

Selection and characterization of elite walnut (*Juglans regia* L.) clone from seedling origin trees in North Western Himalayan region of India

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Abstract

Western Himalayan region of India provides agroclimatic conditions suitable for producing high quality walnuts. Jammu and Kashmir State of India produces major share of export quality walnut. The nuts brought to market are generally a mixture of variable size and shape as they are harvested from different seedling trees. This study aimed to identify a clone, which has high export quality. We identified 63 walnut seedling trees and found GL0109 tree as best meeting all the export standards like nut weight (20.10g), nut size (45.45mm x 42.07mm), nut grade (Jambo), shell thickness (1.24mm), kernel recovery (61.40%), proportion of light coloured kernel (83.40%), protein content (15.66%) and oil content (68.42%). It was also found highly resistant to anthracnose disease as it showed 5-10% incidence and 0-5% severity against 60% incidence and 75% severity in other 63 seedling trees. Overall, it scored 9.25 numerical rating on 10 point scale based on the parameters prescribed by Jammu and Kashmir Walnut Exporters Association. Thus, GL0109 was identified and recommended for cultivation in different walnut growing areas of the state.

Keywords: GL0109; walnut selection; nut; kernel; Jammu and Kashmir Walnut Exporters Association.

Abbreviations: AGMARK_Agriculture Marketing; AOAC_Association of Official Agricultural Chemists; CITH_Central Institute for Temperate Horticulture; GL_Galhar; GC-MS_Gas Chromatography-Mass Spectrometry; J and K_Jammu and Kashmir; Per.Com_Personnel Communications; SKAU-W_Sher-e-Kashmir University of Agricultural Sciences and Technology Walnut; UPOV_International Union for the Protection of New Varieties of Plants.

Introduction

Persian or English walnut (*Juglans regia* L.) is the only species widely cultivated for nut production for table purpose. Walnut is a high energy food, rich in oil including omega-3 fatty acids, vitamins and minerals, and valued as healthy snack food and bakery ingredients (Rana et al., 2007). Its alpha linolenic acid has substantial cardio protective effects as it increases the ratio of high-density lipoprotein cholesterol to total cholesterol, reducing inflammation and improving arterial function (Hu et al., 1999; Diousse et al., 2001; Patel, 2005). It contains 'melatonin' an antioxidant produced by pineal gland and responsible for inducing and regulating sleeps (Reiter et al., 2005). It also reduces the incidence of cancer and, delays neurodegenerative diseases of aging (McGranahan and Leslie, 2012). Western Himalayan region of India produces high quality walnuts. The Jammu and Kashmir State of India alone accounts for >98% of India's total production with an average productivity of 2.69 metric tonnes/ha from an area of 83613.80 ha and production of 224595.85 metric tonnes (Sharma, 2012). India export around 5000 metric tonnes walnut kernel of worth US \$ 260-300 million annually to France, Germany, Spain, Portugal, Austria, United Kingdom, Kuwait, Bahrain, Dubai and Saudi Arabia. Besides, domestic market of worth of US \$ 140-200 million for kernel and in shelled walnuts is also fulfilled by the state (Per. Com. with J and K Walnut Exporters Association in 2012). Interestingly,

the major share of walnut production comes from the trees of seedling origin, which has lot of variability in nut size, shape, shell thickness and kernel quality (Rouskas and Zakynthinos, 2001; Solar et al., 2002; Zeneli et al., 2005). Walnuts grown at higher elevations (>1500 m) and monsoon free areas are generally superior in quality than those growing at lower elevation and high rainfall areas (Rana et al., 2007). Breeding new walnut varieties through hybridization is both difficult and time consuming. It is therefore, convenient to exploit existing variability by making appropriate selections based on characters like climatic adaptations, precocity, high productivity, good quality of nut and kernel and resistance to major diseases. Anthracnose disease affect all leaves, leaf petioles, shoots, nuts and peduncles, and has been reported to infect several cultivars of English (*J. regia* L.) as well as black (*J. nigra* L.) walnuts severely (Berry, 1997; Coates, 2012; Michailides et al., 2012). An ideal walnut variety must have late leafing, both terminal and lateral bearing, low incidence of pistillate flower abscission, high yielding nuts (>6 MT/ha) with jambo size, relatively smooth, >50% kernel recovery, plump and light coloured kernel and at least moderately resistant to pest and diseases (Botu et al., 2010; Cosmulescu et al., 2010a; McGranahan and Leslie, 2012). Scientists from different parts of world have practiced simple selection in the natural seed population and selected trees of high nut quality (Germain, 1997; Sharma and Das, 2003;

Cosmulescu and Botu, 2012). This research paper aimed to select a clone out of existing seedling populations, which has high nut production and quality as well so as to supply quality planting material to walnut growers.

Results

Tree characters

The elite clone, selected from 63 naturally occurring trees was designated as GL0109 and described in Table 1 based on UPOV descriptors (UPOV, 1988). The vigour of tree was medium with spreading growth habit in which side branches were spreading upward and not competing with central leader. The branching density was <4 per meter shoot length on main limbs, thus characterized as sparse. Shoots were slightly pubescent and green brown in colour. Leafing out was early and occurred in the 3rd week of February when lowest temperature recorded to be -3^oC, even there was no spring frost injury. The colour of fully expanded leaves was green, number of leaflets per leaf ranged from 5 to 9 and average leaf area was 512 cm². The clone expressed the dichogamy (protandry: male flowers emerge first followed by female) and bore nuts in clusters having 2-5 nuts/cluster (Fig.1a). The nuts matured during late September yielding 30-35kg nuts/ tree. Anthracnose caused by a fungus *Gnamonia leptostyla* (now *Ophiognomonia leptostyla*, an imperfect stage of *Marssonina juglandis*) was found major disease in the study area. It is characterized by circular, brown spots with yellow halos formed on the ventral side of leaflets, as they attain full size. It weakens the tree that leads to poor kernel filling, low yield and poor quality nuts. In our study area, the incidence of disease was observed to be 60% with 75% severity in most of the trees. The clone GL0109 showed very low incidence (5-10%) and severity (0-5%) of anthracnose disease (Fig.1e-f), which was otherwise uniformly prevalent in other trees nearby growing trees.

Nut characters

The nut characteristics of GL0109 have been presented in Table 1. The hull of nut was green with partially red pigmentation. The nuts have round shape with smooth surface (Fig.1 b). The apical tip of nut has medium prominence, whereas prominence of pad on suture was weak. Nut shell had most desirable light colour, which is an important quality parameter (Fig.1 c). The average nut was 20.10g, nut diameter 42.07mm and nut length 45.45 mm. The average shell thickness was 1.24 mm, which classify GL0109 under medium shell thickness group.

Kernel characters

Kernel characters of GL0109 have been described in Table 2. It showed kernel recovery of 61.40% with 83.40% proportion of light coloured kernel (Fig.1 d). The average protein content of kernels was 15.66% while oil content was 68.42%. The composition of fatty acids showed highest content of linoleic acid (58.88%) followed by oleic (21.30%), linolenic (12.55%), palmitic (5.02%) and stearic (2.26%) acids.

Discussion

In order to designate any variety or clone as superior, it is essential to compare its performance and characteristics with already available commercial varieties. We also compared

characters of GL0109 with many such commercial varieties mentioned in the subsequent text. The medium tree vigour of GL0109 resembled with Franquette, Hartley and Marbot cultivars. The growth habit of walnut cultivars have been categorized into erect, semi-erect and spreading types (UPOV, 1988; Gupta, 1997; Solar et al., 2002). The growth habit of GL0109 was spreading type as its side branches were spreading and not competing with central leader, similar to walnut cvs. Gustine, Payne and Vina (UPOV, 1988). The shoot colour is an important morphological descriptor for characterization of walnuts was greenish brown in GL0109, however, it varied from greenish brown, black to pale brown (UPOV, 1988; Sharma, 2002). Late leafing considered an ideal character in walnut to escape the spring frost injury as shown by other varieties like 'Chico', 'Serr', 'Ashley' and 'Sunland' (Barone et al., 1990). Despite of early leafing character, GL0109 also proved resistant against frost injury. Floral characters such as bearing habit, abundance of male and female flowers coupled with dichogamy are also important traits used in characterization and variation studies in walnut (Sharma, 2004). Based on bearing habit, GL0109 was categorized as intermediate bearing genotype. Walnut is a hetero-dichogamous species demonstrating protandry, protogyny and homogamy in different genotypes (Sharma, 2004). GL0109 showed protandry, which is similar to 'Hartley', 'Payne' and 'Pedro' cultivars (Sounduri et al., 2005). These kinds of varieties need planting of varieties with early, mid and late blooming traits with sufficient overlapping period of blooming for effective pollination. Maturity of nuts also plays an important role to catch market demand. GL0109 matures in 3rd week of September i.e. just after the rainy season is over thereby make post-harvest operations like drying, transportation and storage easy particularly in areas where winter onset is early (Rana et al., 2007). It is interesting to note that clone GL0109 was growing naturally without supplying any additional nutrition, nevertheless the productivity (30-35 kg/tree) was comparable with many cultivated varieties of Jammu and Kashmir State like CITH 6, 7 and 10 (Sofi et al., 2012). There are other factors also that influence productivity of a tree such as age of tree, agro-ecology of area, number of pistillate flowers, per cent fruit set, nut size and kernel percentage (Serr, 1962; Forde, 1979; Akça, 2009). The susceptibility of majority of the seedling tress to anthracnose was also found responsible for low productivity and poor quality nuts. Owing to field resistance to anthracnose disease and good agronomic attributes, GL0109 can also be used an appropriate parent in genetic improvement programme. The nut shape and surface of GL0109 was comparable to cultivar 'Meylannaise'; prominence of apical tip like 'Chico', 'Corne' and 'Hartley' and prominence of pad on suture were like 'Chico', 'Grandjean' and 'Mayette' cultivars (UPOV, 1988). Above all, it is nut and kernel characters that decide the superiority of any variety in the domestic and International market (Bhat et al., 1999; Sundouri and Sharma, 2005, Verma et al., 2009). The Jammu and Kashmir Walnut Exporters Association has specified the grade of walnuts on the basis of nut diameter viz., India-1-AGMARK (28-30mm), India-Special (30-32mm), India Super (32-34 mm) and Jambo (>34mm). The nut diameter of GL0109 was 42.07mm classifying it into Jambo group. Medium thickness of shell is more acceptable in the market than ultra-thin shelled varieties as later are prone not only to breakage during inshelled export but easily damaged and eaten by birds and monkeys (Beyhan and Demir, 2006; Rana et al., 2007). The thick or hard shelled nuts are difficult to crack and not preferred in the market.

Table 1. General tree and nut characters of GL0109 selection (Mean of two years).

Character	Description	Character	Description
Origin	Seedling origin	Number of leaflets	7 or 9 (range 5-9)
Tree vigour	Medium	Leaf area	512.00 cm ²
Growth habit	Spreading	Bearing habit	Intermediate
Branching density	Sparse (<4 per meter shoot length on main limbs)	Number of fruits/ cluster	2-3 (range 1-5)
Shoot pubescence	Slightly pubescence	Time and duration of male flowering	Very early (13 th February) and 14 days
Shoot colour	Green brown	Time and duration of female flowering	Early (21 st February)
Leafing time	Very early (3 rd week of February)	Dichogamy	Protandry
Leaflets color	Green	Time of harvesting	3 rd week of September
Leaflets shape	Broad elliptic	Time of leaf fall	Second fortnight of November
Leaflets margins	Entire	Pattern of leaf fall	Individual leaflet
Rachis colour	Brown	Hull colour	Green with red blush
Leaf and rachis pubescence	Slightly pubescent	Anthracnose disease Incidence	5-10% (60% in local seedlings) 0-5% (50% in local seedlings)
		Severity	
Hull colour	Green with red blush	Yield	30-35 kg
Nut shape	Round	Pad on suture	Weak
Nut surface	Smooth	Nut weight (g)	20.10
Nut colour	Light	Nut diameter (mm)	42.07
Apical tip of nut	Medium	Nut length (mm)	45.45
Grade	Jambo	Shell thickness (mm)	1.24

Table 2. Kernel characters of GL0109 selection (Mean of two years).

Character	Mean	Character	Mean
Recovery (%)	61.40	Stearic acid (%)	2.26
Proportion of kernel colour (%)		Oleic acid (%)	21.30
Light			
Amber	83.40		
Brown	16.61		
rancid	0.00		
Protein Content (%)	15.66	Linoleic acid (%)	58.88
Oil Content (%)	68.42	Linolenic acid (%)	12.55
Palmitic acid (%)	5.02	Numerical rating (out of 10)	9.25

Also, a negative correlation (-0.34) exist between shell thickness and kernel ratio (Arzani et al., 2008). Kernel recovery of GL0109 was very high (61.40%) and better than many international varieties like Payne (50%), Hartley (46%), Franquet (45%), Chico (46%), Sunland (57%), Vina (49%), Chandler (50%), Pedro (46%), Cisco (46%), Kaplan (40%), Yalova-2 (50.37%) and Bilecik (56%) (McGranahan and Leslie, 1990; Akca, 1999) and Indian walnut selections like CITH-1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, Sulaiman, Hamdan, SKAUW0003 and SKAU-W-14 for which it ranged from 50-59% (Sounduri et al., 2005; Verma et al., 2011). The color of kernel also hold premium price in the market and its light colour which is preferred over amber and brown color. Apart from morphological characters, the biochemical characters particularly protein and lipids also play an important role in deciding the market value. The protein content in walnut may vary from 15.00 to 24.00% (Akca and Ozogun, 2004; Ali et al., 2010) and it was 15.66% in GL0109. The high contents of unsaturated fatty acids comparison saturated fatty acids provide walnut oil with many nutritional and medicinal properties including its wide use in cosmetic industry (Cunnane et al., 1993; Abbey et al., 1994; Sabate et al., 1993). The oil content generally ranges from 61.97-70.92 %

with 21.18-40.20% oleic acid, 43.94-60.12% linoleic acid, 6.91-11.52% linolenic acid, 5.24-7.62% palmitic acid and 2.56-3.67% stearic acid (Ozkan and Koyuncu, 2005). The present clone has very high oil content with moderate amounts of fatty acids.

Materials and Methods

Survey and selection of plant materials

Fieldwork was done from 2006-2012 in different parts of Ramban, Doda and Kishtwar districts of Jammu and Kashmir state of India. Seedling trees occurring in these areas were studied using both random and selective samplings. From 2006-2008, we visited 790 seedling tress growing over 18 locations between 1500m-1750m a msl. All the trees yielding inferior nuts were rejected. Finally, 63 promising walnut seedlings trees were marked and nuts were collected between 2009- 2012 to identify the best clone. We zeroed our selection to one seedling tree named as 'GL0109'. It was of ~20 years old and collected from 35 km away from Kishtwar



Fig 1. (a-f) : (a) Bearing habit of GL0109; (b-c) Harvested nuts; (d) Nuts with kernel quality; (e) GL0109 showing resistance to leaf anthracnose; (f) Tree adjacent to GL0109 showing severe infection of leaf anthracnose.

town on Padder road at an elevation of 1710m at N 33°20'18.7" and E 075°55'52.0". In this locality, winter season prevails from October to March and prone for heavy snow fall, being peak in the month of December and January. Frost concurrence is very common in the month of February and March. Temperature ranges from -6°C (winter) to 37°C (summer) during various parts of the year.

Characterization

A total of 50 nuts were collected for recording the data over two seasons. The clone was characterized based on

descriptors developed by International Union for the Protection of New Varieties of Plants (UPOV, 1988) and the standards prescribed by J and K walnut Exporters Association.

Biochemical analysis of kernel was done in the Bio Chemistry Lab of National Bureau of Plant Genetic Resources (NBPGR), New Delhi (India) and Quality Control Lab of Indian Institute of Integrated Medicine, Jammu. GC-MS chromatography of the methyl esters of extracted oils was used to measure the kernel fatty acids. Supelcowax-10 column was used and column was programmed from 150°C to 240°C at the rate 3°C/min. Helium gas was flowed through

the column at the rate of 1ml/min. Oil and protein estimation were done as per AOAC official method (950.48). To arrive at overall rating, the mean value of characters was compared on 10 scale with standards laid down by Jammu and Kashmir Walnut Exporters Association for exporting walnuts. The disease severity and incidence were recorded as per the methods of Claudia et al. (1997) and Masood et al. (2010). In order to compare the agronomic and biochemical characters of GL0109 with standard varieties, five International varieties viz. local, Franquette, Hartley, Marbot, Gustine, Payne and Vina and 14 recently released National selections were used. Since we have discussed the data of only one elite clone, therefore we did not use any specific statistical tool for analyses. The comparison with other standard varieties was based on the values available in secondary literature and UPOV descriptors.

Conclusions

The clone GL0109 has overall score of 9.5 out of 10 helped us to designate it as one of the most superior clone among several available in the study area. The clone has been recommended for walnut growers and planting material has been multiplied for testing its performance at other locations. The clone has also been proposed to be registered with NBPGR (an institution responsible to register plant material in India) as unique plant material. The clone would also be a useful breeding material due to its excellent combination of agronomic, quality and biochemical traits in one genetic background.

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