Arab Gulf Journal of Scientific Research, 20 (2), pp 92-95 (2002)

ORIGINAL PAPER (REF. 2141)

H M Alwadie

Ultrastructure of *Withania somnifera* (L.) Dunal Pollen Grains

Abstract: Light, scanning and transmission electron microscopy were used to study the morphology and ultrastructure of *Withania somnifera* (L.) Dunal pollen grains. Light microscopic examination revealed that the pollen grains are tri- or tetrazonocolpate grains, approximately as long as broad, measuring 29-µm. Scanning electron microscopic observation showed that surface sculpturing of the pollen is scarbate-granulate. Ultra thin sections as examined by transmission electron microscope showed that the pollen contained numerous starch grains, lipid droplets, endoplasmic reticulum and vesicles of dictyosomes. Two layers of the pollen wall were also distinguished, the outer wall (exine) divided into ektexine and endexine as well as the inner layer (intine). The nutritive values of *Withania* pollen are discussed. The importance of studying the ultrastructure of pollen grains as a new tool in palynology is also discussed.

Key words: Pollen grains, ultrastructure, *Withania Somnifera*.

Introduction

Withania somnifera (L.) Dunal is an important medicinal plant belonging to the family Solanaceae and is described under many common names such as Indian ginseng and Ashwagandha. It is widespread in many places in the world e.g. in India, Africa and the Mediterranean region (Mabberly, 1997) and Saudi Arabia (Migahid, 1978; Collenette 1985, 1998 & Doaigey, 1991). Many active substances have been extracted from this plant. Alkaloids have been used for calming, withanolides for their anti-tumor effects and sitoindosides for their anti-stress activity (Elsakka *et al.* 1989; Bhattacharya *et al.* 2000).

Hussien M. Alwadie
Department of Biology
Faculty of Science
King Khalid University
P.O. Box 9004, Abha
Saudi Arabia
Tel: (07) 2246072. Ext. 1092
Fax: (07) 2313321
E-mail: HussienAlwadie@hotmail.com

التركيب الدقيق لحبوب لقاح نبات العبب

حسين العوضي

المستخلص: في هذه الدراسة تم إستخدام المجهر الضوئي والإلكتروني، بنوعيه الماسح والنفاذ، لدراسة الشكل الخارجي والتركيب الداخلي لحبوب اللقاح الناضجة لنبات العبب. أوضح المجهر الضوئي أن حبة لقاح نبات العبب تحتوي على 3 أو 4 ثقوب للإنبات، وكان حجمها حوالي 29 ميكروميتر. كما أوضح المجهر الإلكتروني الماسح، أن سطح حبة اللقاح يحتوي على حبيبات. وعند فحص القطاعات الرقيقة لحبة اللقاح الناضجة بالمجهر الإلكتروني النفاذ، تبين أن جدار حبة اللقاح يتكون من طبقتين الأولى تعرف بالخارجية (Exine)، والتي بدورها تنقسم إلى طبقتين (Ektexine & Endexine). ثم الطبقة الداخلية (Intine). تبين أيضاً أن الطبقة الخارجية تصبغ بكثافة عن الطبقة الداخلية، وذلك عند إستخدام الصبغات الخاصة بالمجهر الإلكتروني، مما يؤكد أن الطبقة الخارجية تختلف في تركيبها الكيميائي عن الطبقة الداخلية. عند مقارنة هذا الإختلاف في حبوب لقاح نباتات أخري من الأبحاث السابقة، إتضح أن طبقات الجدار تختلف من نبات إلى آخر، مما يعطي دلالة على أن هذه الخاصية قد تكون أداة جديدة تساهم في التصنيف الحديث للنباتات الراقية. من ناحية أخري أوضح المجهر الإلكتروني النفاذ، أن حبة اللقاح تحتوي على حبيبات نشوية، قطرات زيتية، شبكة إندوبلازمية وحويصلات من المرجح أن تكون خاصة بجهاز جولجي. من خلال هذا البحث تم التعرف أيضاً على المواد الغذائية الموجودة داخل حبة اللقاح، إذ أنه من المعروف أن حبوب اللقاح تأخذ كأقراص مقوية منذ فترة غير قصيرة.

كلمات مدخلية: نبات العبب، حبوب اللقاح، الشكل الخارجي، التركيب الداخلي.

Pollen characteristics have received attention in taxonomy and pollen morphology, but still very little is known about the ultrastructure and cytochemistry. Pollen grains are three-dimensional objects, spherical to elliptical in shape. They consist of a wall, called "exine", that has different types of apertures (pores, furrows) and sculptures (echinae, verrucae, etc.) on the surface (Moore & Webb, 1978). The mature pollen grains contain nutrients for the growing pollen tube (Jensen *et al.* 1974; Cresti *et al.* 1975). Pollen wall stratification and internal structure can hardly be studied by light microscopy (Zavada, 1990); therefore, scanning and transmission electron microscopy become necessary in examining these characters. El-Ghazaly (1990) and Harley *et al.* (2000) reported the morphology of the pollen grains of many plant species.

Ultrastructural studies on pollen grains of higher plants may add new information about the taxonomy of these plants, particularly regarding the pollen wall. So far, no ultrastructural studies of pollen grains of *Withania somnifera* (L.) Dunal have been reported in Saudi Arabia. The aim of this paper is to describe the ultrastructural features of the mature pollen grain of this plant.

Materials and Methods

Light microscopy (LM). Pollen grains of *Withania somnifera* (L.) Dunal were placed in glacial acetic acid for three minutes, acetolysed according the method of Erdtman (1960) and then mounted in glycerin gel for observation by light microscopy.

Scanning electron microscopy (SEM). Acetolysed pollen grains were placed on aluminum stubs, freeze dried and then coated with gold (Moore & Webb, 1978). The stubs carrying the pollen grains were examined and photographed using a JEOL JSM-T200 SEM at 25 KV.

Transmission electron microscopy (TEM). 1 mm^3 cubes of agar containing fresh pollen grains were fixed for 24 hours in 2.5% glutaraldehyde with 0.05 M cacodylate buffer at pH 7.4 and post fixed in 1% OsO_4 in the same buffer for 2 hours (Cresti *et al.* 1985). The cubes were then dehydrated in graded series of ethanol, and embedded in Spurr's resin (Spurr, 1969). Ultra thin sections were cut using a diamond knife on ultramicrotome, stained with uranyl acetate followed by lead citrate (Reynolds, 1963). The stained grids were examined and photographed with a JEOL JEM 100 B TEM.

Results and Discussion

Pollen grains of *Withania somnifera* (L.) Dunal are always seen attached to the style hairs (Fig.1). Pollen grains of *Withania somnifera* are trizonocolpate, with 3 pores (Fig.2) or tetrazonocolpate, with 4 pores (Fig.3) approximately as long as broad, with a diameter of 28-30 μm. SEM observation showed that surface sculpturing of the pollen is scarbate-granulate (Fig. 4). These observations are more or less in agreement with the results obtained by Moore & Webb (1978) and Ayyad (1988) in the case of the pollen of Solanaceae members.

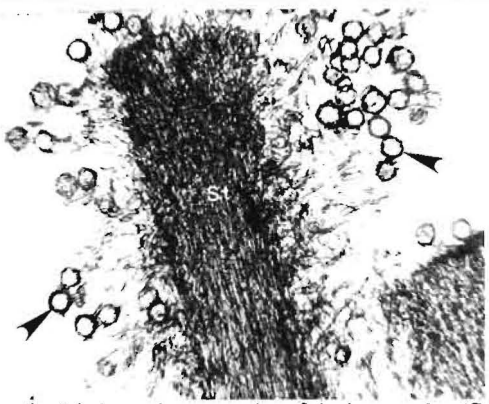

Figure 1. Light micrograph of hairy style (St) of *Withania somnifera* showing many attached pollen grains (arrowheads). Bar=50.0μm.

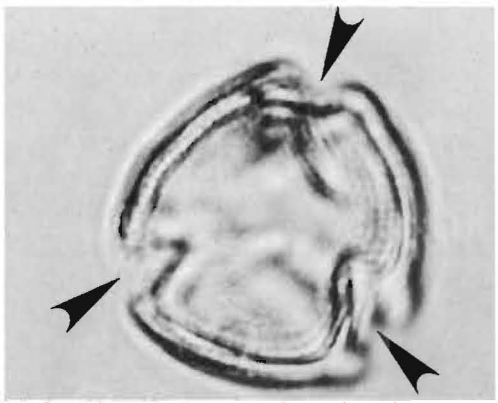

Figure 2. Light micrograph showing a pollen grains of *Withania somnifera* with 3 pores (arrowheads). Bar=1.0μm.

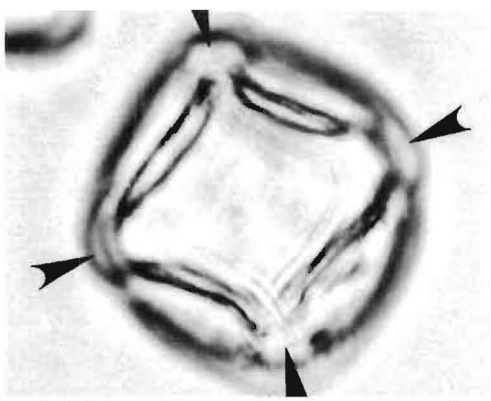

Figure 3. Light micrograph showing another view of a pollen grains of *Withania somnifera* with 4 pores (arrowheads). Bar=1.0μm.

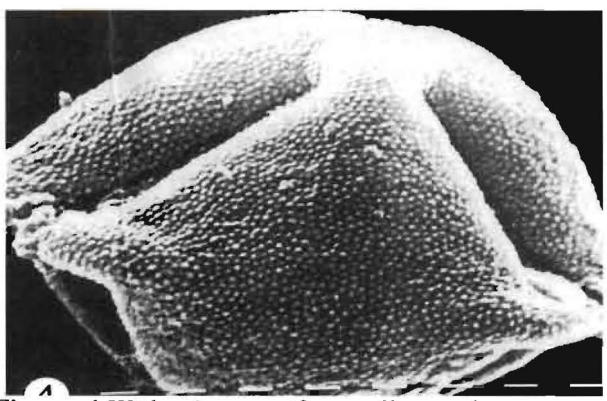

Figure 4.*Withania somnifera* pollen grains as seen by scanning microscope.Note the pores and the granulation of the surface. Bar=5.0μm.

Examination by TEM revealed that the pollen wall of *Withania somnifera* consists of two distinct layers, an outer (exine) and inner (intine) layer. The exine consists of a very unusual substance, called "sporopollenin" (i.e. polymers of carotenoids) (Moore & Webb, 1978), although the exact chemical composition of the exine is a topic of continuing research and debate. The chemical composition of the exine in Withania pollen needs further investigation using ultracytochemical methods. The exine is divided into two layers, ektexine and endexine (Figs. 5 & 6). The ektexine is fairly uniform, of which three layers were visible: tectum, collumellae and foot layer (Faegri, 1956). The inner layer (intine), formed of fibrillar materials such as cellulose, is thick and similar to an ordinary plant cell wall (Figs. 5 & 6). By using the conventional staining method (uranyl acetate/lead citrate) in TEM, the exine appeared to be stained more intensely than the intine (Figs. 5 & 6). These results indicated that the exine is composed from materials different from those of intine. Ultracytochemical methods may recover this point. The type of pollen wall may vary from one plant species to another (Weber, 1999).

The cytoplasm of the mature pollen was found to be non-vacuolated and containing plastids with several starch grains, lipid droplets, endoplasmic reticulum, mitochondria and small vesicles probably belonging to the dictyosomes (Figs. 5 & 6). This description confirmed the findings of Kozar (1974) and Ayyad & Baka (1993). An increase of lipid droplets and vesicles of dictyosomes was observed during the initiation of pollen tubes (Ayyad & Baka, 1993). Fawcette (1966) suggested that these lipid droplets serve as a reservoir of high-energy material and as a potential source of short-chain hydrocarbons for the synthesis of membranes and other lipid-bearing cellular components necessary for the construction of a germ tube in the germinating pollen grain.

Ayyad & Baka (1993) have used a periodic acid-thiocarbohydrazide-silver proteinate technique (PATCHSP) to localize the polysaccharides in the pollen grains of *Plantago major* L. They reported that silver was deposited on endexine of exine, intine, starch grains, vesicles of dictyosomes and lipid droplets indicating that these structures contain polysaccharides. Positive staining of lipid droplets by PATCHSP method could explain the fact that these pollen grains contain lipid droplets mixed with polysaccharides. The intine is mainly formed of cellulose as the normal plant cell wall (Moore & Webb, 1978) or of pecto-cellulose substance (Kozar, 1974). El-Ghazaly & Jensen (1987) reported that the exine of the mature pollen of Triticum aestivum was positively stained when the PATCHSP method was used, indicating that the nature of the material of exine in Triticum pollen is different from that in Plantago pollen. These differences may give support for using ultracytochemical methods as new tools in palynology. Cytochemical studies are needed to study Withania pollen. More advanced techniques such as localization of enzymes and isoenzymes are needed to clarify this point.

The present results indicate that *Withania somnifera* (L.) Dunal pollen contains different nutrients. It is well known that pollen grains are used as robust nutritive supply (as tablets) in Europe. The present investigation is one of a series of studies about the types of nutritive materials, which are found in the pollen grains and also was intended to throw light on new criteria in palynology for studying the ultrastructure of pollen.

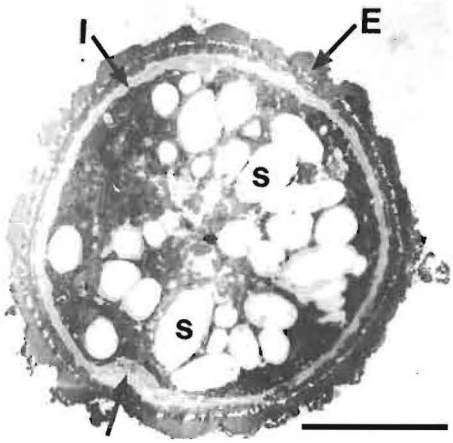

Figure 5. Transmission electron micrograph of a cross section of *Withania somnifera* pollen showing exine (E), intine (I), and starch grains (S). Note the pore (arrow). Bar = 5.0 μm.

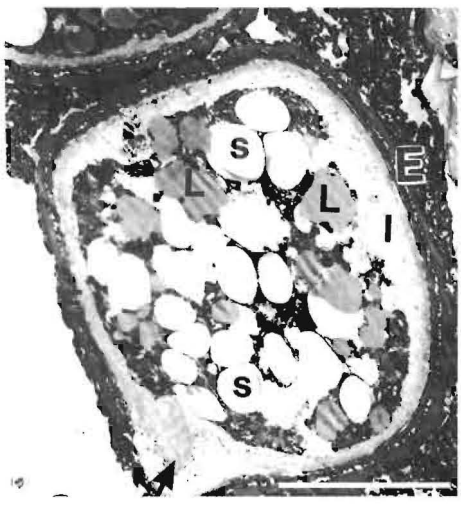

Figure 6. Transmission electron micrograph of a cross section of *Withania somnifera* pollen grain showing starch grains (S), lipid drops (L), electron-dense exine (E), and electron-lucent intine (I). Note the initiation of pollen tube (arrow). Bar = 5.0 μm.

References

Ayyad, S.M., (1988) Pollen grain ecology of the Mediterranean Sea coast, Egypt. PhD thesis, Faculty of Science, University of Mansoura, Egypt.

Ayyad, S.M., and Baka, Z.A.M. (1993) Ultrastructure and localization of polysaccharides in pollen grains and young pollen tubes of Plantago major L. J. of Environmental Sciences,**5**: 221-234.

Bhattacharya, S.K., Bhattacharya, A., Sairam, K. and Ghosal, S. (2000) Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine **6**: 463-469.

Collenette, S. (1985) An illustrated guide to the flowers of Saudi Arabia. MEPA Flora Pub. 1. Scorption Publ. Ltd., London.

Collenette, S. (1998) A checklist of botanical species in Saudi Arabia. International Asclepiad Society.

Cresti, M., Pacini, E., Sarfatti, G., and Simoncioli, C. (1975) Ultrastructural features and storage function of Lycopersicum peruvianum pollen. In: Mulcally, D. L. (ed) Gamete Composition in Plants and Animals, North-Holand Publications Company, Amstrdam.

Cresti, M., Clampolini, F., Mulcahy, D.L., and Mulcahy, G. (1985) Ultrastructure of Nicotiana alata pollen, its germination and early tube formation. American J. of Botany **72**: 719-727.

Doaigey, A.R. (1991) Occurrence, type, and location of calcium oxalate crystals in leaves and stems of 16 species of poisonous plants. American J. of Botany **78**: 1608-1616.

El-Ghazaly, G. (1990) An illustrated key to endoaperture morphology. Review of Palaeobotany and Palynology **63**: 227-231.

El-Ghazaly, G. and Jensen, M. A. (1987) Development of wheat (Triticum aestivum) pollen. II. Histochemical differentiation of wall and ubisch bodies during development. American J. of Botany **74**: 1396-1418.

Elsakka, M., Pavelescu, M., and Grigorescu, E. (1989) *Withania somnifera*, a plant with great therapeutical future. Review of Medical and Chir. Society Med. Nat. Iasi. **93**: 349-350.

Erdtman, G. (1960) The acetolysis method, revised description. Sven. Bot. Tidstr. **54**: 651-564.

Faegri, K. (1956) Recent trends in palynology. Botanical Review **22**: 639-664.

Fawcette, D.W. (1966) An atlas of fine structure. The cell, its organelles and inclusion. W.B. Saunders Co., Philadelphia.

Harley, M., Morton, C. and Blackmore, S. (2000) Pollen and Spores, Morphology and Biology. Royal Botanic Garden, Kew, London.

Jensen, W.A., Ashton, M. and Heckard, L. R. (1974) Ultrastructural studies of the pollen subtribe Castilleine, family Scrophulariaceae. Botanical Gazette **135**: 210-218.

Kozar, F. (1974) Ultrastructure of pollen of Opuntia polycantha. Canadian J. of Botany **52**: 313-315.

Mabberly, D.J. (1997) The plant-book. A dictionary of the vascular plants. 2nd ed. Cambridge University Press.

Migahid, A.M. (1978) Flora of Saudi Arabia. 2nd ed. Riyadh University Press.

Moore, P. D. and Webb, J. A. (1978) An Illustrated Guide to Pollen Analysis. Hodder and Stroughton, London.

Reynolds, E. S. (1963) The use of lead citrate at high pH as an electron- opaque stain in electron microscopy. J. of Cell Biology **17**: 208-212.

Spurr, A. R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J. of Ultrastructural Research **26**: 31-43.

Weber, M., Halbr, H. and Hesse, M. (1999) The basic pollen wall types in Araceae. International J. of Plant Science **160**: 415-423.

Zavada, M.S. (1990) A contrbution to the study of pollen wall ultrastructure of orchid pollinia. Annales Mo. Botanical Garden. St. Louis **77**: 785-801.

Received 04/12/2001, in revised form 08/06/2002