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Adaptive Fruit Structural Mechanisms of Asiatic *Salsola* Species and Its Germplasm Conservation and Utilization

K.N. TODERICH*[1], E.V SHUYSKAYA[2], Faisal TAHA[1], Shoaib ISMAIL[1], L.G. GISMATULLINA[3] and E.V. LI[3]

Abstract: We summarize the predicted effects of flower and fruit adaptive traits on the genetic structure of some annual and perennial species of Asiatic *Salsola* populations in harsh desert environments. Results show that the Asiatic *Salsola* complex is an example of evolutionary convergence of ecological, structural, and physiological mechanisms, which are determined genetically. Phenotypic plasticity in the sexual expression of flower organs affects interspecies and intrapopulation genetic structure both for annual and perennial species. Fruit diversity in the form and color of wings, structure of columns, presence, shape and spatial arrangement of the papillous protuberances, numbers of collenchymatous layers, degree of sclerification and parenchymatization determine seed dispersal and plant survival. Availability of pigments, tracheid-like moisture holding cells, and abundance of crystals in the perianth tissues also promote the protection of the embryo from unfavorable desert environments. Wings or fruit tepals determine anemochorous seed dispersion into open pastures spaces. Micro-morphology of epicuticular secretion and presence of crystals in the pericarp may play a role in changing soil pH, and providing more favorable conditions for plant survival. Salt gland secretion products on the surface of fruit tepals and perianth might protect reproductive organs and provide a strategy for germplasm conservation. Small and isolated populations of the majority of the annual species with a predominantly wind-crossed mode of reproduction exhibit low levels of genetic variation and in return the ability of plants to adapt to frequent droughts and climate changing conditions. Appropriate conservation and sustainable utilization measures are suggested to protect natural habitats of *Salsola* species.

Key Words: Adaptive traits, Chenopodiaceae, Flower dimorphism**,** Genetic polymorphism, Germplasm conservation

1. Introduction

More than 140 species of the genus *Salsola* (Chenopodiaceae), which includes shrubs, semishrubs, perennial and annuals herbs, have been described in Central Asia (Botschantsev, 1969; Freitag, 1997). These species have a greater tolerance to water, heat, and salt stresses. They colonize marginal and desert lands and constitute up to 45% of all Chenopodiaceae species in Central Asian Flora. They have developed adaptive features that give them more competitive advantage over sensitive glycophyte species. Perennial species represent valuable germplasm resources for applications such as fixation of shifting sands and improvement of degraded rangelands and salt affected marginal lands, and serve as an excellent component for the creation of multiple-use protected areas. Stems, fruits, and leaves can be used as a year-round feed for camels, and as summer feed for sheep and goats. Annual *Salsola* species may be used as partial substitute for feed concentrates, especially in autumn and winter. *S. richteri*, has medicinal, industrial and commercial uses (Gintzburger *et al*., 2003). Currently, there is a gradual but serious decrease in the size and the number of natural populations of many *Salsola* species, which ultimately lowers their surviving rates under current conditions of climate change.

Successful establishment of wild *Salsola* species appears to be exclusively dependent on sexual reproduction. Plant reproductive strategies, based on structural features and genetic diversity in different ecological *Salsola* groups within Central Asia are not well documented in literature (Butnik, 1981, Wolfe, 1998, Toderich *et al*., 2009). Traditionally, descriptive fruit morphology studies were done mainly on the family or genus level. Several key findings, however, are unreliable since polymorphism within taxa often exceeds interspecific boundaries. The extent of vulnerability and the degree of adaptability of *Salsola* taxa are difficult to evaluate, and the roles of different species in providing ecosystem services is not known. It is impossible to define appropriate conservation measures and sustainable use of Asiatic *Salsola* species without knowledge of genetic information at all taxonomic levels (Lewontin, 1991).

Natural populations serve as repositories of genetic diversity, which can be useful for ecosystem rehabilitation and/or restoration, after a loss of species has occurred. Populations of

* Corresponding Author: ktoderich@yahoo.com
PFU-CGIAR at ICARDA sub-office in Tashkent, 6a, Osie str., P.O. Box 4564, Tashkent 100000, Uzbekistan
[1] International Center for Biosaline Agriculture (ICBA) [2] K.A. Timiryazev Institute of Plant Physiology RAS, Russia
[3] Department of Desert Ecology and Water Resources Research, Samarkand Division of the Academy of Sciences of Uzbekistan, Uzbekistan

wild Central Asian *Salsola* species may become extinct due to ongoing climate change and increasing anthropogenic pressure. Annual species, usually represented by small, geographically isolated populations are evidently sensitive to changing environments and are under threat of habitat destruction. Under current conditions of rapid climate change a catastrophic loss of genetic diversity, is likely to occur (Safriel, 1994).

This paper considers the adaptive features of flower and fruit morphology of annual and perennial Asiatic *Salsola* taxa, and interspecies genetic variation in order to identify adaptive potential, and considers conservation.

2. Materials and Methods

Flowers and fruits were collected from different populations of annual (*S.aperta, S.pestifer, S.paulesenii*) and perennial (*S.orientalis, S. gemmascens, S. arbuscula* and *S. richteri*) species during several field expeditions (2005-2010) across the Aral Sea Basin and Kyzylkum desert. Morphology of fruit and seeds was investigated according to Kaden and Smirnova (1971) and Dudik (1971). Samples for anatomical studies of fruit covers were fixed in alcohol: glycerine : water (1:1:1). Sections were stained with methylene blue. Two methods of sample preparation were used for Scanning Electron Microscopy (SEM) as described by Bozolla and Russell (1998). For isoenzyme determination with electrophoresis, seeds were randomly collected from 10-25 plants from each population. After a 12-hour germination of seeds on wet paper, proteins were extracted by homogenisation of single embryos in 80 μl of extraction buffer (EDTA, KCl, $MgCl_2$, TRITON, PVP, TRIS-HCl). Isoenzymes were separated on 10% starch gel using two buffer systems systems I and II (Muona and Szmidt, 1985). Eight enzymatic systems were studied: 6-phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), superoxide dismutase (SOD, 1.15.1.1), glutamate dehydrogenase (GDH, E.C. 1.4.1.2), glucose-6 -phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic-enzymes (Me, E.C. 1.1.1.40) and NADH diaphorase (DIA, E.C. 1.6.99). Staining of the enzymes as well as the genetic interpretation of the results followed standard techniques (Soltis and Soltis, 1990; Goncharenko *et al.*, 1989). Loci were numbered sequentially with the most anodally migrating enzyme designated "1". Alleles at a locus were designated accordingly to the mobility of allozymes where the maximum difference in migration of individual allozymes was set at 100%. Based on electrophoretic data, the percent of polymorphic loci (P_{99}p and P_{99}s) were determined as described by Nei and Roychoudhury (1978).

3. Results and Discussion

3.1. Flower morphology adaptive traits related to reproduction

Flowers of all investigated species are bisexual, single, sepaloid (5-lobed perianth), alternate or assembled in a spike inflorescence. Anthers are tetrathecae, elongated with different forms of appendage; stigma is thick and short for perennials; long papilliform and deeply bifurcate for annuals (**Fig. 1.1**). Distinct sexual dimorphism of flowers (co-existence of functionally androecious/protandrous and protogynous types), and temporal heterogeneity during flowering period supports a high plasticity of the modes and mechanisms of pollination, pollen dispersal, and timing of reproduction. Self-fertilization, including autogamy (within one flower), geitenogamy (within-plant pollen transfer by wind) and cleistogamy (in closed flowers) was described mainly for annual species. Perennial *Salsola* species have both anamophylous and enthomophylous cross-pollination mode of reproduction, which include different development times and differentiation of male and female flower organs, and varying duration of pollen grain viability and embryo sac maturation. A somewhat unusual feature was described for *S. orientalis, S. arbuscula* and *S. gemmascens* in which large, colorful (bright yellow) anthers that protrude from an inconspicuous perianth might serve as additional pollinator attractants. Additionally, flowers of *S. orientalis* and *S. arbuscula* have a pronounced hypogynous disk and a surprising amount of nectar that might be considered as an apparent mode of insect cross-pollination. Formation of viable seeds as a result of sexual and apomictic (development of embryo from somatic reproductive organs) mode of reproduction was found in *S. orientalis* and *S. richteri*.

3.2. Fruit morphology and germination ecology

Comparison of fruit morphology showed that in all studied species fruit is dry, monospermous, lyzicarpous, indehiscent, with well-developed wings. The embryo is large, peripheral, spirally coiled; usually two cotyledons present with apical cleft or deeply divided; without perisperm. A full development of embryo organs and tissue differentiation indicates germination readiness (**Fig. 1.2**). Embryo development and organ differentiation takes 4.5-5.5 months for perennials and 2.0-2.5 months for annual species. The weight of 1000 seeds varies from 2.4 to 6.98 g. Fruits measured on the horizontal axis ranges between 1.5-3.0 mm in diameter. Fruits are variable in size, shape and color (from light yellow to reddish - *S. richteri*; pale yellow to grayish *S. gemmascens, S.orientalis, S. paulsenii*). Comparative analysis of fruit morphology by means of SEM revealed that taxonomic diagnostic features are expressed in the upper leaflets (above the wings) and vertical

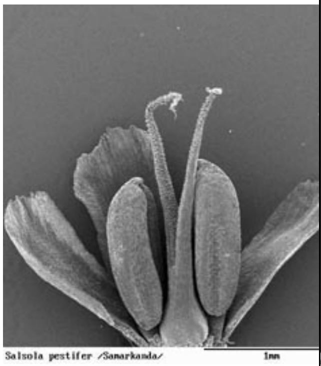

Fig. 1.1. Bisexual flower with bilobed long style, *S. pestifer,* adapted to anemophylous type of pollination.

Fig. 1.2. Micro-morphology of mature embryo, *S. pestifer* ready to germinate.

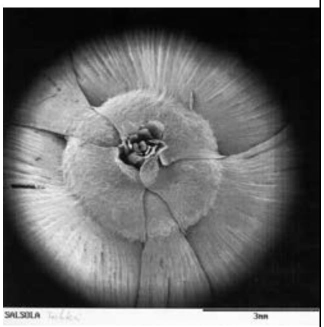

Fig. 1.3. Widely fan-shaped wings of *S. richteri* strongly pressed to the surface and almost accreted, in the basis fruit tepals.

Fig. 1.4. Mature fruit with five unequal and narrow horizontally spreading , wings of *S. pestifer*.

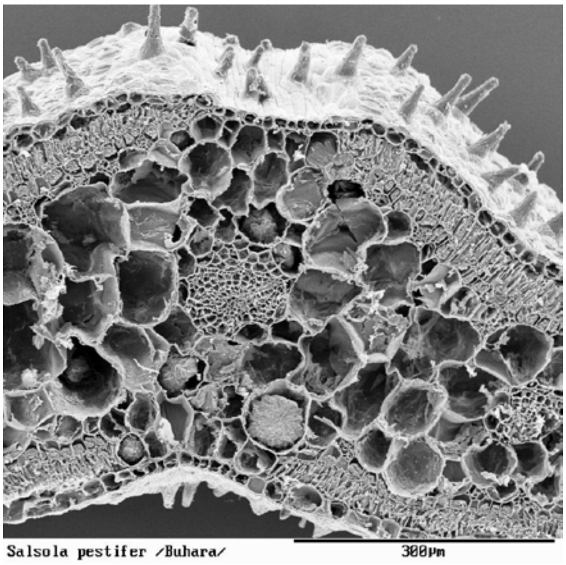

Fig. 1.5. Specialized adaptive structure of fruit tepals: Salt excreting trichomes and epicuticular wax at the epidermis; hydracitic cells, pigments and tracheid-like cells; abundance of crystals; large celled parenchyma.

Fig. 1. Flower, embryo and fruit morphology and anatomy of annual and perennial species; Source: Toderich , 2008

column (open funnel), and less so on the bottom leaflets (under the wings). Among the species studied, fruit variation is displayed in the form and color of wings, structure of columns, and the presence, shape and spatial arrangement of the papillous protuberances (**Figs 1.3 and 1.4**). Fruit tepals played an essential role in embryo protection due to high level of sclerification. Fruit tepals ensure anemohorous seed dispersion into the extensive open pastures, providing self-propagation of *Salsola* populations, which in turn ensure ecosystem functioning and stability. Significant differences between perennial and annual species were found. Perennial species are characterized by presence of two rows of chlorenchyma, which consist of palisade bundle sheath cells and small vascular tissue. Also present is high parenchymatization of perianth segments during fruit maturation, radially elongated cells of thin-walled parenchyma, absence of sclerenchyma in the upper tepals, cholenchymatous thickening of lower epidermis and 1-2 row parenchyma that closely adhere to the epidermis. Annual species are characterized by a thick-walled parenchyma and large thin-walled epidermis cells; presence of a pronounced crystal-salt bearing cell and a pigmented layer. Micromorphology of the epicutical, salt gland/trichome secretion and presence of crystals in pericarp may play a role in changing soil pH, thereby providing a more favorable condition for plant survival (**Fig. 1.5**).

Our experiments showed that perennial *Salsola* species has a low rate (48-72%) of seed germination both under laboratory and field conditions despite a high proportion of viable seeds (with fully a developed embryo), while annual species are characterized by a high rate (90-94%) of seed germination and seedling survival. A high degree of perianth sclerification is one of the main reasons for lower seed germination rate in perennials (Toderich, 2008). Destruction of intact of seed coat or removal of fruit covers resulted in increased germination rate. Short-term dormancy occurs in woody *Salsola* species, lasting from 8-10 months, up to 1.5 years depending on storage conditions. Dry storage conditions alternating with low temperatures usually stimulate seed germination (Gintzburger *et al*., 2003).

3.3. Genetic variation and conservation measures

The significant genetic polymorphism detected in this study indicates that perennial *Salsola* species possess an abundant source of variation required for evolution in a changing environment. Genetic variation of perennial species at population level is lower than at species level, but significantly higher than that of annual species (**Table l**). This evidence indicates a high genetic differentiation of populations and, consequently, a genetic isolation of populations.

Habitat fragmentation in annual *Salsola* species, resulting in smaller and more isolated populations, can pose a significant threat to genetic diversity. For wind-pollinated species, geographic isolation can increase in-breeding. Genetic erosion can affect the ability of plants to adapt to changing

Table 1. Genetic intra-species ($P_{99}s$) and population ($P_{99}p$) variation.

	Life form	Number of population	Sample size	$P_{99}p$	$P_{99}s$
S. orientalis	p*	5	143	0.29	0.64
S.gemmascens	p	2	59	0.18	0.29
S. arbuscula	p	9	291	0.36	0.73
S. richteri	p	3	85	0.29	0.53
S. aperta	a	1	19	0.00	----
S. paulsenii	a	9	265	0.05	0.13
S pestifer	a	2	68	0.22	0.31

*p – perennial, a - annual

conditions. This is likely to increase the potential impact of threats, such as climate change, as plants are unable to adapt quickly enough to change. The greater genetic heterozygosity of cross-pollinated perennials, at species and population levels, may give them a selective advantage in unstable environments (Hamrick *et al*., 1992). Appropriate measures for conservation of genetic diversity should be developed, especially for locally adapted populations for majority of annual *Salsola* species. Phenotypic plasticity in sexual expression, diversity of mode and mechanism of pollination, and of pollen and seed dispersal detected by these studies could also affect genetic structure by altering the distribution of individuals in space and time. Therefore, conservation of fragmented populations is crucial to ensuring their survival in a changing environment. Disappearance of this unique germplasm will lead to the loss of a significant proportion of the total genetic diversity of genus *Salsola* within Central Asian Flora.

Surveys and seed collections show that *Salsola* has potential uses in soil remediation affected by severe salinity and also in improvement of arid fodder pasture production. *Ex-situ* and *in-situ* conservation of economically valuable salt-and drought tolerant *Salsola* species has been initiated by International Center for Biosaline Agriculture (ICBA) at the Gulistan State University, Uzbekistan. It is imperative to accurately assess the threat status of individual species of these valuable arid and semiarid fodder plant resources. Results will help in setting priorities for conservation, and providing the guidance needed to protect the diversity of desert hotspots from land degradation and intensive use.

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