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SEED STRUCTURE, POLYMORPHISM, GERMINATION AND ONTOGENESIS OF SALSOLA KALI SUBSP. TRAGUS (CHENOPODIACEAE) UNDER GREENHOUSE CONDITION

A. Ammarellou & B. Maleki Zanjani

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Protection of desert soil vegetation is one of the key requirements for environmental protection programs. Survival of desert plants is related to dispersal characters and germination mechanisms that ensure germination and successful establishment of seedlings in suitable time and place. Salsola kali subsp. tragus is annual halophyte found in some dry land and rangeland of Tarom-Abbar (Province of Zanjan). In this area it names as Chogan and in ancient times, was used as laundry soap. In this research, seed structure, polymorphism, germination and growing of S. kali subsp.tragus were studied under greenhouse conditions. Three types of seeds were observed: a, dark; b, light yellow and c, yellow color. All seeds had a spiral-shaped embryo. Spiral-shaped embryo gives quick ability for fast germination in good condition. Some of the yellow seeds had a transparent shield which causes gradual penetration of water into the seed. These features cause the seeds that germinate later than the others. Comparison of germination between scarified and not scarified seeds showed that non scarified seeds had more than 2 mounts dormancy. The stages of germination process and morphological characters of plant in different growing life cycle are presented. The special characters of this plant, such as the fast opening of compressed spiral shape embryo, which we report in this research, is a creative natural mechanism for rapid germination and immediately establishes on unreliable conditions. Study of halophytes life cycle, discovery of germination mechanisms and biological structures involved in stress conditions will be very important and necessary for improving of desertification programs, soil improvement, soil erosion control and forage production.

Ali Ammarellou (correspondence <ammarellou@yahoo.com>, Research Institute of Modern Biological Techniques, University of Zanjan, Zanjan, Iran.- Bahram Maleki Zanjani, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

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ساختار بذر، چند شکلی، جوانهزنی و روند رشد و نمو در (Chenopodiaceae) Salsola kali subsp. tragus تحت شرایط گلخانهای علی عمارلو: استادیار، پژوهشکده فناوریهای نوین زیستی دانشگاه زنجان، زنجان بهرام ملکی زنجانی: دانشیار دانشکده کشاورزی دانشگاه زنجان، زنجان، ایران حفاظت از پوشش گیاهی یکی از نیازهای اساسی در موفقیت برنامه های حفاظت از محیط زیست می باشد. استمرار رویش و توسعه گیاهان بیابانی معطوف به مکانیسمهای سهل پراکنش، جوانه زنی و استقرار در خاک در زمان و مکان مناسب است. گیاه چوغان (اصطلاح محلی که در نواحی خشک روستایی استان زنجان بدان نامیده میشود) با نام علمی Salsola kali subsp. tragus یکی از گیاهان یکساله بیابانی شهرستان طارم – آ ب بر بوده و در زمان قدیم برای شستشوی البسه مورد استفاده قرار می گرفته است. در این تحقیق ساختار بذر، پلی مورفیسم، جوانه زنی و روند رشد و نمو این گیاه تحت شرایط گلخانه ای مورد بررسی قرار گرفت. سه تیپ رنگ بذر شامل: تیره، زرد و زرد روشن در این گیاه مشاهده گردید. تعدادی از بذور زرد دارای حفاظ شفاف رویی بوده که باعث جذب تدریجی رطوبت در این تیپ و بالتبع موجب جوانه زنی کند میشود. تمام مراحل رشدی در این گیاه از جوانه زنی تا گیاه کامل مورد بررسی دقیق قرار گرفته و مکانیسمهای سازگاری این گیاه با شرایط بیابانی که بخشی از آن مدیون ساختار حلزونی فنر پیچ فشرده جنین می باشد و جوانه زنی سریع در بازه زمانی ۲۰ دقیقه تا یکساعت را تضمین می کند، تشریح گردیده است. شناخت کامل از مکانیسمهای سازش با شرایط بیابانی در این گیاهان خشکی پسند می تواند در ایجاد زیرساختهای اکوفیزیولوژیکی در اجرای برنامه های بیابانزدایی کشور و جهان بسیار مورد استفاده قرار گیرد.

INTRODUCTION

About one third of the world's land area is drought affected (Kozlowski, 2002; Panahi & al. 2012). Soil salinity in dry lands is one of the most important and serious environmental problems facing arid and semiarid environments on a global scale (Szabolcs, 1994). Saline and alkaline soils are distributed in some provinces of Iran and cover about 12.5 % of the total area of this country (Dewan and Famouri 1964; Akhani and Ghorbanli 1993; Panahi & al. 2012). The halophytic communities of Iran are among the most poorly known vegetation units (Akhani and Ghorbanli, 1993), so study of saline soil plant species is necessary for desertification programs, soil improvement, soil erosion control and forage production. Moreover, many of the Iranian saline soil vegetations have different genera of medicinal plants, locally used for life requirements. Breckle (1983) studied halophytic vegetation of Iran and Afghanistan and classified them to three classes: (1) saline flats (very sparse vegetation, soil with very high salt content); (2) euhalophytic vegetation (halohammada on gravel-sandy but probably on clayey soil); and (3) mesohalophytic vegetation, with less salt in the soil profile. Akhani and Ghorbanli (1993) classified Iranian halophyte vegetations to 10 vegetation units. Seed polymorphism means production of seeds in different shapes, size, color, and/or external structure, as well as in dispersal, dormancy, germination and seedling growth (Drysdale, 1973; Baskin and Baskin, 1998; Wei & al. 2007). Seed polymorphism has been reported for several species of Salsola (Negbi and Tamari 1963; Wei & al. 2007). About 53 % Iranian halophytic flora belong to Chenopodiaceae and Salsola is one of the important genera in this halophytic family (Akhani and Ghorbanli, 1993). Kali L. subsp. tragus (L.) Nyman = Salsola tragus L. is a species of flowering plant in the family Amaranthaceae (previously known as

Chenopodiaceae). It is known by various common names such as prickly Russian thistle, windwitch, or common saltwort (Akhani & al. 2007). It is an annual halophyte found in some dryland and rangeland of Iran such as Alborz Mountains, Azerbaijan, , special parts of Zanjan (Tarom-Abbar). , Fars and Tehran Provinces (Assadi 2001)In Tarom two species of Chenopodiaceae are named as Chogan and in ancient times, were used as laundry soap. According to today's trend of people to nature and plant products, cultivation, production and breeding of this natural detergent and medicinal plant will be in priority. Many of halophyte studies are based on wild habitats and dry samples of herbaria that have not crucial information of their life cycle and other important agricultural requirement data. Despite numerous studies that have been conducted on Salsola spp., nothing is known about the mechanisms and adaptations of seed germination and dispersal of S.kali subsp. tragus to the unpredictable small amounts, timing of rain, and salinity conditions. In this research, seed germination strategies and drought tolerance mechanisms of S. kali subsp. tragus were studied.

MATERIALS AND METHODS Study area

The climate of Tarom is characterized by low annual rainfall ranging from 150 to 400 mm and summer rainfall from 10 to 20 mm. Temperature fluctuations are very pronounced throughout the year, with an average annual temperature between 15 and 25 °C, mild winters and warm summers. The study area (Tarom- Abbar) is located in the north of Zanjan province in longitude 48° 30' to 49° 14' and latitude 36° 38' to 37^{\circ} 13'. The range of annual temperature is between 11.2 and 23.8 °C. The absolute minimum temperature is -13.2 and the absolute maximum temperature is 45 °C.



Fig. 1. Map of the studied and seed collection area in north west of Iran, Zanjan province, Tarom-Abbar.

Plant habitats and seed collection

The rangeland experiments were conducted in Tarom state, which is the northernmost of Zanjan province of Iran (Fig.1). Freshly matured *S. kali* subsp. *tragus* dispersal units were collected in September 2014 from natural habitats of plant populations growing in the salt desert located at Tarom-Abbar.

Seed morphology and polymorphism

Fifty seed structures of *S. kali* subsp. *tragus* were selected from every mother plant randomly to observe under a stereomicroscope (Nikon SMZ1500) and photographs were taken using Sony H9 camera.

Scarification of seed covers and germination

All types of freshly matured seeds were separated from perianth and divided to 2 groups:

In group I the seeds (pericarp and seed coat) were

scarified with a scalpel. Seed surface was sterilized by using 2.5 % commercial bleach (Sodium hypochlorite) for 5 minutes, rinsed with distilled water, placed on three layers of filter paper in Petri dishes, incubated at 20° C in light and used for germination experiments.

In group II, normal seeds were used without any scarification for germination experiments as mentioned above. Seedling of *S. kali* subsp. *tragus* were transferred to plastic pots after 10 days for more growing until seed reproduction in greenhouse conditions.

RESULTS

Seed morphology and polymorphism

Mature fruits of *S. kali* subsp. *tragus* contained embryo of varying color and size, prevailing dark brown and some light vellow seeds with 2 mm dimensions, with lower moisture. Based on shape, color, size (Fig. 5 A) on the embryo, different types of seeds were recognized (Figs. 2 & 3).



Fig. 2. The location of dispersal unit of S. kali subsp. tragus on the plant branches (B) and 3 matured fruits (A).



Fig. 3. The color differences (A: a,b,c) in seed of S. kali subsp. tragus and spiral-shaped embryos (B).



Fig. 4. Comparison of germination between scarified (A) and non scarified (B) seeds.



Fig. 5. Seed with transparent shield (A), seed enclosed in perianth (fruit) (B), exit of embryo from seed perianth (C), magnified spiral- shaped complete embryo (D) opening out of the seed germ (E) and 3 stages of seed germination (F).



Fig. 6. The germination of *S. kali* subsp. *tragus* seeds on mother branches (A: a1: rooting and a2: shooting); seedlings after 10 days of germination (B); moving the seedlings to pots (C); two months old plants (D) and plant in bloom (E).



Fig. 7. The structure of flowers in S. kali subsp. tragus. a: five anthers; b & c: bifurcate stigma; d: immatured seed.

Based on plant growth behavior and fruit structure the studied species were identified as *Salsola tragus* L. Three types of seed polymorphism were observed: a:dark, b:light yellow and c: yellow. All seeds had a

spiral-shaped embryo. Spiral-shaped embryo gives quick ability for fast germination in good condition. Some of the yellow seeds had a transparent shield which causes gradual penetration of water into the seed. The late germination of some types are due to lack of this feature. Comparison of germination between scarified (Fig. 4. A) and nonscarified (B) seeds showed that non scarified seeds had more than 2 mounts dormancy.

The embryo rootletgrow in the beginning and gradually spiral embryo open circular structure (Fig. 5). Under stress conditions such as extreme heat associated with this structure is very important. Some times in germination experiments, at temperatures above 40 °C opening of the fetal spiral rings were observed in less than 20 minutes. This mechanism enables seeds to germinate rapidly and leads to complete the germination process in the shortest possible time and come well in advance to be deployed in soil. Some seeds had germination ability on mother branches at the end of the season (Fig.6.A.). In this case, also the rootlet of the seeds grow out firstly and if sufficient moisture is available, embryonic spiral rings open quickly. The young seedlings are silver puberulent that form on leaf axils (Fig.6.C). All stages of germination process and morphological characters of plant in different growing life cycle is presented in Fig. 6. The two months old and flowering plants are shown in Fig.6: D & E. The anthers are 5 and yellow with white appendages. (Fig.7.a), a bifurcate style (Fig.7.b) and seeds (Fig.7.d) were formed after four mounts growing in August.

DISCUSSION

Salsola kali L. subsp. tragus (L.) Nyman (synonym: Salsola tragus L.) (Assadi 2001), specifically has salsolin and salsolidin as active compounds and has been effectively used in the treatment of hypertonia by stimulating sleep activity (Borkowski and Wrocinski, 1959; Al-Saleh & al. 1993). Germination is a critical stage in the life cycle of plants and tends to be highly unpredictable over space and time (Baskin and Baskin, 2001; Carvalho & al. 2013). Successful establishment of plants largely depends on successful germination and is affected by environmental and internal seed factors and frequently, ideal conditions are species-specific and need to be determined through experimentation (Carvalho & al. 2013). Special combinations of survival strategies are rather common in desert plants (Gutterman, 1993; Wei & al. 2007). Adaptation to the desert environment via special seed dispersal and germination mechanisms is often the key to the survival and development of these plants (Gutterman

1993; Baskin and Baskin 1998; Wei & al. 2007). Seed heteromorphism is common in the Asteraceae, Chenopodiaceae, Poaceae and Brassicaceae (Harper & al. 1970; Manda' k, 1997; Imbert, 2002), and among annuals, often pioneer species or plants faced with stochastic environments such as deserts or semideserts (Manda' k, 1997). Fruit structre makes it obvious that there is a sufficiently wider range of carpological features that could be used in species identification (Sukhorukov, 2007). Our observation showed that dispersal unit of S. kali subsp. tragus have dead woody perianths of the mother plants from maturation until the time when rain or wind disperse them. The developed perianth wings allow these dispersal units to be dispersed only near the mother plant or within a short distance from it (Wei & al. 2007). The fast open compressed spiral shape embryo, which is reported in this research, firstly is a creative natural mechanism for rapid germination and immediately establish on unreliable conditions. Study of halophytes life cycle, discovery of germination mechanisms and biological structures involved in stress conditions are very important and necessary for improvement of desertification programs, soil improvement, soil erosion and forage production.

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³⁶ Seed characteristics of Salsola kali subsp. tragus