

Original Research Article

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D² Statistic Techniques used for Analysis of Genetic Divergence among Litchi Hybrids

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ABSTRACT

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Litchi (*Litchi chinensis* Sonn.) fruit is generally recognized as “Queen of the fruits”. It is highly prized for its excellent quality, pleasant flavour, juicy pulp and attractive red peel colour. Despite of its commercial importance, the study on breeding aspect particularly genetic improvement of available germplasm is lacking. The current study on the D²-based divergence analysis using Euclidian and Tocher’s methods indicated the existence of appreciable amount of genetic diversity in the eighteen hybrids of litchi. Test hybrids were grouped into five clusters based on different quantitative traits using above methods with variable number of entries in each cluster. In Euclidian’s method, cluster 1 had maximum number of hybrids (7) followed by cluster 3 (4), 2 (3), 4 (2) and 5 (2), while in Tocher’s method, cluster I had utmost number of hybrids (10), while the cluster III, IV and V contained the least (1). Maximum inter cluster distance indicate that hybrids falling in these clusters had wide diversity and can be used for improvement programme to get better recombinants in the segregating generations. The hybrids H-400, H-515 and H-711 recognized good for genetic improvement in litchi hybrids for commercial purposes.

Introduction

Litchi (*Litchi chinensis* Sonn.), member of sapindaceae family and sub family nepheleae, have originated in southern china, northern Vietnam, and the Malayan peninsula, where it has been cultivated for centuries (Menzel and Simpson, 1990). It is an important subtropical evergreen tree adapted to the areas of cool dry winters and warm wet summers is grown for its brilliant fruit quality, characteristic pleasant flavour and attractive red colour. Currently the leading litchi-producing countries in the world are China, India, Thailand, and Vietnam

(Menzel, 2002). India is the second largest litchi producing country next to China with an area of 84.2 thousand hectare, production 5.85 lakh tones and productivity 7 MT/ha (NHB 2014). Variability is the basic requirement within the population for the success of any breeding programme. As genetically diverse parents when crossed, it may bring diversity among gene combinations either to cultivate heterosis or to obtain superior recombinants. Therefore, prior to initiation of any breeding programme they should be tested and extent of variability present must be adequately assessed, so that the breeding programme

could yield the desirable results with maximum possible characters. To employ or exploit the available variability present in the genetic material, the divergence studies based upon some desirable/suitable parameters is very essential and of highest significance. Keeping in view, the above genetic divergence and cluster analysis using D^2 statistics was undertaken with the objectives to assess the variability present among the eighteen litchi hybrids and potential use of this variability for improvement programmes. Use of Mahalanobis D^2 statistics to evaluate the net divergence in breeding for crop improvement has been indicated by number of workers in different fruit crops (Saran *et al.*, 2007). The use of genetically divergent parents in hybridization under transgressive breeding programme is dependent upon categorization of breeding material on the basis of appropriate criteria (Santos *et al.*, 2011). Apart from providing requisite assistance in selection of divergent parents in hybridization, D^2 statistics also adequately assists in the measurement of diversification and in the contribution of the relative proportion of each component trait towards the total genetic divergence.

Materials and Methods

The present investigations were carried out in the Department of Horticulture, Bihar Agricultural University, Sabour- Bhagalpur (Bihar) during 2015-16. This particular zone is the hot spot for litchi production. Eighteen hybrids of litchi evaluated in the present study (Table 1a). The hybrids were grown in a randomized complete block design (RCB). Focusing on these twenty quantitative and qualitative characters viz., fruit weight (FW), aril weight (AW), seed weight (SW), peel weight (PW), fruit length (FL), fruit diameter (FD), aril percentage (A%), seed percentage (S%), peel percentage (P%), aril seed ratio (A/S), fruit length width ratio (FL/FW),

inflorescence length (IL), inflorescence width (IW), leaflet-length (LL), leaflet-width (LW), leaf let length width ratio (LL/LW), number of leaflets leaf⁻¹ (LPL), total soluble solids (TSS) were given and observed (Table 1b). Mahalanobis D^2 statistic was used for assessing the genotypic variance among the populations (Mahalanobis, 1936). The generalized distance between any two populations is given by formula:

$$D^2 = \sum \sum \lambda_{ij} \sigma_{ai} \sigma_{aj}$$

Where, D^2 = Square of generalized distance; λ_{ij} = Reciprocal of the common dispersal matrix; $\sigma_{ai} = (\mu_{i1} - \mu_{i2})$; $\sigma_{aj} = (\mu_{j1} - \mu_{j2})$; μ = General mean.

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized character mean (Xs) to standardized uncorrelated variable (Ys) was done to shorten the computational procedure.

The D^2 values were calculated as the sum of squares of the differences between pairs of corresponding uncorrelated (gs) values of any two uncorrelated genotype of D^2 value. All $n(n-1)/2$ D^2 value were clustered using Toucher's method described by Rao (1952). The intra cluster distances were calculated by the formula given by Singh and Choudhary (1997):

$$\text{Square of the inter cluster distance} = \sum D_i^2 / n$$

Where, $\sum D_i^2$ is the sum of distance between all possible combinations of the entries included in a cluster and n is number of all possible combinations.

The inter cluster distances were calculated by the formula described by Singh and Choudhary (1997):

Square of the intra cluster distance = $\Sigma D^2_i/n_i n_j$
Where, ΣD^2_i is the sum of distances between all possible combinations ($n_i n_j$) of the entries included in the clusters under study. n_i is number of entries in cluster i and n_j is number of entries in cluster j . The criteria used in clustering by this method was that any two genotype belongs to the same cluster, at least on an average, show a small D^2 value than those belonging to two different clusters.

Results and Discussion

On the basis of D^2 analysis, all the eighteen hybrids of litchi were grouped into five clusters by Euclidean's and Tocher's methods (Table 2). The intra cluster distances ranged from 86.836 to 287.703 by Euclidean's methods indicating that the hybrids in clusters have dissimilarity for morphological features and performance. The members of cluster 5 and 2 exhibited maximum divergence (inter cluster distance 519.599) followed by the members of cluster 4 and 2 (inter cluster distance 488.119) and cluster 4 and 1 (inter cluster distance 446.275). The members of cluster 3 and 1 were least divergent (inter cluster 210.645). The mutual relationships among five clusters are represented pictorially (Fig. 1a). The inter cluster distances were larger than the intra cluster distances indicating a wider genetic diversity among genotypes of clusters with respect to trait considered. Maximum inter cluster distance is indicative that hybrids falling in these clusters had wide diversity and can be used for improvement programme to get better recombinants in the segregating generations. Inter and intra cluster divergence values (D^2) by Tocher's method between and within five clusters are presented in the Table 2. Highest value of intercluster distance were existed between member of cluster IV and IV (328.740), followed by member of cluster V and IV (217.19) and cluster V and III (209.742) while it was lowest (101.859) between cluster III and I. The intra cluster

distance was maximum (92.284) for cluster II and minimum (0.00) for cluster III, IV, and V. The mutual relationships among five clusters are presented in diagram (Fig. 1).

Lowest inter cluster between clusters were indicative of close relationship and similarity for most traits in the genotypes hence selection of parents from these clusters is to be avoided. However, in all cases the inter cluster distance were greater in studied conducted earlier on other fruit crops viz. Rajan *et al.*, (2009) in mango, Rajamanickam and Rajmohan (2010) in banana, Barua and Sharma (2003) in apple, Singh *et al.*, (2003) in pomegranate and Rai and Misra (2005) in bael. It is concluded that hybrids of litchi with wide genetic variation accompanied with useful characteristics could be efficiently employed in specific crosses with the hope that this would lead to the transmission of higher genetic gain for different reputed traits with more emphasis on yield related traits from practical utility point of view. On the basis of relative magnitude of D^2 values, the test hybrids were grouped into five clusters (Table 3) based on different quantitative traits using Euclidian's (1-5) and Tocher's (I-V) method with variable numbers of entries in each cluster, indicating the presence of genetic diversity among the genotypes of present study. Cluster-1 included highest number of hybrids comprising (7): H-98, H-524, H-526, H-594, H-518, H-609, H-515 followed by cluster-3 comprising (4): H-141(E), H-517, H-140, H-573 while lowest number of hybrids included in cluster-4 and 5 comprising (2): H-400, H-520 and H-580, H-711 respectively. Cluster-I had maximum number of hybrids comprising(10): H-98, H-524, H-526, H-594, H-141(E), H-517, H-140, H-573, H-520, H-609 followed by cluster-II comprising (5): H-104, H-141(W), H-518, H-503, H-580 whereas, cluster-III, IV and V had minimum number of genotypes comprising (1): H-515, H-400 and H-711 respectively.

Table.1a List of litchi hybrids used in the present study

S. No.	Treatments (Hybrids)	Parentage
1.	H-98	Purbi X Early Bedana
2.	H-104	Purbi X Early Bedana
3.	H-140	China X Bedana
4.	H-141(W)	China X Bedana
5.	H-141(E)	China X Bedana
6.	H-400	Dehrrarose X Purbi
7.	H-503	Ojhauri X Purbi
8.	H-515	Purbi X Bedana
9.	H-517	Purbi X Bedana
10.	H-518	Purbi X Bedana
11.	H-520	Purbi X Bedana
12.	H-524	Late Bedana X China
13.	H-526	China X Bedana
14.	H-573	Purbi X Kasba
15.	H-580	Purbi X Bedana
16.	H-594	Purbi X Bedana
17.	H-609	Purbi X Early Bedana
18.	H-711	Purbi X Bedana

Table.1b Characters of litchi considered for evaluation

S. No.	Characters
1.	Fruit weight (gm)
2.	Aril weight (gm)
3.	Seed weight (gm)
4.	Peel weight (gm)
5.	Fruit length (mm)
6.	Fruit diameter (mm)
7.	Aril %
8.	Seed %
9.	Peel %
10.	Aril/seed ratio
11.	Fruit length/width ratio
12.	Inflorescence length (cm)
13.	Inflorescence width (cm)
14.	Leaf blade length (cm)
15.	Leaf blade width (cm)
16.	Leaf length/width ratio
17.	leaflets/leaf
18.	TSS %
19.	Acidity %
20.	TSS/Acid ratio

Table.2 Average of intra (diagonal) and inter cluster distance of litchi among 18 hybrids by Euclidian's and Tocher's methods

Clusters [1-5(Euclidian's method)/ I-V(Tocher's method)]					
	Cluster 1/I	Cluster 2/II	Cluster 3/III	Cluster 4/IV	Cluster 5/V
Cluster 1	137.932	378.343	210.645	446.275	369.606
Cluster I	47.217	102.714	101.859	135.280	109.448
Cluster 2		219.871	347.572	488.119	519.599
Cluster II		92.284	179.768	189.398	164.932
Cluster 3			86.836	225.402	292.338
Cluster III			0.000	328.740	209.742
Cluster 4				205.935	407.586
Cluster IV				0.000	217.119
Cluster 5					287.703
Cluster V					0.000

Table.3 Mean inter and intra cluster distance among four clusters in litchi hybrids on the basis of D² Statistics by Euclidian's method (1-5) and Tocher's method (I-V)

Cluster Euclidian's method	No. of hybrids	Name of hybrids	Cluster Tocher's method	No. of hybrids	Name of hybrids
C1	7	H-98, H-524, H-526, H-594, H-518, H-609, H-515	CI	10	H-98, H-524, H-526, H-594, H-141(E), H-517, H-140, H-573, H-520, H-609
C2	3	H-104, H-141(W), H-503	CII	5	H-104, H-141(W), H-518, H-503, H-580
C3	4	H-141(E), H-517, H-140, H-573	CIII	1	H-515
C4	2	H-400, H-520	CIV	1	H-400
C5	2	H-580, H-711	CV	1	H-711

Table.4 Cluster means for different characters of litchi among 18 hybrids by Euclidian's and Tocher's methods

Characters	Clusters [1-5(Euclidian's method)/I-V(Tocher's method)]				
	1/I	2/II	3/III	4/IV	5/V
Fruit weight	17.30	16.91	18.07	17.92	13.20
	16.92	15.85	26.27	18.47	13.09
Aril weight	11.44	10.68	11.01	10.46	9.04
	10.62	10.29	18.79	10.22	8.60
Seed weight	2.70	2.23	2.76	3.13	1.78
	2.68	2.09	3.54	3.31	2.28
Peel weight	2.68	3.40	3.87	3.82	2.03
	3.15	2.94	3.43	4.50	1.98
Fruit length	32.90	31.77	34.92	35.44	29.67
	33.58	31.98	35.60	37.37	26.88
Fruit diameters	30.48	29.13	31.28	30.51	28.20
	30.54	28.77	35.22	30.20	28.62
Aril percentage	65.03	64.09	60.80	58.58	68.22
	62.38	65.71	71.20	55.57	65.14
Seed percentage	15.99	12.80	15.20	17.37	13.68
	15.98	12.84	13.69	17.81	17.75
Peel percentage	16.00	19.48	21.61	21.15	15.53
	18.73	18.06	13.05	24.23	15.31
Aril seed ratio	4.29	3.60	2.89	2.89	4.45
	3.55	3.88	5.46	2.34	4.32
Fruit length and width ratio	1.08	1.10	1.12	1.16	1.05
	1.10	1.11	1.01	1.24	0.94
Inflorescence length	19.09	26.94	20.67	17.42	19.33
	19.03	24.60	22.00	17.33	18.00
Inflorescence width	12.59	18.22	14.71	13.67	8.54
	13.03	16.05	14.00	13.83	7.67
Leaf-blade length	10.19	12.17	12.58	16.00	17.75
	11.83	12.47	7.00	19.67	18.33
Leaf-blade length and width ratio	3.35	4.94	2.81	2.87	3.67
	3.05	4.44	3.25	2.88	3.74
No. of leaflets per leaf	7.05	6.89	7.17	7.00	7.33
	7.07	7.07	7.33	6.67	7.33
Total soluble sugar	17.98	21.33	19.81	19.27	18.10
	18.81	19.93	17.30	20.43	18.37
Titration acidity	0.61	0.46	0.56	0.26	0.72
	0.52	0.61	0.53	0.28	0.79
TSS and acid ratio	34.36	56.09	36.31	74.17	25.62
	40.61	43.02	33.34	75.05	24.07

Fig.1a Cluster diagram depicting intra and inter-cluster distances based on Euclidian's method

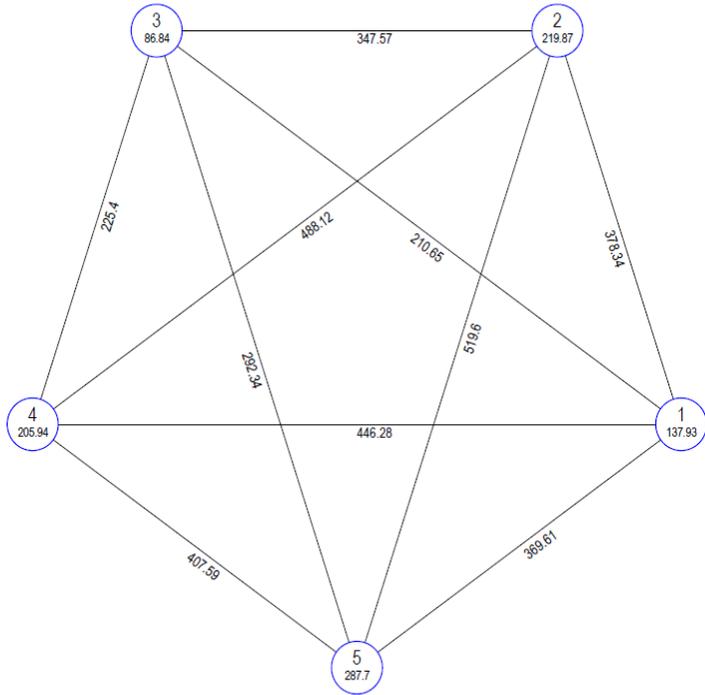


Fig.1b Cluster diagram depicting intra and inter-cluster distances based on Tocher's method

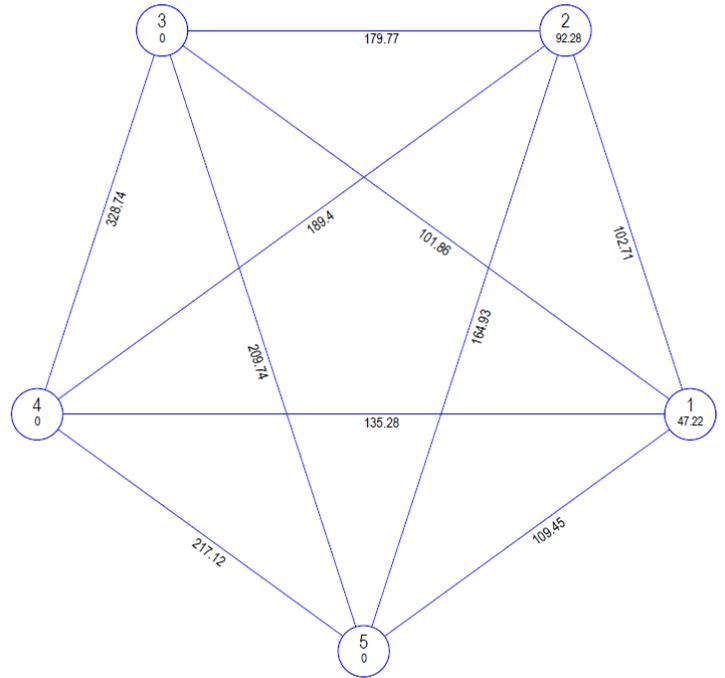


Fig.2a Clustering pattern of 18 litchi hybrids by wards minimum variance dendrogram (Euclidian's method)

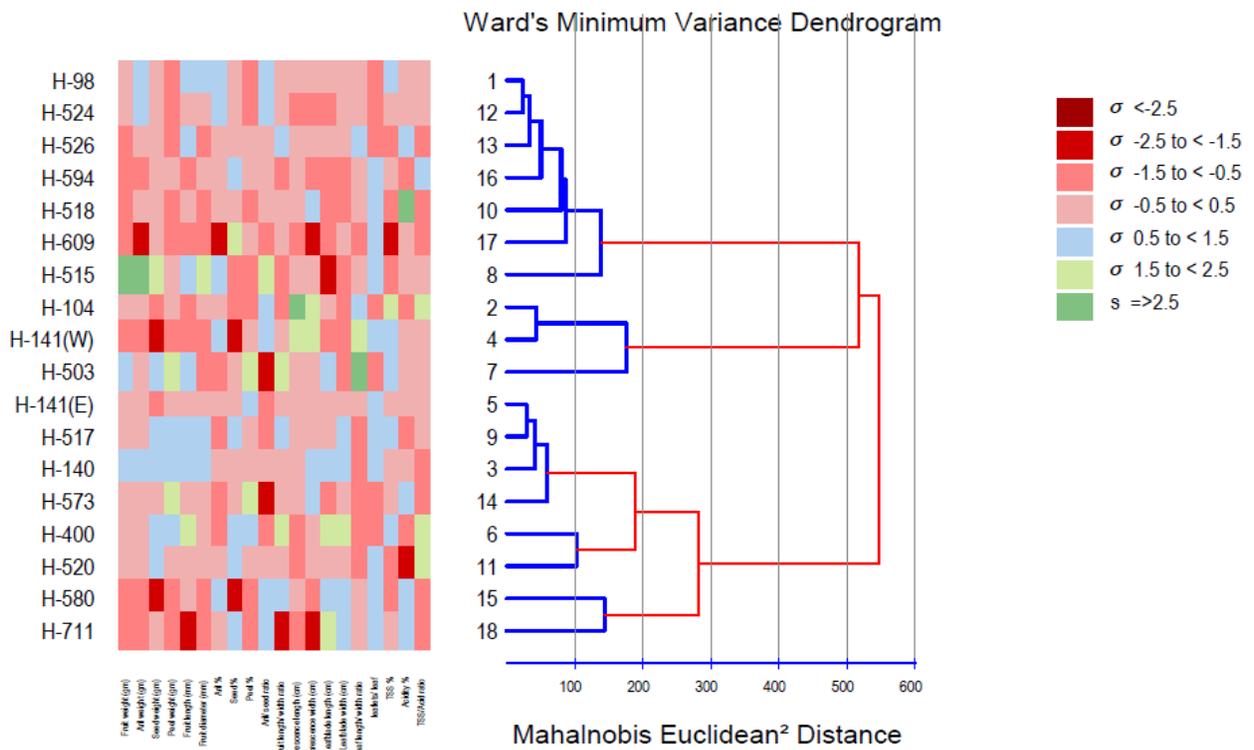
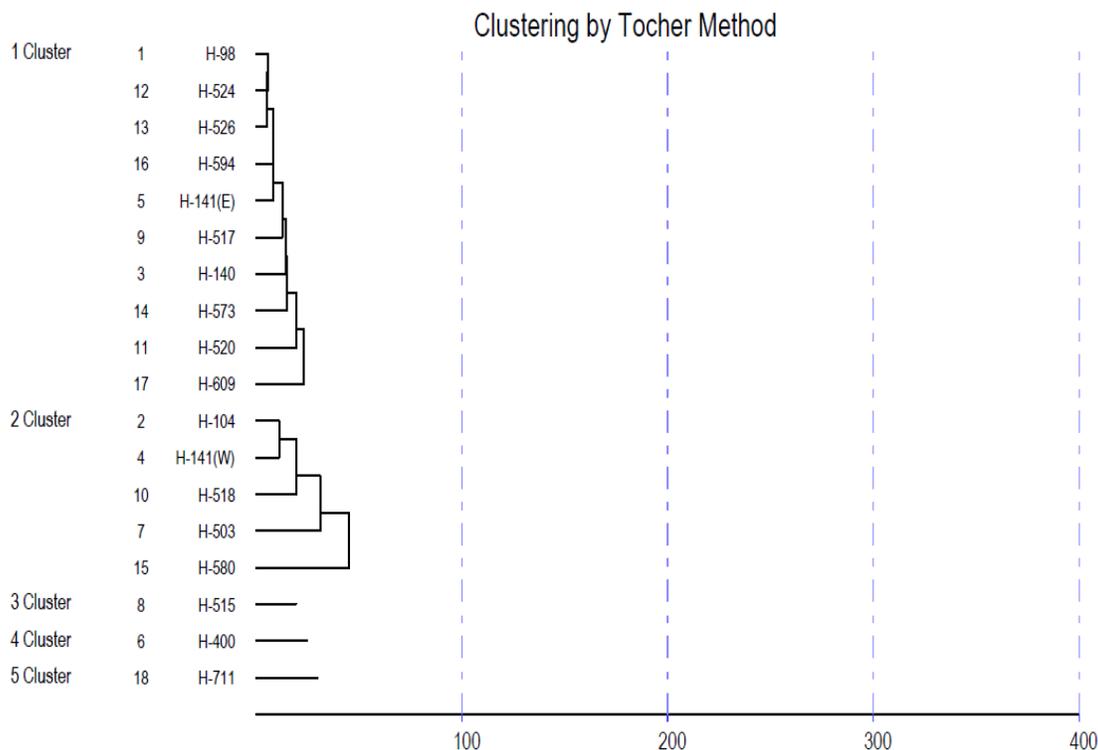


Fig.2b Clustering of 18 litchi hybrids based on Tocher's method



The dendrogram of 18 litchi hybrids was constructed based on morphological data in order to examine the genetic diversity by Euclidean's and Tocher's method (Fig.2a and 2b). Hybrids grouped within the same cluster in the dendrogram were basically related to the original sources of the hybrids used as parents in the breeding program. In general, the hybrids sharing common parents or with at least one of the parents tend to group together.

The cluster mean value for twenty characters is presented in Table 4. Considerable differences in cluster mean values were evident for all the characters. It can be seen from the cluster means that each cluster has its uniqueness that separated it from other characters. Cluster 1 was characterized by maximum aril weight (11.44) and low mean value for leaf-blade length (10.19) and total soluble sugar (17.98). Maximum mean value for inflorescence length (26.94), inflorescence

width (18.22), leaf-blade length and width ratio (4.94) and total soluble sugar (21.33) whereas minimum mean value for seed percentage (12.80) and no. of leaflets per leaf (6.89) were represented by cluster 2. Cluster 3 was characterized by highest mean value for fruit weight (18.07), peel weight (3.87), fruit diameters (31.28) and peel percentage (21.61) and least value for aril seed ratio acidity (2.89) and leaf-blade length and width ratio (2.81). Cluster 4 was characterized by maximum mean value for seed weight (3.13), fruit length (35.44), seed percentage (17.37), fruit length and width ratio (1.16), TSS and acid ratio (74.17) and least mean value for aril percentage (58.58), aril seed ratio (2.89), inflorescence length (17.42) and titrable acidity (0.26). Cluster 5 was characterized by maximum mean value for aril percentage (68.22), aril seed ratio and acidity (4.45), leaf-blade length (17.75), no. of leaflets per leaf (7.33), titrable acidity (0.72) whereas minimum mean value for fruit weight (13.20),

aril weight (9.04), seed weight (1.78), peel weight (2.03), fruit length (29.67), fruit diameter (28.20), peel percentage (15.53), fruit length and width ratio (1.05), inflorescence width (8.54) and TSS and acid ratio (25.62).

Maximum and minimum mean value was not recorded in cluster-I. Cluster-II had maximum mean value for inflorescence length (24.60), inflorescence width (16.05) and leaf-blade length and width ratio (4.44) while minimum mean value was observed in seed weight (2.09) and seed percentage (12.84). In cluster-III, maximum mean value observed for the characters fruit weight (26.27), aril weight (18.79), seed weight (3.54), fruit diameter (35.22), aril percentage (71.20), aril seed ratio (5.46), no. of leaflets per leaf (7.33) whereas minimum mean value observed for the character peel percentage (13.05), leaf-blade length (7.00) and total soluble sugar (17.30).

Cluster-IV was characterized by maximum mean value for peel weight (4.50), fruit length (37.37), seed percentage (17.81), peel percentage (24.23), fruit length and width ratio (1.24), leaf-blade length (19.67), total soluble sugar (20.43) and TSS and acid ratio (75.05) and minimum mean value for aril percentage (55.57), aril seed ratio (2.34), inflorescence length (17.33), leaf-blade length and width ratio (2.88), no. of leaflets per leaf (6.67) and titrable acidity (0.28). Cluster- V was characterized by maximum no. of leaflets per leaf (7.33) and titrable acidity (0.79) and low mean value for fruit weight (13.09), aril weight (8.60), peel weight (1.98), fruit length (26.88), fruit diameters (28.62), fruit length and width ratio (0.94), inflorescence width (7.67) and TSS and acid ratio (24.07).

In above investigation, the hybrids H-400, H-515 and H-711 recognized best on the basis of both Euclidian's as well as Tocher's methods to contribute in future breeding efforts aimed

at genetic improvement in litchi hybrids for commercial achievement of production.

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