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Research article

Analysis of storage compounds and inorganic ions in dimorphic seeds of euhalophyte *Suaeda salsa*

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ABSTRACT

Suaeda salsa is an annual euhalophytic herb that produces dimorphic seeds, such as small black seeds and big brown seeds. In the present study, the fatty acid composition, content of total phenols, flavonoids, carotenoid and inorganic ions in dimorphic seeds of the species collected in the field were measured. There was no significant difference in total oil content between black and brown seeds. Seed total oil content was approximately 19% based on dry weight. The most abundant fatty acid was linoleic acid, and the content was 76.3 and 70.5% of total fatty acids in black and brown seeds, respectively. Furthermore, the contents of total phenols, flavonoids, carotenoids and inorganic ions in brown seeds were higher than those in black seeds, which might be the mechanism of higher salt tolerance of brown seeds than black seeds. The ecological, physiological and genetic mechanisms of the different abilities of nutrition accumulation in black and brown seeds of *S. salsa* are also discussed and worthy to be investigated in the future.

1. Introduction

With the rapid development of human population and soil salinization in arid and semiarid regions, the competition for arable land among the agriculture and domestic use and industrial use of fresh water has greatly increased (Rozema and Flowers, 2008; Fedoroff et al., 2010). The rational use and domestication of halophytes that grow in arid and semi-arid areas of plants to alleviate this conflict is the best option. Many salt-adapted plants can be developed as oilseed crops (Weber et al., 2007; Rozema and Schat, 2013; Duan et al., 2018). Some halophytes, such as *Suaeda* spp., produce seeds that contain edible oil with high quality, and these are likely to be developed as oilseed crops (Zhao et al., 2018a). For example, the seed oil content of *Suaeda aralocaspica* (*S. aralocaspica*) was 29% on a dry weight basis, and its dimorphic seeds contain approximately 93% unsaturated fatty acids of total fatty acids (Wang et al., 2012). Therefore, *S. aralocaspica* can be regarded as a promising halophytic oilseed crop (Wang et al., 2012).

Many *Suaeda* species are also promising sources of pharmaceuticals due to the high content of polyphenols, carotenoid and other bioactive compounds. For example, the edible halophyte *Suaeda fruticosa* is a valuable source of antioxidants that have novel anti-cancer and anti-inflammatory capacities (Oueslati et al., 2012). Astragalin (a kind of flavonoids), which can be isolated from extracts of *Suaeda asparagoides*, can play a role as an antioxidant in biological systems, and protect cellular membranes against reactive oxygen species (Park et al., 2012). However, it remains unclear how dimorphic seeds of halophytes accumulate certain bioactive compounds.

Suaeda salsa L. (*S. salsa*) is an euhalophytic herb. The species occurs in both the intertidal zone and inland saline sites in China (Song et al., 2011; Li et al., 2012; Chen et al., 2016; Liu et al., 2018). *S. salsa* produces dimorphic seeds, such as soft brown seeds and hard black (Li et al., 2005), and brown seeds have higher salt tolerance than black seeds during seed germination and seedling stages (Li et al., 2005, 2016; Chen et al., 2014; Zhou et al., 2016; Sui et al., 2017; Song et al., 2017). Furthermore, it has been reported that the oil content of dry seeds of *S. salsa* is approximately 20% (Song and Wang, 2015). However, it is still not clear whether dimorphic seeds of *S. salsa* differently accumulate fatty acids and certain bioactive compounds that may be related to the nutritional value and salt tolerance of dimorphic seeds of the species. Due to the high content of unsaturated fatty acids, the seed oil of *S. salsa* can be used to decrease blood sugar and blood pressure, dilate blood vessels, prevent heart disease, and develop disease immunity in traditional Chinese medicine (Song and Wang, 2015). Xu et al. (2017) reported that different the expression of genes may be associated with fatty acid and osmotic regulation substances in brown and black seeds. However, it remains unclear how dimorphic seeds of *S. salsa* differently accumulate these substances. Therefore, in the present study, the oil content, fatty acid composition, total phenols, flavonoids,

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carotenoid and inorganic ions in dimorphic seeds of *S. salsa* collected from the saline inland sites of the Yellow River delta in China were analyzed, in an attempt to further evaluate the nutritional value, and determine whether these substances are correlated to the salt tolerance of dimorphic seeds in the studied species.

2. Materials and methods

2.1. Seed collection

Mature seeds of *S. salsa* in inland saline soils were collected from 50 plants in the Yellow River Delta (N37°26′, E118°51′) of Shandong in late November 2015. These seeds were used for the experiment just after these were air dried for 15 days.

2.2. Extraction of seed oil

In order to analyze seed oil contents and fatty acid compositions, powdered seeds (7 g) were extracted in a Soxhlet apparatus using pure ethyl ether for 8 h. Then, the solvent was recycled through the Soxhlet apparatus. Finally, the seed extracts were freeze-dried and stored at −20 °C in sealed tubes prior to analysis. Three replicates were used for black and brown seeds.

2.3. Fatty acid analysis

The fatty acid compositions in samples were determined by gas chromatography of the fatty acid methyl esters (FAMEs), according to the method of Zhao et al. (2018b). Three replicates were used for black and brown seeds.

2.4. Analysis of total phenolic content

The powdered seeds (0.2 g) were extracted in an ultrasonic cleaner using 80% alcohol (5 ml) for 80 min (Wang et al., 2014). The supernatant of the extracts was collected and maintained at 4 °C for further analysis.

The total phenolic content of the seeds extracts was determined using Foline-phenol reagent, which was slightly modified by Wang et al. (2014) using gallic acid as a standard. A sample extract (0.2 ml) was added to 1 ml of Foline-phenol reagent. Then, the mixture was shaken, and allowed to stand for 5 min before the addition of 3 ml of 7.5% Na_2CO_3. Subsequently, the solution was maintained at ambient temperature in the dark for 30 min. After that, the absorbance at 765 nm was recorded. The total phenolic content was expressed as micro-gramme of gallic acid equivalents per gramme of dry seed weight (μg g^{-1} DW) through the calibration curve with gallic acid. Three replicates were used for black and brown seeds.

2.5. Analysis of flavonoid content

The dry and powdered seeds (0.2 g) were extracted in shaker using 50% methanol (10 ml) for 10 h (Zhang, 2007). Afterwards, the extracts were filtered, and 50% methanol was added up to a final volume of 25 ml for each extract. The extracts were stored in the dark prior to further analysis.

The flavonoid content of the seed extracts was determined by the colorimetric assay developed by Zhang (2007), using rutin as a standard. Each sample (1 ml) was added to 0.5 ml of $NaNO_2$ solution (5%), and mixed for 5 min before the addition of 0.5 ml of $Al(NO_3)_3$ solution (10%). After 6 min, 2 ml of NaOH solution (4%) was added. Finally, each sample was thoroughly mixed and fixed for 10 min. The absorbance of the mixture was determined at 510 nm. Flavonoid content was expressed as micro-gramme rutin equivalent per gramme of dry seed weight (μg g^{-1} DW). Three replicates were used for black and brown seeds.

2.6. Analysis of carotenoid content

The dry and powdered seeds (0.2 g) were extracted with acetone and petroleum ether (1:1) using the ultrasonic cleaner (Xue, 2009). The extract for each oil sample was condensed with nitrogen in the dark at ambient temperature prior to further analysis. Three replicates were used for black and brown seeds.

The seed oil was analyzed for contents of carotenoid, according to Xue (2009). The volume of each sample was adjusted to 10 ml with acetone. The absorbance was read at 446 nm, and used to calculate the carotenoid content. The results were expressed as μg of carotenoid per g of dry seed. The contents of carotenoid were calculated using the following equation:

$$X(\mu g \cdot g^{-1}) = \frac{A \times y(mL) \times 10^6}{A_{1cm}^{\%} \times 100 \times g}$$

Where A is the absorbance at 446 nm, y is the volume of the extracting solution, $A_{1cm}^{\%}$ is the average absorption coefficient of carotenoid molecules (2,500), and g is the weight of the sample.

2.7. Measurement of inorganic ions

Seed samples (0.3 g) were digested in a HNO_3-H_2O_2 solution and heated by microwave for several hours, and ultrapure water was added to obtain a final volume of 25 ml (Zhang et al., 2012). Then, the contents of K, Mg, P, Cu, B, Fe, Mn and Zn were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES; Optima 7300 DV, Perkin-Elmer, USA) (Xu et al., 2017). Three replicates were used for brown and black seeds.

2.8. Statistical analysis

The results were reported as means and standard deviation. Differences between black and brown seeds in all analyses were tested using one-way ANOVA for statistical analysis. SAS software (SAS Institute, Inc. 1989) was used for statistical analysis.

3. Results

3.1. Seed total oil content

The total seed oil content of black and brown seeds was 19.7 and 17.5% of seed dry weight, respectively. There was no significant difference in seed oil content between black and brown seeds (Table 1).

3.2. Fatty acid composition

The fatty acids of black and brown seeds included saturated, monounsaturated and polyunsaturated fatty acids (Table 1). Brown seeds contained more saturated and less unsaturated fatty acids than

Table 1
Contents of total seed oil (% of seed dry weight), saturated, monounsaturated, polyunsaturated, and total unsaturated fatty acids (% of total fatty acids) in black and brown seeds of *S. salsa* in field condition.

	Black seeds	Brown seeds
Total seed oil	19.66 ± 2.73 a	17.52 ± 1.02 a
Saturated	8.61 ± 0.48 b	10.32 ± 0.22 a
Monounsaturated	10.97 ± 0.58 b	13.50 ± 0.29 a
Polyunsaturated	80.43 ± 0.20 a	76.18 ± 0.50 b
Total unsaturated	91.40 ± 0.48 a	89.68 ± 0.22 b
Unsaturated/saturated	10.66 ± 0.67 a	8.69 ± 0.20 b
Polyunsaturated/saturated	9.37 ± 0.53 a	7.38 ± 0.20 b

Mean values with the same letter are not significantly different at the 0.05 level between black and brown seeds.