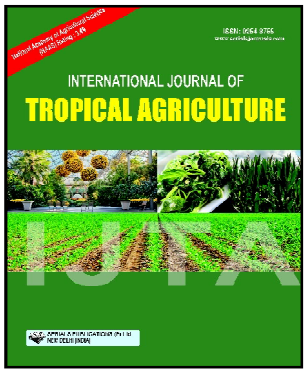

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Phenotypic variation in native Persian walnut (Juglas regia L.) collection from Himachal Pradesh

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Abstract: Walnut (Juglans regia L) has various microelement and nutritious substances and is one of the important nut crops in the world. India has significant Persian walnut (Juglans regia L.) trees, most of which are seedling grown. Due to the extensive variability of biological material, the result of cross-pollination and generative propagation prevailed by natural dissemination in Himalayan region of India has rich phenotypic diversity in walnut germplasm. In the present research, 8(eight) walnut genotypes were selected from different parts of Himachal Pradesh, India and evaluated to determine phenotypic diversity and to detect superior trees. Thus, morphological diversity of 8(eight) walnut accessions was studied and trees with promising fruits characteristics were identified. There was high variability for the measured phenological and pomological characteristics. Walnut fruits are particularly demanded on various markets due to their nutritional value and the walnut timber is valued for furniture. *Juglans regia* species has a vast habitat, but is more and more affected by genetic erosion and vulnerability and for this reason the necessity of saving its genetic variability is very important. Among the accessions studied, the ranges of 10.1–12.2 g for dry nut weight,33.2–40.1mm nut size index and 41.2–51.1 % for kernel percentage were observed. The best accessions were Chamba Collection,Kinnaur Collection,Lahaul & Spiti Collection and Soghi Collection which had thinner shell, heavier kernel, light kernel, more fruitfulness and late flowering. The most promising trees will be used to the benefit of conservation research, breeding and cultivation.

Keywords: Walnut, Variability, morphological, phenologocal, horticultural traits, Promising trees

INTRODUCTION

The Juglans genus consists of 21 species and Persian walnut (*Juglans regia* L.) is the only species that widely cultivated for production of edible nuts 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 high density lipoprotein cholesterol to total cholesterol ratio, decreasing inflammation and improving arterial function (Patel 2005). It contains 'melatonin' an antioxidant produced by pineal gland and responsible for inducting and regulating sleeps. It also reduces the incidence of cancer and, delays neurodegenerative diseases of aging (McGranahan and Leslie 2012). Interestingly, the major share of walnut production comes from the trees of seedling origin, which has a lot of variability in nut size, shape, shell thickness and kernel quality (Berry 1997; Solar *et al.* 2002; Zeneli *et al.* 2005). Walnuts grown at higher elevations (above 1500 m) and monsoon free areas are generally superior in quality than those growing at lower elevation and high rainfall areas (Rouskas and Zakynthinos 2001; Rana *et al.* 2007). Breeding new walnut cultivars through hybridization is both difficult and time consuming. Therefore, it is 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 affects all leaves, leaf petioles, shoots,nuts and peduncles, and has been reported to infect several cultivars of Persian (J. regia) as well as black (J. nigra) walnuts severely (Coates 2012; Michailides *et al.* 2012).

Persian walnut has been probably domesticated in Afghanistan and Iran and then introduced to Eastern Europe, Russia and Indo-China (Bayazit *et al.* 2007). An ideal walnut cultivar must has late leafing, both terminal and lateral bearing, low incidence of pistillate flower abscission, high yielding nuts with large size, relatively smooth,50 % kernel recovery, plump and light colored kernel and at least moderately resistant to pest and diseases (Cosmulescu *et al.* 2010; Botu *et al.* 2010; McGranahan and Leslie 2012). Scientists from different parts of world have practiced simple selection in the natural seed populations and selected trees with high nut quality (Germain 1997; Sharma and Das 2003; Cosmulescu and Botu 2012). The aims of the current work were to evaluate phenotypic diversity of walnut trees of Himachal Prdesh in North Western Himalayan region, presents an interesting genetic resource; and to identify superior trees with late flowering date and high nut and kernel quality.

MATERIALS AND METHODS

The present study was conducted on 8 (eight) Persian walnut tree accessions selected from different parts of Himachal Pradesh, India. Trees were selected after evaluation on the basis of regular fruit production according to interviews with orchard owners and observed phenotypic diversity. The selected trees were healthy, mature and had a full crop. Diverse horticultural practices, fertiliser application, irrigation and other cultural practices were applied at regular intervals each year.

Phenotypic evaluation, Morphological characterization was performed according to walnut descriptor (IPGRI 1994). Kernel ratio was estimated using formulas "kernel weight/nut weight X 100".

Besides, agronomic traits evaluated included dates of leaf and flowering emergence, dichogamy and harvest date. Phenological and flowering traits included leafing (budbreak) date, female and male flowering date and period, dichogamy kind and flowering habit. The leafing (budbreak) date was recorded in early spring, when over 50 % of terminal buds had enlarged and bud scales had split to expose the inside green leaves (IPGRI 1994). Since phenological traits show variability due to environmental conditions (McGranahan and Forde 1985), observations on all of these characters were adjusted to the standard accession. According to this, the earliest leafing accession(s) was regarded as a control or reference standard and was rated as zero; for other trees, the number of days after the reference

tree was recorded. The accessions with having a minimum 6 days overlapping in their pistil receptivity and pollenshedding period; were regarded to be homogamous (Arzani *et al.* 2008). Moreover, the lateral bud flowering data were recorded according to the percentage of current season lateral shoots with female flowers. For recording harvest time, when nuts from the first accession were ripened, its date was scored as zero and then other trees were recorded on the bases of the number of days after the reference standard (Arzani *et al.* 2008).

Different characteristics were used to assess the range of variation among the accessions during two successive years (Table 1). Measurements of each nut and kernel trait were based on 30 replicates and the mean values were used. Some variables were measured by laboratory equipment. Nut dimensions (length and width) were measured using a digital Vernier caliper. The weight for nut and kernel was measured using electronic balance with 0.01 g precision. Also, some characteristics such as nut shape, kernel traits (filled, plumpness, shriveling and color), shell traits (color, seal, texture and hardiness), kernel removal from nuts and susceptibility to disease were determined based on rating.

RESULTS AND DISCUSSION

In India, the maximum walnut trees are grown on own roots i.e. seedling origin. The evaluations conducted for all the 8(eight) walnut selections showed that 4 of them are vigorous in tree size, 3 selections have medium vigor and only one selection has low vigor (Table 1). Phenological stages of the walnut selections have been recorded during the study period. Blooming of the female flowers occurred between March 31 and April 24. The male flowers started blooming in from March 20 and April 16. Walnut blooming is characterized by dichogamy. Protandrous selections proved dominant in (87.5 %), the rest being homogamous (12.5%). Ripening time of walnut fruits vary within the region. Walnut selections from the beginning of September till beginning of October (Table 2).

There were significant differences for bud break and flowering dates among the studied accessions. Late leaflng is an ideal character in walnut to escape the spring frost injury as shown by other walnut cultivars such as 'Chico', 'Serr', 'Ashley' and 'Sunland' (Barone *et al.* 1990) and the same also reported by Akca and Ozongun (2004).. Leaflng date in the selected trees ranged from 20 March to 30 March. Walnut cultivation is limited by low temperatures in early autumn and late spring at higher altitudes (Aslantas 2006). 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 and Das 2003). Date of flowering (both catkin and female flower) ranged from 20 March to 22 April. The dichogamy was not all the same in the selected trees. But, protandry was the most common among the studied trees. Most of walnuts have genetically protandry (Germain 1997). Ecological condition may effect on dichogamy in walnut (Sen 1998). A high variation of adaptation characters such as earliness and length of the flowering period and dichogamy offer the possibility of adapting the crop to diverse agro-ecological environments. Out of 8,7 accessions were terminal fruitfulness, so that all shoots from terminal generated female flowers and fruits. The one accession (Chamba Collection) was mixed and had terminal and lateral fruitfulness. Yield capacity is an important trait for economic walnut production. Productivity of walnut depends on flowering time and habit, fruit number on lateral and terminal shoots, lateral fruitfulness, nut and kernel weights, and kernel percentage (Hendricks *et al.* 1998).

Nut size is the determining factor for the market. Nut size index varied from 33.2 to 40.1 mm. Previous research (Cosmulescu and Botu 2012) in 109 walnut accessions of seedling origin growing

naturally in Oltenia region of Romania showed the diversity of the fruit: nut length (28.20–49.70 mm) and nut diameter (25.70–40.60 mm). Nut weight is one of the most common important parameters influencing the quality. Dry Nut weight varied from 10.1 to 12.2 g. The highest value of nut weight among the studied accessions here (12.2 g) was less than nut weight reported by Sen and Tekintas (1992) for walnuts in Adilcevaz, Turkey (23.81 g); and more than nut weight by Atefi(2001) for walnuts in Kamal-Abad, Iran (20 g); Sharma and Sharma (1998) for Himachal Pradesh, India (18.60 g); Yarilgac *et al.* (2001) for east Anatolia, Turkey (17.04 g); Aslantas (2006) for North-eastern Anatolia, Turkey (16.01 g) and Cosmulescu and Botu (2012) for Oltenia region, Romania (18.40 g). Weight of kernel ranged from 1.32 to 10.00 g. Desirable nut and kernel weight should range from 12 to 18 g and 6–10 g, respectively, or kernel weight should be at least 50 % of the entire nut weight, and the kernel should have a light color (Arzani *et al.* 2008). Percentage of kernel is a feature of great importance in setting the amount of selections and an important character for improvement. This character is related to nut and kernel weight. Accordingly, kernel percentage varied from 41.2 to 51.1%. The higher the kernel percentage, the lower the nut weight, while the ratio kernel/nut is higher and increases the value of the fruit. The highest kernel percentage (83.88 %) was higher than the data reported by Zeneli *et al.* (2005) (63.80 %), Aslantas (2006) (67.14 %), Arzani *et al.* (2008) (79.60 %) and Cosmulescu and Botu (2012) (71.70 %). Among all of the studied accessions, 204 accessions proved to be promising for new selections owing to higher kernel percentage i.e. [50%. Of them, accession no. 573 had the highest kernel percentage (83.88 %), followed by accessions no. 95 (71.82 %), 309 (68.91 %), 374 (68.91%) and 539 (68.75%). Walnut accessions with a kernel percentage higher than 48–50 % are more desirable (Germain 1997). It has been reported that fruit characteristics are not affected by tree age (Sharma and Sharma

Table 1
Descriptor of the qualitative characters utilized for the studied walnuts

Flowering habit	*Lateral*	*Mixed*	*Terminal*		
Dichogamy	Protandry	Homogamy	Protogyny		
Anthracnose susceptibility	Very low	Low	Intermediate	High	very high
Yield	Very low	Low	Intermediate	High	Very high
Nut shape	Round	Broadly ovate	ovate	Broad elliptic	Elliptic
Shell texture	Very smooth	Smooth	Intermediate	Rough	Very rough
Shell color	Very light	Light	Dark	Very dark	
Shell seal	Excellent seal	Slightly open	Intermediate	Wide	Very wide
Shell hardiness	Paper	Soft	Intermediate	Hard	Extremely hard
Shell surface serration	Low	Intermediate	High	Very high	
Ease of kernel removing from nuts Very easy	Easy	Intermediate	Difficult	Very difficult	
Kernel filled	Very low	Low	Intermediate	Filled	
Kernel plumpness	Very low	Low	Intermediate	Very plump	
Kernel shriveling	Slightly wrinkled	Intermediate	Wrinkled		
Kernel color	Very light	Light	Light amber	Amber	

2001). In the present study, kernel color in all accessions was very light to light. Generally, the most interesting accessions are those whose kernels can be easily removed from the shell and those whose kernels have a light color (Chamba Collection, Kinnaur Collection and Soghi Coolecton. These traits have been used for selection of superior walnut genotypes (Sharma and Sharma 2001; Yarilgac *et al.* 2001; Zeneli *et al.* 2005; Aslantas 2006; Arzani *et al.* 2008; Cosmulescu and Botu 2012).

Out of 8 walnut selections, 7 have terminal bearing type, while those of Chamba Collection have intermediate bearing. The fruit characteristics exhibit variability in both of the selection areas due to a range of morphological and biochemical elements. Fruit size and weight varies very much from one selection to another. Walnut fruit size is one of the main determinants for international trade (Table 3). Nut Size Index (N.S.I.) as an average of the widest fruit width (D), narrow width (d) and height (h) have

Table 2
Tree characteristics of walnut selections from Himachal Pradesh, India

No.	*Selection*	*Tree vigour*	*Fruit bearing type*	*Fruit yield*	*Resistance to Blight and Anthracnose*
1	Soghi Collection	Medium	Terminal	High	Less susceptible
2	Kullu Collection	Medium	Terminal	High	Less susceptible
3	Chamba Collection	Low	Mixed Lateral & Terminal	High	Less susceptible
4	Lahul & Spiti Collection	High	Terminal	High	Less susceptible
5	Kinnaur Collection	High	Terminal	Medium	Less susceptible
6	Kotkhai Collection	Medium	Terminal	Medium	Less susceptible
7	Shimla Collection	High	Terminal	High	Less susceptible
8	Rohru Collection	High	Terminal	Medium	Less susceptible

Table 3
Phenological characteristics of walnut selections from Himachal Pradesh, India

No.	*Selection*	*Bud break*	*Male flower blooming time*	*Female flower blooming time*	*Dichogamy type*	*Fruit ripening time*
1	Soghi Collection	Medium	29 March-8 April	6-11 April	Protandrous	10-20 September
2	Kullu Collection	Medium	29 March-7 April	6-12 April	Protandrous	20-30 September
3	Chamba Collection	Medium	27 March-11 April	31March-11 April	homogamous	01-15 September
4	Lahul & Spiti Collection	Very early	23 March-10 April	5 April - 10 April	Protandrous	15-30 September
5	Kinnaur Collection	Very early	20 March-31 March	5 April - 10 April	Protandrous	10-25 September
6	Kotkhai Collection	Late	8 April - 16 April	14-24 April	Protandrous	05-20 September
7	Shimla Collection	Very early	26 March - 3 April	10 April - 20 April	Protandrous	01-10 September
8	Rohru Collection	Medium	26 March - 5 April	12 April - 22 April	Protandrous	20 September-05 October

Table 4
Fruit characteristics of the walnut selections from Himachal Pradesh, India

No.	*Selection*	*Nut Size Index (mm)*	*Dry Nut weight (g)*	*Kernel ratio (%)*	*Fruit wt.(g)*	*Nut Shell Thickness (mm)*
1	Soghi Collection	34.8	10.1	48.0	42.05	1.13
2	Kullu Collection	34.3	10.5	45.2	42.12	1.29
3	Chamba Collection	35.1	12.2	51.1	43.72	1.20
4	Lahul& Spiti Collection	33.2	10.8	48.5	51.22	1.38
5	Kinnaur Collection	40.1	10.5	47.0	54.01	1.28
6	Kotkhai Collection	39.6	11.2	46.0	56.25	1.39
7	Shimla Collection	34.3	11.3	41.2	37.61	1.37
8	Rohru Collection	35.1	10.2	46.0	31.22	1.36

been used (Table 3). The good quality walnut fruits of 9.0 to 10.0 g weight can be used for shelled market while those exceeding 11 grams can be used also for in-shell market. Another important element for defining the walnut quality is the percentage of kernel. In case of selections, the percentage of kernel ranged from 41.2% to 51.1%. Of the 8 selections, only 1 (12.5) yields more than 50% kernel. Nut shell of the walnut selections is thin or medium (0.8 to 1.2 mm in thickness). Taking into account the growth and fruiting characteristics of the walnut genotypes Chamba Collection,Kinnaur Collection, Lahaul & Spiti Collection and Soghi Collection have been selected for further evaluation and direct use in the walnut farms, but only after clonal propagation.

CONCLUSIONS

The north-western Himalayan region particularlyHimachal Pradesh,India have important biodiversity of the Juglans regia L. species, wide variability, but increasingly subject to genetic erosion and genetic vulnerability pressure. The two areas have geographical and ecological characteristics differentiated between them and they have made their mark on the adaptability of walnut populations and selections. Chamba Collection,Kinnaur Collection, Lahaul & Spiti Collection and Soghi Collection have greater agro biological value similar to commercial cultivars and can be introduced, after clonal propagation, into the new walnut orchards established in the two areas. The best accessions were Chamba Collection,Kinnaur Collection,Lahaul & Spiti Collection and Soghi Collection which had thinner shell, heavier kernel, light kernel, more fruitfulness and late flowering. The most promising trees will be used to the benefit of conservation research, breeding and cultivation. More over, Chamba Collection could be used in high-density orcharding in walnut.

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