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PHYSIOLOGICAL CHARACTERISTICS OF THREE WILD *SONCHUS* SPECIES TO PROLONGED DROUGHT TOLERANCE IN ARID REGIONS

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Abstract

Drought is one of the main abiotic factors determining plants growth and productivity in arid and semiarid regions. Understanding the physiological responses of wild plants to drought in different growth stages is essential to evaluate their ability of drought tolerance and allow identification and selection of valuable tolerant plants to be cultivated and introduced in arid and semiarid regions. Three wild *Sonchus* species, *Sonchus oleraceus* L., *Sonchus wightianus* DC. and *Sonchus uliginosus* M. B. were compared regarding some physiological indexes in leaves such as antioxidant enzymes (superoxide dismutase and peroxidase), malondialdehyde, osmotic solutes (proline, soluble sugar and soluble protein), photosynthetic pigments (total chlorophyll, chlorophyll a, chlorophyll b and carotenoid) under the natural condition at seeding stage, flowering stage and maturation stage respectively. Comparing to *S. uliginosus* and *S. wightianus*, *S. oleraceus* had the higher peroxidase (POD) and superoxide dismutase (SOD) activities and total chlorophyll (Chl_{a+b}) and carotenoid (Car) content in three growth stages, and the higher proline content at flowering and maturation stage and the lower malondialdehyde (MDA) content at seeding stage and flowering stage. But the ratio of Chl_a/Chl_b and Car/Chl_{a+b} in *S. uliginosus* were significantly higher than that in *S. oleraceus* and *S. wightianus*. These findings suggested that *S. oleraceus* had the higher tolerance to prolonged drought than *S. wightianus* and *S. uliginosus* due to the better capacity to prevent oxidative damage to cellular components and osmoregulation and photosynthetic ability and *S. uliginosus* were more photo-protected under drought. The research results were instructive for cultivation and introduction of *S. oleraceus* in arid and semiarid regions.

Key words: *Sonchus* ssp., Drought tolerance, Growth stage, Physiological traits, Arid regions.

Abbreviations: AOS, active oxygen species; SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; FW, fresh weight.

Introduction

Wild plants have more potential in stress resistance than cultivated plants, which has been proved in many crops such as beet (Bor *et al*., 2003), barley (Ahmed *et al*., 2013), rice (Xie *et al*., 2010), common bean (Cortés *et al*., 2013) and so on. Wild plants can be use as an abundant source of resistance genes and genetic variation for crop improvement (Marok *et al*., 2013). Less precipitation and prolonged dry spells during growth stage are the main severe limitation of plant growth and productivity in arid and semiarid regions (El-Sharkawy, 2007). The most effective and economical way to cope with water resource shortage and drought environment in arid and semiarid areas is to exploit plants and understand their resistance mechanisms (Ahmed *et al*., 2013). The performance of genetic potential of plants is expressed by the physiological realization in fields (Shao *et al*., 2005a, 2007). So, the accurate field evaluation of wild plants physiological traits is pivotal in understanding the plant stress resistance (Iannucci *et al*., 2002; El-Sharkawy, 2007). Meanwhile, drought causes various physiological changes at different growth stages (Hu & Xiong, 2014). Field research at different growth stages can verify findings in controlled environments and provide insights into the real potential of plants under the natural conditions (El-Sharkawy, 2007).

One of the earliest responses of plants to drought is the accumulation of active oxygen species (AOS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Shao *et al*., 2005a). The formation of AOS can cause oxidative injury among plants when the defensive ability is overwhelmed, which can lead to damage to important cellular components, including membrane lipids, proteins and nucleic acids (Zhang & Kirkham, 1996; Tan *et al*., 2006). Membrane lipid peroxidation can directly influence cell membrane functions and exacerbate the oxidative damage through the production of lipid-derived radicals (Gill & Tuteja, 2010). Malondialdehyde (MDA) is a product of lipid peroxidation and has been regarded as an indicator of cell membrane damage of plants under oxidative stress (De Azevedo Neto *et al*., 2006). The increased MDA led to increase of ion leakage, which meant that lipid peroxidation could cause enhanced membrane permeability in plants under drought (Liu *et al*., 2011).

Plants have evolved various enzymatic and nonenzymatic activities to detoxify harmful AOS (Zhang & Kirkham, 1996). Antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), are contributing to reduce the concentrations of O_2^- and H_2O_2. SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2. H_2O_2 is broken down to H_2O and O_2 by peroxidase (POD) and catalase (CAT)

(Zhang & Kirkham, 1996; Reddy *et al*., 2004a). Researchers find that POD, SOD, CAT and MDA are significant indicators to evaluate the status of plant redox under drought stress. The higher activities of POD, SOD, CAT and the lower content of MDA indicate the higher anti-oxidative capacity and drought tolerance (Shao *et al*., 2005a, 2005b, 2007).

Osmotic adjustment, defined as lowering osmotic potential in plants due to accumulation of net solutes, enables plants to endure moderate water deficits and grow in regions of limited water availability (Ackerson, 1981; Girma & Krieg, 1992). Synthesis and accumulation of compatible solutes such as proline, soluble sugar and soluble protein are important physiological indicators to evaluate the ability of plant osmotic adjustment (Shao *et al*., 2006; Kadkhodaie *et al*., 2013). It is well described that these solutes accumulate in plants under drought (Shao *et al*., 2006; Shinde *et al*., 2016). The more accumulation of compatible solutes can maintain the lower intracellular water potential, thus promoting the absorption of water from environment and maintaining proper cell turgor and normal life functions (Kadkhodaie *et al*., 2013).

Proline has been long considered as an osmolyte due to the evidence that it accumulates predominantly to the high concentrations in the relatively small volume of the cytoplasm and organelles (Reddy *et al*., 2004a; Shinde *et al*., 2016). However, proline is also considered as an AOS scavenger and to be more important in overcoming stress than simply working as an osmolyte (Reddy *et al*., 2004a). Researchers have suggested that both proline synthesis and catabolism are necessary and pivotal to promote plant growth and drought tolerance (Sharma *et al*., 2011). Besides, researchers found other roles of proline such as protecting mitochondrial complex II and behaving as a component of cell wall synthesis under stresses (Hamilton & Heckathorn, 2001; Ueda *et al*., 2007). Although the synthesis of proline is a stress-induced response, the role of proline act in drought is still controversial (De Diego *et al*., 2013; Habash *et al*., 2014).

Photosynthetic cells, characterized by high oxygen concentration, are likely to be particularly tending to oxidative stress under drought (Zhang & Kirkham, 1996). O_2^- and H_2O_2 are accumulated during drought, which results from the increased rate of O_2 photoreduction in chloroplasts (Reddy *et al*., 2004a). The accumulated AOS lead to dramatic loss of photosynthetic pigments and disorganization of thylakoid membranes (Reddy *et al*., 2004b). Chlorophyll stability has been considered as a promising criterion for drought tolerance in plants (Nayyar & Gupta, 2006; Jaleel *et al*., 2008). On the other hand, chlorophyll loss is regarded as an adaptation to prolonged drought. Because it reduces the amount of photons absorbed by leaves and enhances the photoprotective of plants under drought (Munné-Bosch & Alegre, 2000). Carotenoids act as light harvesting pigments and assimilate the light that chlorophyll molecules can not absorb (Siefermann-Harms, 1987). Besides, the carotenoid is well known for its antioxidant activity within the chloroplasts, scavenging singlet oxygen and lipid peroxy radicals, as well as preventing lipid peroxidation (Munné-Bosch & Alegre, 2000; Reddy *et al*., 2004a).

Sonchus spp., belonging to the family Compositae, is widely distributed in China and of great stress resistance. *Sonchus oleraceus* L., *Sonchus wightianus* DC. and *Sonchus uliginosus* M.B. are traditional medicinal and edible plants in China (Jimoh *et al*., 2011). Researchers have found that the extracts of these plants have antioxidant, antidiabetic, anti-ageing and anti-tumor ability (Teugwa *et al*., 2013; Ou *et al*., 2015). Physiological responses to drought and evaluations of drought tolerance have been studied in field crops (Tan *et al*., 2006). However, there are few researches about physiological traits of *Sonchus* species tolerance to prolonged drought. We analyzed SOD and POD activities, soluble sugar, soluble protein, proline, MDA, total chlorophyll, chlorophyll a, chlorophyll b and carotenoid content in leaves of *S. oleraceus*, *S. wightianus* and *S. uliginosus* collected at seeding stage, flowering stage and maturation stage respectively under the natural condition. We are concerned about physiological indexes changes and attempt to analyse protective mechanisms of three plants under drought, from which evaluate their ability to withstand prolonged drought and make a decision for the selection of wild plant species in arid and semiarid regions. It contributes to our knowledge of wild plants tolerance to drought and provides a scientific basis for selection of wild plants in arid and semiarid regions. Moreover, the research establishes an efficient platform for explaining wild plants physiological and molecular mechanisms under drought and provides access to untapped resistance genes.

Materials and Methods

Plant materials: Three wild *Sonchus* species were studied (Table 1): *S. oleraceus*, *S. wightianus* and *S. uliginosus*. They were collected from Wushan County, Gansu province (34°38′N, 104°47′E) and identified by Prof Zhenhai Wu from the College of Life Science, Northwest Agriculture and Forestry University. They were planted with roots and the line spacing was 25 cm. Experimental plot area of each plant was 3.0 m x 1.5 m with three replications and used randomized block design. The leaves of *S. oleraceus*, *S. wightianus* and *S. uliginosus* were collected respectively at seeding stage, flowering stage and maturation stage under natural condition. The growth stage and sampling time of three plants is given in Table 2.

Study area description: The study area is affiliated to the Key Field Observation Station of Ecological Environment of the Ministry of Agriculture on the Loess Plateau, which is located in the Gongjiawan of Lanzhou city of Gansu province in China (36°02′N, 103°44′W). This area belongs to the arid hilly and gully regions on the Loess Plateau. The altitude of study area is 1719 m. Mean annual precipitation is 324.5 mm, most of which falls between July and September. The annual evaporation is approximately 1450.0 mm and the sunshine duration is 2651.4 h. The mean annual air temperature is 9.3ºC, the lowest temperatures being -23.1ºC and the highest temperatures being 39.1ºC.

Table 1. *Sonchus* species with some general information in this study.

Species	Growth form	Description
S. oleraceus	Annual or biennial herb	Herbs 40–150 cm tall. Stem below synflorescence simple or branched, glabrous. Basal and lower stem leaves with basal portion petiole-like and attenuate. Middle and upper stem leaves extremely variable, elliptic, oblanceolate, or lanceolate. Synflorescence shortly corymbiform or racemiform, with few to several capitula. Capitula with many florets. Achene obcolumnar, 2.5–4 mm
S. wightianus	Perennial herb	Herbs 30–150 cm tall, with a taproot. Stem branched from base or higher, glabrous below synflorescence. Basal and lower stem leaves oblanceolate to elliptic. Middle and upper stem leaves elliptic to lanceolate. Synflorescence corymbiform, with several to many capitula. Capitula with very many (usually 180–300) florets. Achene narrowly ellipsoid, 3.5–4.5 mm
S. uliginosus	Perennial herb	Herbs 30-100 cm, with a taproot. Basal and lower stem leaves oblong-ovate, oblong-oblanceolate, oblong-lanceolate. Middle and upper stem leaves similar to lower leaves. Synflorescence corymbiform, with many capitula. Achene elliptic, 3 mm

Table 2. Growth stage and sampling time of three *Sonchus* species.

	Seeding Stage		Flowering Stage		Maturation Stage	
	Month/day	Sampling time	Month/day	Sampling time	Month/day	Sampling time
S. oleraceus	4/19-6/2	5-15	6/2-8/14	7-25	8/14	9-11
S. wightianus	4/20-6/5	5-15	6/5-8/16	7-25	8/16	9-11
S. uliginosus	4/25-6/15	5-15	6/15-8/2	7-25	8/28	9-11

Table 3. The two-way ANOVA result on effect of species and growth stage on physiological traits.

Subset		SOD	POD	MDA	Pro	SP	SS	Car	Chl_{a+b}
Species	F	99.718	650.487	717.683	83.830	171.405	749.442	62.097	149.853
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Growth Stage	F	4.363	213.292	189.070	60.247	14.431	67.004	7.935	13.568
	p	0.031	0.000	0.000	0.000	0.000	0.000	0.004	0.000

Note: SOD: superoxide dismutase activities; POD: peroxidase activities; MDA: malondialdehyde content; Pro: proline content; SP: soluble protein content; SS: soluble sugar content; Car: carotenoid content; Chl_{a+b}: total chlorophyll content

Experimental method

Antioxidant enzymes: Fresh leaves 0.5 g were ground with a mortar and pestle under chilled conditions in the presence of 5 ml phosphate buffer (50 mmol/L pH 7.8). The homogenate was centrifuged at 10000 rpm for 20 min at 4°C. The resulting supernatant was used for the assay of antioxidant enzymes.

The superoxide dismutase (SOD) activities was measured according to Shao *et al*. (1983). One unit of SOD activity was defined as the amount of enzyme required to produce a 50% inhibition of reduction of NBT at 560 nm. The reaction mixture contained 0.01 ml of enzyme extract. Peroxidase (POD) activities was according to Giannopolitis & Ries (1976). The oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min. One unit of POD activity was defined as an absorbance change of 0.01 units per min. The reaction mixture contained 0.5 ml of enzyme extract. The activity of each enzyme was expressed on protein basis.

Lipid peroxidation: Lipid peroxidation level in leaves was determined in terms of malondialdehyde (MDA). Malondialdehyde (MDA) content was measured by the thiobarbituric acid (TBA) reaction as described by Zhao *et al*. (1994).

Proline, soluble protein and soluble sugar content: The content of proline was measured by the ninhydrin method (Troll & Lindsley, 1955). Soluble protein content was measured by the Bradford protein assay (Jones *et al*., 1989). Soluble sugar content was according to anthrone method (Yemm & Willis, 1954).

Pigments: Total chlorophylls (Chl_{a+b}), chlorophylla (Chl_a), chlorophyll$_b$ (Chl_b), and carotenoids (Car) were determined spectrophotometrically using 80% acetone as a solventby referring to Lichtenthaler (1987).

Data Statistical analysis: We used one-way ANOVA and multiple comparisons (Duncan's test at $p<0.05$ level) to compare the differences in the means of physiological indexes among different species and growth stages. Moreover, two-way ANOVA was used to evaluate effects of species and growth stages on physiological indexes as presented in Table 3. Statistical analysis were performed with SPSS 18.0.

Results

Changes of SOD and POD activities, MDA content: The two-way ANOVA results clearly showed that there were significant effects of species and growth stages on the physiological indexes researched (Table 3). *S. oleraceus* had the higher SOD and POD activities at three growth stages and the lower MDA content at seeding stage and flowering stage compared to *S. wightianus* and *S. uliginosus*. SOD and POD activities in *S. oleraceus* were lower at seeding stage (115.360 U mg^{-1} protein and 75.020 U min^{-1} mg^{-1} protein) than that at flowering stage (144.130 U mg^{-1} protein and 104.195 U min^{-1} mg^{-1} protein) and maturation stage (137.163 U mg^{-1} protein and 169.734 U min^{-1} mg^{-1} protein). But MDA content in *S. oleraceus* at seeding stage (30.764 nmol g^{-1} FW) was significantly lower than that at flowering stage (35.325 nmol g^{-1} FW) ($p<0.05$, Fig. 1A, B, C).

Changes of proline, soluble sugar and soluble protein content: *S. oleraceus* had the higher proline content at flowering stage and maturation stage compared to *S. wightianus* and *S. uliginosus*. It significantly increased with maturation and reached the highest content at maturation stage (0.279 mg g^{-1} FW). The lowest proline content was in *S. wightianus* (0.039 mg g^{-1} FW) at flowering stage (Fig. 1D). The soluble sugar and soluble protein content of *S. uliginosus* were singificantly higher than that of *S. wightianus* and *S. oleraceus* during the growth period. And these two solutes in *S. wightianus* and *S. oleraceus* did not change obviously (p<0.05, Fig. 1E, F).

Changes of total chlorophyll and carotinoid content, the ratio of Chl_a/Chl_b and Car/Chl_{a+b}: Comparing to *S. wightianus* and *S. uliginosus*, *S. oleraceus* had the higher total chlorophyll content at three growth stages and the higher carotinoid content at seeding stage and maturation stage. And the highest chlorophyll content of *S. oleraceus* was at flowering stage (2.21 mg g^{-1} FW). The chlorophyll content and carotinoid content in *S. uliginosus* was significantly lower than that in *S. oleraceus* and *S. wightianus*. But the ratio of Chl_a/Chl_b and Car/Chl_{a+b} in *S. uliginosus* was significantly higher than that in *S. oleraceus* and *S. wightianus* at three growth stages (p<0.05, Fig. 2A, B, C, D).

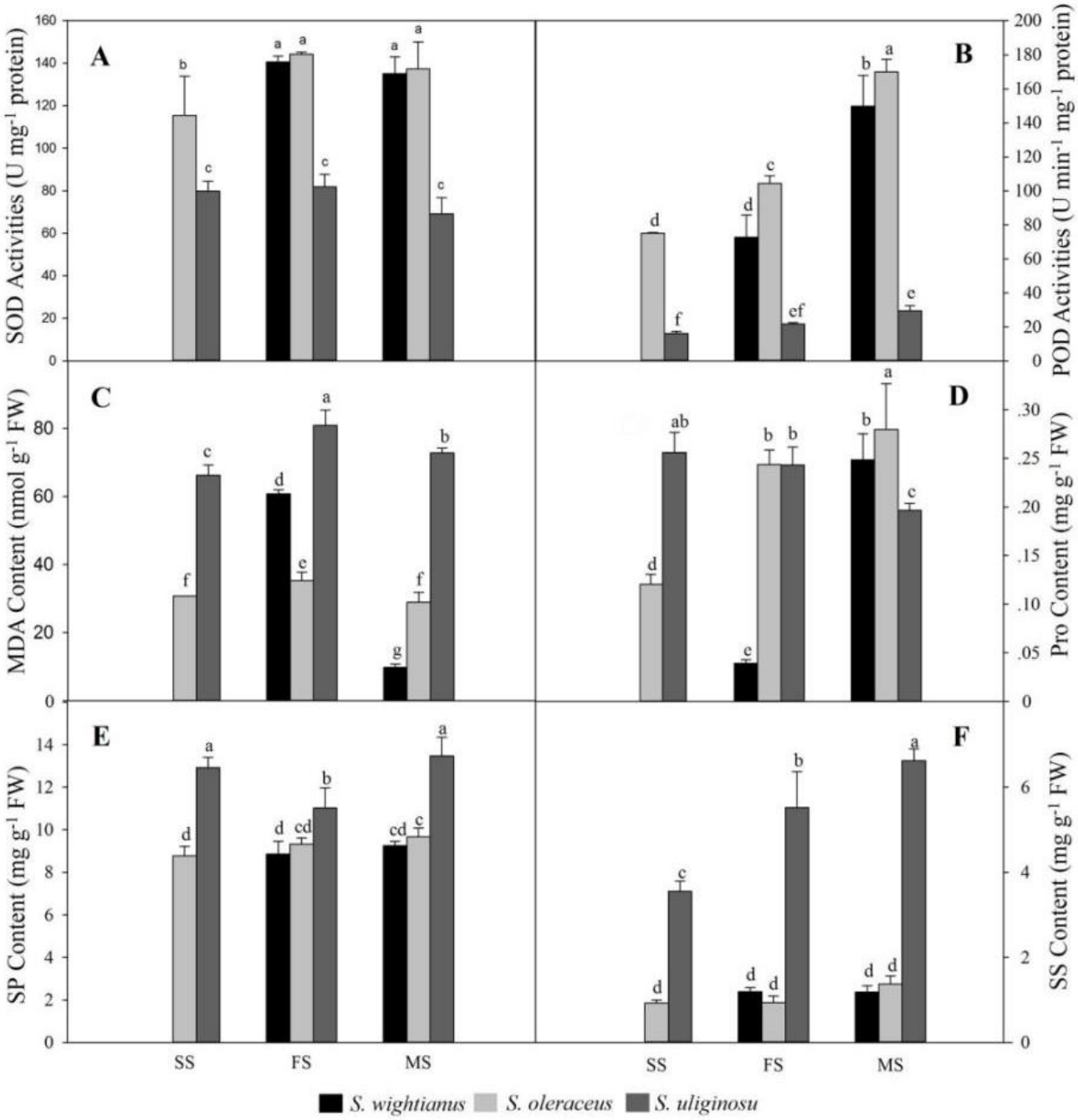

Fig. 1. Superoxide dismutase (SOD) activities (A), peroxidase (POD) activities (B), malondialdehyde (MDA) content (C), proline (Pro) content (D), soluble Protein (SP) Content (E) and soluble sugar (SS) content (F) of *S. oleraceus*, *S. wightianus* and *S. uliginosus* at seeding stage (SS), flowering stage (FS) and maturation stage (MS) (Mean ± SE; n = 3). The different letters represent significant difference by Duncan's test at p<0.05.

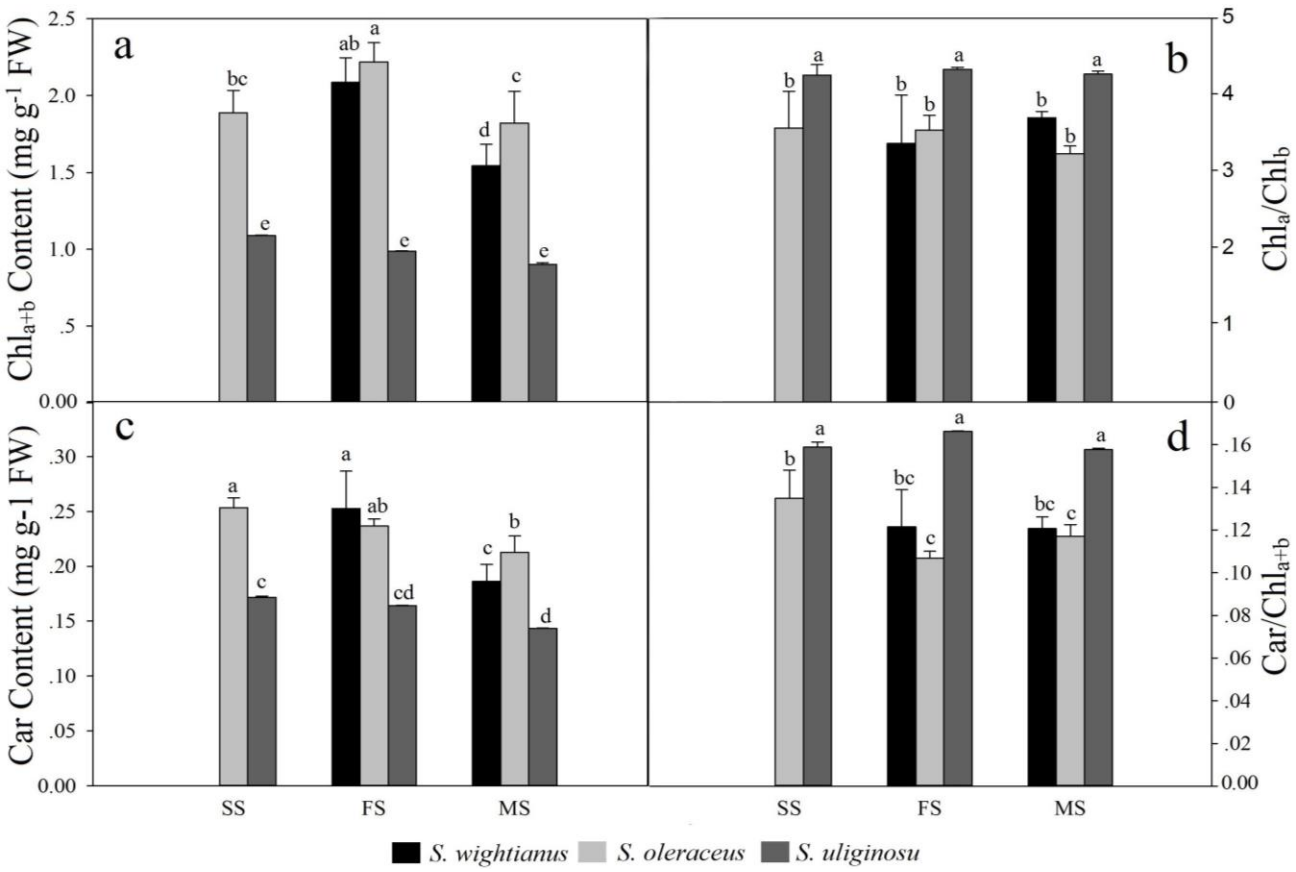

Fig. 2. Chlorophyll (Chl$_{a+b}$) content (A), carotinoid (Car) content (C), the ratio of chlorophyll a to chlorophyll b (Chl$_a$/Chl$_b$) (B) and the ratio of carotenoids to total chlorophylls (Car/Chl$_{a+b}$) (D) of *S. oleraceus*, *S .wightianus* and *S. uliginosus* at seeding stage (SS), flowering stage (FS) and maturation stage (MS) (Mean ± SE; n = 3). The different letters represent significant difference by Duncan's test at $p<0.05$.

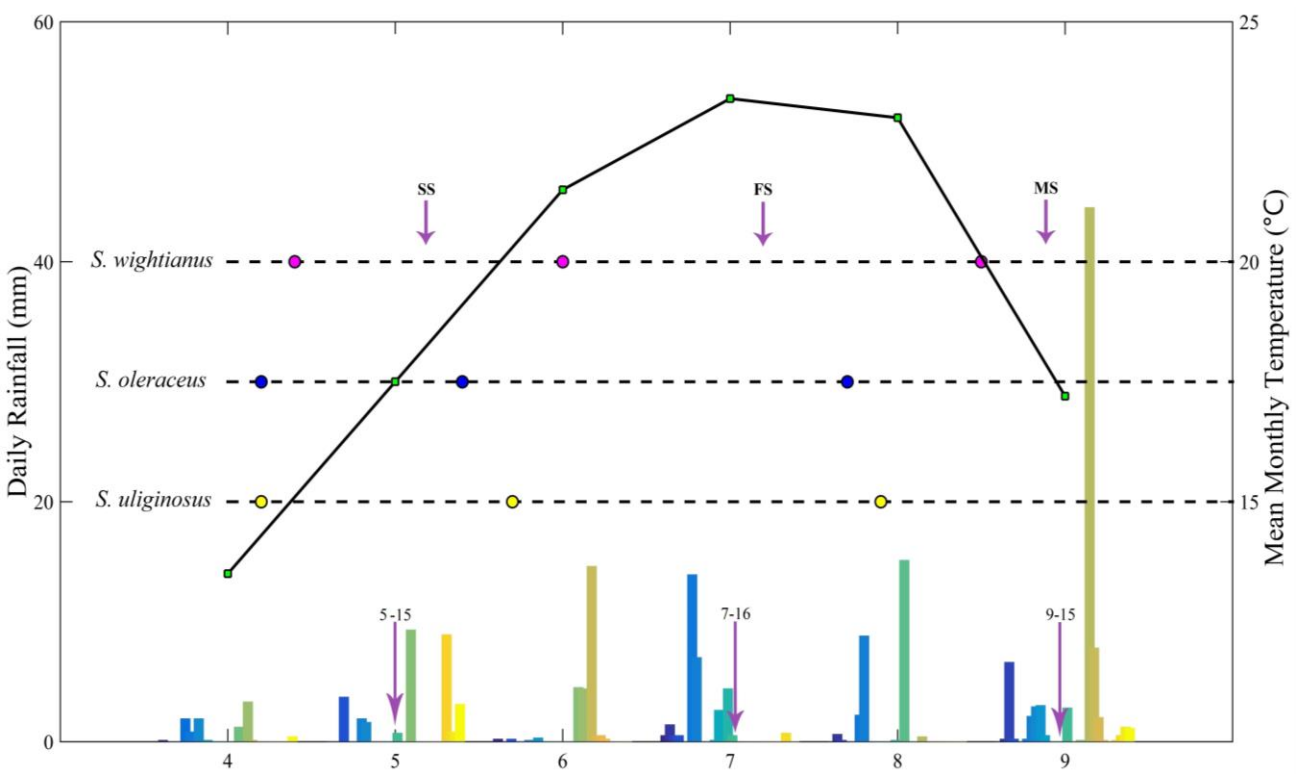

Fig. 3. Daily rainfall and mean monthly temperature from April to September in research area. 5-15 (month-day), 7-16 (month-day), 9-15 (month-day) is the sampling time of *S. oleraceus*, *S. wightianus* and *S. uliginosus* respectivly at seeding stage (SS), flowering stage (FS) and maturation stage (MS).

Discussion

The environmental conditions of arid and semiarid regions (less rainfall and high evaporative demand) often cause drought stress in plants during growth season (El-Sharkawy, 2007). Meteorological data of research area during growth stage was showen in figure 3 (Meteorological data was from Key Field Observation Station of Ecological Environment of the Ministry of Agriculture on the Loess Plateau). Changes of osmotic potential across the plasma membrane result in an oxidative burst in plants under drought (Reddy *et al*., 2004a). Plants postpone dehydration and allow water uptake from environment by promoting water influx as a result of compatible solutes accumulation (De Diego *et al*., 2013). These so-called osmolytes, such as soluble sugar, soluble protein and proline lower cell sap osmotic potential and prevent turgor loss, so that turgor and turgor-related processes, such as cell expansion, photosynthesis, growth and gas exchange, may be maintained during drought (Girma & Krieg, 1992; Iannucci *et al*., 2002; Liu *et al*., 2011; Kadkhodaie *et al*., 2013). Osmolytes are also involving in scavenging AOS and protecting macromolecules by maintaining around them a water shell (Costa França *et al*., 2000; Reddy *et al*., 2004a). Osmotic adjustment has been considered as a major indicator that is related to drought tolerance in plants (Hu & Xiong, 2014).

The perturbations are observed in carbon metabolism that complex carbohydrates and proteins are broken down by enzymes into the simpler sugars and amino acids in plants under drought (De Diego *et al*., 2013; Habash *et al*., 2014). Habash *et al*. provided some evidences at molecular level that drought stress altered transcripts for sugar and amino acid metabolism, an increase in expression for sucrose and starch breakdown and proline biosynthesis (Habash *et al*., 2014; Yang *et al*., 2015). Osmotic adjustment has been associated with drought tolerance in many plant species. Proline concentrations were significantly higher in drought-tolerant cultivars than that in drought-sensitive cultivars, such as wheat (Shao *et al*., 2006), peanut (Quilambo, 2004) and mulberry (Reddy *et al*, 2004b). The accumulation of soluble sugars also contributed to drought tolerance of canola (Nosrati *et al*., 2014) and sugar beet (Chołuj *et al*., 2008). The higher proline content in *S. oleraceus* and the higher soluble sugar and soluble protein content in *S. uliginosus* at three growth stages might confer them the better osmotic adjustment ability and cell membrane stability, revealed the higher tolerance to drought than *S. wightianus.* And the proline content in *S. oleraceus* significantly increased with maturation. The result was consistent with the previous research that proline content was higher at later stage than that at early stage of plants (Yamada *et al*., 2005). It is proposed that the proline content varies depending on the age of plants.

The varieties of solutes accumulated and their relative contributions to osmotic adjustment differ in plant species (Iannucci *et al*., 2002; De Diego *et al*., 2013). Proline accumulation is traditionally considered to be significant and essential for plants to response to drought (De Diego *et al*., 2013; Hu & Xiong, 2014). However, some controversies about the roles of proline in plants under drought have been reported (De Diego *et al*., 2013; Habash *et al*., 2014). Proline has been long regarded as a compatible osmolyte due to its high concentrations in the relatively small volume of chloroplasts and cytoplasmic compartments under drought (Reddy *et al*., 2004a; Shinde *et al*., 2016). Nevertheless, proline accumulation can contribute to stress tolerance in multiple ways. Proline has been reported to act as a molecular chaperone that protects structures and activities of different proteins and enzymes (Costa França *et al*., 2000; Reddy *et al*., 2004a). Liu *et al*. reported positive relationships between contents of proline and antioxidant enzyme activities (SOD and POD) in two shrubs, suggesting that proline accumulation largely permitted the high activities of antioxidant enzymes under drought (Liu *et al*., 2011). Proline is also involved in reducing the photodamage of the thylakoid membranes by scavenging and/or reducing the production of $^1O_2$ (Reddy *et al*., 2004b). Vendruscolo *et al*. demonstrated that proline contributed to drought tolerance to wheat by increasing the antioxidant activities rather than increasing osmotic adjustment ability (Vendruscolo *et al*., 2007). Reddy *et al*. thought proline could act as a free radical scavenger and might be more important in overcoming stress than in working as a simple osmolyte (Reddy *et al*., 2004b). Free radical levels decreased in transgenic tobacco and wheat plants resulting from hyperaccumulation of proline by P5CS overexpression (Hong *et al*., 2000; Vendruscolo *et al*., 2007). Since proline can stabilize the structures and activities of enzymes, the more accumulation of proline in *S. oleraceus* seems to largely permit its high activities of SOD and POD, promote the growth of seedlings and enhance the development of flowers compared to *S. uliginosus* and *S. wightianus* (Kavi Kishor *et al*., 1995; Hong *et al*., 2000; Liu *et al*., 2011). However, the actual biological functions of proline in *Sonchus* species remain unclear.

Drought stress usually causes the high formation of AOS in chloroplasts and mitochondria due to stomatal closure (Liu *et al*., 2011). The excess AOS are highly reactive and toxic to plants, which causes damage to proteins, lipids, nucleic acids, photosynthetic pigments and enzymes (Zhang & Kirkham, 1996; Tan *et al*., 2006). Membrane lipid peroxidation can led to damage to the membrane system and cell ultrastructure and causes a decrease in photosynthesis and respiration (Bai *et al*., 2006). Since MDA has been considered as an indicator of oxidative damage for membrane lipid, the lower MDA content in *S. oleraceus* at seeding stage and flowering stage may suggest that *S. oleraceus* is more protected from oxidative damage and has the better membrane integrality than *S. wightianus* and *S. uliginosus* (De Azevedo Neto *et al*., 2006; Liu *et al*., 2011).

The lower MDA content in *S. oleraceus* appear to be related to the higher SOD activities as well as POD activities, which facilitate the reduction in O_2^- and H_2O_2 concentration and therefore minimize lipid peroxidation in leaves of *S. oleraceus* (Liu *et al*., 2011). Researchers have reported that drought tolerant genotypes had less accumulation of AOS and MDA compared to sensitive

genotypes because of the higher antioxidant enzyme activities (De Azevedo Neto *et al*., 2006). High activities of antioxidant enzymes also improved drought tolerance of mulberry (Reddy *et al*., 2004b), alfalfa (Luo *et al*., 2009) and maize (Bai *et al*., 2006). SOD, POD and CAT and MDA had been used as a selection criterion for drought tolerant plants, whose higher activities of the antioxidant enzymes and lower content of MDA reflected the higher anti-oxidative ability and drought tolerance (Shao *et al*., 2005a; Shao *et al*., 2005b; Tan *et al*., 2006; Shao *et al*., 2007). However, both antioxidant enzyme activities (SOD and POD) and MDA content in *S. oleraceus* were lower at seeding stage. It demonstrated that there were other important antioxidant enzymes and nonenzymatic antioxidants attributing to the lower membrane injury, such as the accumulated proline.

Chandrasekar *et al*. thought that leaf chlorophyll content and carotenoid levels might be considered as a valuable indicator to assess the plant stress intensity and genotype tolerance to water deficit (Chandrasekar *et al*., 2000). Besides acting as light-harvesting pigments, carotenoids are well known as non-enzymatic antioxidants to quench AOS in chloroplasts, stabilize photosynthetic complexes and prevent lipid peroxidation (Chandrasekar *et al*., 2000; Munné-Bosch & Alegre, 2000; Liu *et al*., 2011). In higher plant chloroplasts, carotenoids might also play an important structural role to stabilize the lipid phase of thylakoid membranes (Havaux, 1998). Decrease in pigments content is a typical symptom of oxidative damage in plants under drought (Liu *et al*., 2011). Less extent of MDA and the smaller reduction of pigments content in *S. oleraceus* may due to the higher proline accumulation and SOD and POD activities under prolonged drought, revealing the higher protection against oxidative stress and drought tolerance (Liu *et al*., 2011). Since carotenoids play an important role in antioxidant, light-harvesting as well as photoprotection functions, the higher carotenoid levels may largely enhance its drought tolerance in *S. oleraceus* under drought (Maghsoodi & Razmjoo, 2015).

Although chlorophyll loss is a negative result of stress, it has been regarded as an adaptive response to drought in plants (Munné-Bosch & Alegre, 2000). Lowering of photosynthetic biosynthetic transcripts has been found in plants under drought, which reflects a down-regulation of light capture (Habash *et al*., 2014). Some researchers explain this phenomenon as a photoprotective mechanism through which reducing light absorbance (Munné-Bosch & Alegre, 2000; Baquedano & Castillo, 2006; Elsheery & Cao, 2008; Liu *et al*., 2011). Inhibition of photosynthetic activity results from imbalance between light capture and utilization can cause oxidative damage in plants under drought (Zhou & Yu, 2010). Chlorophyll loss decreases the light capture and prevents the further photo-oxidative damage to photosynthetic apparatus caused by AOS under excess excitation energy (Elsheery & Cao, 2008). Photoinhibition is also associated with the maintenance of reversible energy dissipating mechanism, which is mediated by a particular group of carotenoids. Excess energy in plants can led to oxidative stress to photosynthetic apparatus and other cell components (Demmig-Adams & Adams, 1996). The ratio of Chl_a/Chl_b and Car/Chl_{a+b} in *S. uliginosus* was significantly higher than that in *S. oleraceus* and *S. wightianus*. This result was related to an increase in excess energy dissipation through carotenoids and a decrease in absorption of energy through chlorophylls, affording the photo-protection against oxidative stress (Demmig-Adams & Adams, 1996; Baquedano & Castillo, 2006). And the higher Car/Chl_{a+b} ratio would imply a higher need of photoprotection by carotenoids in *S. uliginosus* (Liu *et al*., 2011).

Conclusion

In arid and semiarid regions, water deficit is the main factor that limits plants performance. Under such natural condition, *S. oleraceus* accumulated more proline while *S. uliginosus* accumulated more soluble sugar and soluble protein in three growth stages, which conferred them the better osmotic adjustment ability and cell membrane stability. Although the solutes detected may be important in the adaptation of each species to water-stressed environments. Osmotic adjustment is a highly regulated process, involving metabolites besides those analyzed in this experiment. Less extent of MDA and the smaller reduction of pigment content in *S. oleraceus* may due to the higher proline accumulation as well as the higher activities of SOD and POD under prolonged drought, revealing the higher protection against oxidative stress. And accumulated proline in *S. oleraceus* seems to largely permit its higher activities of SOD and POD. All of these responses in *S. oleraceus* may contribute to relieve the negative effects of drought on plants by alleviating damage to cellular components and enhancing osmoregulation and photosynthetic capacity. It is suggested that *S. oleraceus* has the higher tolerance to prolonged drought compared to *S. uliginosus* and *S. wightianus.* With its inherent capacity to adapt to the prolonged drought, *S. oleraceus* should be expected to insure the basis seedling number, promote the growth of seedlings and the development of flower, thus providing more essential food, medicinal materials and feed than *S. uliginosus* and *S. wightianus* in arid and semiarid regions.

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