



Exploiting genetic diversity of walnut (*Juglans regia*) from Jammu region of India for export related traits

R M SHARMA¹, K KOUR², B SINGH³, N KOTWAL⁴, J C RANA⁵, N SHARMA⁶,
J D BANDRAL⁷ and M JAMWAL⁸

Regional Horticulture Research Sub-Station, Bhaderwah, SKUAST Jammu, Jammu and Kashmir

Received: 28 November 2013; Revised accepted: 9 May 2014

ABSTRACT

The present field work was done to study the existing genetic diversity and its exploitation through identifying elite walnut (*Juglans regia* L.) mother trees from the large number of naturally growing seedling trees in erstwhile Doda district of Jammu region of Jammu & Kashmir state. The field work was done from 2006 to 2012. A total of 63 walnut trees were marked as elite, out of 790 trees visited at 18 locations situated at 1500m-1750m above mean sea level. Out of total collections, 49.20% samples have attractive colour influencing the consumer acceptability. Five collections (BS1207, BT1807, MR0309, MR0509 and GL0109) had kernel recovery of >50%. Wide range of variability was recorded for various nut characters being highest in kernel recovery (18.54-61.40%) with highest coefficient of variation. Nut weight was positively correlated with nut size. The total variance in first principal component was due to nut size and weight while, in second component, it was contributed by kernel recovery. Further, analysis based on export quality traits (prescribed by J&K walnut grower association) lead to the identification of 8 most promising walnut clones, viz. BS1207, PK2707, MR0309, MR0509, MR0809, GL0109, PR0309 and NZ0112. Of these, GL0109 proved best in respect of export related traits and can be developed as a good table cultivar.

Key words : Clones, Genetic diversity, Nut and kernel characteristics, Walnut

Persian walnut (*Juglans regia* L.) belongs to family Juglandaceae, consisting of seven genera and about 60 species. The genus *Juglans* has 20 species and *Juglans regia* is most widely cultivated and economically important (McGranahan and Leslie 1990, Arzani *et al.* 2008). The distribution of Persian walnut ranges from the Carpathian mountains of Eastern Europe, all through Western Asia, the Himalayan region of Pakistan, India, Nepal, Bhutan and East China (Khan *et al.* 2010). Related species of *J. regia* are not found in India, but wild forms do exist throughout Himalayan region at an elevation of 1100 to 3000m (Rana *et al.* 2007a). The Western Himalayan region of India especially the dry region of states of Jammu & Kashmir and Himachal Pradesh produces excellent quality walnuts. This region alone contributes >98% of walnut production in India with an average productivity of 2.69 metric tonnes/ha. Presently, walnut is being cultivated on 83613.80 ha in state with an annual production of 224595.85 metric tonnes. In Jammu region, it occupies 36858.80 ha area and contributes 52044.85 metric tonnes production annually

(Anonymous 2012a). It exports about 5000 metric tonnes walnut kernel annually to the Europe and Asia, which is worth \$260-300 million. Besides, the domestic demands of kernel and in shelled nuts worth \$140-200 million/annum is also fulfilled (personnel communication J&K Walnut Exporters Association 2012) by this region. However, the nuts sold in the market invariably are mixtures of different nut size, shape, shell type (thin/hard) and kernel quality as these were collected from different seedling origin trees (McGranahan and Leslie 1990, Cosmulescu and Botu 2012). Being perennial, monoecious, wind pollinated and growing in a wide range of different soils and climatic conditions, walnut shows high variability in both pomological and phenological traits. Majority of the walnut grown worldwide is of seedling origin, therefore, depict high genetic variability in the nut, kernel and tree characters (Paunovic 1990, Solar 1990, Hemery 1998, Malvolti *et al.* 1993, Malvolti *et al.* 1996, Botu *et al.* 2001, Sharma and Sharma 2001, Akcedila and Sen 2001, Zeneli *et al.* 2004; Rana *et al.* 2007b, Ghasemi *et al.* 2012, Xu *et al.* 2012).

The walnuts found growing at 1500m altitude or above and free from monsoon are superior in quality compared with those found at lower altitudes and have high rainfall (Rana *et al.* 2007a, Verma *et al.* 2011). These walnut trees are important source of genetic diversity for *J. regia* presenting many opportunities for walnut breeding (McGranahan and Leslie 1990). Walnut selection has a

¹Principal Scientist (e mail: rmsharma345@gmail.com);
²Junior Scientist (e mail: kirranhort@gmail.com); ³Junior Scientist (e mail: brijeshbhau@yahoo.co.in); ⁴Junior Scientist (e mail: neerajkotwal@gmail.com); ⁵Head NBPGR (e mail: ranajc2003@yahoo.com); ⁶Assistant Professor, FS (e mail: nirmalsharma.77@rediffmail.com); ⁷Assistant Professor, FST(jdbandral@gmail.com); ⁸Assistant Professor, FS (drmahitaljamwal@gmail.com)

long history, and it was carried out by method of simple selection out of natural seedling populations having intended traits with high quality walnuts to assure the sustainability of walnut growers, and so far, this method is the basic one (Germain 1997, Sharma and Das 2003, Botu *et al.* 2010, Cosmulescu *et al.* 2010, Cosmulescu and Botu 2012). In India, though systematic exploration of walnut germplasm has been done in Kashmir, Uttarakhand and Himachal Pradesh (Rana *et al.* 2007a, Sharma and Sharma 2001, Verma *et al.* 2011), however, higher elevations of Jammu region (Doda, Ramban and Kishtwar) are still unexplored. We attempted the present study for around seven years to study the existing genetic variability and to select elite clones from the naturally occurring seedling trees for further commercialization.

MATERIALS AND METHODS

The field work was done for seven years (2006-2012) at 18 sites of Jammu region of Western Himalayan region. The sites, viz. Bhaderwah, Premnagar, Khelani, Bankut, Darshipura, Chapnari, Thalthar, Banihal, Kundal, Dharai, Nowgam, Hajwag, Quderana, Ashangam, Patsgam Yourdu, Nazzla, Sigdi, Sarkote and Galar were located between 1500m-1750m amsl. Initially, a total of 790 naturally growing seedling trees were marked and data on nut characters were recorded to select the elite clones. The samples (5 nuts/tree) were jointly inspected by the experts from J & K Walnut Exporters Association and Regional Horticultural Research Sub-Station, Bhaderwah. On the basis of nut data the samples of 727 trees were rejected and finally 63 samples were selected to collect larger sample size (50 nuts) to collect data as per walnut descriptor developed by National Bureau of Plant Genetic Resources, India (Mahajan *et al.* 2002) and export standards in order to find out the most promising walnut seeds for export related traits like appearance, shape, size and weight of nuts, kernel colour and recovery and overall acceptability in terms of numerical rating out of 10 (5 for nut appearance + 5 for kernel colour and taste) prescribed by J&K Walnut Export Association keeping in view the standards of international markets. These promising walnuts were analysed keeping in view the export related traits such as appearance, shape, size and weight of nuts, kernel colour, kernel recovery and overall acceptability. In order to quantify the genetic variability of walnut germplasm available in the area and character association between the traits, mean, range, coefficient of variation and correlation coefficient for nut characters were also determined. Coefficients of variability, heritability and genetic advance were calculated as per formulae given by Burton and De Vane (1953) and Johnson *et al.* (1955). The coefficients of correlation were computed by the method proposed by Al-Jibouri *et al.* (1958). To find out the most significant traits to establish genetic relationship among collected accessions, principal component analysis for nut size and nut weight was also done using SYSTAT-12 statistical software. Frequency distribution table for nut/kernel characters were prepared in order to identify the

dominance in particular characters and most prevalent ranges.

RESULTS AND DISCUSSION

In walnut, a nut and kernel character influences the nut quality and its marketability (Fig 1). The nut surface which is considered a primary character for enhancing nut attractiveness and the marketability was smooth for 46.03% clones followed by rough (28.57%), very smooth (14.29%) and very rough (11.11%). However, on the contrary Rana *et al.* (2007) reported smooth shell surface as 13% compared to rough (42%) and medium smooth (45%) in 182 walnut clones. Among different walnut shapes, round type was dominant (42.86%), while for other shapes such as ovate, elliptic and trapezoid, it ranged between 15.87 to 20.63%. Nut shell colour, which also influence the consumer acceptability of inshelled nuts dominated by very light and light coloured (49.20%) followed by medium (38.10%) and dark colour (12.70%). These cosmetic traits primarily show lot of marketing concern, however, preference may vary regionally but largely smooth and light coloured shells are preferred by the consumers. Apart from these cosmetic traits, kernel recovery and kernel colour have major influence in the international trade of shelled walnut. We observed kernel recovery percentage varied from 10 to 61.4% and good number of clones (39.68%) fall in the range of 40-50% kernel recovery. Five clones, viz. BS1207, BT1807, MR0309, MR0509 and GL0109 found most outstanding clones not only for nut traits but also showed kernel recovery >50%. A clone GL0109 had highest kernel recovery of 61.40% not only among the selected clones of present study but also among most prevalent varieties, viz. CITH walnut 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 in the region (Anonymous 2012b, Verma *et al.* 2011). The selected clones have all traits of an ideal kernel, i.e. easy to remove from the shell, uniform light in colour, clean, plump and weighs at least 50% of nut weight (McGranahan and Leslie 1990, Sunduri *et al.* 2005). In another study, Sundouri and Sharma (2005) recorded kernel recovery of 54.76% in Hamdam clone followed by Sulaiman (52.37%) and SKAU-W-0003 (50.94%). Extra light and light coloured kernels fetch higher returns as compared to light amber coloured kernels in the International market (Sundouri *et al.* 2005). Of the total collections, maximum number of accessions (52.38%) had >50% light coloured kernels including those 05 accessions (BS1207, BT1807, MR0309, MR0509 and GL0109) which were having >50% kernel recovery too. In the present study, 41.27% accessions were without light coloured kernel, however, only 6.34% accessions were found having 25-50% light coloured kernel. Only four accessions (BS2207, BK1307, BG0606 and BB2307) showed the higher proportion (>50%) of amber coloured kernel, while eight accessions (BR0407, BR0607, BR0707, BR0907, BK0807, BG1506 and BC2407) were found in the next best category (25-50%) as compared to rest of accessions studied. Majority of the clones were found free from any trace of brown (76.19%) and rancid (80.95%) colours. The numerical rating

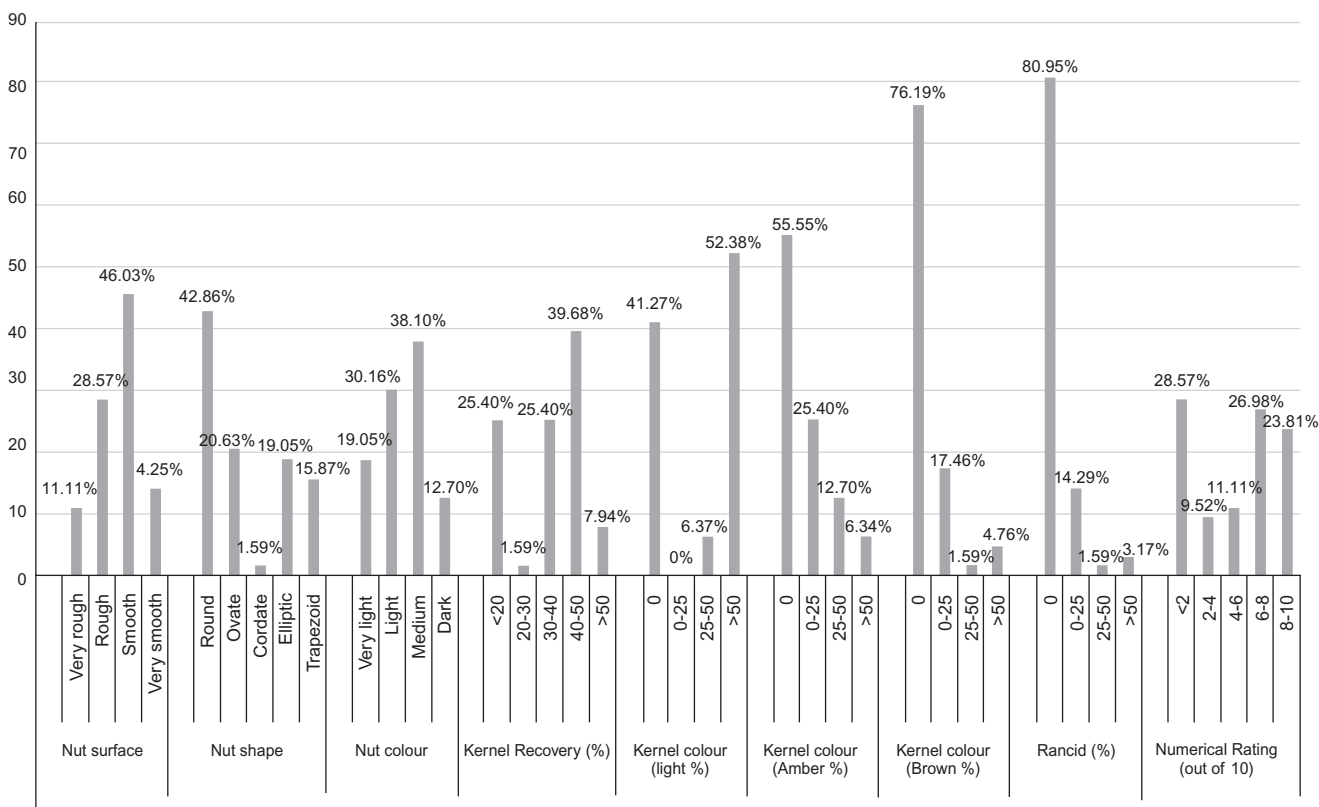


Fig. 1 Diagram showing distribution for various traits among walnut germplasm

done on 10 scale showed that 23.81% clones scored highest rating (8-10), followed by 26.98% clones, which had rating between 6-8, while 49.21% clones had numerical rating <6. Seven clones collected from Bhaderwah (BS1207), Padder (PK2707), Marwah (MR0309, MR0509 and MR0809), Premnagar (PR0309) and Nazzla Sigdi (NZ0112) had rating 9 out of 10, while one clone collected from Galar (GL0109) had rating 9.25/10 (Fig 2 & 4).

Wide range of variability was recorded on nut weight, nut length, nut diameter and kernel recovery and it varied from 7.70 - 20.10g, 3.13 - 4.83cm, 2.84-4.54cm and 18.54%-61.40%, respectively (Table 1). The coefficient of variation

was high for kernel recovery (35.43) followed by nut weight (22.45), nut length (11.15) and nut diameter (9.56). Similarly wide range of variation for nut length (2.82-4.97cm), nut diameter (2.57- 4.06cm) and nut weight (6.8-18.4g) were also observed in 109 walnut genotypes of seedling origin by Cosmulescu and Botu (2012) in Oltenia region of Romania, while range was 6.33-16.89g for nut weight, 27.94-42.20 mm for nut length, 26.62-35.98 mm for nut width and 31.76-60.51% for kernel recovery in 70 seedling genotypes in Iran (Ghasemi *et al.* 2012). In the present study, heritability (broad sense) was high for nut weight (83.72) followed by kernel recovery (81.32), nut length (79.56) and nut diameter (70.43). Genetic advance expressed

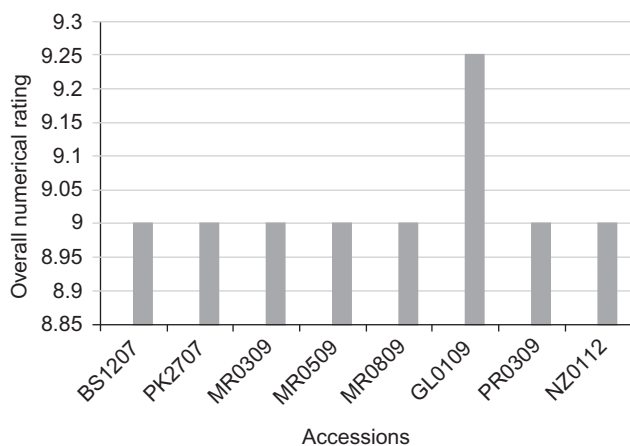


Fig 2 Overall numerical rating (out of 10) of most promising walnut accessions

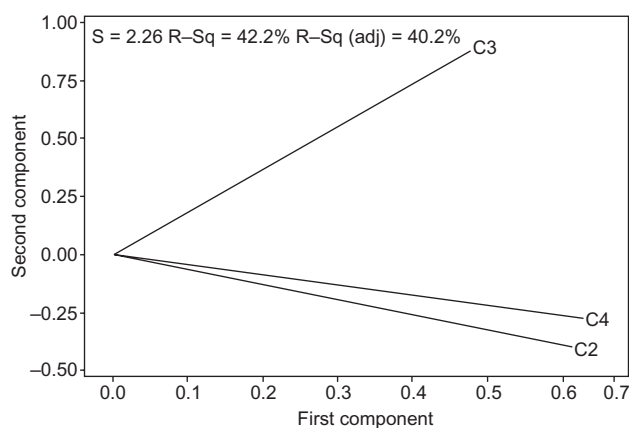


Fig 3 Biplot of first two components in PCA

Table 1 Extent of variation, heritability and genetic advance among walnut genotypes for different nut characters

	Range	Coefficient of variation	Heritability (Broad sense)	Genetic advance
Nut weight (g)	7.70–20.10	22.45	83.72	38.82
Nut length (cm)	3.13–4.83	11.15	79.56	34.25
Nut diameter (cm)	2.84–4.54	9.56	70.43	31.98
Kernel recovery (%)	18.54–61.40	35.43	81.32	42.56

as per cent of mean was high for kernel recovery (42.57), nut weight (38.82), nut length (34.25) and nut diameter (31.98). Since range and coefficient of variation was high for kernel recovery and nut weight hence, the selection for the trait could be done in the walnut accessions to select heavier nut. Nut weight, although an easily measured character, varies with the extent of drying, and is also influenced by shell thickness and degree of filling, and therefore, not entirely a reliable indicator of kernel weight (Rana *et al.* 2007a) hence, it must be measured in conjunction with the kernel percentage which is fairly high in the present study. The high range of variability coupled with high

Table 2 Correlation coefficient for different nut characters among walnut genotypes

Characters	Nut weight (g)	Nut length (cm)	Nut diameter (cm)	Kernel recovery (%)
Nut weight (g)	1.00			
Nut length (cm)	0.33**	1.00		
Nut diameter (cm)	0.64**	0.38**	1.00	
Kernel recovery (%)	0.18	0.015	0.11	1.00

**Significant at 1% level of significance

values of heritability and genetic advance indicate good scope for practicing simple selection methods to improve walnut seedlings (Rana *et al.* 2007b).

Nut weight showed significant positive correlation (Table 2) with nut length (0.33) and nut diameter (0.64) 1 per cent level of significance. Similarly nut length exhibited significant correlation coefficient (0.38) with nut diameter at 1 per cent level of significance. Significant positive correlations among nut characters have been reported in other walnut populations in the Indian Himalayan region (Sharma and Sharma 2001 , Rana *et al.* 2007). In other studies, Arzani *et al.* (2008) found significant positive correlation of nut weight with nut length (0.57), nut width (0.68), nut thickness (0.67), kernel weight (0.75) and shell thickness (0.32). No significant correlation was observed in nut weight and kernel recovery in the present studies. Similarly Ghasemi *et al.* (2012) also observed that nut weight has significant positive correlations with nut diameter (0.706) and nut length (0.485), however, they observed non-significant association between nut weight and kernel recovery, indicating that nut weight alone may not give clear picture of kernel recovery.

Principal component analysis is used identify most significant traits in the data set and to establish genetic relationship among clones in the germplasm. The inference drawn from principle component analysis (Table 3 & Fig 3) may correspond to genetic linkage between loci controlling the traits (Iezzoni 2008 , Rakonjac *et al.* 2010). The correlation matrix revealed two principal components with Eigen value >1 and accounted for 75.14% of the total variation. First principal component explained 49.50% of the total variance and was mainly loaded on nut weight, nut diameter and nut length, whereas second component accounted for 25.64% of the total variance, and it was mainly contributed by kernel recovery.

Wide range of genetic variability was observed in the seedling origin walnut growing in the Western Himalayan region of India. This offers greater scope for walnut

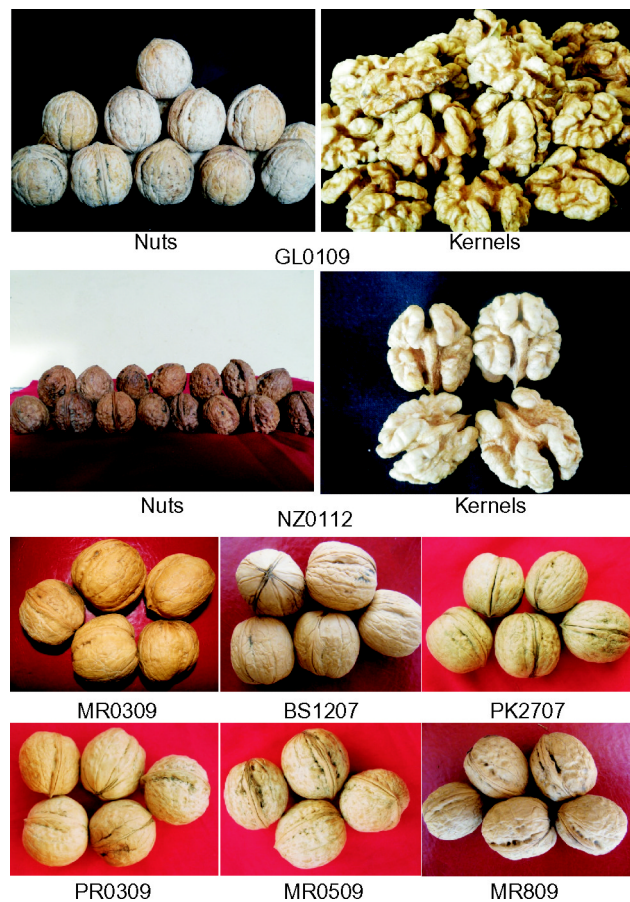


Fig 4 Promising selections

Table 3 Principal component analysis (PCA) of walnut genotypes

Character	PC1	PC2
Nut weight	0.85	0.34
Nut diameter	0.66	0.75
Nut length	0.87	0.24
Percent of total variation	63.81	24.37

improvement. The clones rated as most outstanding clones in the present work would be of immense use not only to develop variety *per se* but will serve as useful donors in the walnut breeding programmes. One best clone (GL0109) has been established at various locations (government and private farms) after multiplication for its large scale promotion to bring more and more area under quality planting materials.

REFERENCES

- Akcedila Y and Sen S M. 2001. Study on the selection of superior walnut trees in Hizan (Bitlis) populations. *Acta Horticulturae* **544**: 115–8.
- Al-Jibouri H A, Miller P A and Robinson H F. 1958. Genotypic and environmental variance in an upland cotton crosses of interspecific origin. *Agronomy Journal* **50**: 633–7
- Anonymous 2012a. *Area and Production Database of Fruit Crops*. State Department of Horticulture, Jammu, Government of Jammu and Kashmir, India
- Anonymous 2012b. New Horizons. *Indian Journal of Horticulture* **69** (2): iv–vi.
- Arzani K, Mansouri Ardakan H, Ali V and Reza R M. 2008. Morphological variation among Persian walnut (*Juglans regia* L.) genotypes from central Iran. *New Zealand Journal of Crop and Horticulture* **36**: 159–68.
- Botu M, Botu I, Achim G H and Godeanu I. 2001. Genetic variability of the *Juglans regia* L. natural populations from Oltenia–Romania. *Acta Horticulturae* **544**: 149–54.
- Botu M, Tudor M, Botu I, Cosmulescu S and Papachatzis A. 2010. Evaluation of walnut cultivars in the conditions of the Oltenia's hill area regarding functioning potential. *Analele Universitatii din Craiova, Biologie, Horticultura, Tehnologie prelucrarii produselor agricole, Ingineria mediului* **15**: 94–103.
- Burton G W and Devane E H. 1953. Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal* **45**: 478–81.
- Cosmulescu S and Botu M. 2012. Walnut biodiversity in south-western Romania Resource for perspective cultivars. *Pakistan Journal of Botany* **44** (1): 307–11.
- Cosmulescu S, Botu M and Trandafir I. 2010. Mineral composition and physical characteristics of walnut (*Juglans regia* L.) cultivars originating in Romania. *Selçuk Tarým ve Gýda Bilimleri Dergisi* **24**(4): 33–7.
- Germain E. 1997. Genetic improvement of the Persian walnut (*Juglans regia* L.). *Acta Horticulturae* **442**: 21–32.
- Ghasemi M, Arzani K and Hassani D. 2012. Evaluation and identification of walnut (*Juglans regia* L.) genotypes in Markazi province of Iran. *Crop Breeding Journal* **2**(2): 119–24.
- Hemery G 1998. Walnut (*Juglans regia* L.) seed-collecting expedition to Kyrgyzstan in Central Asia. *Journal of Forestry* **92** (2): 153–7.
- Iezzoni AF 2008. Cherries. *Temperate Fruit Crop Breeding*, pp 151–75. Hancock J F (ed.). Springer, Berlin.
- Johnson H W, Robinson H P and Comstoc R E. 1955. Estimation of genetic and environmental variability in soybeans. *Agronomy Journal* **47**: 314–8.
- Khan M W, Ahmad I A, Ali H, Haidar G S, Afzal M, Khan F A, Shah M and Afridi S G. 2010. Estimation of genetic diversity in walnut. *Pakistan Journal of Botany* **42**(3): 1 791–6
- Mahajan R K, Gangopadhyay K K, Gunjeet K, Dhobhal V K, Srivastava U, Gupta P N and Pareek S K. 2002. *Minimal Descriptors of Agri-Horticultural Crops Part III: Fruit Crops*. NBPGR, Pusa Campus, New Delhi.
- McGranahan G H and Leslie C. 1990. Walnuts (*Juglans*). *Acta Horticulturae* **290**: 905–51.
- Malvolti M E, Beritognolo I and Spada M. 1996. Diversita' genetica in *Juglans regia*, valutazione delle risorse genetiche in Europa per uno studio agricolo e forestale sostenibile – Sherwood. *Foreste ed Alberi Oggi* **15**: 11–7.
- Malvolti M E, Paciucci M, Cannata F and Fineschi S. 1993. Genetic variation in Italian populations of *Juglans regia* L. *Acta Horticulturae* **311**: 86–94.
- Paunovic S A. 1990. The walnut cultivars selected from indigenous population of *Juglans regia* L. in SR Serbia, SFR Yugoslavia. *Acta Horticulturae* **284**: 135–42.
- Rakonjac V, Fotiric AM, Nikolic D, Milatovic D and Colic S. 2010. Morphological characterization of 'Oblacinska' sour cherry by multivariate analysis. *Scientia Horticulturae* **125**: 679–84.
- Rana J C, Pradheep K, Verma V D. 2007a. Naturally occurring wild relatives of temperate fruits in Western Himalayan region of India: an analysis. *Biodiversity and Conservation* **16**: 3 963–91.
- Rana J C, Singh D, Yadav S K, Verma M K, Kumar K and Pradheep K. 2007b. Genetic diversity collected and observed in Persian walnut (*Juglas regia* L.) in the Western Himalayan region of India. *Plant Genetic Resources News Letter* **151**: 68–73.
- Sharma, A K and Das B. 2003. Genetic variation study on nut and kernel characters of walnut seedlings. *Progressive Horticulture* **35**: 11–3.
- Sharma O C and Sharma S D 2001. Correlation between nut and kernel characters of Persian walnut seedling trees of Garsa valley in Kullu district of Himachal Pradesh. *Acta Horticulturae* **544**: 129–32.
- Solar A. 1990. Phenological and pomological characteristics of walnut cultivars in northeastern Slovenia. *Acta Horticulturae* **284**: 67–174.
- Sundouri A S, Sharma A K and Mir M A. 2005. Performance of some indigenous walnut selections compared to exotic cultivars. *Environment and Ecology* **23**(Spl.3): 441–4.
- Sundouri A S and Sharma A K. 2005. Pomological characterization of some walnut selections. *Horticulture Journal* **18**(3): 157–60.
- Verma M K, Ahmad N, Sofi A A, Verma R K, Mir J I, Singh D B, Kumar S, Singh D, Srivastava S R, Bhat K K, Pandit H A, Sheikh M A and Shah S. 2011. High yielding of better quality walnut released. www.scribd.com.
- Xu Zheng, Tingxing Hu and Fan Zhang. 2012. Genetic diversity of walnut revealed by AFLP and RADP markers. *Journal of Agricultural Science* **4**(7): 271–6.
- Zeneli G, Kola H and Dida M. 2004. Phenotypic variation in native walnut populations of Northern Albania. *Scientia Horticulturae* **105**(1): 91–100.