

## CHEMOTAXONOMIC RELATIONSHIP AMONG JUJUBE (*ZIZIPHUS MAURITIANA* LAMK.) CULTIVARS AND SOME RELATED SPECIES

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'Ber' or jujube, *Ziziphus mauritiana* Lamk. (Rhamnaceae) is a highly cross pollinated fruit tree. Its cultivars have developed as a result of selection from the diverse natural variability. Although cultivars can be differentiated by morphological characters, these characters cannot always indicate inherent relationships between them. Flavonoids, being the most stable plant constituents as taxonomic markers, were studied in the leaves of a fairly large group of jujube cultivars to establish resemblances and relationships among them.

The studies were conducted on 4-5 year old trees of 59 jujube cultivars and two *Ziziphus* species i.e. *Z. nummularia* and *Z. mauritiana* var. *rotundifolia* (Bhansali, 1975) at research farm of Central Arid Zone Research Institute, Jodhpur (N 26°20' latitude and E 73°5' longitude) in the Thar desert of India. Cultivars collected from different parts of India were budded on *Z. mauritiana* var. *rotundifolia* rootstock. The trees received 2 or 3 protective irrigations every year.

Mature leaves, collected after fruit set during December, were washed and air dried. The dried leaf material (50 g) was ground and refluxed with 180 ml of methanol. Filtration and evaporation of each methanol extract yielded a sticky green residue which was washed into petroleum ether to remove chlorophyll. Stock solution for application on thin layer chromatography plate was prepared by dissolving about 0.1 g of residue in 1 ml methanol. The two dimensional thin layer chromatographic analysis was carried out on a microcrystalline cellulose (Merck) plate using butanol-acetic acid-water (BAW, 4 : 1 : 5) and acetic acid-water (AcOH, 15 : 85) as solvents. The dried developed plates were first viewed in UV light (long wave length) alone and then in the presence of ammonia fumes and after spraying with a 2% solution of aluminium chloride in methanol. Rf values in each solvent system were calculated for each of the numbered spots. Spots with similar colour changes and Rf values in the two solvent systems were regarded as identical.

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Cultivars showing distinct distribution pattern of major flavonoid spots were recognised as chemical races. Morphological characters of leaf and fruit were used to support the chemotaxonomic studies.

## RESULTS AND DISCUSSION

The results of flavonoid distribution pattern of 59 cultivars of jujube (*Z. mauritiana* and two other species i.e. *Z. nummularia* and *Z. mauritiana* var. *rotundifolia* have been presented in Tables 1, 2 and 3 respectively.

### A. Intra-group relationship

#### 1. Cultivars with erect growth habit

Cultivars 'Seb', 'Khatti', 'Sua' and 'Ilayachi' showed distinct flavonoid pattern within the group having no spot common with other cultivars (Table 1). 'Seb' and 'Khatti' had round fruit shape but differed in other characters. Styler end of fruit in 'Seb' had a typical apple-like depression unlike the obliquely beaked end in 'Khatti'. Chemotaxonomically also, 'Khatti' was distinct in its flavonoid pattern and was thus entirely different from other cultivars. All the flavonoid spots in 'Khatti' showed similar colour changes in UV, UV/NH<sub>3</sub> and UV/AlCl<sub>3</sub>. This indicated the presence of a particular class of flavonoids (Harborne, 1976) in this cultivar. Similarly cv 'Sua', although grouped with 'Tikadi' and 'Sanaur-1' having ovate fruit shape, differed markedly in other morphological characters. Distinct flavonoid pattern in 'Sua' further lends support to the morphological variations.

In this group, spot number 2 was common in 'Thornless', 'Sanaur-1' and 'Nari-keli', spot number 7 in 'Nimaj' and 'Thornless', spot number 6 in 'Chhuhara' and 'Narma', spot number 8 in 'Tikadi' and 'Thornless', spot number 17 was common in 'Lam', 'Nimaj' and 'Kaithali' and the spot number 27 was common in 'Gobindgarh Selection' and 'Kaithali'.

In the 'erect' group, 'Ilayachi' had its independent identity. Its fruit is oblate, very small with distinct ridges on the stem end. Khoshoo and Singh (1963) reported 'Ilayachi' to be an octaploid. Its independent identity was also supported by the presence of a distinct leaf flavonoid pattern.

#### 2. Cultivars with semi-erect growth habit

The presence of an equal number of spots with similar colour changes and identical flavonoid pattern in cultivars 'Ajmeri', 'Umran' and 'Katha' was in agreement with the similarity observed in their morphological characters although this cultivar is named differently at different places i.e. 'Katha' in Rajasthan, 'Umran' in the adjoining Haryana and Punjab States (Chadha et al 1972) and 'Ajmeri' in Gujarat.

Table 1. Characteristics of flavonoid spots on 2-dimensional chromatograms in jujube cultivars

Rf value in		Spot No	*Colour change			Cultivars
BAW	ACOH		UV	UVNH <sub>3</sub>	UV-AlCl <sub>3</sub>	
0.12	0.03	58	Br	Br	Br Y	Sua
0.14	0.03	56	Y	Y	Br Y	Mirchia
0.15	0.24	60	Br	Br	Br Y	Sua
0.17	0.46	61	Br	Br	L Y	Sua
0.19	0.01	62	Br	Br	Br Y	Sua
0.19	0.50	63	Br	Br	Br Y	Willayati
0.19	0.61	64	DBr	DBr	Le Y	Willayati
0.20	0.39	65	Bl	Bl	DBl	Thornless
0.22	0.66	66	Br	Br	BrBl	Sua
0.24	0.67	67	Y	Y	Br Y	Chencho
0.24	0.93	68	Y	Y	Br Y	Dandan
0.24	0.29	69	DBr	Br	Le Y	Willayati
0.26	0.04	70	Y	Y	Le Y	Willayati
0.27	0.10	71a	Bl	Bl	DBL	Thornless
0.27	0.20	71b	Bl	Bl	DBL	Thornless
0.27	0.85	72	Y	Y	Br Y	Dandan
0.28	0.63	73	Br	Br	Br Y	Banarasi kadaka
0.29	0.29	74	DBr	DBr	Y	Ilagachi
0.30	0.05	30	Y	Y	Br	Kali, Narma
0.30	0.07	75	Y	Y	Br	Jhajjar selection
0.32	0.06	76	Y	Y	Bl	Bahadurgarh
0.33	0.60	31	Br	Br	YBr	Rewa selection, Narikeli
0.34	0.07	77	DBr	DBr	Y	Ilaichi
0.35	0.65	32	Br	Br	Le Y	Seb, Meharun
0.36	0.61	33	Br	Br	Br Y	Popular Gola, Nimaj
0.36	0.76	78	Br	Br	Br Y	Rewa selection
0.38	0.28	79	DBr	DBr	LY	Sua
0.38	0.68	80	Br	Br	Bl	Madhuri
0.38	0.18	81	Bl	Bl	BrBl	Aliganj
0.39	0.61	1	Br	Br	YBr	Narma, Ajmeri, Katha, Umran Kala Gola, Chonchal, Maharwali Mundia
0.39	0.20	82	Bl	Bl	DBl	Thornless
	0.57	83	Br	Br	YBr	Noki
0.39	0.72	84	Br	Br	YBr	Kaithali
0.39	0.89	85	DBl	DBl	Bl	Jhajjar selection
0.40	0.52	34	DBr	DBr	LY	Katha phal, Sua
0.41	0.54	35	Br	Br	Br Y	Sanaur-3, Triloki
0.41	0.61	14	Br	Br	Br Y	Chuhara, Madhuri
0.41	0.65	86	Br	Br	BrBl	Ponda
0.41	0.81	87	Bl	Bl	YPl	Sua
0.42	0.76	88	Bl	Bl	YBr	Kala gola
0.42	0.55	89	DBr	Br	Le Y	Madhuri



## CHEMOTAXONOMY OF JUJUBE CULTIVARS : 127

1	2	3	4	5	6	7
0.52	0.55	121	DBr	DBr	Le Y	Pebandi Alwar
0.52	0.32	122	DBr	Br	DBr	Gola, Gurgaon-1
0.52	0.52	123	DBr	Br	Le Y	Maharwali
0.53	0.61	124	Bl	Bl	Le Y	Aliganj
0.53	0.67	125	Br	Br	DBr	Chonchal
0.53	0.39	126	Br	Br	Br Y	Noki
0.53	0.62	20	Br	Br	Br Y	Pebandi Alwar, Narma, Maharwali
0.53	0.91	127	Br	Br	Br Y	Mundia
0.54	0.56	128	DBr	Br	YBr	Rewa selection
0.54	0.02	129	Y	Y	Le Y	Gola, Gurgaon-1
0.54	0.23	130	Br	Br	Br Y	Banarasi pebandi
0.55	0.96	131	Y	Y	Br	Dandan
0.56	0.33	7	DBd	DBr	Le Y	Pathan, Thornless, Nimaj, Mirchia, Kakrola Gola
0.56	0.56	21	DBr	DBr	Le Y	Narma Gola, Gurgaon, Jogia
0.56	0.27	132	DBr	DBr	Le Y	Lam
0.56	0.59	133	DBr	DBr	Le Y	Akrota
0.56	0.25	134	Br	Br	YBr	Narikeli
0.56	0.62	9	Br	Br	YBr	Kala gola, Gola Gurgaon, Pebandi Alwar, ZG-3
0.56	0.55	135	Br	Br	YBr	Deedwana
0.56	0.89	136	Br	Br	YBr	Dandan
0.56	0.31	22	Br	Br	DBr	Umran, Ajmeri. Katha
0.56	0.5	39	Bl	Bl	YBl	Gola, Safed Rohtak
0.56	0.70	137	Br	Br	YBl	Jhajjar selection
0.57	0.62	40	DBr	DBr	Le Y	Bahadurgarh, Seb
0.57	0.56	4	DBr	DBr	Le Y	Chuhara, Ponda, Kheera, Dandan; Mundia, Triloki
0.57	0.25	41	DBr	DBr	Le Y	Kaithali, Chonchal
0.57	0.55	138	DBr	Br	Br Y	ZG-3
0.57	0.7	42	Br	Br	Br Y	Thornless, Bahadurgarh
0.57	0.68	139	Bl	Bl	Le Y	Noki
0.58	0.04	43	Y	Y	Br Y	Pathan, Gola
0.58	0.64	140	Y	Y	Br Y	Safed Rohtak
0.58	0.61	141	Br	Br	Bl	Bagwadi
0.59	0.39	23	DBr	DBr	Le Y	Jhajjar selection, Popular Gola, Meharun
0.59	0.76	44	Bl	Bl	YBl	Gola, Laddu
0.59	0.67	142	Bl	Bl	YBl	Sua
0.60	0.61	45	Br	Br	Le Y	Gobindgarh selection Jullundhari
0.60	0.50	143	Br	Br	Le Y	JS II
0.60	0.55	46	Br	Br	Le Y	Thornless, Pebandi Alwar
0.60	0.34	10	DBr	DBr	YBr	Seb, Meharun. Popular gola, Kala gola

1	2	3	4	5	6	7
0.60	0.58	144	DBr	DBr	YBr	Sanaur-2
0.60	0.30	11	DBr	DBr	Le Y	Ilayachi, Banarasi, Kadaka, Deedwana, Noki
0.60	0.38	145	DBr	DBr	Le Y	Narma
0.60	0.41	146	DBr	DBr	DBr Y	Aliganj
0.60	0.27	147	DBr	Br	Le Y	Maharwali
0.60	0.05	47	Y	Y	Le Y	Jhajjar Selection, Jullundhari
0.61	0.88	148	Bl	Bl	DBl	Sanaur-2
0.61	0.35	149	DBr	BrY	DBr Y	Gola, Gurgaon
0.61	0.57	150	DBr	BrY	DBr Y	Bagwadi
0.62	0.53	5	Br	Br	Br Y	Nimaj, Safed Rohtak, Ajmeri, Katha, Umran, Kala gola
0.62	0.58	24	Br	Br	Br Y	Pathan, Chonchal, Narma
0.62	0.61	151	Br	Br	DBr	Khatti
0.62	0.34	152	Br	Br	DBr	Gola, Gurgaon-3
0.62	0.32	6	DBr	DBr	LY	Chhuhara, Akrota, Narma, Kheera, Mundia, Jogia
0.62	0.53	48	DBr	DBr	LY	Gola, Sanaur-1
0.62	0.42	49	DBr	DBr	LY	Bal adurgarh, Ponda
0.62	0.29	12	DBr	DBr	LY	Dandan, Sanaur-2, Chonchal, Sanaur-1,
0.62	0.73	153	DBr	DBr	LY	Vikas-2
0.62	0.08	154	Y	Y	Br Y	Ponda
0.62	0.01	13	Y	Y	Br Y	Maharwali, Chhuhara, Gola, Gurgaon, Kala gola
0.62	0.05	25	Y	Y	Br Y	Aliganj, Deedwana, Banarasi kadaka
0.63	0.37	155	DBr	YBr	Le Y	Jullundhari
0.63	0.64	156	DBr	BrY	Br	Kali
0.63	0.26	157	DBr	DBr	YBr	Bagwadi
0.63	0.51	158	Br	YBr	Br Y	Maharwali
0.64	0.71	159a	Br	Br	Le Y	Kali
0.64	0.70	159b	Br	Br	DBr	Khatti
0.64	0.04	50	Br	Br	Le Y	Meharun, Seb
0.64	0.41	160	DBr	DBr	Br Y	Ponda
0.64	0.44	161	Br	Br	DBr	Sananr-3
0.64	0.04	162	Bl	Bl	Br	Meharun
0.64	0.80	163	Bl	Bl	Br	Jullundhari
0.64	0.65	164	Bl	Bl	LY	Noki
0.65	0.38	26	Br	Br	Br Y	Gobindgarh selection, Safed Rohtak, Bagwadi
0.65	0.90	165	DBr	DBr	YBr	Vikas-2
0.65	0.38	51	DBr	DBr	YBr	Triloki, Gola
0.65	0.26	166	DBr	DBr	YBr	ZG-3
0.65	0.65	167	DBr	DRr	YBl	Jhajjar selection
0.65	0.02	168	Y	Y	Le Y	Kheera

1	2	3	4	5	6	7
0.66	0.74	169	Br	Br	LBr	Bahadurgarh
0.66	0.86	170	DBl	Bl	DBl	Aliganj
0.66	0.80	171	Br	Br	DBr	Khatti
0.67	0.56	52	Y	Y	YBr	ZG-3, Gola Gurgaon
0.67	0.06	27	Y	Y	YBr	Gobindgarh selection, Bagwadi, Kaithli
0.67	0.41	172	DBr	BrY	YBr	Kali
0.68	0.62	23	Br	Br	Le Y	Aliganj, Mundia
0.68	0.50	54	Br	Br	Le Y	Banarasi Kadaka, Gobindgarh selection
0.68	0.59	173	Br	Br	Le Y	Bahadurgarh
0.68	0.09	55	LeY	LeY	Br	Triloki, JS II
0.68	0.56	174	LeY	LeY	Br	ZG-3
0.69	0.55	28	Br	Br	YBr	Jogia, Dandan, Triloki
0.69	0.76	175	Bl	Bl	Bl Y	Akrota
0.70	0.35	176	Br	Br	DBr	Khatti
0.70	0.44	29	DBr	DBr	Le Y	Ajmeri Umran, Katha
0.70	0.32	177	DBr	DBr	Le Y	Vikas-2
0.70	0.77	178	Bl	Bl	DBl	Aliganj
0.71	0.52	179	Bl	Bl	Bl Y	ZG-3
0.71	0.83	180	Bl	Bl	Bl Y	Pebandi Alwar
0.71	0.64	181	Bl	Bl	BrBl	Gola
0.72	0.50	182	Y	Y	Le Y	Gola
0.72	0.60	183	DBr	DBr	Br Y	Vikas-2
0.73	0.89	184	DBl	DBl	Br Y	Rewa selection
0.73	0.89	185	Br	Br	Ly	Sua
0.74	0.56	186	Br	Br	DBr	Khatti
0.75	0.44	187	Br	Br	Br Y	Sanaur-2
0.76	0.59	188	DBr	DBr	Y	Safed Rohtak
0.76	0.65	189	Bl	Bl	DBl	Pebandi Alwar
0.77	0.05	190	Y	Y	YBr	Safed Rohtak
0.77	0.86	191	Y	Y	YBl	Jhajjar selection
0.78	0.68	192	Bl	Bl	LBl	Gola
0.78	0.66	193	Bl	Bl	DY	Safed Rohtak
0.79	0.73	56	Bl	Bl	DBl	Triloki, Sanaur-2
0.79	0.80	194	Y	Y	Bl Y	Jhajjar selection
0.80	0.43	195	DBr	DBr	DY	Safed Rohtak
0.81	0.90	196	Y	Y	Br	Dandan
0.86	0.68	197	Bl	Bl	DBl	Kala Gola
0.86	0.71	198	Bl	Bl	DBl	Thornless
0.87	0.73	199	Bl	Bl	DBl	Bahadurgarh
0.89	0.56	200	Br	DBr	YBr	Safed Rohtak
0.90	0.38	201	Br	DBr	Bl	Safed Rohtak
0.92	0.67	202	Bl	Bl	DBl	Gobindgarh selection

\*Colour and Shade abbreviations :

Br — Brown; Bl — Blue; Y — Yellow; L — Light; D — Dark; L — Lemon

In this group spot number 1 was common in 'Umran', 'Ajmeri', 'Katha', 'Chonchal' and 'Mundia', spot number 4 in 'Mundia' and 'Triloki', spot number 6 in 'Mundia' and 'Jogia', 11 and 25 in 'Banarasi kadaka' and 'Deedwana', 12 in 'Chencho' and 'Dandan', 15 in 'JS II', 'Pathan' and 'Mirchia', 24 in 'Pathan' and 'Chonchal', 28 in 'Jogia', 'Dandan' and 'Triloki', 36 in 'Banarasi pebandi' and 'Pathan'. However, within this group the cultivars 'Babadurgarh', 'Madhuri', 'ZG-3', 'Jullundhari' and 'Vikas 2' did not have common flavonoid spots.

### 3. Cultivars with spreading growth habit

Cultivars 'Gola', 'Gola Gurgaon', 'Gola Gurgaon-1', 'Popular gola' and 'Kakrola gola' had round fruit and the leaf shape varied from cordate, oblong to oval. Although 'Gola' (meaning round shape) is common in their nomenclature, the flavonoid pattern did not establish any relationship among these cultivars. 'Kala gola' seemed to be a misnomer since its fruits are oblong. Incidentally 'Kala gola' resembled Maharwali with regard to leaf flavonoid pattern. Cultivar 'Willayati', however, retained its independent identity in its flavonoid pattern and did not resemble any of the cultivars of the group.

Spot number 1 was common in 'Maharwali', 'Kala gola', spot 2 in 'Meharun' and 'Kakrola gola', spot 4 in 'Ponda' and 'Kheera', spot 5 in 'Safed Rohtak' and 'Kala gola', spot 9 in 'Gola Gurgaon' and 'Pebandi Alwar', 10 in 'Popular gola', 'Kala gola' and 'Sanaur-2', 13 in 'Gola', 'Gurgaon' and 'Kala gola', 18 in 'Katha phal' and 'Rewa selection', 19 in 'Kala gola' and 'Sanaur-3', 20 in 'Pebandi Alwar' and 'Maharwali', 23 in 'Jhajjar Selection', 'Popular gola' and 'Meharun', 26 in 'Safed Rohtak' and 'Bagwadi', 39 in 'Gola' and 'Safed Rohtak' and 44 in 'Gola' and 'Laddu'. Other cultivars had no common spots.

### B. Inter-group relationship

Among the cultivars emanating from a village Sanaur in Punjab (Chadha et al., 1972), 'Sanaur-1' had erect growth habit and 'Sanaur-2' and 'Sanaur-3' were of spreading type. 'Sanaur-1' and 'Sanaur-3' had ovate fruit shape but in 'Sanaur-2' fruits were oblong. Chemotaxonomic evidence showed 'Sanaur-1' and 'Sanaur-2' to resemble in their flavonoid pattern while 'Sanaur-3' was different. Similarly 'Kala gola' with spreading growth habit showed affinity with 'Umran', 'Ajmeri' and 'Katha' of the semi-erect group. 'Kala gola' could therefore be a seedling selection from the population of commercial cultivar 'Umran' in Haryana. Flavonoid spot pattern in cultivar 'Nimaj' resembled 'Umran', 'Ajmeri' and 'Katha' on one side and 'Mirchia' on the other. 'Mirchia' also resembled 'Pathan'. This suggests a common lineage of these cultivars. The flavonoid pattern similarities in the intergroup cultivars may be due to their development from cross pollination in an intermixed population. Seedling

progeny of the cultivated tetraploid types are never uniform because of their natural hybridization through entomophily (Khoshoo and Singh, 1963). Such hybrids are likely to be reasonably fertile and the resultant polyploids are segmental allöploids in nature.

### C. Relationship with wild species

Flavonoid analysis of the wild species *Z. nummularia* (Table 2) and *Z. mauritiana* var. *rotundifolia* (Table 3) showed distinct patterns, no flavonoid spots in common and having no possible relationship with the cultivars of *Z. mauritiana*.

Table 2. Characteristics of flavonoid spots in *Ziziphus nummularia*

Spot No.	Rf		Colour change		
	BAW	AcOH	UV	UV/NH <sub>3</sub>	AlCl <sub>3</sub>
1	0.43	3.40	DBr	DBr	Le Y
2	0.36	0.24	Br	Br	Y
3	0.23	0.05	Y	LY	DY
4	0.51	0.35	Br	Br	Le Y
5	0.51	0.44	Br	Br	Le Y
6	0.50	0.48	Br	Br	Le Y
7	0.34	0.48	Br	Br	Y
8	0.49	0.58	Br	Br	YBr
9	0.58	0.61	Br	Br	Y
10	0.55	0.67	Br	Er	Br Y
11	0.50	0.61	Br	Br	YBr
12	0.43	0.60	Br	Br	Br Y
13	0.36	0.56	Br	Br	Y
14	0.24	0.60	Br	Br	Y
15	0.50	0.67	Br	Br	Le Y
16	0.53	0.68	Br	Br	Le Y
17	0.46	0.73	Br	Br	Le Y
18	0.42	0.71	Br	Br	DBr
19	0.21	0.78	Bl	Bl	DBl

Br — Brown; Bl — Blue; Le — Lemon; D — Dark; Y — Yellow; L — Light

Table 3. Characteristics of flavonoid spots in *Zizyphus mauritiana* var. *rotundifolia*

Spot No.	Rf		Colour change		
	BAW	ACOH	UV	UV/NH <sub>3</sub>	AlCl <sub>3</sub>
1	0.48	0.35	DBr	DBr	Le Y
2	0.39	0.38	DBr	DBr	Le Y
3	0.19	0.07	Y	Y	DY
4	0.50	0.45	DBr	DBr	Le Y
5	0.44	0.50	Y	Y	Le Y
6	0.39	0.51	DBr	DBr	Le Y
7	0.48	0.54	DBr	DBr	Le Y
8	0.41	0.57	DBr	DBr	Le Y
9	0.46	0.68	Bl	GrBl	Le Y

Br — Brown; B — Blue; L — Light; Le — Lemon; Gr — Green; Y — Yellow; D — Dark

All round-fruited types had independent identity. 'Katha', 'Umran' and 'Ajmeri' cultivars were found to be identical.

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