African Journal of Biotechnology Vol. 10(69), pp. 15616-15625, 7 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1503
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Aging and some physiological and biochemical characteristics of two Aelorupus species

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Accepted 15 June, 2011

In order to understand the effects of aging on physiological traits involved in abiotic stress tolerance in different species of halophyte plants (Aelorupus lagopoides and Aeloropus littoralis), alteration in weight, malondialdehyde (MDA), sodium and potassium, proline, protein and antioxidant enzymes in these two species after germination and during the increasing of the age were studied. According to these results, ageing in both species result in change of the ion content, proline content and protein levels. Decreased chlorophyll and carotenoids content was followed to reduced photosynthetic capacity. According to the results of this study, the potential of the abiotic stress tolerance in vegetative growth of A. lagopoides is probably higher than A. littoralis and this potential during the aging is less altered, because A. lagopoides compare with the other species in maintaining potassium than sodium ions were more efficient at different time. Although the activity of superoxide dismutase (SOD), peroxidases (POD) and polyphenol oxidase (PPO) enzymes in A. lagopoides in non-stress conditions during normal aging was changed, most of the time, antioxidant activity of this enzyme in A. lagopoides was higher than A. littoralis. Furthermore, the amount of MDA and fluctuations in A. lagopoides on different days was lower than the other species, which is a conformation for better antioxidant system in A. lagopoides.

Key words: Aelorupus lagopoides, Aeloropus littoralis, aging, antioxidant and abiotic stress.

INTRODUCTION

Ageing and senescence like drought and other abiotic stress result in increased free radical production in plants (del Rı´o et al., 1998; Navari-Izzo and Rascio, 1999; Alscher et al., 2002). Free radical production leads to impaired redox homeostasis and play key role insignaling (Foyer and Noctor, 2003). Alteration of the rate of free radicals in plant leads to metabolism and photosynthetic changes such as lipid peroxidation, protein, nucleic acids and photosynthetic damage (Navari-Izzo et al., 1994; Tambussi et al., 2000; Brito et al., 2003). Plants, to protect itself against the adverse effects of free radical, possess the antioxidative systems (enzymatic and non-enzymatic compounds) which are involved in scavenging of different types of active oxygen species (Langridge et al., 2006). Some induced changes in plant antioxidative system by aging and drought stress has been reported which shows that plants drought tolerate will change by age (Gaff and Giess, 1986; Gaff, 1989).

Aeluropus species plants are tolerant to salinity and aridity. This species belongs to the Poaceae family. Two species of Aeluropus littoralis and Aeluropus lgopoides are distributed in semi-desert plateau areas in Iran. These species are C4 halophyte plants. Long roots, small leaves, waxy epicotyls and gland salt secretion result in relatively high salt tolerance in these species to deal with

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abiotic stress. Although, several studies concerning the effect of the salinity and drought stress on growth, physiological and biochemical responses of the Aelorupus have been performed (Waghmode and Joshi, 1982; Golzar and Khan, 2001; Golzar et al, 2003; Mohsenzadeh et al, 2006), considerable information concerning the effect of aging and senescence on halophyte plant responses is not available.

The present study was performed to understand the effects of aging on the physiological and biochemical traits possibly involved in abiotic stress response and tolerance of different species of Aelorupus. The results will not only provide the comparison of the institutions and inherent characteristics of Aelorupus lagopoides and Aeloropus littoralis, but also provide the possible differences in stress tolerance of each species in different time. Study of the effects of aging on characteristics and physiological responses of halophyte plants will provide thoughtful identification of the factors involved in stress tolerance in this plant. Obviously, the first step for transferring of appropriate genes from halophyte plants to salt sensitive crop plants which is salt sensitive to improve their tolerance to abiotic stress is the correct identification of the tolerance factors in the halophyte plants.

MATERIALS AND METHODS

Plant material and drought treatment

Seeds of A. Lagopoides (L.) Trin and A. littoralis were obtained from a population on the vegetative zone of Kashan located in the central region of Iran. The collection site has 34°25´ latitude, 53°15´ longitude, an altitude of 1209 m from sea level and 90 mm annual rainfalls. Seeds were surface- sterilized by 5% sodium hypoclorite + 0.6% triron-100 for 10 min and rinsed 5 times by sterile distilled water. Seeds were germinated in pots containing an equal mixture (1:1:1) sand, vermiculite and soil and placed in phytotron with25°C temperature, 50% humidity and 16/8 h photoperiod. Pots were irrigated every other day for 2 month. Samples gathered for physiological analysis after 65, 71, 75, 78 and 82 days, respectively.

Fresh and dry weight

Plant fresh weight was determined after cutting the roots and dry weight was measured after drying at 70°C oven for 48 days according to Dhanda and Sethi (1998).

Na^+, K^+, free proline and MDA content

Na^+ and K^+ content was measured by flame photometer (model PFP7, UK). Free proline content was measured by using 3% sulfosalicylic acid based on Bates et al. (1973) method. The absorbance was assayed using spectrophotometer (shimadzu UV-160, Japan) at 520 nm.

The lipid peroxidation was assayed by measuring malon-dialdehyde (MDA) content in reaction with thiobarbitoric acid (TBA) according to Heath and Packer (1968). Absorbance was read in 532 nm and corrected for non specific turbidity by subtracting A600. MDA content was calculated using an extinction coefficient of 155 $M^{-1}cm^{-1}$ and corrected for non-specific turbidity by subtracting A_{600}. Measurements repeated for three independent samples and standard error of the means was calculated.

Determination of chlorophyll and carotenoid content

The content of $chlorophyll_{a, b}$ and carotenoids were determined after extraction in 80% acetone at 20°C according to Lichtenthaler (1983). Absorption was measured at 663.2, 646.8 and 470 nm.

Oxidative enzyme extraction

For enzyme extraction we used Sudhakar et al. (2001) method which can be used for peroxidise (POD) and polyphenol oxidase (PPO). POD activity assay was measured according to the method of Abeles and Bites (1991). The assay mixture consisted of 4 ml sodium acetate buffer (pH 4.8, 0.2 M), 0.4 ml H_2O_2 (3%, v/v), 0.2 ml banzidine (0.02 M in 50% methanol). The absorbance was read at 530 nm. PPO activity was measured according to Raymond et al. (1993). The assay mixture consisted of 2.5 ml sodium acetate buffer (0.2 M, pH 6.8), 0.2 ml pyrogallol (0.02 M). The absorbance was measured at 430 nm.

Protein extraction

For protein extraction, the method of Lammeli (1970) was used. After powdering 0.1 g fresh material by liquid nitrogen, 1 ml extraction buffer (0.1 M Tris-HCl pH 8, 5 mM DTT, 5 mM EDTA) plus 52 µl 5mM PMSF (1 M) were added and centrifuged at 13000 g at 4°C for 5 min. Total protein content was determined by protein binding procedure described by Bradford (1976) and bovine serum albumin (BSA) was used as standard. Absorbance was read at 529 nm by UV-visible recording spectrometer (UV-160 Shimadzu, Japan) with 10-nm matched quartz cells.

Statistical analysis

The data in triplicate was analyzed by analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) 13. Nonparametric, Mann-Whitney and Kruskal-Walis tests were applied to compare mean values. In all cases, P value equals 0.05 and Mann-Whitney value is zero.

RESULTS

According to the obtained results, the sodium ion content of A. littoralis did not change with age, while this content in A. lagopoides was increased (Figure 1a). As shown in Figure 1b, potassium ion content in A. littoralis with increasing age was not only increased but also significantly decreased except in 75 day. Unlike the A. littoralis, in A. lagopoides potassium rate was increased from 71 to 82 day (Figure 1c).

As shown in Figure 2a, MDA levels in A. littoralis during the days of the study fluctuated, while in A. lagopoides, except for 75 day, stability of lipid peroxidation were preserved. As shown in Figures 3a to d, pigment rate including chlorophyll a, chlorophyll b and total chlorophyll

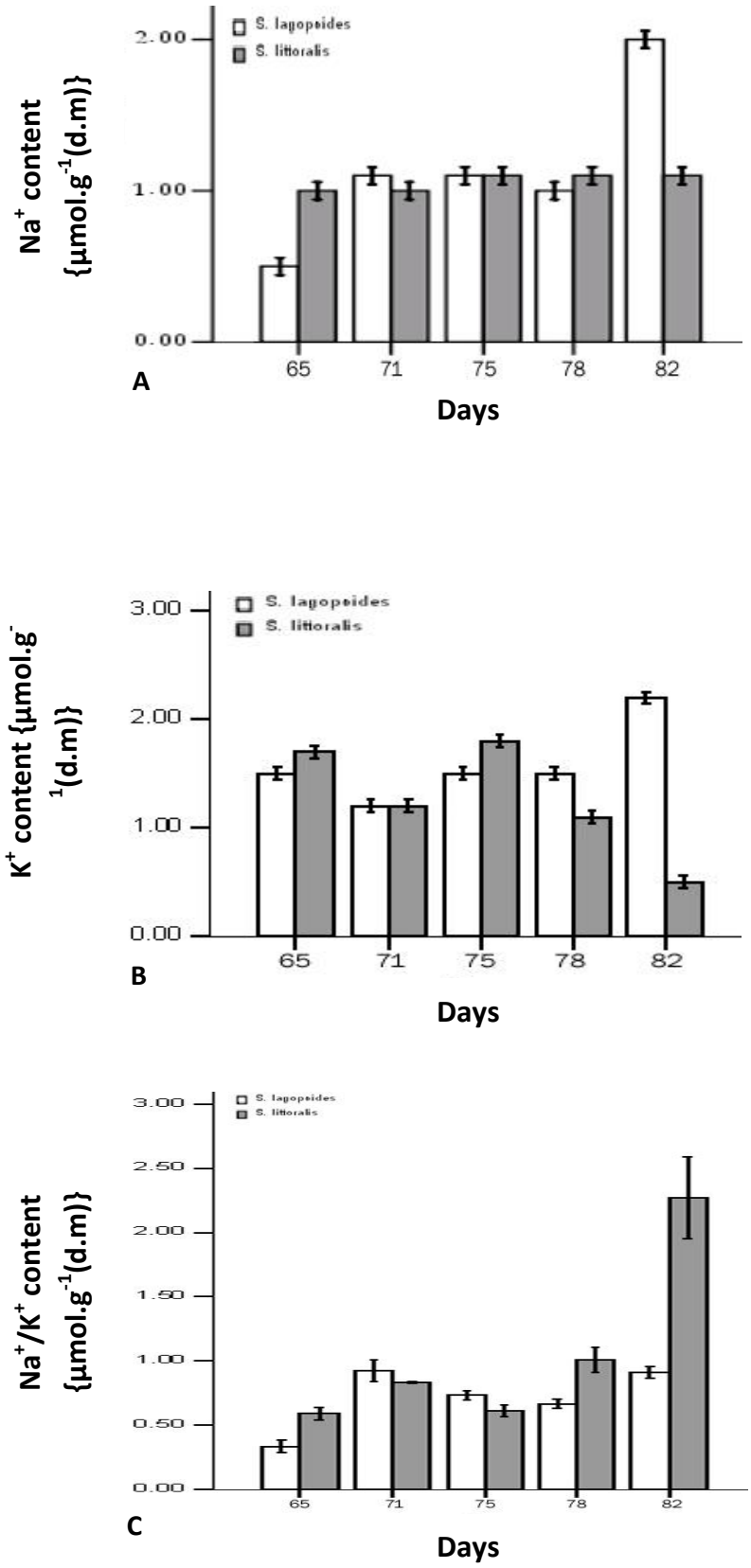

Figure 1. Effect of aging on Na^+ (A), K^+content (B) and Na^+/K^+ ratio (C) in A. lagopoides and A. littoralis in days 65,71,75,78 and 82. N= 3 replicates.

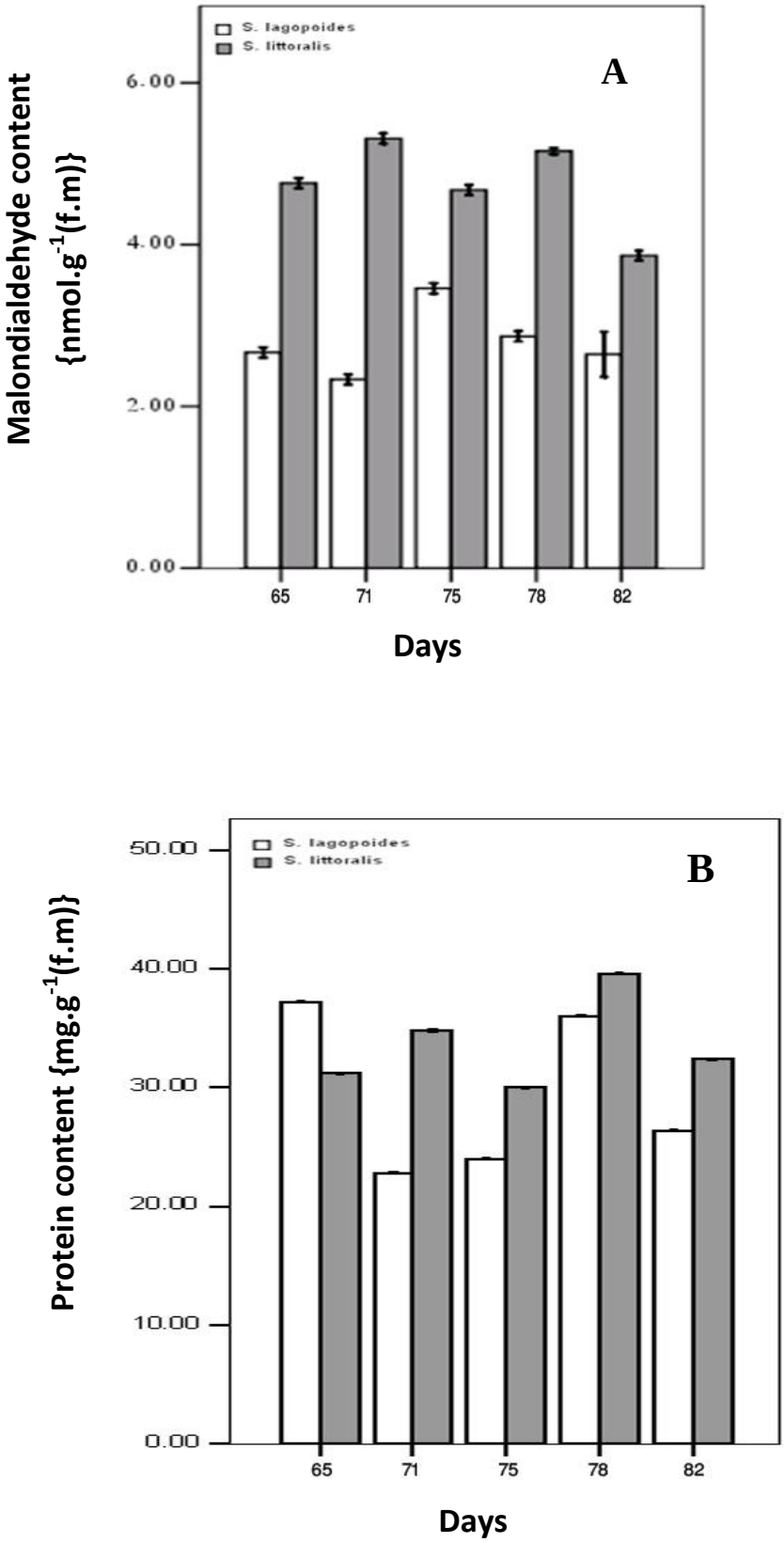

Figure 2. Effect of aging on MDA content (A), total soluble protein (B) and proline content (C) in A. lagopoides and A. littoralis in days 65,71,75,78 and 82. N= 3 replicates.

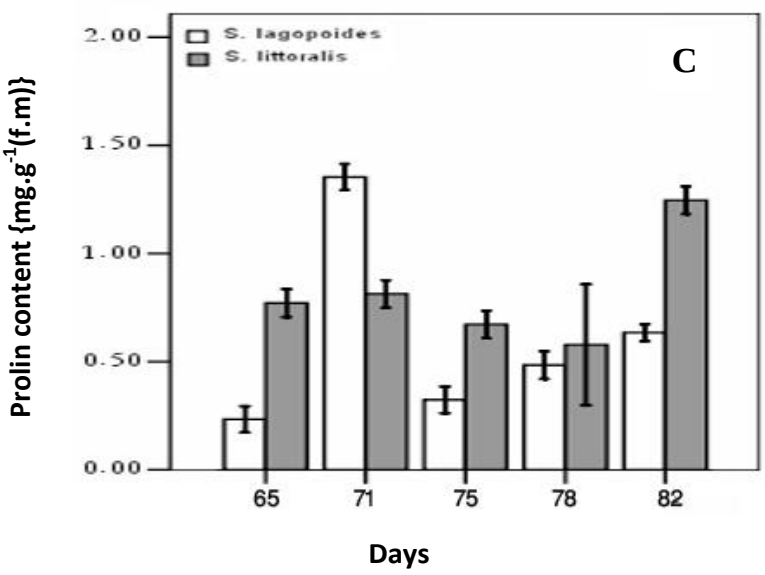

Figure 2. Continued.

of A. littoralis were decreased from 65 to 82 days. The only exception available was a partially increasing chlorophyll a content in 82 days compare with 78 days. In A. lagopoides, the content of measured pigment in days 65 and 71 remained constant. Content of chlorophyll a, chlorophyll b and total chlorophyll on days 65 rather than day 75 and day 82 was decreased and in day 78 was increased. Carotenoids content was gradually decreased from 65 to 75 day in both species and dramatically decreased in 78 day only in A. littoralis species (Figure 3d). Although proline content of A. littoralis did not show significant change from 65 to 78 days, a significant increase was observed in 82 days (Figure 2c). Amount of this amino acid (osmolyte) in A. lagopoides was elevated in day 71 and day 82 when compare with day 65 (Figure 2c). As shown in Figure 2b, protein content of A. littoralis on days 71, 78 and 82 were increased compare with 65 day and only partial reduction was observed in day 75. In contrast to A. littoralis, protein content of A. lagopoides was decreased in 71, 75, 78 and 82 days rather than 65 day.

As shown in Figure 4c, in both species, SOD enzyme activity did not significantly change over time, the only significant reduction was observed in 82 day in A. littoralis. As depicted in Figure 4a, activity of POD enzyme in both species was relatively similar and in all days was increased compared with day 65. Activity of this enzyme in A. littoralis and A. lagopoides species reached to its highest level on 82 and 71 days, respectively.

As illustrated in Figure 4b, activity of PPO enzyme in A. littoralis was increased on 71 day compare with 65 day and was decreased on the following days. In A. lagopoides species the increasing rate of the activity of this -enzyme was continued until 75 day and then decreased with increased time.

DISCUSSION

The aim of this research was the study of the effect of aging on metabolic changes and physiological responses of two Aelorupus species and find out the possible relationship between age and tolerance of these two halophyte species to salinity and drought stress. According to the results of this study, sodium ion content of A. littoralis in different sections was more stable compared with A. lagopoides. The ratio of sodium/potassium ion showed severe reduction in A. littoralis in comparison to A. lagopoides with aging. According to the results of this study, although sodium ion content in A. littoralis was more stable in different sections when compared with A. lagopoides species, the ratio of sodium to potassium ion in A. littoralis showed severe reduction with age compared with A. lagopoides. According to Yeo (1998), ratio of sodium to potassium ion is considered as a key factor in improving performance of plants under drought stress. Increasing NaCl concentration and salinity occurrence results in membrane depolarization and replacement of sodium ions instead of potassium ions which ultimately lead to potassium deficiency in the plant (Cramer et al., 1991). These two cations have similar atomic properties and transporters of potassium ions at high levels of sodium ions are less specific (Castillo et al., 2007). Thus, for adjustment of the harmful effects of sodium ions under osmotic stress, plants depend on the status of potassium ions. According to the results of this study and previous studies, we can suggest that, the reduction in the ratio of sodium/potassium in two species during aging decrease the potential of these plants to maintain ionic homeostasis under osmotic stress. However, this decrease in A. lagopoides compared with A. littoralis was very negligible and referred to higher

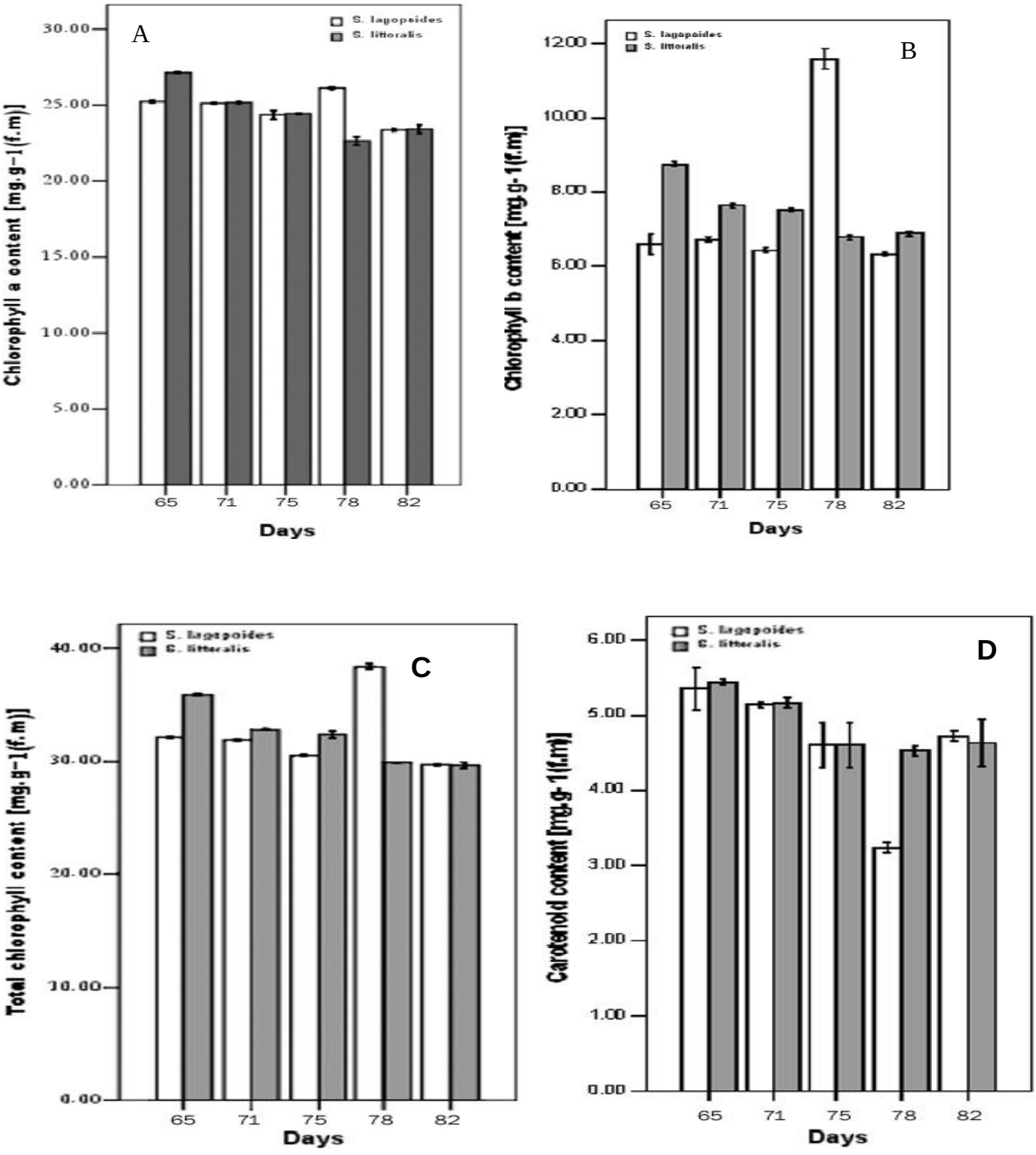

Figure 3. Effect of aging on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) in A. lagopoides and A. littoralis in days 65,71,75,78 and 82. N= 3 replicates.

tolerance of this species to osmotic stress in particular on 82 days. Types of reactive active oxygen (ROS), which can be produced by incomplete reduction of oxygen molecule is by-product and harmful in normal metabolism

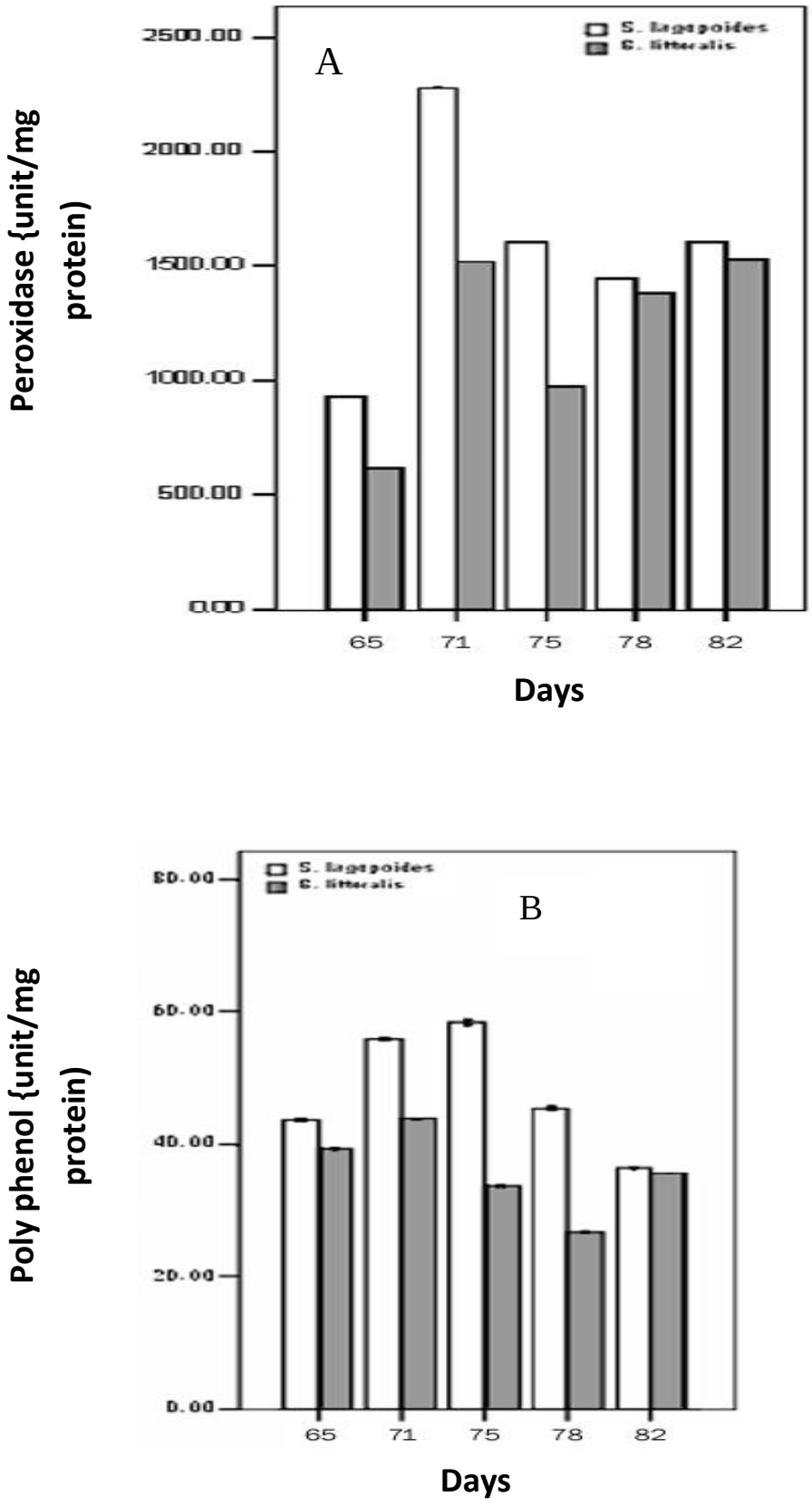

Figure 4. Effect of aging on peroxidise (A) and polyphenol oxidase (B) in A. lagopoides and A. littoralis in days 65,71,75,78 and 82. N= 3 replicates.

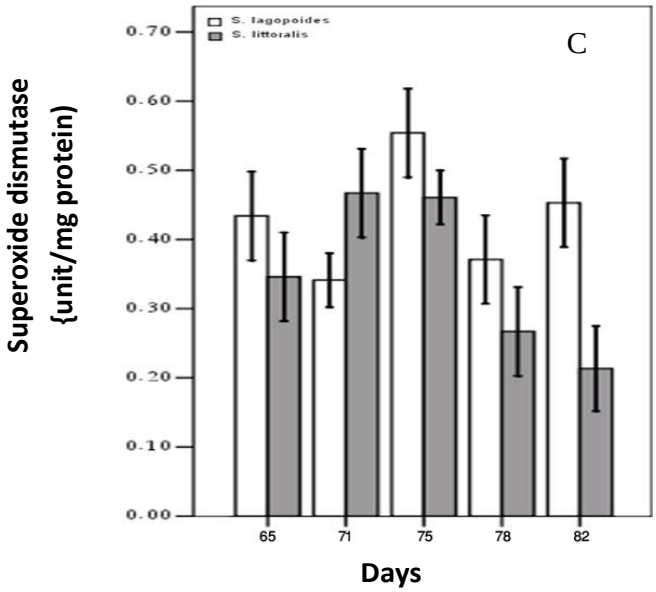

Figure 4. Continued

of aerobic organisms (Halliwell, 2006). Studies have shown that ROS production increases during leaf aging (Wang et al., 2009).

High levels of ROS, including hydrogen peroxide accelerate reactions such as Haber - Vis and results in lipid peroxidation (Neill et al., 2002). In this present study, changes in the rate of lipid peroxidation in A. littoralis in different days of the study showed changes in the rate of hydrogen peroxide radical in different section times. The rate of lipid peroxidation in different days of A. lagopoides with control the ROS production or effective antioxidant factors and free radical scavengers was kept constant. Also, in all days, MDA content in A. littoralis species was relatively higher than A. lagopoides which indicate that, lipid peroxidation in A. lagopoides was intrinsically lower than A. littoralis. This could be due to higher activity of enzymatic and non-enzymatic factors in different developmental stages of A. lagopoides, which results to increase the ability of the halophyte plants under abiotic stress conditions.

In general, age increase reduced the photosynthetic pigment in both species. However, this effect in A. littoralis was observed from the first day of the study (day 65); this effect was visible in A. lagopoides in day 71. Carotenoids are known as an efficient non-enzymatic antioxidant against oxidative damages (Jimene'z and Pick, 1993). Chlorophyll stability can be considered to evaluate the stress tolerance in plants, because photosynthetic pigment rate could affect the photosynthetic capacity (Sinha and Patil, 1986). Decreased in chlorophyll content is an index for oxidative stress (Smirnoff, 1993) and probably could be due to increase of its chlorophyll degradation or decrease in chlorophyll production (Brito et al., 2003; Santos, 2004). The obtained results suggest that, age increase with reduced photosynthetic pigment increased sensitivity and vulnerability of two Aelorupus species to abiotic stress. Negative effect of the aging on the rate pigment was started later in A. lagopoides than A. littoralis (71 days) and the exception for this process was chlorophyll content in day 78.

Increasing proline content (on 82 and 71 day in A. littoralis and in A. lagopoides, respectively) could be due to more synthesis of amino acids or protein degradation. There are conflicting reports regarding the role of proline in salt or drought stress tolerance. Some scientists considered positive effect for proline accumulation with salt and drought tolerance (Ahmad et al. 2007, Su and Wu 2004, Ghoulam et al. 2002; Handa et al., 1986).

Proline accumulation under stress conditions could be considered as a defensive mechanism against osmotic stress (Ghoulam et al., 2002). Therefore, one could suggest that the higher rate of proline in some days in Aelorupus species can increase the potential of the plants against the stress. Proline is a compatible solute which is effective on osmotic protection and scavenging of free radicals. Proline probably will interact with macromolecules to maintain their native form and their activity under stress conditions. For example, proline can cover the protein and removing of the material from the surface of the protein can maintain their accurate folding and their active form. Proline also act as a protector for many cytoplasmic enzymes and play roles in preventing enzyme denaturation, stability of protein synthesis, adjustment of the acidity of the cytosol and as a sources of nitrogen and carbon (Su and Wu, 2004; Pandey et al., 2004; Hoekstra et al., 2001, Bandurska, 1993; Paleg et al. 1981; Solomon et al., 1994; Ahmad et al., 2007). The role of proline in plant stress tolerance is not clearly

certain because, as Su and Wu (2003) reported, induced expression of P5CS gene under stress condition is more effective in stress tolerance than its constitutive expression. Also, some scientists believe that proline accumulation under osmotic stress is a marker for stress-induced damage and its role under stress condition (Ibarra-Caballero et al., 1988; Garcia et al., 1997; Hoai et al., 2003). Researchers show that, plant age is effective on the protein content. Veljovic-Jovanovic et al. (2006) showed that aging decrease the soluble proteins in Ramonda serbica plant. Aging in perennial plants is associated with protein degradation which is due to increases of catabolic oxidative reactions and activation of specific protease (Thomson et al., 1987). Close relationship has been proposed between oxidative changes in proteins and their proteolysis (Davies et al., 1987). According to this study, aging in A. lagopoides is associated with reduction of soluble proteins, which indicates protein degradation. Increase of proline content in this species in some days of study can also be related to increased protein degradation. In A. littoralis, protein content like MDA content fluctuated with age which is due to the inability of A. littoralis in maintaining the oxidative status of the cell. As mentioned, aging and senescence lead to increasing ROS in plants and are also followed by several changes in cell metabolism including the antioxidant defense systems. Antioxidative enzymes such as super oxide desmutase (SOD), peroxidase (POD) and poly phenol oxidase (PPO) are the most important antioxidant factors as their catalytic activities are able to detoxify free radicals (Hsu and Kao, 2003; Agarwal and Pandey, 2004). Veljovic-Jovanovic et al. (2006) reported that, SOD enzyme activity increases in senescent and drought stressed leaves of R. serbica plants. On the other hand, ROS accumulation during aging results in damage to the cell (Kar and Feierabend, 1984; Jime'nez et al., 1998). Perl-Treves and Galun (1991) showed that, the expression level of the Cu SOD and Zn SOD change under both oxidative stress and developmental regulation. According to Kardish et al. (1994) promoter region of plastidic SOD gene contains a motif which is controlled with developmental stages and a motif which is controlled with light or oxygen radicals. Antioxidants which are expressed under developmental stages in comparison with events which are induced by stress have more important role in resistance to stress (Donahue et al., 1997). Transformed plants which have constitutively high expression level of Cu SOD and Zn SOD are more tolerant to oxidative stress (Donahue et al., 1997). According to the results of this study, SOD activity did not show significant change from 65 to 78 days in both species. Activity of this enzyme in A. lagopoides remained constant on day 82, but was reduced in A. littoralis which can be as a result of reduced stress tolerance at this age. Also, in most days of the study, the SOD activity in A. lagopoides was higher than A. littoralis which is indicative of higher oxidative stress tolerance in this species. According to the results of researchers, POD enzyme activity will also be affected by ageing. Veljovic-Jovanovic and colleagues (2006) suggested that, increasing POD activity during aging in R. serbica plant is considered as a part of antioxidant defense under these conditions. They also mentioned that, those POD isoforms which is induced under senescence are anionic and contributed to cell wall lignifications. According to the result of this study in both species, the lowest POD activity observed on day 65 showed that, the role of this enzyme in increasing stress tolerance is important. Comparison of two Aelorupus species shows that, POD of A. littoralis in 82 day and in A. lagopoides in 71 day has the most important role in increasing stress tolerance. The results indicate that, activity of this enzyme in A. lagopoides is higher than A. littoralis which could imply a higher tolerance in A. lagopoides when compared with other species. According to results of PPO enzyme, activity of this enzyme is similar to two other antioxidant enzymes in the A. lagopoides which was higher than A. littoralis. It is worth noting that, the prominent role of this enzyme in A. littoralis on day 71 and in A. lagopoides on day 75 was expected.

Conclusion

Based on the results obtained from comparison of the time and aging on physiological and biochemical characteristics of two Aelorupus species, one can suggest that, although, the expression of coding gene or regulation of activity of the enzymatic antioxidant in A. lagopoides in non-stress conditions during normal aging will vary, but in all the time studied, antioxidant enzyme activities mentioned in this species was higher than A. littoralis. This can cause a higher tolerance of A. lagopoides to abiotic stress in different age, which lower MDA content and its fluctuation during aging in A. lagopoides compared with A. littoralis. Furthermore A. lagopoides was more efficient in maintaining potassium/ sodium ion rate at time different than the other species. Decreasing of chlorophyll content and carotenoids in both species showed that the photosynthetic capacity during aging decreased but for concluding about the increase or decrease of stress tolerance during the aging, further traits must be studied.

Abbreviations

MDA, Malondialdehyde; **TBA,** thiobarbitoric acid; **POD,** peroxidise; **PPO,** polyphenol oxidase; **BSA,** bovine serum albumin.

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