DOI : 10.5958/0974-0112.2016.00009.8

Indian J. Hort. 73(1), March 2016: 1-7

Analysis and utilization of genetic diversity of '*Ambri*' apple (*Malus* × *domestica* Borkh.) in Jammu region

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ABSTRACT

Present investigation was conducted in erstwhile Doda district of Jammu & Kashmir state to study the genetic diversity among '*Ambri*' apple variants and its exploitation to revive this indigenous variety in the state for commercial cultivation and to utilize in apple improvement programme. Among the 34 collected variants, a wide range of diversity was observed in respect of fruit weight (86.90-334.97 g), fruit length (59.13-84.00 mm), fruit width (68.21-94.31 mm), flesh firmness (3.65-9.65 N), seed number (5.00-7.00), TSS (10.90-20.00ºBrix) and titratable acidity (0.31-1.78%). Highest coefficient of variation was recorded for titratable acidity (54.76%). Fruit weight showed significantly positive correlations with fruit length and stalk and number of seeds per fruit. First three factors having eigen value having >1 explained 70.04% of total variation observed in the collected population. Of the total collections, only seven variants attained the numerical rating, being accepted of consumer's acceptability. Variants BM2910 and BC3210 were identified as most acceptable variants for mass multiplication and to revive the '*Ambri*' apple cultivation and their further use in breeding programmes.

Key words: *Ambri* apple, fruit weight, genetic diversity, variants.

INTRODUCTION

Apple, the principal temperate fruit crop of India is being cultivated on 3.12 lakh ha area with an annual production of 9.12 million tonnes and productivity of 6.1 tonnes ha^{-1}, in general and 8.57 tonnes ha^{-1} in Jammu & Kashmir (J&K) state, in particular. Despite of limited growing region, 23806 tones apple worth of US $ 8.32 million, is exported from India annually (Anon, 1). In India, about 33 apple varieties are grown, however, the availability of Indian apple (fresh) in markets is only restricted between July to November, because of poor shelf life. '*Ambri*' (developed naturally either through chance seedling or bud mutation) an excellent dessert variety of apple is indigenous to Kashmir, and continues to keep its superiority by virtue of its crisp texture, sweet flesh and excellent aroma with prolonged storability (up to six months in ordinary storage under typical temperate areas). Despite of long gestation period, biennial bearing habbit and susceptibility to scab disease, '*Ambri*' apple is in great demand because of unparalleled flavour and prolonged shelf-life, which have also made '*Ambri*' variety the choicest parent to use in Indian apple breeding programme for improving the quality and shelf-life of Delicious apples with good success. With the introduction of early maturing cultivars like Starkrimson and Mollies Delicious, the demand of Indian consumers later in the season relies on imported apple at non-affordable price, causing decreased per capita consumption of this nutritious fruit. Hence, the revival of '*Ambri*' apple being a late maturing cultivar, is an excellent alternative for the sustainability of Indian apple industry. The scattered plantations of '*Ambri*' apple can be found in Kashmir valley, and Ramban, Doda and Kishtwar districts of Jammu region. The existence of seedling populations and its highly cross-pollinated nature have contributed towards the tremendous variability in shape, size and colour development thereby providing a platform for exploitation of vast gene pool of '*Ambri*' apple. Due to scant attention of researchers, meagre efforts have been made to analyse the genetic diversity of '*Ambri*' apple and for further exploitation for its revival in J & K state.

Apple germplasm and the maintenance of genetic diversity are important for future breeding because genetic diversity gives species the ability to adapt to changing environments and provide the raw material to breed new cultivars *via* hybridization or selection (Dhillon and Rana, 3). Estimating genetic diversity and determining the relationships among germplasm collections enhance efficiency of its management and genetic improvement (Rana *et al*., 11). Future of breeding programmes depends on the availability of genetic variability to increase productivity. Morphological characterization of trees

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and fruits is the first and the most important step for the description, classification and characterization of germplasm collections (Verma *et al.*, 14). Apple fruits are characterized using both, maturity indices including firmness, sugar, starch, acid content, and ethylene concentration as well as marketability indices such as flesh and background colour and fruit shape which consumer use to differentiate cultivars. In the present study, the attempt has been made to analyse the existing variability of '*Ambri*' apple and exploit the traits that contribute towards fruit weight and consumer acceptability of '*Ambri*'apple.

MATERIALS AND METHODS

The potential areas of erstwhile Doda district (now divided into three districts, *viz.*, Ramban, Kishtwar and Doda) were surveyed personally or through the help of extension personnel of State Department of Horticulture, Jammu (J&K) for selecting and marking the trees of '*Ambri*' populations during first year (2009-10). A random sample of 20 fruits, harvested at commercial maturity, from each tree was taken during second year for recording the data on fruit characters (Table 1). Data on fruit weight (g) was recorded on top pan balance, while fruit size (length and width), average cross diameter, and depth (mm) and width (mm) of stalk and eye basin were measured with Mitutoyo digital Vernier calipers. Locule aperture was described as per the method given in the TG/14/9 document (UPOV, 13). Fruit TSS (ºBrix) was measured with hand refractometer. Flesh firmness was measured with the help of Effegi penetrometer-FT 327(as force in N). Fruit colour was measured with the help of Hunterlab colour meter, and expressed as CIE Hunter colour values. Titratable acidity (as % malic acid) was determined by standard AOAC method (Horwitz, 5). Ascorbic acid was determined as per the method of Roe (12). To assess the consumer preference, organoleptic evaluation of fruits of different variants was done by the panel of judges as per the 9 point hedonic scale (Peryam and Pilgrim, 10).

Cluster and factor analysis was applied to study the relative contribution made by the different fruit characters. The factors having eigen value greater than or equal to one were extracted as variables. Also multiple linear regression equation was fitted to predict the fruit weight of '*Ambri*' apple on the basis of remaining fruit characters. The data were analysed using software SYSTAT-12.

RESULTS AND DISCUSSION

The proportions of different morphological characters of fruits observed in the collected variants of '*Ambri*' apple have been presented graphically in Fig. 1. Among different fruit shapes, conic type was dominant (44.12%) followed by broad conic (20.59%) and globose (17.65%), while for other shapes such as globose conic, broad globose and ovoid, they ranged between 2.94-8.82%. Majority of the variants (97.06%) had creamy-white flesh colour. Closed locule aperture was observed in 41.18% variants, while locule apertures were moderately closed and fully open each in 29.41% variants. The colour frequency for L^* value of variants was equal (29.41% in each) between 27-32 and 32-37 colour values ranges. The a^* value of 50% variants ranged between 37-42 followed by 42-47 (29.41%). In case of b* colour value, the highest frequency (47.05%) of variants ranged between 10-14 b^* value followed by 18.22 (26.47%) and 14-18 (23.53%).

Range, mean, standard deviation and coefficient of variation of different physico-chemical fruit characters of '*Ambri*' apple variants are presented in Table 2. Among the characters studied, fruit weight ranged between 86.90-334.97 g, whereas fruit length, fruit width, fruit diameter, stalk depth and stalk width ranged between 59.13-84.00 mm, 68.21-94.31

Table 1. Location of '*Ambri*' apple variants in Jammu region, J&K.

Variant*	Place	Elevation (m amsl)	Coordinates	
BS0110, BS0210, BS0310, BS0410, BS0510, BS0610, BS0710, BS0810, BC0910, BC1010, BC1110, BC1210, BC1310, BC1410, BC1510, BS1610, BS1710, BS1810, BS1910, BS2010, BD2110, BS2210, BS2310, BS2410, BC2510, BC2810, BM2910, BC3210, BC3310	Bhaderwah	1,618	32°58'58.95"N	75°42'39.45"E
BN3110	Banihal	2,832	33° 25' 0" N	75° 12' 0" E
BL3410	Bhalessa	1,644	33°01'55.96"N	75°54'36.11"E
DD2610, DD2710	Doda	1,131	33°08'38.12"N	75°32'46.73"E
GL3010	Galhar	2,046	33°20'18.66"N	75°56'02.33"E

*Tree age of variants ranged from 18-20 years

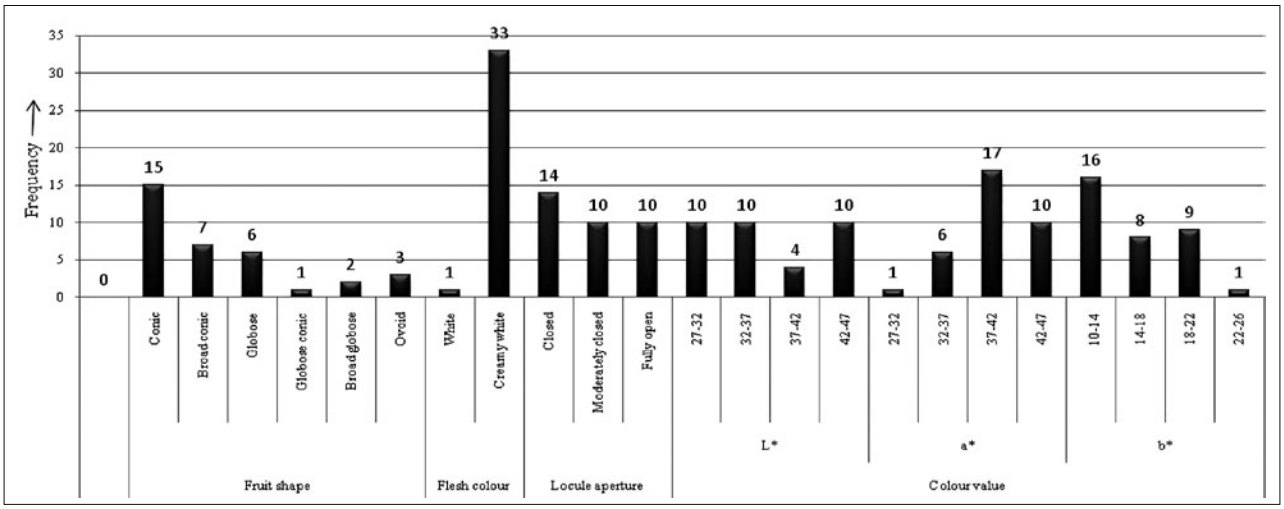

Fig. 1. Frequency distribution of morphological traits in *'Ambri'* variants.

Table 2. Range, mean standard deviation and coefficient of variation in physico-chemical fruit traits in '*Ambri*' variants.

Trait	Range	Mean	Standard deviation	Coefficient of variation
Fruit weight (g)	86.90-334.97	209.90	62.29	29.68
Fruit length (mm)	59.13-84.00	75.67	6.98	9.23
Fruit width (mm)	68.21-94.31	81.48	6.96	8.54
Fruit diameter (mm)	18.30-30.30	24.59	4.24	17.26
Stalk depth (mm)	1.10-2.80	2.06	0.44	21.56
Stalk width (mm)	1.40-4.00	2.71	0.62	22.71
Flesh firmness (N)	3.65-9.65	8.16	1.27	15.55
No. of seeds	5.00-7.00	5.38	0.60	11.22
TSS (°B)	10.90-20.00	14.47	2.13	14.68
Titratable acidity (%)	0.31-1.78	0.60	0.33	54.76
Ascorbic acid (mg/ 100 g)	2.10-6.80	3.72	1.38	37.11

mm, 18.30-30.30 mm, 1.10-2.80 mm and 1.40-4.00 mm, respectively. Values for flesh firmness, number of seeds, TSS, titratable acidity and ascorbic acid ranged between 3.65-9.65 N, 5.00-7.00/ fruit, 10.90-20.00ºBrix, 0.31-1.78% and 2.10-6.80 mg/100 g FW, respectively. Highest values for coefficient of variation was recorded as 54.76% for titratable acidity followed by 37.11% in ascorbic acid, 29.68% in fruit weight, 22.71% in stalk width, 21.56% in stalk depth, 17.26% in fruit diameter, 15.55% in flesh firmness, 14.68% in TSS and 11.22% in number of seeds. Fruit length and width registered lower values for coefficient of variation as 9.23 and 8.24%, respectively.

Correlation coefficients presented in Table 3 revealed that fruit weight had highly significant and positive correlation with fruit length, fruit width, fruit diameter, stalk depth, stalk width and number of seeds. Fruit length was correlated significantly and positively with fruit width, fruit diameter, stalk depth and width. Fruit width had positive and significant correlation with fruit diameter, stalk depth and width. Fruit diameter was correlated significantly with stalk depth and width. Stalk depth was correlated significantly with stalk width and number of seeds and stalk width was correlated significantly with number of seeds.

The dendrogram produced through multivariate analysis performed on 12 quantitatively measured traits showed that 34 accessions were grouped into two clusters in which cluster I had 20, while cluster II had 14 (Fig. 2). In cluster I accession, GL3010 showed high genetic distance (21.51) followed by BC1110 (11.14) and GL3010 (10.06), while in cluster II BS0510 showed high genetic distance (21.51) followed by BS1710 (11.14). The minimum genetic distance was showed by BS0610 (1.34) in cluster I and BS2210 in cluster II. Grouping of accessions into

Table 3. Correlations among different fruit characters of '*Ambri*' apple variants.

	FW	FL	FW	FD	SD	SW	FF	SN	TS	TA	AA
FW	1.000										
FL	0.490**	1.000									
FW	0.840**	0.648**	1.000								
FD	0.726**	0.476**	0.722**	1.000							
SD	0.667**	0.408*	0.732**	0.679**	1.000						
SW	0.667**	0.499**	0.599**	0.564**	.0752**	1.000					
FF	0.183	0.176	0.127	0.079	0.079	0.292	1.000				
SN	0.472**	0.066	0.394	0.354	0.479**	0.452**	0.139	1.000			
TS	-0.072	0.387	-0.031	0.117	-0.113	0.131	0.194	-0.327	1.000		
TA	0.115	0.221	-0.016	-0.084	-0.070	0.058	0.229	-0.202	0.432	1.000	
AA	-0.131	-0.414	-0.238	-0.086	-0.219	-0.158	-0.113	0.078	-0.328	-0.078	1.000

**Significant at 1%; FW = Fruit weight; FL = Fruit length; FW = Fruit width; FD = Fruit diameter; SD = Stalk depth; SW = Stalk width; SN = No. of seeds; FF = Flesh firmness; TS = Total soluble solids ; TA = Titratable acidity; AA = Ascorbic acid

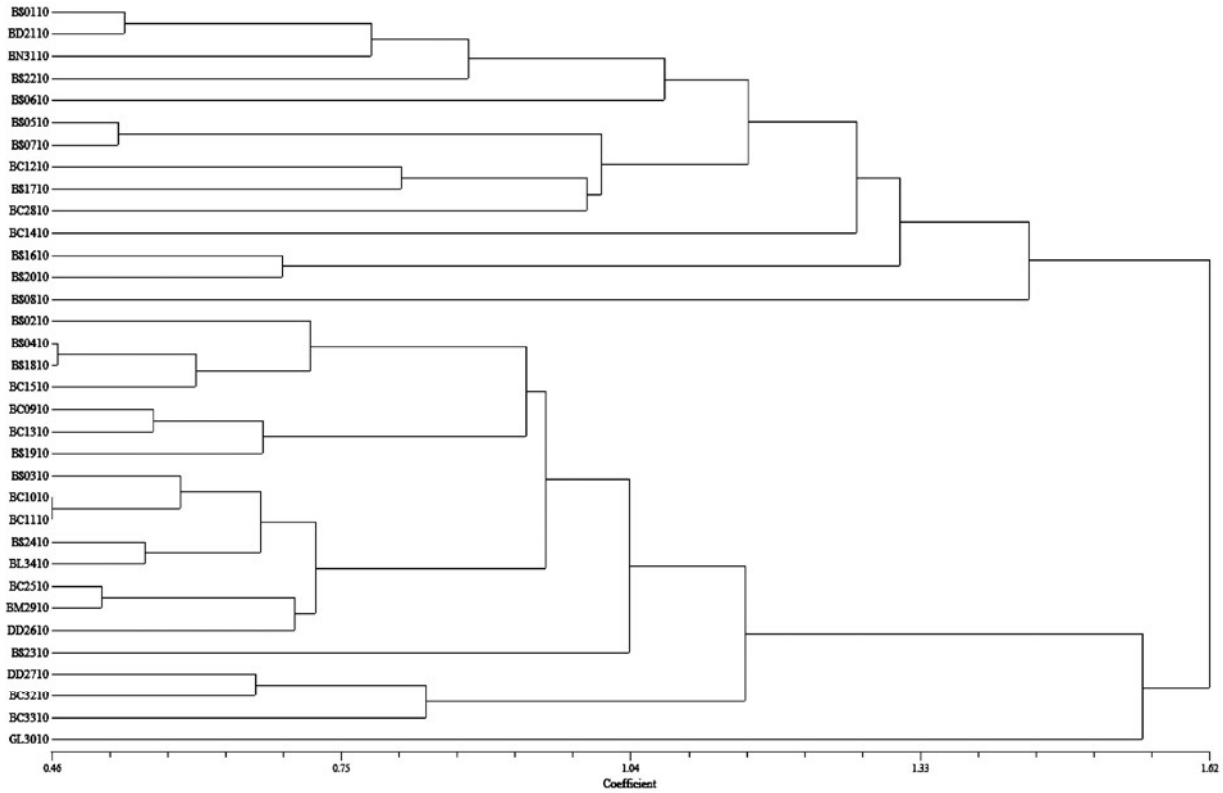

Fig. 2. Dendrogram of '*Ambri*' variants based on quantitative traits.

few numbers of homogenous clusters facilitate the selection of diverse parents and also permits precise comparison among all the possible pair of populations and provide an opportunity for bringing together gene constellation yielding desirable progenies.

Factor analysis technique was applied to extract the basic factors underlying the observed characters of '*Ambri'* apple and values pertaining to it (Table 4). The other factors corresponding to eigen value less than unity (λ<1), were not taken into further consideration, and ignored due to Guttmen's lower bond principle. After ignoring the non-significant correlation, orthogonal factors were extracted. The centroid method of analysis was used in arriving at

Table 4. Loadings of components (having λ<1) extracted through principal component analysis.

Trait	Loading		
	PC1	PC2	PC3
Fruit weight	0.88	-0.10	0.083
Fruit length	0.68	0.47	-0.224
Fruit width	0.90	-0.06	-0.148
Fruit diameter	0.82	-0.08	-0.133
Stalk depth	0.85	-0.20	-0.081
Stalk width	0.83	0.02	0.185
Flesh firmness	0.25	0.32	0.718
No. of seeds	0.53	-0.53	0.312
TSS	0.08	0.84	-0.042
Titratable acidity	0.05	0.66	0.397
Ascorbic acid	-0.29	0.50	0.435
Eigen value	4.55	2.06	1.10
% of total variance	41.34	18.71	9.98
% of cumulative variance	41.34	60.05	70.04

the factors. The following three factors were thus obtained:

Factor 1 : 0.88X1 + 0.68X2 + 0.90X3 + 0.82X4 + 0.85X5 + 0.83X6 + 0.53X8

Factor 2 : 0.84X9 + 0.66X10 + 0.50X11

Factor 3 : 0.72X7

These three factors having eigen values more than one explained 70.04% of the total variation. The first, second and third factors had variance of 4.55 (41.34% of total variation), 2.06 (18.37% of total variation) and 1.10 (9.98% of the total variation), respectively. The rotated factor matrix and the communalities were obtained through orthogonal transformation procedure. The first factor extracted was the combination of fruit weight, fruit length, fruit width, fruit diameter, stalk depth, stalk width and number of seeds in, which all the characters had positive loadings. The contributing characters of first principle component comprised of the characters which are responsible for yield. The second factor was the combination of TSS, titratable acidity and ascorbic acid, which pertained to the quality parameters. Third factor comprised of only one loading, *i.e.* flesh firmness, which was related to the storability quality of fruit.

For exploiting the existing variability to revive the '*Ambri*' apple in J&K, the organoleptic evaluation of all the variants was conducted as per the hedonic scale. Of the collected variants (34), only seven could score the numeric rating to the extent of consumer acceptability (Fig. 3). BM2910 was the most acceptable variant as it scored the highest numerical rating (9.5) followed by BC3210 (8.5). These two variants were found most promising and may be recommended for mass multiplication to revive the '*Ambri'* apple for the sustainability of apple industry of the state. Besides, these variants proved also found superior in respect of fruit length, TSS and good acid content (Table 2) over standard '*Ambri*' (having maximum fruit length upto 77.66 mm, TSS up to 15.90ºBrix and acidity up to 0.09%) as reported by Bisati (2), while studying the variation in '*Ambri'* in Kashmir region.

Variability refers to the differences which develop between individuals may be inherited (genetic) or

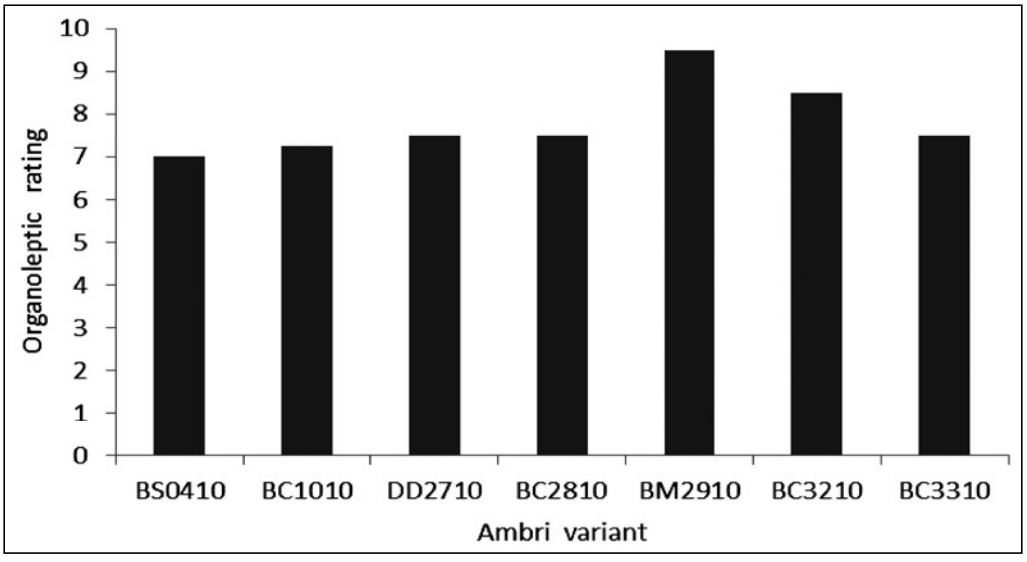

Fig. 2. Overall acceptability of '*Ambri'* apple variants.

un-inherited (environmental). The changes due to genetic causes can be inherited from one generation to the next generation, while the changes arise due to environmental causes cannot. Therefore, there must be genetic variability among the population from which better individuals are expected to be selected. Since naturally growing plants are generally of the seed origin, therefore variability present in them is mostly due to genetic causes. In light of above facts, it is evident that variability of the randomly selected samples of '*Ambri'* apple is due to genetic variation and is heritable. In the present study, considerable variation for different traits was observed particularly for titratable acidity, ascorbic acid content, fruit width, stalk width, stalk depth, fruit diameter, flesh firmness, TSS and number of seeds per fruit. Study undertaken by Kunihisa *et al*. (6) indicated that these traits are controlled by multiple alleles therefore, if variability is available, there is chance for their improvement through selection of these traits. Similarly, Mratinic and Aksic (7) while studying the local apple germplasm in Serbia found large variations for physico-chemical characteristics enabling the selection of some superior clones from the local apple types. Highest coefficient of variation in '*Ambri*' apple variants was recorded for titratable acidity among the studied population. Similarly, Mratinic and Aksic (7) also recorded the highest coefficient of variation (80.42%) for titratable acidity among different physico-chemical characters of apple. Least variable characters among the studied population pertained to fruit length and fruit width. Correlation coefficients revealed the relationship between the characters, and inference can be drawn regarding the indirect selection for a particular character. Correlation coefficients between almost all phenology traits were found significant. Fruit weight, an important character contributing towards yield was significantly and positively correlated with the dimensions of fruit and stalk and number of seeds. In general, fruit diameter proved to be a significant parameter for fruit weight and volume is correlated significantly with stalk depth and stalk width. Stalk depth was correlated significantly with stalk width and number of seeds and stalk width was correlated significantly with number of seeds. Principal component analysis (PCA) has been used previously to evaluate apple germplasm (Pereira-lorenzo *et al*., 9). A multivariate statistical tool, PCA is used to study correlations among fruit traits and to establish genetic relationships among different cultivars. Associations between different traits established by this method may correspond to genetic linkage between the loci controlling those traits or a pleiotropic effect (Oraguzie *et al*., 8). PCA coefficients are functions of the eigen values and eigen vectors of variance/ covariance matrix. Eigen values measure the variance accounted by a given principal component, and is useful for determining the number of significant factors. In the present study, three principal components having eigen value more than one were extracted. Thus, eleven characters of '*Ambri'* apple cultivar were classified into three basic factors by applying the factor analysis technique of multivariate analysis. The highest loading on first component were due fruit weight, fruit length, fruit width, fruit diameter, stalk depth, stalk width and number of seeds. TSS, titratable acidity and ascorbic acid contributed highest loading on second principle component. Flesh firmness contributed highest loading towards principle component 3. These factors could be used in further breeding programmes for getting the transgressive recombinants. Similarly, Farrokhi *et al.* (4) also reported that principle component analysis divided the yield contributing parameters into one principle component and fruit quality parameters into other. Present studies confirmed the utility of fruit weight as an important contributing trait for yield and accession identification. Principal component analysis has also been used previously to evaluate germplasm of apple (Pereira-lorenzo *et al*., 9). Further the prediction model for fruit weight worked through regression analysis is as under:

$$\text{Fruit weight} = -361.00 + 6.15\ (\text{Fruit width}) + 25.94\ (\text{Stalk width}) \quad (R^2 = 0.75)$$

Above equation reveals that with as unit increase in fruit width and stalk width, fruit weight increases 6.15 and 25.94 times, hence wider fruits have high fruit weight. No significant influence of fruit length has been observed on fruit weight, even though they were positively and significantly correlated.

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Received : September, 2015; Revised : January, 2016; Accepted : February, 2016