Biospectra : Vol. 5 (1), March 2010, pp 105-110
An International Biannual Refereed Journal of Life Sciences

Plant Sciences

ISSN : 0973-7057

Differentiation of litchi genotypes based on leaf biochemical parameters: a feasibility study

Archana Prasad[a], Bikash Das* & Manju Gerard[a]

[a]ICAR Research Complex for Eastern Region, Research Centre, Plandu, Ranchi 834010, Jharkhand

[b]Allahabad Agricultural Institute-Deemed University, Allahabad 211007, U.P.

Received 21st December, 2009; Revised 27th February, 2010

Abstract : A study was undertaken at ICAR Research Complex for Eastern Region, Research Centre, Ranchi during 2006 with the objective to differentiate the litchi genotypes statistically, based on content of different biochemical constituents. Leaf samples collected from 25 genotypes of 25 year old litchi trees at 2 stages from fruit set to maturity were analyzed for content of Total free amino acid, total soluble protein, total phenol and total carbohydrate. At both the stages of observation, significant differences among the genotypes could be recorded for all the four parameters. During both the stage of observation, the content of total free amino acids was found to he the maximum in genotype Purbi whereas the genotype CHL-3 contained the maximum leaf carbohydrate content at both the stages of observation. Cluster analysis presented at as dendrograms based on Euclidean Distance coefficients resulted in grouping the entire genotypes in to 5 primary clusters. Although the phenotypically similar genotypes were present in the first cluster, a number of phenotypically distinct genotypes were also found under the same cluster. The genotypes Shahi and China which are distinct from each other in terms of morphological as well as phenological characters, were found to have close affinity. However, the cultivar Swarna Roopa which is phenotypically distinct from other genotypes was found as single member in a different cluster. Hence, classification of genotypes through D^2-analysis based on leaf biochemical characters like total free amino acid, total phenol, total soluble protein and total carbohydrate was found to be insufficient, although the method holds promise by inclusion of more number of biochemical parameters.

Key words: Litchi, Leaf biochemical, D^2 analysis

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a member of Sapindaceae family originated in China and is widely distributed in tropic and sub-tropics. Although a crop of Chinese origin with a narrow genetic base (Rai *et al.* 2001), heterogygosity arising out of sexual method of propagation has given rise to new genotypes. During the course of its dissemination these cultivars were misidentified or renamed i.e. the same cultivar may be known under different names in different countries or even with in a country and different cultivars sometimes may be kept under the same name (Menzel and Simpson, 1990). Cultivars have been classified on the basis of major economic traits (fruit size, shape, pulp thickness sweetness, season of ripening and seed size) in China and on the basis of growth and flowering pattern (flushing pattern, color, flowering ability and fruit characters) in India (Arora *et al.* 1996; Singh, 1998).

Xuequin (1995) reported 20-25 main litchi types possessing characters of larger fruit size, small stone, thick and free aril of good quality with good flavor having canning quality from South China. However, cultivar synonymy does exist due to the insufficient information on characterization for different fruit morphological traits and delineation of the characters in its original name. Only few cultivars can be distinguished based on their fruit characters and maturity period, which creates confusion in identifying the suitable cultivar for any specific region. Rai *et al.* (2002), worked on differentiation of 17 litchi genotypes based on fruit morphological parameters which indicated a very narrow range of criterion for differentiating among the different genotypes. However, using morphological trait

*Correspondent author :
Mobile : 9431169835

for cultivar identification is limited due to interaction with environmental condition. It is therefore important to have biochemical markers for cultivar identification and verification because they exhibit co-dominant expression and do not show environmental effects (Torres and Bergh, 1980). Characterization of litchi cultivar by using different biochemical parameters in plant tissues offers an effective tool for clear cut differentiation of plant genotypes and similar study has also been reported by Singh *et al.* (1987). Although the content of different biochemical constituent in plant tissue is influenced by environmental factors, the pattern of change as influenced by the environmental condition of a particular location will be same for all the genotypes growing in the same locality. Changes in the biochemical composition in leaf have been reported also to be associated with differential phenophases of the plants. Fruits being the major sink in the plant during its developmental stage may induce changes in the leaf biochemical composition. Hence, characterization of leaf biochemical composition at different stages of fruit growth may facilitate genotypic characterization. Multivariate analysis by D^2 statistics is a powerful tool in quantifying the degree of divergence among all possible pair. Keeping this in view, a study was undertaken with 25 litchi genotypes with the objective to differentiate the litchi genotypes statistically, based on content of different biochemical constituents.

MATERIALS AND METHODS

The study was conducted at the ICAR Research Complex for Eastern Region, Research Centre, Ranchi during fruiting season of 2006. Leaf samples were collected from 25 genotypes of 25 year old litchi trees growing at the Farm number 2 of ICAR RCER, RC, Ranchi, at 2 stages from fruit set to maturity. Fully matured leaves below the panicle were collected for analysis of different biochemical constituents. The leaf samples were brought to the laboratory, immediately after collection and processed for extraction of different biochemical (total phenol, total carbohydrate ,total free aminoacid ,total soluble protein) for their analysis.The content of total phenol was estimated by using Folin-Ciocalteau reagent, content of total soluble protein was estimated spectrophotometrically using Lowry's method, content of total free amino acids was estimated spectrophotometrically using ninhydrin reagent and The content of total carbohydrate was estimated spectrophotometrically using Anthrone method. (Thimmaiah, 1999). Amino acid profiling was done by thin layer chromatography (TLC) using silica gel coated plates (Thimmaiyah, 1999)

The experiment was laid out in a Randomized Block Design with 3 replications. For differentiation of the litchi genotypes statistically, based on content of different biochemicals constituents, Under this experiment, the data obtained from different biochemical (total phenol, total carbohydrate ,total free aminoacid ,total soluble protein) were subjected to D^2 analysis for by Toucher method and genotypes were clustered in a Dendrogram using statistical software (WINDOWSTAT).

RESULTS AND DISCUSSION

Morphological similarities existing among the litchi genotypes in India has resulted in emergence of opinion among litchi taxonomists on cultivar synonymy. Segregation of genotypes based on biochemical markers can provide an alternative for overcoming the problem of genotypic clustering due to failure of distinguishing the genotypes based on perceivable morphological differences. In the first part of the experimentation, efforts were made to determine the pattern of variation among the genotypes with respect to content of different biochemical parameters in leaf at two different stages during fruit growth. Data on content of total free amino acids, total phenol ,total soluble protein and total carbohydrate in leaves of the litchi genotypes have been presented in table 1. During both the stages of observation, the genotypes differed significantly with respect to content of total free amino acids in leaf. During first stage of observation, the maximum content of total free amino acid in leaf was observed in case of genotype Purbi (3.65 mg/g) which was at par with that in case of the genotypes China, Trikolia, Longia, Bombaiya I, Bombaiya II, Kasba, Dehradun, Shahi, CHES-2, CHL-4, CHL-5 and CHL-6 and the minimum (0.52 mg/g) was recorded in case of the genotype Ajhauli. During the second stage of observation, an in general increase in the content of total free amino acid could be observed in all the genotypes. As in case of the first stage, the maximum content of total free amino acid in leaf was also recorded in case of the genotype Purbi which was at par with that in case of the genotypes Rose Scented, Yogda Selection,

Trikolia in the second stage. In the present study, the content of total free amino acids was the maximum in late maturing genotype like Purbi and minimum in the genotype Ajhauli which is the earliest maturing genotype under Ranchi conditions.

Plant phenolics have been extensively studied for genotypic identification. Concentration of phenolics has been successfully used by Scalabrelli *et al.* (2004) for identification of groups of presumed clones of grape classifiable into one or more biotypes. In the present study, as in case of total free amino acids, the genotypes differed significantly with respect to content of total phenol in leaves during both the stages of observation. During the first stage of observations, the genotype Trikolia recorded the minimum leaf phenolic content (2.78 mg/g) while the maximum was recorded in case of the genotype CHL-3 (17.30 mg/g). During the second stage of observation, the content of total phenolics in leaves ranged from 2.56 mg/g (Ajhauli) to 8.32 mg/g (Sarguja Selection 1).

Significant differences among the genotypes could also be recorded with respect to the content of total soluble proteins in leaves at both the stages of observation. The content of total soluble protein in leaves ranged from 6.26 mg/g (Sarguja Selection 2) to 37.49 mg/g (Longia) during the first stage of observation whereas during the second stage it ranged from 6.49 (Rose Scented) to 56.97 (CHES II).

Varietal differences in the content of carbohydrate in leaf have been studied by many workers. Popa and Popa (1975) classified grape genotypes based on frost tolerance by considering the content of carbohydrate in dormant buds. In the present experiment, the content of carbohydrate in leaves in different litchi genotypes also differed significantly at both the stages of observation. During the first stage of observation, the maximum content of carbohydrate was recorded in case of the genotype CHL-5 (8.94 %) which was at par with that in case of CHL-3 (8.69 %) the minimum being observed in case of Rose Scented (1.32 %) which was at par with that in case of Yogda Selection (1.83%). During the second stage of observation, the maximum content of carbohydrate in leaves was recorded in case of Shahi (9.52%) which was at par with that in case of CHL-3 (8.37%) the minimum being observed in case of the genotype Green (2.73%).

Consistencies in the rank of the genotypes during both the stages of observation with respect to the content of different biochemical can be a measure of effectiveness of the parameter to be considered for classification of genotypes. An insight into the rank consistencies of different genotypes at both the stages of observation indicated that the maximum number of genotypes ranked under the same group during both the stages of observation was recorded in case of the content of total free amino acid (5 numbers), followed by that in case of the content of carbohydrate (3 numbers), the minimum being in case of the content of total phenol in leaves (0 number). This hinted at the effectiveness of the parameters like total free amino acid content and total carbohydrate content of leaves to be considered as a criterion for classification of litchi genotypes. In their studies on variability among grape genotypes with respect to different biochemical parameters, Giridharan and Jindal (1999) reported high heritability and genetic advances in the content of total free amino acids in grape. However, the variability among the genotypes with respect to fruit growth and maturity stage might also have contributed towards rank inconsistencies of other genotypes at the two stage of observation. Correlation studies among the different parameters indicated significant positive correlation between content of total free amino acid and total soluble protein (0.69) in the leaf during the first stage of observation, between total phenol and total carbohydrate content during both stages of observation (0.57 and 0.42 during first and second stages of observation, respectively).

Pattern of amino acid profile in leaves also provide a useful tool for classification of genotypes. The data on amino acid profile of different genotypes as observed in the present study is presented in table 2. It is evident from the table that an in-general increase in the number of amino acids could be observed during the second stage of observation than that in case of the first stage of observation in all the genotypes. During the first stage, the number of amino acids observed ranged from 4 (Longia, Purbi, Kasba, Dehradun, Shahi and Swarna Roopa) to 10 (CHL-6 and CHL-3) while during the second stage, the maximum number of amino acids was observed in case of the genotype CHL-6 (16). The mean of Rf values recorded during the first stage of observation ranged from 0.24 (Bombaiya 1) to 0.50 (China) while during the second stage of observation the maximum and minimum values of mean

of Rf values was recorded in case of the genotypes Late Bedana (0.24) to CHL-6 (0.45). Measure of coefficient of variation of Rf values indicated the maximum variation in case of the genotype Bombaiya-I (61.14%) during the first stage of observation while during the second stage of observation the maximum variation in Rf values was recorded in case of the genotype CHL-5 (77.36%).

Clustering of genotypes based on D^2 –analysis is an efficient method for genotypic classification. In fruit crops a number of research works had been carried out for genotypic classification based on cluster analysis. The Results of cluster analysis using Toucher method is presented in Figure 1 as dendrograms based on Euclidean Distance coefficients. As evident from the figure, the entire genotypes can be grouped in to 5 primary clusters. In the first cluster, the genotypes, Deshi, Green, Late Bedana, D. Rose, Ajhauli, Bedana, Bombaiya-1, China, Shahi, Rose Scented, Yogda Selection, CHL-4,Sarguja Selection-2, Trikolia, Kasba, Purbi, Bombaiya-II, CHL-3 and Dehradun were observed in ascending order of distance coefficient between the pairs. In the second and third clusters, only one genotype each viz., Swarna Roopa and CHL-5 respectively were found. The fourth cluster consisted of the genotypes Longia, CHL-6 and CHES-2. In the fifth cluster, only one genotype i.e. Sarguja Selection-1 could be observed. It must be noted here that although the phenotypically similar genotypes were present in the first cluster, a number of phenotypically distinct genotypes were also found under the same cluster. The genotypes Shahi and China which are distinct from each other in terms of morphological as well as phenological characters, were found to have close affinity. However, the cultivar Swarna Roopa which is phenotypically distinct from other genotypes was found as single member in a different cluster. Hence, classification of genotypes through D^2-analysis based on leaf biochemical characters like total free amino acid, total phenol, total soluble protein and total carbohydrate was found to be insufficient, although the method holds promise by inclusion of more number of biochemical parameters.

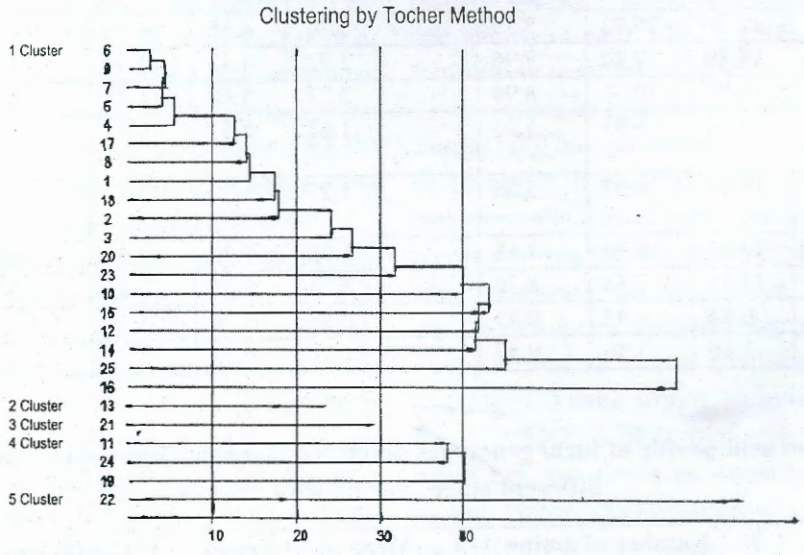
Table 1. Variability among litchi genotypes with respect to leaf biochemical composition at different stages during fruit growth

Genotypes	3rd week of March				2nd week of April			
	Total free amino acid (mg/g)	Total phenol (mg/g)	Total soluble protein (mg/g)	Carbohydrate (%)	Total free amino acid (mg/g)	Total phenol (mg/g)	Total soluble protein (mg/g)	Carbohydrate (%)
China	6.51	14.00	2.10	2.57	5.94	14.38	5.48	8.62
Rose Scented	10.19	12.10	1.33	1.81	6.39	6.41	6.97	13.94
Yogda	11.18	9.70	2.05	1.41	3.69	23.15	6.23	13.99
Ajhauli	4.69	9.91	1.99	0.53	2.57	12.41	4.94	9.26
D.Rose	5.99	9.18	1.94	1.73	5.43	8.29	7.90	8.96
Deshi	8.15	11.06	3.29	1.15	3.03	10.17	3.01	8.14
Late Bedana	5.18	10.52	2.75	1.20	3.28	14.17	3.91	11.78
Bombaiya	3.19	15.86	1.34	2.70	3.47	18.70	5.25	10.56
Green	4.23	14.52	1.84	1.77	4.33	10.22	2.74	9.54
Trikolia	2.79	22.16	2.16	3.33	7.90	13.56	7.98	12.71
Loungia	5.21	37.50	5.14	2.37	4.11	37.25	7.28	8.86
Purbi	7.77	22.58	5.35	3.66	4.05	13.52	3.08	14.30
Swarnroop	5.04	36.23	5.83	3.54	5.52	9.44	3.87	11.97
Bombaiya-II	5.11	23.56	5.08	2.87	4.59	21.47	11.66	4.40
Kasha	9.10	17.73	7.46	2.55	7.62	16.74	7.30	5.94
Dehradun	13.03	20.82	5.04	3.02	8.57	20.62	7.86	3.79
Bedana	7.17	14.25	5.47	1.61	4.07	14.07	4.73	4.03
Shahi	3.59	17.94	6.09	2.66	5.78	7.90	9.53	4.59

CHES-2	16.70	26.72	5.39	3.70	6.65	56.98	9.01	4.19
CHL-4	14.89	9.10	3.96	2.72	4.43	14.99	5.15	2.94
CHL-5	16.95	20.03	8.94	2.27	5.69	11.71	4.93	3.02
Sarjuga Sale-1	10.64	6.85	5.03	1.67	8.33	46.47	7.64	3.23
Sarguja Sale-2	15.17	6.27	5.38	1.27	6.25	22.77	7.34	3.25
CHL-6	17.01	16.70	7.58	3.03	7.26	42.96	3.69	4.65
CHL-3	17.31	7.38	8.70	1.53	5.08	23.85	8.38	3.42
SEm	0.84	1.92	0.43	0.65	0.26	2.34	1.21	0.82
C.D. at 5%	1.69	3.86	0.87	1.31	0.52	4.71	2.43	1.64

Table 2. Leaf Amino acid profile of litchi genotypes developed through Thin-Layer Chromatography at two different stages during fruit growth

Genotypes	Number of amino acids observed		Mean of Rf values observed		Coefficient of variation in Rf values	
	3rd week of March	2nd week of April	3rd week of March	2nd week of April	3rd week of March	2nd week of April
China	6	8	0.50	0.28	37.54	50.97
Rose Scented	7	11	0.48	0.31	28.33	49.64
Yogda Selection	6	7	0.34	0.25	43.33	58.44
Ajhauli	5	5	0.30	0.26	40.91	45.50
D.Rose	5	6	0.30	0.28	41.85	49.16
Deshi	6	7	0.28	0.27	45.26	59.49
Late Bedana	7	7	0.28	0.24	58.31	51.36
Bombaiya I	6	5	0.24	0.24	61.14	53.20
Green	5	9	0.34	0.28	42.11	54.88
Trikolia	6	9	0.31	0.27	58.80	47.61
Longia	4	10	0.30	0.34	44.02	63.76
Purbi	4	6	0.31	0.30	52.01	34.55
Swarna Roopa	4	7	0.33	0.25	46.74	61.93
Bombaiya-II	7	7	0.35	0.25	44.74	61.76
Kasba	4	8	0.37	0.27	35.16	47.10
Dehradun	4	8	0.35	0.35	47.44	54.61
Bedana	5	7	0.41	0.28	40.11	44.57
Shahi	4	8	0.31	0.33	47.99	48.26
CHES-2	5	12	0.37	0.35	44.36	59.17
CHL-4	5	8	0.29	0.25	48.02	76.46
CHL-5	7	7	0.43	0.31	51.30	77.36
Sarjuga Sel-1	5	13	0.47	0.37	47.59	55.67
Sarguja Sel-2	6	9	0.43	0.28	58.61	76.90
CHL-6	10	16	0.41	0.45	48.15	51.18
CHL-3	10	10	0.42	0.34	48.50	62.05



1- China, 2- Rose Scented, 3- Yogda, 4- Ajhauli, 5- D.Rose, 6- Deshi, 7- Late Bedana, 8- Bombaiya I, 9- Green, 10- Trikolia, 11- Longia, 12- Purbi, 13- Swarna Roopa, 14 - Bombaiya-II, 15- Kasba, 16- Dehradun, 17-Bedana, 18- Shahi, 19- CHES-2, 20- CHL-4, 21- CHL-5, 22- Sarjuga Sel-1, 23- Sarguja Sel-2, 24- CHL-6, 25- CHL-3

REFERENCES

1. **Arora, R.K., Rao, V.R. and A.N. Rao** 1996. Status report on genetic resources of litchi in China. *IPGRI Regional Office for Asia, the Pacific and Oceania, Singapore:* 1-18.
2. **Giridharan, M.P. and Jindal, P.C.** (1999) Correlation, regression and path coefficient analysis of biochemical parameters associated with duration of crop growth in grape (*Vitis vinifera*). *Indian Journal of Agricultural Sciences.* 69:261-264
3. **Menzel .C.M. and D.R.Simpson.** (1990)Performance and improvement of lychee cultivars .A review *Fruit Var.J.*44:197-215.
4. **Popa, E and Popa, V.G.** (1975) Biochemical aspects of frost resistance in grape in relation to variety, training and pruning. *Anale Institutul de Cercetari Pentru Viticultura si Vinificatie.*7:185-197
5. **Rai, Mathura, Bikash Das, Vishal Nath, Manoj Kumar and Rajesh Kumar Rai** (2002) Fruit characteristics of litchi cultivars under Chotanagpur conditions. *Orissa J.Hort.*30(2) 1-5
6. **Rai, Mathura, Vishal Nath and P.K. Dey.** 2001. Litchi, C.H.E.S., Ranchi, India.
7. **Scalabreli, G; Loreti, F; Ferroni, G. and D-Onofrio** (2004) Clonal selection of Sangivese in Tuscany. *Acta Horticulturae.*652: 35-43
8. **Singh, Ajay; Abidi, A.B.; Surendra Srivastava and Singh I.S.** (1987) Correlations among physical and biochemical traits in litchi (*Litchi chinensis* Sonn.). *Narendra Deva Journal of Agricultural Research* 2: 2, 160-163.
9. **Singh, H.P.** 1998. Genetic diversity, breeding and utilisation of the gene pool of litchi. *In*: Tropical Fruits of Asia: Diversity, maintenance, conservation and use (R.K. Arora and V.R. Rao eds) IPGRI Office for South Asia, New Delhi: 185-195.
10. **Thimmaiah, S.K.** (1999) Standard Methods of Biochemical Analysis. Kalyani Publishers, Ludhiana, India
11. **Torres, A.M and B.O.Bergh.** (1980)Fruit and leaf isozymes as genetic markers in avocado .*J Amer.Soc Hort.Sci.*105:614-619.
12. **Xuequin, Z.** 1995. Genetic resources of tropical fruit species in China. In: Proceedings of the Expert Consultation on Tropical Fruit Species of Asia (R.K. Arora and V.R. Rao Ed). IPGRI Office for South Asia, New Delhi: 103-111.

* * *