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Short communication

Phylogenetic relationship among Indian jujube cultivars based on flavonoid spectrum

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Indian jujube (*ber*) cultivation has been in vogue in India since Vedic age. It is adapted to the extreme agro-climatic conditions of the arid ecosystem and is, therefore, a popular fruit crop in this region. Genetically, the crop is highly heterozygous and, therefore, a wide genetic variability exists in nature. Apart from this, polyploidy and hybridity has further enriched in the gene pool of this crop species. As a result of systematic evaluation of the gene pool a large number of cultivars have been identified. The classification of these cultivars rests mostly on floral and fruit characteristics which presently becomes overlapping and hence renders the identification difficult. Apart from this, the migration of varieties from one location to other and often with different names has led to greater confusion in identification of varieties. In a pursuit to develop National Gene Bank of *ber*, a total of 318 cultivars has been collected and maintained at CIAH, Bikaner farm.

The cultivars are being evaluated and attempts are underway to develop phyto-chemical markers for their identification and assess the phylogenetic relationships between them. Among the various phyto-chemical markers in recent past, flavonoids have gained importance in varietal identification and assessment of phylogeny. This is on account of the fact that flavonoids are secondary metabolites, species specific and remains unchanged even under environmental fluctuations. Therefore, in the present study an attempt has been made to use flavonoid pulp of eight *ber* cultivars and assess the inter-relationships between them.

Eight genotypes of *ber* (*Ziziphus mauritiana* var. *rotundifolia*) *viz*., Illaichi, Bagwadi, Banarsi Karaka, Seb, Gola, Umran, Reshmi and Mundia constituted the material for the present investigation. The leaf samples, for the extraction of flavonoid 2 g of leaf sample was fixed in 10 ml of methanol containing 1% HCl. The fixed samples were stored at room temperature and were macerated in mortar and pestle before analysis. The whole content was centrifuged at 10,000 rpm for 20 min. at room temperature. The clear fluid was taken and evaporated to dryness in an oven maintained at 60°C. Finally, the sample was taken in 1 ml methanol.

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The flavonoids were separated on thin layer chromatography (TLC) plates coated with 0.6 mm thick layer of cellulose. An aliquot of 10 µl of sample as prepared above was loaded on one corner of the plate. The plate was first developed with 2% formic acid and later, after rotating at 90°, in solvent containing amyl alcohol : acetic acid and water in the ratio of 10: 6: 5. The plates after air drying were viewed for flavonoid spots as: (i) without any spray, (ii) spray with 1% methanolic $AlCl_3$ under UV, and (iii) spray with 1% methanolic NaOH under UV. The spots were marked and pooled chromatogram of each cultivar was prepared. They were then numbered and master chromatogram was prepared for comparison of cultivars. The phylogenetic relationship was ascertained by using NTSYS 2.0 computer software.

Data in Table 1 revealed that the pooled chromatogram of eight *ber* cultivars possessed a total of 28 flavonoid spots. The distribution of these spots among the taxa was highly variable. Spot No. 6 had universal presence being represented in 100% of taxon under investigation. However, Spot Nos. 1, 7, 10 and 16 were present in 75% of cultivars. Similarly, Spot No. 8 and 26 were found in 82.5% cultivars, Spot Nos. 11, 14, 15, 20 and 25 were found in 50% cultivars, Spot Nos. 2, 4, 9, 13, 17, 19, 22, 23, 27 and 28 were recorded in 27.5% cultivars. Spot Nos. 3 and 12 were encountered only in single taxon and hence they proved to be marker spots for respective cultivars, by indicating the presence and absence of a particular spot.

Perusal of Table 1 revealed that cultivars differed with respect to the number of spots in the profile. Maximum number of spots (15) was found in cv. Seb and minimum (8) was recorded in cv. Reshmi. The other cultivars showed a total of 14 spots in cvs. Banarsi Karaka, Umran and Mundia; a total of 13 spots in cvs. Bagwadi and Gola and 11 in cv. Illaichi.

Apart from number of spots in the respective profile, the cultivars also differed with respect to the type of spot present in flavonoid profile of each cultivars. On account of this, the cultivars can be distinguished from each other. For instance Spot No. 3 was found exclusively in cv. Gola and Spot No. 12 in Illaichi. Thus, these spots were characteristics of

Table 1. Flavonoid spectrum of *ber* cultivars.

Cultivar	Flavonoid spot number																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Illaichi	+					+	+	+		+		+		+		+	+					+	+					
Bhagwadi	+					+	+	+	+	+	+				+	+			+					+	+	+		
Banarsi Karaka	+	+		+		+	+			+	+		+	+	+	+			+	+			+					
Seb	+				+	+	+	+	+	+						+	+	+		+	+				+	+		+
Gola	+	+	+	+	+	+	+				+		+			+	+	+									+	
Umran						+		+		+	+			+	+	+			+	+	+	+	+			+	+	
Reshmi						+			+				+	+										+	+	+		+
Mundia	+	+		+		+	+	+		+					+					+		+			+	+	+	+

Table 2. Similarity index between *ber* cultivars.

Cultivar	Illaichi	Bagwadi	Banarsi Karaka	Seb	Gola	Umran	Reshmi	Mundia
Illaichi	1.000							
Bagwadi	0.333	1.000						
Banarsi Karaka	0.388	0.421	1.000					
Seb	0.368	0.473	0.260	1.000				
Gola	0.263	0.238	0.421	0.333	1.000			
Umran	0.388	0.421	0.473	0.3158	0.174	1.000		
Reshmi	0.117	0.312	0.157	0.277	0.105	0.157	1.000	
Mundia	0.315	0.421	0.400	0.450	0.285	0.400	0.222	1.000

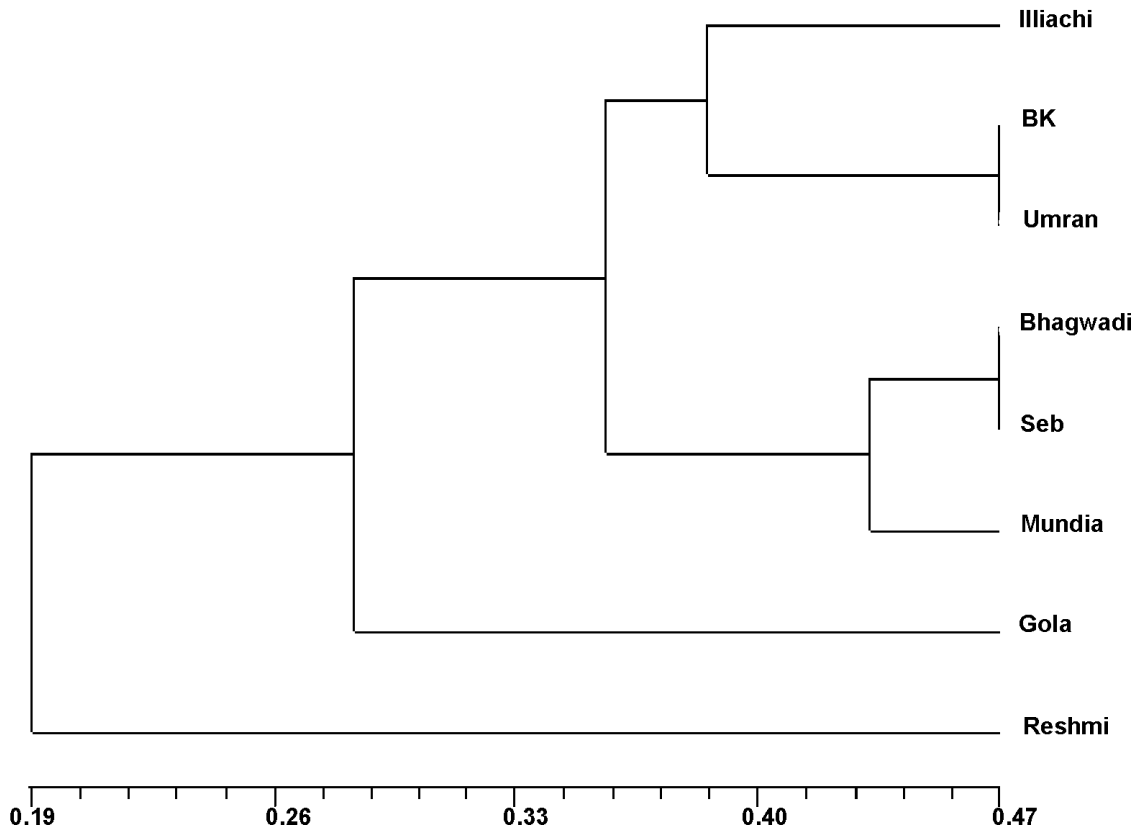

Fig. 1. Dendrogram based on Jaccard's similarity index among *ber* cultivars. (BK= Banarsi Karaka).

respective cultivars. Similarly, the other cultivars under study can also be distinguished based on typical spot combinations. This is illustrated by the fact that cultivars Bagwadi can be distinguished from rest of cultivars by typical spot combination of 9, 15 and 24. Similarly, typical spot combinations for cv. Banarasi Karaka was 2, 11 and 14; for Seb 5, 9, 20 and 21; for Umran 21, 22, 26 and 27; for Reshmi 9, 13 and 14; and for Mundia 2, 22 and 25.

Attempts to describe and characterize *ber* cultivars has been done by various workers using morphological traits (Ram, 8; Chandra, 3), Chadha, *et al*., 2; Vashishtha, 10,11; Vashishtha and Pareek, 12). However, still considerable discrepancies exist in the description of morphological characters. Apart from this, the nomenclature of cultivars grown at different locations has further aggravated the problem and hence, there is a need to develop stable markers for each cultivar so that overlapping in name of cultivar can also be resolved.

Role of flavanoids in biosystematics at species or sub-species level has been demonstrated earlier too by Bhargava (1), de-Kok *et al.* (4), Joshi (6), Koul *et al*. (7), Upson *et al.* (9), Garcia and Oieda (5), and Vysochina (13). The results obtained in the present investigations also demonstrate that *ber* cultivars can be easily distinguished based on flavonoid spectrum. This can be achieved either by use of typical spot or typical spot combination. Thus, cultivar Illaichi can be distinguished from rest of the cultivars by typical spot combination of 12, 22 and 23. The similar typical spot combination for Bagwadi is 9, 15 and 24; for Banarsi Karaka 2, 11 and 14; for Seb 5, 9, 20 and 21; for Gola 5, 9, 20 and 21; for Umran 21, 22, 26 and 27; for Reshmi 9, 13, 14 and for Mundia 2, 22 and 25. Thus, the flavonoid profiles can be used as taxonomic marker in delimitation of cultivars.

The second objective of the present study was to assess the phylogenetic relationship among the ber cultivars. Accordingly, the data was subjected to cluster analysis using NTSYS software. The similarity matrix obtained by Jaccard's similarity coefficient is presented in Table 2 and Fig. 1. Perusal of data revealed that genetic similarity among cultivars differed from a minimum of 0.117 in cvs. Illaichi and Reshmi to a maximum of 0.473 between cvs. Seb and Bagwadi and Umran and Banarasi Karaka. The data generated by Jaccard's similarity coefficient was used to construct

the dendrogram (Fig. 1). The perusal of dendogram classifying the *ber* cultivars could be grouped into 5 major groups. Group A consisted of cv. Reshmi which was clearly distinct from all other cultivars and showed poor affinity with them. The group B is also represented by single cv. Gola which shows low affinity with other cultivars under investigation. The group C has six cultivars which shows more affinity with each other. This group can further be divided into group D consisting of three cultivars (Illaichi, Banarasi Karaka and Umran) having closer affinity and group E consisting of other three cultivars (Bhagwati, Seb and Mundia) having closer affinity. Thus, the dendogram reveals the genetic relationship among the cultivars under investigation.

Thus, from the foregoing results, it is evident that flavonoid spectrum can be used for cultivar identification as well as for assessing phylogenetic relationship among taxa in Indian jujube (*ber*).

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