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BIO-CHEMICAL COMPOSITION OF SOME WALNUT (*JUGLANS REGIA* L.) GENOTYPES OF NORTH-WESTERN HIMALAYAN REGION

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Abstract

Fifteen autochthonous walnut (*Juglans regia* L.) genotypes of the North-western Himalayan region were evaluated for biochemical composition. The moisture content in them ranged from 2.98 to 8.69 % whereas, the oil content varied from 40.00 to 62.43 %. The linoleic acid was found most abundant among the fatty acids values which ranged between 48.50 and 67.30 %. Furthermore, linolenic acid and oleic acid content ranged from 5.70 to 18.58% and 12.14 to 20.60 %. The protein and carbohydrate content extended from 15.00 to 23.00% and 9.03 to 12.96 g/100 g, respectively. Vitamin B1 was the amplest vitamin ranging from 0.74 to 0.52 mg/100g. Results of the present study revealed that the genotype JWSD-59 contained highest percentage of fats, oils and nutrients and genotype JWSP-06 in proteins. These genotypes can be utilized for breeding of nutrient rich cultivars and serve as reserve gene pool for the future walnut improvement programme efforts.

Introduction

Walnut (*Juglans regia* L.) tree belonging to *Juglandaceae* family is one of the most economically significant and widely distributed cultivated species for its timber and nutritious nuts all over the world (Bayazit *et al*. 2007). Walnut is a rich source of many oxidative compounds namely melatonin, ellagic acid, vitamin E, carotenoids, and polyphenols (Ros and Matrix 2006, Rahimipanah *et al*. 2010). These compounds act against aging, cancers, inflammations, and neurologic illnesses (Sen 2011). It is also an important source of vitamins such as Riboflavin, Niacin, Thiamine, Pantothenic acid, Vitamin B6, and Folate/B9 (Kornsteiner *et al*. 2006, Sen 2013). Generally, there are 50 g (47.14 g) of multi-unsaturated fatty acids in 100 g of walnut and 40 g (38.09 g) of it is Omega 6 (linoleic acid), and 10 g (9.08 g) is Omega 3 (linolenic acid) (Sen and Karadeniz 2015) and protein 14.1 g, total oil 68.0 g, total carbohydrates 3.2g, cellulose 9.7 g, ash 1.8 g and moisture 3.2 g (Tonbak *et al*. 2006).The ratio of Omega 6 to Omega 3 in walnut is meagre compared to other hard-shell nuts, and it is a positive quality (Ma *et al.* 2010, Sen 2011). In terms of nutritive value, walnut is superior to pistachio, almond, hazelnut, pine nut, and peanut (Vinson and Cai 2012). In the recent past, many studies have dealt with the biochemical compositions of walnuts from different countries, including India (Saxena *et al.* 2009, Verma *et al*. 2020), Turkey (Simsek *et al*. 2017), Tunisia (Abdallah *et al.* 2015), Spain (Tapia *et al*. 2013), and Romania (Trandafir *et al.* 2016). Jammu and Kashmir is the central walnut producing state in India (Shah *et al.* 2021). Walnuts exist in these production areas of India in naturalized form and have not been explored so much yet. In Jammu, the most prominent areas under walnut cultivation are the Doda and Kishtwar districts. There is much diversity in walnut in these hilly areas of the North western Himalayan region of Jammu as reported by Shah *et al*. (2020). Though

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there are very few citations on the nutritional composition of local walnut genotypes from Kashmir but no information is available on the chemical composition, and kernel quality of nuts harvested from Jammu province. Therefore, the present investigation was conducted to evaluate the quality and bio-chemical composition of regional walnut species (*Juglans regia* L.) growing in Jammu and Kashmir which may provide support to their sustainable use for the development of future breeding programme efforts in walnut and serve as reserve gene pool.

Materials and Methods

The survey was carried out from 2016 to 2018 at the walnut growing areas of Kishtwar, Doda, and Rajouri districts of the North Western Himalayan region of Jammu and Kashmir a (33° 19′ 12″ N and 75° 46′ 12″ E). The sampling area is mainly hilly and mountainous, with valleys and stretches of plains with sub-tropical to temperate climate. Fifteen promising seedling walnut genotypes such as JWSG-43,JWSK-77, JWSP-06, JWSD-01,04,20,25,59 and JWSM-35,36,37,38,50,54,66 of Janmmu region of Jammu and Kashmir were selected for the present study and analyzed at the Quality Control and Quality Assurance Laboratory of Indian Institute of Integrative Medicine (IIIM). The samples from each seedling plant were collected seperately in a plastic bag and labeled at the time of harvest. The bags were immediately brought to the laboratory and held in an oven for 3 days at 30°C to bring the moisture content of the samples to 8%. The closed samples in plastic bags were then flushed with liquid nitrogen and kept in a refrigerator at −4 °C, until the time of tests. Before each of chemical analysis, the walnuts were manually cracked and shelled, and then chopped in a blender.

For the determination of oil content (%) walnuts (2 g) were finely ground using a Moulinex Optiblend 2000. The oil from the finely ground samples was extracted by modifying a procedure previously described by Savage *et al.* (1997). Briefly, the oil was extracted with 6 ml hexane/ isopropanol (3:2, v/v) at room temperature under vigorous stirring for one hour in thin glass beakers to facilitate homogenization of the nuts. The preparations were then filtered through a vacuum, and the residues were washed twice with 4 ml hexane/isopropanol solvent. Later, 7 ml of 6.7% sodium sulphate (w/v) was added, and the samples were vortexed for 30 sec centrifuged at 2000 r.p.m. for10 min. The solvent layer was removed, dried under nitrogen, and the pure oil was weighed to calculate the percentage yield.

To analyse lipid profile (%) fatty acid methyl esters (FAME) were analysed in a Varian CP 3800 gas chromatograph (Palo Alto, CA) coupled with a Varian CP-8200 autosampler and a flame ionization detector (FID). A Varian FAME fused silica capillary column (100 m x 0.25 mm, Varian CP-Select CB) was used to determine lipid profile. The oven temperature gradient was set from 0–30 min at 185 ^{0}C and 30–45 min at 235 ^{0}C with an increase of 20 ^{0}C/min. FID temperature was set at 270 ^{0}C, and helium, air, and hydrogen flow at 1.6, 300, and 35 ml/min, respectively. FAME mixtures were prepared using the protocol described by Misiri *et al.* (1985). Approximately 0.15 g of oil was diluted in 3ml of diethyl ether and 0.2 ml of 20% tetra-methyl ammonium hydroxide in water was added and allowed to react for 5 min. After time elapsed, 0.5 ml of methanol was added and vortexed for 1 min.. After phase separation, 1 ml of the upper organic phase was transferred to a vial and capped for injection into the gas chromatograph.

To estimate protein content (%) a known weight of total fat flour (~0.2-0.25g) produced by soaking of kernels in water for dehulling followed by drying and grinding of kernels into powder form was placed in a micro-Kjeldahl flask. A catalyst mixture of 0.42 g $CuSO_4$ + 9.0 g K_2 SO_4, few steel beads (to prevent sample bumping), and 15 ml concentrated H_2SO_4 (36 N) was added to each sample. Samples were digested at 410 ^{0}C for 45 min or until a clear green solution was obtained, which ensured complete oxidation of all organic matter. The digest was diluted with 50

ml of distilled water, and the micro-Kjeldahl flask was attached to the distillation unit. After adding 45 ml of 15 N NaOH, sample distillation was performed to collect released ammonia into a boric acid solution containing the indicators methylene blue and methyl red. Borate anion (proportional to the amount of nitrogen) was titrated with standardized 0.1 N H_2SO_4. H_2SO_4 was standardized using 0.1 N Na_2CO_3 as a primary standard. A reagent blank was run simultaneously (Chung *et al*. 2013).

Sample nitrogen content was calculated using following formula :

N (%) = ml H_2SO_4 added x Normality of H_2SO_4 x 1.4007 / Weight of sample (g)

Protein (%) = N (%) x 5.32 (AOAC 1995).

The carbohydrate content was estimated by calculating the differences based on the other components and using the following formula :

Carbohydrate content (%) = 100% - (moisture (%) + protein (%) + oil (%) + ash (%) (Gharibzahedi *et al*. 2014)

For the purpose of vitamin analysis 5g of each of the samples were homogenized with 50 ml of ethanolic sodium hydroxide solution. This was filtered into a 100 ml flask. Ten milliliters (10 ml) of the filtrate was pipetted into a beaker, and color developed by the addition of 10 ml potassium dichromate. The absorbance was read at 360 nm. A blank sample was also prepared and read at the same wavelength. The values are extrapolated from a standard curve. Acid extraction was performed to efficiently extract of vitamin B6 vitamers namely, Pyridoxine (PN), Pyridoxal (PL)n and Pyridoxamine (PM) following the protocols mentioned in AOAC (1990). Optimal extraction of vitamin B6 from kernels was achieved by autoclaving the sample in 0.44 N HCl for two hrs at 121°C. Following digestion, the hydrolyzates were cooled and adjusted to pH 4.5 with 6 N KOH and then diluted with water. The digests were then filtered and chromatographed on Dowex AG 50W-X8. Hot 0.02 M potassium acetate at pH 5.5 was used to elute impurities. Potassium acetate (0.04 M) at pH 6.0 was used to elute PL, followed by boiling 0.1 M potassium acetate at pH 7.0, for PN and KCl-K_2HPO_4 at pH 8.0 was boiled for elution of PM. Each vitamin was individually quantitated and summed to obtain total vitamin B6.

To analyse mineral constituents (mg/100g) an amount of 0.5 g of each of the walnut samples were accurately weighed into a Teflon flask. The nut was then mixed with nitric acid and perchloric acid. The concentration of minerals in walnuts, such as calcium, magnesium, potassium, sodium, manganese, and iron, was calculated according to the standard curve, using atomic absorption spectrophotometer (Model Varian 220 AA, Australia).

All the sampling experiments were carried out in triplicate and the results were depicted as a mean of the three values with the standard error.The statistical comparison among the treatment means for significance was done by Duncan's Multiple Range Test (DMRT) using SPSS version 10. The bar diagram with standard error was constructed in microsoft excel.

Results and Discussion

Variation in moisture content among walnut genotypes presented in Table 1 showed the maximum moisture content (8.69 %) in the genotype JWSM-36, followed by the genotypes JWSM-54 (8.21 %), JWSD-01 (8.04 %), and JWSM-50 (5.68 %), whereas minimum moisture content (2.98 %) was recorded in genotype JWSD-25. The mean value of moisture content among all the genotypes was 5.57%. Caglarırmak (2003) reported the moisture 3.2 to 3.9% among 35 walnut genotypes in Turkey whereas, Peng *et al.* (2021) reported moisture content of different

walnut genotypes in China between 1.20 and 9.92% with the average value of 5.55%. These findings are more or less similar to the results reported by Kader (2002) and Amaral *et al*. (2003).

Table 1. Analysis of kernel quality characteristics of different walnut (*Juglans regia* L.) genotypes.

Sl. No.	Genotypes	Moisture content (%)	Linoleic acid (%)	Linolenic acid (%)	Oleic acid (%)	Stearic acid (%)
1	JWSD-01	8.04bc	56.09bc	12.45bcd	19.58cd	2.05c
2	JWSD-04	3.02a*	52.03abc	15.07cde	17.94bcd	1.43b
3	JWSP-06	3.94a*	57.29c	15.80de	18.9bcd	0.20a
4	JWSD-20	3.35a	56.45bc	12.86bcde	16.51abcd	1.50b
5	JWSD-25	2.98 a	53.51abc	14.02bcde	14.22ab	3.07d
6	JWSM-35	3.74 a	49.56ab	13.75bcde	15.73abcd	3.24d
7	JWSM-36	8.69 a	54.10abc	13.31bcde	18.09bcd	0.08a
8	JWSM-37	4.61a	51.6abc	9.61abc	12.67a	1.90b
9	JWSM-38	4.72 a	48.5a	8.90ab	16.7abcd	3.70d
10	JWSG-43	4.39 a	58.43c	18.58e	12.14abcd	3.54d
11	JWSM-50	5.68ab	55.8abc	9.4abc	15.8abcd	0.10a
12	JWSM-54	8.21bc	51.9abc	5.7a	19bcd	0.20a
13	JWSD-59	4.07 a	67.3d	17.83de	20.6d	0.60a
14	JWSM-66	3.91 a	54.9abc	8.6ab	16.2abcd	0.20a
15	JWSK-77	4.24 a	55.6abc	9.2abc	15.1abc	0.50a

*The same characters in the same column show the difference between them is non-significant at $\alpha = 5\%$.

The data presented in Fig. 1 depicts that oil content among different walnut genotypes varied from 40.00 (JWSM-36) to 62.43 % in JWSD-59 with an average value of 57.98%. These values ranged between 62.3 and 66.5 % in six cultivars of walnuts grown in New Portugal (Amaral *et al*. 2003). However, Muradoglu *et al*. (2010) reported it between 49.8 and 66.1 % for 18 genotypes of walnut in Turkey. The oil content of this study was however, lower than those reported by other researchers (Savage 2001, Pereira *et al*. 2008). Who reported total oil contents from 62.6 to 70.3% and 78.83 to 82.4 %, respectively.

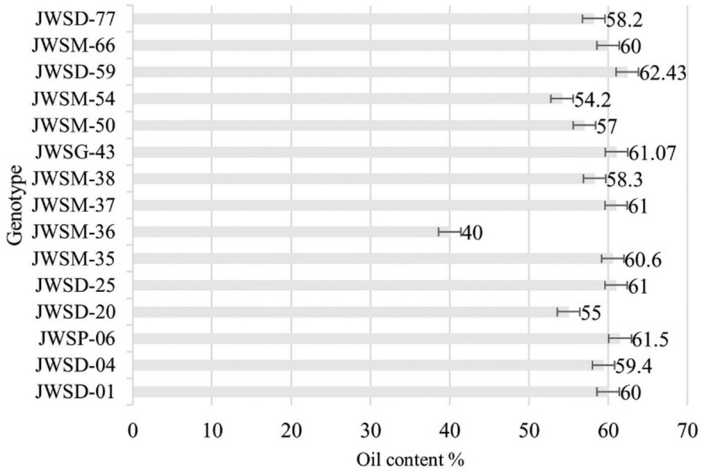

Fig.1. Variation in total oil content (%) among the analyzed walnut genotypes.

The fatty acid composition of walnut samples is essential in human nutrition and biochemistry. The major fatty acids recorded from genotypes were linoleic (C18 : 2), oleic (C18 : 1), α-linolenic acid (C18 : 3), and stearic acid (C17 : 0). C18 : 2 was found most abundant among all the fatty acids with values ranging from 48.5 % to 67.30 (Table 1). The maximum and minimum C18:2 content was observed in genotype JWSD-59 (67.30%) and JWSM-38 (48.5%) with an average value of 54.87 %. The average C18:1 content recorded was 16.61% and ranged from 12.14% (JWSG-43) to 20.60% (JWSD-59) among genotypes. The C18:3 was found in greater range in walnut genotypes from 5.70% (JWSM-54) to 18.58% (JWSG-43) with mean value of 12.33%. C17:0 content ranged from 0.08 to 3.70% with an average value of 1.48%. The percentage range of fatty acid components in this study is close with the findings of Kodada *et al.* (2016). Who found C18:1, C18:2, C18:3 and C17:0 in the range of 12.47 to 22.01%, 55.03 to 60.01 %, 9.3 to 15.87 % and 1.7 to 2.92%, respectively. The results of bio-chemical composition of walnut by other workers (Rabrenovic *et al.* 2011, Bayazit and Sumbul 2012, Tootoonchi *et al*. 2013, Bouabdallah *et al.* 2014) are also consistent with the present results.

Walnut has a high protein contents like legumes and cereals, which usually varied between 6.3 and 22% (Fig. 2). The maximum protein content (23%) was observed in the genotype JWSD-59 whereas the minimum protein content (15.00 %) was recorded in the genotype JWSM-66. The mean protein content of walnut genotypes was 18.78%. These values are similar to the results reported by Savage (2001), Amaral *et al*. (2003) and Muradoglu *et al*. (2010), they reported 13.6-18.1% protein in New Zealand genotypes, 12.2 - 15.2% protein in Portuguese genotypes, 12.8 - 22.3% protein in genotypes of eastern Turkey, respectively. However, Simsek *et al*. (2017), reported that protein content in naturally growing seed-propagated walnut genotypes of Turkey (Beyazsu region) ranged between 13.65 to 18.92%.

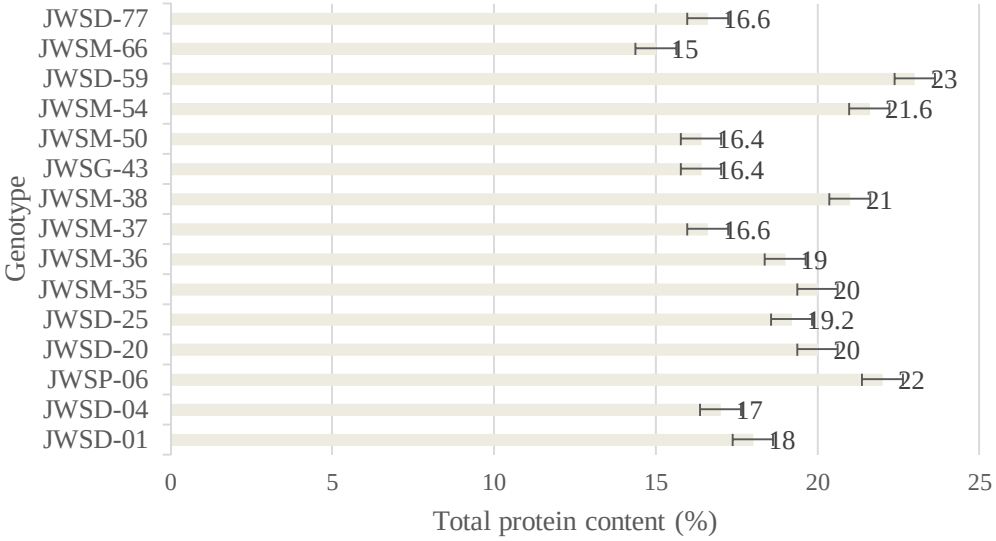

Fig. 2. Variation in total protein content (%) among the analyzed walnut genotypes.

The mean value of carbohydrate content in walnut genotypes was 11.63 g/100g while it ranged between 9.03 and 12.96 g/100 g in rest of the genotypes (Fig. 3). The result strongly corroborate with the findings of Yerlikaya *et al*. (2012), Kodada *et al*. (2016) and Simsek *et al.* (2017).

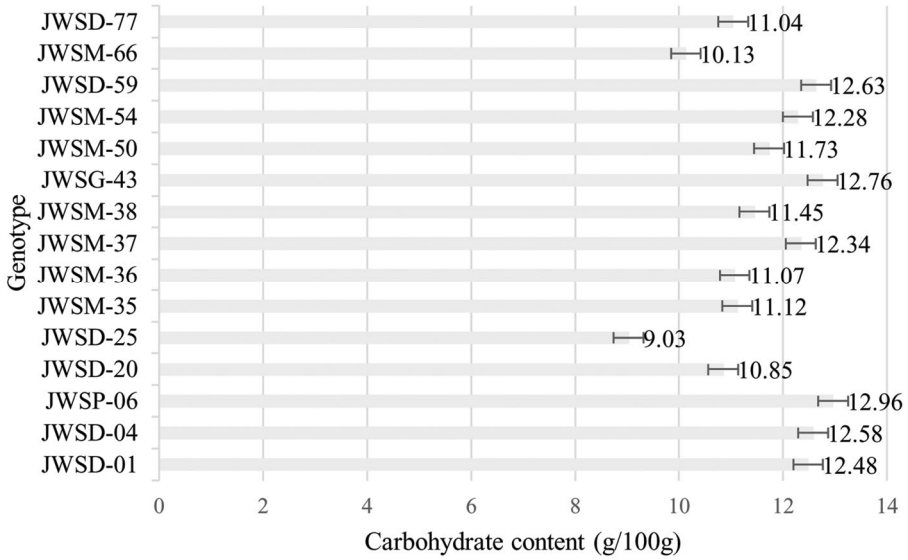

Fig.3. Variation in total carbohydrate content (g/100g) among the analyzed walnut genotypes.

Table 2. Analysis of kernel quality characteristics of different walnut genotypes.

Sl. No.	Genotypes	Vit B1 (mg/100g)	Vit B6 (mg/100g)	Vit B9 (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Cu (mg/100g)	Mn (mg/100g)	Zn (mg/100g)
1	JWSD-01	0.69cde	0.20bc	0.11ab	29.40c	120.23a	10.48bcd	1.02a	0.93a
2	JWSD-04	0.71de	0.10a	0.13abc	21.31a	117.38a	10.10bcd	6.80bc	4.27fg
3	JWSP-06	0.74e	0.25c	0.18c	25.41abc	119.81a	14.83e	8.04c	3.70e
4	JWSD-20	0.63cd	0.21bc	0.15abc	21.64ab	117.81a	11.30cde	7.85c	5.93k
5	JWSD-25	0.63cd	0.22bc	0.13abc	25.43abc	122.14a	6.01ab	6.56bc	4.56gh
6	JWSM-35	0.65cd	0.21bc	0.12ab	22.51ab	120.30a	13.31de	8.40cd	4.22f
7	JWSM-36	0.65cd	0.20bc	0.13abc	24.74abc	121.69a	4.80a	9.47d	1.98b
8	JWSM-37	0.53a	0.15ab	0.16bc	23.47abc	121.73a	15.27e	7.87c	2.92c
9	JWSM-38	0.67cde	0.21bc	0.10a	27.75bc	122.93a	15.50e	5.56b	3.21d
10	JWSG-43	0.61bc	0.20bc	0.11ab	27.45abc	120.11a	10.47bcd	6.80bc	5.39i
11	JWSM-50	0.52a	0.22bc	0.11ab	27.58bc	116.86a	8.73abc	6.69bc	4.90h
12	JWSM-54	0.54ab	0.22bc	0.12ab	24.89abc	119.17a	8.38abc	6.49bc	4.42fgh
13	JWSD-59	0.63cd	0.23c	0.13abc	29.36c	123.75a	10.43bcd	6.55bc	4.46fgh
14	JWSM-66	0.68cde	0.18bc	0.12ab	23.34abc	118.25a	9.78bcd	5.65b	3.39d
15	JWSK-77	0.65cd	0.21bc	0.14abc	22.43ab	119.38a	7.34abc	6.93c	4.38fgh

The same characters (denoted above) in the same column shows the difference between is non-significant at α = 5%.

The major vitamins B1, B6 and B9 found in the analysed genotypes ranged from 0.52 to 0.74 mg/100 g, 0.10 to 0.25 mg/100 g and 0.10 to 0.18 mg/100 g, respectively with their mean values 0.63 mg/100 g, 0.20 mg/100 g, 0.12 mg/100g (Table 2). The mean B1 value found is higher than reported by Muradoglu *et al.* (2010), who reported that vitamin B1 in walnut as 0.04 ± 0.02 mg/100 g.

Kernels of walnut have a remarkable amount of mineral nutrition beneficial to body health, among common foodstuffs. The major minerals found in the selected walnut genotypes were Mg, Ca, Cu, Mn, and Zn (Table 2). Among minerals magnesium was found predominantly in all genotypes and found to range from 116.86 (JWSM-50) to 123.75 mg/100g (JWSD-59) with mean value of 120.10 mg/100 g. Calcium, copper, manganese and zinc content of genotypes were recorded between 21.31 and 29.40, 4.80 to 15.50, 1.02 to 9.47 and 0.93 to 5.39 mg/100g, respectively. Polat *et al*. (2015) found Mg as predominant mineral in superior walnut genotypes but at higher value within the range of 241 to 426 mg/100 g followed by Ca (194.79 to 267.85 mg/100 g), Zn (1.93 to 3.47 mg/100 g) and Cu (0.72 to 1.43 mg/100 g). The present findings on mineral constituents of walnut are also confirmed by the reports of Cosmulescu *et al*. 2010, Muradoglu *et al*. 2010, Yerlikaya *et al*. 2012, Polat *et al*. 2015.

According to the findings of this study, the genotype JWSD-59 had the largest percentage of fats, oils, and nutrients, whereas the genotype JWSP-06 had the highest percentage of proteins. These genotypes can be used to develop nutrient-dense cultivars and serve as a gene pool for future walnut improvement efforts. The current research also highlighted the diversity of walnut genotypes based on geographical sampling of beneficial quality chemicals. It will improve understanding of the nutritional makeup of local walnut genotypes for consumption and export.

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