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Morphological characterization of *Ziziphus* species fruits in the Mid-Zambezi Valley in northern Zimbabwe

[1,2]Innocent Wadzanayi Nyakudya

[1]Department of Crop Science, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe (Permanent Address)
[2]Department of Soil Science and Resource Conservation, National University of Lesotho, P.O. Roma 180, Maseru, Lesotho (Current Address)

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ABSTRACT

Emergence of industrial-scale *Ziziphus* fruits processing in the Mid-Zambezi Valley necessitates selection of superior genotypes for cultivation to meet anticipated increased demand. The study evaluated morphological variation among *Ziziphus* fruits to determine their agronomic value and genetic diversity. Dry fruit samples were collected from bulked fruits and separated into three perceived varieties, which were further separated into 12 collections. Data depicted large coefficients of variation ≈50% for fruit and seed mass. Fruit parameters ranges were: mass (0.56–2.57 g), length (1.14–2.33 cm), width (1.05–2.16 cm), density (0.24–0.38 g/ cm^3), percent pulp (63.03–89.28). Seed parameters were: mass (0.15–0.56 g), length (0.57–1.27 cm), width (0.43–0.66 cm). Fruit mass was positively correlated to yield components. Cluster analysis grouped the collections into two clusters with two sub-clusters each. I concluded that there could be two *Ziziphus* species in the area. The WF-variety is recommended for cultivation and further improvement because of its superior yield and taste variation. The HSDB variety is recommended for its high percent pulp subject to confirmation that hollowness is not a physiological disorder. Research is recommended to determine the *Ziziphus* spp. genotypes; genotypes nutritional composition; effect of ecology on fruit morphology; and evaluate agronomic performance.

Introduction

The genus *Ziziphus* (Rhamnaceae family) has about 100 species distributed in tropical and subtropical regions between latitude 34°S and 51°N and from zero to 2800 m a.s.l. (Razi *et al*., 2013). Major species in the Rhamnaceae family are *spina-christi*, *mucronata*, *nummularia*, *lotus*, *selata*, *spinosa*, *oenoplia*, *vulgaris*, *jujuba* and *mauritiana* (Khan *et al*., 2023). *Ziziphus mauritiana* L. (Indian ber or jujube, Chinese apple, Musawu in Zimbabwe) is native to South Asia and eastern Africa (Bebawi *et al*., 2016) and it is the species that is reported to occur in the Mid-Zambezi Valley where the tree has naturalised. Dhileepan (2017) report on invasive *Z. mauritiana* populations in southern

Corresponding author's E-mail iwnyakudya@buse.ac.zw (Dr. I. W. Nyakudya)

Africa, specifying Zimbabwe and Zambia. However, the species can be positively exploited to produce economic benefits of national importance. *Ziziphus* spp. fruit quality is best under sunny, dry and hot conditions that enhance accumulation of important biochemicals (phenols and flavonoids) and antioxidant activity, which ultimately increases the fruits' medicinal and nutritional value (Riaz *et al.*, 2021). The Mid-Zambezi Valley provides optimum conditions for production of high quality fruits because fruit growth and ripening occurs during the dry season, making the region a high potential area for *Ziziphus* fruits production.

Information on exceptional nutritional, medicinal and cosmeceutical value of *Ziziphus* fruits, and the forms in which they are utilised is widely documented (Butt *et al.*, 2021; Ma *et al.*, 2021; Jailani *et al.*, 2020; Nyanga *et al.*, 2013 and Rathore, 2009). Despite acknowledgement of the significant contribution of *Ziziphus* fruits in the livelihoods of local people in the Mid-Zambezi Valley, the genus has not been adequately researched compared to China and India (Razi *et al.*, 2013). However, emergence of industrial-scale processing of *Ziziphus* fruits through establishment of the Zambezi Valley Masawu Value Addition Plant in 2025 might prompt selection of varieties for cultivation to meet the anticipated fruit demand.

Morphological parameters provide indices for characterization, taxonomic classification, and agronomic value of plants, and information on genetic diversity (Riaz *et al.*, 2021). Therefore, the first step in the cultivar selection process is to characterize the morphological diversity of existing *Ziziphus* fruits gene pool in the Mid-Zambezi Valley, in order to develop a reliable identification key, which helps selection of better genotypes for cultivation. Although the species reported to occur in the Mid-Zambezi Valley is *Z. mauritiana*, there are no published reports on characterisation of genetic and morphological traits of the *Ziziphus* spp., therefore, it is possible that other species exist in the region.

Ziziphus spp. produce stone fruits whose morphological traits include colour, size, shape, flesh (mesocarp) weight, and stone weight. Remarkable variations of fruit morphological traits have been reported in *Z. jujuba* cultivars (Ivanišová *et al.*, 2017). The colour of dried Chinese *Z. jujuba* is an important quality index with darker colours having low market value and quality (Fang *et al.*, 2010). Markovski and Velkoska-Markovska (2015) found that *Ziziphus* fruits seed weight, fruit weight, fruit flesh/seed ratio, and fruit yield showed high heritability estimates and genetic advance. Accordingly, they reported that these traits can be improved through selection.

The objective of the study was to evaluate morphological variation among *Ziziphus* fruits in the Mid-Zambezi Valley in order to determine their agronomic and industrial value, and genetic diversity. The study combined conversational interviews with local *Ziziphus* fruits harvesters and traders (local suppliers); measurements of fruit mass, length, width, percent edible portion (pulp), and density; and seed mass, length, and width; and qualitative assessment of fruit shape, colour, firmness and skin texture. Fruit and seed size and mass are yield components, whilst fruit colour, shape, firmness, and percent pulp are quality attributes.

Material and Methods

Description of the study area

The study was conducted in Muzarabani (16.12°S, 31.15°E, 334 m a.s.l.) in northern Zimbabwe. Soils in the area range from fine-grained loamy sands to sandy clays. Mean annual rainfall is < 650 mm and the area is frost-free with a mean maximum temperature of 28–30°C (Manatsa *et al.*, 2020). The vegetation is dry savanna (Hoare *et al.*, 2002) comprising *Colophospermum mopane* Kirk ex Benth., and *Combretum*, *Sterculia* and *Vachellia* spp. *Ziziphus mauritiana* is dominant along waterways.

Data collection

Conversational interviews

Data on indigenous knowledge about local *Ziziphus* fruit varieties was collected from 112 local fruit suppliers through conversational interviews (West and Conrad, 2018) from September to December 2024. Participants were interviewed individually or in groups. In some cases multiple interviews were conducted with same participants for checking consistency of responses. The main question that I asked participants was: "How many varieties of Musawu are found in your area?" Follow-up questions captured morphological differences among varieties, fruit taste, and the production context that affects fruit size and mass.

Sampling of fruits for measurements

Seven sampling visits were conducted and at each visit fruits were placed in separate containers with the aid of local suppliers based on perceived varieties. Three varieties were identified: (i) wrinkled firm with colours ranging from reddish brown to dark brown, sub-divided into sweet sub-variety and less sweet sub-variety; (ii) hollow soft dark brown; and (iii) small golden-yellow to light brown sweet. At the end of the sampling period, the fruits varietal categories were bulked in separate 50-kg bags. From the varieties, fruits were graded into 12 sub-categories (collections) of different morphologies (Table 1). From the 12 collections, fruit samples measuring about 2000 cm^3 were collected through the quartering technique and placed in plastic sampling bags ready for physical measurements.

Undisturbed fruits quantitative measurements

Quantitative measurements were conducted at the Bindura University Biology Laboratory. Before measurement, all fruit samples were air-dried in the open sun for 5 days to remove moisture that could have been absorbed during handling. The twelve 2000 cm^3 samples were separated into four quarters each using the quartering technique. Three quarters were placed in three 650 cm^3 beakers and filled to the brim using fruits from the fourth quarter forming three replicates per sample. The number of fruits in each replicate 650 cm^3 beaker were counted. Fruit parameters measured were mass, width and length. Fruit mass was measured using a digital analytical balance (Model Adam Nimbus, NBL-254i, USA) with a sensitivity of ±0.0001g). From each replicate, three fruits were randomly selected for measuring width and length using Vernier's caliper (Model 6050926 Shangai Shenhan Measuring Tools Co. Ltd.) with an accuracy of 0.02 mm.

Undisturbed fruits qualitative measurements

Fruit colours were determined using a Munsell Colour Sheet. Fruit skin texture was assessed visually and by hand feel noting features like smoothness or wrinkling. Firmness or softness of fruits was assessed through pressing the fruits between fingers and fruit shape, fruit apex shape and fruit base shape were assessed visually.

Disturbed fruits measurements

After undisturbed fruit measurements, fruits were boiled for 30 minutes in 1.25 litres of water and the pulp [pulp (mesocarp) and skin (exocarp)] was separated from the seed [seed covering (stone) (endocarp) and seed] by pressing using a stainless steel potato masher. The samples were left to cool down before seeds were picked by hand, cleaned

Table 1. Description of varieties of *Ziziphus* spp. fruits identified by local suppliers and sub-categories (collections)

Variety description	Collection	Sub-category description	Remarks
Wrinkled firm	1	Large wrinkled firm sweet	Fruits exhibited similar morphology, grading was based on fruit size except for sample number 1, which was selected for its sweetness
	2	Large wrinkled firm	
	3	Medium wrinkled firm	
	4	Small wrinkled firm	
Hollow soft dark brown	5	Large soft dark brown	Fruits exhibited similar morphology, grading was based on fruit size
	6	Medium soft dark brown	
	7	Small soft dark brown	
	8	Very small soft dark brown	
Small golden-yellow sweet	9	Wrinkled golden-yellow very sweet	Fruits exhibited varying degrees of wrinkling and sweetness with the golden-yellow being very sweet
	10	Soft light brown sweet	
	11	Small wrinkled light brown sweet	
	12	Small soft light brown	

with distilled water and further cleaned to remove pulp with the aid of a kitchen knife for the wrinkled firm variety, which exhibited the 'clingstone' characteristic, where pulp adheres to the seed strongly (Figure 1). After cleaning seeds were sun-dried to a constant weight for five days and the seed length, seed width and seed mass were measured.

Fig. 1. Seeds from *Ziziphus* spp. wrinkled firm variety fruit depicting 'clingstone' behaviour

Data analysis

Qualitative data analysis

Data from conversational interviews was analysed using selective coding, that is, focussing on core terms that described varieties and management of the fruit trees.

Calculation of derived parameters

Fruit and seed shape indices were calculated by dividing the lengths by their respective widths. Individual fruit mass was calculated by dividing the mass of fruits in each sub-sample by the number of fruits in that sub-sample. Fruit bulk density was calculated by dividing the mass of fruits in sub-sample by the volume of the beaker (650 cm^3). Percent pulp on a dry mass basis was calculated as:

Percent pulp = [((Mass of dry fruits – Mass of dry seeds)/Mass of dry fruits) × 100] [Eq. 1]

Statistical analysis

Variability of quantitative parameters was evaluated using descriptive statistics. Fruit mass, density, length, width and shape index; and seed mass, length, width, and arcsine-transformed percent pulp values, and shape index data for the 12 collections were subjected to ANOVA. Pearson correlation analysis was performed to establish the relation between fruit mass and, fruit and seed quantitative parameters. Fisher's LSD test was used to separate significantly different means at P = 0.05. Hierarchical cluster analysis was conducted using Ward's method and the Squared Euclidean distance. Cluster analysis was performed using z-scores for fruit mass, fruit length, fruit width, fruit shape index seed mass, seed length, seed width and seed shape index. All quantitative data analyses were performed using IBM SPSS for Windows Version 20.0.

Results and Discussion

Description of Ziziphus species varieties and agronomic practices

The local people identified three *Ziziphus* spp. varieties based on fruit morphology: wrinkled firm; hollow soft dark brown; and small golden-yellow sweet varieties (Table 1, Figure 2). The large wrinkled firm variety was perceived to have more pulp than other varieties and preferred for dry fruit consumption. Fruit wrinkling and firmness were regarded good quality indicators because they were linked to fruit filling, i.e. having more pulp. The hollow soft dark brown variety was the least preferred because it was regarded as having cavities in the fruit pulp. The small golden-yellow sweet variety was distinguished by its small size and colour. Whilst occurrence of small fruits in the other varieties was reported to be caused by water stress or tree ageing, fruits from this variety were reported to be 'genetically' small.

Some varieties were reported to cause discomfort in the form of sharp, pain in the teeth in response to acidity (dentin hypersensitivity). The wrinkled firm variety, varied in sweetness or sourness, the hollow-soft dark brown variety was generally reported to be sour whilst the small golden-yellow sweet variety was reported to be always sweet to very sweet.

All light brown to golden-yellow fruits were reported to be sweet regardless of variety. Fang *et al.* (2010) state that the colour of dried Chinese *Z. jujuba* is an important quality index; the darker the colour, the less the quality and market value. Due to the underdevelopment of *Ziziphus* fruits value chain in the Mid-Zambezi Valley, the importance of colour did not feature in this study but it could enhance acceptability of the small golden-yellow sweet collections to some users. For evaluated collections fruit colour differed among varieties as reported by local suppliers (Figure 2); wrinkled firm and hollow soft dark brown varieties varied from dark brown to reddish brown; whilst the small golden-yellow sweet variety was golden-yellow to light brown. Fruit skin texture varied from winkled in the wrinkled firm variety and some collections in the small golden-yellow sweet variety, to smooth in some collections in the small golden-yellow sweet variety and slightly smooth in the hollow soft dark brown variety (Figure 2). Fruit shapes in the wrinkled firm variety varied from oval, obovate, oblong, and round whilst those in the other two varieties were generally round. Fruit apex shapes were oblate and rounded in all varieties and fruit base shapes were mostly flattened in all varieties but there were some fruits with oval bases in the wrinkled firm variety and the hollow soft dark brown variety.

Fig. 2. *Ziziphus* fruit collections from three perceived varieties identified with the help of local suppliers in the Mid-Zambezi Valley

The wrinkled firm variety and wrinkled collections in the small golden-yellow sweet variety were firm whilst the hollow soft dark brown variety and non-wrinkled collections in the small golden-yellow sweet variety were soft. The hollow soft dark brown variety cracked when pressed between fingers creating a popping sound most of the times. These variations suggest that there could be at least three varieties as reported by local suppliers. Conversational interviews revealed that the only management practice that was implemented on *Ziziphus* trees was pruning to increase fruit size.

Quantitative characteristics of *Ziziphus* species fruits

The twelve collections depicted large variation for the major yield parameters, fruit mass and seed mass, coefficients of variation (CV) 56.6% and 49.2% respectively and moderately large variations for fruit length, fruit width, and seed length CV ≥ 25% (Table 2). The variations were also confirmed by cluster analysis, which showed existence of two divergent genotypes with sub-clusters (Figure 3). Clustering of the two clusters at a long genetic distance, ± 25, signifies large genetic divergence probably at species level. High fruit weight in *Ziziphus* plants is a supreme characteristic in breeding programmes Norouzi *et al.* (2017), but it can be affected by ecological conditions and cultivars (Riaz *et al.*, 2021). Therefore, differences in mass of fruits from similar geographical areas may be due to genotypic effects (Karadeniz, 2002). This implies that the large variations in fruit mass observed in this study may be due to genotypic variations among the collections supporting local suppliers' perceptions. Results from this study agree with previous studies on *Ziziphus* spp., which showed high genetic CVs in fruit mass (Norouzi *et al.*, 2017; Islam *et al.*, 2010).

Fruit density had a moderate CV of 15.1%. The densest collections from the wrinkled firm variety were 1.6 times denser than the least soft light brown sweet collection (Table 2). Coefficient of variation for percent pulp showed low variability (10.4%) being lowest in the small wrinkled light brown sweet collection (63.03%) and highest in the large soft dark brown collection (89.28%).

Fruit mass was positively related ($P<0.001$) to: fruit length, $r = 0.852$; fruit width, $r= 0.823$; seed mass, $r =0.941$; seed length, $r = 0.836$; and seed width, $r = 0.810$. Similarly seed mass was positively correlated with seed length, $r = 0.800$ and width, $r = 0.733$). High positive correlations of these traits with fruit mass implies that they may be suitable criteria for selecting *Ziziphus* genotypes for cultivation and breeding. Grygorieva *et al.* (2014) also obtained high correlations between the fruit mass and the fruit width ($r = 0.971$) and fruit mass and fruit length ($r = 0.771$).

Hurtado *et al.* (2012) highlighted the significance of investigating phenotypic diversity in determining uniqueness and distinctness of genotypes. Ward's method resulted in two clusters at a short genetic squared Euclidean distance of 6 (Figure 3) showing that within each cluster there are genetically related varieties with shared ancestry and adaptation to similar ecological conditions. Clustering of three wrinkled firm and two hollow soft dark brown collections into one cluster at a relatively short distance of 6 (Figure 3) suggests that part of the observed hollowness may be a physiological disorder. It is important to note that, although hollowness ("fruit shrink") is a genetic trait, in *Ziziphus* fruits it is generally considered a physiological disorder caused by poor ovule fertilization, high temperatures, drought, nutrient imbalance, or pests and diseases Kumar *et al.* (2015). Ranked using fruit (mass, length, width, density, percent pulp) and seed (mass, length, width) the large soft dark brown, large wrinkled firm sweet, and medium soft dark brown collections were the top three (Table 2).

Conclusion

Twelve collections of *Ziziphus* spp. fruits in three perceived varieties were evaluated quantitatively and qualitatively. The collections exhibited large variation in yield and quality parameters suggesting that there were genotypic variations. Cluster analysis separated the collections into two clusters at a relatively short Squared Euclidean distance of 6 that combine ±25, implying huge genotypic divergence suggesting that there could be two species in the area. Wrinkled firm and large soft dark brown collections depicted superior characteristics for yield components. The golden-yellow sweet variety exhibited inferior yield attributes but its unique colour may be desired in industrial production. Overall, this study provides a basis for selection of cultivars for cultivation but genetic studies will be required for fruit improvement.

Table 2. Measured quantitative characteristics of Ziziphus species fruits collected from the Mid-Zambezi Valley in northern Zimbabwe

Variety description	Collection identity	Description of fruits in sub-category (collection)	Mean fruit dimensions/ parameters (SD)[1]				Mean seed dimensions/ (SD)			
			Length (cm)	Width (cm)	Shape index	Mass (g)	Mass (g)	Length (cm)	Width (cm)	Shape index
Wrinkled firm	1	Large wrinkled firm sweet	2.07 (0.11)^{a}	1.81 (0.13)^{ac}	1.14 (0.05)^{ac}	2.33 (0.03)^{a}	0.52 (0.05)^{a}	1.27 (0.15)^{a}	0.61 (0.06)^{ac}	2.09 (0.28)^{aci}
	2	Large wrinkled firm	1.96 (0.18)^{a}	1.68 (0.20)^{ad}	1.18 (0.14)^{abd}	2.05 (0.09)^{b}	0.47 (0.02)^{a}	1.15 (0.15)^{bd}	0.62 (0.06)^{ac}	1.86 (0.29)^{abif}
	3	Medium wrinkled firm	1.72 (0.12)^{b}	1.38 (0.18)^{bf}	1.27 (0.20)^{d}	1.34 (0.02)^{c}	0.34 (0.03)^{b}	1.19 (0.11)^{ab}	0.56 (0.04)^{ab}	2.11 (0.14)^{cde}
	4	Small wrinkled firm	1.49 (0.15)^{c}	1.33 (0.10)^{bg}	1.12 (0.07)^{abe}	1.11 (0.10)^{d}	0.33 (0.03)^{b}	0.99 (0.05)^{c}	0.51 (0.07)^{bd}	1.92 (0.29)^{adf}
Hollow soft dark brown	5	Large soft dark brown	2.33 (0.11)^{d}	2.16 (0.10)^{e}	1.08 (0.03)^{abe}	2.57 (0.16)^{e}	0.56 (0.07)^{c}	1.06 (0.05)^{de}	0.66 (0.07)^{ce}	1.62 (0.166)^{bg}
	6	Medium soft dark brown	2.09 (0.51)^{a}	1.92 (0.40)^{c}	1.08 (0.07)^{abe}	1.68 (0.12)^{f}	0.41 (0.01)^{d}	1.03 (0.10)^{ce}	0.61 (0.05)^{ae}	1.69 (0.13)^{bfj}
	7	Small soft dark brown	1.65 (0.19)^{bc}	1.51 (0.23)^{dfg}	1.10 (0.12)^{abe}	1.21 (0.04)^{d}	0.15 (0.04)^{e}	0.98 (0.12)^{c}	0.52 (0.06)^{d}	1.91 (0.30)^{ef}
	8	Very small soft dark brown	1.51 (0.33)^{c}	1.40 (0.31)^{fg}	1.08 (0.09)^{abe}	0.78 (0.02)^{g}	0.22 (0.01)^{f}	0.80 (0.11)^{f}	0.50 (0.04)^{df}	1.64 (0.30)^{bk}
Small golden-yellow sweet	9	Wrinkled golden-yellow very sweet	1.15 (0.09)^{e}	1.08 (0.06)^{h}	1.07 (0.03)^{ce}	0.56 (0.00)^{h}	0.16 (0.01)^{ef3}	0.67 (0.07)^{gh}	0.46 (0.04)^{fh}	1.47 (0.09)^{gjkl}
	10	Soft light brown sweet	1.14 (0.08)^{e}	1.05 (0.06)^{h}	1.09 (0.10)^{abe}	0.56 (0.02)^{hi}	0.15 (0.01)^{ef}	0.57 (0.07)^{hi}	0.43 (0.06)^{gh}	1.35 (0.16)^{hl}
	11	Small wrinkled light brown sweet	1.22 (0.10)^{e}	1.10 (0.10)^{h}	1.11 (0.07)^{abe}	0.70 (0.04)^{gij}	0.20 (0.03)^{ef}	0.75 (0.10)^{fg}	0.47 (0.08)^{dh}	1.60 (0.21)^{gjk}
	12	Small soft light brown	1.24 (0.09)^{e}	1.12 (0.07)^{gh}	1.10 (0.11)^{abe}	0.63 (0.02)^{hj}	0.17 (0.02)^{ef}	0.68 (0.07)^{gi}	0.43 (0.07)^{h}	1.62 (0.24)^{gjk}
Coefficient of variation (%)			26.8	27.0	9.9	56.6	49.2	25.3	13.3	17.7

[1]Values in the same column with a common letter in the superscript are not significantly different ($P > 0.05$)

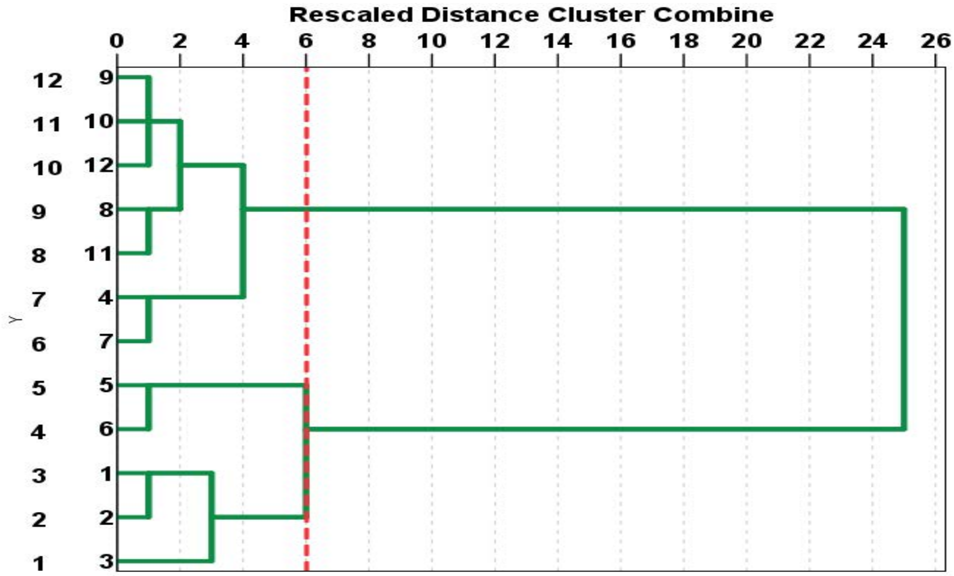

Fig. 3. Cluster analysis of 12 *Ziziphus* spp. collections based on fruit mass, length, width, shape index; and seed mass, length, width and shape index using squared Euclidean distances (1-Large wrinkled firm sweet, 2-Large wrinkled firm, 3-Medium wrinkled firm, 4-Small wrinkled firm, 5-Large soft dark brown, 6-Medium soft dark brown, 7-Small soft dark brown, 8-Very small soft dark brown, 9-Wrinkled golden-yellow very sweet, 10-Soft light brown sweet, 11-Small wrinkled light brown sweet, 12-Small soft light brown)

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Conflict of Interest

The authors declare no conflict of interest.

Data Sharing

All relevant data are within the manuscript.

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