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Seed Coat Morphology Differentiates Blackberry Cultivars

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Abstract

Determining the cultivar identity of blackberry (*Rubus* L.) fruit may be problematic when the parent plant is not available for examination. The ability to correctly identify commercial cultivars is important to the industry. Less desirable cultivars may be mistaken or substituted for more desirable ones, resulting in mislabeled products or economic losses. The objective of this study was to develop a simple and effective method to distinguish blackberry cultivars using seed morphology. Seeds of 17 commercially important blackberry cultivars ('Black Diamond', 'Black Pearl', 'Boysen', 'Cacanska Bestrna', 'Chester Thornless', 'Hull Thornless', 'Kotata', 'Loch Ness', 'Marion', 'Navaho', 'Nightfall', 'Obsidian', 'Newberry', 'Silvan', 'Tupy', 'Wild Treasure', and 'Young') were collected from the United States Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR) and Horticultural Crops Research Laboratory (HCRL), Corvallis, Oregon. Seeds were examined with a dissecting light microscope and scanning electron microscopy. A key characteristic was the shape of the raphe on the seed: straight, concave or convex. The 17 cultivars could be divided into three groups based on raphe shape. Cultivars within each group could be differentiated by seed shape, size, color and seed-coat sculpturing. Cultivars originating from the same parents (full sibs) or the same maternal line could be distinguished as well. Although scanning electron microscopy showed the seed coat sculpturing in greater detail, all cultivars could be distinguished successfully using a dissecting microscope.

Blackberry (*Rubus* L.) cultivar fruit identity may be difficult to discern in the harvested product. Fruit variations such as shape and size occur in many cultivars. The ability to correctly identify the cultivars of commercially produced fruit is important to the industry. Economic losses may result when less desirable cultivars are mistaken or substituted for more desirable ones. Cultivar verification using molecular techniques is expensive for general use and requires special training if it is going to be done in-house.

Seed shape and structure are used to identify many plants (2, 6). The structures and surface sculpturing on seed coats are characteristic of the maternal parent, so they are distinctive for each species. Satomi and Naruhashi (8) used seed coat characteristics such as seed shape, shape of reticulations, height of ridges and shape of valleys, for taxonomic repositioning of *Rubus trifidus* from genus *Rubus* subg. *Anoplobatus* to subg. *Idaeobatus*. Wada and Reed (10) studied seeds of 56 types of blackberry and raspberry (*Rubus* species and hybrids) and found that the seed coat structures, especially seed coat microsculpturing, were distinctive for the 12 subgenera with the scanning electron microscope (SEM). A standard low-power dissecting light microscope (10-40X) revealed the shape and major surface features of the seed coat.

SEM examination shows detail that is not clearly visible with light microscopy and can be used to highlight important structural characteristics and make definitive classifications. At higher magnification (80-400X) with SEM, it is possible to see specific cell types and fine seed coat structures. Dowidar et al. (4) studied the ultrastructure of seed coats and achenes of 47 taxa of the Rosaceae using SEM and identified many taxonomically important characters. Seed-coat sculpturing can differentiate even closely

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related cultivars and species. The patterns of seed-coat sculpturing of the many species of *Rubus* used in breeding cultivars are very diverse (10). The combination of light and SEM images provides definitive identifying features for distinguishing cultivars using a simple key (9).

The objective of this study was to develop a systematic method for distinguishing economically important blackberry cultivars in the US based on key anatomical features of seed morphology using a combination of light microscopy and SEM.

Materials and Methods

Fruit of 17 commercial blackberry cultivars was collected in the summer of 2007 from plants identified as true to type in the NCGR collections or from the breeding collection at HCRL, both near Corvallis, Ore. (Table 1). For seed extraction, fruit (200 g) was placed in a beaker with 400 ml water and 15 ml pectinase (Novozymes, Fresno, Cal.) for 24 h at 25 ˚C. Seeds were extracted in a blender with the blades covered with plastic tubing and set at a low speed for 2 min with a 3:1 water-to-berry ratio. Floating seeds and fruit were discarded. The main mass of fruit pulp was added to a strainer and washed thoroughly in running water. Seeds were mashed against the strainer as needed to completely remove the fruit pulp and clean seeds were spread on paper towels to air dry at ambient temperature (~25˚C).

The light microscope study of clean dry seed employed a Nikon SMZ 1000 stereomicroscopic Zoom Microscope (Nikon Instruments, Tokyo, Japan), Infinity digital microscope camera, and Infinity image capture and analyze software (Luminera Corporation, Ottawa, Canada). Seeds were measured for length and width with the microscope and software (10 seeds/cultivar), and weighed (3 replicates of 100 seed). Raphal shape and seed color were determined.

SEM images were taken using an AmRay3300 (Amray, Bedford, Mass.) or Quanta FE Field Emission SEM (FEI Company, Hillsboro, Ore.). Seeds were mounted on aluminum stubs using two-sided carbon conducive-adhesive tape and sputter coated for 20 seconds with either a thin layer of an alloy (60% gold and 40% palladium) or with 100% gold (Edwards S150B, Crawley, U.K.). All supplies for the SEM were purchased from Ted Pella, Inc. (Redding, Cal.). Terminology for describing the seeds is based on Barthlott's SEM observations of epidermal and seed coat surfaces of 5000 species of seed plants (1) and the terminology of Koul (5). Further terms were added to describe seed coat morphology specific to blackberries. Cultivar identifying numbers (PI numbers) for plants from NCGR are shown in Table 1. Information on pedigrees was obtained from Clark et al. (3) and personal communication with Dr. Chad Finn (HCRL, Corvallis, Ore.).

Data were analyzed with SAS /STAT software (Cary, NC) version 9.2 (7). Means were compared with t tests using an ANOVA table and mean separation was determined with Duncan's multiple range test (α=0.05).

Results and Discussion

Blackberry cultivars are often complex hybrids of several species and many cultivars (Table 1). This complexity is evident in the structure of the seed coat: characteristics of the raphe (the portion of funiculus that is attached to the ovule wall), the shape of the ridge on the back of the seed, and the sculpturing on the sides of the seed coat. Low power light microscopy (dissecting microscope) confirmed that the 17 cultivars could be distinguished by seed color, size and shape as well as by seed coat characteristics (Table 2). The cultivars could be divided initially into groups based on the raphe shape (concave, straight, and convex). Once divided into these groups the individual cultivars could be differentiated by seed size, shape and color.

Seeds can be distinguished by color when compared together (Table 2). Three groups (pink, purple, and brown) were observed. Within the pink group, the darkest was 'Marion' a rosy pink, followed by 'Nightfall',

Table 1. Pedigree and background information for 17 commercial blackberry cultivars.

Cultivar	Plant introduction number	Pedigree	Background information
Black Diamond	638257	Kotata x NZ 8610L-163 (E90 x N-71)	Popular northwestern US processing cultivar becoming a standard
Black Pearl	638260	ORUS 1117-11 (OSC 1122 x OSC 2009) x ORUS 728-3 (Olallie x ORUS 728-3)	New and likely to be a popular northwestern US processing cultivar
Boysen	553336	A selection of *R. ursinus* var. *loganobaccus*	Industry standard of Boysen group
Cacanska Bestrna	643970	Dirksen Thornless x Black Satin	Popular Serbian cultivar with increasing acreage
Chester Thornless	553322	SIUS 47 (US 1482 x Darrow) x Thornfree	Popular fresh cultivar that is often sold to processors
Hull Thornless	553299	SIUS 47 (US 1482 x Darrow) x Thornfree	Popular fresh cultivar that is often sold to processors
Kotata	553293	OSC 743 (Pacific x Boysen) x OSC 877 (Jenner 1 x Eldorado)	Popular northwestern US processing cultivar, often mixed with ‘Marion’
Loch Ness	638182	Complex hybrid derived from Comanche, Chehalem, Early Harvest, Thornfree and selections from Illinois and N.C.	Popular fresh cultivar in Europe that is often sold to processors
Marion	553254	Chehalem x Olallie	Industry standard
Navaho	553343	Ark. 583 (Thornfree x Brazos) x Ark. 631 (AR550 x Cherokee)	Popular fresh cultivar in US often sold to processors
Nightfall	638263	Marion x Waldo	New and likely to be popular northwestern US processing cultivar
Obsidian	638259	ORUS 828-42 (OSC 1122 x OSC 1683) x ORUS 1122-1 (Olallie x ORUS 728-3)	New and Popular northwestern US processing cultivar
Newberry	658340	ORUS 834-5 (OSC 1826 x OSC 2024) x ORUS 1045-14 (ORUS 880-1 x Austin Thornless)	Being planted as “firm Boysen for fresh market” in California; concern about blending.
Silvan	553308	ORUS 742 (Pacific x Boysen) x Marion	Popular northwestern US processing cultivar
Tupy	638226	Wild Uruguayan blackberry (suspected to actually be Boysen) x Comanche	Popular fresh cultivar in Mexico often sold to processors
Wild Treasure	638265	*Rubus ursinus* (GP4-9) x Waldo	New northwestern US small-fruited processing cultivar
Young	553266	Mayes x Phenomenal	Popular processing cultivar often mixed with ‘Boysen’

'Black Pearl', 'Black Diamond', 'Tupy'. 'Young' was the lightest as salmon pink. The purple group included dark purple 'Silvan', 'Boysen', and 'Navaho', to the light purple of 'Wild Treasure', 'Kotata', and 'Newberry'. For the brown group, 'Obsidian' and 'Hull Thornless' were dark brown, 'Loch Ness', 'Chester Thornless', medium brown and 'Cacanska Bestrna' was tan.

Seed sizes and weights were also characteristic for each cultivar (Table 2). Lengths ranged from 4.30 mm for 'Boysen' to 2.49 mm for 'Wild Treasure' and width from 3.01 mm for 'Hull Thornless' to 1.63 mm for 'Wild Treasure'. Weight of 100 seeds varied from 0.32 g for 'Boysen' to 0.08 g for the tiny 'Wild Treasure'. Seeds could possibly be differentiated on the basis of length, width and weight. For example, 'Boysen', 'Cacanska Bestrna', and 'Obsidian' are not significantly different in length, but can be distinguished by width and weight, and they differ greatly in color, shape and sculpturing. 'Black Pearl' and 'Hull Thornless' are similar in length, but significantly different in width and weight as well as color and raphal shape. Some cultivars such as 'Boysen' and 'Cacanska Bestrna' or 'Obsidian' and 'Newberry' cannot be distinguished by length, width or weight but the morphological characters clearly indicate cultivar differences.

From these data we conclude that commonly interchanged and closely related cultivars can be readily separated based on seed characters visible with a dissecting microscope (Table 2, Fig. 1-4). Cultivars from the same maternal line such as 'Cacanska Bestrna' (Fig. 1a), 'Chester Thornless' (Fig.1b) and 'Hull Thornless' (Fig. 1d) are distinguishable by size, shape, color, and depth of reticulations. 'Navaho' (Fig. 4a) is distinguished from 'Tupy' (Fig. 4b) by length, color and the depth of reticulations. 'Boysen' seed (Fig. 3b) are distinctive from 'Newberry' (Fig. 3e) and 'Young' (Fig. 3g) based on color, shape, seed length and width. 'Marion' seed (Fig. 3c) can be separated from 'Kotata' (Fig. 2d) and 'Silvan' (Fig. 3f) based on size, shape and color.

This is the first SEM study of *Rubus* cultivar seed morphology. The SEM images show distinct reticulations for each cultivar, even those with similar parentage. Distinctive seed coat and morphological characteristics clearly differentiate the 17 cultivars in this study, even full siblings.

A straight raphe was characteristic for four cultivars (Fig. 1). 'Cacanska Bestrna' exhibits a sharp back ridge and has the largest seed in this group. 'Hull Thornless' is also large-seeded and has prominent reticulations, deep and sharply curved L-shaped valleys, high, narrow walls, and high, sharp back ridges. 'Loch Ness' is a medium sized seed with a high but somewhat rounded back ridge and half-moon shape, with U-shaped valleys, and regular reticulations. 'Chester Thornless' has the smallest seed in this group and has deep reticulations with broad walls, a protruded back ridge, and a triangular outline. Seed coats of cultivars with the same maternal parent are similar, because the seed coat is maternal tissue. This is clearly seen in those cultivars with a straight raphal region that are derived from SIUS 47 (i.e. 'Hull Thornless' and 'Chester Thornless') and as a result have similar shapes and reticulations (Fig. 1a-d).

Concave raphae characterize the largest cultivar group, with a range from deeply concave to very slight. Four cultivars have deeply-concave raphe (Fig. 2). 'Kotata' has a flat seed surface with insignificant surface reticulation, shallow valleys and rounded back ridges. The 'Black Diamond' seed surface is very similar to its maternal parent 'Kotata' as it is relatively flat, with shallow secondary walls, and flattened and rounded back ridges. 'Nightfall' displays shallow U-shaped valleys and low walls. 'Wild Treasure' is a very small, flat seed with insignificant reticulations, very shallow valleys and very low walls.

A moderately or slightly concave raphe is characteristic of seven cultivars (Fig. 3). 'Black Pearl' seed exhibits a protruding back ridge and moderate reticulation with

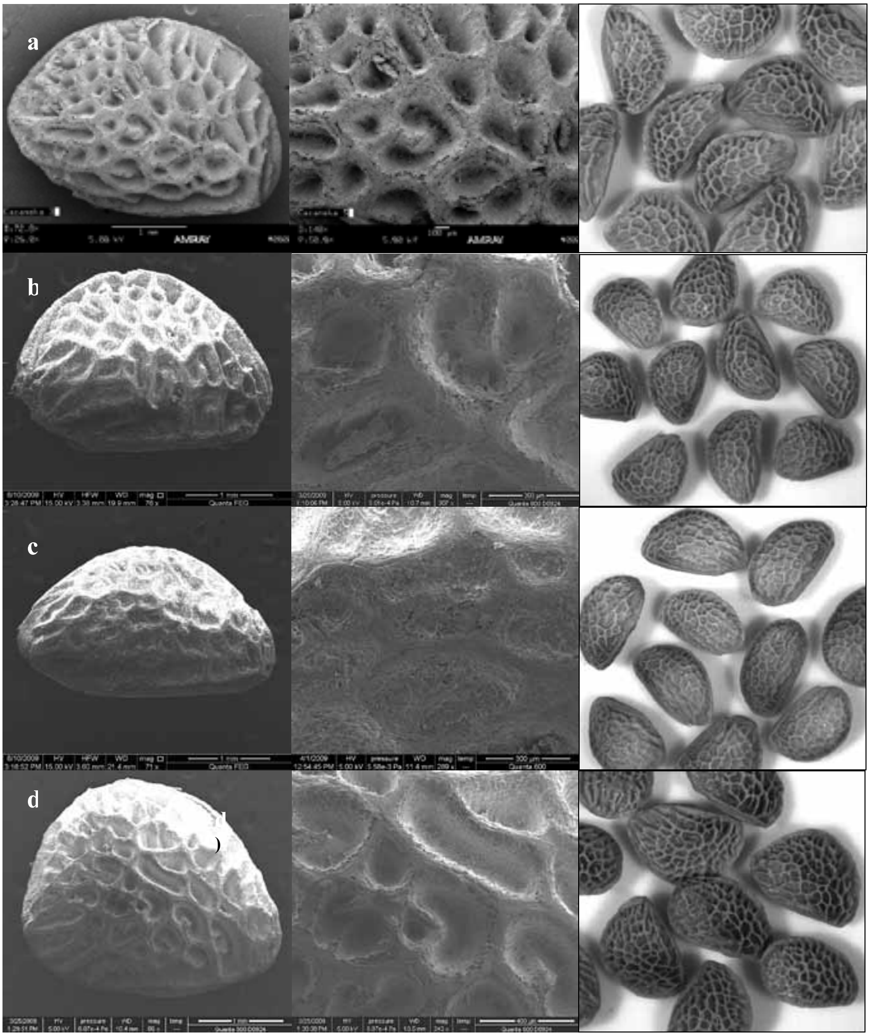

Fig. 1. Blackberry cultivars with a straight raphal region: a) 'Cacanska Bestrna', b) 'Chester Thornless', c) 'Loch Ness', and d) 'Hull Thornless'. Left; SEM whole seed, Center; SEM surface reticulation, Right; group of seeds at 10X with light microscopy.

U-shaped valleys. 'Boysen' has a round back ridge, deep L-shaped valleys with wide walls, and deep valleys with narrow edges. The 'Marion' surface has deep, irregular reticulations (long I- and L-shape, triangular, round rectangular and polygonal). 'Obsidian' seeds are very large with regular reticulations on the center and multiple striations with irregularities on the chalazal region, and broad, high secondary walls. 'Newberry' exhibits regular reticulations and a round back ridge. 'Silvan' displays irregular shaped reticulations, long I- and L-shaped, triangular and round valleys. 'Young' exhibits low reticulation with shallow valleys and flat, low walls, flat and rounded back ridges.

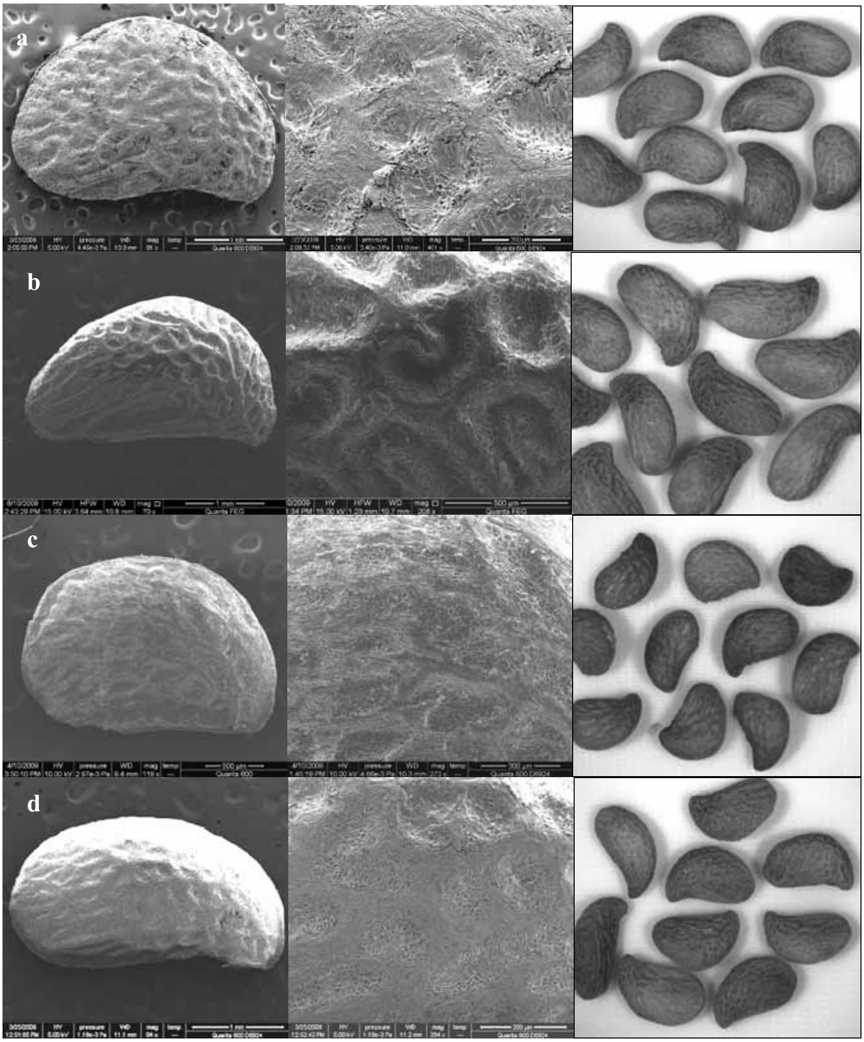

Fig. 2. Blackberry cultivars with a deeply concave raphal region: a) 'Black Diamond', b) 'Nightfall', c) 'Wild Treasure', and d) 'Kotata'. Left; SEM whole seed, Center; SEM surface reticulation, Right; group of seeds at 10X with light microscopy.

Two cultivars displayed convex raphae (Fig. 4). 'Navaho' is a triangular-shaped seed with deep U-shaped valleys, round-topped flat walls and broadly protruded back ridges. 'Tupy' seed has a unique triangular outline, with regular reticulations in the back ridges and irregular sculpturing patterns in the lower half of seed.

We earlier found that wild *Rubus* species exhibit unique seed surface microsculpturing under the SEM (10). Seed samples of blackberry species of subg. *Rubus* have clearly reticulated secondary periclinal walls which appear to the naked eye as a rough seed surface; raspberries of subg. *Idaeobatus* have smoothly curved rounded ridges and lower

Table 2. Characteristics of blackberry seeds used to differentiate the cultivars.

Cultivar (lower edge)	Raphe shape	Seed Size	Color	Length^z (mm)	Width^z (mm)	Weight^z g/100 seed
Cacanska Bestrna	Straight	Large	Light yellow brown	4.20 a^y	2.59 b	0.31 b
Chester Thornless	Straight/broad	Medium	Purple-brown/ mauve	3.19 g	2.33 c	0.28 cd
Hull Thornless	Straight	Large, broad	Dark brown	3.91 c	3.01 a	0.43 a
Loch Ness	Straight	Medium and broad	Medium brown	3.37 ef	2.14 ef	0.22 g
Black Diamond	Deeply concave	Medium/thin	Pink	3.20 g	1.96 g	0.20 h
Kotata	Deeply concave	Medium to small, narrow	Light purple	3.48 e	1.82 h	0.21 gh
Nightfall	Deeply concave	Medium to small	Medium pink	3.60 d	2.00 fg	0.15 i
Wild Treasure	Deeply concave	Very small and thin	Light purple	2.49 i	1.63 i	0.08 j
Black Pearl	Concave	Medium	Deep pink	3.81 c	2.20 de	0.27 de
Boysen	Slightly concave	Large	Purple	4.30 a c	2.54 cd	0.32 b
Marion	Slightly concave	Medium to small	Rosy pink	3.44 e	2.06 fg	0.16 i
Newberry	Slightly concave	Large	Light purple	4.06 b	2.09 efg	0.25 f
Obsidian	Slightly concave	Large, long and narrow	Dark brown purple	4.13 ab	1.96 g	0.26 ef
Silvan	Slightly concave	Medium to small	Deep purple	3.04 h	1.83 h	0.16 i
Young	Slightly concave	Medium and long	Salmon pink/yellow	3.36 ef	1.71 hi	0.27 de
Navaho	Convex	Medium and broad/short	Dark purple	3.28 fg	2.44 c	0.29 c
Tupy	Convex	Medium	Light pink	3.66 d	2.34 c	0.22 g

^{z} Measurements of seed size are the means of 10 seeds.
^{y} Means in a column with the same letter are not significantly different by Duncan's multiple range test (α=0.05).
^{x} Mean weight of 100 seeds

reticulations and appear as a smooth seed surface (10). Seed surface morphology is defined by the ovary wall of the maternal parent. Four groups in the subgenus *Rubus* have distinctive surface sculpture trends caused by cell shapes and the U, or V-shaped or irregular valleys as seen by SEM. The subg. *Rubus* usually has deep and steep-edged truncate or acute ridges with a wide, obvious raphal region like 'Marion (Fig. 3c) or 'Chester Thornless' (Fig. 1a). Many of the 17 complex hybrid cultivars examined here show subg. *Rubus* characteristics. Some cultivars such as, 'Boysen' and 'Young' (Fig. 3b, 3g) have the milder and flatter rounded back ridges of subg. *Idaeobatus* leading to the speculation that they have raspberry species present in the maternal line.

This morphological study of *Rubus* cultivar seeds demonstrates that they can be eas-

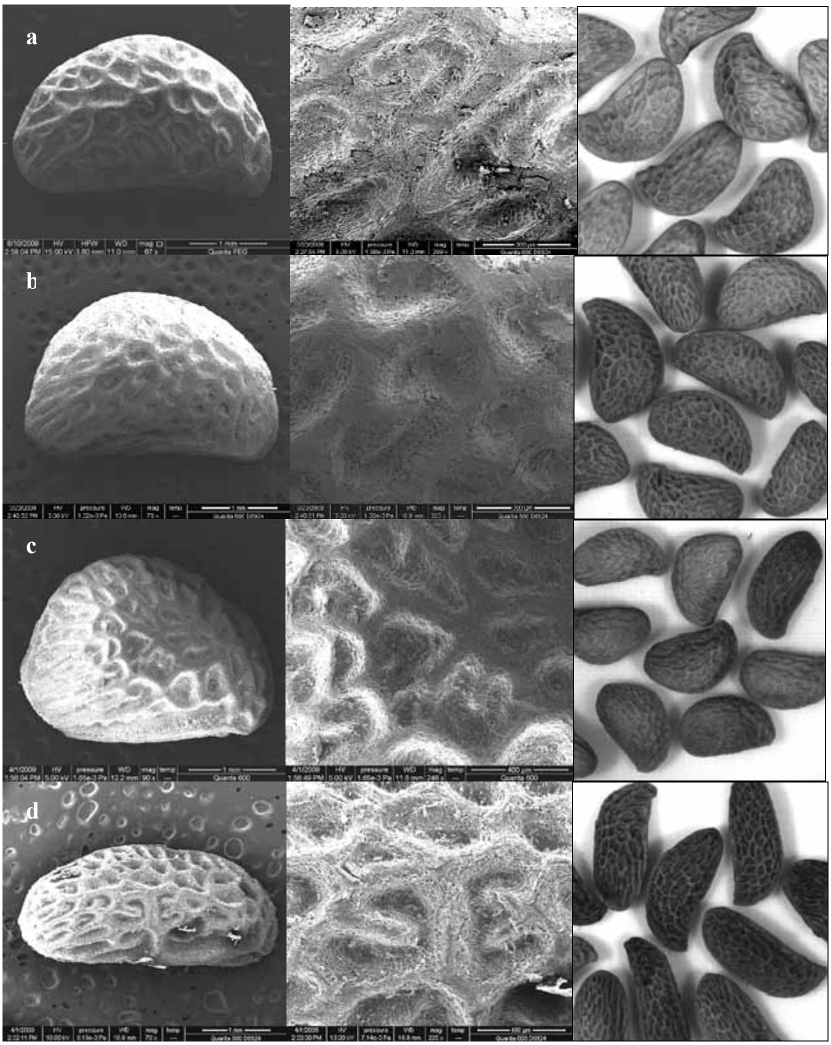

Fig. 3.

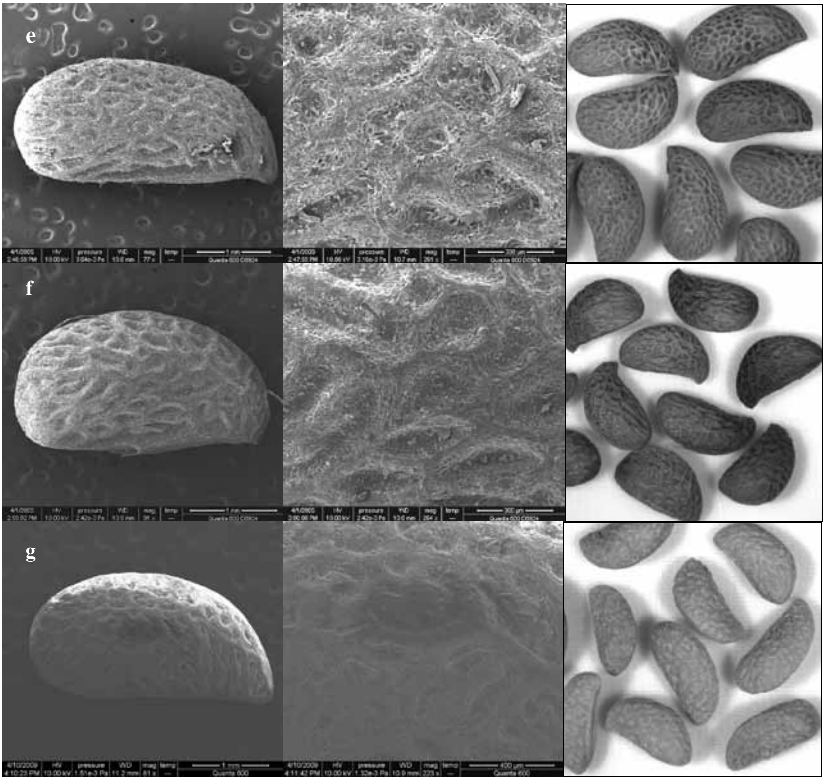

Fig. 3. Blackberry cultivars with a moderate to slightly concave raphal region: a) 'Black Pearl', b) 'Boysen', c) 'Marion', d) 'Obsidian', e) 'Newberry', f) 'Silvan', and g) 'Young'. Left; SEM whole seed, Center; SEM surface reticulation, Right; group of seeds at 10X with light microscopy.

ily identified under a dissecting microscope, although SEM provides a clearer view of the unique reticulations of each cultivar and would be more useful for taxonomic questions. Determining the shape of the raphe, then separating the seeds by size and color provides a technically easy and inexpensive, but dependable way to identify *Rubus* cultivars. A handbook with a dichotomous key and color photos was developed for commercial use by growers and processors as a first step in identifying mislabeled fruit based on the seed color, size and shape of the commonly grown cultivars (9).

Acknowledgements

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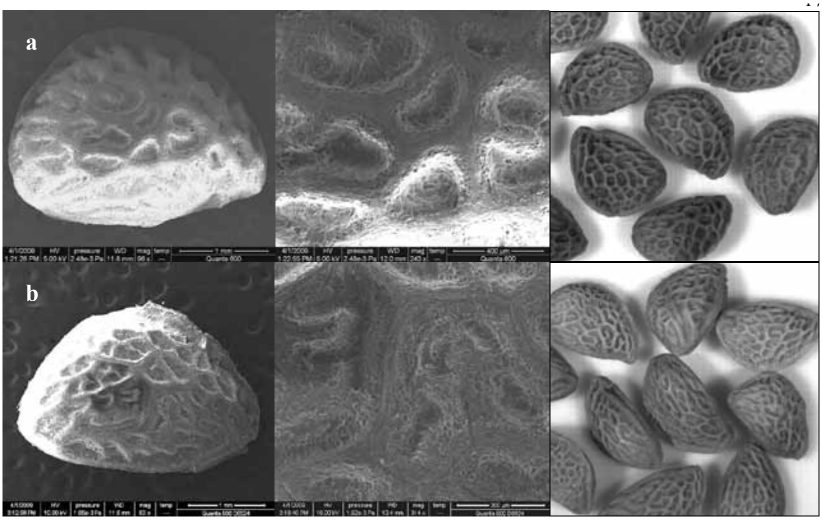

Fig. 4. Blackberry cultivars with a convex raphal region: a) 'Navaho' and b) 'Tupy'. Left; SEM whole seed, Center; SEM surface reticulation, Right; group of seeds at 10X with light microscopy.

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