Flowering Season-Dependent Variation In The Ultrastuctural And Soluble Proteins of Achillea Wilhelmsii Mature Pollen Grains

Leila Amjad, Ahmad Majd, Mozhgan Kavianifar

Abstract:- In plant word, pollen grains have major role in sexual reprodactive cycles. In this research, we compared the ultrastructural and soluble proteins of pollen grains of Achillea wilhelmsii in early-season and late-season flowering. The pollen were collected around the city of Isfahan, Iran. The pollen ultrastructure have studied by scaning electron microscope(SEM). The pollen total proteins in both flowering season were extracted by phosphate-buffered saline and examined using Bradford assay on electrophoresis on SDS-Page. The resulte showed that the pollen grains of early-seasons flowering were oval and mature pollens in late-seasons flowering were spheroid. The pollen protein bands in early-season and late-season flowering are seem in the rang of 14.4(Kda) to 66.2(Kda), 18.4(Kda) protein band exist in early-season pollens and 66.2(Kda) protein band in late-season pollen grains.

Index Terms:- Achillea wilhelmsii, soluble proteins, pollen, flowering season.

1 Introduction

POLLEN grains are male gametophytes and release male cells. Their major function is in genital reproductive cycles in the plant world [1], [2]. Same any other plant cells, pollen grains involve many several types of proteins, which are detected in main regions: in the cytoplasm and at the surface of the exine and intine [3]. The *Achillea wilhelmsii* pollen grains are tricolporate and ornamentations are echinate [4], [5]. The genera Achillea is one of the most important genera of the Asteraceae family, subfamily Asteroideae, tribe Anthmideae. Anthemideae involves 109 genus and almost 1740 species [4], [5]. *Achillea wilhelmsii* C. Koch (Asteraceae) is widely found in different parts of Iran [3]. This plant is full of the flavonoids and sesquiterpene lactones, which have been shown to be effective in lowering blood lipids and hypertension [6], and widely used, in Iranian traditional medicine for gastrointestinal disorders. It has chemical components, including flavonoids, alkaloids (achilleine), cineol, borneol, α- and β-pinen, camphor, car yophyllene, thujene, rutin, sesquiterpenoids and monoterpenoids [6]. Thus, the main aim of the present project was to carry out a biological investigation on *Achillea wilhelmsii* C. Koch from the Iran. In this study, we tried to compare pollen ultrastructural and protein contents in early and late flowering season.

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2 Materials and Methods

2.1 Sampling

Pollen grains were collected around region of Isfahan city. Novel pollen grains were filtrate by passage through mesh with 30(µm) diameter pores and then to keep at freezer -20ºc [7].

2.2 Scanning Electron Microscope

Pollen grains were studied by scanning electron microscopy. The mature pollen grains collected in early and late flowering season were coated with gold, samples were analyzed using a scanning electron microscope(Model SEM-xL30, Philips, Netherlands) [1],[5].

2.3 Preparation of Pollen extracts

Pollen extracts was assembled by incubating pollen grains in 0.1M phosphate buffered saline (PBS) pH 7.4 for 24h at 4ºc while stirring. The suspensions were centrifuged at 10000g for 45min at 4ºc and the supernatants hase removed [8].

2.4 Preparation of Pollen extracts

The total protein contents of extracts were believed in 595nm defined according to Bradford (1976) protein assay [8]. Extraction of pollen protein from early and late flowering season were separated using 12% SDS-PAGE at 70V constant voltage for 3-4h at 20ºc. Soluble proteins were extracted in sample buffer with heating for 3-4 min at 100ºc before loading. The gel was run in Tris-glycine buffer (PH 8.3) with 0.1% SDS and calibrated with a marker protein obtained from Fermentase. Proteins were observed with silver nitrate staining [7], [8].

3 Results

The study of pollen ultrastructure by SEM showed that the mature pollen grains in early flowering season were oval and in late flowering season were spheroid (Fig.1, 2). Exine surface echins in early season pollen grains are short and low accumulation and in end season surface eckins pollen grains are long and full density (Fig.3, 4). The SDS-PAGE protein profiles of early and late flowering season mature pollens are shown that bands are presents in the molecular weight range 14.4 (kDa) to 66.2 (kDa). SDS-PAGE showed that protein

band 18.4(kDa) not observed in late season pollen grains and also protein band 66.2(kDa) not showed in season early pollen grains (Fig.5). Bradford protein analysis communicated a higher total protein content in season late pollens than that of season early pollens. The protein concentration of season early and late mature pollen extracts were 1.11(mg/g) and 1.47(mg/g) respectively.

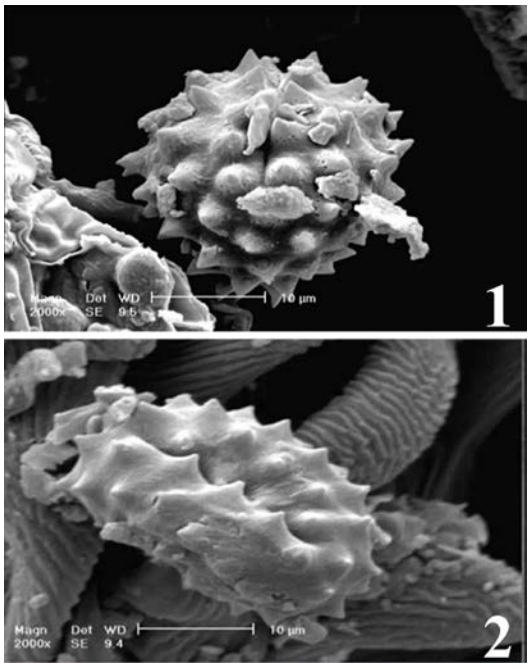

Fig. 1, 2. Scanning electron micrographs of early and late season mature pollen grains
(The pollen grains oval and spheroid)

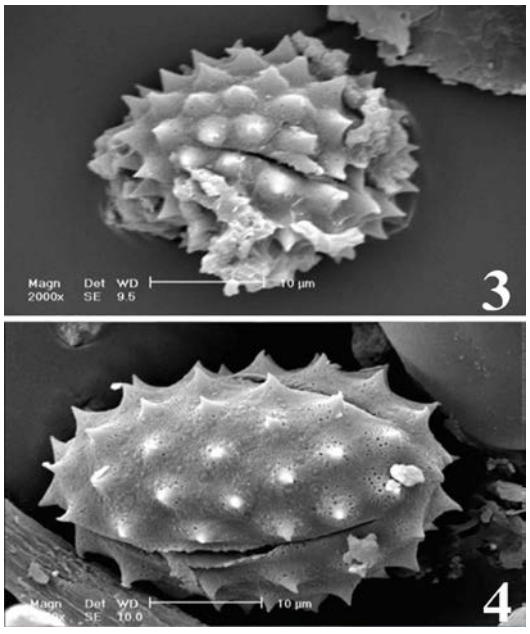

Fig. 3, 4. Scanning electron micrographs of early and late season mature pollen grains
(The pollen grains with short and long echins)

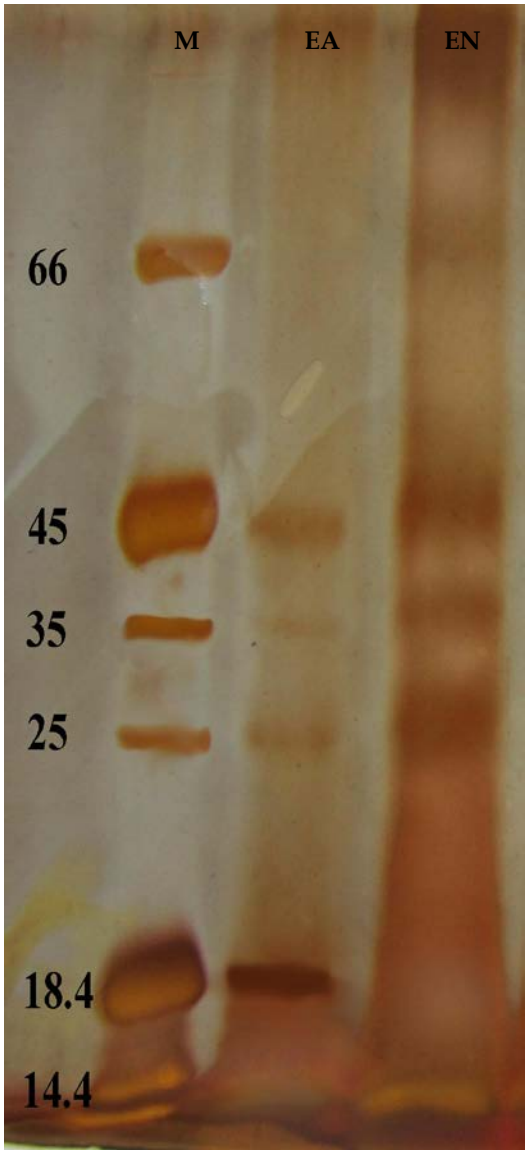

Fig. 5. SDS-PAGE pattern of soluble proteins of early and late season mature pollen extracts (M: protein markers, EA: bands of early season mature pollen, EN: bands of end season mature pollen)

4 DISCUSSION

Many pieces of evidence such as the synchronicity of seasonal variation that cause temperature increase from spring season to summer season, soluble proteins content decrease and also to change enzymes activity in pollen grains, that findings of several researchers support this idea [9], [10]. There is also noticeable variation in pollen structure in relation to place and climate conditions such as relative humidity, photoperiodic changes and sunlight intensity that cause of pollen grains to spherical and so exine with height echins and full accumulation that is from symptoms of mature pollen which is agreement with those reporting of Amjad et al. [5] and is agreement with observation reported by Koti et al. [11]. Flowering cycle Achillea wilhelmsii plant is from middle of the spring until middle of the summer, which collected season early pollens in spring season and late season pollens in summer season, thus sunlight intensity and temperature

increased from spring to summer, as a result decreased number from proteins in season late pollen grains [12]. New proteins synthesis in season late pollen to relate with developmental process of pollen grains that is similar to findings of Amjad et al. [5], that are role this proteins allergenicity, thus suggest that pollen is one of the most possible causes of allergy that are similar to findings of Amjad et al. [3]. The analysis of SDS-PAGE data demonstrated protein band 66.2(kDa) that exist in late season pollens, it seems that proteins synthesis associate to developmental process of pollen grains [13], also this proteins were from effective elements in allergenicity[3]. According to our results, total proteins of pollen grains significantly increase in late season pollen grains, because pollen proteins content to relate with development stages of pollen [5]. There are reports showing that Ca2+ ions are in particular essential regulatory components of all organisms. Being a second messenger, Ca2+ is involved in regulation at all stages of plant growth and development, including growth and differentiation, photo morphogenesis and embryogenesis, the self-incompatibility responses in pollen-pistil interactions, perception of symbiotic signals, hypersensitive responses induced by pathogens and elicitors, gravitropism and phototropism, assembling and disassembling of cytoskeleton elements, perception of red and blue light, cyclosis, and movement of stomatal cells[14]. Ca2+ is an important factor for the bioregulation in plants. The Ca2+-concentration in cell walls and vacuoles is up to 100 times higher than in the cytosol, the cell membranes provide sharp gradients of Ca2+ concentration, along with a electrochemical potential, which is widely modified by the activity of membrane channels. Phytochrome a activity and circadian regulation are driven by Ca2 + oscillations, which are discussed as part of the "biological clock" of plants [15].Therefore, a direct influence of environmental stresses is discussed affecting Ca2+ levels via the ion cyclotron resonance mechanism. It influences the available Ca2+ and thereby regulatory processes. Therefore, our studies on pollen grains showed that climate conditions causing changes of physiology, morphology and ontogeny of pollens. These changes to pollen structure can affect flavonoids and proteins of pollens via Ca2+ changes in development different stages.

5 Conclusion

The generative season environment changes interact with the pollen of plants causing alteration of morphology, biochemistry and physiology of pollens. These changes to pollens structure and ultrastructure can affect proteins of pollens. Researchers have attributed these features to different structures and proteins content of the pollen are Ca2+ ion changes in regulation at all stages of plant growth and development.

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