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Cultivar Effect on Moringa oleifera Glucosinolate Content and Taste: A Pilot Study

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Cultivar Effect on *Moringa oleifera* Glucosinolate Content and Taste: A Pilot Study

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Leaves of the tropical tree Moringa oleifera *are widely promoted in areas of chronic malnutrition as nutritional supplements for weaning infants and nursing mothers. Adoption, in these circumstances may hinge upon taste, which can vary greatly amongst cultivars. It is widely assumed that this taste variation is primarily germplasm-dependent, and results from the breakdown of glucosinolates to isothiocyanates. Leaves of 30 accessions, grown at a single field plot, were sampled 3 times over the course of a year. Taste, assessed in a masked protocol, was not related to glucosinolate content of the leaves.*

KEYWORDS glucosinolate, biomass, biofumigation, nutrition, taste, isothiocyanate, tropic, tree

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INTRODUCTION

Moringa oleifera is a pan-tropical tree of great potential value in the tropics due to its nutritional, biomass, water-purification (seeds), agro-forestry, bio-fumigation (leaves), and medicinal qualities (Palada, 1996; Fahey, 2005). It produces large quantities of seedpods within the first few years of growth. By tradition, it is almost exclusively propagated by seed in certain regions (e.g., the Sudan) and by vegetative methods in other areas (e.g., India) (Jahn et al., 1986). As a result of the rapid dissemination of germplasm from its point of origin in the sub-Himalayan region of India, Pakistan, and Afghanistan (Olson, 2002), development of hybrid cultivars has not occurred, nor has germplasm distribution been well documented (Olson and Rosell, 2006). As a result, performance evaluations of multiple accessions of this single species have not been well documented by objective measures, nor has the content of specific potentially valuable phytochemicals (e.g., glucosinolates) been compared across accessions. Correlating horticultural and phytochemical characteristics (phenotype) with genotype will be a critical task as increasingly detailed scientific research focuses on the phytochemicals of *M. oleifera* and their contribution to the widely reported disease-preventive and therapeutic properties of this edible plant.

As with all foods, palatability is a very important parameter to those interested in the utilization of Moringa for its nutritional and medicinal properties. It has been widely promoted by a great many non-governmental organizations (NGOs), and even by some governmental agencies, as a possible weaning food to prevent starvation in chronically malnourished populations (Fahey, 2005). Regional preferences regarding taste and other organoleptic, horticultural, and agronomic characteristics vary greatly. For example, in one part of India the pods of the tree are cherished (and they are quite hot and spicy) and the leaves are avoided, and in another part of India the opposite is true. The taste of fresh leaves is known to vary greatly – some of them are quite "radishy", hot, and pungent, while others are very mild. To the extent that the leaves (typically dried and powdered) are suggested for use in weaning porridges, it would seem to be important to be able to suggest or provide sources of less harsh or less radishy tasting leaves. Although the potential aversion of infants to a very pungent gruel has not been tested scientifically, from a commonsense standpoint it would seem logical that having taste options would be beneficial.

Cells throughout the tissues of *Moringa* spp. contain glucosinolates (Bennett, 2003; Fahey et al., 2001; Fahey, 2005; Kjaer, 1979) – primarily 4-(rhamnopyranosyloxy)benzyl glucosinolate [4RBGS], and monoacetyl-(rhamnopyranosyloxy)benzyl glucosinolate [MAGS], and much lesser quantities of benzyl glucosinolate. These glucosinolates are converted to isothiocyanates by an enzyme called myrosinase. This enzyme resides in the microflora

within the gastrointestinal tracts of human beings, and it also co-exists in the tissues of plants containing those glucosinolates. It is released upon maceration of that plant tissue (e.g., chewing) and it acts very rapidly to convert the benign glucosinolates into their much more active and pungent, spicy or hot tasting isothiocyanates. The isothiocyanates produced from the reaction of glucosinolates and myrosinase in Moringa are responsible for at least some of the medicinal properties that have been ascribed to Moringa (Fahey, 2005), and we expect that they are responsible for some portion of the taste profile of the distinctive leaves and pods of this species (*Moringa oleifera*) – the horseradish tree, as it's commonly known. We have thus evaluated the palatability of 30 accessions of *M. oleifera*, and we have measured the levels of the primary glucosinolates found in leaves harvested from all 30 accessions.

MATERIALS AND METHODS

Thirty accessions of *Moringa oleifera* were grown from seeds in blocks of 10 trees, in a single field plot in North Fort Myers, Florida, USA. *M. oleifera* accessions were obtained from sources in North and Central America, the Caribbean, Africa, and India. The breeding line PKM1 was developed by Tamil Nadu Agricultural University, and has been widely distributed via Horti Nursery Networks (Erode, TN, India). Technically, although PKM1 has been called a variety, it appears to be a population that has been allowed to intercross to establish panmixia equilibrium. It is thus a "synthetic population" produced by repeatedly culling off-types from an open-pollinated population grown in isolation for several generations (Anbarassan et al., 2001). A bushy growth habit can be attained with this breeding line by repeated pruning, resulting in some of the branches even growing in a horizontal orientation. Similarly, a line called PKM2 was selected in India for its heavy production of singularly large and fleshy seedpods ("drumsticks"). Both the PKM1 and PKM2 lines can be intensively managed to produce seedpods after as little as six months from planting. They have become quite popular with growers in India and other parts of the developing world, and have also been the subject of at least one taste test in which the samples were evaluated after cooking, rather than being tasted fresh (Pasternak, 2004). Seed source and country of origin is provided in Table 1.

On July 10, 2003, seedlings were transplanted from a seedling nursery to an irrigated plot on a poorly drained, deep sand soil. The soil is characterized as a sandy, siliceous, hyperthermic Arenic Haplaquod soil in the Immokalee Series. The test site is level and poorly drained, with deep sand dominated by medium and fine grain sand, having a low natural fertility, an organic matter content of 1-2%, and a pH of 7.5.

TABLE 1 Total (Rhamnopyranosyloxy)benzyl Glucosinolate (RBGS) Content and Taste of 30 *Moringa oleifera* Accessions.
There was a highly significant trend for ranking of the 30 accessions by RBGS across harvest dates ($z = 5.06$, $p < 0.001$, by nptrend analysis), and there was no correlation between RBGS and taste (hot-, or overall-) ($p > 0.05$, by ANOVA) at either of the sample dates in which both were evaluated.

		Total RBGS (μmol/g fresh wt.)			Taste (1–3 scale)			
					Hot		Overall	
Source	Accession	May 05	Dec 05	Apr 06	May 05	Apr 06	May 05	Apr 06
North America								
Bradenton, FL	02099-021D	76.5	108	93.5	2.1	2.0	1	0
ECHO Farm	92028-991E	14.1	60.2	62.0	2.7	2.1	2	0
Ft. Myers	92026	39.1	62.8	82.3	2.4	1.4	1	0
N. Wood, FL	00099-001D	30.8	68.5	75.5	2.1	1.4	2	0
Central America								
Villoria, Belize	03051-031D	18.3	37.4	52.7	2.7	2.0	0	1
Mexico	01084-011D	56.9	53.0	61.2	2.6	1.9	0	1
Caribbean (Haiti)								
K. Flanagan	01046-011A	44.7	65.5	65.9	2.2	2.0	2	2
Bohoc	02055-021H	45.0	72.2	66.5	2.0	1.6	3	0
La Gonave	02073-021H	71.4	56.0	93.0	2.6	2.2	0	0
Les Cayes	02057-021H	26.0	43.8	82.3	2.9	2.5	0	1
Port Au Prince	02056-021H	57.9	46.1	67.1	2.4	1.8	0	1
C. Thede	03064-031H	85.0	75.1	98.8	2.7	2.1	0	0
C. Thede	03065-031H	50.1	65.8	79.4	2.1	1.7	2	1
C. Thede	03067-031H	43.1	68.0	56.4	2.1	2.0	1	1

C. Thede	03068–031H	35.4	66.0	65.2	2.7	1.6	1	1
C. Thede	03069-031H	51.4	67.2	71.6	2.3	1.5	0	1
Archai	03070-031H	49.0	47.4	80.2	3.2	2.2	0	1
C. Thede	03071-031H	79.3	63.8	81.2	2.3	1.5	1	2
Titayen	02058-021H	44.7	56.3	90.2	2.9	1.8	1	2
South Asia (India)								
PKM-1 Horti	00045-011A	28.7	46.0	77.3	2.3	1.7	1	2
PKM-2 U. Asmar	03005-031A	62.8	63.1	74.0	2.0	1.5	2	3
Pocha Exports	91070	8.7	49.9	54.9	2.8	1.6	0	0
Trust Hospital	03056-031D	25.6	73.0	50.0	2.4	1.6	0	1
Africa								
CWS Senegal	03052-031D	67.0	78.6	87.5	2.6	1.5	0	0
Tanzania/Malawi	98018	23.1	101	99.1	2.0	1.9	2	1
Msingi, Tanzania	03034-031D	68.1	98.2	83.7	2.4	1.8	1	0
Optima, Tanzania	01088-011D	28.1	61.0	43.4	2.4	1.9	1	0
Optima, Tanzania	03066-031H	59.9	55.9	86.9	2.6	1.6	0	1
Groves, Mozambique	03055-031D	79.4	50.8	81.2	2.3	1.5	1	2
Binga Trees, Zimbabwe	03053-031D	21.6	78.7	70.9	2.1	2.2	2	1

A total of 283 trees representing 30 accessions were planted in 10 rows, with 3 accessions per row and 10 trees per accession (except for 3 accessions which together total 13 trees). Young trees were trimmed to a height of either 0.45 or 0.9 m during the spring of 2004, and were again trimmed to a height of either 1 m or 2 m in the fall of 2004. Samples from 3 trees per plot were taken on May 5, 2005 for taste evaluations. This plot of trees was established in order to evaluate differences in leaf production (biomass and nutritional analysis not reported herein), and the trees were planted at relatively close spacings (90 cm within a row and 180 cm between rows). The primary intention of the test plot was to maintain a regular pruning schedule that would permit us to compare yield and taste by accession, and to provide large quantities of leaves with which to make dried Moringa leaf powder for other uses. In southwest Florida, *M. oleifera* grows very rapidly during the hot rainy season and goes into a semi-dormant stage during the cooler winter months. Leaf collections were made twice a year – once in the fall to collect the large quantity of leaves produced over the peak growing season, and once in the spring. Leaf samples were taken for taste and phytochemical analysis immediately following each of these biomass harvests from branches that were tagged so that they could be repeatedly accessed for this purpose. Thus, harvests were performed in May 2005 (the hot, rainy season), December 2005 (cooler, dry season), and April 2006 (spring period of vigorous new growth).

Leaf samples removed at 2 of the 3 harvest dates were used for immediate taste testing, and at each date subsamples containing 3 full leaves (each leaf of *M. oleifera* contains between 20 and 40 individual leaflets) were immediately chilled by gently packing in a styrofoam cooler with "blue" ice-packs, and shipped by overnight freight to Baltimore for immediate extraction and evaluation of glucosinolate content as described elsewhere (Troyer et al., 2001; Fahey et al., 1997; Wade et al., 2007). Organoleptic evaluation (*overall taste* and *relative hotness*) was performed at the first and third harvest dates. Tree height was measured at only the first harvest date and girth was measured at all harvests. Glucosinolate content was measured for all samples.

DATA COLLECTION

Four randomly tagged trees of each accession were used throughout the trial (the same trees at each measurement period) to collect direct measurements of tree height (May 2005 only) and tree girth (at 0.3 m above ground). One branch (from 1 tree) was tagged in each accession at the first taste evaluation, and this branch was used as a source of fresh leaves for this and subsequent taste tests.

Organoleptic quality (taste and hotness or pungency) of the fresh leaf was evaluated for all 30 accessions of *M. oleifera*. Data were collected by teams of unpaid volunteers (9 in 2005 and 10 in 2006), who received instructions on methodology guided by commonly accepted protocols (Schonhof, 2004; Hough, 2006) at the beginning of each data collection session. Two taste scores were provided. The first, was a rating of "*taste hotness*" in which scores were scaled from "1" (mild), to "3" (hot, radishy, and pungent), and the second was an overall and highly subjective evaluation of palatability (*overall taste*) in which scores ranged from "1" (least desirable) to "3" (most desirable). Tasters were isolated from each other, and a study supervisor recorded scores. Each taster rinsed their mouth with fresh water between samples.

Glucosinolate Analysis

Glucosinolate levels were determined by HPLC analysis of extracts (1:1:1:1, dimethyl sulfoxide: acetonitrile: dimethyl formamide: water) made using leaf samples as described by Troyer et al. (2001). Briefly, samples were injected onto a PolyhydroxyethylA column (PolyLC, Columbia, MD) and eluted with isocratic 30 mM ammonium formate pH 5.4 in 85:15 acetonitrile:water (vol:vol) at a flow rate of 2 ml/min. Detection was via photodiode array with absorbance monitored at 235 nm. All solvents (Fisher Scientific, Fairlawn, NJ; Sigma-Aldrich, Inc., St. Louis, MO; and J. T. Baker, Inc., Phillipsburg, NJ) were ACS or HPLC grade and all of the HPLC components were purchased from Waters (Milford, MA).

A sub-sample of the extracts was subject to myrosinase digestion to confirm, by difference, the identity of chromatographic glucosinolate peaks (Fahey et al., 1997). Briefly, a small amount of extract was evaporated to dryness using a Savant Speedvac Concentrator (Savant Instruments, Inc., Farmingdale, NY), and redissolved in an aqueous system comprised of 20 mM sodium phosphate buffer, pH 6.0, 500 μM ascorbic acid (freshly prepared), and enough myrosinase to completely hydrolyze the glucosinolates during a 2 hour incubation at 37°C. Following hydrolysis, an aliquot was chromatographed as outlined in the previous section, and the results were compared to the unhydrolyzed sample chromatogram. Peaks that disappeared following myrosinase digestion were glucosinolates. As a further validation of peak identity, peaks were also regularly checked by electrospray mass spectroscopy against authentic standards.

Statistics

ANOVA, nptrend, and Spearman's rank correlations were performed using Stata 7 (Stata Corp., College Station, TX).

RESULTS AND DISCUSSION

There was no correlation between total glucosinolate content (RBGS), or either of the 2 major glucosinolates found in fresh leaves (4RBGS and MAGS), and the taste of fresh Moringa leaves as evaluated by masked taste panels at 2 separate harvest times, May 2005 (n = 9 judges) and April 2006 (n = 10 judges). There were strong, positive correlations between glucosinolate content and sample date (Spearman's rho = 0.6111; $P < 0.00001$), as well as girth, which is a proxy for sample date (see supplemental data Table S1). Mean glucosinolate content increased with each of the 3 harvests in a manner consistent with findings in other glucosinolate containing crops (Cartea et al., 2007; Charron et al., 2005; Farnham et al., 2000, 2004; Rosa 1996). Some of the 30 accessions had consistently high glucosinolate content, some were consistently low, and the commercially important cultivar PKM2 had consistently moderate levels of glucosinolates (Figure 1). There was a highly significant trend for ranking of the 30 accessions by glucosinolate content across harvest dates (Table 1) ($z = 5.06$, $p < 0.001$, by nptrend analysis), and by the individual glucosinolates 4RBGS ($z = 4.72$, $p < 0.001$, by nptrend analysis) and MAGS ($z = 4.28$, $p < 0.001$, by nptrend analysis) (data in Table S1). There was no correlation between taste (pungency) and any of the other parameters measured ($p > 0.05$ in all cases).

Neither of the 2 major glucosinolates found in these leaves (4RBGS and MAGS), nor the sum of these 2 (RBGS), were correlated with taste (by ANOVA), and there were no correlations between glucosinolate levels and any of the other parameters measured. Levels of the 2 major glucosinolates did not vary inversely, and were therefore correlated with total glucosinolate content as expected. As mentioned earlier, "taste hotness" is highly subjective, and more importantly, it is viewed differently by different cultures. What is mild to a group of tasters in the U.S. may be perceived quite differently elsewhere in the world. Nonetheless, if the leaf powder of Moringa is to be used as a nutritional supplement in weaning foods, one might expect better acceptance regardless of cultural differences, if it were milder, rather than harsh, acrid, or hot.

These findings suggest that deliberate selection for agronomic, taste, or quality factors can be made without jeopardizing the content of one of the more important phytochemicals in Moringa. Leaf samples were taken at 3 different times of the year, designed to encompass the range of seasonal influence on glucosinolate content. However, this field trial has not been replicated across multiple soil types, climates, or geographic environments. It is also entirely possible that levels of myrosinase vary even more than glucosinolate levels do. Myrosinase is the enzyme which is responsible for converting the biologically inactive glucosinolates into the quite reactive isothiocyanates – these lachrymose metabolites contribute to the hot, spicy taste of foods which contain them. Thus the effect of myrosinase might

TABLE S1 Major Glucosinolates of 30 *Moringa oleifera* Accessions: 4-(rhamnopyranosyloxy)benzyl glucosinolate (4RBGS) and Monoacetyl-(rhamnopyranosyloxy)benzyl glucosinolate (MAGS).
There was a highly significant trend for ranking of the 30 accessions by individual glucosinolate content across harvest dates: 4RBGS ($z = 4.72$, $p < 0.001$, by nptrend analysis) and MAGS ($z = 4.28$, $p < 0.001$, by nptrend analysis). There were strong, positive correlations between glucosinolate content and both girth, and sample date (Spearman's Rho = 0.611; $p < 0.00001$).

		4RBGS (μmol/g fresh wt.)			MAGS (μmol/g fresh wt.)			Girth (in.)		Ht. (ft.)
Source	Accession	May 05	Dec 05	Apr 06	May 05	Dec 05	Apr 06	May 05	Apr 06	May 05
North America										
Bradenton, FL	02099-021D	30.3	66.6	43.8	46.2	41.7	49.6	7.56	9.00	12.6
ECHO Farm	92028-991E	7.3	47.0	37.6	6.8	13.2	24.4	8.94	11.50	14.6
Ft. Myers	92026	13.8	21.7	33.1	25.2	41.1	49.2	7.13	8.81	9.6
N. Wood, FL	00099-001D	14.9	45.5	34.6	15.8	23.0	40.9	7.44	10.5	10.1
Central America										
Villoria, Belize	03051-031D	3.2	13.8	18.6	15.1	23.6	34.0	5.06	6.06	9.2
Mexico	01084-011D	37.7	26.2	46.3	19.3	26.8	14.9	8.44	10.31	12.4
Caribbean (Haiti)										
K. Flanagan	01046-011A	33.0	43.3	42.8	11.7	22.2	23.0	8.69	10.13	13.6
Bohoc	02055-021H	23.0	37.1	38.3	22.0	35.1	28.2	8.44	10.19	12.9
La Gonave	02073-021H	35.7	33.9	55.8	35.8	22.1	37.2	8.25	10.56	9.9
Les Cayes	02057-021H	6.4	15.1	35.4	19.6	28.7	46.9	5.69	6.69	9.3
Port Au Prince	02056-021H	44.9	23.3	42.1	13.0	22.8	25.0	9.63	11.25	12.8
C. Thede	03064-031H	39.6	33.2	55.1	45.4	41.9	43.7	8.56	10.56	12.6
C. Thede	03065-031H	18.0	46.6	35.6	32.1	19.2	43.8	7.63	9.69	12.4
C. Thede	03067-031H	10.0	15.9	14.5	33.1	52.1	41.8	7.06	8.88	11.2
C. Thede	03068-031H	8.5	22.8	22.7	26.9	43.2	42.5	8.56	10.56	12.8
C. Thede	03069-031H	23.2	41.6	43.7	28.2	25.6	27.9	8.75	9.88	11.8
Archai	03070-031H	20.3	26.0	34.8	28.7	21.4	45.4	6.31	8.06	10.4
C. Thede	03071-031H	45.7	23.9	41.8	33.6	39.9	39.4	9.63	11.5	15.0
Titayen	02058-021H	26.5	27.9	38.7	18.2	28.4	51.5	10.9	14.25	13.1

(*Continued*)

TABLE S1 (*Continued*)

Source	Accession	4RBGS (µmol/g fresh wt.)			MAGS (µmol/g fresh wt.)			Girth (in.)		Ht. (ft.)
		May 05	Dec 05	Apr 06	May 05	Dec 05	Apr 06	May 05	Apr 06	May 05
South Asia (India)										
PKM-1 Horti	00045-011A	8.2	21.0	31.9	20.5	25.0	45.4	7.00	8.81	9.8
PKM-2 U. Asmar	03005-031A	20.7	27.2	35.5	42.1	35.9	38.5	9.81	13.38	12.5
Pocha Exports	91070	2.1	25.5	27.4	6.6	24.4	27.5	4.06	8.06	6.9
Trust Hospital	03056-031D	5.3	44.1	17.8	20.3	28.9	32.2	8.38	11.13	15.0
Africa										
CWS Senegal	03052-031D	34.8	44.5	44.9	32.2	34.1	42.6	8.44	10.50	12.0
Tanzania/Malawi	98018	8.3	73.3	54.5	14.8	27.3	44.7	9.63	13.56	12.9
Msingi, Tanzania	03034-031D	28.7	38.4	32.5	39.4	59.8	51.1	7.69	10.00	11.9
Optima, Tanzania	01088-011D	12.4	31.3	18.0	15.7	29.7	25.4	8.69	10.94	10.9
Optima, Tanzania	03066-031H	21.0	29.5	44.1	38.9	26.4	42.9	8.13	10.25	12.9
Groves, Mozambique	03055-031D	40.3	23.6	48.6	39.1	27.2	39.3	4.88	8.25	6.8
Binga Trees, Zimbabwe	03053-031D	6.4	52.0	22.1	15.2	26.7	48.7	5.25	6.81	7.6

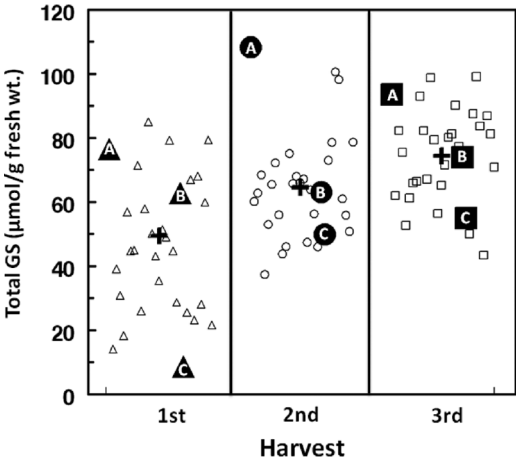

FIGURE 1 Glucosinolate content for 30 Moringa accessions at 3 harvest dates: 1st – May 2005 (Δ), 2nd – December 2005 (○), 3rd – April 2006 (□). Overall means for each harvest date are plotted as large crosses. Representative high-, medium-, and low-glucosinolate accessions are indicated by "A" (Bradenton accession 02099-021D), "B" (PKM-2), and "C" (Pocha Exports accession 91070), respectively.

actually overshadow the effect of glucosinolate levels. Although the phytochemical benefit to be derived from Moringa's glucosinolates would still accrue to people eating plants high in glucosinolates and low in myrosinase, such a conclusion is not warranted by our pilot study.

If intelligent efforts are to be directed toward producing and disseminating plants with a specific taste (e.g., mild) for specific purposes (e.g., weaning food), baseline information on these qualities must first be developed. Ultimately, it will be necessary to determine the degree to which harshness of taste is controlled by the genetics of a cultivar, variety, or accession, and by the environmental conditions in which the plants are grown (soil type, water status, amount of heat, drought, pathogen stress, etc.). Leaf or foliage production (e.g., biomass) must also be better characterized. Foliage production can be expected to vary across cultivars or breeding lines based upon their genetics, and by environmental conditions and it is even possible that these factors could influence glucosinolate localization within the leaf canopy. The genetics of a cultivar determine the potential of that cultivar for biomass production, growth habit, and phytochemical content, whereas environmental conditions modulate that potential. There is considerable experimental evidence that glucosinolate levels vary widely among cultivars of a particular species grown in the same environment (Farnham et al., 2000, 2004; Rosa, 1996). Part of the variation in glucosinolate content is also correlated to the stage of plant growth and due to environmental variables. For example, higher temperatures and longer days lead to higher glucosinolate production in plant leaves (Cartea et al.,

2007; Charron et al., 2005). The detection and plant extraction methodologies used herein are well established and widely used.

Evidence for the positive influence of these glucosinolates on health has been addressed in the literature (reviewed by Fahey et al., 2001). We have identified cultivars from an open germplasm collection that have high-, medium-, or low glucosinolate content over the course of a year's growing cycle (Figure 1). Our data suggest that it is unlikely that there is a significant relationship between pungency (hot taste) and glucosinolate content. If these preliminary indications hold up to further experimental scrutiny, then selection based on taste should not place negative selection pressure on glucosinolate content of the germplasm in a breeding program. This conclusion would support greater efforts to cultivate varieties that have both a mild taste and higher levels of health-promoting glucosinolates.

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