Assessment of Variability, Correlation and Path Analysis for the Selection of Elite Clones in Litchi Based on Certain Traits

Narayan Lal[1] · Awtar Singh[2] · Abhay Kumar[3] · Sheshdhar Pandey[3]

Abstract

In the present study, 131 litchi clones were assessed for variability, heritability, genetic advance and association studies. The phenotypic and genotypic coefficient of variation was high for TSS acid ratio (47.19% and 47.10%), acidity (44.77% and 44.70%), skin weight (29.38% and 29.26%), pulp weight (24.93% and 24.79%) and seed weight (23.81% and 23.66%). Heritability in the broad sense was more than 80% for all parameters except length:breadth (78%). The higher heritability estimates accompanied by higher genetic advance were observed in all parameters except fruit length, fruit width, length:breadth of fruit and total soluble solids (TSS). A highly significant and positive association at genotypic and phenotypic levels was found between fruit weight and length, fruit width and pulp weight. Path analysis showed that the maximum positive direct effect on fruit weight was through pulp weight (0.9715), skin weight (0.1998) and seed weight (0.1978). The traits of fruit length and fruit width imparted a positive indirect effect on fruit weight. From principal component analysis (PCA) and variability estimates, fruit weight, acidity, skin weight and fruit length were identified as important traits for identifying high-yielding clones. This study identified weight of fruit, pulp, peel and seed as important characteristics for identifying high-yielding clones.

Keywords Genetic variability · Genetic advance · Heritability · Litchi chinensis · Path analysis

Introduction

Litchi (*Litchi chinensis* Sonn.) is an evergreen fruit tree of the family Sapindaceae. Very few cultivars are commercially grown in India, which indicates a narrow genetic base. Genetic variations have been assessed in litchi (Singh and Nath 2012). Selecting clones from different regions of ecological conditions may be useful to widen the diversity in litchi. The selection of promising clones from the existing population is the best and fastest way to develop new cultivars in litchi. Significant variations in various traits such as morphology as well as biochemical and yield parameters can be used for identification and breeding purposes

✉ Narayan Lal
  narayanlal.lal7@gmail.com

[1] ICAR-Indian Institute of Soil Science, 462038 Bhopal, Madhya Pradesh, India

[2] ICAR-Indian Agricultural Research Institute, 110012 New Delhi, Delhi, India

[3] ICAR-National Research Centre on Litchi, 842002 Muzaffarpur, Bihar, India

(Chandola and Mishra 2015). Although litchi is an introduced crop in India, variations in yield and quality traits have been found (Lal et al. 2018). Variability among the population of breeding material is an essential prerequisite for a successful breeding program. The presence of variability coupled with higher heritability provides greater scope for further improvement in the crops. Such improvements in important traits such as fruit weight, quality and yield contribute to the possibilities for increasing quality in litchi (Gupta et al. 2017). The knowledge of variability, heritability and genetic advance is very important and provides information on various contributing traits that govern yield. Thus, studies of diversity based on genotypic coefficient of variation (GCV), heritability and genetic advance are of the utmost importance for successful breeding (Mishra et al. 2015). The approaches of correlation and path analysis provide information about the nature and magnitude of variation present in quantitative traits to make significant improvements. Trait association analysis gives a clear picture of interrelationships and the relative contribution of independent characteristics on dependent variables, thereby enabling breeders to make selection procedures for crop improvement. Principal component analysis (PCA) simplifies

the complexity of high-dimensional data while retaining trends and patterns. It does this by transforming the data into fewer dimensions, and cluster analysis helps in grouping similar objects into respective categories so that diverse parents can be selected for further breeding programs. Thus, the present study was conducted to confirm genetic variability among litchi clones and identify potential traits for the selection of superior clones.

Materials and Methods

In the present study, 131 litchi clones (1–131), were collected from different sources and evaluated for economically important fruit traits. The selected plants were 25–30 years old, and the plants were raised under similar conditions and uniform orchard practices. The observations were recorded for 2 consecutive years (2018 and 2019) in Eastern India on fruit physico-chemical parameters and each clone consisted of three replicates. The weight of fruits, pulp, seed and skin was measured on a digital weighing balance. Total soluble solids (TSS) was measured with a HANNA digital refractometer (range 0–52%) by putting 3 ml juice on the prism-plate, and reading was recorded as total soluble solids (°Brix). For evaluation, 10 fruits per replication were randomly collected from all directions. After testing homogeneity, the mean values of 11 traits for both years were pooled and subjected to analysis of variance (ANOVA) to test the presence of significant differences among clones (Panse and Sukhatme 1954) following a randomized block design. The phenotypic coefficient of variation (PCV), GCV, heritability and genetic advances were estimated using WASP2 statistical software. The correlation coefficient analysis among all the possible combinations at the phenotypic and genotypic levels and estimates of direct and indirect effects of

component characters on fruit weight were estimated using standard methods. Principal component analysis (PCA) was carried out to simplify the complexity in high-dimensional data, and neighbor joining cluster analysis to build diverse groups was performed using R software (Everitt and Dunn 2010).

Results and Discussion

The presence of genetic variability is a prerequisite for breeding programs, and assessment of the existing variability is essential for selecting desirable clones. The minimum, maximum and mean performance, PCV and GCV, heritability and genetic advance (Table 1) exhibited a wide range of variability for most of the characteristics studied. PCV and GCV were high for TSS acid ratio (47.19% and 47.10%), acidity (44.77% and 44.70%), skin weight (29.38% and 29.26%), pulp weight (24.93% and 24.79%) and seed weight (23.81% and 23.66%) and moderate to low for other traits. The estimates of GCV were lower in magnitude than PCV, with the range indicating that there is much more diversity in the population for most of the traits. The current study suggests that PCV was higher than GCV for all traits. This was also the case for all traits observed in another study (Osman et al. 2012), which reported that the effect of environment on any trait is indicated by the magnitude of differences between GCV and PCV; large differences reflect a large environmental influence, while small differences reveal a high genetic influence. In this study, the small differences between PCV and GCV for most traits represented some degree of environmental influence on the phenotypic expression of these traits (Tuhina-Khatun 2015). It also suggests that selection based on these traits would be effective for future breeding programs. These observations were in agreement with the findings of Marboh et al.

Table 1 Estimates of various genetic parameters of litchi clones

Characteristics	Min	Max	Mean	SD	CV	GCV	PCV	h2	GA	GA (%)
Fruit weight (g)	10.14	29.65	19.33	3.57	18.47	18.41	18.61	97.86	7.25	37.51
Fruit length (mm)	3.04	4.02	3.61	0.21	5.89	5.71	6.24	83.78	0.39	10.77
Fruit width (mm)	2.51	3.80	3.24	0.24	7.33	7.16	7.65	87.74	0.45	13.82
Length:breadth	0.92	1.32	1.12	0.06	5.10	4.87	5.52	78.00	0.10	8.86
TSS (°Brix)	12.34	20.88	16.95	1.58	9.33	9.20	9.58	92.10	3.08	18.18
Seed weight (g)	0.59	4.59	3.16	0.75	23.71	23.66	23.81	98.77	1.53	48.44
Skin weight (g)	1.29	4.92	2.68	0.79	29.30	29.26	29.38	99.17	1.61	60.02
Pulp weight (g)	3.77	24.70	13.48	3.35	24.84	24.79	24.93	98.87	6.85	50.78
Pulp (%)	35.76	83.17	68.86	7.38	10.72	10.62	10.93	94.40	14.63	21.25
Borer attack (%)	1.20	2.00	1.89	0.21	11.38	11.29	11.56	95.43	0.43	22.72
Acidity (%)	0.16	1.28	0.47	0.21	44.72	44.7	44.77	99.66	0.43	91.91
TSS:acid	14.24	106.69	44.09	20.78	47.13	47.10	47.19	99.61	42.69	96.83

Min minimum, *Max* maximum, *SD* standard deviation, *CV* coefficient of variation, *GCV* genotypic coefficient of variation, *PCV* phenotypic coefficient of variation, *h2* heritability, *GA* genetic advance, *TSS* total soluble solids

(2018), who reported high PCV and GCV for pulp weight (32.86 and 31.11%) and seed weight (30.56 and 27.93%). Thus, these characteristics would be useful to select good clones in litchi. The high values of PCV and GCV for different traits such as fruit weight, seed weight, pulp weight and acidity indicated that greater improvement can be anticipated through selection based on these characteristics. However, the GCV does not offer full scope to estimate the variations that are heritable in nature, and thus estimation of heritability becomes necessary.

Yield is the most desirable quantitative trait complex in inheritance and is highly influenced by the environment. Heritability plays a useful role in estimating the scope of improvement by selection, and selection would be very effective for the trait with high heritability because there would be close affinity between clones and phenotypes due to lower environmental influence. Heritability in the broad sense was more than 80% for all the parameters except length:breadth (78%). The high heritability indicates that the characteristics under study had great scope for further genetic improvement. Rajan et al. (2009) also found higher heritability for various traits in many fruit crops. Moderate to low estimates indicate a limited scope of improvement through selection. Hence, computation of heritability alone would not be sufficient to bring adequate improvement in fruit traits unless there is a high genetic gain, involving additive gene action which can be achieved through selection. Estimated heritability associated with genetic advance is much more reliable than heritability alone to predict the impact of selection. High heritability along with higher genetic advance provides the most effective criteria for selection (Johnson et al. 1955). In the present study, higher heritability estimates with high genetic advance were found for all parameters except fruit length, fruit width, length:breadth of fruit and TSS, indicating that these characteristics are exhibiting additive gene action and phenotypic selection may be more fruitful for all these traits. The higher values of heritability for the particular traits revealed that traits were least affected by environment and selection based on phenotypic performance would be reliable. Similar findings were also reported by several workers (Rajan et al. 2009; Srivastava et al. 2014) who have found high heritability with greater genetic gain for various attributes in other fruits crops.

Correlation at genotypic and phenotypic levels was worked out among 131 litchi clones for all studied traits to know the nature of association existing between the individual traits (Table 2). The correlation coefficient between different traits of litchi clones included in the study revealed significant correlations among the traits. A highly significant and positive association at phenotypic and genotypic levels was found between fruit weight, fruit length, fruit width and pulp weight, while a negative association was found with length:breadth of fruit. The value of genotypic correlation was higher than phenotypic correlation, which indicated that the strong association among the traits was genetic. Knowledge of the relationship between the characteristics may be useful to select suitable clones for litchi breeding. In addition, positive associations among the characteristics reveal that an improvement in one characteristic can improve another characteristic (Yucel et al. 2006). For example, a positive relationship between fruit and quality traits reveals that an improvement in fruit quality traits can also improve yield in litchi.

The correlation coefficient provides information about the relationship between traits, but a dependent trait is a product of interaction amongst several mutually associated component traits, and a change in any one trait may disturb the whole. Path coefficient analysis reveals an effective means of partitioning as direct and indirect association. Path analysis was performed to assess the direct and indirect effects of different characteristics on fruit weight (Table 3). Correlation analysis quantifies only the degree of relationship between two characteristics, it does not provide a reason for such an association. Thus, a non-significant correlation value cannot be used to imply the absence of functional association between two traits. However, path coefficient analysis reveals this by breaking the total correlation coefficient into components of direct and indirect effects. The result of path analysis gives the relative contribution of different traits towards fruit weight. The maximum positive direct effect (Table 3) on fruit weight was through pulp weight (0.9715), skin weight (0.1998) and seed weight (0.1978). The traits of fruit length and fruit width imparted a positive indirect effect on fruit weight. High magnitude of residual effect at the phenotypic level indicated the limitations of characteristics included in the present study that need to be supplemented by more morpho-physiological traits so as to describe the whole range of variation (Table 3). Based on genetic variability and trait association studies (correlation and path analysis), it could be concluded that pulp weight exhibited maximum positive direct effect on fruit weight and could be relied upon for selection of clones to improve fruit weight of litchi. Hence, the utmost importance should be given to these traits during selection for fruit weight. Selection of litchi clones on the basis of these traits would certainly lead to improvement in fruit weight. Furthermore, high genetic variability for studied traits indicated that these clones might be valuable breeding material for future litchi hybridization programs.

PCA with biplot reduces the dimensionality of interrelated variables and visualizes trait–germplasm relationships. The first (PC1: 33%), second (PC2: 17%), third (PC3: 14%), fourth (PC4: 11%) and fifth (PC5: 8%) principal components with eigen values above unity explained the total variability. Together, they explained 85.91% of the total variation (Table 4). The traits that contributed

Table 2 Genotypic (G) and phenotypic (P) correlation coefficients of some important characteristics in litchi on fruit weight

Characteristics		Fruit length (mm)	Fruit width (mm)	Length:breadth	TSS (°Brix)	Seed weight (g)	Skin weight (g)	Pulp weight (g)	Pulp (%)	Borer attack (%)	Acidity (%)	TSS:acid	Fruit weight (g)
Fruit length (mm)	G	1.000	–	–	–	–	–	–	–	–	–	–	–
	P	1.000	–	–	–	–	–	–	–	–	–	–	–
Fruit width (mm)	G	0.767**	1.000	–	–	–	–	–	–	–	–	–	–
	P	0.656**	1.000	–	–	–	–	–	–	–	–	–	–
Length:breadth	G	0.109*	−0.645**	1.000	–	–	–	–	–	–	–	–	–
	P	0.092NS	−0.529**	1.000	–	–	–	–	–	–	–	–	–
TSS (°Brix)	G	−0.042NS	−0.028NS	−0.025NS	1.000	–	–	–	–	–	–	–	–
	P	−0.033NS	−0.016NS	−0.022NS	1.000	–	–	–	–	–	–	–	–
Seed weight (g)	G	0.418**	0.107*	0.320**	0.147**	1.000	–	–	–	–	–	–	–
	P	0.386**	0.098NS	0.281**	0.144**	1.000	–	–	–	–	–	–	–
Skin weight (g)	G	0.395**	0.415**	−0.121*	0.132**	−0.142**	1.000	–	–	–	–	–	–
	P	0.360**	0.386**	−0.104*	0.123*	−0.140**	1.000	–	–	–	–	–	–
Pulp weight (g)	G	0.667**	0.757**	−0.339**	−0.129**	0.102*	0.004NS	1.000	–	–	–	–	–
	P	0.606**	0.705**	−0.296**	−0.120**	0.101*	0.004NS	1.000	–	–	–	–	–
Pulp (%)	G	0.178**	0.408**	−0.412**	−0.159**	−0.269**	−0.396**	0.784**	1.000	–	–	–	–
	P	0.159**	0.371**	−0.348**	−0.151**	−0.258**	−0.383**	0.757**	1.000	–	–	–	–
Borer attack (%)	G	0.062NS	0.147**	−0.125*	−0.120**	−0.149**	−0.090NS	0.357**	0.396**	1.000	–	–	–
	P	0.055NS	0.139**	−0.119**	−0.119**	−0.145**	−0.087NS	0.349**	0.372**	1.000	–	–	–
Acidity (%)	G	0.023NS	−0.069NS	0.120*	0.176**	−0.056NS	0.189**	−0.079NS	−0.122*	−0.033NS	1.000	–	–
	P	0.021NS	−0.066NS	0.106*	0.169**	−0.055NS	0.188**	−0.079NS	−0.117*	−0.031NS	1.000	–	–
TSS:acid	G	0.077NS	0.143**	−0.107*	0.032NS	0.183**	−0.167**	0.164**	0.141**	0.087NS	−0.845**	1.000	–
	P	0.071NS	0.130**	−0.093NS	0.031NS	0.182**	−0.166**	0.163**	0.137**	0.086NS	−0.842**	1.000	–
Fruit weight (g)	G	0.801**	0.827**	−0.283**	−0.060NS	0.277**	0.191**	0.964**	0.594**	0.283**	−0.044NS	0.160**	1.000
	P	0.727**	0.770**	−0.248**	−0.059NS	0.269**	0.189**	0.950**	0.573**	0.273**	−0.044NS	0.157**	1.000

NS non-significant, *, ** $p < 0.05$ and 0.01, respectively

TSS total soluble solids

Table 3 Direct (diagonal) and indirect effect of important characteristics on fruit weight in litchi

Traits		Fruit length (mm)	Fruit width (mm)	Length:breadth	TSS (°Brix)	Seed weight (g)	Skin weight (g)	Pulp weight (g)	Pulp (%)	Borer attack (%)	Acidity (%)	TSS:acid
Fruit length (mm)	G	-0.0123	0.0088	-0.0001	0.0000	0.0827	0.0789	0.6478	-0.0065	-0.0002	0.0004	0.0012
Fruit width (mm)	G	-0.0094	0.0115	0.0005	0.0000	0.0211	0.0829	0.7354	-0.0149	-0.0006	-0.0011	0.0023
Length:breadth	G	-0.0013	-0.0074	-0.0008	0.0000	0.0633	-0.0241	-0.3290	0.0151	0.0005	0.0019	-0.0017
TSS (°Brix)	G	0.0005	-0.0003	0.0000	0.0004	0.0290	0.0264	-0.1253	0.0058	0.0005	0.0028	0.0005
Seed weight (g)	G	-0.0051	0.0012	-0.0002	0.0001	0.1978	-0.0284	0.0989	0.0098	0.0006	-0.0009	0.0029
Skin weight (g)	G	-0.0049	0.0048	0.0001	0.0001	-0.0281	0.1998	0.0042	0.0145	0.0004	0.0031	-0.0027
Pulp weight (g)	G	-0.0082	0.0087	0.0003	-0.0001	0.0201	0.0009	0.9715	-0.0287	-0.0014	-0.0013	0.0026
Pulp (%)	G	-0.0022	0.0047	0.0003	-0.0001	-0.0531	-0.0792	0.7618	-0.0365	-0.0015	-0.0020	0.0022
Borer attack (%)	G	-0.0008	0.0017	0.0001	-0.0001	-0.0295	-0.0180	0.3471	-0.0145	-0.0038	-0.0005	0.0014
Acidity (%)	G	-0.0003	-0.0008	-0.0001	0.0001	-0.0110	0.0378	-0.0772	0.0045	0.0001	0.0161	-0.0134
TSS:acid	G	-0.0010	0.0016	0.0001	0.0000	0.0361	-0.0334	0.1597	-0.0052	-0.0003	-0.0136	0.0158

G Residuals are –0.00870, P Residuals are 0.02176

Table 4 Eigen values and percent of variation with regard to 20 characteristics of litchi as explained by the first five principal components

Traits	PC1	PC2	PC3	PC4	PC5
Fruit weight (g)	0.474	0.111	–0.057	0.117	–0.033
Fruit length (mm)	–0.360	0.330	–0.258	0.116	0.173
Fruit width (mm)	–0.440	0.166	0.014	–0.266	0.019
Length:breadth	0.219	0.143	–0.320	0.507	0.201
TSS (^{0}Brix)	0.056	0.190	–0.105	–0.213	–0.850
Seed weight (g)	–0.064	0.179	–0.557	0.298	–0.210
Skin weight (g)	–0.076	0.461	0.020	–0.485	0.301
Pulp weight (g)	–0.473	–0.027	0.058	0.174	–0.055
Pulp (%)	–0.335	–0.315	0.288	0.219	–0.178
Borer attack (%)	–0.178	–0.200	0.229	0.217	0.034
Acidity (%)	0.085	0.481	0.395	0.285	–0.185
TSS:acid	–0.129	–0.423	–0.459	–0.262	–0.031
Eigen Value	2.015	1.444	1.341	1.167	1.002
Proportion of variance (%)	33.830	17.370	14.990	11.35	8.370
Cumulative variance (%)	33.830	51.210	66.200	77.55	85.910

most weight to the first principal component axis were fruit weight (0.474), length:breadth ratio of fruit (0.219) and acidity in pulp (0.085). These characteristics are relevant criteria for the selection of clones with large fruit size. The second principal component axis was associated mainly with acidity (0.481), skin weight (0.461) and fruit length (0.330). This component reflects that there is a strong

Fig. 1 A plot showing the association among traits correlated with first and second principal components, accounting for 33.83% and 17.37% of total variation, respectively. *Skin_wt* Skin weight, *Fruit_lt* Fruit length, *Fruit_wd* Fruit width, *Fruit_wt* Fruit weight, *Pulp_wt* Pulp weight, *Seed _wt* Seed weight, *Pulp_pc* Pulp percentage, *Borer_pc* Borer percentage, *Lth_breath* Length breath ratio, *TSS_Acid* TSS acidity ratio

genetic correlation between traits responsible for higher yield. The traits that contributed most weight to the third principal axis were pulp content (0.288) and borer attack (0.229). Figure 1 shows the association among various traits. The angle size between any two or more traits is directly proportional to correlation between these characteristics. Higher correlation is indicated by the closer traits (Marboh et al. 2018). Consequently, a high correlation was observed between pulp percentage and borer attack and maximum clones were found clustering in PC2 under the influence of these traits. A high correlation was also observed between TSS and acidity. The traits contributing to maximum divergence should be more focused upon for selection in breeding. These results were consistent with the findings of Marboh et al. (2015) and Ranpise and Desai (2003) in citrus in which genotypes were classified together on the biplot based on fruit attributes. Cluster analysis was performed based on 31 traits (Fig. 2). The dendrogram depicted the phylogenetic relationship among 131 litchi clones. These clones were classified into two main clusters (Cluster I and Cluster II), which were further categorized into different clusters. Basic statistics, i.e., standard deviation (SD), mean, range and coefficient of variation (CV %) of the variables are given in Table 1. The hierarchical clustering method is relevant for analyzing the phenotypic and genetic diversity of germplasm. The clustering method grouped clones of litchi based on the characteristics they possessed. The results of cluster analysis on 131 litchi clones suggested that there are enough variations among the studied clones for different traits (Fig. 2). Clones with greater similarity for morphological traits were placed in the same cluster. Cluster analysis has great practical importance for plant breeders as it distributes the clones into different clusters. Representative clones from each cluster
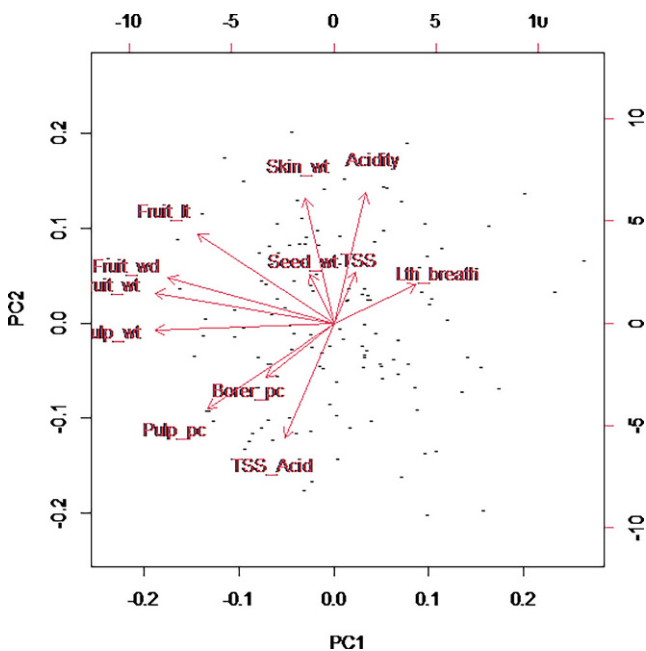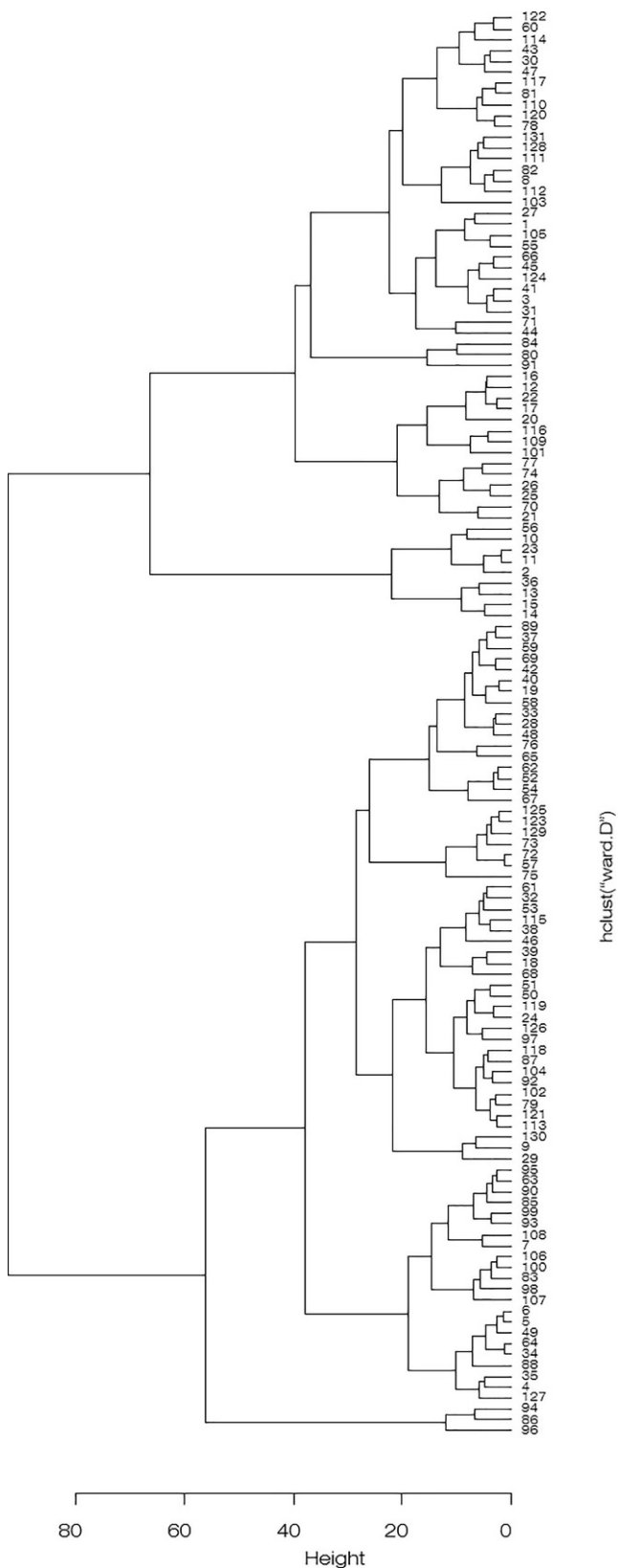
Fig. 2 Neighbor joining cluster analysis of 131 different litchi (*Litchi chinensis* Sonn.) clones

can be selected for use in crop improvement programs. Maximum variation in the populations can be achieved by selecting and using clones from diverse clusters. The information on relationships obtained from these studies may be useful in the exploitation of the available germplasm resources. Cluster I consisted of 75 clones, while Cluster II comprised 56 clones. Clones 86, 94 and 96 were separated from Cluster I. Clone 86 had low seed weight and borer infestation and high TSS:acidity ratio. Clone 94 had high skin weight and acidity, while clone 96 had higher fruit width and borer infestation and lower TSS, pulp content and acidity. Similarly, clones 13, 14, 15 and 36 were separated from Cluster II. Clone 15 had larger fruit size as well as high TSS and pulp content.

Conclusion

The study revealed that PCV and GCV were high for TSS acid ratio, acidity, skin weight, pulp weight and seed weight. Fruit weight was positively correlated with fruit length and width as well as pulp weight. Fruit length and width imparted a positive indirect effect on fruit weight. Therefore, indirect selection practiced on these characteristics will result in the improvement of respective characteristics and ultimately fruit yield. The present studies also indicated that pulp weight, skin weight and seed weight have a direct effect on fruit weight. Therefore, emphasis should be put on the selection of these characteristics for the improvement of yield in litchi, which was the main aim of the present study. Crossing can be done between clones from clusters that are genetically distant from one another to obtain new recombinant litchi, since the size of heterosis depends largely on the level of genetic diversity of parents.

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