FACTA UNIVERSITATIS
Series: **Physics, Chemistry and Technology** Vol. 2, N° 2, 2000, pp. 93 - 96

THE OXYGEN RADICALS IN ASTRAGALUS ONOBRYCHIS AND OXYTROPIS PILOSA

UDC 582.4+546.21]:613.15

D. Miladinović[1], Lj. Miladinović[2]

[1]Department of Chemistry, Faculty of Medicine, B. Tasković 81, 18000 Niš, Yugoslavia
[2]High school "B. Stanković", Voždova 27, 18000 Niš, Yugoslavia

Abstract. *The quantities of $O_2^{\cdot-}$ and $^{\cdot}OH$ of* Astragalus onobrychis Var Chlorocarpus *(Gris.) Stoj et Stef. (Leguminosae) and* Oxytropis pilosa *(L.) DC. produced from three different growth stages during 1995 at the locations Korvin grad (near Doljevac) and Subotinac (near Aleksinac), Serbia, were examined. The highest quantity of $O_2^{\cdot-}$ was found in the root of* Astragalus *in the seed forming stage (3053.49 nmol/g fresh material). The highest quantity of $O_2^{\cdot-}$ in* Oxytropis *was also found in its root but in the blooming stage (2894.56 nmol/g fresh material). The root of* Astragalus *contained the highest quantity of $^{\cdot}OH$ (30.20 nmol/g fresh material) in the blooming stage. The leaves of* Oxytropis *in the same stage of growth contained 15.90 nmol/g fresh material.*

Key words: Astragalus onobrychis *Var.* Chlorocarpus *(Gris.) Stoj. et Stef. (Leguminosae),* Oxytropis pilosa *(L.) DC., oxygen radicals, leguminosae.*

1. INTRODUCTION

Plant leaves intercept light, transform it into chemical energy, i.e., ATP and NADPH, and use these primary products of photosynthesis for assimilatory processes, mainly the reduction of CO_2 [1]. If plants are exposed to excess light that cannot be utilized for production of cellular reductant, photosynthetic antennae may transfer excitation energy to ground state oxygen ($^3O_2$) yielding singlet oxygen ($^1O_2$) [2]. Furthermore, light-driven electron transport systems may divert electrons to O_2 instead of $NADP^+$, resulting in superoxide radical ($O_2^{\cdot-}$) production (known as Mehler reaction) [3]. This light-dependent production of reactive oxygen species is generally termed photooxidative stress. Primary oxidants such as $O_2^{\cdot-}$ give rise to secondary oxidants, namely H_2O_2. In the presence of transition metals, these two oxidants together initiate the production of hydroxyl radicals ($^{\cdot}OH$) (Fenton reaction) [4]. Damage by extremely toxic $^{\cdot}OH$ can only be prevented by controlling the concentrations of their precursors [5].

Received April 10, 2001

Environmental conditions that induce or favor photooxidative stress are part of plants' everyday life. Thus, generation of reactive intermediates of oxygen metabolism is inevitable in aerobic organisms [6]. Drought, high temperatures and chilling alone and in combination with high light intensities etc. are such environmental factors that plants have to overcome to survive. Plants suffering from drought stress decrease their stomatal conductance to reduce water loss and, thus, restrict the flux of CO_2 into chloroplasts [7]. Under these conditions, the demand for NADPH in photosynthetic carbon reduction is decreased. It has been suggested that O_2 might serve as an alternative electron acceptor when NADPH utilization is limited, thereby resulting in an increased $O_2^{\cdot-}$ production and an enhanced potential for oxidative injury [8,9]. Oxidative damage in plants might be the result of enhanced production of $^{\cdot}OH$ through the Fenton reaction because severe drought results in increased tissue concentrations of transition metals such as Fe^{2+} and Cu^{+} [10].

The objective of this work was to study the $O_2^{\cdot-}$ and $^{\cdot}OH$ productions in two leguminosae: *Astragalus onobrychis* Var. *Chlorocarpus* (Gris.) Stoj. et Stef. (Leguminosae) and *Oxytropis pilosa* (L.) DC. during vegetative period.

2. EXPERIMENTAL

Plant leaves were collected in 1995 during the active vegetative period (from April to October) in two locations in southeastern Serbia: *Astragalus onobrychis* Var. *Chlorocarpus* (Gris.) Stoj. et Stef. (Leguminosae) in Korvin grad (near Doljevac), and *Oxytropis pilosa* (L.) DC. in Subotinac (near Aleksinac). The plant material was collected at three stages of growth (SG) as follows:

– 1st stage-the initial vegetation stage (April 1995)
– 2nd stage-the blooming stage (June 1995)
– 3rd stage-the seed forming stage (October 1995)

A herbarium specimen was deposited in the Botany Department, Faculty of Science, University of Niš, Yugoslavia.

One g of plant material was ground with quarz sand in cold mortar. The ground material was suspended in 5 ml 0,1 mol/l phosphate buffer (K_2HPO_4/KH_2PO_4, pH 7), centrifuged for 10 min at 15000 g. After the centrifugation at 4 ^{0}C the aliquots of the supernatant were used for biochemical determinations. Superoxide radical was studied by the inhibition of adrenaline autooxidation [11]. Hydroxyl radical was measured by the inhibition of the deoxyribose degradation [12].

The values are expressed as mean ± standard error.

3. RESULTS AND DISCUSSION

The results of the determination of superoxide and hydroxyl radicals are summarized in Tables 1 and 2. The highest quantities of $O_2^{\cdot-}$ in both investigated plants were observed in their roots but in different stages of growth. The highest quantity of $O_2^{\cdot-}$ in the root of *Astragalus* was in the seed forming stage (3053.49 nmol/g fresh material), while the highest quantity of $O_2^{\cdot-}$ was in the root of *Oxytropis* in the blooming stage (2894.56 nmol/g fresh material). Quantities of $O_2^{\cdot-}$ in cultivated and wild species of garlic were

been 2000 nmol/g fresh material approximately [13].

Table 1. Quantity of $O_2^{\cdot-}$ (nmol/g fresh material) in *A. onobrychis* L. and *O. pilosa* L.

SG	Plant organs	*A. onobrychis* L.	*O. pilosa* (L.) DC.
1st	Leaf	1484.69±21.02	1531.14±26.47
	Stalk	2130.35±25.47	2572.18±20.54
	Root	2330.68±23.98	2810.72±18.82
2nd	Leaf	2012.22±23.57	1657.97±24.80
	Stalk	2189.17±32.46	2334.67±29.05
	Root	2560.64±26.86	2894.56±20.62
3rd	Leaf	2824.50±31.33	2000.80±20.01
	Stalk	2244.40±28.83	2554.14±22.24
	Root	3053.49±22.07	2831.74±18.54

As shown in Table 2 the root of *Astragalus* contained the highest quantity of $^{\cdot}OH$ (30.20 nmol/g fresh material) in the blooming stage. The highest quantity of $^{\cdot}OH$ in *Oxytropis* (15.90 nmol/g fresh material) was measured in the same stage of growth in its leaves. In comparison with cultivated and wild species of garlic (10 nmol/g of $^{\cdot}OH$ fresh material approximately) *Oxytropis* was contained higher quantity of most dangerous free radical [13].

In the chloroplast, $O_2^{\cdot-}$ is rapidly converted to H_2O_2 through the action of superoxide dismutase [14]. There are also a number of nonenzymatic mechanisms that convert $O_2^{\cdot-}$ into H_2O_2 [15]. The capacity of protective enzymes and antioxidants are not constant but responds to intrinsic as well as exogenous factors, such as light or developmental determinants. Therefore, antioxidative systems show large changes during the life cycle of a leaf, including the stages of germination, emergence of young foliage from buds, expansion growth, maturity, senescence, and death. [16]. Young leaves of a range of plant species including tobacco, pea, poplar, and maize were found to contain higher antioxidative capacities than mature leaves [17]. The observed general increase of quantities of $O_2^{\cdot-}$ and $^{\cdot}OH$ from the initial vegetation stage to the seed forming stage, agree with previous concludes. Namely, in all plant organs in the last stage of vegetation, the quantities of $O_2^{\cdot-}$ and $^{\cdot}OH$ are higher than in the initial vegetation stage.

Table 2. Quantity of $^{\cdot}OH$ (nmol/g fresh material) in *A. onobrychis* L. and *O. pilosa* L.

SG	Plant organs	*A. onobrychis* L.	*O. pilosa* (L.) DC.
1st	Leaf	18.23±2.31	15.10±2.75
	Stalk	15.40±1.14	13.73±2.70
	Root	21.90±3.22	12.77±3.45
2nd	Leaf	20.60±2.09	15.90±2.60
	Stalk	16.90±1.56	14.47±3.31
	Root	30.20±2.36	12.13±3.79
3rd	Leaf	20.10±2.56	15.80±3.66
	Stalk	14.93±2.06	13.87±3.96
	Root	22.40±3.35	11.33±3.11

The roots of *Astragalus* and *Oxytropis* are highly exposed to the negative influence of $O_2^{\cdot-}$. On the other hand, root of *Astragalus* and leaves of *Oxytropis* contained highest quantities of $^{\cdot}OH$.

Acknowledgments: We thank Prof. Dr N. Ranđelović, The University of Niš, Yugoslavia, for the botanical identification of *Astragalus onobrychis* Var. *Chlorocarpus* (Gris.) Stoj. et Stef. (Leguminosae) and *Oxytropis pilosa* (L.) DC.

REFERENCES

1. Niyogi, K.K., (1999) Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**, 333.
2. Yruela, I., Pueyo, J.J., Alonso, P.J. and Picorel, R ., (1996) J Biol. Chem. **271**, 27408.
3. Mehler, A.H., (1951) Arch. Biochem. Biophys. **33**, 65.
4. Davies, K.J.A., (1995) Biochem. Soc. Symp. **61**, 1.
5. Hirayama, S., Ueda, R. and Sugata, K., (1996) Free Radic. Res. **25**, 247.
6. Toivonen, P.M.A. and Sweeney, M., (1998) J. Agric. Food Chem. **46**, 20.
7. Christov, K. and Bakardjieva, N., (1998) Plant Peroxid. **11**, 35.
8. Lamb, C. and Dixon, R.A., (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol. **48**, 251.
9. Rainwater, D.T., Gossett, D.R., Millhollon, E.P., Hanna, H.Y., Banks, S.W. and Lucas, M.C., (1996) Free Rad. Res. **25**, 421.
10. Iturbe-Ormaetxe, I., Escuredo, P.R, Arrese-Igor, C. and Becana, M., (1998) Plant Physiol. **116**, 173.
11. Misra, H.P. and Fridovich, I., (1972) J. Biol. Chem. **247**, 3170.
12. Cheesman, K.H., Beavis, A. and Esterbaurer, H., (1988) Biochem. J. **252**, 649.
13. Štajner, D., Milić, N., Mimica-Đukić, N., Lazić, B. and Igić, R., (1998) Phytother. Res. **12**, S13.
14. Miladinović, D., Najman, S., Ranđelović, N and Gašić, O., (1995) Facta Universitatis, **1**, 183.
15. Noctor, G. and Foyer, C.H., (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**, 249.
16. Cakmak, I, Strbac, D. and Marschner, H., (1993) J. Exp. Bot. **44**, 127.
17. Foyer,C., Souriau, N., Perret, S., Lelandais, M., Kunert, K.J., Pruvost C. and Jouanin L., (1995) Plant Physiol. **109**, 1047.

KISEONIČNI RADIKALI U ASTRAGALUS ONOBRYCHIS I OXYTROPIS PILOSA

D. Miladinović, Lj. Miladinović

Ispitivana je količina O2·- i ·OH u korenu, stablu i listu vrsta Astragalus onobrychis Var. Chlorocarpus (Gris.) Stoj. et Stef. (Leguminosae) i Oxytropis pilosa (L.) DC. sa staništa Korvin grad (u blizini Doljevca) i Subotinac (u blizini Aleksinca) tokom vegetacionog perioda u 1995. godini. Najveća količina O2·- je ustanovljena u korenu Astragalus-a (3053,49 nmol/g, svež uzorak) u fazi formiranja semena. U korenu Oxytropis-a je takođe utvrđena najveća količina superoksid radikala (2894,56 nmol/g, svež uzorak) ali u fazi cvetanja. Koren Astragalus-a u fazi cvetanja sadrži najviše ·OH (30,20 nmol/g, svež uzorak). List Oxytropis-a u istoj fazi vegetacije sadrži 15,90 nmol/g, svež uzorak, ·OH.