On the Genetics and Histology of the Hull-less Character of Styrian Oil-Pumpkin (*Cucurbita pepo* L.)

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Introduction: The seeds of *Cucurbita pepo* possess normally a thick, leathery seed coat (hull) due to the strong lignification of some of its testa layers. The mutant Styrian oil-pumpkin exhibits a complete lack of lignification of the testa. The proto-chlorophyll content of the fifth testa, chlorenchym, layer gives the seeds an olive-green color. This mutation emerged probably in the 1880's in the South-East of the then Austro-Hungarian-Monarchy (8).

Several studies on the seed coat character and on its genetics were carried out in the early 1950´s (1, 2, 3, 4, 5, 6). There is a general agreement on the existence of a major dominant gene responsible for a strong lignification of some of the testa layers of the hulled seed type. If both alleles in this locus are homozygous recessive, the seeds are basically of hull-less type. Nevertheless, they show a varying amount of lignification from a complete lack up to a clearly lignified margin and a thin lignified layer on the seed surface. For this variation different genetic interpretations were put forward, from a single gene with modifiers (6) up to 9 minor genes (8).

Histological investigations demonstrate that seed-coat development is identical in both seed types up to 15 to 20 days post-anthesis, resulting in five clearly distinguishable cell layers. The effect of the mutation becomes visible when lignification starts. While in the hulled type the second, third and fourth cell layers become strongly lignified, in the hull-less type, due to the lack of lignin deposition, these cell layers collapse (4, 7).

In this report we are reexamining the variation of the seed coat character of the hull-less segregants in three different crosses, using visual and histological investigations.

Materials and Methods

Crosses: The three crosses were: "SZG1 x True French", "Retzer Gold x Tigress" and "Lady Godiva x Bianco friulano". We used two Austrian and a French oil-pumpkin varieties as the hull-less parents, and two zucchini varieties and one crock-neck genotype, as the hulled parents. Crosses were made in the green house in the winter 1999/00. In summer 2000 F1 plants from each cross were grown and selfed. From a single F1 fruit per cross more than 100 F2 seeds were planted in summer 2001 and selfed to produce F3 seeds. To further follow segregation of the hull-less character, 10 F3 seeds from each of the F2 hull-less plants from the first cross (SZG1 x True French) were planted in the summer 2002 to produce F4 seeds. It should be noted that the seed coat of a F(n) plant can only be evaluated after harvesting the F(n+1) seeds, since the seed coat is of maternal origin.

Segregation and statistical analysis: Ripe seeds of F2-plants from each of the three crosses were harvested and dried. The hull-less seed types were then categorized based on the visual appearance of the seed coat: Category 1: completely hull-less phenotypes, similar to the hull-less parents (Fig. 1b). Category 3: the most lignified seeds among the hull-less segregants, with a thin but definitely lignified layer on the surface of the seed (Fig. 2b). Category 2: all the other seed coat phenotypes: seeds with margin but clearly less than in category 3 or even without margin, generally with a thin layer on the surface clearly less then in category 3, not necessarily

covering the whole seed surface (Fig. 2a as an example). To check Mendelian segregation, results were submitted to a χ^2 test.

Histological observation: Seeds from the parents and from the different categories established visually were fixed right after opening the fruit in the field in a formaldehyde (40%) - glacial acetic acid - ethanol (70%) mixture (2:1:7) for three days, then transferred to 96% ethanol. The material was then embedded in technovit (embedding solution). Cross sections of 5 µm from the seeds were mounted on slides, stained specifically for lignin with safranine-astrablue and toluidine blue which gives blue-greenish staining in colored pictures. Investigation was done under the light microscope.

Results and Discussion

Segregation ratios: In the three crosses, the seed coat of the F1 plants was completely hulled. Seed coats of the F2s segregated 3:1 hulled versus hull-less (Table 1). According to the data in Table 1, complete lignification of the seed coat is basically controlled by a single major gene with two alleles, with the genotypes "HH" and "Hh" for the completely hulled seed type (Fig. 1a) and "hh" for the hull-less seed type (Fig. 1b) as was postulated first by Schöniger (4). Nevertheless, the F2 hull-less progenies include a number of segregants the seeds of which exhibit a residual lignification, that appear to vary between the F2-segregants.

The segregation pattern of the three categories in the three crosses did not fit a 1:2:1 ratio (Table 2), assuming that an incompletely dominant secondary major gene is responsible for this residual lignification (5). This finding supports the critics on the two genes hypothesis of Schöniger (4, 5) by Grebenščikov (1) and Prym von Becherer (3). We studied the F3 progeny (10 plants) derived from the hull-less F2-plants of the cross (SGZ1 X True French) for further segregation of the three categories. If a secondary major gene is responsible for the phenotype of these categories, category 1 and 3 will not further segregate but category 2 will produce all three phenotypes (5). This was not the case, and from the 26 hull-lees F2-progenies only 5 progenies (1 from category 3, and 4 from category 1) appeared to be non-segregating. All other progenies showed a seemingly random segregation.

Histological analysis: The five layers described in the literature namely, epidermis (E), hypodermis (H), sclerenchyma (S), aerenchyma (Ae) and chlorenchyma (C) are present in the testa of the ripe parental hulled seeds, the layers H, S and Ae being strongly lignified on the seed surface (Fig. 3a) and in the margin (Fig. 3b). In the parental hull-less ripe seed testa, the four upper layers are collapsed into a hyaline without any trace of lignin (Fig. 4a and b).

The different degrees of the residual lignification observed in the testa of the hull-less seeds are shown in Figures 5 and 6. While the visual classification gave rise to three categories, the histological observations show a more or less continuous variation of lignin deposition.

In the testa of category 1 seeds like in the hull-less parent, only a thick hyaline of the four collapsed layers can be seen without any trace of lignin even in the margin (not shown). The seed coat of category 2 shows sclerenchyma cells in a single continuous (Fig. 5a and b) or discontinuous layer (Fig. 5c and d), the shape of the sclerenchyma cells is either normal (Fig. 5c) or half collapsed as if the amount of lignin would not be enough to support cell architecture (Fig. 5a and d). Category 3 representing the most lignified hull-less progeny, showed a clear lignin deposition in the sclerenchyma which consisted of one to three continuous cell layers (Fig. 6a and c) with characteristic elongated cells in the margin (Fig. 6b and d).

Further extended histological studies comprising all F2-segregants of hull-less type from the three crosses will disclose whether residual lignification is controlled by several genes with minor effects producing a continuous variation or a discontinuity controlled by a manageable number of genes.

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Fig. 1: Seed types of the hulled (a) and hull-less (b) parents

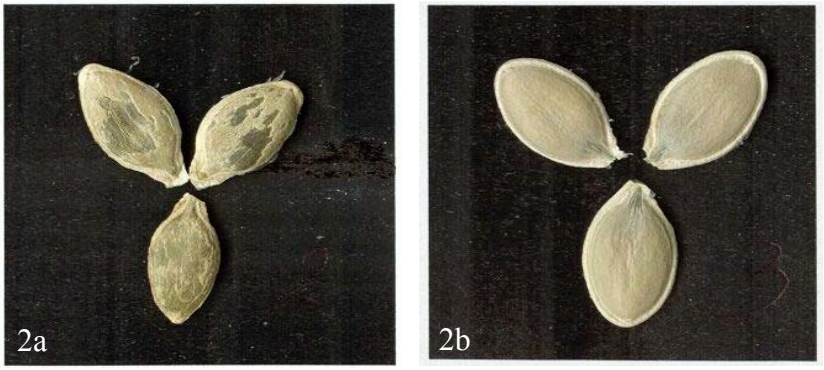


Fig. 2: A sample of category 2 seeds exemplifying heterogeneity (a) and seeds typical for category 3 (b)

Table 1: The segregation of the seed coat character in the F2s in the three crosses

	Total	Hulled	Hull-less	Ratio	P
SZG1 x True French	195	148	47	3.1 : 0.9	0.96
Lady Godiva x Bianco friulana	190	140	50	2.8 : 1.2	0.90
Retzer Gold x Tigress	117	84	33	2.6 : 1.4	0.72

Table 2: χ^2 test of segregation ratios of the three categories in the three crosses.

	Total	Categ. 1	Categ. 2	Categ. 3	P
SZG1 x True French	25	9	10	6	0.52
Lady Godiva x Bianco friulana	25	12	10	3	0.02
Retzer Gold x Tigress	25	13	12	1	0.003

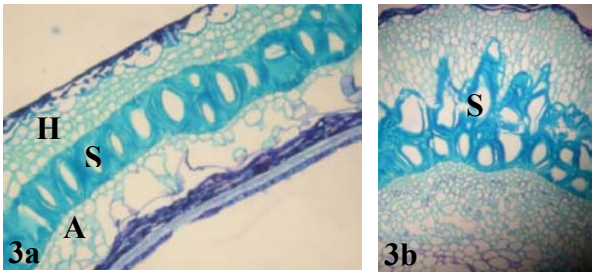


Fig. 3: Cross sections of testa of a hulled zucchini seed on the surface (a) and in the margin with typically elongated sclerenchyma cells (b). The strong lignification of the sclerenchyma (S), hypodermis (H) and aerenchyma (A) is clearly visible.

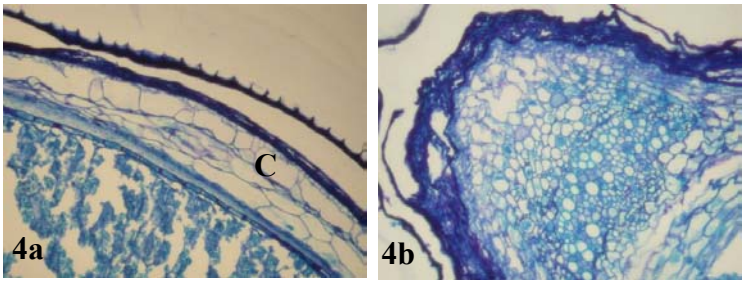


Fig. 4: Cross sections of the testa of a hull-less seeded Styrian oil pumpkin. Hypodermis, sclerenchyma and aerenchyma above chlorenchyma (C) on the seed surface (a) and in the margin (b) are collapsed to a thin hyaline. Cross sections of category 1 seeds are undistinguishable from those of the hull-less parent.

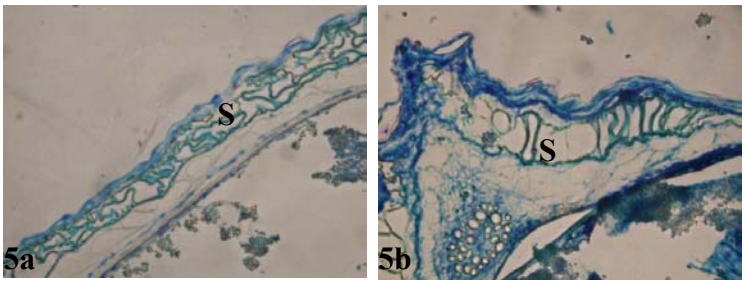
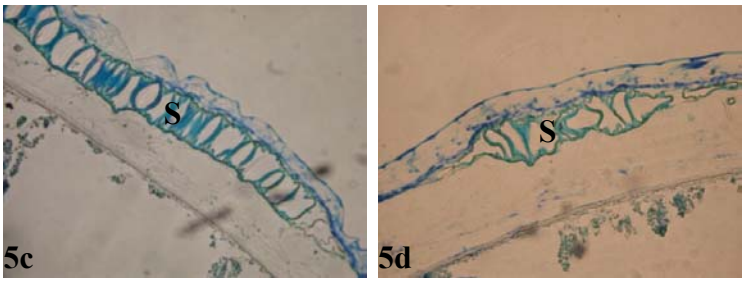


Fig. 5: Cross sections of category 2 seeds, showing sclerenchyma cells in a single continuous (a, b) or discontinuous (c and d) layer, the shape of the cells is either normal (c) or half collapsed (a).



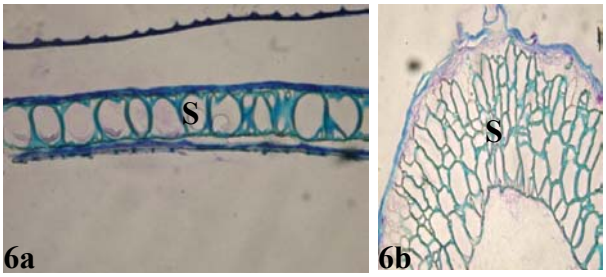
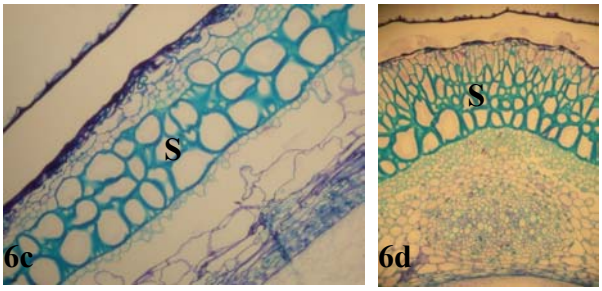


Fig. 6: Cross sections of category 3 seeds with one or more sclerenchyma cell layers (a and c) and with elongated cells characteristic for the margin (b and d). The degree of lignification appears to be higher in the seeds c and d.



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