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Research article

Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity

Xuejun Yang [a,b], Ming Dong [a], Zhenying Huang [a,*]

[a] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Artemisia sphaerocephala (Asteraceae) is one of the pioneer species in moving and semi-stable sand dunes in the deserts of northwest China. The outer surface of *A. sphaerocephala* achenes contains a pectinaceous mucilage layer that can imbibe a large amount of water when wetted. We hypothesized that the mucilage can aid achene germination in heterogeneous environments. Germination of both intact achenes and those from which the mucilage had been removed (demucilaged) declined with increasing osmotic potential and NaCl concentration. However, the germination percentage of intact achenes was significantly higher than that of demucilaged achenes. The early seedling growth of intact achenes did not differ significantly from that of demucilaged achenes in either osmotic potential or NaCl solutions. Achene mucilage presumably plays an ecologically important role in the life cycle of *A. sphaerocephala* by aiding germination in osmotically- and saline-stressful habitats of the cold desert environment.

1. Introduction

The outer seed coat of angiosperms participates in many important processes during seed development and germination, e.g. transport of nutrients, protection of the embryo and control of seed dormancy [2,20]. The morphology of the external surface of the seed coat in angiosperm is extremely diverse, reflecting multiple adaptations to seed dispersal and germination in different environments [5]. The seeds of many species produce pectinaceous mucilage upon imbibition of water (myxospermy), and the mucilage is thought to play an important role in maintaining seed viability and ensuring seed germination by: (1) permitting seeds in different stages of development within fruit to attain a sufficient level of maturation that keeps them in a state of readiness to germinate until the beginning of rainy season [7,24]; (2) protecting the seed against drying during germination and early seedling growth by retaining moisture [7,14,23] and increasing the area of contact of seed with soil, thus increasing the moisture supply to the seed and minimizing water loss [8,13,31]; (3) preventing further dispersal of the seed by rain and wind and of collection by ants or other seed predators by forming strong adherence to the soil surface once the mucilage is dry [10,16,17]; and (4) initiating or enhancing germination [4,11,13,31]. In addition, mucilage formed by wetting the achenes with dew at night or a small amount of rain can enable the embryo to repair DNA and thus help maintain its viability under harsh desert conditions [15].

Artemisia sphaerocephala Kraschen. (Asteraceae) is one of the most important pioneer plants of the moving and semi-stable sand dunes in the deserts and steppes of northwest China [3]. Other than its sand stabilization value, *A. sphaerocephala* can also be used for pasture and for fuel. Due to its wide distribution, the habitat of *A. sphaerocephala* can be very heterogeneous. Na^+ is the predominant soluble cation in the soils of arid and semi-arid areas. Mean (±s.e.) concentrations of available Na^+ in the soils where *A. sphaerocephala* grows can be as high as 265.33 ± 6.15 μmol (100 g d w soil)$^{-1}$, which is equal to 432 mmol L^{-1} NaCl solution when water content of the soil is 0.61% [27]. Thus, salt stress, together with low water availability, is the environmental stress factor that this species encounters during achene germination. The outer layer of the thick pericarp of *A. sphaerocephala* achenes becomes mucilaginous when wetted, and mass of the achenes can increase to 589 times its original mass when they absorb water [18]. The pectinaceous mucilage of *A. sphaerocephala* is a stable polysaccharide, consisting of D-glucose, D-mannitol, D-galactose, L-arabinose and xylose, which can strongly adhere to sand [28]. Studies on germination strategies of *A. sphaerocephala* achenes have identified the suitable conditions (e.g. temperature, light, soil moisture and sand burial depth) for germination of the achenes of this species [18]. Nevertheless, little

* Corresponding author. Fax: +86 10 82595899.
E-mail address: zhenying@ibcas.ac.cn (Z. Huang).

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is known about the role of mucilage in achene germination, especially in a stressful environment.

Development of mucilage seems to be one of the evolutionary adaptations of plants to desert environments, and it provides certain ecological benefits in the extreme desert conditions [10,15]. We hypothesized that the mucilage on the outer surface of *A. sphaerocephala* achenes can enhance seed germination and seedling growth in conditions of drought and salinity. Thus, we asked the following questions: (1) can mucilage aid the germination of *A. sphaerocephala* achenes? and (2) can mucilage promote early seedling growth under drought stress and salinity? Answers to these questions would advance our understanding of the ecological role of seed mucilage in germination in the natural environment.

2. Materials and methods

2.1. Study species

A. sphaerocephala is a shrub that is mainly distributed in the deserts along an altitudinal gradient of 1000–2850 m a.s.l. in northwest and north-central China, including southwest Inner Mongolia, north Shanxi and Shaanxi, Ningxia, northwest Gansu, north Qinghai and east Xingjiang [3]. Throughout the range of the species, average annual precipitation is very low, and in the western and northern parts it is less than 100 mm [15]. The species has strong resistance to wind erosion, drought, cold and saline-alkali soil conditions [3]. In its natural habitats, *A. sphaerocephala* flowers from July to August, and the achenes mature from mid-September to early November. During maturation, a polysaccharide is secreted through the epidermal cells of the fruit wall and accumulates in layers of mucilage. Involucral bracts of inflorescences protect achenes from wetting by rain until after dispersal. The achenes are dispersed by wind, after which they adhere to sand particles by the mucilaginous layer that forms on the achene surface upon imbibition [3,15].

2.2. Achenes

Freshly mature achenes of *A. sphaerocephala* were collected from dry unopened infructescences in December 2007 from natural populations near the Ordos Sandland Ecological Research Station of the Chinese Academy of Sciences (39°29′N, 110°11′E; 1296 m a.s.l.) on the Ordos Plateau in Inner Mongolia, north-central China. This area has a typical continental semi-arid climate with mean annual precipitation of 250–490 mm, primarily from June to August. Mean annual temperature is about 6.0–9.0 °C, and most of the region is covered by sand layers of varying thicknesses [32]. In the laboratory, infructescences were manually shaken to detach the achenes, which were stored dry in a closed cotton bag at 5 °C until used in experiments.

Achene mass was gravimetrically determined by weighing four replicates of 1000 achenes each and achene size by measuring the length and width of 10 randomly chosen achenes using a light microscope (Eclipse E200 POL, Nikon Instruments Inc., Japan) equipped with a micrometer.

To determine mucilage mass, mucilage on achene surfaces was removed carefully by a scalpel from four replicates of 10 achenes each, before and after which achenes were weighed to the nearest 0.0001 g using an analytical balance (BS221S, Sartorius Group, Germany). Mucilage mass was calculated by subtracting the mass of achenes without mucilage from that of intact achenes.

2.3. Mucilage removal

To remove the mucilage, intact achenes were submerged in water for 5–10 min and then rubbed gently and rapidly on filter paper several times, until no mucilage was released when they were imbibed with water [15]. Hereafter, the achenes with mucilage removed are termed “demucilaged achenes”.

2.4. Effect of osmotic potential on germination and seedling growth of demucilaged and intact achenes

To test the effect of drought stress on germination of intact and demucilaged achenes, polyethylene glycol (PEG) was used to generate osmotic stress. PEG 6000 (analytical grade) solutions were used in concentrations of 0 (distilled water control), 2.5, 5, 10 and 20% (w/v) solutions. Osmotic potentials, calculated using the formula from Money for PEG 6000 [19], of 0.0, −0.003, −0.027, −0.155 and −0.87 MPa were obtained for 0, 2.5, 5, 10 and 20% PEG solutions, respectively.

For each water potential and each of the two achene types (i.e. with and without mucilage), there were four Petri dishes (replicates) with 25 achenes each. Two and one-half milliliters of solution were added to each Petri dish (5-cm-diameter), after which they were sealed with Parafilm to minimize evaporation of water from the solutions. The achenes were incubated at optimal conditions [18], i.e. constant temperature regime of 25 °C in continuous fluorescent light (about 100 μmol m^{-2} s^{-1}). Germination (radicle emergence) was monitored every 24 h. On the 15th d of incubation when germination percentage in most dishes reached a peak, three newly germinated seedlings in each Petri dish were selected to monitor seedling growth (25 °C, filter paper as substrate, continuous fluorescent light and the same osmotic and salinity stress as for germination). After 30 d of incubation, germination tests were terminated. Also, lengths of seedlings from both intact and demucilaged achenes that had been growing for 15 d were measured to the nearest millimeter with a ruler.

2.5. Effect of salinity on germination and seedling growth of demucilaged and intact achenes

The effect of NaCl on germination was tested because it is the main soil salt in *A. sphaerocephala* habitats [27]. The effects of 0 (distilled water control), 5, 10, 50, 100 and 200 mmol L^{-1} NaCl (analytical grade) on intact and demucilaged achenes also were tested at a constant temperature regime of 25 °C in constant fluorescent light (about 100 μmol m^{-2} s^{-1}). Four replicates of 25 achenes in 5-cm-diameter plastic Petri dishes with 2.5 mL solutions were used for each treatment; the Petri dishes were sealed with Parafilm. Germination was monitored every 24 h for 30 d and germination percentages calculated. Seedling lengths were determined as previously described.

Ungerminated achenes from the 30-d NaCl incubation experiment were rinsed three times with distilled water and then incubated for another 10 d in Petri dishes that contained 2.5 mL distilled water. Recovery percentage was calculated by the following formula: $[(a - b)/(c - b)] \times 100$, where a is the total number of achenes that germinated in salt solution plus the number that had germinated in distilled water after 10 d, b is the number of achenes that germinated in a salt solution and c is the total number of achenes tested [9]. Final germination was recorded as $(a/c) \times 100$. Viability was expressed as $[(a + d)/c] \times 100$, where d is the number of embryos that stained pink in the TTC solution after the germination test. Viability of achenes that did not germinate in the experiments was tested by the TTC method [1], and achenes with embryos stained pink were evaluated as viable.