

A “Hull-less” Seed Trait of *Cucurbita maxima* Duch. in Accession BGH 7653

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The consumption of seeds of *Cucurbita* sp. as food is common in many cultures throughout the world. The hull-less seeds are served as snackseed and their oil can be used in salads. An assessment of pumpkin (*Cucurbita maxima* Duch.) from the Germplasm Bank of Vegetables (BGH) of the *Universidade Federal de Viçosa* (Federal University of Viçosa) revealed the production of hull-less seeds by a mutation in accession BGH 1518 (designated BGH 7653). Thus, this work aimed at the physical-chemical characterization of the hull-less seeds identified, by comparing them with seeds with normal integument from the original accession. The weight of one thousand seeds was similar among the genotypes; however, the dry seed yield per fruit of the original sample was higher. The chemical composition of the normal seeds was 41.75% of oil, 28.64% of proteins and 3.74% of ash, whereas the corresponding composition of the mutant BGH 7653 was respectively 46.70%, 37.93%, and 4.5%. The accession BGH 7653 has potential for breeding programs concerning the consumption of roasted seeds, the oil production for salads, and the use for medicinal and industrial purposes.

Keywords: oil, protein, ash, pumpkin, naked seed.

Introduction

The seeds of *Cucurbita* sp. have been used as food and medicines (1,3,14), because they are excellent sources of proteins (32-44%), oil (34-50%) and vitamin E (1,2,3,5,6,7,8,10,12, 14). In Austria, the extraction of oil from the seeds of *Cucurbita pepo* L. has been carried for more than 100 years. Extraction from hulled seeds is difficult, but the extraction became easier and more efficient with the use of a natural mutant, which hindered the lignification of the hull (3,6,7,8,11,12). Currently, the

oil production for salad using hull-less seeds or naked seeds of *C. pepo* are found mainly in Austria, Hungary, Slovenia and Yugoslavia (3,4,8,12,13). The seeds of *C. maxima* contains over 70% of unsaturated fatty acids comprised mainly by linoleic and oleic acids (1,10). The commercial exploitation of genotypes that produce hull-less seed has not been reported for Brazil. However, broad genetic variability is preserved in the Germplasm Bank of Vegetables (BGH) from the Federal University of Viçosa (UFV), which preserves more than 295 accessions of pumpkins (*C. maxima* Duch.) (9). Thus, this paper aims to describe hull-less seeds of *Cucurbita maxima* from the accession BGH 7653, which has potential for integrating the breeding program for human nourishment as well as for oil production. To our knowledge, this is the first report of the hull-less trait in a Cucurbit species other than *C. pepo*.

Materials and Methods

The experiment was carried out at *Horta Velha* located at 600 m of altitude (latitude 42° 52' 53" S, longitude 20° 45' 14" W), Viçosa, Minas Gerais state, Brazil from February to August 2009. An assessment of the seeds of BGH 1518 accession from the Germplasm Bank of Vegetables from the Federal University of Viçosa revealed a hull-less type (designated BGH 7653) apparently arising by spontaneous mutation. The sowing occurred on February 18th 2009 and the transplantation was carried on March 09th 2009. During the culture, normal production and phytosanitary treatments were applied. Plants were sprinkler irrigated. Controlled pollinations were carried out between plants from the same accession, with their flowers protected immediately after pollination using paper bags. The fruits were harvested

45 and 55 days after pollination during the winter, and stored for 45 days. They were weighted before the manual removal of the seeds, and immediately gently washed on a steel sieve with running water to remove the mucilage. After drying, they were solar irradiated for one day, and plump seed were separated from shrivelled seeds. The seeds with moistures around 10% humidity were packed into plastic bags and stored at 5°C. One sample was used to measure the physical characteristics and chemical composition of the seeds. Regarding the physical characteristics of the seeds, ten seeds were randomly chosen from each fruit for length, width and thickness measurements using a digital calliper. Additionally, some seeds were randomly measured to determine hull thickness. We used twelve fruits harvested from different plants from the original accession with hulled seeds of BGH 1518 and only two fruits from the same plant of the hull-less mutant (BGH 7653).

The chemical composition determination of the seeds were carried out in duplicate through the following methods: (a) the dry matter, by drying the seeds at 105 °C for 24 hours; (b) the protein content, by the method of micro Kjeldahl using the factor 6.25; (c) lipid content, by the Soxhlet device using hexane as solvent; and (d) the ash incinerating at 600 °C in a muffle furnace for 4 hours.

The software Microsoft Excel was used for the data analysis and the results expressed the mean and standard deviation (SD) from different accessions.

Results and Discussion

Variation was observed for the seed yield, physical characteristics and for chemical composition of hull-less seeds when compared to the seeds with normal integument (Table 1) therefore demonstrating the potential for the breeding program to enhance the value of the product and to create new niches in the market, which would create more jobs and would provide more income to farmer.

The weight of the fresh fruit was 2.819 kg (SD ± 1.54) for the accession BGH 1518 and 3.139 kg (SD ± 0.04) for the mutant BGH 7653.

The number of seeds obtained per fruit, the dry weight of seeds per fruit, and the weight of one thousand seeds were 498.38 (SD ± 248.76), 78.94g (SD ± 36.42) and 164.91g (SD ± 37.30) for the accession BGH 1518 and 268.04 (SD ± 51.36), 42.707g (SD ± 10.90) and 158.34g (SD ± 51.36) for the mutant BGH 7653, respectively. The difference between the total dry weight of the accession and the mutant is mainly due to the number of seeds per fruit. The values regarding the number of seeds per fruit and the total dry weight of the mutant BGH 7653 were

higher than those obtained by (7) for the four selected lines of *C. pepo* "hull-less", showing therefore the potential of hull-less seeds as snackseeds. The weight of one thousand seeds of the genotypes assessed was lower than the 12 cultivars of *C. maxima* used by Stevenson *et al.* (10).

The ratio between the dry weight of the seeds and the fresh weight of the fruits was 2.95% (SD ± 0.94) for the accession BGH 1518 and 1.36% (SD ± 0.36) for the hull-less mutant BGH 7653. Such difference mainly reflects the number of seeds obtained per fruit, where the mutant BGH 7653 produced almost half of the number of the original genotype. A 20-year program focused on selection in *C. pepo* to increase hull-less dry seeds mass compared to fresh fruit weight achieved an increase from 1.5% to approximately 3.0% (13).

The length, width and thickness of the seeds were 16.8mm (SD ± 1.39), 8.96mm (SD ± 0.96) and 3.13mm (SD ± 0.49) for the accession BGH 1518 and 15.5mm (SD ± 0.42), 9.16 mm (SD ± 0.62) and 2.81 (SD ± 0.16) for the mutant BGH 7653, respectively. The genotypes have similar measurements.

The thickness of the seed depends on its hull and embryo thickness. According to Andres (3), the testa of seed varies in thickness and hardness, and thinner testa is tolerated when ground or roasted. Stuart and Loy (11) measured several layers of the testa and found that the dry hull-less seeds have minimal dimensions. Thus, the measures from the normal type showed 0.43 mm in dimension while the hull-less mutant variety 'Tricky Jack' was 0.20 mm. We observed variable thickness in different plants of BGH 1518, whose white hulled seeds showed a mean thickness of 0.26 mm (SD±0.03) and the brown hulled seeds 0.63 mm (SD±0.18).

The accession BGH 7653 had similarities with the hull-less mutant described by Stuart & Loy (11), Winkler (12) and Fruhwirth & Hermetter (6). The mutant showed the embryo cover comprised of a rudimentary testa (Figure 1).

The moisture level of the seeds was 8.77% (SD ± 1.03) for the accession BGH 1518 and 8.97% (SD ± 2.32) for the mutant BGH 7653. Such values were similar to those observed for *C. maxima* by Cerqueira *et al.* (5), but considerably higher than those obtained by Alfawaz (1) and Amoo *et al.* (2). The seed moisture reflects the drying system and the equilibrium of the relative humidity with the environment in which the seeds are kept.

The mean protein level of normal seeds of the accession BGH 1518 was 28.64%, whereas for BGH 7653 hull-less mutant it was 37.93% (SD ± 0.88). Such values were lower than those observed for *C. maxima* by Alfawaz (1), but higher than those obtained by Amoo *et al.* (2) and Cerqueira *et al.* (5). They were also lower than most of

lines of hull-less *C. pepo* assessed by Idouraine et al. (7), but similar to those obtained by Younis et al. (14) when using African accessions cropped in areas of altitude above 2100 m.

The mean oil level of the seeds of hull-less mutant BGH 7653 was 46.70%, and higher than the mean of the seeds from the original accession BGH 1518 [41.75% (SD \pm 0.59)], in accordance with Alfawaz (1) who showed an oil percentage increase after removing the hull of the seeds. Such genotypes were higher than those of *C. maxima* used by Alfawaz (1), Stevenson et al. (10) and Cerqueira et al. (5). The potential of the genotype BGH 7653 must be assessed when compared to the genotypes obtained by breeding programs of *C. pepo* for oil production. Thus, its oil concentrations are close to the best lineages selected by Idouraine et al. (7) and to the varieties used by Winkler (13) in breeding programs in Austria. Therefore, the high percentage of oil observed for hull-less mutant BGH 7653 supports the proposal regarding its usage as progenitor in breeding programs for oil extractions from seeds of *C. maxima*.

The mean level of ash obtained for the genotype BGH 7653 was 4.50% (SD \pm 0.64), higher than that observed for the mean of original genotypes BGH 1518 (3.74%), shown in the Table 1. The ash levels observed for the mutant BGH 7653 were lower than those obtained for *C. maxima* by Alfawaz (1) and Cerqueira et al. (5), as well as for *C. pepo* by Idouraine et al. (7).

The mutant BGH 7653, rich in oil and protein, may increase the use of seeds as food supplement and the development of genotypes destined for food, medicinal or industrial purposes (oil extraction). The development of varieties and hull-less hybrids may result in additional income to farmer without increasing their current cropping area.

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Table 1. Characteristics of seed of the normal accession (BGH 1518) and hull-less mutant (BGH 7653) of *C. maxima*

Characteristic	BGH 1518	BGH 7653
Mean weight of fresh fruits (kg) (a)	2.819 (SD ± 1.54)	3.139 (SD ± 0.04)
Mean number of seeds per fruit	498.38 (SD ± 248.76)	268.04 (SD ± 51.36)
Mean weight of dry seeds per fruit (b)	78.94 (SD ± 36.42)	42.707 (SD ± 10.90)
Ratio (b/a)	2.95 (SD ± 0.94)	1.36 (SD ± 0.36)
Weight of one thousand dry seeds (g)	164.91 (SD ± 37.30)	158.34 (SD ± 10.32)
Seed length (mm)	16.8 (SD ± 1.39)	15.5 (SD ± 0.42)
Seed width (mm)	8.96 (SD ± 0.96)	9.16 (SD ± 0.62)
Seed thickness (mm)	3.13(SD ± 0.49)	2.81 (SD ± 0.16)
Humidity (%)	8.77 (SD ±1.03)	8.97 (SD ± 2.32)
Protein (%)	28.64 (nd)	37.93 (SD ± 0.88)
Oil (%)	41.75 (SD ± 0.59)	46.70 (nd)
Ash (%)	3.74 (nd)	4.50 (SD ± 0.64)

nd = not determined

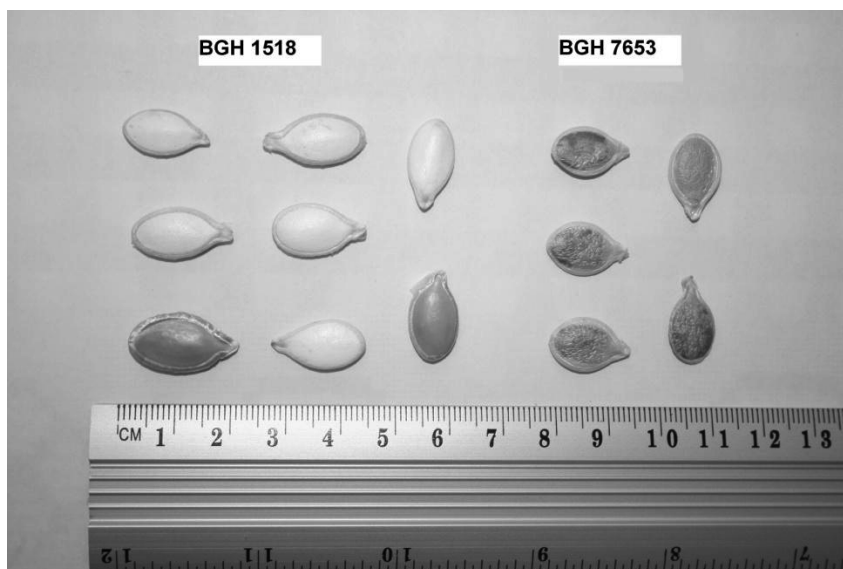


Figure 1. Morphological aspects of normal seeds of the accession BGH 1518 and mutant hull-less seeds BGH 7653.