

ORIGINAL ARTICLE

Agave Seeds: Physical and Chemical Characterization and Identification of Storage Proteins

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ABSTRACT

Changes in the physical and chemical characteristics, isoelectric point and protein fractions due to variety and variant were evaluated in agave seeds. The results showed that protein and fiber are the major components of all agave seeds. The salmiana variant had the hardest seed, while higher breaking point were observed in A. atrovirens. The isoelectric point and proportion between protein fractions was different in all seeds. The protein of all the seeds showed similar electrophoretic pattern with mixture of components and conserved regions among genotypes of the same species. In conclusion, variant and variety showed influence on the characteristics of Agave seeds.

KEYWORDS: agave, variety, variant, seed, protein

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INTRODUCTION

Because of the rich assortment of varieties found in the nominated Mesoamerica region (272 species of 310 species reported), Mexico has been considered the center of origin and biodiversity of the *Agave* genus (García-Mendoza and Galván, 1995). Agave plants have two reproduction types, sexual reproduction and cloning (asexual reproduction), the first is carried out by seed production and the second can be performed in different species through clonal shoots in different parts of the rosette (Arizaga and Ezcurra, 2002). Sexual reproduction is an efficient way to enhance heterogeneity, which is crucial for the survival of agave plants, however, has several problems. First the low probability of falling into sites with appropriate conditions for germination and seedling establishment (García, 2002), generating seed desiccation stress, especially in low rainfall regions (Arizaga and Ezcurra, 2002). Second, traditional farmers' practice to avoid sexual reproduction, consisting in apical inflorescence elimination, diminishing the relative abundance of viable seeds. For these reasons, the asexual propagation is generally used, avoiding genetic variation, producing a high number of plants genotypically similar with a common susceptibility. Actually, some agave species are at risk of extinction due to clonal reproduction, climatic change and reduction in the distribution, number and availability of wild agaves, for this reason the sexual reproduction is very important for maintaining a rich gene pool and preventing genetic drift and may also diminish damage resulting from pests and diseases (Ramírez-Tobias et al., 2012). However, the seed is not only important for propagation and dispersal, in some cases is the highest part of the plant harvested by humankind due to protein content in seeds, which can vary in the range of 10% (cereals) to 40% (legumes and oilseeds). The vast majority of proteins present in mature seeds has metabolic or structural roles, and contains one or more groups of proteins that are present in high amounts and that serve to provide a store of amino acids for use during germination and seedling growth. These storage proteins are important due that they determine not only the total protein content of the seed but also its

quality for various end uses (Shewry, Napier and Tatham, 1995). Despite the importance of agave seeds, currently are few studies of the morphological and physicochemical characteristics of agave seeds, for this reason the aim of this study was to characterized two varieties and two variants of agave seeds.

MATERIALS AND METHODS

Vegetative material

The seeds of *A. atrovirens* var. *mirabilis* and *A. salmiana*, salmiana variant were obtained from Tulancingo Valley and *A. salmiana*, xamini variant seeds were collected from Mezquital Valley, both located in the Hidalgo State (Mexico). The seeds were ground in a manual blender, the material was passed through number 40 mesh (aperture size 425 μ m).

Physical and chemical characteristics

The chemical analysis was performed by AOAC (1990) methods: protein using factor 6.25 (AOAC 955.04D), fiber crude (AOAC 962.09), fat crude (AOAC 920.39C), moisture (AOAC 931.04), and ash (AOAC 923.03). Carbohydrates were obtained by the difference to 100% dry basis.

The physical characteristic evaluated were hectolitic weight, dimensions size (long and width), and texture properties. The texture characteristics (hardness and breaking point) of the seeds were determined, using a texture analyzer TA 500, with an acrylic cone of 30°.

A scanning electron microscope JEOL (JEOL, type EX-1200, Japan) operated at 15 kV was used to visualize the structure in the seeds. The samples were mounted in a double-sided carbon tape and covered with roughly 10 nm of gold in a Denton sputter coater.

Isoelectric Point (pI)

The Bernardino-Nicanor *et al.*, (2000) method was used. The pI of seed proteins was determined, in two steps: first as the pH value of maximal solubility and second the pH value of maximal precipitation. Twenty grams of seed meal was extracted water to solids ratio 20:1 (v/w), pH (10 to 11.5 maximal solubility) was kept constant during the extraction by adjusting with 0.1N NaOH, to 40°C for 30 min. The pI was found by titrating aliquots collected extracted to specific pH values and determining the protein content (Kjendhal) of the supernatant after centrifugation.

Seeds protein fractions

The seeds protein fractions were obtained by Osborne's method, modified by Bernardino-Nicanor *et al.*, (2006).

Albumins: Seeds meal, was first extracted with distilled water (0.1 g/mL) by two stirring steps of 1 h at 4°C and then centrifuged at 10 000g for 30 min at 4°C. Supernatant was freeze-dried.

Globulins: The residue from albumins extraction was extracted under magnetic stirring for 1 h with NaCl (10 g/100g) at 4°C and centrifuged at 10 000g for 30 min at 4°C. Supernatant was dialysed against distilled water for 5 days, changing water dialysis every day and freeze-dried.

Prolamins: The residue resulting from globulins extraction was extracted under magnetic stirring for 1 h at 4°C with 70 ml/ 100 ml aqueous 2-propanol and centrifuged at 10 000g for 30 min at 4 °C. Supernatant was dialysed against acetic acid (1 mL/ 100 mL) for 5 days, with daily changes of solution, and freeze-dried.

Glutelins: The residue resulting from prolamins extraction was extracted under magnetic stirring for 1 h at 4°C with NaOH (pH 12) and centrifuged at 10 000g for 30 min at 4 °C. Supernatant was dialysed against acetic acid (1 mL/ 100 mL) for 5 days, with daily changes of solution, and freeze-dried.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), using the Mini Protean 3 Cell (Bio-Rad Laboratories, Hercules, CA 94547 USA) vertical unit. Molecular weight of the polypeptides were calculated using the following standard proteins (Bio-Rad Laboratories Hercules, CA 94547, USA): myosin (195.514 kDa), β -galactosidase (111.754 kDa), BSA (58.631 kDa), carbonic anhydrase (30.619 kDa), soybean trypsin inhibitor (25.226 kDa), lysozyme (13.130 kDa) and aprotinin (6.404 kDa). 1 mg/mL of each sample were dissolved in sample buffer (0.1 mol/L Tris, pH 6.8, 20 mL/100 mL glycerol, 2 g/100 mL SDS, and 0.05 g/100 mL bromophenol blue). Gels were fixed and stained with Coomassie Brilliant Blue.

Statistical Analysis

The quantitative data were expressed as the mean \pm standard deviation, and the analysis of variance (ANOVA) was carried out followed by a Tukey's test. SAS v.8 (Statistical Analysis System, SAS Institute Inc., Cary, NC) software was used for the data analysis, and all experimental determinations were assayed in triplicate.

RESULTS AND DISCUSSION

Physical and chemical characteristics

Agave seeds showed significant differences in the chemical composition (Table 1) between variant and varieties, being the salmiana variant seeds, the samples with higher protein and fiber content, however the moisture content were lowest in both *A. salmiana* variants seeds, 3% lower in comparison with *A. salmiana* Chino variant (9.8%) and 1% lower in relation to *A. atrovirens*, the results obtained are within the range of variability reported for other Agave seeds (Ramírez-Tobías et al., 2012).

The protein content of agave seeds is higher than cereal grains (Brand et al., 2003, Grausgruber et al., 2004) and pseudocereals like amaranth (Juan et al., 2007), and lies within range reported for some legumes (peas, rapeseed) (García and Medina, 2006) and oilseeds (sesame, peanuts, sunflower) (Prakash and Narasinga, 1986). These results show the potential of agave seeds as protein source for human and animal feed.

On the other hand, the fiber content of agave seeds show a higher value (32.6 to 55.5%) than other seeds (cereals and legumes), (Grausgruber et al., 2004, Li, Andrews and Pehrsson, 2002; De Almeida et al., 2006, Li et al., 2002), in both groups do not exceed 15%.

The moisture content from *A. salmiana* was lower than *A. atrovirens* (Table 1), however the moisture content was kept between 6.8 and 7.8, which fell within values reported in the literature for agave seeds (Ramírez-Tobías et al., 2012). This moisture content in the *A. salmiana* and *A. atrovirens* seeds can permit a long-time storage. In relation to low fat content (2.68% to 3.69%), this concentration would be an advantage to prevent oxidation reactions of lipids in long-time storage of the seeds (Ladeji, Solomon and Egbomeadeh, 2000).

Table 1. Chemical analysis of agave seeds

Component	<i>A. salmiana</i>		<i>A. atrovirens</i>
	xamini variant	salmiana variant	variety mirabilis
Moisture	6.80 ± 0.22 ^b	6.86 ± 0.13 ^b	7.87 ± 0.10 ^a
Ash	3.83 ± 0.01 ^a	2.09 ± 0.03 ^b	1.51 ± 0.05 ^c
Fat	3.56 ± 0.10 ^b	3.69 ± 0.11 ^a	2.68 ± 0.14 ^c
Fiber	32.65 ± 0.58 ^c	55.50 ± 34 ^a	50.17 ± 1.03 ^b
Protein	22.25 ± 0.21 ^c	29.49 ± 0.29 ^a	27.61 ± 0.33 ^b
Carbohydrates	30.91 ± 0.80 ^a	2.37 ± 0.70 ^c	10.16 ± 1.20 ^b

Data are presented as mean ± standard error of three replications. Data are expressed as g/100g on a dry weight basis for ash, fat, fiber, protein and carbohydrates. Different letters in a row denote significant difference, $p < 0.05$.

The physical characteristics of agave seeds showed significant differences among varieties and variants (Table 2), the *A. salmiana*, salmiana variant seeds were the smallest. The length and width of the *A. salmiana*, xamini variant and *A. atrovirens* seeds are within the reported range (7.1 to 8.4 and 5.4 to 6.5, length and width respectively) for *A. salmiana* seeds from San Luis Potosi (Vázquez et al., 2011).

The hectolitic weight was negatively associated with seed size, *A. salmiana* have the biggest seeds and smallest hectolitic weight while *A. atrovirens* seeds showed an inverse behavior. These results showed the wide range of seed size among Agave species; which, together with seed humidity, are affected by the environment within which they exist and interaction between species (Ramírez-Tobías et al., 2012).

The results showed that hardness of the seeds are significantly different between variety and variants (Table 2), the salmiana variant seeds are the hardest seeds, while xamini variant seeds are the softest seeds of all the studied seeds. Respect to breaking point, the highest values were obtained from *A. atrovirens* seeds, considering that hardness and breaking point of the seeds are adaptive properties of seeds under adverse environmental conditions (Castillo and Gueni, 2001), for this reason the results obtained showed great variations in breaking point within all variants and varieties which depend of climatic conditions, however in the germination process the hardness of the seeds could be a disadvantage so generally performed scarification processes (Wencomo, 2004).

Table 2. Physical characteristics of agave seeds

Parameter	<i>A. salmiana</i>		<i>A. atrovirens</i>
	xamini variant	salmiana variant	variety mirabilis
Hectolitic weight (kg/hL)	0.204	0.241	0.157
Long (cm)	0.833±0.080 ^a	0.656±0.089 ^c	0.796±0.062 ^b
Width (cm)	0.621±0.078 ^a	0.475±0.072 ^b	0.623 ±0.067 ^a
Thickness (cm)	0.075±0.075 ^a	0.069 ±0.096 ^a	0.052 ±0.073 ^a
Hardness (kgf/mm)	0.275±0.014 ^c	0.460±0.031 ^a	0.356±0.042 ^b
Breaking point (kgf)	0.208±0.007 ^b	0.295±0.039 ^a	0.306±0.041 ^a

Data are presented as mean \pm standard error of three replications. Different letters in a row denote significant difference, $p < 0.05$.

The agave seeds are flat, ovoid with pointed terminal (Figure 1), with similar color (black) to the *A. potatorum* seeds (Martinez 2004), The salmiana variant seeds showed a coat roughest surface than the xamini variant and *A. atrovirens* seeds.

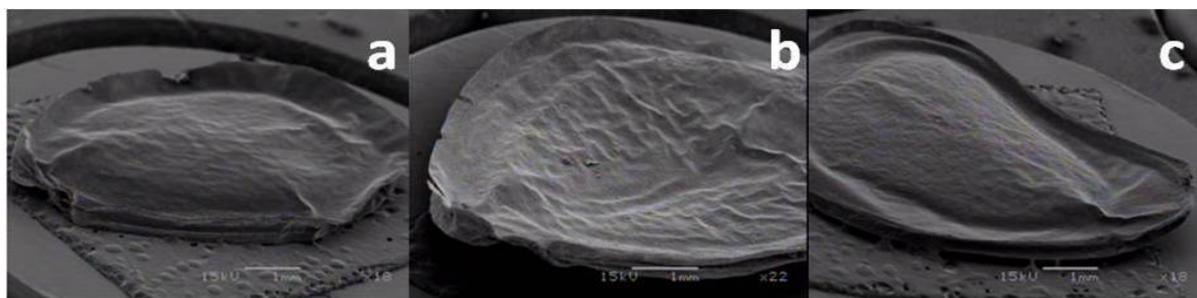


Figure 1. Micrographs of agave seeds surface (a) xamini variant (*A. salmiana*). (b) salmiana variant (*A. salmiana*) variant salmiana (c) *A. atrovirens* var. *mirabilis*.

The hilar region of all seeds responds to environmental cues that cause dormancy break with better definition of the hilar region in the xamini variant seeds in comparison with others variants and varieties analyzed (Figure 2).

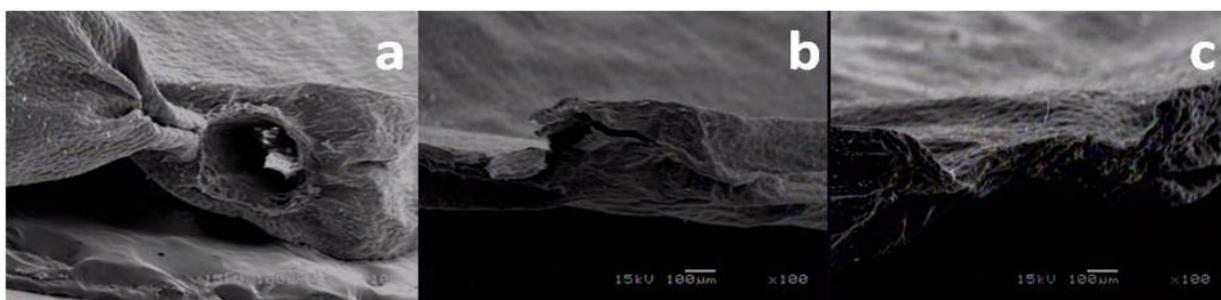


Figure 2. Morphologic characteristics from agave seeds (a) xamini varian (*A. salmina*). (b) salmiana variant (*A. salmiana*)(c) *A. atrovirens* var. *mirabilis*.

Isoelectric Point (pI)

The isoelectric point (pI) of the protein from agave seeds showed significant difference between variants and varieties. The range of pI obtained for the protein of variants and varieties of agave seeds was 3 to 4.5 (Table 3), being the protein of xamini variant which showed the lowest pI value in comparison with the protein of the other agave seeds analyzed in this study. The pI obtained of the agave seeds protein is within the range reported for other seeds. However, in relation with food seeds only the protein of *A. atrovirens* seeds showed an isoelectric point within range reported for legumes and cereals (4.0 to 4.5), such as soybean (Rickert et al., 2004, Cyde, 2002), bean (Chi-Fai, Cheung and Yum-Shing, 1997), chickpea (Sánchez-Vioque et al., 1999); and wheat germ (Hettiarachchy, Griffin and Gnanasambandam, 1996). Respect to oilseeds and fruit seeds too *A. atrovirens* seeds are the only whose protein is within the range obtained for pI values of oilseeds and fruit seeds (3.9 to 5.5) such as sunflower (Paredes-López and Ordorica-Falomir, 1986), safflower (Madrigal and Ortega, 2002), rapeseed (Goncalves et al., 1997), peanut (Ferreira et al., 2007), sesame (Bandyopadhyay and Ghosh, 2002), guava seed (Bernardino-Nicanor et al., 2000) and tomatoe seed (Liadakis et al., 1995).

Table 3. Isoelectric point of the proteins from agave seeds

Parameter	<i>A. salmiana</i>		<i>A. atrovirens</i>
	xamini variant	salmiana variant	variety mirabilis
Isoelectric point (pI)	3.0 \pm 0.002 ^c	3.5 \pm 0.002 ^b	4.5 \pm 0.006 ^a

Data are presented as mean \pm standard error of three replications. Different letters in a row denote significant difference, $p < 0.05$.

Seeds protein fractions

The content of each protein fractions for all agave seeds is summarized in the Table 4, which shows that the globulins and albumins are the more abundant proteins in all seeds analyzed while the prolamins and

glutelins content is within the range of 10 to 20%, the insoluble residue is the higher component in the *A. atrovirens* and salmiana variant, this results showed that the proportion of protein fractions is not similar to protein fractions content of cereals, where prolamins and glutelins are predominant fractions (Shewry and Halford, 2002), however, in comparison with protein of legumes (Gallegos et al., 2004), oilseeds (Goncalves et al., 1997) and other seeds, the proportion of the protein fraction of agave seeds are similar, being albumins and globulins the predominant protein fractions.

Apparently the hardness and breaking point of the agave seeds influence on the protein fractions extractability, considering that seeds with higher textural values showed the most low-yielding (42.5% and 46.5%; *A. salmiana* salmiana variant and *A. atrovirens* var. *mirabilis* respectively), in these two seeds were obtained the highest concentration of insoluble residue (Table 4).

Table 4. Protein fraction of the agave seeds

Protein fraction	<i>A. salmiana</i>		<i>A. atrovirens</i>
	xamini variant	salmiana variant	variety mirabilis
Albumins	21.60±0.74 ^a	10.34±0.43 ^c	11.89±0.61 ^b
Globulins	36.51±0.21 ^a	25.93±0.87 ^b	20.68±0.13 ^c
Prolamins	9.96±0.04 ^a	3.54±0.31 ^b	3.69±0.55 ^b
Glutelins	10.37±0.10 ^a	6.78±0.16 ^b	6.28±0.62 ^b
Insoluble residue	21.56	53.41	57.46

N × 6.25. Data are presented as mean ± standard error of three replications. g of protein fraction/100g of total protein. Different letters in a row denote significant difference, $p < 0.05$.

Electrophoretic characteristics of Agave seed fractions

According with SDS-PAGE, apparently the protein fractions of *A. salmiana* and *A. atrovirens* seeds are a mixture of polypeptides of different sizes (Figures 3A, 3B and 3C).

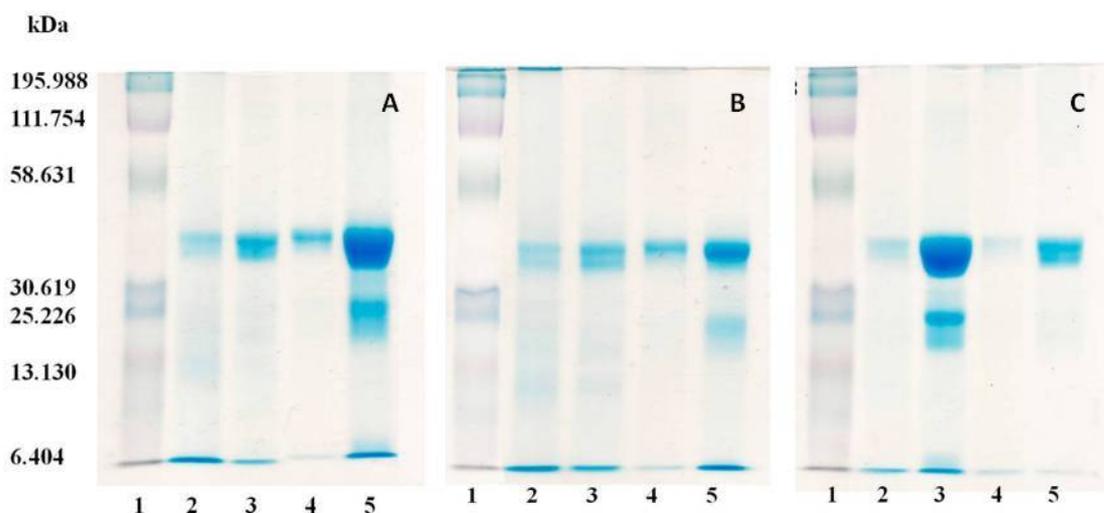


Figure 3. SDS-PAGE from agave seeds proteins A) *A. atrovirens* var. *mirabilis* seeds, B) *A. salmiana*, salmiana variant; C) *A. salmiana*, xamini variant; (1) Molecular weight standards, (2) Albumins, (3) Globulins, (4) Prolamins, (5) Glutelins.

The albumin fractions of salmiana and xamini variants (*A. salmiana*) showed two similar polypeptides around 46 kDa (46.4 and 45.8 kDa respectively) and 39 kDa (39.4 and 38.8 kDa respectively), this could indicate that both fractions share conserved regions among genotypes of the same specie, on the other hand respect to protein of *A. atrovirens* was observed that the albumin fraction had subunits with different molecular weight (48.2, 43 and 14.4 kDa) which could indicate that in the *Agave* genus exist differences between members of different species with respect to the albumin fraction.

In comparison with the albumin fraction of other seed proteins the number of subunits from albumin fraction of Agave seeds protein was lower than the number of subunits from *Akebia trifoliata*, oat and chickpea (Du et al., 2012; Chang et al., 2011), however, the molecular weight of the subunits of Agave albumin fraction are within the range of values found for albumin fraction of other seeds protein (oat, chickpea and storage protein of legume seeds), (Chang et al., 2011).

The globulin fraction from variants of *A. salmiana* share a polypeptide with molecular weight of approximately 46 kDa, however, in comparison with globulin fraction from *A. atrovirens* seed protein only the salmiana variant shared a polypeptide with molecular weight of approximately 20 kDa, which could indicate that the fractions contain conserved regions or that the polypeptide with molecular weight of approximately 46 kDa could be the same observed in the albumin fraction due to that many albumins are extracted simultaneously with the globulin fraction (Kortt and Caldwell, 1990, Du et al., 2012).

In concordance with the concentration of the globulin fraction from both species, the polypeptides with high molecular weight showed very well defined bands with high intensity, while the polypeptides with lower molecular weight were only present in low amounts. The subunits of globulin fraction from Agave seeds showed molecular weight similar to reported in other studies for globulin fraction of chickpea (22.3 kDa and 26.3 kDa), oat (22.3, 39.4 kDa, 35.8 kDa and 45.8 kDa) and sesame (range from 45 to 48 kDa), (Chang et al., 2011, Orruño and Morgan, 2011, Achouri, Nail and Boyce, 2012)

The electrophoretic pattern of prolamin fraction was similar for all seeds showed only one band, however, the apparent molecular weight was different in each prolamin fraction (45.8 kDa for xamini variant; 39.42 kDa for salmiana variant and 48.25 kDa for variety *mirabilis*). The polypeptide of approximately 46 kDa was observed in both fractions, albumin and prolamin, which could be due to that derived from a functionally similar ancestral protein common (Shewry et al., 1995). The prolamin fraction of agave seed have a molecular weight higher than prolamin fraction from *Akebia trifoliata* (Du et al., 2012).

The glutelin fraction of two variants of *A. salmiana* have a common polypeptide with apparent molecular weight of 43 kDa, while salmiana variant and *A. atrovirens* glutelin fraction shared a polypeptide of around of 39 kDa, all three glutelin fraction of Agave seeds share a polypeptide with molecular weight of 24 kDa, suggesting that this subunits to be an inherent protein of Agave species, for this reason like all fractions (albumin, globulin and prolamin) contain conserved regions. In relation to protein to others seeds the glutelin fraction of Agave seeds are similar to oat glutelin (23.6 kDa), in the case of xamini variant, while that glutelin fraction of *A. atrovirens* seeds is comparable to rice glutelin (48.25kDa) (Chang et al., 2011).

CONCLUSION

The chemical composition, physical characteristics and protein characteristics are influence by the variant or variety analyzed, however all seeds have similar characteristics such as the high proportion of protein, crude fiber, albumin and globulin fraction. On the other hand, Agave seeds exhibit some differences respect to protein characteristics such as isoelectric point (is between 3 and 4.5) and electrophoretic pattern. All Agave seeds showed similar polypeptides which could be due to that derived from a functionally similar ancestral protein common in the Agave genus.

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