

SEED COAT DEVELOPMENT AND ITS EVOLUTIONARY IMPLICATIONS IN DIPLOID AND AMPHIDIPOID BRASSICA SPECIES

CHANG-LI ZENG^{1,2}, XIAO-MING WU³, AND JIAN-BO WANG^{1*}

¹Key Laboratory of MOE for Plant Developmental Biology, College of Life Sciences,
Wuhan University, Wuhan, 430072, China

²College of Medicine and Life Sciences, Jiangnan University, Wuhan, 430056, China

³Institute of Oil Crop Research, Chinese Academy of Agricultural Sciences,
Wuhan, 430062, China

Received January 2, 2006; revision accepted March 5, 2006

Variations in seed coat patterns are successfully employed in the establishment of evolutionary relationships. This research addressed the evolutionary implications of the anatomy of the developing seed coat in amphidiploid *Brassica* species. Light microscopy was used to study the development of seed coat structure in six species (15 accessions): three amphidiploids and their three diploid parents. Four types of epidermis layer, six types of subepidermis and nine types of palisade layer could be recognized during the course of the seed coat developmental process. The types of epidermis and subepidermis layers in diploids and amphidiploids changed similarly during seed development. Although there was little difference in the types of palisade layer among the accessions of diploids and amphidiploids at the early stages, many particular types appeared in these species at middle and later developmental stages. Palisade layer development varied in complicated ways in amphidiploids. Some accessions showed palisade layer types intermediate between the two putative parents, while others resembled only one of the two diploid ancestors. The developmental types of epidermis and subepidermis did not show the relationships between amphidiploids and diploids. However, the development of types of palisade layer apparently can serve as an excellent character indicating the seed coat evolution of amphidiploids.

Key words: *Brassica*, diploids and amphidiploids, seed coat anatomy, seed coat development.

INTRODUCTION

The development of seed coat patterns is successfully employed in the identification and classification of taxa, and in establishing phylogenetic and evolutionary relationships (Koul et al., 2000; Zeng et al., 2004; Karcz et al., 2005). The seed coat or testa is the interface between the embryo and its exterior environment, and it has many important functions (van Dongen et al., 2003). A large body of literature describes the anatomy of mature seed coats in *Brassica* species (Mulligan and Bailey, 1976; Iwanowska et al., 1994; Ren and Bewley, 1998). The developing seed coats of the family Cruciferae have also been studied (Harris, 1991; van Caseele et al., 1982; Beeckman et al., 2000; Western et al., 2000; Windsor et al., 2000). However, up to now there has been very little literature that takes a phylogenetic approach to the comparative developmental anatomy of seed coat

to explore the evolutionary relationships between polyploids and their putative parents.

Polyploidy is an important evolutionary process in plants (Liu and Wendel, 2003). Angiosperms, and even nearly all organisms, are polyploids to some extent; many aspects of research on polyploids have been reported (for review see: Soltis, 2005; Pires et al., 2004). It is well known that the developmental traits of polyploids are the product of gene expression and regulation. Therefore the comparative developmental morphology of a trait that exhibits great diversity on both evolutionary and human time scales is an important area for study (Richardson et al., 1999; Applequist et al., 2001). *Brassica* species not only have great economic significance in agriculture (Kumar, 1995) but also are regarded as the perfect material for studying evolutionary relationships because of their abundant amphidiploids and parent diploids (Schranz and Osborn, 2004). Based on interspecific hybridization and cytogenetic evidence,

*e-mail: jbwang@whu.edu.cn

TABLE 1. List of *Brassica* accessions studied for seed coat anatomy

Species	Accession no.*	Common name	Ploidy level	Genome	Source
<i>B. rapa</i> (syn. <i>B. campestris</i>)	0074	Oil rape	2x	AA (n=10)	IOCR
	0113	Oil rape	2x	AA (n=10)	IOCR
	0265	Oil rape	2x	AA (n=10)	IOCR
<i>B. nigra</i>	3518	Black mustard	2x	BB (n=8)	IOCR
<i>B. oleracea</i>	930	Cabbage	2x	CC (n=9)	VIW
	6111	Flowering kale	2x	CC (n=9)	WU
<i>B. napus</i>	2685	Rapeseed	4x	AACC (n=19)	IOCR
	1256	Rapeseed	4x	AACC (n=19)	IOCR
	1219	Rapeseed	4x	AACC (n=19)	IOCR
<i>B. juncea</i>	2194	India mustard	4x	AABB (n=18)	IOCR
	2316	India mustard	4x	AABB (n=18)	IOCR
	0857	India mustard	4x	AABB (n=18)	IOCR
<i>B. carinata</i>	3529	Ethiopian mustard	4x	BBCC (n=17)	IOCR
	3534	Ethiopian mustard	4x	BBCC (n=17)	IOCR
	3524	Ethiopian mustard	4x	BBCC (n=17)	IOCR

**B. oleracea* 930 was supplied by the Vegetable Institute of Wuhan (VIW), China. *B. oleracea* 6111 was supplied by Wuhan University (WU), China, and the other accessions were supplied by the Institute of Oil Crop Research, Chinese Academy of Agricultural Sciences (IOCR, CAAS). The accession numbers mentioned are the same as used by the suppliers.

U (1935) proposed interrelationships among six *Brassica* species, a scheme known as the U triangle. There are three amphidiploids, *Brassica napus*, *B. carinata*, and *B. juncea*, and their putative diploid relatives, *B. rapa*, *B. nigra* and *B. oleracea* (Warwick et al., 1993). Most seed coats of *Brassica* are derived from the outer and inner integuments of the ovule, composed of an epidermis layer which sometimes is mucilaginous, a palisade layer, several layers of crushed parenchyma cells and a single layer of aleurone cells (Bouman, 1975; Corner, 1976; van Caseele et al., 1982). Although there is a large body of literature describing seed coat surface features in *Brassica*, little has been known about the change of patterns in the three other layers of seed coat during all of seed development, and there are few studies on the relationships between amphidiploid and their putative diploid *Brassica* relatives in terms of evolutionary developmental biology. The present study aimed (i) to analyze the changes of seed coat structure during all its developmental stages in diploid and amphidiploid *Brassica* species, and (ii) to address the evolutionary implications of the anatomy of the developing seed coat in amphidiploid *Brassica* species.

MATERIALS AND METHODS

PLANT MATERIAL

A list of species along with the accessions investigated is given in Table 1. Seeds of these accessions were selected and planted in the field at Wuhan

University. The accessions of *B. oleracea* and *B. napus* were planted in the last decade of September 2003, and the others in the last decade of October 2003. They all began to flower at the end of March 2004. During flowering stages, nylon netting was used to cover the plants of each accession in order to isolate them from each other and prevent pollen transfer between accessions.

Flowers were tagged on the days of full anthesis; each accession had a hundred flowers tagged at the same time. Seed production was ensured by hand-pollination. Immature seeds were collected at 5, 10, 20 and 30 days after pollination (DAP), and mature seeds were also examined.

METHODS

Seeds for light microscopy were fixed in 2.5% glutaraldehyde buffered with 0.1 mol l⁻¹ phosphate buffer (pH 7.2) for 3 h at room temperature and then fixed in 1% osmium tetroxide for 2 h. The material was dehydrated in an acetone series. The acetone was gradually replaced with Spurr's resin and the samples were embedded in rubber pans and polymerized at 70°C for 16 h. Thick sections (1–2 µm) were prepared with an MT-X microtome using glass knives. Sections were stained with 0.5% toluidine blue solution and photographed through a Leica Dmirb microscope with a Digital 60675-DEI-750 camera. For uniformity, the areas of mid-seed at the adaxial side according to the established seed coat designations (Sumner and van Caseele, 1987; Ren and Bewley, 1998) were studied, a minimum of five seeds from each accession.

RESULTS

DEVELOPMENT OF EACH SEED COAT CELL LAYER
IN DIPLOID BRASSICA SPECIES

In this paper, the terminology of Buth and Ara (1981), Vaughan and Whitehouse (1971) was followed, and some extra types were added to describe the anatomical changes of each layer of seed coat during the whole developmental process (Tab. 2). Based on the seed coat sections, a total of five types of epidermis layer were recognized: ellipse, flattened, intestine-shaped-n, intestine-shaped-m, and polygonal. The types of subepidermis layer could be named giant size, flattened, polygonal, circular, ellipse, and crushed. The palisade layer could be separated into nine types: ladder-shaped, polygon-a, polygon-b, polygon-c, U-shaped-a, U-shaped-b, U-shaped-c, U-shaped-d, and threadlike (Figs. 1–5; Tab. 3).

There were six accessions of diploid *Brassica* species in this study: three accessions of *B. rapa*, one accession of *B. nigra*, and two accessions of *B. oleracea*. Seeds of all three accessions of *B. rapa* matured at 35 DAP. There were four types of epidermis, two types of subepidermis and five types of the palisade layer in this species. The types of the epidermis layer in the three accessions of *B. rapa* changed very similarly: ellipse at 5 DAP, polygonal at 10 DAP in *B. rapa* 0074 and *B. rapa* 0113, but ellipse at 10 DAP in *B. rapa* 0265. Then the types changed to intestine-shaped-n at 20 and 30 DAP, and finally to flattened at the mature stage (Fig. 1a–e; Tab. 3). The development of the subepidermis and palisade layer of seed coat structure followed similar courses in the three accessions of *B. rapa*; for example, the subepidermis types were ellipse at 5, 10, 20, and 30 DAP, and flattened at the mature stage in all three. The types of palisade layer were polygon-a at 5 and 10 DAP, polygon-b at 20 DAP, polygon-c at 30 DAP, and U-shaped-a at the mature stage in this species (Fig. 1a–e; Tab. 3).

The maturity time for *B. oleracea* seed was 40 DAP. Variation in the types of epidermis in the two accessions of *B. oleracea* was almost the same: both were ellipse at 5 DAP and 20 DAP, intestine-shaped-n at 30 DAP and flattened at the mature stage; the exceptions were that at 10 DAP the epidermis cell type was ellipse for *B. oleracea* 930 and polygonal for *B. oleracea* 6111 (Fig. 1f–j; Tab. 3). There was no difference between the progressions of type of subepidermis and palisade layer cells in the two accessions of *B. oleracea*. For example, in the subepidermis layer the cells of both accessions were ellipse type at 5, 10, 20 and 30 DAP, and flattened at the mature stage (Fig. 1f–j; Tab. 3). The palisade layer cell types in this species changed from poly-

TABLE 2. Terminology terms adopted to describe the anatomical features of each testa layer during seed development (modified from Buth and Ara, 1981; Vaughan and Whitehouse, 1971)

Seed coat layer	Shape of cells	Explanation in detail	
Epidermis	Flattened	A kind of long strip; thin and flat	
	Intestine-shaped-n	Long tubular shape, sometimes rough or even on the surface; no mucilage in the cells	
	Intestine-shaped-m	Long tubular shape; sometimes rough or even on the surface; mucilage in the cells	
	Polygonal Ellipse	4-6-sided; angular Shape close to elongated circle	
Subepidermis	Giant size	Very large in volume; irregular in shape	
	Flattened	A kind of long strip; thin and flat	
	Polygonal Ellipse Circular Crushed	4-6-sided; angular Cell looks like an ellipse Cell looks like a circle Parenchymatous cells were compressed and indiscernible.	
	Palisade layer	Ladder-shaped Threadlike	Cell looks like a trapezoid Structure originates from the radial walls of the palisade; short and thick threads
		Polygon-a Polygon-b Polygon-c U-shaped-a U-shaped-b U-shaped-c U-shaped-d	Composed of one or two layers of 4-6-sided cells Composed of many layers of 4-6-sided shape; only edges of cells are thickened 4-6-sided; all cell walls are thickened Cell walls are thick; U-shaped; bulbous or conical Cell walls are thin; U-shaped; bulbous or conical Shape intermediate between U-shaped-a and U-shaped-b in the thickness of cell walls Shape intermediate between U-shaped and threadlike

gon-a at 5 and 10 DAP to ladder-shaped at 20 DAP, polygon-b at 30 DAP and U-shaped-b at the mature stage (Fig. 1f–j; Tab. 3).

TABLE 3. Overview of the variation of seed coat cell type in *Brassica* species at different DAP. E – epidermis; S – subepidermis; Pal – palisade layer; El – ellipse; Po – polygonal; Poa – polygon-a; Pob – polygon-b; Poc – polygon-c; Ci – circular; Fl – flattened; Gi – giant size; Inn, Inm – intestine-shaped-n, intestine-shaped-m; Th – thread-shaped; La v ladder-shaped; Cr – crushed; Usa – U-shaped-a; Usb – U-shaped-b; Usc – U-shaped-c; Usd – U-shaped-d

Taxon	DAP	5 DAP			10 DAP			20 DAP			30 DAP			Mature stage		
	Accession	E	S	Pal	E	S	Pal	E	S	Pal	E	S	Pal	E	S	Pal
<i>B. rapa</i>	0074	El	El	Poa	Po	El	Poa	Inn	El	Pob	Inn	El	Poc	Fl	Fl	Usa
	0113	El	El	Poa	Po	El	Poa	Inn	El	Pob	Inn	El	Poc	Fl	Fl	Usa
	0265	El	El	Poa	El	El	Poa	Inn	El	Pob	Inn	El	Poc	Fl	Fl	Usa
<i>B. nigra</i>	3518	El	El	Poa	Po	Ci	Poa	Inm	Gi	Pob	Inm	Gi	Th	Inm	Gi	Th
<i>B. oleracea</i>	930	El	El	Poa	El	El	Poa	El	El	La	Inn	El	Pob	Fl	Fl	Usb
	6111	El	El	Poa	Po	El	Poa	El	El	La	Inn	El	Pob	Fl	Fl	Usb
<i>B. napus</i>	2685	El	El	Poa	Po	El	Poa	El	El	La	Po	El	Poc	Fl	Cr	Usc
	1256	El	El	Poa	El	El	Poa	El	El	Pob	Inn	El	Pob	Fl	Cr	Usc
	1219	Po	El	Poa	El	El	Poa	El	El	Pob	El	El	Poc	Fl	Cr	Usa
<i>B. juncea</i>	2194	El	El	Poa	El	El	Poa	In	Gi	Pob	Inn	Gi	Poc	Fl	Cr	Th
	2316	El	El	El	El	El	Poa	In	Gi	Pob	Inn	Gi	Pob	Fl	Cr	Usa
	0857	El	El	Poa	El	El	Poa	El	Gi	Pob	Inn	Gi	Pob	Inn	Cr	Usa
<i>B. carinata</i>	3529	El	El	Poa	El	El	Poa	Inm	Gi	Pob	Inm	Gi	Pob	Inm	Gi	Usd
	3534	El	El	Poa	Po	El	Poa	Inn	Gi	Pob	Inm	Gi	Pob	Inm	Gi	Th
	3524	El	El	El	Inn	El	Poa	Inn	Gi	La	Inm	Gi	Pob	Inm	Gi	Usb

Seeds of the wild diploid *B. nigra* matured at 30 DAP. Only four types appeared in the epidermis layer, two types in the subepidermis layer and three types in the palisade layer during the course of seed development: ellipse in epidermis and subepidermis at 5 DAP; polygonal in epidermis and circular-shaped in subepidermis at 10 DAP; intestine-shaped-m in epidermis and giant size in the subepidermis layer from 20 DAP to mature stage; and in the palisade layer, polygon-a at 5 and 10 DAP, polygon-b at 20 DAP, and threadlike at 30 DAP and mature stage (Fig. 1k–o; Tab. 3).

There was less difference between the accessions of *B. rapa* or *B. oleracea* in the change of subepidermis and palisade layer cell types during the whole developmental process. Some interesting types were observed during the development of each layer of seed coat. The ellipse was the main type in these diploids in the development of the epidermis and subepidermis layer. The intestine-shaped-n type was a very important one in *B. rapa* at the middle and later stages, and the flattened type at the mature stage in *B. rapa* and *B. oleracea*. The intestine-shaped-m type characterized the epidermis layer in *B. nigra* for all of its later development, and circular and giant size were important types for its subepidermis layer. For the palisade testa layer, polygon-c and U-shaped-a were characteristic types during the development of seed in *B. rapa*, whereas threadlike was the special type in *B. nigra*, and ladder-shaped and U-shaped-b in *B. oleracea*.

DEVELOPMENT OF EPIDERMIS AND SUBEPIDERMIS IN AMPHIDIPOID *BRASSICA* SPECIES

Seed coat development was studied in the nine accessions of amphidiploid *Brassica* species: three accessions of each amphidiploid *Brassica* species. *B. napus* matured at 40 DAP, as did *B. oleracea*. The seeds of *B. juncea* and *B. carinata* matured at 35 DAP; for *B. juncea* that was the same time as one of its ancestral parents, and the maturity time of *B. carinata* was intermediate between its ancestral parents, *B. oleracea* and *B. nigra*.

For *B. napus*, the epidermis cell type was ellipse in accessions 2685 and 1256 but polygonal in 1219 at 5 DAP; later, it was polygonal in accession 2685 but ellipse in 1256 and 1219 at 10 DAP. They were all typified by ellipse at 20 DAP and flattened at maturity, but varied markedly at 30 DAP: polygonal in *B. napus* 2685, intestine-shaped-n in *B. napus* 1256, and ellipse in *B. napus* 1219 (Fig. 2d,i,n). The type of subepidermis in the three accessions of *B. napus* had the same developmental process: ellipse shape at 5, 10, 20 and 30 DAP, and distinctly crushed at the mature stage (Tab. 3).

For the three accessions of *B. juncea*, the types of epidermis cells changed very similarly. They all had ellipse patterns at 5 DAP and 10 DAP, and intestine-shaped-n at 30 DAP. At 20 DAP, however, intestine-shaped-n appeared in accessions 2194 and 2316, but the ellipse type in 0857. Similarly, at the mature stage the type of epidermis in accessions 2194 and 2316 was ellipse, but intestine-shaped-n type in *B. juncea* 0857 (Fig. 3e,j,o; Tab. 3). The subepidermis layer changed similarly in the three

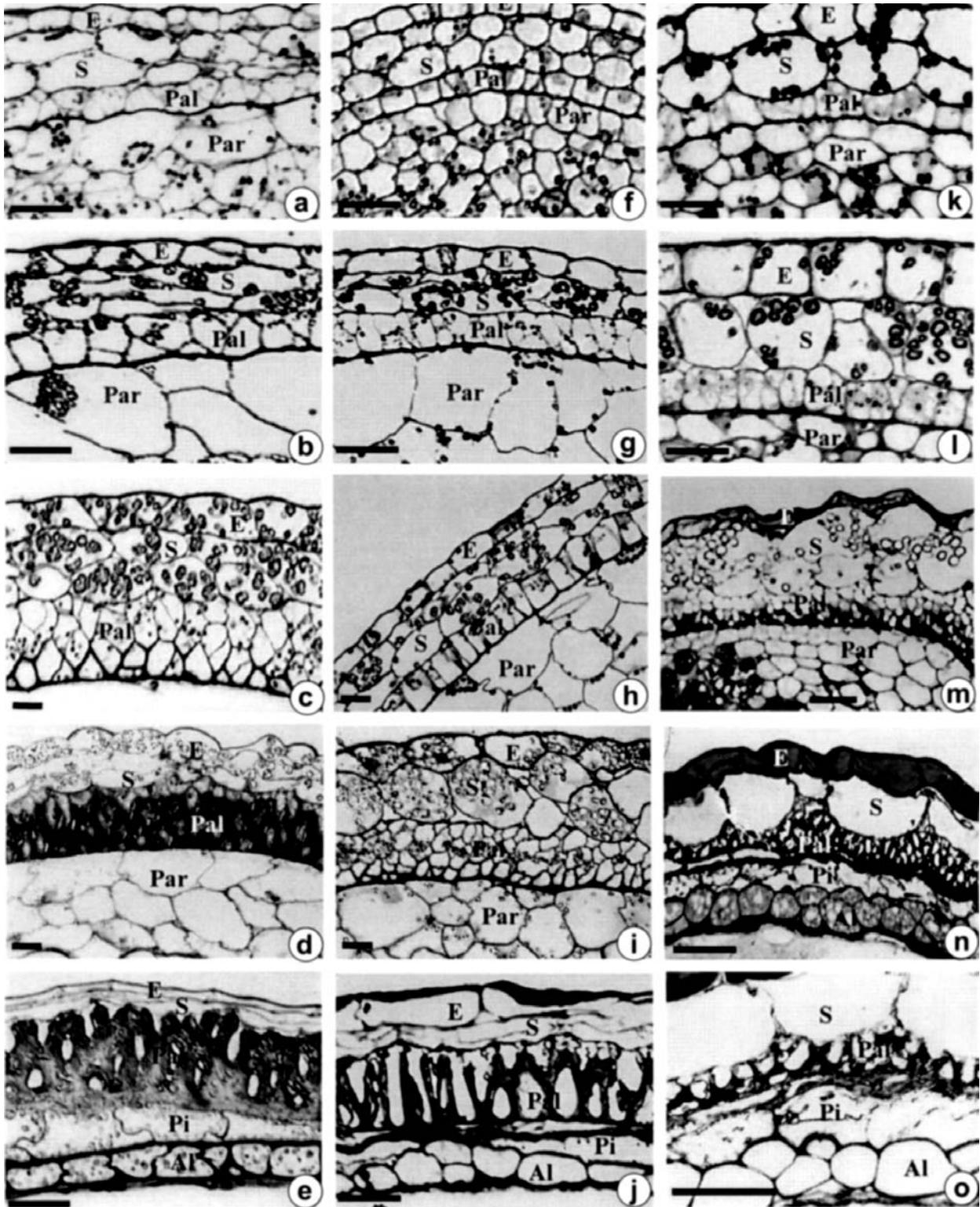


Fig. 1. Transsections of developing seed coat structure of diploid *Brassica* species. (a-e) *B. rapa* 0074. (a) At 5 DAP, (b) At 10 DAP, (c) At 20 DAP, (d) At 30 DAP, (e) At mature stage. (f-j) *B. oleracea* 930. (f) At 5 DAP, (g) At 10 DAP, (h) At 20 DAP, (i) At 30 DAP, (j) At mature stage. (k-o) *B. nigra* 3518. (k) At 5 DAP, (l) At 10 DAP, (m) At 20 DAP, (n,o) At 30 DAP. E - epidermis; S - subepidermis; Pal - palisade layer; Par - parenchymatous layer; Pi - pigmented layer; Al - aleurone layer. Bars = 25 μ m.

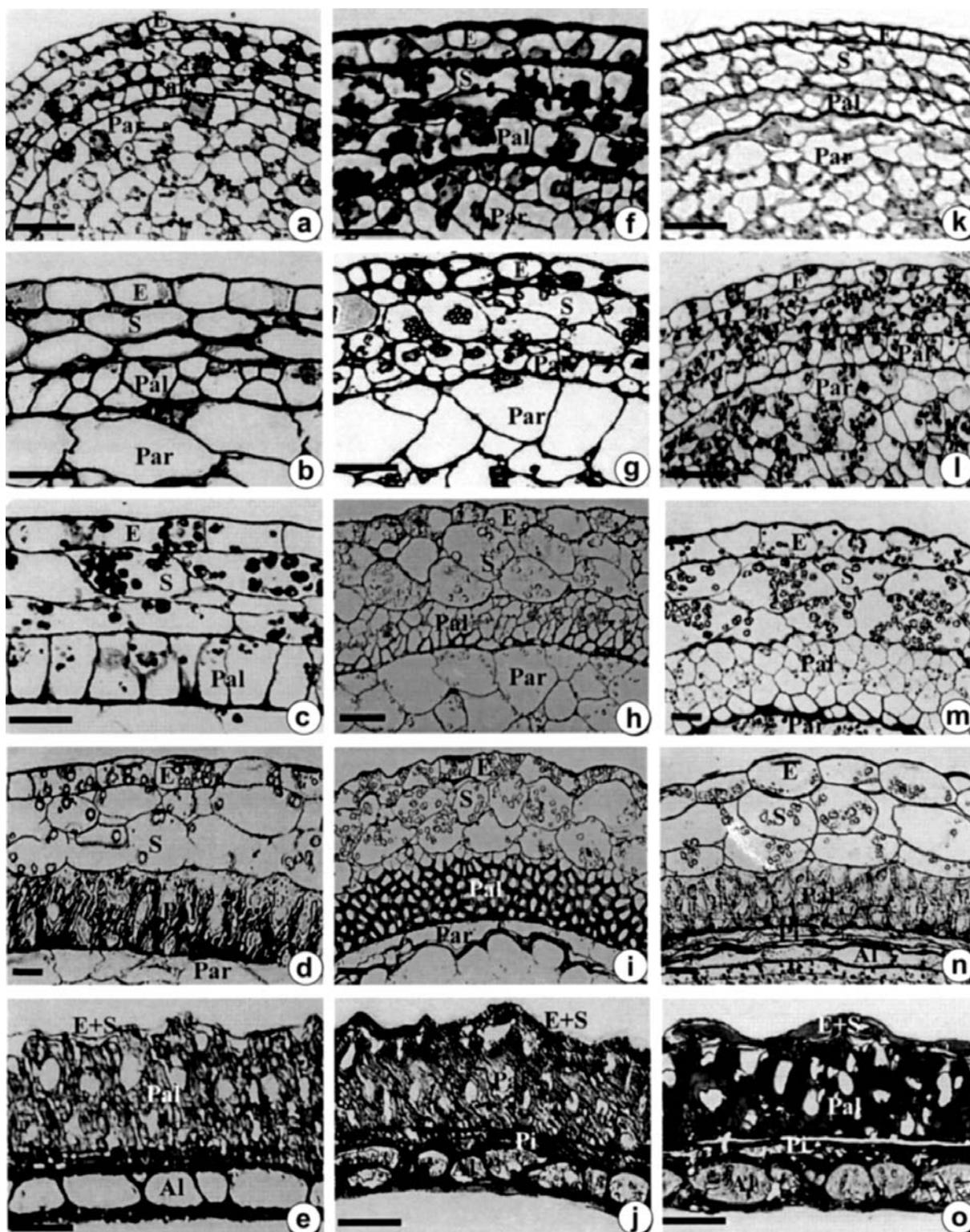


Fig. 2. Transsections of developing seed coat structure of *B. napus*. (a–e) *B. napus* 2685. (a) At 5 DAP, (b) At 10 DAP, (c) At 20 DAP, (d) At 30 DAP, (e) At mature stage. (f–j) *B. napus* 1256. (f) At 5 DAP, (g) At 10 DAP, (h) At 20 DAP, (i) At 30 DAP, (j) At mature stage. (k–o) *B. napus* 1219. (k) At 5 DAP, (l) At 10 DAP, (m) At 20 DAP, (n) At 30 DAP, (o) At mature stage. E – epidermis; S – subepidermis; Pal – palisade layer; Par – parenchymatous layer; Pi – pigmented layer; Al – aleurone layer. Bars = 25 μ m.

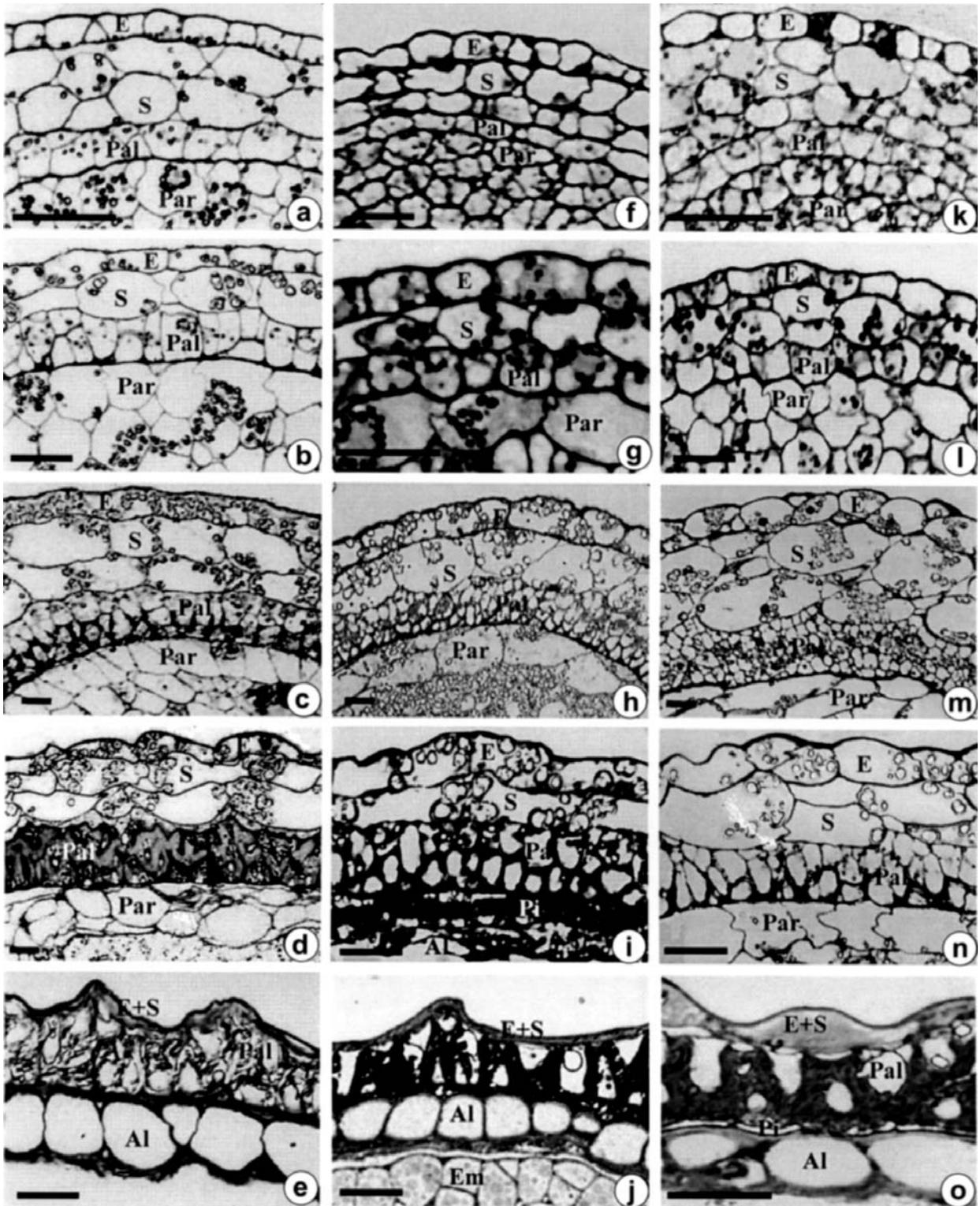


Fig. 3. Transsections of developing seed coat structure of *B. juncea*. (a-e) *B. juncea* 2194. (a) At 5 DAP, (b) At 10 DAP, (c) At 20 DAP, (d) At 30 DAP, (e) At mature stage. (f-j) *B. juncea* 2316. (f) At 5 DAP, (g) At 10 DAP, (h) At 20 DAP, (i) At 30 DAP, (j) At mature stage. (k-o) *B. juncea* 0857. (k) At 5 DAP, (l) At 10 DAP, (m) At 20 DAP, (n) At 30 DAP, (o) At mature stage. E – epidermis; Em – embryo; S – subepidermis; Pal – palisade layer; Par – parenchymatous layer; Pi – pigmented layer; Al – aleurone layer. Bars = 25 μ m.

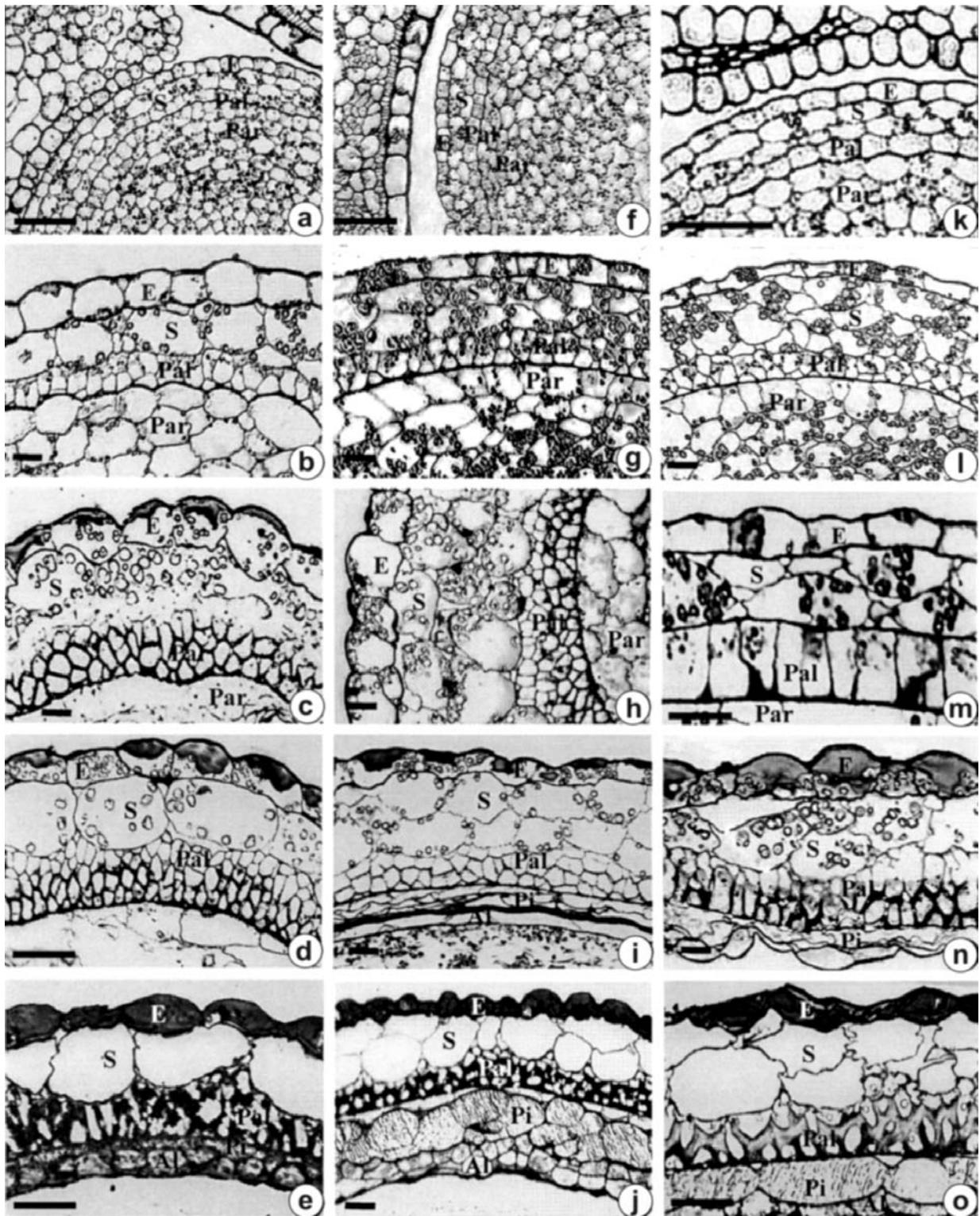


Fig. 4. Transsections of developing seed coat structure of *B. carinata*. (a–e) *B. carinata* 3529. (a) At 5 DAP, (b) At 10 DAP, (c) At 20 DAP, (d) At 30 DAP, (e) At mature stage. (f–j) *B. carinata* 3534. (f) At 5 DAP, (g) At 10 DAP, (h) At 20 DAP, (i) At 30 DAP, (j) At mature stage. (k–o) *B. carinata* 3524. (k) At 5 DAP, (l) At 10 DAP, (m) At 20 DAP, (n) At 30 DAP, (o) At mature stage. E – epidermis; S – subepidermis; Pal – palisade layer; Par – parenchymatous layer; Pi – pigmented layer; Al – aleurone layer. Bars = 25 μ m.

accessions of *B. juncea*, such as ellipse at 5 and 10 DAP, giant size at 20 and 30 DAP, and completely crushed at the mature stage (Tab 3).

There were four types of epidermis layer cell in the three accessions of *B. carinata*. The type was ellipse at 5 DAP, and intestine-shaped-m at 30 DAP and mature stage. There were differences in epidermis cell types between the three accessions of *B. carinata* at 10 and 20 DAP. At 10 DAP the ellipse type appeared in *B. carinata* 3529, but polygonal in accession 3534 and intestine-shaped-n in 3524 (Fig. 4b,g,l; Tab. 3). At 20 DAP, *B. carinata* 3529 showed intestine-shaped-m cell type in the epidermis layer, and accessions 3534 and 3524 showed intestine-shaped-n. Two types of subepidermis layer were observed in *B. carinata* during the course of development: ellipse and giant. Here there were no differences between the three accessions of *B. carinata*. They all had ellipse type at 5 and 10 DAP, and giant size at 20 and 30 DAP and mature stage (Tab. 3).

DEVELOPMENT OF PALISADE IN AMPHIDIPOID BRASSICA SPECIES

The palisade layer occurs next to the subepidermis and is the most characteristic layer of the *Brassica* seed coat. There was no difference between the three *B. napus* accessions in early developmental stages but distinct differences in the middle and later stages. For *B. napus* 2685, four types of cell in the palisade layer were observed: first the polygon-a type at 5 and 10 DAP, followed by ladder-shaped, polygon-c and U-shaped-c (Fig. 2a-e; Tab. 3). The types of palisade layer cells in *B. napus* 1256 were polygon-a type at 5 and 10 DAP, polygon-b at 20 and 30 DAP, and U-shaped-c at the mature stage (Fig. 2f-j; Tab. 3). *B. napus* 1219 was completely consistent with *B. rapa*, one of its ancestral parents, with changes from the polygon-a type to polygon-b, polygon-c and U-shaped-a (Fig. 2k-o; Tab. 3).

For *B. juncea*, the developmental types of palisade layer cells in the three accessions were largely similar in the early and middle stages: polygon-a at 10 DAP and polygon-b at 20 DAP, and polygon-a in *B. juncea* 2194 and 0857, but ellipse in *B. juncea* 2316 at 5 DAP. The palisade layer cell types differed markedly in later stages. For example, accession 2194 had the polygon-c type at 30 DAP and threadlike at the mature stage, but 2316 and 0857 shared polygon-b at 30 DAP and U-shaped-a at the mature stage (Fig. 3d,e,i,j,n,o; Tab. 3). In *B. carinata*, all three accessions had polygon-a at 10 DAP and polygon-b at 30 DAP. (Fig. 4b,d,g,i,l,n). However, *B. carinata* 3529 and 3534 had polygon-a at 5 DAP and polygon-b type at 20 DAP, but accession 3524 featured ellipse and ladder-shape types in the respective stages. The differences were conspicuous at the mature stage: U-shaped-d type for accession 3529,

threadlike for 3534 and U-shaped-b for 3524 (Fig. 4e,j,o).

In summary, complicated changes in testa layer cell types each appeared during seed development in amphidiploid *Brassica* species. The ellipse type was the main one in the epidermis and subepidermis layer at early developmental stages, while intestine-shaped-n, intestine-shaped-m and flattened types played important roles in the epidermis at middle and mature stages. The giant size and crushed types were important ones in the subepidermis layer at middle and mature stages. Furthermore, the three accessions of each amphidiploid did not show differences in variation of the palisade layer at early developmental stages, but distinct changes appeared at middle and later developmental stages.

DISCUSSION

COMPARISON OF EPIDERMIS AND SUBEPIDERMIS LAYER DEVELOPMENT BETWEEN AMPHIDIPOIDS AND THEIR PUTATIVE PARENTS

Vaughan and Whitehouse (1971) remarked that the testa is probably of the greatest value in determining relationships among the Cruciferae, and distinguished fifteen basic types in mature epidermis cells. In the present study, the changes of cell type in the epidermis layer varied little between the three amphidiploids at early developmental stages, but there were obvious differences between them at middle and later developmental stages. At 30 DAP, for example, the polygonal type appeared in *B. napus* 2685, and intestine-shaped-n in *B. napus* 1256, but ellipse type in *B. napus* 1219. Intestine-shaped-n appeared in *B. juncea* and intestine-shaped-m in *B. carinata* at that same stage. Intestine-shaped-m appeared in *B. carinata* at middle and later developmental stages, but not in the other two amphidiploid *Brassica* species. Among the diploids, therefore, the intestine-shaped-m type characteristic of the epidermis layer in *B. nigra* at those stages should draw special attention.

Mucilage is commonly found in the epidermal cell layer of Cruciferae during seed maturation (Buth et al., 1987; Vaughan and Whitehouse 1971; Windsor et al., 2000; Western et al., 2000), forming a gel-like capsule surrounding the seed upon imbibition (Western et al., 2001). Some *Brassica* species such as *B. campestris* (van Caseele et al., 1981), *B. nigra* (Bouman, 1975) and *B. juncea* (Setia and Richa, 1989) were reported to produce epidermal mucilage, but there are conflicting findings. Vaughan and Whitehouse (1971) reported that *B. campestris*, *B. juncea* (Indian and European materials), *B. oleracea* and *B. napus* did not produce mucilage. Our results were completely consistent with the observa-

tions of Vaughan and Whitehouse (1971). Of the three diploid *Brassica*, only *B. nigra* had a mucilaginous epidermal cell layer; the intestine-shaped-m type of epidermis cell was observed only in *B. nigra* but not in the other two diploids. The three accessions of *B. carinata* also produced mucilage at middle and later developmental stages. Apparently this amphidiploid inherited the characteristic of one of its putative parents, *B. nigra*.

In a study of seed coat anatomy of mature seed, Buth and Ara (1981) found the subepidermis layer cells of *B. napus* to be crushed, and flattened in *B. juncea*. Our observations were consistent with that result for *B. napus*, but not for *B. juncea*; Vaughan and Whitehouse (1971) also found crushed type for that species. There was little difference in the types of subepidermis layer among these amphidiploids at early developmental stages. For example, all amphidiploid accessions had the ellipse type of cell shape at 5 and 10 DAP, like five of the six diploids at those stages. However, distinct differences appeared at the middle and later stages: *B. napus* had the same type as that of *B. rapa* and *B. oleracea* at 20 and 30 DAP, but *B. juncea* and *B. carinata* had giant size type at 20 and 30 DAP, like that of *B. nigra*, one of their putative parents. *B. napus* accessions had their own particular type at the mature stage which did not appear in their corresponding parental ancestors at the same stage, but *B. carinata* shared the giant size type with *B. nigra* at the mature stage.

Although there was some information implying the relationship between amphidiploids and their putative diploids, such as *B. carinata* and *B. nigra* having the characteristic of producing mucilage (or intestine-shaped-m type in the epidermis layer), and *B. carinata* and *B. juncea* sharing the same type of subepidermis with *B. nigra*, one of their putative ancestors, at 20 and 30 DAP, the type of epidermis and subepidermis layer probably changed in the accessions (such as in the conflicting reports above). Thus, in this study it was difficult to determine the relationships between amphidiploids and their ancestors based on the variation of types of epidermis and subepidermis cell layers.

EVOLUTIONARY IMPLICATIONS OF THE PALISADE LAYER IN AMPHIDIPOIDS

The type of epidermis and subepidermis testa layer had no significance for the phylogenetic and evolutionary relationships in *Brassica*. The palisade layer, on the other hand, is the prominent cell layer of the mature seed coat (Rahman et al., 2001), and its variations have often been utilized in taxonomic studies (Vaughan and Whitehouse, 1971). Some characteristics of this layer at the mature stage, such as cell type, have played an important role in ana-

lyzing the relationships among *Brassica* species (Buth and Ara, 1981). Very interestingly, the types of palisade layer in amphidiploid *Brassica* were very similar at corresponding early and middle developmental stages. In later development, however, the palisade layer in the three amphidiploids displayed cell types either intermediate between the two putative parents or resembling only one of them. For *B. napus* 1219, the types of palisade layer matched those of one of its ancestral parents, *B. rapa*, during the whole developmental process (Tab. 3). Similarly, the palisade cell patterns of *B. juncea* 2316 and 0857 resembled one of their putative ancestors, *B. rapa*; they shared the same U-shaped-a type at the mature stage (Figs. 1e, 3j,o). *B. carinata* 3524 resembled one of its putative parents, *B. oleracea*, not *B. nigra*; it had the same ladder-shape at 20 DAP and U-shaped-b at the mature stage as *B. oleracea* (Figs. 1h,j, 4m,o). On the other hand, *B. carinata* 3534 was more similar to *B. nigra* during the whole developmental process. For example, it shared threadlike type at the mature stage, regarded as the characteristic type of *B. nigra* (Figs. 1o, 4j). These cases suggest that the accessions showed the characteristics of only one of their ancestral parents.

The palisade layer of some accessions of *Brassica* amphidiploids showed an intermediate pattern between the two putative parents. For example, *B. napus* 2685 had the ladder-shaped pattern at 20 DAP, like *B. oleracea* at the same developmental stage (compare Figs. 1h and 2c), but shared the characteristic pattern of *B. rapa* at 30 DAP (compare Figs. 1d and 2d). The palisade cell type at the mature stage was U-shaped-c, intermediate between *B. rapa* and *B. oleracea*, its two ancestral parents. The palisade types of *B. carinata* 3529 and *B. juncea* 2194 also resembled those of both their putative ancestors. *B. carinata* 3529 had the U-shaped-d type, synthesizing the characteristics of the palisade layer in *B. nigra* and *B. oleracea* at the mature stage (Figs. 1j,o, 4e). The above results are generally consistent with Zeng et al.'s (2004) observations of seed coat microsculpture in *Brassica*.

Seed coat microsculpture and seed coat structure in mature dry seeds are useful taxonomic features of the family Cruciferae. In the present study we comparatively analyzed the development of the three outer layers of seed coat testa in amphidiploid and diploid *Brassica* species, and found that the type of epidermis and subepidermis cells had little significance for evolutionary relationships. The developmental type of palisade layer, however, showed characteristic diversity among the studied *Brassica* species, suggesting that the dynamic development of the palisade layer can serve as a new parameter for examining the relationships between amphidiploids and their ancestral diploids.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 30521004, 30170063) and the Program for Changjiang Scholars and Innovative Research Teams at Universities.

REFERENCES

- APPLEQUIST WL, CRONN R, and WENDEL JF. 2001. Comparative development of fiber in wild and cultivated cotton. *Evolution and Development* 3: 1–13.
- BEECKMAN T, DE RYCKE R, VIANE R, and INZE D. 2000. Histological study of seed coat development in *Arabidopsis thaliana*. *Journal of Plant Research* 113: 139–148.
- BOUMAN F. 1975. Integument initiation and testa development in some Cruciferae. *Botanical Journal of the Linnean Society* 70: 213–299.
- BUTH GM, and ARA R. 1981. Seed coat anatomy of some cultivated Brassica. *Phytomorphology* 31: 69–78.
- BUTH GM, ARA R, and NARAYAN A. 1987. Seed and seed coat anatomy of some members of tribe Arabideae (Brassicaceae). *Phytomorphology* 37: 341–348.
- CORNER EJH. 1976. *The seeds of dicotyledons*, vol. II, Cambridge University Press, London.
- HARRIS WM. 1991. Seed coat development in radish (*Raphanus sativus* L.). *Phytomorphology* 41: 341–349.
- IWANOWSKA A, TYKARSKA T, KURAS M, and ZOBEL A. 1994. Localization of phenolic compounds in the covering tissues of the embryo of *Brassica napus* (L.) during different stages of embryogenesis and seed maturation. *Annals of Botany* 74: 313–320.
- KARCZ J, KSIAZCZYK T, and MALUSZYNSKA J. 2005. Seed coat patterns in rapid-cycling Brassica forms. *Acta Biologica Cracoviensia Series Botanica* 47: 159–165.
- KOUL KK, NAGPAL R, and RAINA SN. 2000. Seed coat microsculpturing in Brassica and allied genera (subtribes Brassicinae, Raphaninae, Moricandiinae). *Annals of Botany* 86: 385–397.
- KUMAR D. 1995. Salt tolerance in oilseed Brassicas: present status and future prospects. *Plant Breeding* 65: 1438–1447.
- LIU B, and WENDEL JF. 2003. Epigenetic phenomena and the evolution of plant allopolyploids. *Molecular Phylogenetics and Evolution* 29: 365–379.
- MULLIGAN GA, and BAILEY LG. 1976. Seed coat of some Brassica and Sinapis weedy and cultivated in Canada. *Economic Botany* 30: 143–148.
- PIRES JC, ZHAO J-W, SCHRANZ ME, LEON EJ, QUILJADA PA, LUKENS LN, and OSBORN TC. 2004. Flowering time divergence and genomic rearrangements in resynthesized Brassica polyploids (Brassicaceae). *Botanical Journal of the Linnean Society* 85: 675–688.
- RAHMAN MH, JOERSBO M, and POULSEN MH. 2001. Development of yellow-seeded Brassica napus of double low quality. *Plant Breeding* 120: 473–478.
- REN CW, and BEWLEY JD. 1998. Seed development, testa structure and precocious germination of Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). *Seed Science Research* 8: 385–397.
- RICHARDSON MK, MINELLI A, and COATES ML. 1999. Some problems with typological thinking in evolution and development. *Evolution and Development* 1: 5–7.
- SCHRANZ ME, and OSBORN TC. 2004. De novo variation in life history traits and responses to growth condition of resynthesized polyploid Brassica napus (Brassicaceae). *American Journal of Botany* 91: 174–183.
- SETIA RC, and RICHA M. 1989. Anatomical studies on siliqua wall and seed coat development in Brassica juncea (L.) Czern & Coss. *Phytomorphology* 39: 371–377.
- SOLTIS P S. 2005. Ancient and recent polyploidy in angiosperms. *New Phytologist* 166: 1–5.
- SUMNER MJ, and VAN CASEELE L. 1987. Ovule development in Brassica campestris: a light microscope study. *Canadian Journal of Botany* 66: 2459–2469.
- U N. 1935. Genome analysis in Brassica with species reference to the experimental formation of B. napus and peculiar mode of fertilization. *Japanese Journal of Botany* 7: 389–452.
- VAN CASEELE L, MILLS JT, SUMNER M, and GILLESPIE R. 1981. Cytology of mucilage production in the seed coat of Candle canola (Brassica campestris). *Canadian Journal of Botany* 59: 292–300.
- VAN CASEELE L, MILLS JT, SUMNER M, and GILLESPIE R. 1982. Cytological study of palisade development in the seed coat of Candle canola. *Canadian Journal of Botany* 60: 2469–2475.
- VAN DONGEN JT, ANKIE MH, WOUTERLOOD AM, VAN AELST AC, and BORSTLAP AC. 2003. Structure of the developing pea seed coat and the post-phloem transport pathways of nutrients. *Annals of Botany* 91: 1–9.
- VAUGHAN JG, and WHITEHOUSE JM. 1971. Seed structure and the taxonomy of the Cruciferae. *Botanical Journal of the Linnean Society* 64: 383–409.
- WARWICK SI, and BLACK LD. 1993. Molecular relationships in subtribe Brassicinae (Cruciferae, tribe Brassiceae). *Canadian Journal of Botany* 71: 906–918.
- WESTERN TL, SKINNER DJ, and HAUGHN GW. 2000. Differentiation of mucilage secretory cells of the Arabidopsis seed coat. *Plant Physiology* 122: 345–355.
- WINDSOR JB, SYMONDS VV, MENDENHALL J, and LLOYD AM. 2000. Arabidopsis seed coat development: morphological differentiation of the outer integument. *Plant Journal* 22: 483–493.
- ZENG CL, WANG JB, LIU AH, and WU XM. 2004. Seed coat microsculpturing changes during seed development in diploid and amphidiploid Brassica species. *Annals of Botany* 93: 555–566.