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Morphological characterization of walnut genotypes of diverse origin

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ABSTRACT

Walnut (*Juglans regia* L.) is a major nut crop of temperate region and the existing germplasm available in the country is of seedling origin, thus, contributing towards the large variability in this crop. Therefore, a research study was carried out ICAR-CITH, Srinagar to characterized and decipher the genetic variability among 27 genotypes of Indian walnut (*Juglans regia* L.) based on morphological characters, *viz*., growth habit, bearing habit, foliage, fruit and kernel characteristics for further improvement, conservation and utilization. The Erect growth habit was noticed in genotype, *viz.,* CITH-W-12, while semi erect growth habit was noticed in majority of the genotypes. Three types of leaf shapes were recorded *i,e* narrow elliptic, elliptic, and broad elliptic and based on leaf characteristics all the genotypes could also be categorized *viz.* pubescence as glabrous, slightly pubescent and pubescent. The genotype was categorized into early, mid and late group based on their fruit maturity duration. High variability was also recorded for fruit shape *viz*, round, cordate, ovate, long trapezoid, and elliptic. The current findings clearly characterized each genotype and can be identified or grouped individually based on this descriptor. Present study provides the detailed morphological descriptor of walnut which can be utilised for DUS testing of walnut, varietal identification, characterization, registration, documentation *etc*. The database generated may be useful for comparison against the candidate varieties developed in future

Key words: *Juglans regia*, DUS descriptor, Kernel, Nut.

INTRODUCTION

Walnuts are members of the family Juglandaceae and genus *Juglans* L. containing about 60 species, 21 of which are placed in the genus Juglans. Walnut is a monoecious species that is pollinated by the wind (Westwood, 13). The most commonly grown tree for nuts is the English or Persian Walnut, (*Juglans regia* L.) as it is rich source of energy, protein, fibre, minerals, anti-oxidants and vitamins which are essential for optimum health. This alpha-linolenic acid has substantial cardio protective effects as it surges the ratio of high-density lipoprotein cholesterol to total cholesterol, thereby, plummeting the inflammation and mending arterial function. It is grown mainly in Jammu & Kashmir, Himachal Pradesh, Uttarakhand and Arunachal Pradesh. However Jammu & Kashmir is the major walnut producing state contributing 80.58% of total area and 91.16% total production of the country. The most important walnut growing districts in Kashmir are Anantnag, Pulwama, Kupwara, Budgam, Baramulla and Srinagar, while in Jammu region Doda, Kistwar, Poonch, Udhampur are major region whereas minor plantation also exist in Rajouri and Kathua. Walnut germplasm has been extensively used in the selection studies for producing the superior walnut clones (Botu *et al.,* 2) .A wide range of variability exists in walnut in India for all important economic characters suggesting substantial scope for improvement. Varietal identification has attained critical importance in view of the intellectual property rights (IPR) regime enforced in the country as per trade related aspects of intellectual property rights (TRIPS) agreements under WTO to protect plant breeders and farmer's rights. The UPOV convention provides DUS testing of crop varieties, and has been adopted worldwide. The testing for distinctness, uniformity and stability (DUS) is the basis for grant of protection of new plant varieties under the protection of plant varieties and Farmer's Right Act. 2001 in India. The act has provision to compare the candidate variety with the genotypes of common knowledge on a set of relevant characteristics prescribed in the Draft National Test Guidelines for DUS testing of walnut. Therefore, the present study was undertaken with the objective to characterize 27 walnut genotypes on the basis of morphological characters to validate the distinctness of the walnut germplasm. This investigation may also be helpful to the researchers with respect to improvement of walnut genotypes for particular traits in the targeted regions.

MATERIALS AND METHODS

To investigate the morphological diversity a total of 27 walnut genotypes *viz.*, CITH-W-1, CITH-W-2, CITH-W-3,

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CITH-W-4, CITH-W-5, CITH-W-6, CITH-W-7, CITH-W-8, CITH-W-9, CITH-W-10, CITH-W-11, CITH-W-12, CITH-W-13, CITH-W-14, CITH-W-15, CITH-W-16, CITH-W-17, CITH-W-18, CITH-W-19, CITH-W-20, Opex Caulchry, Sulaiman, Hamdan, Nugget, Franquette, Tutle, and Cheinova were evaluated at field gene bank of ICAR-Central Institute of Temperate Horticulture, Srinagar (J&K), India in a randomized block design with three replications (Gomez and Gomez, 6). The spacing adopted was 6 m × 6 m. The genotypes were evaluated for 29 characters at specific stage of crop growth when characteristics had full expression. To establish distinctiveness among cultivars, the descriptors of essential characters (Table 1 and Fig. 1) were used in sequential manner as per the National Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on walnut (UPOV, 12) Accordingly, for the assessment of distinctiveness and stability, observation were made on 6 plants or 18 parts taken from each of 6 plants with the exception of the observations on nut and kernel which were made on 20 nuts. All observations on the tree and the branches were made during dormancy. Observations on the mature fruit/nut were recorded when fruit was ready for harvesting and packaging tissue was turning brown. Observations on the leaf were made on fully developed leaves of the middle third of current season's shoot.

RESULTS AND DISCUSSION

Considerable variations were recorded among 27 walnut genotypes for various morphological characters. Variation across the genotypes was observed with respect to growth habit, bearing habit, leaf characteristics, kernel characteristics etc. The chief characteristics of different walnut varieties under study are presented in Table 1. The frequency distribution of each character along with the example genotypes are given in Table 1. In the present study, the erect growth habit was noted in one variety, *viz.* CITH-W-12, while semi-erect growth habit was noticed in 16 genotypes, and ten genotypes showed spreading growth habit. Substantial variation was observed for leaflet shape. Three types of leaf shapes were recorded in walnut, *viz.,* narrow elliptic (CITH-W-4 and CITH-W-10), elliptical,CITH-W-5, CITH-W-2, CITH-W-3, CITH-W-8, CITH-W-9, CITH-W-12, CITH-W-17, CITH-W-19, CITH-W-20, CITH-W-15, Franquette, and Opex Caulchry are broad elliptic CITH-W-1,CITH-W-6, CITH-W-7, CITH-W-11, CITH-W-13, CITH-W-16, CITH-W-18, CITH-W-20, Tutle, Hamdan, Nugget, Sulaiman, and Cheinova. The dichogamy is one of the important biological characteristics of walnut. In this study 11 genotypes, *viz.,*CITH-W-4, CITH-W-5, CITH-W-7, CITH-W-8, CITH-W-10, CITH-W-11, CITH-W-12, CITH-W-18, CITH-W-15, Franquette and Opex Caulchry showed protandrous nature and 15 genotypes, *viz.,*CITH-W-1,CITH-W-2,CITH-W-3,CITH-W-6,CITH-W-9,CITH-W-13,CITH-W-16, CITH-W-17,CITH-W-19, CITH-W-20, Tutle, Sulaiman, Nugget, Hamdan and Cheinova, were protogynous. Lebidenets and Bulgakova (8) while

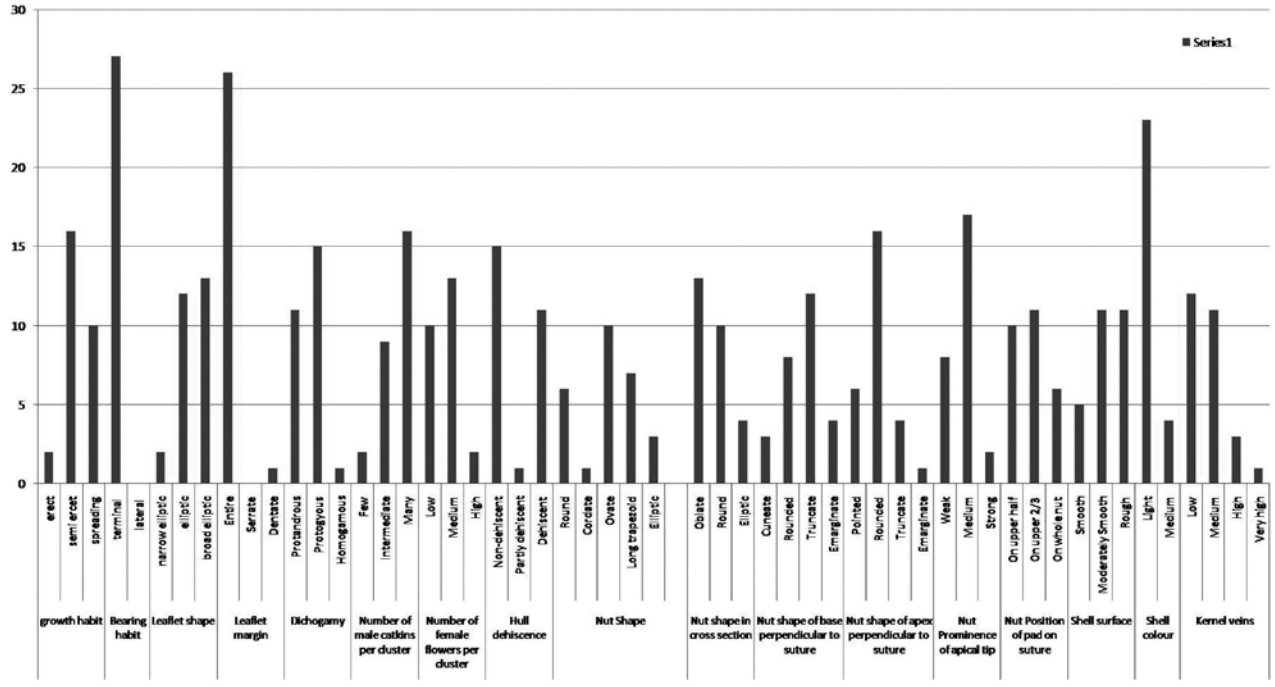

Fig. 1. Frequency distribution of plant characteristics along with range in expression across 27 walnut genotypes.

Table 1: Characterization of walnut genotypes based on DUS descriptor.

Genotype \ Character	Tree vigour (TV)	Growth habit (GH)	Density of branches (DB)	Bearing habit (B H)	Leaf let length (LL)	Leaflet shape (LS)	Leaflet margin (LM)	Leaflet colour (LC)	Rachis colour (RC)	Leaflet rachis persistence (LRP)	Shoot pubescence (SP)	Shoot colour (SC)	Dichogamy (D)	No. of male catkins per cluster (NMC)	No. of female catkins per cluster (NFC)	Stigma colour (SC)	Hull dehiscence (HD)	Nut shape (NS)	Shape in cross section (SCS)	Shape of base perpendicular (SB)	Nut diameter (ND)	Nut Length (NC)	Nut Weight (NW)	Shell surface (SS)	Shell colour (SC)	Shell seal (SS)	Shell thickness (ST)	Kernel weight (KW)	Kernel colour (KC)
CITH-W-1	7	5	7	1	7	3	3	7	3	7	2	5	5	7	5	3	7	6	3	5	7	7	7	3	3	5	1	7	1
CITH-W-2	5	5	5	1	3	2	3	7	3	5	3	5	5	7	5	3	7	4	7	5	7	7	5	7	3	5	2	7	2
CITH-W-3	3	7	5	1	3	2	3	7	3	7	1	5	5	5	5	3	7	4	7	3	5	5	7	7	3	9	2	7	2
CITH-W-4	5	7	7	1	7	2	3	5	5	3	3	5	3	7	5	3	7	6	3	3	7	7	7	7	3	7	2	7	2
CITH-W-5	7	7	5	1	3	2	3	5	3	7	3	5	5	7	5	3	7	4	5	5	7	7	7	3	3	7	2	7	1
CITH-W-6	5	5	5	1	7	3	3	5	3	3	3	5	5	7	5	3	7	4	5	7	7	7	7	7	3	5	2	7	2
CITH-W-7	5	5	5	1	5	2	3	5	5	2	1	5	3	7	3	3	7	8	7	1	7	7	7	7	5	7	2	7	4
CITH-W-8	5	5	5	1	3	1	3	5	5	7	3	5	3	7	5	3	7	6	3	1	7	7	7	7	3	5	2	7	2
CITH-W -9	5	5	7	1	5	2	3	7	3	7	1	5	5	7	5	3	7	1	3	5	7	7	7	5	3	5	2	7	2
CITH-W-10	5	5	7	1	3	1	3	7	3	5	2	5	3	5	5	3	7	6	3	3	7	7	7	5	3	5	2	7	2
CITH-W-11	5	5	7	1	7	3	3	7	3	7	1	5	3	7	7	3	7	1	3	3	5	5	5	5	3	5	2	5	1
CITH-W-12	5	3	5	1	3	2	3	7	7	7	2	5	3	5	5	3	7	5	5	7	5	5	5	5	3	7	2	5	4
CITH-W-13	5	7	7	1	7	3	3	7	3	7	2	5	5	7	3	7	7	6	5	5	7	5	7	3	3	7	2	5	2
CITH-W-14	5	7	7	1	7	3	3	7	3	7	2	5	5	7	3	3	7	6	3	3	7	5	7	3	3	7	2	5	2
CITH-W-15	5	5	7	1	7	3	3	3	3	7	1	5	3	7	3	3	7	6	5	5	7	5	7	5	3	7	2	7	1
CITH-W-16	5	7	7	1	7	3	3	7	3	7	1	5	5	5	7	3	7	4	5	5	5	5	7	5	3	7	2	7	2
CITH-W-17	5	7	7	1	7	3	3	7	3	7	1	5	5	5	7	7	7	8	3	5	5	5	7	7	3	7	2	7	4
CITH-W-18	7	5	7	1	5	2	3	7	3	5	3	5	3	5	5	7	7	1	3	5	7	7	7	7	3	5	2	7	2
CITH-W-19	5	7	7	1	7	2	3	7	3	5	1	5	5	5	3	5	7	3	3	3	7	7	7	7	3	7	5	5	2
CITH-W-20	5	5	7	1	7	3	3	7	3	5	2	5	5	5	3	3	7	4	3	7	7	7	7	5	3	3	2	5	2
Opex Caulchry	3	7	1	1	2	2	3	7	7	3	3	5	3	7	5	3	7	1	5		5	5	3	5	5	7	2	3	2
Sulaiman	5	5	7	1	7	3	3	7	3	5	2	5	5	7	3	3	7	1	5	5	5	5	5	7	5	5	1	5	1
Hamdan	7	7	7	1	7	3	3	5	3	5	2	5	5	3	3	3	7	4	7	7	5	7	5	3	3	3	1	5	1
Nugget	7	5	5	1	7	3	3	5	3	5	2	5	5	7	5	3	7	3	3	7	5	5	3	5	3	9	2	3	2
Franquette	5	5	5	1	3	2	7	5	7	7	2	5	3	5	5	3	7	8	3	3	3	3	3	5	5	9	1	3	2
Tutle	3		5	1	3	3	3	7	3	3	1	5	5	7	5	7	7	1	3	3	3	3	3	5	5	7	1	7	7
Cheinova	5	5	5	1	5	3	3	7	7	7	1	5	5	7	3	3	7	4	7	1	5	7	3	5	5	7	2	5	2

TV: Low (3), Intermediate (5), High (7)	**GH:** Erect (3), Semi erect (5), Spreading (7)	**DB:** Sparse (3), Intermediate (5), Dense (7)	**BH:** Terminal (1), Lateral (9)
LLG: Short (3), Medium (5), Long (7),	**LS**: Narrow elliptic (1), Elliptic (2), Broad elliptic (3)	**LM:** Entire (3), Serrate (5)	**LC:** Light green (3), Green (5), Dark green (7), Purplish (9)
RC: Green (3), Yellow (5), Red (7)	**LRP**: Few (3), Intermediate (5), Many (7)	**SP:** Glabrous (1), Slightly pubescent (2), Pubescent (3),	**SC:** Green (3), Brown (5), Dark Brown (7)
D: Protandrous (3), Protogynous (5), Homogamous (7),	**NMC:** Few (3), Intermediate (5), Many (7)	**NFC**: Low (3), Medium (5), High (7),	**SC:** Green (3), Yellow (5), Red (7)
HD: Non–dehiscent (3), Partly dehiscent (5), Dehiscent (7)	**NS:** Round (1), triangular (2), cordate (3), Ovate (4), Short trapezoid (5), Long trapezoid (6), Broad Elliptic (7)	**ND:** Small (3), Medium (5), Large (7)	**NL:** Small (3), Medium (5), Large (7)
NW: Light (3), Medium (5), Heavy (7)	**SS:** Smooth (3), Moderately (5), Smooth Rough (7)	**SC:** Very light (1), Light (3), Medium (5), Dark (7)	**SS:** Weak (3), Intermediate (5), Strong (7), very strong (9)
ST: Thin (1), Medium (2), Thick (3)	**KW:** Light. (3), Medium (5), Heavy (7)	**KC:** Extra light (1), Light (2), Amber (4), Dark Amber (7)	**SCS:** Oblate (3), Round (5), Elliptic (7)
SBP: Cuneate (1), Rounded (3), Truncate (5), emarginated (7)			

studying the floral biology of 83 walnut genotypes reported that majority of the population (67.06%) was protogynous in nature while as (Yadrow and Zinin, 14) reported that higher proportion (60%) of walnut genotypes were protoandrous in nature. In our study 40.70% genotypes showed protoandry and 55.5% genotypes were protogynous. Nut shape was found to be one of the key characters which categorize the walnut genotypes into five groups. The nut shape showed great diversity within the population. Nut shape range from round (Sulaiman, Opex Caulchry, Tutle,CITH-W-9, CITH-W-11,CITH-W-18), cordate (Nugget), ovate (Cheinova, Hamdan, CITH-W-2, CITH-W-3, CITH-W-5, CITH-W-6, CITH-W-12, CITH-W-16, CITH-W-19, CITH-W-20), long trapezoid (CITH-W-1,CITH-W-4, CITH-W-8, CITH-W-10, CITH-W-13, CITH-W-14, CITH-W-15) and elliptic (Franquette, CITH-W-7,CITH-W-17). Eskandari *et al*. (5) also selected some genotypes according to yield and nut characteristics from natural populations in different provinces. High variability in nut traits has been reported in walnut trees in different countries. In many countries, selection of walnut was carried out by method of simple selection out of natural seedling populations with high quality walnuts. A great range of variability was observed for various nut and kernel characters on 23 bearing seedling trees in Ladakh region of India by Sharma *et al*. (11). Nut shape in cross section and nut shape of base perpendicular to suture also varied among different walnut genotypes (Fig. 1). Thirteen genotypes *viz*., Franquette, Nugget, CITH-W-1, CITH-W-4, CITH-W-8, CITH-W-9, CITH-W-10, CITH-W-11, CITH-W-14, CITH-W-17, CITH-W-18, CITH-W-19, and CITH-W-20 had oblate nut shape in cross section while ten genotypes *viz* Sulaiman, Opex Caulchry, Tutle, CITH-W-2, CITH-W-5, CITH-W-6, CITH-W-12, CITH-W-13, CITH-W-15 and CITH-W-16 had round shape and four genotypes viz Cheinova, Hamdan, CITH-W-3 and CITH-W-7 have elliptic nut shape in cross section, Nut shape of base perpendicular to suture also showed variation across the genotypes, maximum number of genotypes were having truncate shape of base perpendicular to suture. Other nut characters like nut shape of apex perpendicular to suture, nut prominence of apical tip, nut position pad on suture also varied among different walnut genotypes. Shell character like shell colour and shell surface were also different across walnut genotypes. Maximum genotypes Hamdan, Nugget, Tutle, Sulaiman, CITH-W-1, CITH-W-2, CITH-W-3, CITH-W-4, CITH-W-5, CITH-W-6, CITH-W-8, CITH-W-9, CITH-W-10, CITH-W-11, CITH-W-12, CITH-W-13, CITH-W-14, CITH-W-15, CITH-W-16, CITH-W-17, CITH-W-18, CITH-W-19 and CITH-W-20 had light shell colour. Shell surface also varied from smooth, moderately smooth to rough. Hamdan, CITH-W-1, CITH-W-13, CITH-W-14 and CITH-W-5 have smooth shell surface

while as Nugget, Cheinova, Opex Caulchry, Tutle, CITH-W-10, CITH-W- 11, CITH-W-12, CITH-W-15, CITH-W-16 and CITH-W-20 have moderately smooth shell surface and genotypes like Sulaiman, CITH-W-2, CITH-W-3, CITH-W-4, CITH-W-6, CITH-W-7, CITH-W-8, CITH-W-9, CITH-W-7, CITH-W-18 and CITH-W-19, have rough shell surface. Twelve genotypes (Tutle, Opex Caulchry, CITH-W-1, CITH-W-2, CITH-W-3, CITH-W-10, CITH-W-11, CITH-W-12, CITH-W-15, CITH-W-17, CITH-W-18, and CITH-W-20) had low kernel veins, while Sulaiman have very high kernel veins. Enormous variability has been reported in nut traits e.g., nut sizes (small to very large), shape, shell thickness (very thin to very thick), the degree of shell seal, the colour of kernels, taste and appearance of kernels. High variability in nut traits *e.g*. nut sizes, shape, shell thickness, kernel percent, colour of kernels and taste of kernels, has been reported in walnut trees in different regions (Casal *et al,*. 4 and Khan *et al.,* 7) .The variability found in the present study is in agreement with that reported for the Eurasian walnut distribution range from Iran (Atefi, 1) and India (Sharma and Sharma, 10). The descriptor will benefit the users of plant genetic resources working with walnut and in general will make access to diversity of the walnut crop by the researchers or users of genetic resources (Biodiversity International. 3). Development of morphological descriptor in walnut for DUS testing will be useful for varietal identification, registration, characterization, documentation etc. The detailed descriptor will be useful for creating plant genetic resource database (Singh *et al*., 9).

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