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ANATOMICAL STUDY OF SEED AND FRUIT MORPHOLOGY OF AN INVASIVE WEED BUFFALOBUR (*SOLANUM ROSTRATUM* DUNAL)

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Abstract

Solanum rostratum Dunal, commonly known as buffalobur, is an invasive species in China. It caused significant damage to natural ecosystem, agricultural production and human health in many countries. Seed and fruit morphology of *S. rostratum* were examined using Scanning Electron Microscopy (SEM) and stereomicroscope. The seed surface of *S. rostratum* was characterized by palisading hair-like structures which surrounded each depressed cellular reticula. Two types of ultrastructure were firstly observed on the surface of reticula, differing in arrangements of fingerlike projections and the number of tilted holes. A visible cavity was found between endosperm and micropyle region. Knowledge obtained in this study would provide useful information in identification of *Solanum* species in plant quarantine and understanding its wide adaption to the environments.

Key words: Solanaceae, *Solanum rostratum*, Seed coat, Reticula, Ultrastructure.

Introduction

Solanum rostratum Dunal is a member of *Solanaceae* family and is commonly known as buffalobur, Kansas thistle and Texas thistle (Anon., 2018). Because of its rapid growth, high adaptability and large seed production, it has been disseminated throughout the world including China (Zhao & Lou, 2017; Wei *et al*., 2007; Qiu *et al*., 2013; Thomas & Hassan, 2002). Invasive species could profoundly alter ecosystem structure, cause significant economic losses and lower the biodiversity and function of invaded ecosystems (Wan & Yang, 2016). Zhong *et al*., (2009) predicted that *S. rostratum* had the potential to invade into the vast area of the Central, North, and East China. Based on weediness and potential harm, *S. rostratum* has been considered as a highly risk quarantine pest in China (Wei *et al*., 2007), and further introduction of this invasive species should be minimized. Seed is the primary source of colonization and dissemination, *S. rostratum* overwinters mainly by seeds. Strategies to reduce seed production and decrease the size of seedbank in the soil can effectively control the weeds (Wei *et al*., 2005; Oreja *et al*., 2017). It could provide useful information for further studies of the reproductive characteristics and assist in understanding the relationship between seed structure and function, thus would be helpful in developing more effective weed control strategies. Seed coat structures have been used to provide important information for identification of weed specie (Khaniki, 2003; Stern *et al*., 2010; Kanwal *et al*., 2016). The hair-like structures covering the seed surface of some *Solanum* species had been reported (Lilian *et al*., 2007), but the ultrastructure, the fingerlike projections and tilted holes in epidermal pore of seed coat of *S. rostratum* were not known. The objectives of this study were to present morphological and anatomical characterization of fruit and seed of *S. rostratum*, especially the anatomical microstructure of seed coat and inner structures related to germination and to provide useful guidance for identification and classification of this species.

Materials and Methods

Seed materials: Mature seeds and fruits of *S. rostratum* plants were obtained growing in Beijing-China, where it grows in open uncultivated land contiguous to railways. The seed from berries of *S. rostratum* were separated, crushed, cleaned and stored for experimental purpose.

Morphological characterization of fruit: The shape and colour of *S. rostratum* were examined visually. To observe interior structure of the fruit, epicarp and persistent calyx were dissected. Images were taken with a digital camera (Sony Super SteadyShot DSC-H50; Sony, Japan), which was connected to a stereomicroscope (Olympus SZ61; Olympus, Tokyo, Japan).

Morphological characterization of the seed surface: Seed sterilization was done by soaking it for 10 minutes in 5% (v/v) sodium hypochlorite solution, dried under room temperature and then were mounted on aluminium stubs. Finally, seeds were observed with a scanning electron microscope (Hitachi S-3400N, Hitachi Instrument, Tokyo, Japan).

Anatomical characterization of the seed in germination process: Seeds were evenly placed in a 9-cm petri dish, moistened with water, sealed and incubated in a thermostat-controlled darkened chamber (70% relative humidity and 30°C). For observation of germination process, the seeds were sampled at different developmental stages and cut longitudinally, then observed under a stereomicroscope which equipped with a reflex digital camera.

Results and Discussion

Fruit morphology: The ripe fruits of *S. rostratum* ranged 1-1.2 cm in diameter and looks black or black brown at maturity (Fig. 1A, E). Seeds were embedded in a solid fleshy axile placenta (Fig. 1F) and the pericarp was enclosed by a sharp spiny persistent calyx (Fig. 1C, D), containing 33 to 81 seeds per fruit, and an average of 1635-43809 seeds per plant (Wei *et al*., 2010). The thickness of pericarp was decreased gradually, which changed from thick succulent to black thin membranous and were attached to the prickly persistent calyx (Fig. 1A, E). The fruit was almost completely enclosed by the prickly persistent calyx at maturity (Fig. 1A). An aerial space wasformed between the seeds and the pericarp when the fruit was matured (Fig. 1A). Moreover, the persistent calyx could split into five regular cracks or continue enclose the berry (Fig. 1B), with the help of the persistent calyx. Moreover, the pericarp of *S. rostratum* can easily rupture, thus scattering the seeds at maturity.

Seed morphology: Seed shape of *S. rostratum* was spheric to reniform, with lateral face convex, and the testa of radicle was thinner and prominent (Fig. 3A, C). The black or dark brown seeds were 2.47±0.18 mm in length, 2.02±0.13 mm in width, and 1.00±0.09 mm in thickness (Wei *et al*., 2010). The testa, which consists of two or three layers of cells (Qu & Zhang, 2009), was osseous with metallic luster, rough, thick, uneven, and covered by different sizes of depressed cellular reticula (Fig. 3B, D, F, H). The surface of side had varying thickness (Fig. 3G). The semi-closed micropyle region of *S. rostratum* was uniform in shape, which is located in seed base coated with thin horny film (Fig. 3E).

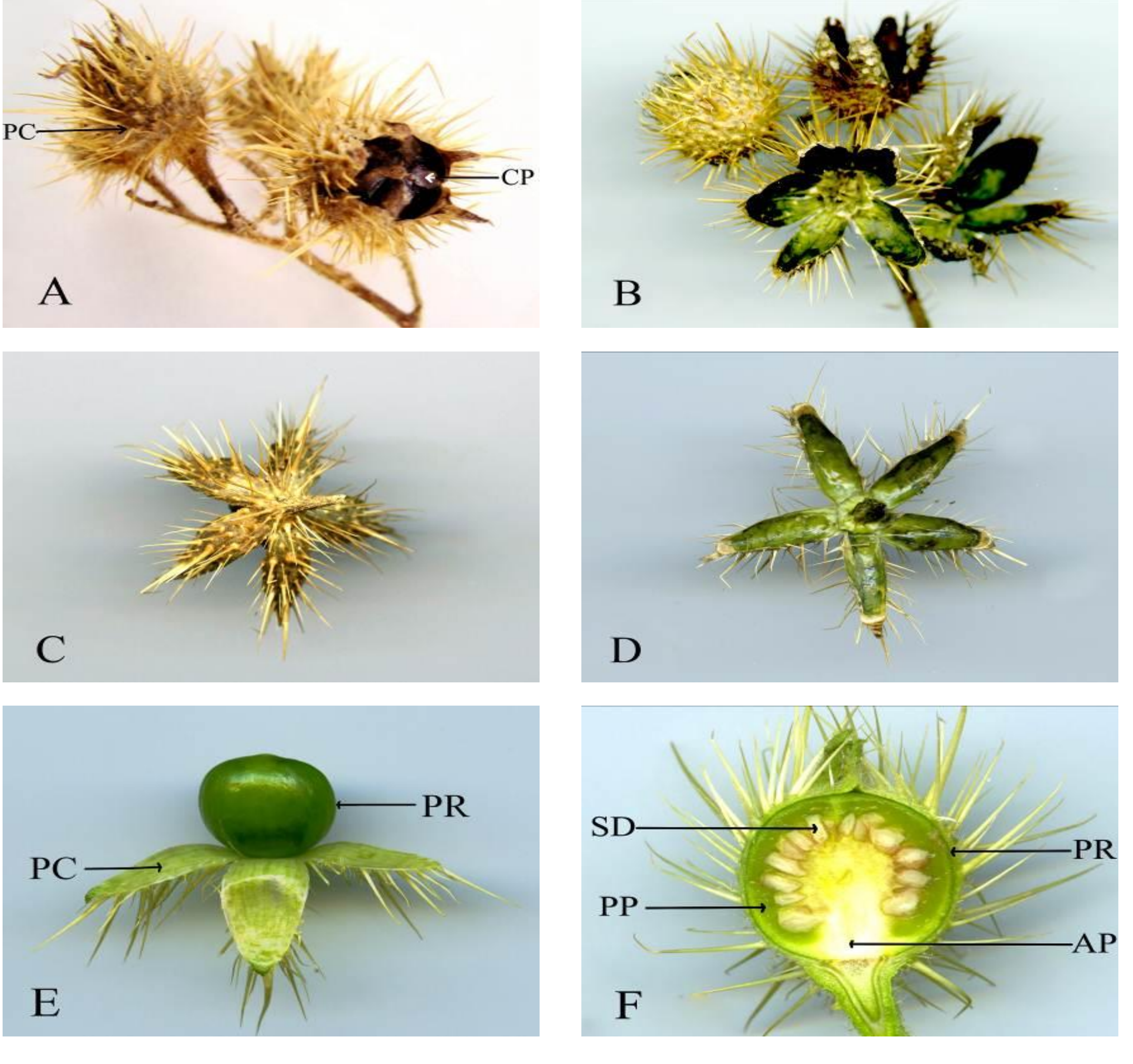

Fig. 1. Morphological characterization of the fruit and persistent calyx of *Solanum rostratum*.
(A) Fruits with persistent calyx (PC) and cracked pericarp (CP). (B) Fruits after seed dispersing, with open persistent calyx (PC) (C) Obverse side of the persistent calyx. (D) Reverse side of the persistent calyx. (E) Mature fruit with persistent calyx (PC), showing the green pericarp (PR). (F) Inner structure of fruit including axile placentation (AP), seed (SD), pulp (PP).

The embryo of *S. rostratum* was dicotyledonous surrounded by abundant endosperm cells (Fig. 2C). The shape of embryo was spiraled into number “6”, and the radicle tip was covered by a cap-like structure (Fig. 2A, B). This endosperm cap of Solanaceae-type seeds was the area where radicle protrusion would occur and there was a visible gap between testa and endosperm (Fig. 2C, D). The shape of transverse section of micropyle region was also uniform (Fig. 2C, D). The space between testa and endosperm can be a channel connected to semi-closed micropyle region, which may accelerate transportation of water and oxygen required for seed germination, thus speeding up embryo expansion and assisting radicle protrusion.

The microstructural feature of the seed surface was areolated with many depressed cellular reticula (Fig. 3A). The palisading hair-like structures covering the seed surface were clearly revealed by scanning electron microscope (Fig. 3B, D, F). Meanwhile, these characters proved to have taxonomic value in discriminating within the entire *Solanum* section (Stern *et al*., 2010). A range of ultrastructure was observed on testa surface. Each depressed cellular reticula had a lumina which was encircled by palisading lines of reticula (Liu *et al*., 2004). The surface of depressed cellular reticula was covered by fingerlike projections arranged in a ring structure except the top of the seed (Fig. 3B, D, F). Varying amounts of tilted holes were also observed along lines of reticula (Fig. 3B, D, F, H).

Germination process: The germination process of *S. rostratum* begins with seed imbibition, hypocotyl elongation and ends with radicle penetration (Fig. 4A-D). The endosperm cap of *S. rostratum* resemble to that of tomato and coffee seeds, it acts as a constraint (Fig. 2C) to regulate seed germination suggesting that the region, where the radicle will protrude, is predestined in the seed (Finch-Savage & Leubner-Metzger, 2006; Oreja *et al*., 2017; Bewley *et al*., 2013). On the contrary, micropyle region can meet the demand of water uptake and govern the rate of water penetration to the embryo and serve as a reservoir of water and air for the seed to complete germination process. With increasing permeability of the testa, the restraint of endosperm cap weakened and the expansion of embryo accelerated, these changes can result in testa rupture. As the mechanical resistance of the endosperm cap decline, which appears to be a prerequisite for radicle protrusion and the radicle could protrude through the barrier and then complete germination. Meanwhile, the sol-gel phase transition had taken place in the endosperm cell around radicle which changed from liquid state to solid state (Fig. 4B-D). The endosperm provides nutrition and micropyle region provides water and air and the seed completes the germination process (Fig. 4D).

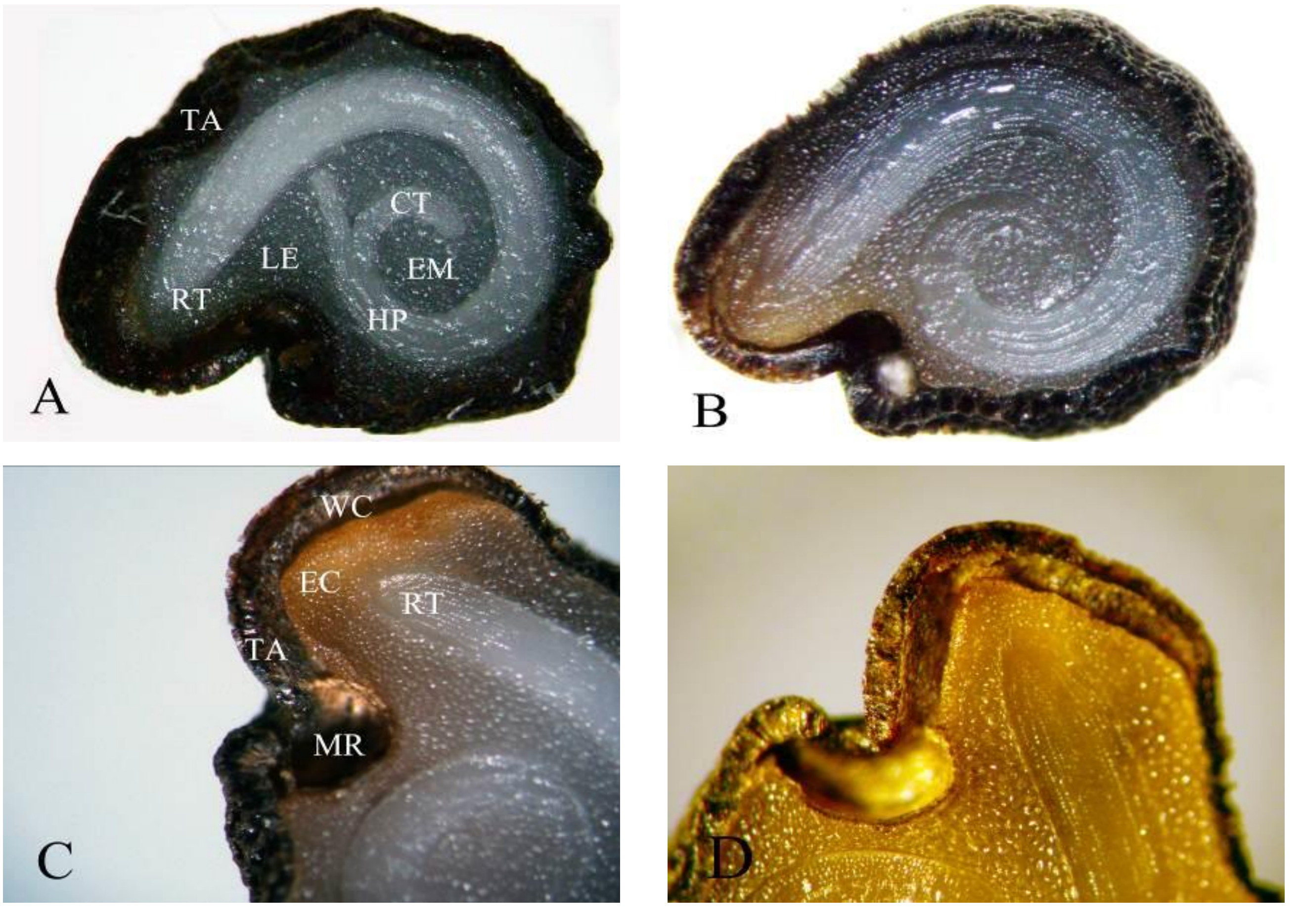

Fig. 2. Seed morphological characterization of *Solanum rostratum*.
(A) Longitudinally cut seed imbibed in water, showing the radicle tip (RT), testa (TA), lateral endosperm (LE), hypocotyl (HP), embryo (EM), and cotyledon (CT). (B) Longitudinally cut seed of the reverse side. (C) Longitudinally cut seed imbibed in water, showing the micropyle (MR), water channel (WC), endosperm cap (EC), testa (TA) and radicle tip (RT). (D) Longitudinally cut dry seed.

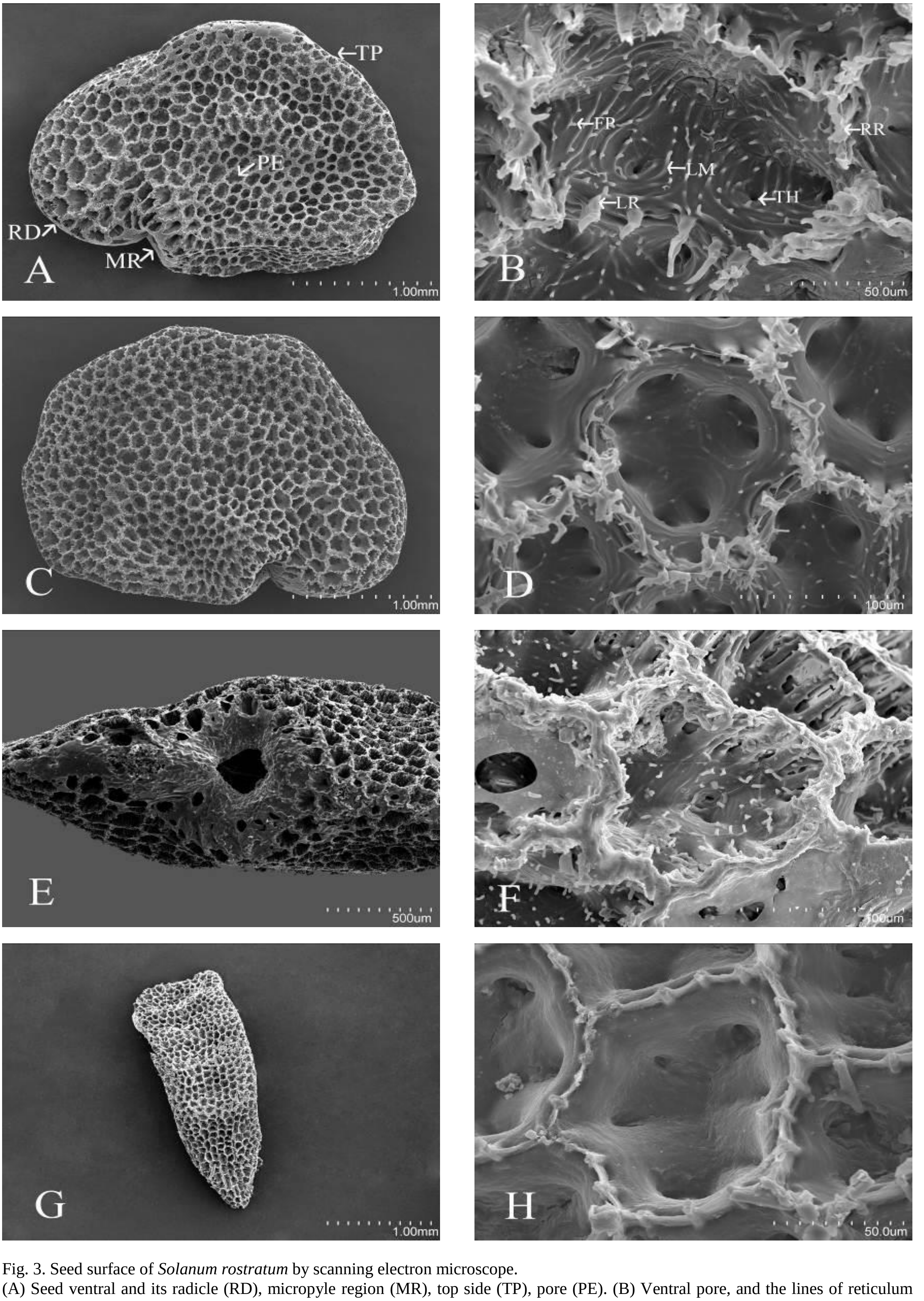

Fig. 3. Seed surface of *Solanum rostratum* by scanning electron microscope.
(A) Seed ventral and its radicle (RD), micropyle region (MR), top side (TP), pore (PE). (B) Ventral pore, and the lines of reticulum (LR), fingerlike projection (FP), lumina (LM), ridge of reticulum (RR), tilted hole (TH). (C) Seed dorsal (D) Dorsal pore. (E) Micropyle. (F) The pore near micropyle. (G) Top side. (H) Top pore.

S. rostratum compete with crops and pastures for space, water, nutrients, and light throughout the growing season. It was listed as the most troublesome annual weed species and the top ten invasive alien species in China (Wei & Yang, 2013). *S. rostratum* could cause great economic losses and considerable damage to natural ecosystem. Fruit and seed characters could be the basis of classification at different levels of taxa and could provide valuable information in the delimitation and identification of *S. rostratum*. The fruit of *S. rostratum* are indehiscent, small to medium sized, black or black brown and enclosed by calyx at maturity. In *S. rostratum* fruits, the protective or mechanical function of pericarp is strengthened by the persistent calyx. Changes in temperature and humidity can lead the persistent calyx to crack and the pericarp to rupture which help in seeds disperse under suitable conditions.

Compared with multiple modes of reproduction and dissemination of other invasive plant, like *Eupatorium adenophorum* (Wang *et al*., 2011), seeds are the only means of reproduction and overspreading for *S. rostratum*. The morphology of seed coat is usually stable and is little influenced by external environmental conditions during the seed development and ripening within the fruit (Oreja *et al*., 2017; Luan *et al*., 2017; Mondo *et al*., 2010; and Martínez *et al*., 1997). The seed surface of *S. rostratum* like other *Solanaceae* seeds, was characterized by palisading hair-like structures. Development of hair-like structures on the anticlinal walls of seed epidermis has been recorded in a number of members of the Solanaceae (Oreja *et al*., 2017; Qu & Zhang, 2009). Seed epidermis structures have been used to provide valuable information in the delimitation and identification of *Solanum* species (Lilian *et al*., 2007; Stern *et al*., 2010). Two kinds of ultrastructure, fingerlike projections and tilted holes, were differently arranged and numbered in the depressed cellular reticula, respectively. Apart from contributing to identification of *S. rostratum*, the ultrastructure may have the function of water retention and air exchange which can accelerate the softening of seed coat, thus allowing the radicle to penetrate at lower pressure compared to dry seed.

Our study indicated that the endosperm cap surrounded the radicle was the main physical barrier for seed germination, like other Solanaceae species e.g. tomato, pepper, tobacco and so on (Finch-Savage & Leubner-Metzger, 2006; Oreja *et al*., 2017; Qu & Zhang, 2009; Wei *et al*., 2010). Meanwhile, at seed maturation a cavity was formed between radicle and testa which was connected to micropyle region. The micropyle region may play a major role in water absorption and air exchange which is vital to seed germination. The cavity can be a passage that affects delivery of oxygen and water to embryo. These particular structures may provide evidence for elucidating the mechanism of *S. rostratum* seed dormancy and germination. Information on the germination processes of *S. rostratum* seed may give clues pertaining to the nature of physiological and biochemical processes. The testa ruptures and sol-gel phase transition of endosperm cap can be a prerequisite for radicle protrusion.

Knowledge obtained in this study would provide useful information in identification and classification of *Solanum* species. New discoveries in our experiments would be helpful in identification of *Solanum* species in plant quarantine. A systematic research about the morphological and anatomical characters of *S. rostratum* could help us to understand the relationship between structure and function and may contribute to a better understanding of general invasion mechanisms.

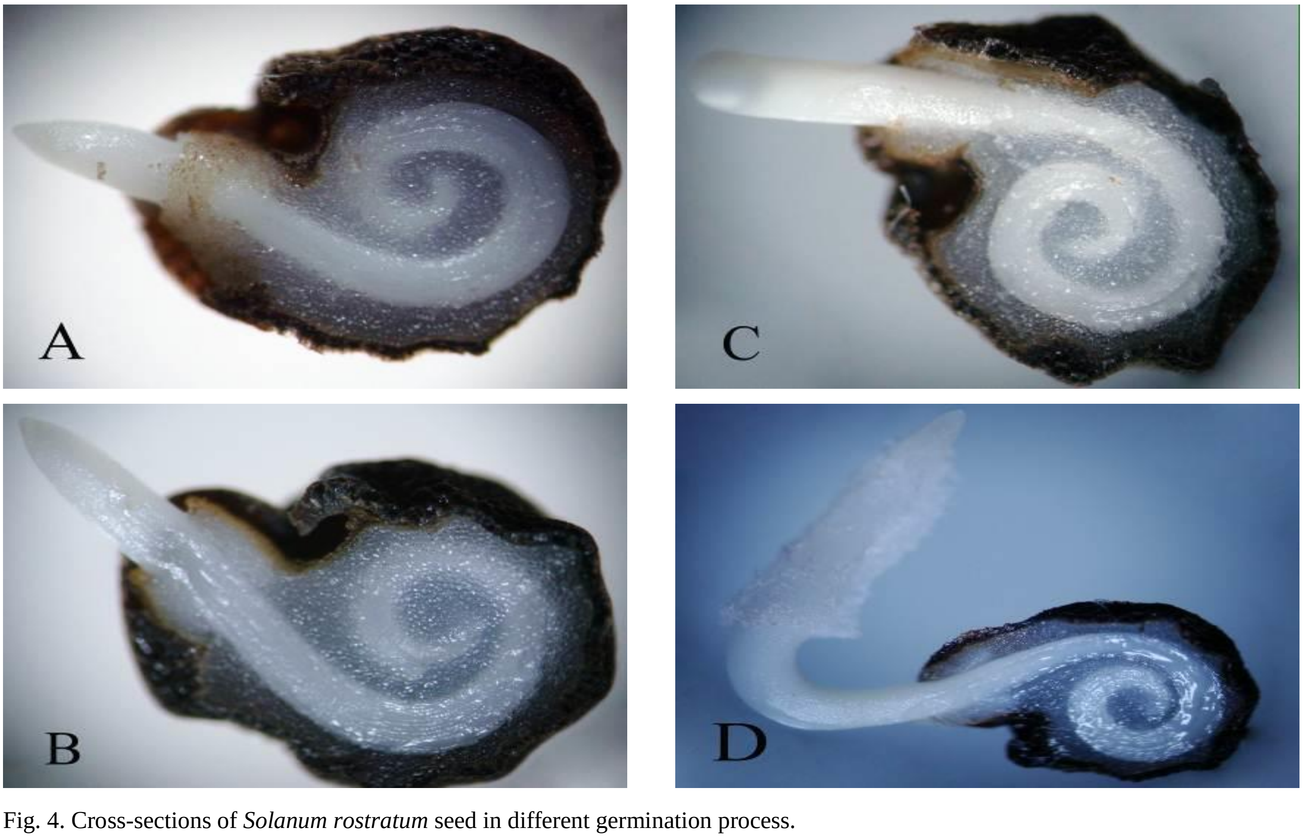

Fig. 4. Cross-sections of *Solanum rostratum* seed in different germination process.
(A) Radicle protruding through the endosperm cap; (B-D) Seedling development incubated in a thermostat-controlled dark chamber (70% relative humidity and 30 C).

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