GLUCOSINOLATE SURVEY OF CULTIVATED AND FERAL MASHUA (*TROPAEOLUM TUBEROSUM* RUÍZ & PAVÓN) IN THE CUZCO REGION OF PERU[1]

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*Se identificó y cuantificó mediante "High Performance Liquid Chromatograph" los glucosinolatos (GSL) presentes en mashua cultivada (*Tropaeolum tuberosum *Ruíz & Pavón) y sus parientes silvestres. Los compuestos principales descubiertos fueron los siguentes glucosinolatos aromáticos: 4–Hydroxybenzyl GSL (OHB, Glucosinalbin), Benzyl GSL (B, Glucotropaeolin) y m–Methoxybenzyl GSL (MOB, Glucolimnathin). El contenido total de GSL fluctuó entre 0.27 a 50.74 μMol/g de tejido del tubérculo seco. La mayoría de las accesiones con bajo contenido de GSL estuvieron distribuidos dentro de la población cultivada con una concentración total de GSL menor a 5.00 μMol/g de tejido del tubérculo seco, mientras que el mas alto contenido total (más de 25.00 μMol/g de tejido del tubérculo seco) y la mas alta concentración de GSL individuales (OHB, B y MOB) se observó en la población silvestre con pocas excepciones. Además, seis fenotipos de GSL diferentes fueron determinados: Sólo MOB; sólo B; OHB y B; OHB y MO; B y MOB; y OHB, B y MOB.*

Key words: *Tropaeolum tuberosum;* mashua; glucosinolate; benzyl; methoxybenzyl; anticarcinogens; secondary metabolites; Andean tuber crops; isothiocyanates; glucotropaeolin.

Glucosinolates (GSL) are secondary metabolites found in several families of dicotyledonous angiosperms, including the Brassicaceae and a large number of other edible species (Wang et al. 2002, Robbelen et al. 1989). When plant tissue is disturbed by crushing, cutting, or other physical damage, GSLs are brought into contact with the enzyme myrosinase (thioglucoside glucohydrolase), which rapidly hydrolyzes them. This hydrolysis yields unstable intermediates that, as dictated by chemical conditions as well

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as by the presence of various accessory proteins, spontaneously release a variety of aglycone derivatives, such as isothiocyanates, thiocyanates, nitriles, oxazolidine–2–thiones (Wang et al. 2002, Mithen 2001, Halkier 1999), and epithionitriles (Lambrix et al. 2001) plus elementary sulfur, depending on the concentration of H+ and other factors (Bones and Rossiter 1996).

Mashua (*Tropaeolum tuberosum* Ruíz & Pavón spp. *tuberosum*) is a member of the family Tropaeolaceae (the Nasturtium familly) (Sparre 1973, Sparre and Anderson 1991, Ruíz and Pavón 1802). It is also known as isañu (Aymara-Bolivia), cubio (Colombia), añu, and ysaño, (Quechua in Peru and Bolivia) (Grau et al. 2000, Hernandez and León 1994, Herrera 1941). It ranks fourth in importance in the Andean region after potato, oca, and ulluco (National Research Council [NRC] 1989); however, mashua is the least popular tuber crop because of its bitterness due to the presence of isothiocyanates released by GSL hydrolysis (Johns and Towers 1981, Kjær et al. 1978). Isothiocyanates are well known for their biological activity as diuretics, as agents that can offer protection against cancer, and as antibiotics, insecticides, and nematocides (Halkier and Du 1997, Stoner and Morse 1997, Sugie et al. 1994, Wattenberg 1987 and 1992). These reports corroborate the extensive use of mashua in Andean folk medicine to treat kidney aliments, skin ulcers, kidney stones, and to kill parasites. In addition, mashua is often used by Andean growers as a pest repelling plant in border rows surrounding potato fields because of its putative fungicidal, bactericidal, nematocidal, and allelopathic properties. These properties are probably conferred by its isothiocyanates (Brabban and Edwards 1995, Johns et al. 1982, NRC 1989). Two different kinds of GSLs are found in the family Tropaeolaceae; (1) aliphatics derived from methionine, isoleucine, and valine and (2) aromatics derived from phenylalanine and tyrosine (Fahey et al. 2001). As reported by Johns (1981), mashua releases benzyl and p–methoxybenzyl isothiocyanates, derived from aromatic benzyl (B) and p–methoxybenzyl (MOB) GSL, respectively. Indeed, additional phytochemical analyses of isothiocyanates by Kjær et al. (1978) and Johns and Towers (1981) support the recognition of two subspecies in *T. tuberosum*: cultivated mashua and a tuber-bearing wild form, referred to by these authors as *T. tuberosum* ssp. *silvestre*. Mashua produces *p*–methoxybenzyl isothiocyanate, which gives mashua its characteristic piquant flavor, whereas the wild material is characterized by benzyl isothiocyanate, 2–propyl isothiocyanate, and 2–butyl isothiocyanate (Johns and Towers 1981). In particular, Benzyl isothiocyanate, which seems to be the main isothiocyanate of mashua, is also found in Nasturtium *Tropaeolum majus* (Lykkesfeldt and Møller 1993, Wielanek 1999). This compound has been reported as a potent chemo-protective agent that blocks chemically induced carcinogenesis and prevents several types of cancer in rodents (Halkier and Du 1997, Stoner and Morse 1997, Sugie et al. 1994, Wattenberg 1987 and 1992), including liver tumors (Sugie et al. 1994, Rosa et al. 1997). Isothiocyanates cause carcinogen detoxification prior to induction of carcinogenesis and act as suppressing agents to inhibit the neoplastic effects of carcinogens, possibly by inducing apoptosis of pre-cancerous cells or by adjusting key enzymes to reduce exposure of tissues to DNA damage (Shapiro et al. 1998, Stoner and Morse 1997). In addition, some recent studies of this Andean crop done by Ramallo et al. (2004) confirm the presence of p–methoxybenzyl GSL in six varieties of mashua from Bolivia.

The main objective of this paper is to determine the GSL content of mashua cultivars grown in the Cusco region of Peru, of wild forms of mashua collected in the same region, and a sample of cultivated mashua from the germplasm collection maintained at the International Potato Center (CIP). This sample is a good representation of the genetic variability of the species *T. tuberosum*. Such information will be useful for (1) future selection of genotypes with high content of beneficial GSL that could be used as a source of functional foods or medicine and (2) selection of low content GSL mashua, thus removing the bitterness produced by some of these compounds, to produce lines with improved palatability thus helping increase the acceptability of this high protein crop.

Materials and Methods

Plant Material

In June 2002, mashua tubers were harvested in the following communities of the Cuzco region of Peru: (1) Matinga, Picol, and Qquecca-

yoc (Zone 1 located on the District of Taray in the Province of Calca) and (2) Ch'umpe, Poq'ues, and Sayllafaya (Zone 2 located on the District of Lamay in the Province of Calca). Five mature and healthy mashua tubers per accession were sampled for each of the following locations: 20 accessions from Matinga, 17 from Picol, two from Qqueccayoc (District of Taray-Cusco–Peru); 57 from Ch'umpe, 92 from Poq'ues, 152 from Sayllafaya (district of Lamay-Cusco–Peru); 102 from the cultivated mashua collection held by CIP (Peru); and 39 feral accessions from different provinces of Cuzco (Peru), maintained by Centro Regional de Investigación en Biodiversidad Andina (CRIBA)—Universidad Nacional San Antonio Abad del Cusco (Ortega 2000). The feral accessions are most likely the result of escapes from cultivation (Ortega 2005). Additionally, in order to test for possible environmental effects on glucosinolate concentration, we established three replicated trials, of three replications each, for four accessions ranging from low to high GSL concentration. The locations of these trials were Kayra, Sayllafaya, and Qqueccayoc.

The tubers of all the samples were then cut in 50-millimeter (mm) sections and lyophilized for GSL analysis. Tuber morphology characters (predominant tuber skin color, secondary skin color, predominant tuber flesh color, and secondary tuber flesh color) were used to test for possible associations with GSL content.

Sample Preparation

A modified protocol reported by Kraling et al. (1990), was used for GSL analysis. For this purpose approximately 0.5 grams (g) of lyophilized tuber tissue was ground (roughly equivalent to 2 g of fresh tissue) and stored at –20 degrees Centigrade (°C) to preserve the GSL intact, as myrosinase remains inactive until the addition of water to the dry material (Rosa et al. 1997). The tissue was extracted with 5 milliliters (ml) of 70% methanol at 80°C for 15 minutes (min), and then centrifuged at 3,500 rpm for 5 min, and then the supernatant was removed. After applying the supernatant to an ion exchange DEAE–Sephadex A–25 (SIGMA) column, the GSL were converted into desulfoglucosinolates with sulfatase (0.5% enzyme in water for 16 hours at room temperature, Sigma H–I type). The desulfoglucosinolates were then eluted by adding 1.5 ml water.

HPLC Analysis

From the resulting mixture (1.5 ml), 5 microliters (μl) were injected and separated using a Agilent 1100 HPLC (High Performance Liquid Chromatograph) equipped with a Diode Array Detector set at a wave length of 229 nanometers (nm) for detection, using a RP–18 column (X Terra) and a linear solvent gradient from 1% to 15% acetonitrile in water over 4 min, and washing of the column with a linear gradient of 15% to 95% acetonitrile for 6 minutes. The flow rate was set at 0.9 ml/min, with a stop time of 8.5 min and a post time of 1.5 min, at 32°C.

Glucosinolate Identification and Quantification

The chromatogram was compared to the desulfoglucosinolate profile of maca (*Lepidium meyenii* Walp.) (Dini et al. 2002, Li et al. 2001, Piacente et al. 2002), which shares the same aromatic GSL content with mashua: Benzyl GSL (glucotropaeolin) and p–methoxybenzyl GSL (glucoaubreitin) (Johns 1981). The presence of Benzyl GSL was confirmed by using pure glucotropaeolin (Merck Co.) as a standard. The quantification of GSL was obtained by performing a regression analysis using a mashua accession containing Benzyl GSL (accession number W–24) at various concentrations (0.05, 0.10, 0.50, 1.00, and 5.00 ml. of extracted tissue) using 0.5 g of lyophilized tuber tissue for this accession. Then, based on the areas of the peaks and the W–24 Benzyl GSL slope = 386.43, the concentration of GSLs in μMol/g of dried tuber tissue was calculated for the rest of the samples in Benzyl GSL equivalents. By dividing the area by the Benzyl GSL slope, the GSL concentration is given in ηMol in 5 μl of mixture injected. Finally, using the following formula the GSL concentration was converted into μMol/g of dried tuber tissue given in Benzyl–GSL equivalency.

$$\text{GSL concentration} = \left[\left[\left(\frac{\text{AREA}}{\text{Benzyl Slope}}\right)\times 300\right]\div 0.5\right]\div 1000$$

Results and Discussion

GSL Identification

The main compounds detected in lyophilized tubers of mashua were the following aromatic GSL (Figs. 1 and 2). (1) 4–Hydroxybenzyl GSL

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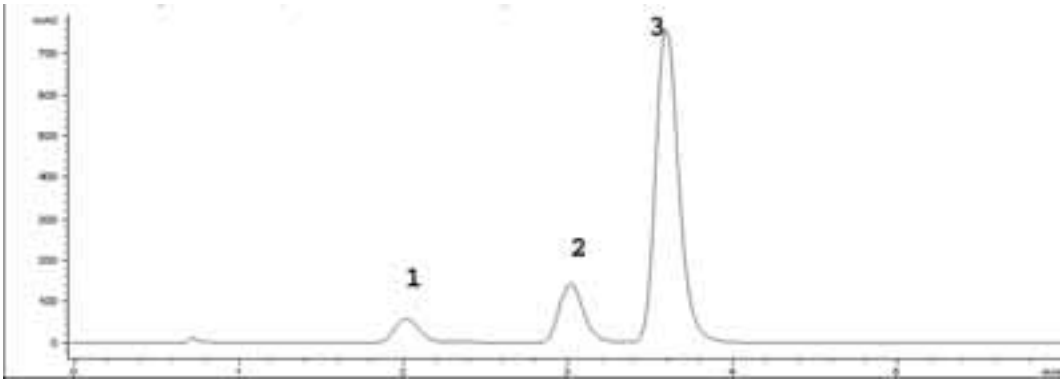

Fig. 1. Typical HPLC profile for mashua aromatic GSL displaying the migration of (1)4-Hydroxybenzyl Glucosinolate—OHB GSL, (2)Benzyl Glucosinolate—B GSL, and (3)m—Methoxybenzyl Glucosinolate—MOB GSL.

(Glucosinalbin) (OHB), previously reported by Fahey et al., (2001) in the Tropaeolaceae family. (2) Benzyl GSL (Glucotropaeolin) (B) identified using tropaeolin as an internal standard. This GSL was previously reported in mashua by Johns (1981). (3) m–Methoxybenzyl (tentative identification) GSL (Glucolimnathin) (MOB) has the same migration as the equivalent GSL found in maca by Piacente et al. (2002). This compound was previously reported as p–Methoxybenzyl GSL (Johns 1981, Johns and Towers 1981, Johns et al. 1982, Li et al. 2001). Similar to the cultivated ones, we only found aromatic GSL in the feral forms (B, OHB, and MOB GSL), which is in contrast with the report of Johns and Towers (1981), who found B GSL, 2–propyl GSL, and 2–butyl GSL in wild mashua (*Tropaeolum tuberosum* spp. *silvestre*).

Glucosinolate Quantification

The total amount of GSL observed in mashua ranges from 0.27 to 50.74 μMol/g of dried tuber tissue (Table 1, Fig. 3). The highest total GSL content was observed in the accession S-36 from Sayllafaya (Zone 2) followed by feral accession W-19. MOB GSL is the highest and more frequent GSL among all mashua accessions and consequently among all different populations. The highest concentration of MOB GSL is present in Sayllafaya, followed by the feral population. The CIP collection is the population with the lowest GSL content in total and individual GSL. Individually OHB and B GSL are higher and more frequent in Zone 2 and in the feral accessions.

Of the cultivated mashua population (most of them from the CIP collection: 99 out of 102 accessions), 41 percent had low-GSL content, that is, with less than 5.00 μMol/g of dried tuber tissue, which we propose to call "sweet mashuas" (Table 1, Fig. 3). In contrast, only 13 percent (five out of 39 accessions) of feral mashuas have total GSL amount lower than 10.00 μMol/g of dried tuber tissue. Most of the wild accessions probably have resulted from escapes to cultivation as reported by Leon (1964), based on morphological study and by Ortega (2005) based on molecular marker analysis. The trend of higher GSL content observed in these accessions might have originated as a defense mechanism in response to insect pests in the harsher non-cultivated environment where they are found. However, high GSL content is not restricted to feral accessions since the cultivated accessions S-26, S-32, S-35, S-36, S-37, S-40, S-41, S-47, S-68, S-90, S-32 (Sayllafaya), CH-1017, CH-1020, CH-1021 (Ch'umpe), and Q-2501 (Qqueccayoc) also have a considerable total GSL content (above 30 μMol/g of dried tuber tissue). Morphological tuber trait inspection failed to disclose any association with GSL content. For example, the accessions with dark skin color (black and grayish purple) exhibited a moderate GSL concentration (between 6 and 29 μMol/g of dried tuber tissue), while accessions with orange skin color and yellow flesh color,

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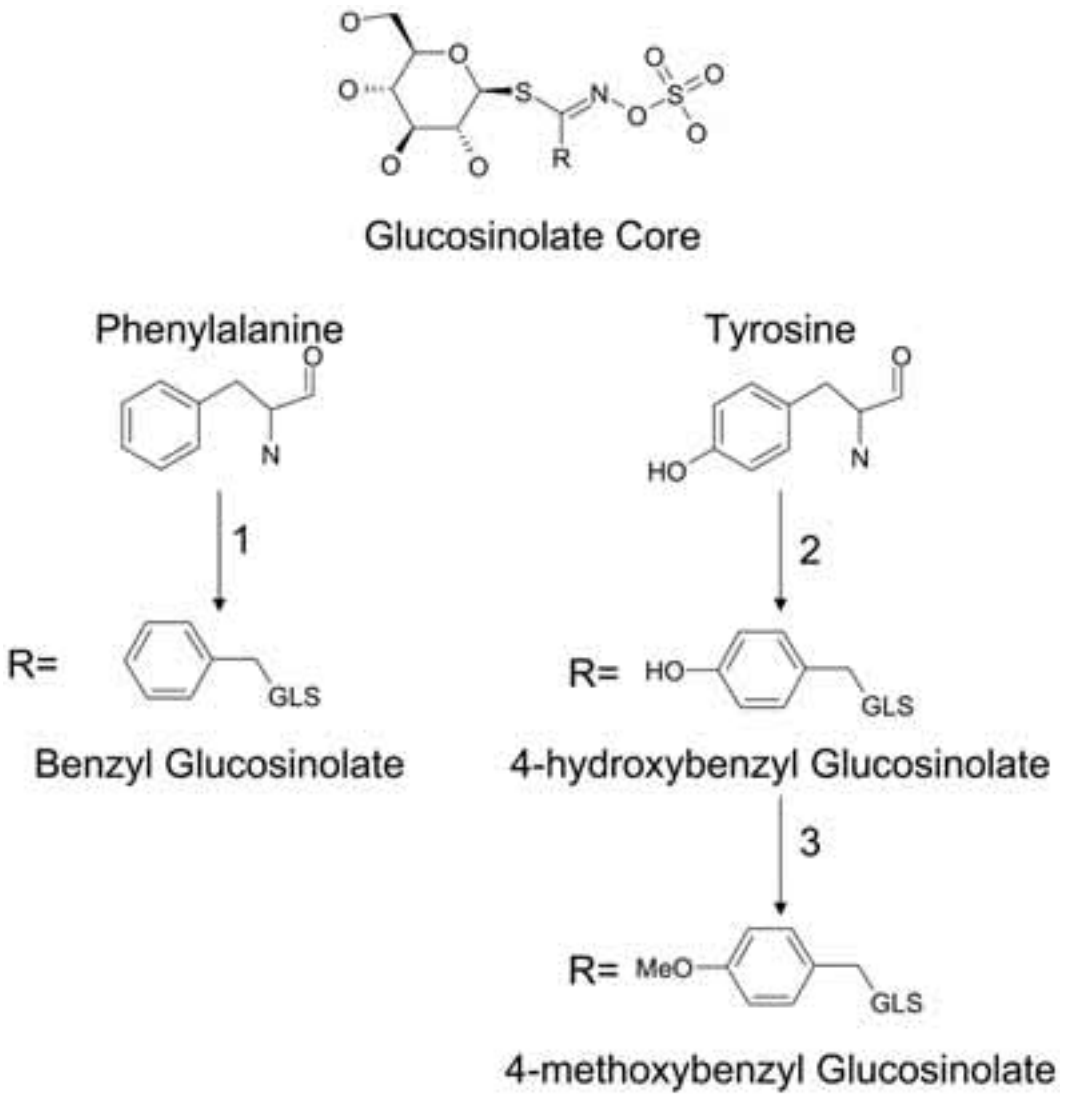

Fig. 2. Top—Chemical structure of the glucosinolate core, showing the location of the variable side chains (R). Bottom—Pathways from tyrosine and phenylalanine as precursors of Benzyl GSL; 4–hydroxybenzyl GSL, and m–methoxybenzyl GSL, respectively.

such as cultivated accession S-36, or yellow skin color and yellowish white flesh color, such as feral accession W-19, showed the highest GSL concentration. These observations, where neither tuber skin color nor tuber flesh color are related with total or individual GSL concentration, are not consistent with other reports. For example, Ramallo et al. (2004), which was based on the study of a few accession of mashua and was in agreement with folk beliefs, reported that dark color in mashua is related to medicinal value and consequently has higher GSL content.

Statistical analysis of the same-environment trials for the accession sampled indicated a lack of significant environmental effect among communities for specific and total GSL concentration: OHB (P=0.686), B (P=0.587), MOB (P=0.998), and total GSL (P=0.590). This finding was not surprising considering that the en-

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TABLE 1. INDIVIDUAL AND TOTAL GSL CONCENTRATION IN MASHUA ACCESSIONS EXPRESSED IN μMOL/G OF DRIED TUBER TISSUE. (MIN=MINIMUM, MAX=MAXIMUM).

Region	Community	Number of Accessions	OHB GSL		B GSL		MOB GSL		Total GSL	
			Min	Max	Min	Max	Min	Max	Min	Max
Zone 1	Matinga	20	0.09	0.86	0.00	0.60	1.44	21.62	1.54	22.56
	Picol	17	0.10	0.80	0.00	0.61	10.13	26.26	10.53	26.61
	Qqueccayoc	2	0.33	0.45	0.00	0.00	14.64	30.11	14.97	30.56
Zone 2	Ch'umpe	57	0.00	5.33	0.00	4.16	0.27	30.28	0.27	31.74
	Poq'ues	92	0.00	0.73	0.00	3.84	0.36	27.62	0.36	27.77
	Sayllafaya	152	0.00	4.26	0.00	8.72	0.11	50.01	0.37	50.74
CIP		102	0.00	0.40	0.00	1.97	0.00	5.89	0.33	5.93
Feral		39	0.00	2.36	0.00	19.54	0.00	44.81	4.11	46.78

GSL concentration for each accession is available upon request to the corresponding author. These data can be found also in Ortega 2005.

vironments of the different locations sampled are not very different from each other.

POSSIBLE GLUCOSINOLATE PATHWAYS IN MASHUA

The following potential pathways for aromatic GSL syntheses in mashua can be postulated from the observed variation in the populations sampled in the present study (Fig. 2):

- Phenylalanine as a precursor, catalyzed by a CYP79-like enzyme, leads to the synthesis of Benzyl GSL.
- Tyrosine as a precursor, catalyzed by a separate CYP79-like enzyme, leads to the synthesis of Hydroxybenzyl GSL and then, by the action of a methyl transferase OHB, is converted into MOB GSL.

Six different GSL profiles where found in the mashua collection and the frequencies of these are shown in Fig. 4. Phenotype OHB-B–MOB: 4–Hydroxybenzyl, Benzyl and m–Methoxybenzyl GSL; is divided in four sub-groups, which are described below. The correlation analysis was used to determine whether there is a positive or negative association between specific GSLs (level of significance: 0.05 and 0.01) in accessions that contain more than one of these compounds in their profile according to the postulated pathway listed above (Table 2).

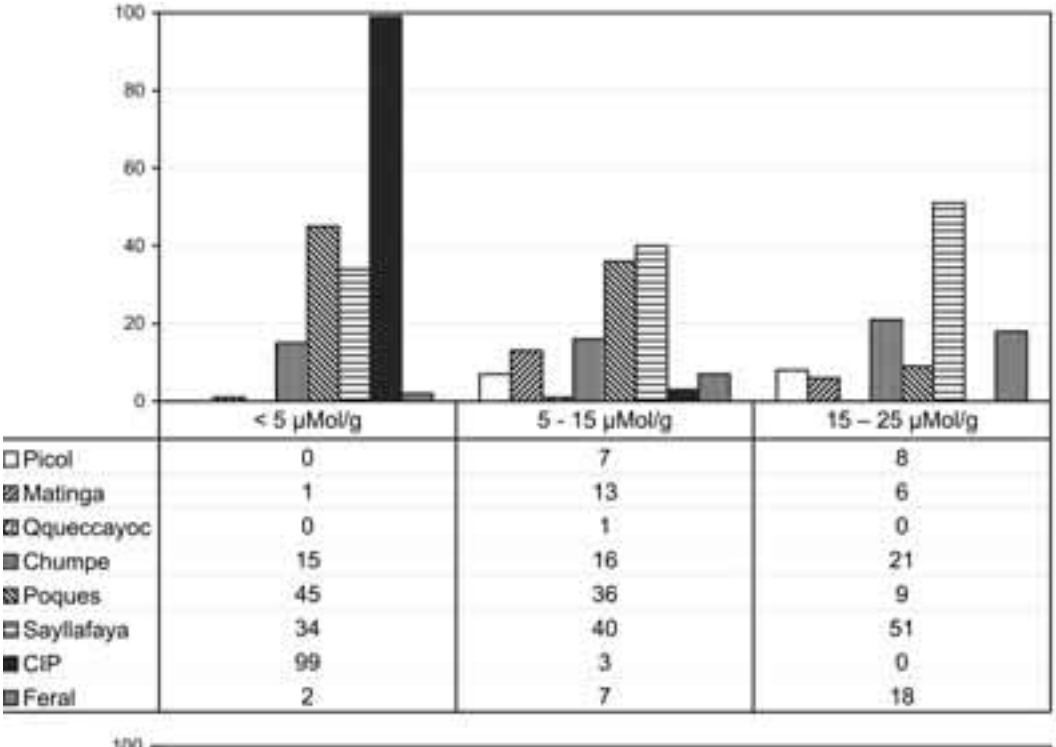
DESCRIPTION OF GSL PHENOTYPES

Only Benzyl GSL (B): Typical of the feral accessions, although it was also found in two cultivated accessions from Cajamarca. In this case, B is synthesized from phenylalanine. The lack of OHB production might be due to the absence of catalysis from tyrosine, and consequently absence of production of MOB.

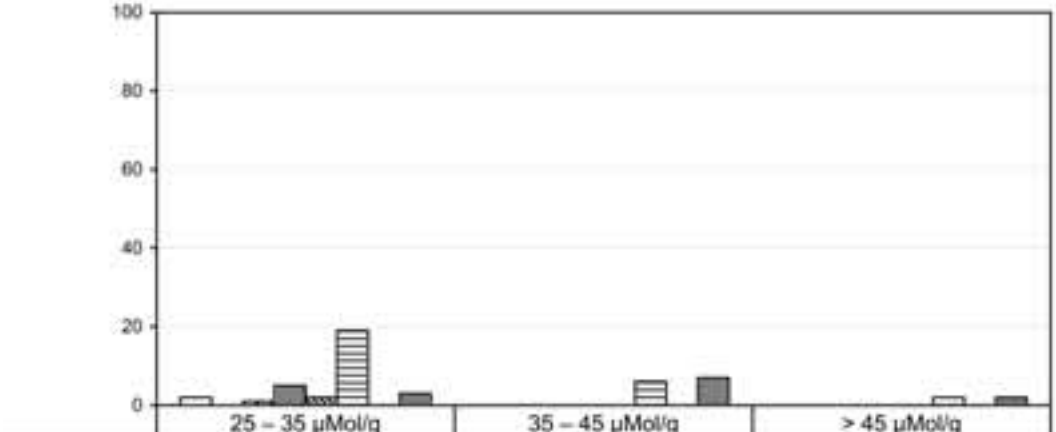
Only m–Methoxybenzyl GSL (MOB): Present only in cultivated mashua. Phenotype characteristic of the accessions from Zone 2 (Ch'umpe, Poq'ues, and Sayllafaya), and 19 accessions from the CIP cultivated mashua collection (spread all over Peru). This phenotype is probably due to the absence of B production from phenylalanine, while tyrosine yields OHB but it is used up completely by a highly efficient MOB forming enzyme.

4–Hydroxybenzyl and Benzyl GSL (OHB–B): Present only in a single feral accession, W-18. In addition, the amount of B in this accession is the highest among the entire mashua collection. In this case, it is possible that there is a high production of B from phenylalanine and an inefficient production of OHB from tyrosine, followed by a complete absence of MOB synthesis. There are not enough accessions to calculate a possible correlation between these two GSLs in this phenotype.

4–Hydroxybenzyl and m–Methoxybenzyl GSL (OHB—MOB): This is the most common phenotype present in both cultivated and feral accessions. In most cases, there is a positive and highly significant association ($p<0.01$) between these two GSLs, indicating that they are produced in the same pathway. For example, in most of the accessions tested, the correlation coefficient for the presence of these two GSL were above 0.48. The exception was found in the accession of the CIP collection, where $r=0.1727$. It is still a direct (positive) relationship, but too low and not significant to



	< 5 µMol/g	5 - 15 µMol/g	15 – 25 µMol/g
Picol	0	7	8
Matinga	1	13	6
Qqueccayoc	0	1	0
Chumpe	15	16	21
Poques	45	36	9
Sayllafaya	34	40	51
CIP	99	3	0
Feral	2	7	18



	25 – 35 µMol/g	35 – 45 µMol/g	> 45 µMol/g
Picol	2	0	0
Matinga	0	0	0
Qqueccayoc	1	0	0
Chumpe	5	0	0
Poques	2	0	0
Sayllafaya	19	6	2
CIP	0	0	0
Feral	3	7	2

Fig. 3. Frequency distribution for total GSL concentration in cultivated accessions for different locations and for feral accessions of mashua. Top: < 5 to 25 µMol/g; bottom: 24 to > 45 µMol/g.

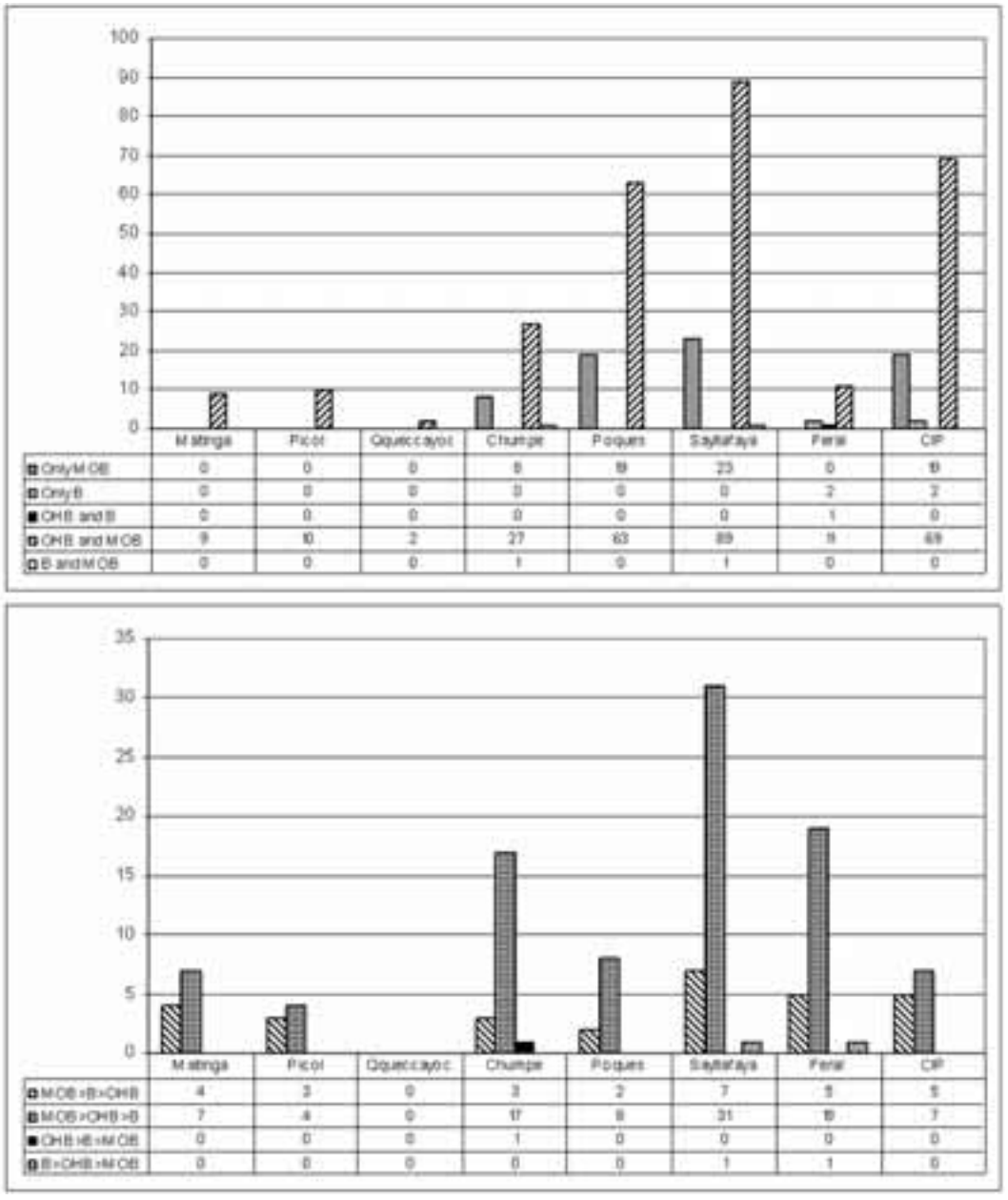


Fig. 4. (A) Number of mashua cultivated accessions from different locations and feral accessions per GSL phenotype Accessions containing one and two GSL. (B) Accessions containing all three GSL: MOB–B–OHB, distinguished in four different phenotypes on the basis of predominant GSL concentration.

lead us to any assumption. This supports the assumption that the synthesis of these GSL involves tyrosine, which produces OHB followed by a constant and efficient methylation in order to produce MOB. B synthesis from phenylalanine is non-functional in these accessions.

Benzyl and m–Methoxybenzyl GSL (B–MOB): Present only in two accessions: CH-1040 (Ch'umpe) and S-64 (Sayllafaya). Both of

TABLE 2. CORRELATIONS BETWEEN SPECIFIC GSL IN DIFFERENT GSL PHENOTYPES.

		GSL PHENOTYPE					
		MOB>B>OHB			MOB>OHB>B		
Population	OHB-MOB	OHB-B	OHB-MOB	B-MOB	OHB-B	OHB-MOB	B-MOB
Matinga	0.81**	0.66	0.50	0.79	−0.20	0.57	−0.34
Picol	0.50	0.67	−0.16	0.62	0.98*	0.52	0.33
Qqueccayoc	1.00	—	—	—	—	—	—
Ch'umpe	0.62**	−0.23	0.94	−0.54	0.39	0.60**	0.32
Poq'ues	0.55**	1.00	1.00	1.00	−0.18	0.55	−0.22
Sayllafaya	0.48**	0.91**	0.88**	0.63	0.69**	0.33	0.21
Feral	0.78**	0.90*	0.54	0.25	0.59**	0.69**	0.41
CIP	0.17	0.97**	0.68	0.64	0.69	0.34	0.65

* = $P<0.05$ significance level,
** $P<0.01$ significance level.

these GSL are present in low amounts, but not low enough to consider them sweet. The synthesis of B is from phenylalanine, and MOB by complete methylation of OHB produced from tyrosine.

4–Hydrozybenzyl–Benzyl and m–Methoxybenzyl GSL (OHB–B–MOB): This phenotype was prevalent in all the sampled locations except on the individuals from Picol in Zone 1 and the feral population. According to the relationship among individual concentrations for each GSL, the following four different subclasses could be defined: (a) MOB>B>OHB, (b) MOB>OHB>B, (c) OHB>B>MOB, and (d) B>OHB>MOB. These sub-classes were found in both cultivated and feral accessions. According to the correlation analysis ($P<0.01$) and variation in concentration of these GSLs shown in Table 1, the maturity of the tuber might cause the increase and/or decrease in total and individual GSL concentration. A direct and significant correlation ($P<0.01$) observed between GSLs synthesized from a different amino acid such as B and OHB, could be explained by the fact that phenylalanine and tyrosine production is biochemically related and possibly coregulated.

CONCLUSIONS

The total amount of GSL in the mashua accessions sampled in the present study has a wide range of variation, with the upper range of ~51 μMol/g, which is considerably higher than that found in other edible GSL-containing crops (Li *et al.* 2001). Mashua contains mainly three aromatic GSLs: 4–Hydroxybenzyl GSL (Glucosinalbin), Benzyl GSL (Glucotropaeolin), and m–Methoxybenzyl (tentative identification) GSL (Glucolimnathin). This confirms previous reports by Johns (1981) and Ramallo et al. (2004). An extensive variability in GSL content was observed among the cultivated and feral accessions, but a general trend for higher concentrations was observed for the feral accessions suggesting a possible role of GSL and its derivatives on adaptation of this species to harsh environmental conditions. Therefore, some of the feral accessions could serve as an important genetic resource to increase the GSL content in cultivated mashua. Through breeding, varieties with high GSL content could be produced for consumption as "functional foods" or for GSL extraction for medicinal purposes. However, accessions like S-36, which is the cultivated accession with the highest total GSL content, provides evidence that mashua with high GSL content is already being cultivated by farmers in the Andean fields of Cuzco in Peru. On the other hand, "sweet mashuas," with GSL concentrations of less than 5.00 μMol/g, were widespread among the cultivated accessions from the all communities and the CIP collection. Selection of these accessions could be useful in the future to develop mashua varieties with better palatability to promote the consumption and increase the market acceptance of this crop considering its high protein content and rusticity under poor growing conditions.

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"Strengthening the On-Farm Conservation and Food Security of Andean Tubers in the Fragile Ecosystems of the Southern Peruvian Highlands."

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