, the plant yields a fiber which is used in indigenous medicines as an antihistaminic and expectorant (Farnsworth, 1996). Furthermore, it is used for treatment of gout and rheumatism (Cioffi *et al.*, 2006). The family Compositae (Asteraceae) contained the highest number of plants followed by the Cucurbitaceae, Malvaceae and Solanaceae. Sixty eight percent of the documented vegetables are cultivated, eleven percent is usually obtained in the wild while twenty one percent is either cultivated or obtained from the wild (Ayodele, 2001). Many medicinal uses of *Launaea spinosa* are used in folk medicine in bitter stomach, skin diseases and reported to have antitumers, insecticide and cytotoxic activities. (El-Bassuony and Abdel-Hamid, 2006). Drought stress induced free radicals cause lipid peroxidation and membrane deterioration in plants. Drought stress leads to an imbalance between antioxidant defenses and the amount of active oxygen species (AOS) resulting in oxidative stress. Active oxygen species are necessary for inter and intracellular signaling, but at high concentration can cause damage at various levels of organization including chloroplasts (Smirnoff, 1993). These AOS have the capacity to initiate lipid peroxidation and degrade proteins, lipid and nucleic acids (Hendry, 1993). Mechanism of retardation of lipid peroxidation consists of free radical scavenging enzymes such as catalase, peroxidase and superoxide dismutase (Fridovich *et al.*, 2000). A number of enzymatic and non-enzymatic antioxidants are present in chloroplasts that serve to prevent AOS accumulation (Srivalli *et al.*, 2003).

The aim of this study is to evaluate the role of some antioxidants in drought tolerance of two non-succulent xerophytic species.

MATERIALS AND METHODS

The plant materials used in the present investigation are *Launaea spinosa* and *Leptadenia pyrotechnica* growing naturally at wadi Hagul eastern desert of Egypt the study sites were selected 15& 25 Km in the extension of the wadi east of the high way where main habitats of the two studied species represented. The plant species were collected during summer of 2008 and winter of 2009.

Plant analyses:

Concerning antioxidants, enzymatic antioxidant (catalase, peroxidase, polyphenol oxidase) was assayed after the method of Chance and Maehly, (1955). The results were expressed as enzymatic activity/g f wt./h. Non-enzymatic antioxidants, ascorbic acid content was assayed as described by Omaya *et al.* (1979). The results were expressed as mg/g fresh weight. The alpha tocopherol (α-tocopherol) content was assayed as described by Backer *et al.* (1980). The results were expressed as mg/g fresh weight.Variance analysis of data was done using ANOVA program for statistical analysis. The differences among means for all treatments were tested for significance at 1% level by using Duncan (1955) new multiple range tests as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The changes in enzymatic activity (catalase, peroxiduse and poly phenol oxidase) of *Leptadenia pyrotechnica* and *Launaea spinosa* during wet and dry seasons are recorded in Table 1. It is observed in both species that, the enzymatic activities of catalase, peroxidase and poly phenol oxidase, were significantly higher in dry than in wet season. Antioxidants are substances that when present at low concentration compared to that of an oxidable substrate markedly, delay or prevent its oxidation (Torres *et al.*, 2008) Antioxidants protect cells against the effects of harmful free radicals. There exists scientific evidence that the excessive production of free radicals in the organism, and the imbalance between their concentrations and the antioxidant defenses, may be related to processes such as aging (Calabrese and Maines., 2006). Peroxidase activity of *L. spinosa* was higher in dry season by about 2.9 fold than in wet one. The highest catalase activity was recorded for *L. pyrotechnica*, in dry season.It was doubled than that in wet season. Catalase and peroxidase are oxidoreductases involved in the molecular mechanisms of defence against reactive oxygen species. There are two abundant groups of enzymes posses striking similarities their reaction mechanism but have different residues in the heme activity. An increase in peroxidase activity is a common response to oxidative and biotic stress. Plants produce H_2O_2 in metabolic processes and cause damage to cell oxidation function, while catalase eliminate H_2O_2 and plays a key role in the elimination of O_2 (Jaleel *et al.*, 2007). Catalase is an important enzyme that converts H_2O_2 to water in the peroxysomes (Fridivich, 1989). In this organelle, H_2O_2 is produced from B-oxidation of fatty acids and photorespiration (Morita *et al.*, 1994). Higher activity of catalase decrease H_2O_2 level in the cell and increase the stability of membranes and CO_2 fixation because several enzymes of Calvin cycle within chloroplasts are extremely sensitive to H_2O_2. Peroxidase is one of the key enzymes controlling plant differentiation and development (Sakharov *et al.*, 2001). It is known that this enzyme is involved in the construction and rigidification of cell walls by contributing to the formation of lignin and cross-link between cell wall proteins, in the biosynthesis of H_2O_2, in the protection of tissue from damage and infection by pathogenic micro-organisms, in wound healing and in oxidation of indoleacetic acid (Farrell *et al.*, 1989; Dunford, 1991). Polyphenol oxidases are a copper-containing enzymes which catalyze the hydroxylation of monophenols to o-diphenols and the oxidation of o-dihydroxyphenols to o-quinines utiliziging molecular oxygen. These quinines covalently modify and crosslink to a variety of cellular constituents (Partington and Bolwell 1996). Polyphenol oxidases are involved in the oxidation of poly phenol into quinines (antimicrobial compounds) and lignification of plant cells during the microbial invation. These enzymes are also involved in reactions culminating in wound-induced tissue browning and erecting physical barriers against parasites (Mohamadi and Kazemi, 2002).

Table 1: Seasonal variation in some antioxidant enzymes of the studied plant species.

Plant species	Enzymatic activity / g F. wt./ hour)								
	Catalase			Peroxidase			Polyphenol oxidase		
	Dry	Wet	LSD 1%	Dry	Wet	LSD 1%	Dry	Wet	LSD1%
Launaea spinosa	675 a	450 b	152	346 a	121 b	77	84 a	65b	16
L. pyrotechnica	1050 a	525 b	424	265 a	139 b	55	191a	50b	44

Non-enzymatic antioxidant

As showen in Table 2 ascorbic acid concentration was significantly increased during the wet season in both studied species, while concentration of α tocopherol was significantly increased during the dry season in both species. In dry season, total concentration of α tocopherol of *Launaea spinosa* and *Leptadenia pyrotechnica* exceeded that in winter by 1.5 and 1.6 fold, respectively. Ascorbate and α tocopherol are extremely effective antioxidants because they are relatively poor electron donors in physiological conditions and act primarily by transfer of single hydrogen atoms (Njus and Kelley, 1991). Ascorbic acid (AsA) is a highly abundant metabolite and has important roles in physiology of plant stress as well as its growth and development. In detoxification of reactive oxygen species, AsA is a key antioxidant. As an enzyme cofactor, AsA plays significant parts in photoprotection, the wounding response and insect herbivory as well as cell expansion and division. Finally, AsA is the precursor for oxalate and tartarate (Conklin, 2001).

Tocopherols (a membrane bound compound) are essential components of biological membranes, where they play both antioxidant and non-antioxidant functions (Kagon, 1989). Alpha-tocopherol has the highest antioxidative activity due to presence of three methyl groups in its molecular structure (ELdin and Appelqvist, 1996). Tocopherols prevent the chain propagation step in lipid autooxidation and this makes it an effective free radical trap (Serbinova and Packer, 1994). In addition, tocopherols act as scavengers of oxygen radicals, especially $^1O_2$ (Fryer, 1992). According to an estimate, one molecule of α tocopherol can scavenge up to 120 $^1O_2$ molecules by resonance energy transfer (Bosch, 2005).

Ascorbic acid and α tocopherol can scavenge hydroxyl radicals, singlet oxygen and super oxide radicals (Arora *et al.*; 2002). Vitamins could be considered a bio-regulator compounds which relatively in low concentrations exerts a profound influences upon plant growth regulating factors that influence many physiological processes, such as synthesis of enzymes, act as coenzymes (Hathout, 1995) and to protect plant from harmful effects of higher temperature and positively increase their metabolic processes (Hathout, 1995).

Table 2: Seasonal variation in some non-enzymatic antioxidant of the studied plant species.

Plant species	Ascorbic acid (mg/g f.wt)			□ tocopherol (mg/g f. wt)		
	Dry	Wet	LSD $_{1\%}$	Dry	Wet	LSD$_{1\%}$
Launaea spinosa	0.595b	0.911a	0.12	4.84 a	3.22 b	0.16
L. pyrotechnica	1.49 b	2.26 a	0.22	7.6 a	4.8 b	0.24

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دور مضادات الأكسدة لنوعين من النباتات الصحراوية الغير عصيرية (المرخ و الكباث) في مقاومة الجفاف

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تم دراسة بعض مضادات الأكسدة الانزيمية وغير الانزيمية لنوعين من النباتات الجفافية هما الكباث *Launaea spinosa* و المرخ *Leptadenia pyrotechinca* و التي تنمو طبيعيا بوادى حجول- الصحراء الشرقية المصرية وقد اوضحت النتائج الأتى:
زيادة نشاط كل من أنزيم الكتاليز والبيروكسيديز والبولي فينول أوكسيديز إضافة إلى ألفاتوكوفيرول أثناء موسم الجفاف بينما ارتفع مستوى حمض الأسكوربيك خلال موسم الرطوبة.

قام بتحكيم البحث

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