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SPORE MORPHOLOGY OF CHEILANTHES PERSICA (BORY) METT. EX KUHN AND ITS DEVELOPMENTAL STAGES

S. M. Masoumi, H. R. Ghasempour & J. Sobhi

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Cheilanthes persica (Bory) Mett. ex Kuhn. (Pteridaceae) is one of the resurrection ferns that fully dries during drought stress and after re-watering will be revived. Fresh material obtained from Kermanshah province, west of Iran (Tagh-e-bostan) and cultured in growth chamber and its developmental stages and life cycle were examined. The spore morphology was studied. The spore had tetrahedral tetrad with discontinuous microreticulate ornamentation.

Seyed Mohammad Masoumi (correspondence <sm.masoumi@razi.ac.ir>*), Hamid Reza Ghasempour, Razi University of Kermanshah & Javad Sobhi, PhD. graduate Razi University of Kermanshah, Iran.*

Key words: Distribution; *Halongia*; Life cycle; Monocot; Pteridaceae; Palynology; Kermanshah

ریخت‌شناسی هاگ سرخس .Cheilanthes persica (Bory) Mett. ex Kuhn و مراحل نموی آن

سید محمد معصومی: استادیار گروه زیست شناسی دانشگاه رازی، کرمانشاه، ایران

حمید رضا قاسمپور: دانشیار گروه زیست‌شناسی دانشگاه رازی، کرمانشاه، ایران

جواد صبحی: دانش آموخته گروه زیست شناسی دانشگاه رازی، کرمانشاه، ایران

Cheilanthes persica یکی از سرخس‌های رستاخیزی است که در طول تنش خشکی کاملاً خشک و بعد از آبیاری احیا می‌شود. این نمونه از کرمانشاه (طاق بستان، غرب ایران) جمع‌آوری و در اتاق رشد کشت و چرخه زندگی آن مورد بررسی قرار گرفت. هاگ به حالت تتراد چهارگوشه‌ای با تزیینات مشبک ریز ناپیوسته است.

INTRODUCTION

Cheilanthes is a large and diverse genus with 150 species having worldwide distribution (Sen & Mukhopadhay, 2011). *Cheilanthes persica* (Bory) Mett. ex Kuhn. synonym *Notholaena persica* Bory. A desiccation tolerant plant, belong to Pteridaceae family, but it already belongs to Adiantaceae family (Parsa, 1978). The geographical distribution of this plant is in Afghanistan (Fraser-Jenkins, 1992), Asia: in the southern regions of the former Soviet Union (Grushvitsky & Zhilina, 1978), Iran (Parsa 1978), India (Kashmir Himachal Pradesh), Pakistan (Mir, 2015), Europe such as: Greece, Italy, Turkey (Kaynak & al. 2008). *C. persica* found in Khuzestan, Shahpur, Kazeroun, dalaki, Lorestan, Kermanshah: Taqbstan, Shahbazi mountains of Iran (Ghahraman, 1978).

Ferns that can grow in difficult conditions such as tolerance to drought for several years and to continue their life back. Tissue culture requires to understanding the cause of this tolerance. Resurrection plants have different taxes (Gaff & Latz, 1978). Thirty species of Pteridophytes in the Big Bend National Texas Park were surveyed (Stanley & al. 1997). These plants often have a degree of drought tolerance. One of these species is *Notholaena sinuata* (Lag. ex Sw.) Kaulf., that has drought stress response in different situations. Among vascular plant there are a small proportion of ferns and flowering plant which are

desiccation tolerant, which can survive water deficits down to completely air dry, when they rehydrate, resume normal tissue function (Gaff & Okong'o-Ogola 1971). Most species of ferns grow where the rocks around the edge of the gap or less are in the shade (Gaff 1977). The morphological and anatomical characteristics in plants due to widespread drought, which in effect result in their physiological processes occur, the loss of water and hold it for critical periods (Levitt 1972). The general ways for drought tolerant plant is wastewater storage and the ability to keep cell membranes and proteins (Gaff, 1989). In higher plants, seeds and pollen are resistant to drought. That is rare drought tolerance in the vegetative tissues. Resurrection plants are small numbers of plants that are capable of losing water up to 95% in the tissues of growing them for a prolonged period of tolerance and physiological activity themselves during rehydration (Alpert 2006). Plants are always dependent on the water in the absence of regular rainfall or regular irrigation losses are incurred. Some factors in the *Cheilanthes persica* (Bory) Mett. ex Kuhn made this fern resistant against dehydration stress (Ghasempour & al. 2003; Sobhi & al. 2015).

Two *Adiantopsis* species from Pteridaceae were observed with Light Microscopy (LM), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopes (TEM). The spores of both species are trilete with an echinate surface (Raquel & al. 2006). Stomatal morphotypes, epidermal characteristics and spore morphology of 27 species of *Cheilanthes* examined by LM and SEM (Sen K. & Mukhopadhay R. 2011). The spore morphology of 9 species of 5 genera in Pteridaceae family were examined under SEM (Salimpour & al. 2011). Also spore morphology of 16 species of *Adiantum* L. were studied by SEM (Kovalska 2013).

Consequently, the aim of the current study was to elucidate the palynomorphological characteristics of spore and life stages by Scanning Electron Microscopy, Olympus Loop and UV microscopy in order to find evolutionary similarities. Also, by tissue culturing in growth chamber and cultivating in pots different stages of life cycle of the *C. persica* (Bory) Mett. ex Kuhn were studied.

MATERIALS AND METHODS

Plant material

Sporophytes of the *C. persica* were obtained from intact plants in Taq-e Bostan, Shahbazi Mountains of Kermanshah at 1750 meters above sea level with geographical location, Longitude 34°23'28.82"N , Latitude 47° 8'14.91"E (fig.1). The plant specimen was identified with using flora of Iran (Parsa 1978) using Razi University Herbarium (RUH), Kermanshah, Iran. Then, was tissue cultured in growth chamber (Conviron CG 72) and allowed to grow under the same conditions as plastic pots in greenhouse. Pots contained a wetted mixture of clay, peat, and perlite (2:3:1, v/v/v), providing 40–50% relative humidity and 25 ˚C in day and 22˚C in night, and grown under cool-white light (16h photoperiod, 26µl $m^{-2}s^{-1}$ light intensity) at the Razi University, Kermanshah, Iran.

Spore germination and gametophyte development

Fresh spores of ferns were domesticated in the laboratory; the best way was to shake the frond of this fern on the paper to collect the brown dust which contains enough spores. By using Olympus Loop and UV microscopy (SZ X12) the collected spores were examined. Mature sporophylls (fronds) were collected, warped in paper bag, and dried in room temperature for one week to release spore. Then, these spores were transferred to centrifuge tubes and stored at 4 ˚C in the dark for further use. Spores were wetted (6 mg) with 0.1 g/l Tween solution for about 30 min, collected on filter paper, dipped in 70% (v/v) ethanol for 30s, surface-sterilized in 0.1 g/l aqueous mercuric chloride solution for 2–3 min, and rinsed six times with sterile distilled water (3 min per rinse). Finally, spores were collected with filter paper and then suspended in 40 ml sterile distilled water (as in Wu & al., 2009). About 1 ml of spore suspension was distributed with a sterilized pipette onto each Petri plate (9 cm diameter), which contained 20 ml culture medium, and then sealed with saran wrap strips. The medium consisted of different strengths of 0.2 MS macronutrients. The medium was solidified with 6.5 g/l Agar (Amreso Biosharp, USA), adjusted to pH 5.8 before being autoclaved at 121˚C and 1.1 kg $cm^{-2}$for 20 min. The Petri dishes were wrapped in foil, sterilized, and the spores were sterilized by Sampler sterile medium in to Petri dishes under laminar flow device. Then, transferred to germinator under controlled conditions (16h during the day and night), day temperature of 27 degrees, night temperature at 23 degrees, 372 watts per square meter of light, with relative humidity 35 %. Further, three replications were used and grown either under cool-white light (16 h photoperiod, 25µl $m2s^{-1}$ light intensity) or dark conditions. All cultures were incubated in a controlled environment room at 25 ±2˚C.

SEM studies

Fresh material obtained from Kermanshah area. For scanning electron microscope (SEM), not

acetolysed spores were used on sample stubs and coated with gold (Sputter coater BAL-TEC model SCDOOS manufacturing companies of Switzerland). The micrographs were prepared using scanning electron microscope (SEM) Hitachi model S-405, using magnification of 1000-10000 to study details of exine surface ornamentation, wall thickness and the pore structure (fig. 4).

RESULTS & DISCUSSION

The mature plant (sporophyte) consists of 3 major parts: the rhizome, the fronds (figs. 2-A, 2-B, 3-A, 3-B) and the sporangia (Clustered in sori of lower surface of fronds) (figs. 2-C, 3-B). The mature fern plant was the sporophyte that produced spores which were released from sporangia (figs. 2-C, 2-D). The observed lines may be aggregations of the sporangia (fig. 2-B, 3-C). A young sporangium contains spore mother cells. The next step was the opening of the sporangium with the help of mechanical layer (figs. 2-E, 3-D) and the spores were scattered and spread out (fig. 4). After meiosis, the haploid spores fall from the sporophyte (fig. 3-E), giving the right conditions, into the gametophyte stage, the prothallus. Figs. 2-F & 3-F show gametophyte (prothallus) being developed and multicellular rhizoids appeared. Also, the rhizoids in lower level of sexual organs produced high amount of prothallus (figs. 2-H, 3-H). Further, the heart-shaped prothallus developed independently after several weeks (figs. 2-G, 2-H, 2-I, 3-G, 3-H). Then, sex organs grow that produces ova (archegonia) and flagellated sperm (antheridia). In fig 2-J and 3-I sex organs, archegonium (AR), (female) and antheridium, (An), (male) are shown. The sperm were able to swim toward the ova to fertilize the egg and form a diploid zygote. Then after division by mitosis a multicellular sporophyte was formed (figs. 2-K, 2-L, 2-M, 3-J). Finally, sporophyte developed and produced a mature plant (figs.1-B, 2-A, 3-A). Spores were trilete, circular shapes (figs. 4-B, 4-C), the tetrads are tetrahedral (fig. 4-A) and the ornamentation on lumen is discontinuous, microreticulate (fig 4-D).

Exine ornamentation of this studied spore is similar to C. *pseudofarinosa* (Ching & Wu 2009) and Indian species of *Cheilanthes* (Sen & Mukhopadhay, 2011). Exine ornamentation in *C. persca* is discontinuous microreticulate, but Salimpour & al. (2011) reported wizened surface spore for this species. The trilete mark in this species is similar to *Adiantum capillus-veneris* L. (Kovalska, 2013). Our tissue culture life cycle procedures passed through different stages in which some stages were similar with previous findings i.e. Kerr (2016).

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Fig. 1. *Cheilanthes persica*: A, habitat observed in the Tagh -e Bostan; B, fern in the spring season.

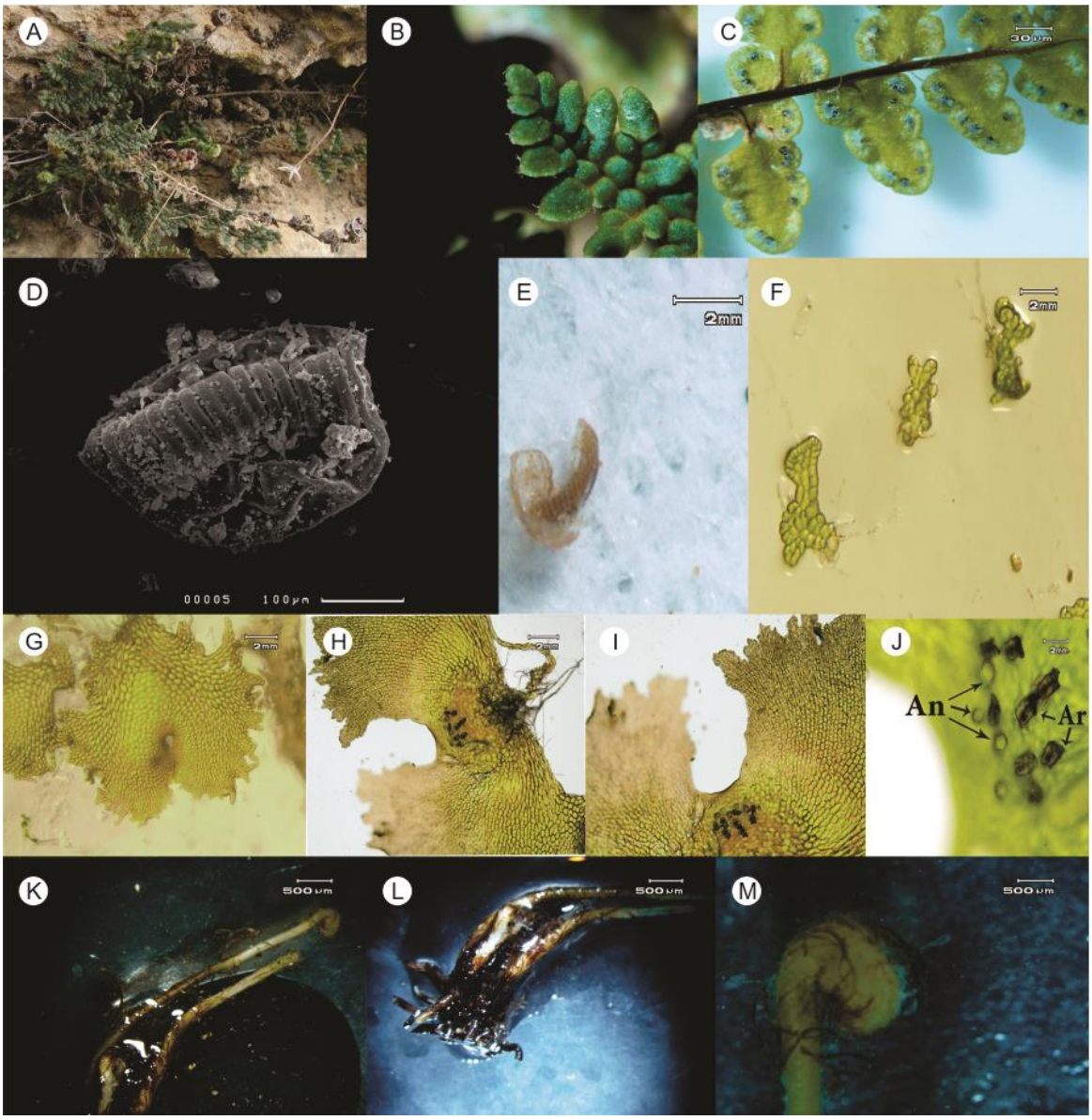

Fig. 2. Morphological feature of *Cheilanthes persica*: A, The beginning of the growth in the remains of the previous year; B, the upper surface of leaflet in frond; C, the position of the sorus is upon the abaxial or lower surface on the edge leaflet in frond; D, scanning electron micrographs of the mechanical construction in wall of the sporangium; E, the opening of the sporangium with the help of mechanical layer; F, gametophyte being developed with multicellular rhizoids; G, gametophyte development and heart-shaped prothallus; H, rhizoids in lower level and the sexual organs produced in high level of prothallus; I, a part of gametophyte development with sex organs in high level prothallus; J, the sex organs: Ar, -archegonium (female) and An, -antheridium (male); K, young sporophyte; L, lower part of sporophyte with underground parts; M, zoom more of sporophyte.

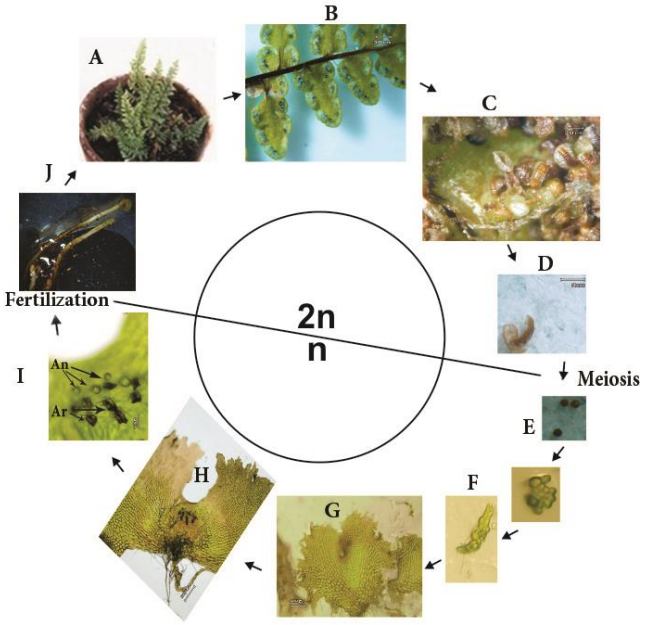

Fig. 3. Lifecycle of *Cheilanthes persica*. A, The mature plant; B, a part of frond; C, the sporangium; D, the opening of the sporangium; E, spores spread; F, the gametophyte (prothallus) develops; G, a heart-shaped prothallus; H, a mature prothallus; I, zoom more sex organ in upper part of prothallus. Ar: archegonium; An: antheridium; J: Young sporophyte.

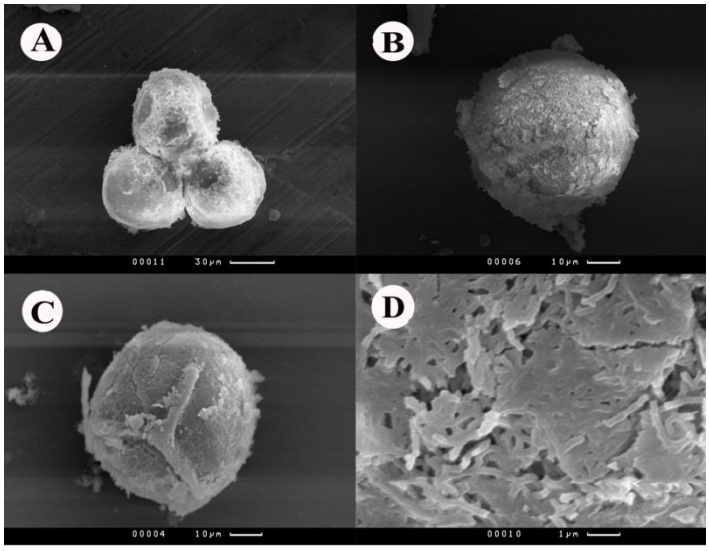

Fig. 4. Scanning electron micrographs of the investigated species spore of *Cheilanthes persica:* A, The general viewof 3 spores sticking together with low magnification as tetrahedral tetrad without one spore (300X); B, the general view of spore from the distal surface with more magnification (1000X); C, the trilete mark on the proximal face of a spore with more magnification (1000X); D, discontinuous microreticulate ornamentation on a piece of lumen with high magnification (10000 X).

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