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Genetic variability, correlation and path-coefficient studies among Persian walnut (*Juglans regia*) genotypes

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ABSTRACT

The present study was conducted during 2015 and 2016 at ICAR-CITH, Rangreth, Srinagar to corroborate genetic variability among walnut genotypes and determine the potential traits for selection of superior genotypes. Genetic variability in any crop is a pre-requisite to initiate the breeding programme for the selection of superior entries over the existing cultivars. The efficiency of selection largely depends upon the magnitude of the genetic variability present in the plant population. Thus, large genetic variability offers better scope for the crop improvement. The experimental material comprised 136 walnut genotypes. Nut samples of each genotype were collected randomly in five replications and each replication consisted of five nuts. Quantitative traits were recorded as per walnut descriptors developed by IPGRI. The highly significant differences ($P = 0.01$) existed between genotypes for the nut and kernel traits studied. The range of some economically important traits varied widely for nut weight (6.08–24.23 g), kernel weight (3.30–11.16 g), and kernel recovery (36.27–60.19 %). The estimates of GCV and PCV were very high for nut weight (25.07, 26.05 respectively) and kernel weight (23.59, 25.35 respectively) indicating the importance of these traits in selection of superior genotypes. Trait association analysis also revealed mutual relationships and degree of interrelationships among all the nut and kernel traits. Thus, ample genetic variability observed among the studied genotypes for nut and kernel traits indicated effectiveness of direct selection for improvement of these commercially important traits.

Key words: Genotypes, Heritability, *Juglans*, Quantitative traits, Selection

Persian walnut (*Juglans regia* L., 2n=32) belongs to family Juglandaceae. It is an economically important species cultivated worldwide for its nutritious kernel and timber quality wood. Walnut kernels generally contain about 60-70% fat. Walnut fat is rich in omega-3 fatty acid called alpha-linolenic acid (ALA) which makes up around 8–14% of the total fat content (Pereira *et al.* 2008).

In India, the North-Western Himalayan (NWH) region is well known for walnut production and has maximum variability for the species (Rana *et al*. 2007) because of the area being a part of center of origin of walnut. Genetic variability is the backbone of any crop improvement program and the effectiveness of selection depends upon the nature and magnitude of genetic variability in the genetic material at the disposal of plant breeders (Singh *et al.* 2012). Genetic variability for nut and kernel characteristics in walnut has been reported in different regions/states by various researchers in India (Sharma *et al*. 2010, Verma *et al*. 2014, Dogra *et al*. 2018, Shamlu *et al*. 2018). But the majority of these studies were based on region specific collections. The Indian Council of Agricultural Research's Central Institute of Temperate Horticulture (ICAR-CITH), Srinagar made a large collection of indigenous walnut genotypes from various walnut growing pockets of the NWH region. This collection presented enough possibilities for utilization of genetic variability to identify yield contributing quantitative traits. The approaches of correlation and path analysis provide information regarding the nature and magnitude of genetic variation present in quantitative traits to make significant improvement in any crop. Trait association analysis gives a clear picture of interrelationships and relative contribution of independent characters on dependent variables, which enables a breeder to make selection procedures for crop improvement. Thus, present study was conducted with the objective to corroborate genetic variability among walnut genotypes and determine the potential traits for selection of superior genotypes.

MATERIALS AND METHODS

The present study was conducted at ICAR-CITH, Rangreth, Srinagar during 2015 and 2016. The experimental site is situated at 33°58'N latitude, 74°48'E longitude and at an altitude of 1643 m amsl. The soil type was alluvial. The climate of Kashmir valley is humid temperate. The average annual rainfall was 710 mm and the average summer

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*Corresponding author email: godara.lal2009@gmail.com temperature was 24.1°C and winter temperature was 2.5°C. The experimental material comprised 136 walnut genotypes. All the genotypes were grafted on *J. regia* seedlings and planted at a spacing of 7 m × 7 m in triplicates using randomized block design at walnut germplasm block of the institute and managed as per advocated package of practices. All the selected genotypes were in age group of 14-15 years. Fruits were harvested when hull started splitting and few nuts dropped naturally from the tree. Hull was removed immediately after harvest and dehulled nuts were sun dried. Nut samples of each genotype were collected randomly in five replications and each replication consisted of five nuts. Measurements of nut and kernel traits were performed one month after drying, during that time nuts were kept at room temperature. Observations on seven quantitative traits, *i.e.* nut weight (g), nut thickness (mm), nut diameter (mm), nut length (mm), shell thickness (mm), kernel weight (g) and kernel recovery (%) were recorded as per walnut descriptors developed by International Plant Genetic Resource Institute (IPGRI 1994). The data recorded on above mentioned traits were statistically analyzed using the Windostat version 9.2 package program. The genetic estimates, viz. phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic gain were computed. The correlation coefficient analysis among all the possible combination at phenotypic (rp) and genotypic (rg) level and estimates of direct and indirect effect of component characters on kernel weight were estimated using standard methods.

RESULTS AND DISCUSSION

Analysis of variance revealed that highly significant difference (P= 0.01) existed among genotypes for the nut and kernel traits studied. The estimates of range, population mean, variance and genetic parameters, viz. phenotypic, genotypic and environmental coefficient of variation, heritability (broad sense) and genetic advance for nut and kernel traits are presented in Table 1. The range of some economically important traits varied widely for nut weight (6.08-24.23 g), kernel weight (3.30-11.16 g), and kernel recovery (36.27-60.19 %) among studied genotypes. Ahadani *et al.* (2014) reported nut weight between 10.10-13.60 g, kernel weight between 4.94-7.50 g and kernel recovery between 48-59% in walnut genotypes from Northern Iran. Also, large differences were observed for general coefficient of variation (2.53-9.28%) for all the traits studied. The presence of wide range of variation in nut and kernel traits indicates divergent nature of genotypes under study. The results are in conformity with findings of Sharma *et al.* (2010), Mosivand *et al.*(2013), Khadivi-Khub *et al.* (2015) and Hussain *et al.* (2016) with respect to nut weight, kernel weight and kernel recovery reported from various countries.

The estimates of genotypic and phenotypic variances were highest in kernel recovery and nut length; moderate in nut weight, nut thickness and nut diameter and low in shell thickness and kernel weight (Table 1). The high value of genotypic variance indicated more contribution of genetic components for the total observable variation. Therefore, traits with high genotypic variance could be considered and exploited for selection, whereas high phenotypic variance indicated strong influence of environmental factors on trait expression during crop growth. The difference between phenotypic and genotypic coefficient of variation (PCV and GCV) was found to be narrow for all traits suggesting least influence of environment on trait expression. Further, contribution of these traits to the total variability across the genotypes indicated the greater contribution of additive gene action on the expression of these traits (Verma *et al.* 2014). High PCV and GCV for nut and kernel weight pointed towards importance of these traits in the total variability. These results indicated that traits with higher magnitudes of coefficient of variation offer a better opportunity for improvement through selection. The nature and extent of genetic variability is one of the most important criteria in formulating an efficient breeding programme. Similarly, knowledge of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) is much helpful in predicting the amount of variation present in a given genetic stock. Therefore, these traits could be considered and exploited for selection purpose.

The estimates of heritability (%) in the broad sense for all the traits was high. The high heritability indicates that the traits were less influenced by environmental factors and a major part of the phenotypic variability in these traits was contributed by the additive gene action. Hence, such traits can be improved by simple selection. The high heritability coupled with high genetic gain was observed for nut weight, nut diameter, nut length, shell thickness and kernel weight. The high heritability with moderate genetic gain was recorded for nut thickness and kernel recovery. Dogra *et al.* (2018) observed high heritability, moderate genetic gain for nut length and kernel weight; high heritability and low genetic gain for nut weight and moderate heritability and low genetic gain for shell thickness and kernel percentage. Earlier studies of Johnson *et al.* (1955) have proved that heritability estimates along with genetic gain is more useful than heritability alone in predicting the resultant effects of selection. It would be worthwhile to resort to breeding methodologies other than conventional pedigree or backcross techniques as these would leave the non-fixable component unexploited. Hence, improvement of nut and kernel traits would be effective through phenotypic selection.

Correlation at genotypic and phenotypic level was worked out among 136 Persian walnut genotypes for all studied traits to know the nature of association existing among the individual traits (Table 2). Correlation coefficient between different traits of walnut genotypes included in the study revealed significant correlations among nut and kernel traits. A highly significant and positive association at genotypic and phenotypic level was found between kernel recovery and kernel weight. However, negative but significant association was found with nut weight, nut thickness, nut length, nut diameter and shell thickness.

Table 1 Genetic parameters for nut and kernel traits in walnut genotypes

Trait	Mean ± SE	Range		Variance			Coefficient of variability				H^2 (Broad sense) (%)	Genetic advance (GA)	GA as % of means
		Minimum	Maximum	PV	GV	EV	General CV (%)	PCV (%)	GCV (%)	ECV (%)			
Nut weight (g)	13.64±0.431	6.08	24.23	12.62	11.69	0.93	7.07	26.05	25.07	7.07	93.00	6.78	49.71
Nut thickness (mm)	33.19±0.375	26.87	41.64	11.20	10.50	0.70	2.53	10.08	9.76	2.53	94.00	6.46	19.47
Nut length (mm)	39.62±0.549	29.70	53.36	28.54	27.03	1.50	3.10	13.48	13.12	3.10	95.00	10.42	26.31
Nut diameter (mm)	34.57±0.429	26.17	43.97	14.51	13.59	0.92	2.77	11.02	10.66	2.77	94.00	7.35	21.26
Shell thickness (mm)	1.71±0.045	1.01	2.36	0.10	0.09	0.01	5.85	18.56	17.62	5.85	90.00	0.59	34.45
Kernel weight (g)	6.62±0.275	3.30	11.16	2.82	2.44	0.38	9.28	25.35	23.59	9.28	87.00	2.99	45.22
Kernel recovery (%)	48.89±1.112	36.27	60.19	29.16	22.98	6.18	5.08	11.04	9.80	5.08	79.00	8.77	17.93

Nut weight had significant positive correlation with nut thickness, nut length, nut diameter and shell thickness, whereas a negative correlation was determined between nut weight and kernel recovery. Similarly, nut thickness, nut length, nut diameter, shell thickness observed significant positive correlation among them and a negative correlation with kernel recovery. Nut and kernel traits exhibited highly significant association with kernel weight while shell thickness and kernel recovery shows poor association with kernel weight. This indicated that kernel recovery is negatively correlated with all the traits under study as it is highly influenced by genotype, environment and orchard practices. The significant and positive correlation found between nut weight and kernel weight indicates that bigger nuts can yield more kernel on a dry weight basis. In the walnut breeding program, increasing the proportion of kernel weight in nut weight is a priority. Kernel content is highly influenced by thickness, length, diameter and weight of nut. Eskandari *et al*. (2005) and Cosmulescu and Botu (2012) found that there was a positive correlation between kernel weight and nut weight. Arzani *et al*. (2008) found that nut weight showed positive correlations with nut length, nut width, shell thickness, and kernel weight. Similarly, other workers (Amiri 2010, Mosivand *et al.* 2013, Ahadani *et al.* 2014, Khadivi-Khub *et al*. 2015, Hussain *et al.* 2016, Dogra *et al*. 2018) also reported significant positive and negative correlation between nut and kernel traits.

A negative correlation has been observed between shell thickness and kernel recovery. It revealed that an increase in the shell thickness leads to reduction in kernel recovery. Dogra *et al.* (2018) found negative correlation between shell thickness and kernel recovery. Kernel recovery showed significant and positive correlations with kernel weight which is in agreement with findings of others (Arzani *et al*. 2008, Ebrahimi *et al*. 2011, Cosmulescu and Botu 2012 and Khadivi-Khub *et al*. 2015).

The value of genotypic correlation coefficient is higher than phenotypic correlation coefficient which indicated that the strong association among the traits is genetic. Knowledge of the relationship between nut and kernel traits and other tree traits can guide appropriate selection schemes for walnut breeding programs. Besides, positive correlation among different traits shows that an improvement of one character can improve the other desired character (Yucel *et al*. 2009). For instance, positive correlation among nut and kernel traits shows that an improvement in kernel quality can improve qualitative yield in walnut.

The correlation coefficient measures the relationship existing between pairs of traits. But a dependent trait is an interaction product of many mutually associated component traits and a change in any one component will disturb whole network of cause and effect system. Path coefficient analysis provides an effective means of partitioning direct and indirect causes of association. The result of path analysis gives relative contribution of different traits towards kernel weight. By partitioning the phenotypic and genotypic correlations, the direct effect of a chosen trait on kernel weight and its

Table 2 Estimate of genotypic and phenotypic correlation among nut and kernel traits in walnut genotypes

Trait		Nut thickness (mm)	Nut length (mm)	Nut diameter (mm)	Shell thickness (mm)	Kernel weight (g)	Kernel recovery (%)
Nut weight (g)	G	0.8509**	0.7187**	0.8393**	0.6094**	0.9171**	-0.3128**
	P	0.8087**	0.6829**	0.7927**	0.5677**	0.9023**	-0.2498**
Nut thickness (mm)	G		0.5602**	0.9313**	0.3085**	0.8592**	-0.0744**
	P		0.5578**	0.9168**	0.2822**	0.7868**	-0.0674**
Nut length (mm)	G			0.4878**	0.3430**	0.6575**	-0.2409**
	P			0.4896**	0.3192**	0.6065**	-0.2093**
Nut diameter (mm)	G				0.3456**	0.8491**	-0.0695**
	P				0.3188**	0.7772**	-0.0604**
Shell thickness (mm)	G					0.3556**	-0.7016**
	P					0.3159**	-0.6186**
Kernel weight (g)	G						0.0834**
	P						0.1647**

indirect effect through other traits were computed and are presented in Table 3.

Nut weight had a very high positive direct genotypic effect on kernel weight followed by kernel recovery, shell thickness, nut length, nut thickness and nut diameter (Fig 1). Nut weight, nut thickness, nut length, nut diameter and shell thickness had a positive indirect effect and only kernel recovery had negative indirect effect on kernel weight. Islam *et al.* (2005) in hazelnut and Amiri *et al*. (2010) in walnut reported a positive direct effect of kernel recovery on kernel weight which was confirmed through our results. However, a negative direct effect was shown by shell thickness on kernel recovery.

The direct effect of kernel recovery, nut diameter, nut thickness, nut length and shell thickness on kernel weight was relatively low (Fig 1) and it was found to affect kernel weight indirectly through nut weight. As a result, increase in nut weight increased the kernel weight. But it is not necessary that larger size nuts will produce well filled kernels as kernel weight is influenced by shell thickness, length, diameter and degree of fill (McGranahan and Lesile 1991). Amiri *et al.* (2010) also mentioned shell thickness as one of the major factors acting upon walnut kernel percentage. Among the studied traits, nut weight was found to have most significant indirect effect at genotypic and phenotypic level (2.7062 and 2.5560).

Based on genetic variability and trait association studies (correlation and path analysis), it could be concluded that, nut weight exhibited maximum positive direct effect on kernel weight and could be relied upon for selection of genotypes to improve kernel weight of walnut. Hence, utmost importance should be given to these traits during selection for kernel weight. Selection of walnut genotypes on the basis of these traits would certainly lead to improvement

Table 3 Direct (diagonal) and indirect effects of component characters contributing to kernel weight in walnut genotypes

Trait		Nut weight (g)	Nut thickness (mm)	Nut length (mm)	Nut diameter (mm)	Shell thickness (mm)	Kernel recovery (%)	Total indirect effect
Nut weight (g)	G	**1.0002**	0.8511	0.7189	0.8395	0.6096	-0.3129	**2.7062**
	P	**0.9822**	0.7943	0.6708	0.7786	0.5576	-0.2453	**2.5560**
Nut thickness (mm)	G	0.0101	**0.0118**	0.0066	0.0110	0.0036	-0.0009	**0.0304**
	P	0.0143	**-0.0177**	0.0099	0.0162	-0.0050	0.0012	**0.0366**
Nut length (mm)	G	0.0143	0.0111	**0.0198**	0.0097	0.0068	-0.0048	**0.0371**
	P	0.0111	0.0091	**0.0162**	0.0079	0.0052	-0.0034	**0.0299**
Nut diameter (mm)	G	0.0067	0.0075	0.0039	**0.0080**	0.0028	-0.0006	**0.0203**
	P	0.0235	0.0272	0.0145	**0.0296**	0.0095	-0.0018	**0.0729**
Shell thickness (mm)	G	0.0183	0.0092	0.0103	0.0104	**0.0300**	-0.0210	**0.0272**
	P	0.0044	0.0022	0.0025	0.0025	**0.0078**	-0.0048	**0.0068**
Kernel recovery (%)	G	-0.1325	-0.0315	-0.1020	-0.0295	-0.2971	**0.4235**	**-0.5926**
	P	-0.1046	-0.0282	-0.0877	-0.0253	-0.2591	**0.4188**	-0.5049

G: R Square = 0.9933 Residual effect = 0.0820 P: R Square = 0.9766 Residual effect = 0.1529

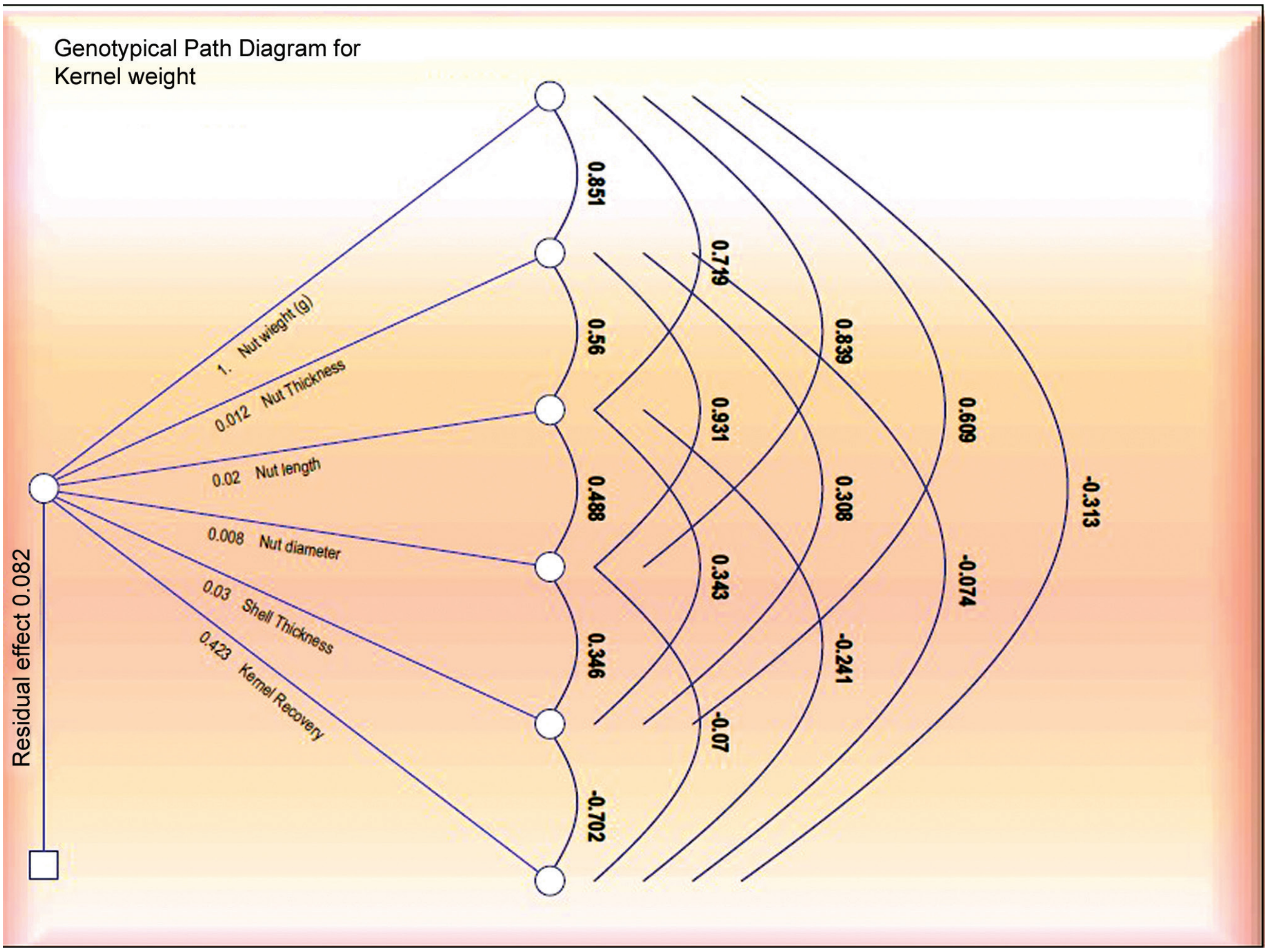

Fig 1 Genotypic path diagram for kernel weight of walnut genotypes.

in kernel weight. Furthermore, high genetic variability for studied traits indicated that this germplasm includes rich and valuable plant material for future walnut hybridization programme.

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