



Horticulture

Studies on genetic diversity of citrus in east Siang district of Arunachal Pradesh

Archan Rabha, L. Wangchu* and B. Singh

Department of fruit science, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India

*Email: lobsang1974@gmail.com

Paper no: 95 Received: 09th November 2012 Received in revised form: 19th November 2012 Accepted: 27th February 2013

Abstract

The present study on variability, heritability, genetic advance as percentage of mean and isozyme analysis in 32 citrus genotypes were carried out for yield and yield attributing characters. The genotypes exhibited significant differences for all the characters under study. A wide range of variability in PCV and GCV was observed for leaf lamina length, leaf lamina width, ratio (leaf lamina length; leaf lamina width), leaf thickness, fruit length, fruit breadth, fruit weight, rind weight, rind thickness, juice content, no. of seed per fruit, seed weight, seed length, seed breadth, TSS%, acidity%, ascorbic acid, reducing sugar, total sugar and yield. High heritability and high genetic gain were observed for leaf lamina length, leaf lamina width, ratio (leaf lamina length and leaf lamina width), leaf thickness, fruit length, fruit breadth, fruit weight, rind weight, rind thickness, juice content, no. of seed per fruit, seed weight, seed length, seed breadth, TSS%, acidity%, ascorbic acid, reducing sugar, total sugar and yield, where as rind weight showed low genetic gain. The result revealed that genotypic correlation coefficient values were higher in magnitude than phenotypic correlation coefficient values. Moreover, peroxidase isozymes analysis on selected 32 Citrus genotypes revealed 12 polymorphic kochi and 52 alleles with Rm values ranging from 0.20 to 0.90.00.

©2013 New Delhi Publishers. All rights reserved

Keywords: Citrus, Genetic variability, heritability, isozyme

Citrus (*Citrus sp.*) is the most important fruit crops of the family Rutaceae grown commercially throughout the country and known to world over for their characteristics flavour, attractive range evergreen foliage and flowers as well as the extraordinary fragrance are the added aesthetic value of citrus trees and is considered as third most important fruit crops after mango and banana. It is grown in acreage of 846 (000) hectares with the production of 7464 (000) MT and productivity of 8.8 MT/ha respectively (NHB 2010-11). Though production of citrus in Indian plains is high, it is reduced to a great extent in North Eastern region due to lack of quality planting materials and poor orchard management, which necessitates to explore for advance techniques that can manage higher demand of this crop.

North-Eastern region is considered as one of the bio-diversity hot spot, has a good diversity of citrus species (Hazarika, 2012). The state of Arunachal Pradesh is one of the major centres of diversity of citrus in the North eastern region of India (Singh, 2010). The main citrus belts of the Arunachal Pradesh are East Siang, West Siang, Upper Siang and parts of Lower Subansari districts. The role of genetic variability, its transmissibility into the progeny and extent of the inheritance are of paramount importance in selecting the best breeding approach. Biometrical techniques are used for systematic assessment of variability instead of age-old visual method (Frey, 1966). Therefore, the present investigation was initiated with the aim to study the variability with respect to the physio-chemical and biochemical markers with the presumption that the results might be of

practical use to those who are engaged in citrus breeding programme.

Materials and methods

The present experiment was carried out at college of Horticulture and Forestry, Central Agricultural University, Pasighat during 2009-2011. The selected genotypes were collected from five villages of East Siang district of Arunachal Pradesh viz., Balek, Boying, Rengging, Ledum, Tekang and experimental farm of College of Horticulture and Forestry, Central Agricultural University. Thirty two diverse genotypes viz., T₁ (Khasi mandarin), T₂ (Hill mandarin), T₃ (Nagpur mandarin), T₄ (Cleopatra mandarin), T₅ (Kinnow mandarin), T₆ (Trifoliate orange), T₇ (Valencia -42), T₈ (Valencia -5), T₉ (Valencia -47), T₁₀ (Lucknow Mosambi), T₁₁ (Pummelo - 1), T₁₂ (Pummelo - 2), T₁₃ (Samphola - 4), T₁₄ (Pummelo -27), T₁₅ (Pummelo - 4), T₁₆ (Grape fruit - 85), T₁₇ (Acid lime - 2), T₁₈ (Acid lime - 15), T₁₉ (Acid lime - 22), T₂₀ (Acid lime - 59), T₂₁ (Acid lime - 60), T₂₂ (Acid lime - 1), T₂₃ (Acid lime - 4), T₂₄ (Assam Lemon), T₂₅ (Pant Lemon), T₂₆ (Citron - 37), T₂₇ (Citron - 23), T₂₈ (Kumquat), T₂₉ (Kamala Australia), T₃₀ (*Citrus volkamiriana*), T₃₁ (Citron - 3)

and T₃₂ (*Citrus latipes*). The experiment was done in Randomized Block Design (RBD) with three replication. Observations were recorded on twenty economic characters of selected genotypes. The data were subjected to analysis of variance as per procedure described by Panse and Sukhatme (1978). The genotypic and phenotypic coefficient of variations was calculated by Burton (1952). Heritability in broad sense and genetic gain was expressed as percentage according to Allard (1960). The correlation coefficients were undertaken as per procedure suggested by Al-Jibouri *et al.*, (1958) and Miller *et al* (1958). Polyacrylamide gel electrophoresis was performed in a vertical slab apparatus following the standard method given by Zuber and Manibhushan (1982). Similarity index percentage was calculated by Subhadrabandhu and Suriyapananont (1998).

Results and discussion

The analysis of variance for all the characters indicated highly significant variation among the genotypes which revealed the existence of sufficient variability in the germplasm (Table 1). The range of variation was highest in fruit weight followed by

Table 1: Analysis of variance for different character in citrus

Sl No.	Source of variation	Genotype	Mean square	Error
	Replication			
	df	2	31	62
1	Leaf lamina length	16.478	49513.717**	209.937
2	Leaf lamina width	0.966	11190.355**	141.178
3	Ratio (L/W)	0.012	9.532**	0.746
4	Leaf thickness	0.000	12.296**	0.046
5	Fruit length	1.873	66266.19**	110.05
6	Fruit breadth	0.576	6180.04**	170.511
7	Fruit weight	2448.678	2702617.123**	63299.345
8	Rind weight	199.474	752912.114**	7228.997
9	Rind thickness	0.114	703.970**	1.273
10	Juice content	1.137	333171.167**	236.342
11	No. of seed per fruit	11.732	11752.918**	245.174
12	Seed weight	0.036	142.59**	0.957
13	Seed length	0.465	1631.951**	7.407
14	Seed breadth	0.444	720.170**	7.042
15	Total soluble solids (%)	0.187	457.166**	5.012
16	Acidity %	0.001	5.566**	0.120
17	Ascorbic acid	3.176	2089.028**	250.694
18	Reducing sugar	0.064	134.971**	4.674
19	Total sugar	0.187	457.166**	5.012
20	Yield per tree	864.074	159677.301**	6025.583

**Significant at 0.01%.



rind weight, juice content, yield per tree, fruit length and fruit breadth indicating good variability among genotypes used in present study. These results are in agreement with those of Hossain and Haque (1977), Kumar *et al.*, (1996) and Mitra and Maity (2000) in jackfruit. The magnitude of phenotypic coefficient of variation (PCV) was greater than the corresponding genotypic coefficient of variation (GCV) for all the characters (Table 2). High PCV and GCV, were recorded for rind weight, leaf thickness, reducing sugar, fruit weight, juice content, number of seed per fruit, acidity, seed weight, seed breadth, rind thickness, seed length, fruit breadth and fruit length. Similar, observation was also made by Maity *et al.*, (2001) and Saikia (2004). This reflects greater genetic variability among genotypes for these characters for making further improvement. The magnitude of heritability ranged from 65.63 to 99.89%.

Maximum heritability was found for juice content followed by fruit length, rind thickness, fruit breadth, leaf thickness, leaf lamina length and seed length which showed that the selecting in these characters would be effective (Table 2). The values of genetic advance as percentage of mean (genetic gain) ranged from 24.49 to 228.51.

High heritability couple with high genetic gain was observed in leaf lamina length, leaf lamina width, fruit length, fruit weight,

fruit breadth, rind thickness, juice content, number of seed per fruit seed weight, acidity, reducing sugar and total sugar indicating that these characters are more reliable for effective breeding selection. Similar results were reported by Prasad and Rao (1989) in acid lime, in litchi by Sarkar *et al.*, (1991), in guava by Bandopadhyay *et al.*, (1992) and Mitra and Maity (2000) in jackfruit.

The phenotypic and genotypic correlation coefficients among different characters were worked out in all possible combinations. Phenotypic correlation coefficient (Table 3) Fruit yield per plant was found to be a positive and significantly correlated with leaf lamina length, leaf lamina width, fruit length, rind weight, rind thickness and seed length; while positive correlation was found with ratio (leaf lamina length/leaf lamina width), leaf thickness, fruit breadth, fruit weight, juice content, number of seed per fruit, seed weight, seed breadth, TSS and reducing sugar. Similar finding was reported by Kumar *et al.* (1986) in sapota; Shamsundaran *et al.*, (1993) in banana and Dwivedi (1998) in papaya.

While genotypic correlation coefficient (Table 4) indicated significant and positive correlation of fruit yield/tree with leaf lamina length, leaf lamina width, ratio (leaf lamina length/leaf lamina width), fruit length, rind thickness and seed length. While a positive correlation was found in leaf thickness, fruit

Table 2: Genetic parameters for yield and yield attributing characters in citrus

Characters	Mean	Range	Coefficient of variability (%)		Heritability (%)	Genetic advance	Genetic advances % of mean
			PCV %	GCV %			
Leaf lamina length	82.51	53.24 - 134.00	28.04	27.95	99.37	47.33	57.37
Leaf lamina width	41.17	20.18 - 63.37	26.81	26.56	98.13	22.31	54.20
Ratio L/W	2.02	1.46 - 3.12	16.42	15.65	90.91	0.61	30.15
Leaf thickness	0.33	0.14 - 2.23	111.56	111.24	99.43	0.74	228.51
Fruit length	69.52	30.17 - 123.00	38.43	38.39	99.75	54.92	78.98
Fruit breadth	63.19	7.20 - 109.81	40.85	40.76	99.58	52.95	83.80
Fruit weight	185.00	21.56 - 712.53	93.22	91.61	96.57	343.06	185.43
Rind weight	74.18	3.20 - 268.28	121.88	121.01	98.57	183.58	24.49
Rind thickness	6.41	2.88 - 12.89	42.95	42.89	99.74	5.65	88.27
Juice content	66.04	3.13 - 219.43	90.66	90.61	99.89	123.21	186.56
No. of seed/fruit	21.29	5 - 48	53.35	52.53	96.94	22.68	106.54
Seed weight	2.72	0.04 - 5.12	45.54	45.31	98.99	2.53	92.87
Seed length	10.27	3.10 - 19.23	40.86	40.72	99.32	8.59	83.60
Seed breadth	6.27	1.91 - 11.73	44.57	44.25	98.55	5.67	90.48
TSS%	8.35	4.50 - 12.32	23.19	22.82	96.80	3.85	46.20
Acidity%	0.48	0.11 - 1.45	51.35	50.19	95.52	0.48	101.06
Ascorbic acid	20.24	11.78 - 31.00	24.77	22.69	83.92	8.67	42.82
Reducing sugar	1.20	0.05 - 4.00	102.13	99.53	94.98	2.39	199.81
Total sugar	2.21	0.20 - 10.21	18.33	14.85	65.63	3.69	166.48
Yield	289.71	212.56 - 432.27	14.57	14.17	94.55	82.21	28.38



Table 3: Phenotypic coefficient of correlation for different characters of citrus

Character	Leaf length mm	Leaf width mm	Leaf area cm ²	Leaf thickness mm	Fruit length mm	Fruit width mm	Fruit weight g	Fruit volume cm ³	Pulp content %	No. of seed per fruit	Seed length mm	Seed width mm	Seed volume cm ³	Seed weight g	Acidity %	Alkalinity %	Fasting sugar %	Total sugar %	Total acid %
Leaf length mm	1.00	0.86**	0.24	0.52*	0.72*	0.45**	0.72**	0.72**	0.32*	0.32	0.77*	0.32*	0.32*	0.32*	0.17*	-0.42**	0.25*	-0.15*	0.69**
Leaf width mm		1.00	-0.26	0.44	0.77*	0.41*	0.68**	0.62**	0.21	0.18	0.77*	0.31*	0.31*	0.31*	0.36*	-0.46**	0.17*	-0.16*	0.49**
Leaf area cm ²			1.00	0.66*	0.68*	0.31*	0.25*	0.21*	0.36*	0.20	0.78*	0.33*	0.33*	0.33*	0.20	0.16*	0.76*	-0.05*	0.62*
Leaf thickness mm				1.00	0.72*	0.34*	0.73**	0.35*	0.41**	0.32	0.78*	0.33*	0.33*	0.33*	0.12*	-0.12*	0.11*	-0.05*	0.42*
Fruit length mm					1.00	0.73**	0.87**	0.65**	0.33*	0.31*	0.85**	0.45**	0.45**	0.45**	0.41*	-0.53**	0.39*	0.10	0.34*
Fruit width mm						1.00	0.62**	0.70**	0.24	0.21	0.76*	0.34*	0.34*	0.34*	0.33*	-0.36*	0.09*	0.09*	0.26*
Fruit weight g							1.00	0.80**	0.72**	0.42**	0.85**	0.41**	0.41**	0.41**	0.41*	-0.73**	0.38*	0.10	0.25*
Fruit volume cm ³								1.00	0.73**	0.42**	0.85**	0.41**	0.41**	0.41**	0.41*	-0.73**	0.38*	0.10	0.25*
Pulp content %									1.00	0.36**	0.77*	0.33*	0.33*	0.33*	0.20	0.16*	0.76*	-0.05*	0.62*
No. of seed per fruit										1.00	0.77*	0.31*	0.31*	0.31*	0.36*	-0.46**	0.17*	-0.16*	0.49**
Seed length mm											1.00	0.77*	0.31*	0.31*	0.36*	-0.46**	0.17*	-0.16*	0.49**
Seed width mm												1.00	0.77*	0.31*	0.36*	-0.46**	0.17*	-0.16*	0.49**
Seed volume cm ³													1.00	0.77*	0.36*	-0.46**	0.17*	-0.16*	0.49**
Seed weight g														1.00	0.77*	0.36*	-0.46**	0.17*	0.49**
Acidity %															1.00	0.77*	0.36*	-0.46**	0.17*
Alkalinity %																1.00	0.77*	0.36*	0.17*
Fasting sugar %																	1.00	0.77*	0.36*
Total sugar %																		1.00	0.77*
Total acid %																			1.00

*Significant at P = 0.05, ** Significant at P = 0.01

Table 4: Genotypic correlation of different characters in citrus

[illegible]*Significant at $P=0.05$; +Significant at $P=0.01$

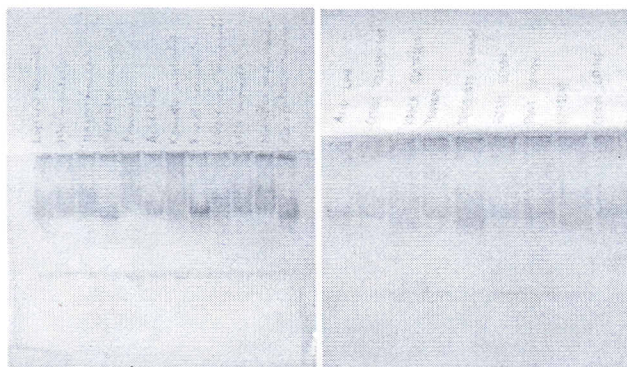


Figure 1: Photographs of polymorphic bands of peroxidase isozymes of selected citrus genotypes



Figure 2: Photograph of selected citrus genotypes

breadth, fruit weight, rind weight, juice content, number of seed per fruit, seed weight, seed breadth, TSS and total sugar. This finding is in consonance with the findings of Dwivedi (1997) in litchi and Dwivedi and Mitra (1995) in litchi.

Peroxidase isozymes analysis on selected citrus genotypes revealed 12 polymorphic loci and 52 alleles with R_m values of 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.70 and 0.90 (Table 5). The genotypes having single banding patterns were T_8 , T_9 , T_{12} , T_{13} , T_{14} , T_{17} with 0.30; T_{11} , T_{16} with 0.35; T_{27} with 0.40 and T_7 with 0.45 R_m values, whereas, the R_m values of genotypes with double banding patterns were T_2 , T_3 and T_4 with 0.25 and 0.50; T_{10} with 0.25 and 0.55; T_{19} , T_{20} , T_{24} , T_{25} , T_{28} , T_{29} , T_{31} and T_{32} with 0.30 and 0.60; T_1 , T_6 , T_{18} , T_{23} and T_{30} with 0.35 and 0.60; T_5 with 0.40 and 0.90; T_{22} with 0.40 and 0.65 and T_{15} with 0.20 and 0.70 R_m values (Table 5). Maximum genotypes had relative mobility value of 0.30, 0.35 followed by 0.60 and least in 0.20, 0.75 and 0.90 (R_m value). Cabrita *et al* (2001) studied four isozyme systems in citrus, which revealed seven loci, four of which were polymorphic. Gogorcena *et al* (1990) also identified nine mandarin genotypes with the aid of isozyme

(peroxidase) analysis in extracts from the rind and leaves. Thus from the present study it has been observed that there is wide variability among the 32 selected genotypes with respect to physio-chemical and biochemical characters, which can be effectively utilized in citrus improvement programme.

References

- Allard, R.W., 1960. Principles of Plant Breeding. John Wiley and Sons. Inc., New York.
- Al-Jibouri, H.A., Miller, P.A., and Robinson, H.P., 1958. Genetic and environmental variance and covariance in upland cotton cross of interspecific origin. *Agron J* **50**: 633-637.
- Bandopadhyay, A., Sarkar, T.K., and Gayen, P., 1992. Variability, heritability and correlation studies in physical characters of leaf and fruit in guava. *Crop Res* **5**:107-111.
- Burton, G.W., 1952. Quantative interaction in grasses. In: *Proc. 6th Inter Grassland Congress* 1: 277-283.
- Cabrita, L., Elisario, P., Leitao, J., Guerreiro, A., Dore C (ed.), Dosba, F (ed.) and Baril, C., 2001. Assessment of the genetic relationships among Citrus species and varieties by isozyme and RAPD markers. *Acta Hort* **546**: 177-181.
- Chandra, A., and Bhatnagar, P., 2000. Physico-chemical characteristics of Kagzi lime fruits. *Agri Sci Digest* **22**: 117-119.
- Chadha, K.L., 1993. Strategy for optimization of productivity and utilization of sapota (*Manilkara achras*, (Mill) Forberg). *Ind J Hort* **49**: 1-17.
- Desai, U.T., Ranpise, S.A. and Kale, P.N., 1993. Improvement of acid lime (*Citrus aurantifolia* Swing.) through selection. (In) Abst. Golden jubilee Symposium Horticulture Society India 21-28 May 1993, Bangalore, p. 18.
- Dwivedi, A.K., and Mitra, S.K., 1995. Genotypic correlation and path analysis in litchi (*Litchi chinensis* Sonn.). *Ind Agri* **39**: 57-61.
- Dwivedi, A.K., 1998. Correlation and regression study of fruit weight and its component in papaya (*Carica papaya* L.). *Orissa J Hort* **26**:50-52.
- Frey, K.L., 1966. Plant Breeding. Iowa state University Press, Iowa, USA.
- Gogorcena, Y., Zubrzycki, H., and Ortiz, J.M., 1990. Identification of mandarin hybrids with the aid of isozymes from different organs. *Scientia Horticulturae* **4**(14): 285-291.
- Hossain, M., and Haque, A., 1977. Studies on the physical characteristics of jackfruit. *Bangladesh Horticulture* **5**: 9-14.
- Jature, S.D., and Chakrawar, V.R., 1981. Variability studies in kagzi lime strains. *Punjab Hort J* **21**: 157-160.
- Kumar, V.R., George, K.C., and Nair, N.K., 1986. Comparative study of the contribution of the biometric characters on yield in dessert varieties of banana. *AgriRes J Kerala* **22**:155-160.
- Lu, L.X., Ye, H., and Simmonds, N.V., 1992. The genetic difference of peroxidase isozymes among some cultivars and wild forms of banana in Fujian, China. *Junior Fujian Agricultural College* **21**:177-180.
- Maity, C.S., Samanta, A.K., and Mitra, S.K., 2001. Genetic variability, heritability and genetic advance relating to some fruit



- characters in pummelo (*Citrus grandis* Osbeck.) genotypes. *Indian Agricul* **45**: 19–23.
- Mandal, A.B., Chowdhury, B., Aparna, M., Elanchezian, R., Maiti, A., and Chowdhury, B., 2001. Isoenzyme markers in varietal identification of banana. In *Vitro Cellular and Developmental Biology Plan* **37**:599–604.
- Mitra, S.K., and Maity, C.S., 2000. Genetic resources of jackfruit (*Artocarpus heterophyllus* Lamk.) in West Bengal, India. *Acta Hort* **575**:269–271.
- Miller, P.A., William, C.V., Robinson, H.F., and Comstock, R.E., 1958. Estimates of genotypes and environmental variance and covariance in upland cotton and their implication in selection. *Agron J* **50** : 126–131.
- Panse, V.G., and Sukhatme, P.V., 1978. Statistical Methods for Agricultural Workers. Publication Information Division, ICAR, New Delhi.
- Prasad, A., 1987. Correlation studies on growth behaviour and fruit characters with yield components in mango. *Ind J Hort* **44**:188–193.
- Prasad, M.B.N.V., and Rao, G.S.P., 1989. Genetic variability, correlation and path coefficient analysis for some morphological and biochemical constituents of acid lime fruit. *Scientia Hort* **41**: 43–53.
- Rai, M., Gupta, P.N., Rana, R.S., and Dwivedi, R., 1993. Variability and characters associated analysis in identified aonla (*Emblica officinalis* Gaertn.) germplasm. (In) Abst. Golden Jubilee Symposium Horticulture Society of India 24–28 May 1993, Bangalore, p. 22.
- Sarkar, T.K., Bandaphadhyay, A., and Gayen, P., 1991. Studies on genetic variability and heritability of some characters in litchi (*Litchi chinensis* Sonn.). *Haryana J Hort Sci* **20**:56–59.
- Shamsundaram, K.S., Reddy, B.M., and Rao, G.S.P., 1993. Correlation studies in robusta banana. (In) Abst. Golden jubilee Symposium Horticulture Society India 24–28, May 1993, Bangalore, p. 424.
- Subhadrabandhu, S., and Suriyapananont, S., 1998. Peroxidase isozymes pattern of some sweet tamarind (*Tamarindus indica* L) varieties in Thailand. *J Issaas* **4**:42–56.
- Zambrano, J., Quintero, I., Alvarez, R., Hortegano, R., and Saenz, M., 2001. Evaluation of fruit quality of 'Valencia' oranges in three altitudinal levels in Trujillo State, Venezuela. *Agronomia Tropical Maracay* **51**: 107–117.
- Zuber, M., and Manibhushan, Rao., 1982. Studies on comparative gel electrophoretic patterns of protein and enzyme from isolates of *Rhizotonia solani* causing sheath blight disease of rice. *Can. J. Microbio* **8**:762–771.
- Hazarika, T.K., 2012. Citrus genetic diversity of north-east India, their distribution, ecogeography and ecobiology. *Genet Resour Crop Evol* **59**: 1267 – 1280
- Singh, I.P., 2010. Exploration, collection and characterization of Citrus germplasm from Meghalaya, Assam and Arunachal Pradesh. *Progre Hort* **42** : 39–43.