J. Hortl. Sci.
Vol. 6(2):148-155, 2011

Short communication

Assessment of genetic diversity in wild raspberry (*Rubus ellipticus* Smith) native to North-Western Himalayan region

Dinesh Singh, K. Kumar and Vikas Kumar Sharma
Department of Fruit Science, University of Horticulture & Forestry
Nauni, Solan - 173230, India
E-mail: dinesh_hort@yahoo.com

ABSTRACT

Nature and magnitude of genetic diversity was assessed in 170 wild raspberry genotypes based on eight quantitative characteristics, viz., fruit weight, fruit length, fruit breadth, TSS, acidity, reducing sugars, non-reducing sugars and Vitamin C. A survey was conducted in three north-western Himalayan states of Himachal Pradesh, Jammu & Kashmir and Uttarakhand. The species was found to be distributed between 760 and 1950m AMSL, 30^{O}10’159" to 33^{O}04’693"N and 74^{O}44’076" to 78^{O}25’681"E. The non-hierarchical cluster analysis resulted in 12 clusters of genotypes. The cluster pattern did not exhibit any interrelation between geographical isolation and genetic diversity. Of the 170 genotypes, 31 fell in Cluster XII, 27 in Cluster V, 19 in Cluster I, 17 in Cluster IX, 16 in Cluster VIII, 15 in Cluster XI, 13 in Cluster II, 12 in Cluster VII, 10 in Cluster III, six in Cluster X, three in Cluster VI and one genotype in Cluster IV. Genotypes falling under Clusters III, VI , VI can be used as parents in hybridization programmes for improving important traits like TSS, fruit weight and acidity respectively.

Key words: Raspberry, genetic diversity, cluster analysis

Using genetic resources for developing sustainable solutions to basic crop-constraints has been suggested from time to time but these resources have not been exploited fully due to the inherent problem of their large size and lack of adequate evaluation and classification (Dahlberg, 1995). Germplasm exploration, maintenance, evaluation and characterization with reference to economically important traits is a pre-requisite for improvement programmes in any crop.

The yellow Himalayan raspberry (*Rubus ellipticus* Smith) is one of the tastiest wild fruits, growing in abundance throughout the North-Western Himalayas. Besides providing essential nutrients required in the human diet, this fruit has great potential in agro-processing industries as squash, jam, yoghurt and ice-cream (Singh and Kumar, 2001; Singh *et al*, 2009). Due to cross-pollination coupled with predominance of sexual propagation, the wild population exhibits a high degree of genetic diversity for economically important characters. Improvement in yield and quality of highly cross-pollinated crops like wild raspberry is generally achieved by selecting from naturally occurring populations genotypes with desirable characters.

Mahalanobis (1936) D^{2} statistics based on multivariate analysis of quantitative traits is a powerful tool for measuring divergence in a set of population. Therefore, an attempt was made to study geographical distribution *vis-a-vis* multivariate analysis of genetic divergence of wild raspberry naturalized in the North-Western Himalayas.

An exploration in the North-Western Himalayas was undertaken during the years 2007 and 2008 in the sub-tropical to wet-temperate areas of Solan, Shimla, Mandi, Kullu and Chamba districts of Himachal Pradesh; Kathua, Udhampur, Samba, Jammu and Reasi districts of Jammu and Kashmir; and, Dehradun, Rishikesh, Uttarkashi and Tehri Garhwal districts of Uttarakhand. GIS data of the areas surveyed were recorded with the help of GPS (GPS MAP-76, Germin, Taiwan) (Table-1). As many as 170 raspberry genotypes, growing scattered, were marked in different geographical regions (Fig.1) for exploration with the local inhabitants. A random sample of 30 fruits in three replicates were taken from each genotype marked and observations were recorded on fruit quality characters, viz., berry weight (g), berry length (mm), berry breadth (mm), TSS (^{0}B), acidity (%), reducing sugars (%), non-reducing sugars (%), Vitamin

Table 1. Geographical distribution of *Rubus ellipticus* genotypes in some location of North-Western Himalayas

Genotype	Location	Altitude	Latitude	Longitude	Distribution
Majhgaon-1	Majhgaon	1482	30°53’115"	77°07’081"	Abundant
Majhgaon-2					
Majhgaon-3					
Oachhghat-1	Oachhghat				
Kiar-1	Kyar	1258	30°52’076"	77°07’646"	Frequent
Dhillon-1	Dhillon	1411	30°52’816"	77°04’216"	Frequent
Kumarhatti-1	Kumarhatti	1590	30°53’431"	77°03’127"	Frequent
Shaktighat-1	Shaktighat	1442	30°56’266"	76°57’739"	Frequent
Garkhal-1	Garkhal	1621	30°54’085"	76°58’922"	Occasional
Garkhal-2					
Gangli-1	Ganglimour	1415	30°56’847"	76°57’199"	Frequent
Khariyana-1	Khadiyana	1328	30°56’356"	77°01’589"	Frequent
Khariyana-2					
Khariyana-3					
Khariyana-4					
Khariyana-5					
Bhimboot-1	Bhimboot	1330	30°56’276"	77°01’641"	Abundant
Deothi-1	Deothi	1390	30°56’447"	77°02’300"	Frequent
Deothi-2					
Deothi-3					
Dedhghrat-1	Dedhghrat	1482	30°57’314"	77°06’827"	Abundant
Dedhghrat-2					
Dedhghrat-3					
Dedhghrat-4					
Dedhghrat-5					
Dedhghrat-6					
Dedhghrat-7					
Salogra-1	Salogra	1443	30°56’005"	77°07’906"	Frequent
Salogra-2					
Salogra-3					
Padag-1	Padag	1480	30°55’411"	77°06’320"	Occasional
Padag-2					
Kiarighat-1	Kyari	1627	30°59’488"	77°05’670"	Occasional
Kiarighat-2					
Kiarighat-3					
HRSK-1	HRSK	1476	30°57’320"	77°06’850"	Abundant
HRSK-2					
HRSK-3					
Vaknaghat-1	Vaknaghat	1697	31°00’520"	77°05’519"	Abundant
Vaknaghat-2					
Vaknaghat-3					
Kandaghat-1	Kandaghat	1415	30°58’154"	77°06’334"	Frequent
Kandaghat-2					
Kandaghat-3					
Kandaghat-4					
Kandaghat-5					
Kandaghat-6					
Kaithleghat-1	Kaithleghat	1710	30°58’198"	77°06’206"	Frequent
Kaithleghat-2					
Kaithleghat-3					
Shamlech-1	Shamlech	1513	30°54’650"	77°06’591"	Frequent
Shoghi-1	Shoghi	1804	31°02’519"	77°07’631"	Frequent
Shoghi-2					
Shoghi-3					
Shoghi-4					
Shoghi-5					
Shoghi-6					

Contd.

Genotype	Location	Altitude	Latitude	Longitude	Distribution
Panthaghati-1	Panthaghati	1979	31°04’214"	77°10’930"	Occasional
Panthaghati-2					
Panthaghati-3					
Hiranagar-1	Hiranagar	1805	31°07’119"	77°06’104"	Occasional
Hiranagar-2					
Hiranagar-3					
Ghanahatti-1	Ghanahatti	1700	31°08’127"	77°05’285"	Abundant
Ghanahatti-2					
Ghanahatti-3					
Nadokhar-1	Nadokhar	1529	31°09’093"	77°01’352"	Frequent
Kalani-1	Kalani	1591	31°09’782"	77°02’178"	Abundant
Kalani-2					
Kalani-3					
Bithari-1	Bithari	1700	31°24’540"	77°08’074"	Abundant
Bithari-2					
Sanarali-1	Sanarali	1610	31°20’265"	77°11’130"	Occasional
Sanarali-2					
Nalagali-1	Nalagali	1750	31°21’345"	77°12’016"	Occasional
Nalagali-2					
Badhu-1	Badhu	1813	31°29’535"	77°00’580"	Abundant
Badhu-2					
Badhu-3					
Badhu-4					
Kukarigalu-1	Kukarigalu	1764	31°31’518"	76°59’648"	Abundant
Kukarigalu-2					
Gumma-1	Gumma	1506	31°57’916"	76°51’155"	Abundant
Gumma-2					
Maigal-1	Maigal	1171	31°45’702"	76°56’973"	Frequent
Maigal-2					
Chhaprahan-1	Chhaprahan	1138	31°37’962"	77°03’712"	Frequent
Chhaprahan-2					
Chhaprahan-3					
Chailchowk-1	Chailchowk	1435	31°33’985"	77°00’013"	Abundant
Pandoh-1					
Pandoh-2					
Ghatasani-1	Ghatasni	1650	31°56’705"	76°50’000"	Frequent
Ghatasani-2					
Balichowki-1	Balichowki	1115	31°41’723"	77°16’617"	Abundant
Balichowki-2					
Chihuntapul-1	Chihuntapul	1276	31°39’125"	77°20’315"	Frequent
Chihuntapul-2					
Chihuntapul-3					
Targali-1	Targali	1238	31°39’593"	77°19’081"	Frequent
Targali-2					
Targali-3					
Sidhwan-1	Sidhwan	1283	31°39’021"	77°20’419"	Frequent
Seobag-1	Seobagh	1225	31°59’460"	77°08’056"	Occasional
Seobag-2					
Karasu-1	Karasu	1432	32°02’108"	77°08’218"	Frequent
Karasu-2					
Raison-1	Raison	1460	32°03’234"	77°08’209"	Frequent
Raison-2					
Chhattenseri-1	Chhattenseri	1373	32°03’213"	77°07’722"	Occasional
Bandrol-1	Bandrol	1342	32°01’506"	77°07’393"	Occasional
Bandrol-2					
Bandrol-3					
Bandrol-4					

Contd.

Genotype	Location	Altitude	Latitude	Longitude	Distribution
Sarali-1	Sarali	1060	32°17’549"	76°06’986"	Abundant
Sarali-2					
Sarali-3					
Sarali-4					
Sarali-5					
Lahru-1	Lahru	770	32°24’343"	75°58’607"	Occasional
Lahru-2					
Lahru-3					
Koti-1	Koti	834	32°39’704"	77°01’736"	Frequent
Koti-2					
Koti-3					
Koti-4					
Daintha-1	Daintha	914	32°19’664"	76°03’956"	Frequent
Sihunta-1	Sihunta	928	32°18’084"	76°05’383"	Frequent
Sihunta-2					
Patka-1	Patka	1086	32°20’292"	76°02’250"	Frequent
Patka-2					
Drumnalla-1	Drumnalla	1096	32°17’337"	76°07’337"	Frequent
Drumnalla-2					
Bhatoli-1	Bhatoli	1360	32°31’273"	75°56’460"	Frequent
Kudera-1	Kudera	1126	32°37’755"	75°53’968"	Frequent
Kudera-2					
Saroga-1	Saroga	1277	32°37’249"	75°52’166"	Frequent
Koharnala-1	Koharnala	1298	32°37’405"	75°51’454"	Frequent
Pepari-1	Pepari	1370	32°37’812"	75°53’518"	Frequent
Pepari-2					
Ranichauri-1	Ranichauri	1737	30°19’335"	78°24’395"	Frequent
Ranichauri-2					
Ranichauri-3					
Ranichauri-4					
Hill Campus-1	Hill Campus	1846	30°18’834"	78°24’507"	Frequent
Badshahithol-1	Badshahithol	1804	30°20’264"	78°24’326"	Abundant
Badshahithol-2					
Badshahithol-3					
Badshahithol-4					
Dharkot-1	Dharkot	1340	30°23’244"	78°23’685"	Frequent
Chamba-1	Chamba	1598	30°20’711"	78°23’678"	Frequent
Chamba-2					
Chamba-3					
Chamba-4					
Than-1	Than	1470	30°19’911"	78°23’561"	Frequent
Than-2					
Hindola-1	Hindola	1367	30°11’016"	78°18’771"	Frequent
Kot Maniar-1	Kot Maniyar	1430	30°22’017"	78°23’605"	Frequent
Ramgarh-1	Ramgarh	1395	30°24’015"	78°24’831"	Occasional
Baurgaon-1	Baurgaon	1438	30°24’338"	78°24’881"	Occasional
Chopdiyal-1	Chopdiyal	1923	30°22’465"	78°22’296"	Frequent
Chopdiyal-2					
Sabali-1	Sabali	1370	30°19’276"	78°23’702"	Abundant
Sabali-2					
Sabali-3					
Sabali-4					
Chopdiyali-1	Chopdiyali	1290	30°19’866"	78°22’690"	Frequent
Kotigad-1	Kotigad	1355	30°22’383"	78°23’660"	Frequent
Guldi-1	Guldi	1600	30°21’346"	78°23’598"	Frequent
Kirgani-1	Kirgani	1319	30°24’243"	78°23’913"	Occasional

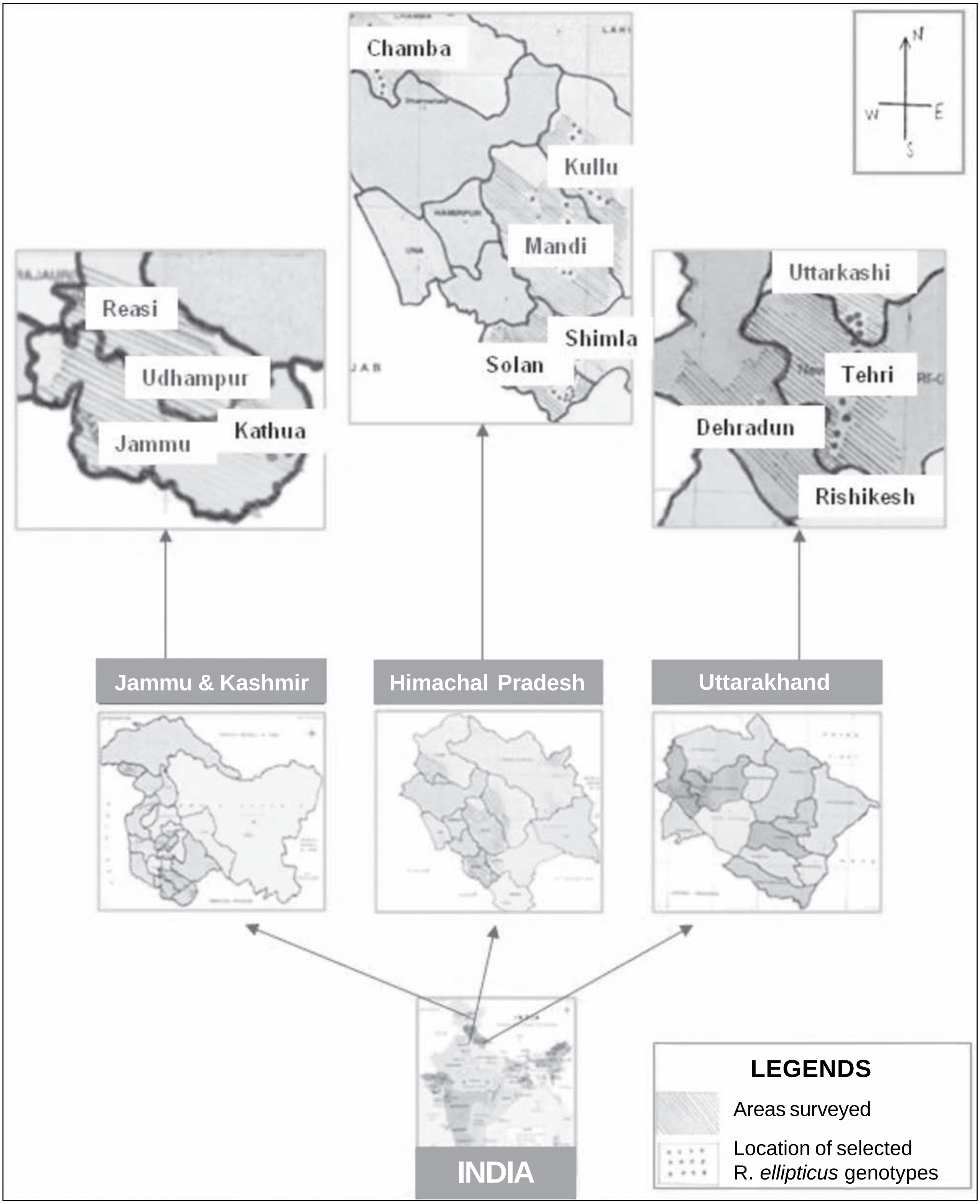

Fig 1. Map showing areas surveyed for biodiversity studies in *Rubus ellipticus* Smith in North-Western Himalayas

C (mg/100g) and berry colour. Berry size was measured with digital Vernier Calipers (Mitutoyo, Japan-CD-6"CS), berry weight with an electronic balance, TSS by digital refractometer and acidity, sugars and ascorbic acid as per standard procedures of Ranganna (1986).

Mean data of eight quantitative traits of 170 genotypes were subjected to non-hierarchical cluster analysis (through the statistical software SPSS 10.0) for studying the pattern of genetic divergence, using Mahalanobis D^2 statistics, and grouping the genotypes as per the method suggested by Tocher (Rao, 1952).

On the basis of the extensive survey conducted in three Indian states of the North-Western Himalayas, the species under study (*Rubus ellipticus* Smith) was found to be geographically located between 760m and 1950m AMSL, at 30^{0}10’159" to 33^{0}04’693"N and 74^{0}44’076" to 78^{0}25’681"E (Table 1).

Analysis of variance for eight quantitative traits showed significant differences among the 170 genotypes, indicating the existence of genetic diversity (Table 5). These 170 genotypes fell into 12 Clusters (Table 2). It was apparent that 31 genotypes were in Cluster XII, 27 in Cluster V, 19 in Cluster I, 17 in Cluster IX, 16 in Cluster VIII, 15 in Cluster XI, 13 in Cluster II, 12 in Cluster VII, 10 in Cluster III, six in Cluster X, three in Cluster VI and only one in Cluster IV. Clustering pattern of the genotypes showed that a genotype from a particular area did not necessarily belong to the same cluster. From a study of genetic divergence among the 170 genotypes, it appears that genetic drift and natural selection under different environmental conditions could cause considerable diversity compared to that caused by geographical distance. Upon divergence, some of the genotypes belonging to different eco-geographic regions could be grouped under one Cluster while genotypes belonging to a particular geographic origin were distributed under different clusters suggesting, that, geographic distances do not necessarily represent genetic diversity (Dwivedi and Mitra, 1995; Maiti *et al*, 2002).

To start a breeding programme involving selection of parents from a large number of genotypes, it is necessary to first classify the genotypes into groups based on their distinguishing characteristics. Such grouping of genotypes is useful for efficient selection of parents in a hybridization programme. Clustering of the 170 genotypes based on eight quantitative traits using non-hierarchical Euclidean cluster analysis gives an idea of extent of similarities and dissimilarities among the genotypes. Inter-and intra-cluster distances thus obtained enable the breeder to decide on the

Table 2. Clustering pattern of 170 pre-selected genotypes of raspberry (*Rubus ellipticus* Smith) based on eight quantitative characters of horticultural importance

Cluster	No. of genotypes	Name of genotype
I	19	Garkhal-2, Deothi-1, Deothi-3, Dedhghrat-1, HRSK-2, Kandaghat-2, Kandaghat-4, Kaithleghat-3, Shoghi-2, Nadokhar-1, Kalani-1, Kalani-2, Sanarali-1, Nalagali-1, Gumma-2, Maigal-1, Pandoh-2, Sarali-1, Ramgarh-1
II	13	Vaknaghat-2, Shoghi-6, Pandoh-1, Targali-2, Sidhwan-1, Sihunta-1, Ranichauri-1, Badshahithol-2, Hindola-1, Chopdiyal-1, Chopdiyal-2, Sabali-1, Sabali-2
III	10	Majhgaon-3, Kandaghat-1, Kandaghat-6, Shoghi-5, Sanarali-2, Ghatasani-1, Chihuntapul-1, Sarali-5, Kudera-2, Pepari-2
IV	1	Dharkot-1
V	27	Majhgaon-2, Dhillon-1, Khariyana-1, Bhimboot-1, Deothi-2, Dedhghrat-5, Dedhghrat-7, Padag-2, Vaknaghat-3, Shamlech-1, Shoghi-1, Shoghi-3, Shoghi-4, Bithari-2, Badhu-3, Maigal-2, Balichowki-2, Targali-1, Seobag-2, Karasu-1, Raison-1, Bandrol-2, Bandrol-3, Sarali-4, Lahru-2, Lahru-3, Drumnalla-1
VI	3	Hill Campus-1, Badshahithol-1, Sabali-4
VII	12	Kumarhatti-1, Kaithleghat-1, Panthaghati-3, Bithari-1, Badhu-1, Badhu-2, Badhu-4, Kukarigalu-2, Chhaprahan-2, Chhaprahan-3, Chailchowk-1, Sarali-3
VIII	16	Oachhghat-1, Khariyana-2, Dedhghrat-4, Salogra-2, Padag-1, Kiarighat-1, Ghatasani-2, Seobag-1, Sarali-2, Daintha-1, Bhatoli-1, Badshahithol-3, Badshahithol -4, Chamba-3, Chamba-4, Kotigad-1
IX	17	Khariyana-4, Khariyana-5, Salogra-1, Kiarighat-2, Kiarighat-3, HRSK-1, Balichowki-1, Koti-3, Kudera-4, Than-1, Than-2, Kot Maniar-1, Baurgaon-1, Sabali-3, Chopdiyali-1, Guldi-1, Kirgani-1
X	6	Shaktighat-1, Garkhal-1, Dedhghrat-2, Dedhghrat-6, Kandaghat-5, Pepari-1
XI	15	Majhgaon-1, Gangli-1, Dedhghrat-3, Vaknaghat-1, Hiranagar-2, Ghanahatti-3, Kukarigalu-1, Chhaprahan-1, Raison-2, Chhattenseri-1, Bandrol-1, Ranichauri-2, Ranichauri-3, Chamba-1, Chamba-2
XII	31	Kiar-1, Khariyana-3, Salogra-3, HRSK-3, Kandaghat-3, Kaithleghat-2, Panthaghati-1, Panthaghati-2, Hiranagar-1, Hiranagar-3, Ghanahatti-1, Ghanahatti-2, Kalani-3, Nalagali-2, Gumma-1, Chihuntapul-2, Chihuntapul-3, Targali-3, Karasu-2, Bandrol-4, Lahru-1, Koti-1, Koti-2, Koti-4, Sihunta-2, Patka-1, Patka-2, Drumnalla-2, Kudera-1, Kudera-3, Ranichauri-4

Table 3. Average inter-and intra-cluster distance (D^2) among 170 genotypes of raspberry (*Rubus ellipticus* Smith)

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	3.92	3.30	2.22	6.92	4.18	5.17	3.93	2.04	4.54	3.61	5.61	2.65
II		3.02	3.97	8.10	2.64	3.45	3.75	2.37	3.34	4.60	3.29	1.97
III			3.16	7.90	5.47	4.73	2.85	3.61	5.94	5.50	5.85	3.35
IV				5.34	6.75	10.77	8.36	6.89	6.35	6.81	7.90	6.50
V					4.17	6.03	4.99	3.11	2.07	4.33	2.94	2.29
VI						6.19	5.03	4.62	6.18	6.66	5.86	4.89
VII							6.10	4.77	6.19	7.06	4.06	3.32
VIII								4.11	2.83	2.52	4.98	2.16
IX									6.26	3.32	4.31	2.94
X										7.02	6.90	4.20
XI											6.40	3.21
XII												1.90

Figures in the diagonal represent intra-cluster distance

Table 4. Cluster means for eight characters in 170 genotypes of raspberry (*Rubus ellipticus* Smith)

Character	Cluster											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Fruit weight (g)	0.51	0.55	0.52	0.51	0.54	0.67	0.59	0.48	0.5	0.45	0.63	0.53
Fruit length (mm)	8.7	10.26	10.19	10.05	9.95	10.96	11.51	9.09	10.05	7.68	12.29	10.63
Fruit breadth (mm)	11.55	12.63	11.8	10.41	12.76	12.01	14.17	10.93	10.85	9.28	14.36	12.12
Fruit TSS (^{0}B)	15.63	13.67	17.2	12.9	11.84	15.51	16.36	13.99	11.39	13.02	12.4	13.92
Acidity (%)	1.34	1.32	1.33	1.42	1.31	1.33	1.29	1.33	1.32	1.34	1.31	1.34
Reducing sugars (%)	2.91	3.27	2.75	4.9	2.81	3.93	2.72	3.46	3.45	2.92	3.11	2.87
Non-reducing sugars (%)	6.69	8.52	7.02	1.03	6.74	11.2	6.95	7.45	6.87	6.87	7.11	6.72
Vitamin C (mg/100g)	3.98	4.01	3.94	5.1	3.68	3.97	3.76	3.74	3.82	3.9	3.92	3.86

best possible combinations of genotypes so that heterosis can be exploited for crop improvement.

Average inter-cluster distance was maximum (10.77) between Clusters IV and VI, whereas, it was minimum (1.97) between Cluster II and XII indicating, that, hybridization among genotypes under Clusters IV and VI can be made to get high heterotic effects, and expect desirable segregants. Whereas, low inter-cluster distance between Clusters II and XII would recommend non-inclusion of genotypes falling under these clusters in any hybridization programme. Similar conclusions were drawn by Rai and Misra (2005), Kaushal and Sharma (2005) and Shah *et al* (2010) in their respective studies based upon cluster analysis of 17 genotypes of bael, 229 genotypes of pecan, and 13 genotypes of almond. Intra-cluster distance was maximum (7.02) within Cluster X, and minimum (1.90) within Cluster XII (Table 3). Low range of distances within a cluster depicts lesser diversity among genotypes.

Highest average fruit weight (0.67g) and non-reducing sugars (11.2%) were observed in Cluster VI. Average fruit TSS was maximum (17.2%) in Cluster III. Cluster IV showed the highest values for reducing sugars (4.9%), acidity (1.42%) and Vitamin C (5.1mg/100g). Fruit acidity was minimum (1.29%) in Cluster VII. Fruit length and breadth were maximum in Cluster XI (12.29 mm) and Cluster V (12.76 mm), respectively (Table 4). Genotypes falling under Cluster VI can be used as parents in breeding for improvement in fruit weight. Similarly, genotypes under Cluster III can be used as a potential source for higher TSS content in berries. Genotypes of Clusters IV and VII can lend themselves as parental sources for the chemical characters of fruit like Vitamin C, sugars and acidity. Conclusions on similar lines were drawn by various workers in their studies (Singh *et al.* 2003 in pomegranate, Roy and Misra, 2005 in bael, Gohil and Pandya, 2006 in salicornia and Nagar and Fageria, 2006 in lehsua).

Table 5. Analysis of variance for some quantitative traits of horticultural importance for pre-selected genotypes of raspberry (*Rubus ellipticus* Smith)

Character	Mean squares		
	Replication	Treatment	Error
Degree of Freedom (d.f.)	2	169	338
Berry weight (g)	0.003	0.048*	0.001
Berry length (mm)	0.062	5.462*	0.039
Berry breadth (mm)	0.053	5.851*	0.044
TSS (^{0}B)	0.0001	10.594*	0.06
Acidity (%)	0.0004	0.050*	0.002
Reducing sugars (%)	0.076	1.079*	0.032
Non- reducing sugars (%)	0.048	2.996*	0.04
Vitamin C (mg/100g)	0.096	0.602*	0.027

CD at (P = 0.05)

ACKNOWLEDGEMENTS

The authors are thankful to Department of Science & Technology, Govt. of India, New Delhi, for providing necessary financial help during the course of this investigation as a pioneering research work conducted under the project entitled "Studies on biodiversity of raspberry (*Rubus ellipticus* Smith) for selection of superior genotypes growing wild in the North-Western Himalayas.

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(MS Received 02 September 2010, Revised 13 April 2011)