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Short Communication

Histological Analysis of Hulled and Hull-less Squash Seed Coat

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Abstract

Differences in the structures of seed coat from one hull-less genotype and three hulled-seeded genotypes of squash (*Cucurbita pepo* and *C. maxima*) were examined in mature seeds using paraffin sectioning and scanning electron microscopy. Histological investigations demonstrated that squash seed coat normally comprise five layers, including the epidermis, hypodermis, sclerenchyma, parenchyma, and chlorenchyma. Three layers of hypodermis, sclerenchyma, and parenchyma cells were strongly or partially lignified and maintained their integrity to form the hard, leathery hull that was characteristic of hulled-seeded squash seeds. On the contrary, three layers of hypodermis, sclerenchyma and parenchyma cells were collapsed in hull-less squash seed coat due to a lack of lignin deposition and this seed coat contained only one or two layers of degenerated parenchymal cells. In addition, the seed margin, a specialised part of the seed coat in mature seeds, exhibited the same structural differences as seed coats in hulled vs. hull-less seed types, except for the presence of a large, circular vascular bundle in the parenchyma of both types of hulled and hull-less seed coats.

Keywords: *Cucurbita* spp.; Naked seed; Seed coat; Seed margin; Anatomical structure

Introduction

The seed coat, which develops from the integument surrounding the embryo and endosperm, protects the embryo and absorbs water during germination. The seed coat is a stable characteristic that is not highly affected by environmental factors and is therefore an important trait for plant classification.

Morphologically, the seeds of most cultigens of *Cucurbita pepo* normally possess a thick, leathery outer layer (hull) due to the strong lignification of some of the testa layers (Latifi *et al.,* 2012). However, some hull-less or 'naked seeded' cultigens of *C. pepo*, which carry recessive mutations for the hull-less trait, have been propagated in Austria, possibly for over 100 years. The first reference to genetically hull-less seeds in *C. pepo* was published in 1934 by an Austrian scientist (Techermak-Seysenegg, 1934). Squash seed oil is highly valued in Asia for use in salad dressings. Therefore, hull-less or 'naked seeded' cultigens have been developed to facilitate the efficient extraction of squash seed oil and to reduce the expense of the decortification process.

Heinisch and Ruthenberg (1950) conducted a detailed anatomical study of the seed coats of hulled and hull-less cultigens of *C. pepo*, which revealed that all seed coat layers are present in hull-less cultigens, but the thickening and lignification of the cell walls are reduced in hull-less seeds. This study, as well as other early studies (Weilung and Prym von Becherer, 1950; Schoniger, 1950, 1952, 1955; Mudr and Neumann, 1952; Grebenssikov, 1954), revealed different genotypes in segregating populations of squash that exhibit different degrees of cell wall thickening and seed coat genetics. The main objective of the present study was to conduct histological analysis to determine differences in seed coat development between hulled and hull-less cultigens of squash.

Materials and Methods

One hull-less squash (*C. pepo*) genotype (0516-2), and three genotypes containing seeds with hulls [Jinyuan (*C. maxima*), Jinhui-2 (*C. pepo*), and Yinhui-1 (*C. maxima*)], were obtained from the Department of Horticulture, Northeast Agricultural University, Harbin, China (Fig. 1). The seeds were fixed in FAA (formaldehyde: acetic acid: ethanol: H_2O, 10:5:50:35, by volume), stained with hematoxylin for at least 24 h, and dehydrated through an ethanol series (Bai *et al.,* 2004). The plant materials were embedded in paraffin and cut into longitudinal and transverse sections. The sections were then observed, measured, and photographed under a light microscope (Nikon-YS100).

The seeds were fixed overnight in FAA and dehydrated in a graded ethanol series. The dehydrated seeds were critical-point-dried using liquid CO_2, mounted on aluminium stubs with double-sided tape, gold-coated with an Edwards S150B sputter coater (Bai *et al.,* 2004), and examined through a scanning electron microscope (S-3400N, at 5 kV).

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Table 1: Morphological indicators in the seed coats of mature squash seeds with four different genotypes

Cell layer	Genotype	Morphological indicators	Sample figures
Epidermis (E)	0516-2	Four upper layers were collapsed into a hyaline, each layer only containing one or two layers of degenerated parenchymal cells	Fig. 2E; Fig. 3E
	Jinyuan	A layer of barrier style of closely spaced parenchymal cells, with wall containing numerous irregular granular structures	Fig. 2F; Fig. 3F
	Jinhui-2	A layer of aligned parenchymal cells	Fig. 2G; Fig. 3G
	Yinhui-1	A layer of barrier style of closely spaced parenchymal cells	Fig. 2H; Fig. 3H
Hypodermis (H)	0516-2	Four upper layers were collapsed into a hyaline, each layer only containing one or two layers of degenerated parenchymal cells	Fig. 2E; Fig. 3E
	Jinyuan	Four or five layers of irregular globular unidiomatic lignified sclerenchymatous cells	Fig. 2F; Fig. 3F
Cell layer	Genotype	Morphological indications	Sample figures
Hypodermis (H)	Jinhui-2	Four or five layers of closely spaced globular unidiomatic lignified sclerenchymatous cells	Fig. 2G; Fig. 3G
	Yinhui-1	Seven or eight layers of closely spaced globular unidiomatic lignified sclerenchymatous cells	Fig. 2H; Fig. 3H
Sclerenchyma(S)	0516-2	Four upper layers were collapsed into a hyaline, each layer only containing one or two layers of degenerated parenchymal cells	Fig. 2E; Fig. 3E
	Jinyuan	Mostly two layers of closely spaced lignified globular sclerenchymatous cells	Fig. 2F; Fig. 3F
	Jinhui-2	One layers of closely spaced lignified sclerenchymatous cells	Fig. 2G; Fig. 3G
	Yinhui-1	One layers of closely spaced globular lignified sclerenchymatous cells	Fig. 2H; Fig. 3H
Parenchyma (P)	0516-2	Four upper layers were collapsed into a hyaline, each layer only containing one or two layers of degenerated parenchymal cells	Fig. 2E; Fig. 3E
Cell layer	Genotype	Morphological indications	Sample figures
Parenchyma (P)	Jinyuan	Five or six layers of irregularly arranged parenchymatous cells, with wall containing microgrooves	Fig. 2F; Fig. 3F
	Jinhui-2	Several layers of irregularly arranged small parenchymatous cells	Fig. 2G; Fig. 3G
	Yinhui-1	Several layers of irregularly arranged parenchymatous cells, with wall containing microgrooves	Fig. 2H; Fig. 3H
Chlorenchyma (C)	0516-2	One to three layers of irregularly arranged parenchymatous cells containing chloroplasts, which was derived from degenerated endosperm	Fig. 2E; Fig. 3E
	Jinyuan	the same as 0516-2	Fig. 2F; Fig. 3F
	Jinhui-2	the same as 0516	Fig. 2G; Fig. 3G
	Yinhui-1	the same as 0516	Fig. 2H; Fig. 3H

Table 2: Morphological indicators in the seed margins of mature squash seeds with four different genotypes

Genotype	Morphological indicators	Sample figures
0516-2	Seed marginal structures were connected to the reduced seed coat. Two layers of thickening parenchymal cells included an obvious circular vascular bundle	Fig. 2A; Fig. 3A
Jinyuan	Seed marginal structures were connected to seed coat. Two fin prominences exhibited at both sides of the top. Hypodermis and sclerenchyma thickened. Sclerenchyma changed from two layers of cells in fin prominences to one layer at the top. Parenchyma contained a large circular vascular bundle	Fig. 2B; Fig. 3B
Jinhui-2	Seed marginal structures were connected to seed coat. Hypodermis and sclerenchyma thickened. Parenchyma consisted of a large circular vascular bundle	Fig. 2C; Fig. 3C
Yinhui-1	Seed marginal structures were connected to seed coat. Hypodermis thickened and sclerenchyma changed from one layer of cells to two layers. Parenchyma comprised a large circular vascular bundle	Fig. 2D; Fig. 3D

Results and Discussion

In this study, the structures of the seed coats of mature seeds from hulled and hull-less squash were compared using microscopic examination of paraffin sections and scanning electron microscopy. Seed coat normally comprise five layers, including epidermis (E), hypodermis (H), sclerenchyma (S), parenchyma (P), and chlorenchyma (C). The H, S and P layers on the seed surface were strongly or partially lignified on the surfaces of both types of hull seeds. However, in hull-less seeds, the three middle layers were collapsed into the hyaline, without any trace of lignin in the testa (Table 1; Fig. 1, 2 and 3), which was in accordance with previous studies (Stuart and Loy, 1983, 1988; Loy, 2000, 2004; Zraidi *et al.*, 2003; Bezold *et al.*, 2005). Moreover, the structural differences in the seed margins between mature hulled and hull-less squash seeds were the same as those of the seed coats, but a large circular vascular bundle existed in the parenchyma of the seed margins of both types of hulled and hull-less seeds (Table 2; Fig. 1, 2 and 3).

The present study indicated that the differences between hulled and hull-less seed coats could be attributed to the different degrees of lignifications that are present in the H, S, and P cell layers. Lignification resulted from the accumulation of lignin in the plant cell wall (Stuart and Loy, 1983). Abnormal lignin synthesis or metabolism in the hull-less seeds might lead to a decrease in lignin accumulation in the cell walls of seed coat, which was also found in the seed coat of *Arabidopsis thaliana* (Liang *et al.*, 2006). Some lignin biosynthetic genes had been cloned and then analyzed deeply and thoroughly from the point of transcriptional regulation (Zhong and Ye, 2009; Zhou *et al.*, 2009; Weng and Chapple, 2010). These researches should facilitate us to clone the key genes regulating lignin synthesis or metabolism in seed coat of squash in the further.

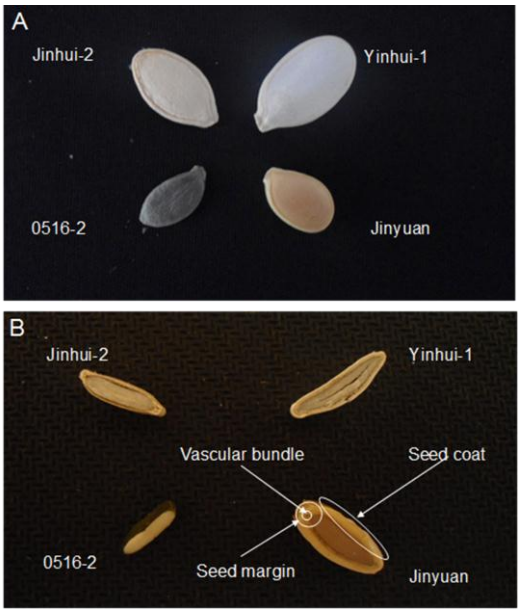

Fig. 1: Seed types of the hulled (Yinhui-1, Jinhui-2, and Jinyuan) and hull-less (0516-2) from surface (A) and longitudinal section (B) observation

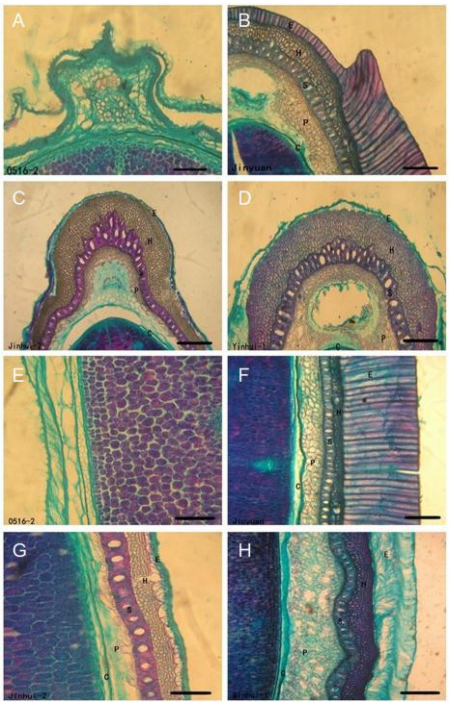

Fig. 2: Morphological structures of five cell layers in the seed coats and seed margins of mature squash seeds with four different genotypes through paraffin sectioning method. Morphological structures of five cell layers in the seed margins of mature squash seeds of 0516-2 (A), Jinyuan (B), Jinhui-2 (C), Yinhui-1 (D) and in the seed coats of mature squash seeds of 0516-2 (E), Jinyuan (F), Jinhui-2 (G), Yinhui-1 (H). The *letters* indicate five cell layers, respectively. *E* epidermis, *H* hypodermis, *S* sclerenchyma, *P* parenchyma and *C* chlorenchyma. Bars = 10 μm (E), 20 μm (A, G) and 40 μm (B-D, F, H)

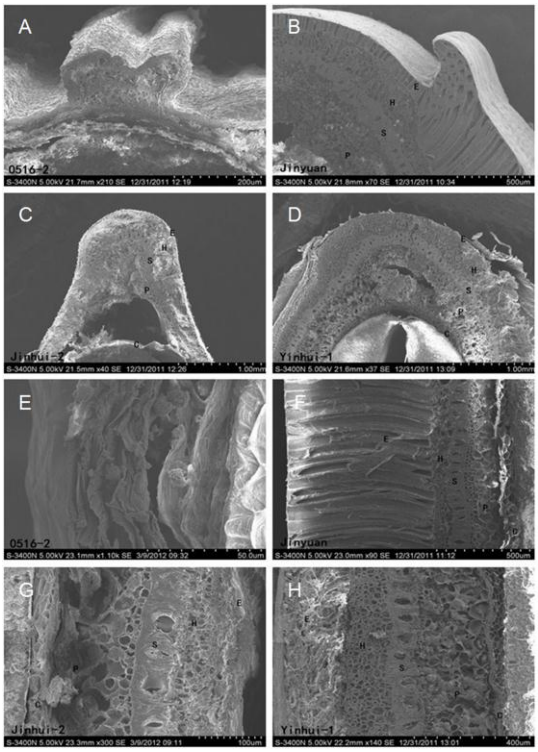

Fig. 3: Morphological structures of five cell layers in the seed coats and seed margins of mature squash seeds with four different genotypes through scanning electron microscopy method. Morphological structures of five cell layers in the seed margins of mature squash seeds of 0516-2 (A), Jinyuan (B), Jinhui-2 (C), Yinhui-1 (D) and in the seed coats of mature squash seeds of 0516-2 (E), Jinyuan (F), Jinhui-2 (G), Yinhui-1 (H). The *letters* indicate five cell layers, respectively. *E* epidermis, *H* hypodermis, *S* sclerenchyma, *P* parenchyma and *C* chlorenchyma. Bars = 50 um (E), 100 um (G), 200 um (A), 400 um (H), 500 um (B, F) and 1000 um (C, D)

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