Indian Journal of Agricultural Sciences **80** (7): 626–30, July 2010

Variability in oil content and fatty acid composition in selected walnut (*Juglans regia*) germplasm

SANGITA YADAV[1], POONAM SUNEJA[2], Z HUSSAIN[3], REKHA CHAUDHARY[4], OMBIR SINGH[5] and S K MISHRA[6]

National Bureau of Plant Genetic Resources, New Delhi 110 012

Received: 13 January 2010; Accepted: 26 May 2010

ABSTRACT

The oil content and fatty acid composition of 16 walnut (*Juglans regia* L.) genotypes collected during 2008 from Phulwama and Budgaon districts of Jammu and Kashmir were determined. Considerable variations between genotypes were observed for all the parameters. The total oil content of the walnut ranged from 65.93% to 75.26%. The oleic acid ranged from 14.77 to 28.29% of the total fatty acid content, while linoleic and linolenic acid ranged from 47.07 to 66.0% and 8.13 to 19.90%, respectively. The saturated fatty acid, i e palmitic acid and stearic acid ranged from 4.76 to 7.63% and 1.74 to 3.34%, respectively. The large variability was observed for all fatty acids and these can act as base for breeding of walnut cultivars for superior varieties with oil of higher quality, satisfying industrial and consumer sectors.

Key words: Fatty acid, Kernel quality, Oil, Variability, Walnut

Walnut (*Juglans regia* L.) tree is native in south-eastern Europe, Asia minor, India and China. The kernel is the edible part of the nut and is considered an important food crop with a high nutritional value. They have been used traditionally to treat cough, stomach and cancer in Asia and European countries (Fukuda *et al.* 2003). Walnut oil is widely used in cosmetic industry where walnut oil is an important ingredient of anti-wrinkle and anti-aging products, dry skin cream because it presents moisturising as well as antioxidant properties (Espin *et al.* 2000). Walnut kernels generally contain about 60% oil (Prasad 1994) but this may vary from 52 to 70% depending upon the cultivar, location of growth and irrigation state. Considerable interest has been generated in walnut as they are believed to possess plasma cholesterol-lowering properties (Sabate *et al.* 2003). Walnut's concentration of omega-3's has many potential health benefits ranging from cardiovascular protection to the promotion of better cognitive function, to anti-inflammatory benefits helpful in asthma, rheumatoid arthritis and inflammatory skin diseases such as eczema and psoriasis. This property is believed to result from the fatty acid profile present in walnut oil. The major fatty acids found in walnut oil are oleic (18:1 ω-9), linoleic (18:2 ω-6) and linolenic (18:3 ω-3) acids. The ratios of these fatty acids are considered important for their economic and nutritional value. For example, lesser the linoleic and linolenic acid contents in the oil, longer the shelf-life, while higher levels of polyunsaturated fatty acids are more desirable because of their potential health benefits (Cunnane *et al.* 1993, Abbey *et al.* 1994).

Major quality loss during storage and transport occurs due to kernel rancidity. Therefore, for oil stability, fatty acid composition essentially the ratio of oleic to linolenic (O/L) acids is considered as an important criterion to evaluate the kernel quality (Kester *et al.* 1993). Walnuts are essential component of human diet because of their biochemical composition of polyunsaturated fatty acids, especially 18:2 and 18:3; therefore, it has very important place in nutritional habits. And for nutritional quality the ratio of linoleic and linolenic (ω-6/ω-3) acid is very important. In the present study nutritive composition of fatty acids has been analyzed in walnut germplasm collected from Jammu and Kashmir, India. Such evaluation would be helpful for characterization of walnut cultivars and would provide selection criteria in terms of quality evaluation and healthy diets that are important for future commercial production in the region.

MATERIALS AND METHODS

Plant materials

Sixteen germplasm of walnut (*Juglans regia* L.) collected from Phulwama and Budgaon districts of Jammu and

[1,3]Senior Scientist (e mail: sangitayadav@nbpgr.ernet.in; zakir24@mailcity.com), [2]Technical Officer (e mail: psuneja @nbpgr.ernet.in), [6]Head, Germplasm Evaluation Division (e mail: 6skmishra_gene@rediffmail.com)

[4,5]Principal Scientist (e mail: rekha @nbpgr.ernet.in; omvir2007@rediffmail.com), Regional Station, NBPGR, Srinagar, Jammu and Kashmir

Kashmir regions were analyzed for their total oil content and fatty acid profile during 2008.

Total oil content of kernel

Kernels of walnut were dried to 4-5% moisture level in oven at 108°C for 16 to 18 hr. The oil content of the seed samples were determined by a non-destructive method using a Newport NMR analyzer (Model-4000) from Oxford Analytical Instruments Ltd, UK after calibrating with pure walnut oil.

Fatty acid analysis by gas liquid chromatograph

Kernels of walnut were freshly grounded (Remi homogenizer) and weighed to obtain 40mg oil when extracted with 10ml solvent mixture consisting of chloroform: hexane : methanol (8:5:2 v/v/v). The extracts obtained were dried at 60°C in nitrogen gas for 30 min. Methyl esters of oil samples were prepared according to the method of Neff *et al*. (1994) with slight modifications. 1µl of the hexane extract was injected into a highly polar HP Innowax capillary column of 30m length (inner diameter: 0.32m, film thickness: 0.5µm, split: 1:80). A Hewlett Packard gas chromatograph, model 6890 equipped with flame ionization detector (FID) was used. The injector and detector temperatures were 260°C and 275°C, respectively. Oven temperature was programmed from 150°C holding at 1 min. to 210°C at the rate of 15°C/min., followed by 210°C to 250°C at the rate of 5°C/min. for 12 min. Peaks of fatty acid methyl esters were identified by comparing their retention times with those of known standards run under similar separation conditions; peak integration was performed applying HP3398A software.

Statistical analysis

Correlation co-efficient was calculated using MS Excel. Pair-wise euclidean genetic distance, UPGM-based clustering, Mentle's test, PCA analysis was calculated using NTSYSpc 2.11X (USA).

RESULTS AND DISCUSSION

The per cent total oil content of the walnut kernel ranged from 65.93 to 75.26% with a mean value of 71.88%. The variability of oil content in walnut kernels confirms the result reported for other walnut cultivars. The highest oil content was found in IC561581 (75.26%), IC561604 (74.45%) and IC561564 (74.36%).

The fatty acid composition along with oil content of walnut is shown in Table 3. The study has shown that walnut has low concentration of the ensemble of saturated fatty acid (SFA) (palmitic and stearic), intermediate for MUFA and high concentration of poly unsaturated fatty acid (PUFA), especially linoleic acid. The values were 4.76–7.63% of total kernel oil for palmitic acid, 1.74–3.34% for stearic acid, 14.77–28.29% for oleic acid, 47.07–66.0% for linoleic acid and 8.13-19.90% for linolenic acid. The high linoleic acid was found in IC561561 (66%), followed by IC561596 (62%), while high oleic acid was found in IC561591 (28.29%), followed by IC561562 (23.96%). The range of variability of each major fatty acid herein reported agrees well with already reported (Greve and Labavitch 1992, Dogan and Akgul 2005, Ozkan and Koyuncu 2005).

The O/L ratio is considered as an important criterion for kernel quality evaluation. There is large variability in the content of oleic acid and linoleic acid. The genotypes with high O/L ratio were IC561591, followed by IC561562, probably because of their high oleic acid. A high oleic acid is very essential from both quality and stability point of view, as it increases the nutritional value and the stability of the fat against rancidity.

Based on the recommendation of FAO/WHO for dietary fat in human nutrition, the fatty acid composition of oil should have significant proportions of two essential poly unsaturated fatty acids, i e ω-6 and ω-3, with a desirable ratio of the two between 5:1 and 10:1. The ω-6/ω-3 ratio ranged from 2.73 to 7.73. The genotypes with high ratio were IC561596 (7.73), followed by IC561561 (7.40).

Table 1 Correlation co-efficient between oil content and fatty acid concentrations in walnut[a]

Component	Oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic	O/L	ω-6/ω-3	IV
Oil		–0.226	–0.046	–0.007	–0.149	0.284	0.060	–0.228	0.282
Palmitic			0.272	–0.473	0.399	–0.290	–0.457	0.310	–0.202
Stearic				–0.282	0.125	–0.063	–0.201	0.218	–0.106
Oleic					**–0.716****	–0.008	**0.968****	–0.235	–0.559
Linoleic						**–0.664****	**–0.851****	**0.807****	–0.032
Linolenic							0.198	**–0.939****	**0.754****
O/L								–0.418	–0.422
ω-6/ω-3									–0.559
IV									

[a]Correlation shown in bold face type are significant at 5% (**) level of significance
O/L, Oleic/linoleic acid ratio; IV, iodine value

Table 2 Pair-wise euclidean genetic distance of 16 genotypes of walnut

	IC 561561	IC 561562	IC 561564	IC 561581	IC 561583	IC 561588	IC 561591	IC 561596	IC 561598	IC 561601	IC 561604	IC 561606	IC 561571	IC 561575	IC 561602	IC 561608
IC561561	0.00															
IC561562	6.16	0.00														
IC561564	5.84	4.31	0.00													
IC561581	4.60	3.59	3.69	0.00												
IC561583	2.65	4.62	5.89	4.14	0.00											
IC561588	5.13	2.15	5.57	3.92	3.11	0.00										
IC561591	7.58	3.20	5.80	6.13	6.32	3.93	0.00									
IC561596	2.46	5.96	6.92	5.12	3.07	4.57	7.02	0.00								
IC561598	4.64	4.14	2.97	2.23	4.69	4.79	6.69	5.50	0.00							
IC561601	4.06	3.26	5.16	4.07	3.25	2.35	3.85	3.26	4.70	0.00						
IC561604	3.35	4.78	5.01	2.25	3.70	4.29	6.65	3.42	3.45	3.52	0.00					
IC561606	3.76	4.91	6.12	4.74	3.76	4.10	6.46	2.79	4.28	3.27	3.94	0.00				
IC561571	5.84	1.96	5.35	4.04	3.95	1.86	4.59	5.55	4.25	3.61	5.00	4.15	0.00			
IC561575	4.73	2.65	3.32	2.84	4.27	3.37	4.84	4.80	2.20	3.08	3.59	3.22	2.95	0.00		
IC561602	3.80	3.08	4.25	3.55	3.36	2.82	4.51	3.53	3.39	1.72	3.41	2.36	3.14	1.69	0.00	
IC561608	4.41	2.77	4.66	3.59	2.41	1.99	4.75	4.86	4.15	3.10	4.31	4.54	2.49	3.40	3.03	0.00
MEGD	5.02	3.43	4.94	4.18	4.37	3.33	5.44	4.17	3.45	2.66	4.20	3.45	2.81	2.54	3.03	0.00
Minimum	2.46	1.96	2.97	2.23	2.41	1.86	3.85	2.79	2.20	1.72	3.41	2.36	2.49	1.69	3.03	0.00
Maximum	7.58	4.91	6.92	6.13	6.32	4.79	7.02	5.55	4.70	3.61	5.00	4.54	3.14	3.40	3.03	0.00

The correlation of co-efficient showed highly significant negative correlation between oleic and linoleic acids (Table 1). Correlation co-efficients greater than 0.71 or smaller than –0.71 have been suggested as biologically meaningful (Skinner *et al.* 1999). In the literature it is reported that oleic acid concentration is controlled by its conversion to linoleic acid, probably as a result of the enzymatic activity of oleic desaturase. In Pistachio, it has been assumed that this enzyme controls the variation in the fatty acid profiles of the nuts (Garcia *et al.* 1992).

The O/L ratio is very essential for stability of oil. Linoleic acid is less saturated and less stable than oleic acid, as shown by strong negative correlation of linoleic acid with O/L ratio and strong positive correlation of oleic with O/L ratio. Similar results have been obtained in almond (Kodad and Company 2008) and pistachio (Garcia *et al.*1992).

Similarly, ω-6/ω-3 ratio is very important for nutritional point of view. It is found that linoleic acid showed strong positive correlation with ω-6/ω-3 ratio while linolenic acid showed a strong negative correlation.

The pair-wise euclidean genetic distance (EGD) among the accessions were calculated using NTSYSpc 2.11X (Table 2). The highest EGD (7.58) was reported among genotypes IC561561 and IC561591, while the lowest (1.69) was among genotypes IC561571 and IC561602. The accession-wise MEGD varied from a maximum (5.02) in IC561561 to minimum (2.54) in IC561575 with the overall MEGD of 3.57. UPGMA cluster analysis was done based in pair-wise euclidean genetic distance matrix (Fig 1). The accession IC561591 was the most distant among accessions with EGC of 5.67. At 4.54 EGD, the remaining 15 accessions were grouped in 2 clusters. Cluster I having only 2 accessions IC561561 and IC591596. At 4.16 EGD the 13 accessions of cluster II was further grouped into 2 sub-cluster IIA with IC561562, IC561588, IC561571, IC561583, IC561608, IC561575, IC561602, IC561606 and with IIB with IC561564, IC561581, IC561598, IC561504.

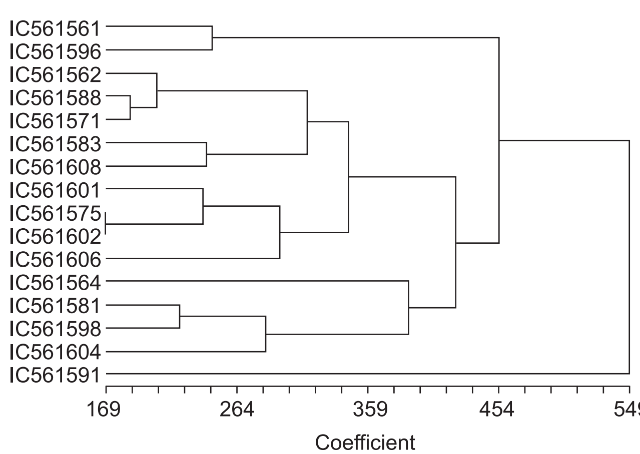

Fig 1 Dendrogram based on UPGMA analysis of Euclidean distance among 16 walnut genotypes

Table 3 Average range of variation and component scores of the first 4 principal components of genetic divergence in 16 genotypes of walnut

Genotype	Min	max	SD	1	2	3	4
Oil	65.9	75.2	2.34	0.28	0.32	0.32	0.78
Palmitic	4.7	7.6	0.77	-0.61	0.05	-0.47	-0.16
Stearic acid	1.7	3.3	0.45	-0.32	0.06	-0.74	0.48
Oleic acid	14.7	28.2	3.82	0.68	-0.73	0.04	0.03
Linoleic acid	47.0	66.0	4.60	-0.94	0.11	0.29	0.01
Linolenic acid	8.1	19.9	3.31	0.71	0.66	-0.22	-0.07
O/L	0.23	0.6	0.10	0.79	-0.59	-0.10	0.06
ω-6/ω-3	2.7	7.7	1.57	-0.84	-0.45	0.19	0.17
IV	152.7	168.6	4.64	0.20	0.95	0.14	-0.10
Varaiance (%)							
Proportion				42.2	28.3	11.9	10.0
Cumulative				42.2	70.5	82.5	92.6

O/L, Oleic /linoleic acid ratio; IV, iodine value

Sixteen populations clustered at two main groups at the linkage distance of 4.54 while one of the accessions IC561591 was most distant. The accession IC561591 had highest O/L ratio and lowest ω-6/ω-3 ratio. Moreover, it also had highest oleic acid content and lowest linolenic acid content. On the other hand, IC561561 and IC591596 of cluster I had highest ω-6/ω-3 ratio and highest linolenic acid. Members in sub-cluster IIA had O/L ratio more than 0.3 and oleic acid concentration was more than 18% while in IIB the O/L ratio was less than 0.3 and oleic acid concentration was less than 18% and this group had higher linolenic acid content. Mantels correlation was 0.69.

To assess the patterns of variation, principal component analysis was done by considering all the nine variables simultaneously, 4 of the principal components accounted for more than 92% of the total variation encountered (Table 3). The first principal component, accounting for more than 42% of the total variation due to oleic acid, linolenic acid, O/L ratio. Oil and iodine value had also relatively positive weight on this component. Conversely, linoleic acid, ω-6/ω-3, palmitic acid, stearic acid had negative weight. The second component accounting for an additional 28% of the total variation depicted primarily the pattern of variation in iodine value, linoleic acid, increased at the expense of O/L and linolenic acid (negative co-efficients).

The pattern of variations illustrated by the PCA was very well substantiated by the genotypic correlation co-efficients determined for pair-wise association of the traits. Consistent to the outputs of the PCA, the traits that contributed most for the 1st principal component (oleic acid) was positively correlated with O/L and negatively with linoleic acid. The contrasting relationship of linolenic acid with ω-6/ω-3 ratio depicted by the 2nd principal component was supported by highly significant negative correlation co-efficient.

Wide variability was observed for the contents of all fatty acids, O/L and ω-6/ω-3 ratio, representing a very promising base to obtain new walnut cultivars for production of healthy snack food high in PUFAs. The variation in fatty acid composition of nuts from different genotypes may affect the final use of the product. For example, the nuts containing high levels of oleic, linoleic and linolenic acid should be preferred if nuts were destined for use in cholesterol-lowering diet. The present study further confirms that walnuts are rich source of essential fatty acids which have positive effect on human health. The superior germplasm identified in the study will be useful for walnut breeding programmes to generate superior genotypes for industrial and consumer needs.

REFERENCES

Abbey M, Noaks M, Belling G B and Nestel P J. 1994. Partial replacements of saturated fatty acid with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. *American Journal of Clinical Nutrition* **59**: 995–9.

Cunnane S C, Ganguli S, Menard C, Liede A C, Hamadeh M J, Chen Z, Wolever T M S and Jerkin D J A. 1993. High linoleic acid flaxseed (*Linum usitatissimum*): some nutritional properties in Humans. *British Journal of Nutrition* **69**: 433–53.

Dogan M and Akgul A. 2005. Fatty acid compositon of some walnut (*Juglans regia* L.) cultivars from east Anatolia. *Grasas y Aceites* **56**: 328–31.

Espin J C, Soler-Rivas C and Wichers H J. 2000. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *Journal of Agricultural and Food Chemistry* **48**: 648–56.

Fakuda T, Ito H and Yoshida T. 2003. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry* **63:**795–801.

Garcia J M, Agar I T and Streif J. 1992. Fat content and fatty acid composition in individual seeds of pistachio varieties grown in Turkey. *Gartenbauwissenschaft* **57**: 130–3.

Greve L C, McGranahan G, Hasey J, Snyder R, Kelly K, Goldhamer D and Labavitch J M. 1992. Variation in polyunsaturated fatty acids composition of Persian walnut. *Journal of the American Society for Horticultural Science* **117** (3): 518–22.

Kester D E, Cunningtam S and Kader A A. 1993. Almonds (*in*) *Encyclopedia of Food Science, Food Technology and Nutrition,* pp 121–6, Academic press, London.

Kodad O and Company R S. 2008. Variability of oil content and of major fatty acid composition in almond and its relationship with kernel quality. *Journal of Agricultural and Food Chemistry* **56**: 4096–101.

Neff W E, Adolf R O, List G R and EL-Agaimy M. 1994. Analysis of vegetable oil triacylglycerols by silver ion high performance liquid chromatography with flame ionization detector. *Journal of Liquid Chromatography* **17**: 3951–68.

Ozkan G and Koyuncu M A. 2005. Physical and chemical composition of some walnut genotypes grown in Turkey. *Federation of African Societies of Chemistry* **56**: 141–6.

Prasad R B N. 1994. Walnuts and pecan (*in*) *Encyclopedia of Food Science, Food Technology and Nutrition*, pp 4828–31, Academic press, London.

Sabate J, Fraser G E, Burke K, Knutsen S, bennett H and Lindsted K D. 2003. Effect of walnut on serum lipid levels and blood pressure in normal men. *The New England Journal of Medicine* **328**: 603–7.

Skinner D, Buchan G R, Auricht G and Hughes A. 1999. A simple method for the efficient management and utilisation of large germplasm collections. *Crop Science* **39**: 1237–42.