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CHAPTER – 3
RESULTS & DISCUSSION

STUDIES CONDUCTED ON THIRTY DIFFERENT VAREITIES

MORPHOLOGICAL STUDIES

This chapter has been divided into two main parts. The first portion includes study on the various aspects of the leaves in thirty different varieties of *M.indica*. The second portion deals with the comparative account of fruit development and biochemical changes associated with the different stages of fruit development in three different varieties growing in two different climatic region of Gujarat.

I.MORPHOLOGICAL FEATURES:

The classification proposed by Gangolly *et al.* (1957), has been used herewith to describe the characteristic morphological features of the of studied *Mangifera indica* L. varieties.

i) Vegetative features

Typical trees in normal healthy growth and of adult bearing age of each variety were selected.These trees have been grown in Orchard of JAU, Junagadh, Gujarat, India. All the samples used in the present study were collected from such trees. Mature shoots and leaves of the current season growth were selected. All the vegetative features are represented in plate 1 and 2.

QUALITATIVE CHARACTERS: Qualitative vegetative characteristic features of the different varieties have been represented in table 2. Leaves of all the varieties are simple, exstipulate and arranged alternately. Petiole has a pulvinus base. When the angle made by the leaf with the shoot is less than 45˚, the leaves are designated as upheld or erect. On the other hand, when the angle is a right angle or wider, the leaves are termed as drooping or down held. The intermediate position entitles the leaves to be termed as spreading or out-held. The angle of leaf to shoot was intermediate in varieties Cowasji, Jhumakhiya 1, Ladvo, Kesar, Jhumakhiya 2, Sopari,

Badshahpasand, Dudhpendo, Alphonso, Fazli, Jahangir, Totapuri, Kaju and Gajariyo. Upheld or erect angle was seen in varieties Batli, Sindoria, Pairi, Goto, Jamadar, Khodi, Langdo, Aambadi, Asadiyo, Neelum, Desi, Rajapuri, Jhamrukhiyo, and Aamirpasand. Varieties Mulgoa and Rucchado showed outheld or spreading type of angle.

a. Folding nature: The nature of folding is a feature of considerable diagnostic value. In some varieties the leaves are flat. In others, the leaves are termed as slightly folded or strongly folded depending upon the extent of the curvature. Nature of folding was seen flat in varieties Cowasji (plate 1a), Jamadar (plate 1g), Rucchado (plate 1i), Aambadi (plate 2a), Asadiyo (plate 2b), Neelam (plate 2c), Badshahpasand (plate 2d), Dudhpendo (plate 2f), Alphonso (plate 2g), Rajapuri (plate 2h), Fazli (plate 2i), Totapuri (plate 2k), Kaju (plate 2m), and Gajariyo (plate 2o). It was found slightly folded in varieties Batli (plate 1b), Jhumakhiya 1(plate 1c), Sindoria (plate 1d), Pairi (plate 1e), Goto (plate 1f), Mulgoa (plate 1h), Ladvo (plate 1j), Kesar (plate 1l), Jhumakhiya 2 (plate 1m), Sopari (plate 1n), Langdo (plate 1o), Desi (plate 2e), Jhamrukhiyo (plate 2l), and Aamirpasand (plate 2n). While in varieties Khodi (plate 1k) and Jahangir (plate 2j), it was strongly folded.

Crinkled leaves form the characteristic features of some varieties, while twisting of the leaves is predominantly observed in others. Wavy margins are also a varietal character. Varieties Cowasji (plate 1a), Rucchado (plate1i), Desi (plate 2e), Dudhpendo (plate 2f), Alphonso (plate 2g), Jhamrukhiyo (plate 2l), Aamirpasand (plate 2n) and Gajariyo (plate 2o), showed slightly wavy condition while wavy condition was seen in Sindoria (plate 1d), Mulgoa (plate 1h), Kesar (plate 1l), Jhumakhiya 2 (plate 1m), Sopari (plate 1n), Langdo (plate 1o), Aambadi (plate 2a), Asadiyo (plate 2b), Badshahpasand (plate 2d). Twisted condition was seen in Batli

(plate 1b), Jamadar (plate 1g), Ladvo (plate 1j), Khodi (plate 1k), Neelum (plate 2c), Rajapuri (plate 2h) and Totapuri (plate 2k). Varieties Jhamakhiya 1(plate 1c), Pairi (plate 1e), Goto (plate 1f), Jahangir (plate 2j), showed a typical crinkled appearance. While in varieties Fazli (plate 2i) and Kaju (plate 2m), the crinkling was not observed, so it was termed straight (table 2).

b.Leaf shape: Leaf shape is a distinct varietal character in mango and furnishes one of the most conspicuous and the simplest diagnostic characters for use by growers. When the leaves are very narrow and lance shaped, they are designated as elliptic lanceolate. When the maximum breadth of the leaf is found at the center of the leaf along with the lance shape are termed as oval lanceolate. Ovate lanceolate is the term applied when the lance shaped leaves have their maximum breadth nearer to the leaf base. Varieties Cowasji, Batli, Jhumakhiya 1, Pairi, Goto, Mulgoa, Rucchado, Ladvo, Khodi, Jhumkhiya 2, Aambadi, Desi, Jahangir, Totapuri, Kaju and Gajariyo showed ovate lanceolate shaped leaf. Sindoria, Kesar, Sopari, Neelum, Dudhpendo, Alphonso showed the elliptic lanceolate leaf shape. A distinct lanceolate shape was seen in Fazli. While an intermediate shape termed oval lanceolate was observed in Jamadar, Langdo, Asadiyo, Badshahpasand, Rajapuri, Jhamrukhiyo and Aamirpasand. The margins of the leaves in all the varieties were entire varying in its crinkling feature.

c.Leaf apex: Leaf apex also called as leaf tip, which has been employed by a number of pomologists as a basis for classification. Leaf tip in mango may be sharp pointed with a long drawn tip (acuminate) and may end in a blunt point (acute) or be intermediate between the above extremes (sub-acuminate) (plate 1 and 2). Leaf tip was acute in Cowasji, Goto, Mulgoa, Jhumakhiya 2, Langdo, Asadiyo, Neelum, Badshahpasand, Totapuri, Jhamrukhiyo, Aamirpasand and Gajariyo while it was acuminate in Batli, Jhumakhiya 1, Khodi, Kesar, Sopari, Desi, Alphonso, Rajaprui

and Jahangir. Sub-acuminate leaf tip was found in Sindoria, Pairi, Rucchado, Ladvo, Dudhpendo and Kaju a while acute to acuminate type was observed in Jamadar and Fazli. In variety Aambadi, it was obtuse type.

d.Leaf base: Leaf base was either obtuse or acute. In varieities Cowasji, Batli, Goto, Ladvo, Khodi, Desi, Jahangir, Kaju and Gajariyo. Acute leaf base was seen in Jhumakhiya 1, Sindoria, Pairi, Jamadar, Mulgoa, Rucchado, Kesar, Jhumakhiya 2, Sopari, Langdo, Aambadi, Asadiyo, Neelam, Badshahpasand, Dudhpendo, Alphonso, Rajapuri, Fazli, Totapuri, Jhamrukhiyo and Aamirpasand.

QUANTITATIVE CHARACTERS: A total of 50 mature leaves were randomly selected from five different trees of the same variety and evaluated for the quantitative characteristics (table 3). Quantitative characteristics of the leaves in the different varieties have been represented in table 3. Length and breadth of lamina of leaf were measured. Length of the leaves in all the varieties varied between 14-30 cm except in Rucchado in which the length was only 8.7cm and in Dudhpendo which showed the highest length 39.37 cm. Highest breadth was seen in Jahangir (8.2 cm) and lowest in Fazli (2.64 cm) variety (table 3). Also the petiole length taken for comparison among varieties showed variations of interest. Highest petiole length was found in Asadiyo (5.4 cm) and lowest in Neelam (0.8 cm) variety. In Fazli, the pulvinus base was very short and the breadth and of the leaf was smallest, so the leaves were very small as compared to the other studied varieties.Highest leaf base length was found in Gajariyo (2 cm) and lowest in Fazli (0.78 cm) variety. Internodal length was highest in Dudhpendo (1.9cm) and Gajariyo (1.85cm) and lowest in Rucchado (0.36 cm), Sopari (0.35 cm) and Jhamrukhiyo (0.36 cm) varieties (table 3). As the internodal length was very less in these three varieties the branches appeared to be densely covered with leaves compared to the other varieties, especially in Dudhpendo and Gajariyo in which the leaves appeared to be widely spaced.The angle of the leaf also varied, ranging from 25-70˚ (table 3). Graphical representation of variation in leaf character is represented in figure 2.

Sr. no.	Variety	Angle of leaf to shoot	Leaf shape	Nature of folding	Crinkling of leaf	Margin	Leaf apex	Leaf base
1	Cowasji	intermediate	ovate lanceolate	flat	slightly wavy	entire	acute	obtuse
2	Batli	upheld or erect	ovate lanceolate	slightly folded	twisted	entire	acuminate	obtuse
3	Jhumakhiya 1	intermediate	ovate lanceolate	slightly folded	crinkled	entire	acuminate	acute
4	Sindoria	upheld or erect	elliptic lanceolate	slightly folded	wavy	entire	sub-acuminate	acute
5	Pairi	upheld or erect	ovate lanceolate	slightly folded	crinkled	entire	sub - acuminate	acute
6	Goto	upheld or erect	ovate lanceolate	slightly folded	crinkled	entire	acute	obtuse
7	Jamadar	upheld or erect	oval lanceolate	flat	twisted	entire	acute - acuminate	acute
8	Mulgoa	out-held or spreading	ovate lanceolate	slightly folded	wavy	entire	acute	acute
9	Rucchado	out-held or spreading	ovate lanceolate	flat	slightly wavy	entire	sub - acuminate	acute
10	Ladvo	intermediate	ovate lanceolate	slightly folded	twisted	entire	sub-acuminate	obtuse
11	Khodi	upheld or erect	ovate lanceolate	strongly folded	twisted	entire	acuminate	obtuse
12	Kesar	Intermediate	elliptic lanceolate	slightly folded	wavy	entire	acuminate	acute
13	Jhum 2	interemdiate	ovate lanceolate	slightly folded	wavy	entire	acute	acute
14	Sopari	intermediate	elliptic lanceolate	slightly folded	wavy	entire	acuminate	acute
15	Langdo	upheld or erect	oval lanceolate	slightly folded	wavy	entire	acute	acute
16	Aambadi	upheld or erect	ovate lanceolate	flat	wavy	entire	obtuse	acute
17	Asadiyo	upheld or erect	oval lanceolate	flat	wavy	entire	acute	acute
18	Neelam	upheld or erect	elliptic lanceolate	flat	twisted	entire	acute	acute
19	Badshahpasand	intermediate	oval lanceolate	flat	wavy	entire	acute	acute

20	Desi	upheld or erect	ovate lanceolate	slightly folded	slightly wavy	entire	acuminate	obtuse
21	Dudhpendo	intermediate	elliptic lanceolate	flat	slightly wavy	entire	sub - acuminate	Acute
22	Alphonso	intermediate	elliptic lanceolate	flat	slightly wavy	entire	acuminate	Acute
23	Rajapuri	upheld or erect	oval lanceolate	flat	twisted	entire	acuminate	Acute
24	Fazli	intermediate	Lanceolate	flat	straight	entire	acute - acuminate	Acute
25	Jahangir	intermediate	ovate lanceolate	strongly folded	crinkled	entire	acuminate	Obtuse
26	Totapuri	intermediate	ovate lanceolate	flat	twisted	entire	acute	Acute
27	Jhamrukhiyo	upheld or erect	oval lanceolate	slightly folded	slightly wavy	entire	acute	Acute
28	Kaju	intermediate	ovate lanceolate	flat	straight	entire	sub - acuminate	Obtuse
29	Aamirpasand	upheld or erect	oval lanceolate	slightly folded	slightly wavy	entire	acute	Acute
30	Gajariyo	intermediate	ovate lanceolate	flat	slightly wavy	entire	acute	Obtuse

Table 2. Qualitative features of leaf of *Mangifera indica* L.

Sr. no.	Varieties	Length (cm)	Breadth (cm)	Pulvinus leaf base(cm)	Petiole (cm)	Interode length (cm)	Angle
1	Cowasji	26.78	6.46	1.96	1.92	1.62	25-40
2	Desi Batli	15.22	4.48	1.06	1.92	0.93	43-49
3	Desi Jhumakhya-1	20.88	5.36	1.5	1.66	1.34	29-40
4	Desi Sindoria	20.8	5.32	1.38	2.42	0.4	30- 33
5	Pairi	24.9	6.38	1.44	2.34	1.26	50-55
6	Desi Goto	28.8	7.1	1.38	1.4	1.11	30-35
7	Jamadar	16.5	3.6	0.98	2.3	0.8	45-58
8	Mulgoa	19.74	5.8	1.64	1.82	1.28	30-40
9	Desi Rucchado	8.7	5.78	1.7	2.06	0.36	33-42
10	Desi Ladvo	13.9	3.9	1.36	2.1	0.68	43-49
11	Khodi	23.72	5.6	1.2	2.1	1	35-44
12	Kesar	30.8	4.77	1.5	2.02	1.15	42-50
13	Desi Jhumakhya-2	27.2	6.06	1.44	2.4	0.44	49-54
14	Sopari	17.38	3.22	1.04	1.32	0.35	40-45
15	Langdo	18.72	5.82	1.66	1.28	0.83	32-45
16	Aambadi	18.14	5.9	1.44	1.36	0.48	38-43
17	Asadiyo	22.32	6.16	1.36	5.4	1.1	30-52
18	Neelam	13.22	4.9	1.26	0.8	0.72	25-40
19	Badshah Pasand	16.78	4.8	1.6	2.46	1.11	31-49
20	Desi	19.31	4.7	1.38	2.28	1.02	36-44
21	Dudhpendo	39.97	7.05	1.46	4.24	1.9	33-35
22	Alphonso	21.52	4.5	1.42	2.08	1.2	30-40
23	Rajapuri	18.68	6.7	1.44	1.84	0.86	30-47
24	Fazli	16.78	2.64	0.78	1.76	0.76	45-50
25	Jahangir	24.3	8.2	1.44	1.6	0.6	25-39
26	Totapuri	23.04	5.7	1.58	1.64	1.06	35-40
27	Jhamrukhiyo	17.62	4.3	1.04	1.36	0.36	30-50
28	Kaju	18.7	3.9	1.5	1.02	1.26	25-45
29	Amir Pasand	23.4	4.7	1.8	1.78	1.2	35-48
30	Gajariyo	21.82	5.1	2	1.83	1.85	30-70

Table 3. Quantitative characteristics in the leaves of different varieties of *Mangifera indica* L.

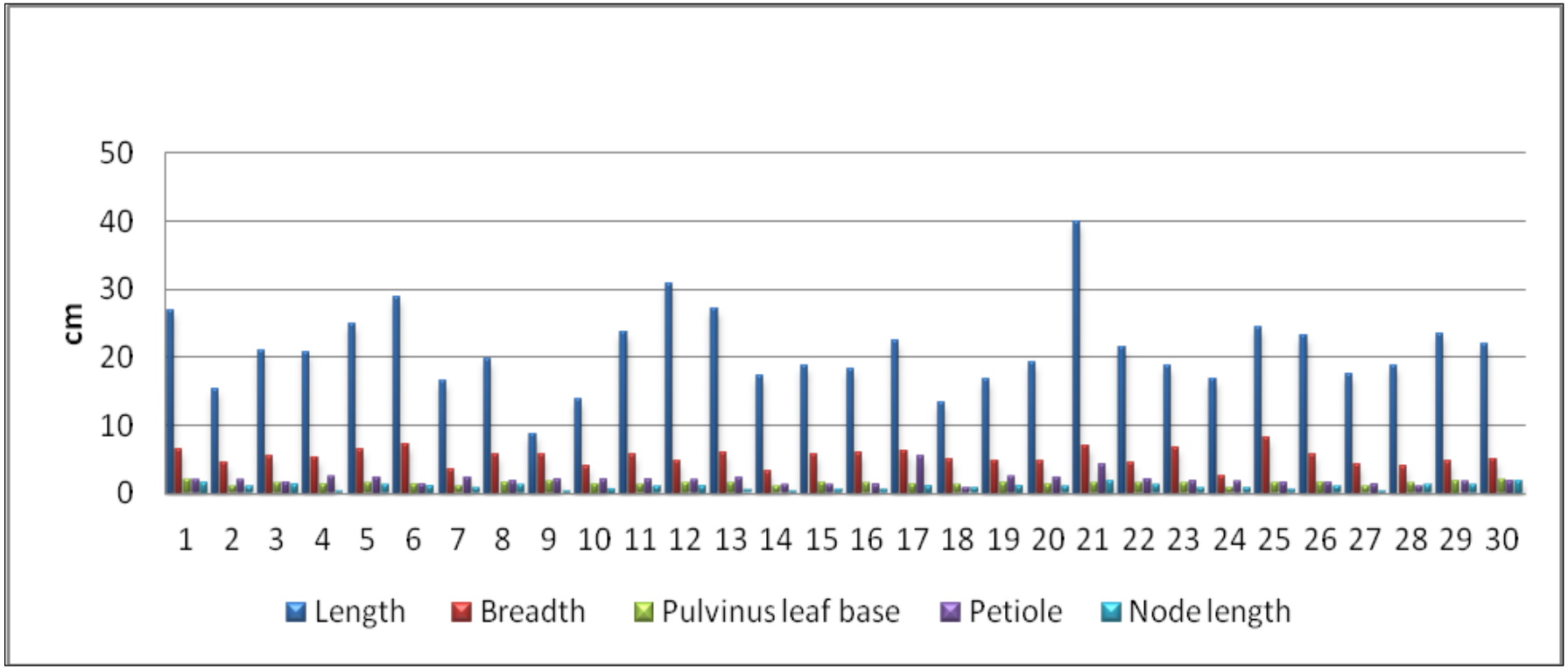


Figure 2: Variation in leaf characters of thirty varieties of *M.indica.*

REPRODUCTIVE CHARACTERISTICS: In the reproductive characteristics, features of the inflorescence (plate 3 and 4) and fruits (plate 5 and 6) were evaluated.

ii) Floral characters

a.Inflorescence: Flowering started in November - December in all the variety except in Mulgoa, Langdo, Asadiyo, Neelam and Gajariyo. Flowers were seen in these vareities during the month of Jan-feb in Mulgoa, Langdo and Gajariyo and March-April in Asadiyo and Neelam.

Inflorescence features included qualitative characters like shape of the panicle, color of inflorescence axis, floral characters and quantitative characters like peduncle length and ratio of male to bisexual flower. Variation in length of the panicle is represented in figure 3.

b. Panicle shape: The shape of the panicle was one of the most important varietal characteristics. Conical panicles with the length of the main axis markedly greater than the spread are a definite character in some varieties. In others, pyramidal inflorescence with the spread approximating the length forms the distinguishing feature. The shape of inflorescence was found pyramidal in varieties Cowasji(plate 3a), Jhumakhiya 1 (plate 3c), Sindoria (plate 3d), Pairi (plate 3e), Goto (plate 3f), Mulgoa (plate 3h), Ladvo (plate 3j), Khodi (plate 3k), Kesar (plate 3l), Jhumakhiya 2 (plate 3m), Langda (plate 3o), Aambadi (plate 4a), Asadiyo (plate 4b), Dudhpendo (plate 4f), Alphonso (plate 4g), Fazli (plate 4i), Totapuri (plate 4k), and Gajariyo (plate 4o). It was conical in varieties Batli (plate 3b), Jamadar (plate 3g), Sopari (plate 3n), Neelam (plate 4c), Badshahpasand (plate 4d), Desi (plate 4e), Rajapuri (plate 4h), Jahangir (plate 4j), Jhamrukhiyo (plate 4l), and Aamirpasand (plate 4n), (Sharma *et al*. 2011). A cylindrical form was evident in Rucchado (plate 3i) and Kaju (plate 4m) variety.

c. Colour: The colour of the inflorescence was also recorded to differentiate the varieties. Colour like, green and red were observed in different varieties. The inflorescence axis was green in Cowasji(plate 3a), Batli(plate 3b), Jhumakhiya 1 (plate 3c), Rucchado (plate 3i), Ladvo (plate 3j), Khodi (plate 3k), Jhumakhiya 2 (plate 3m), Desi (plate 4e), Alphonso (plate 4g), Rajapuri (plate 4h), Jahangir (plate 4j), Aambadi (plate 4a), Asadiyo (plate 4b) and Kaju (plate 4m). Red coloured axis was found in Sindoria (plate 3d), Pairi (plate 3e), Goto (plate 3f), Jamadar (plate 3g), Mulgoa (plate 3h), Kesar (plate 3l), Sopari (plate 3n), Langdo (plate 3o), Badshahpasand (plate 4d), Dudhpendo (plate 4f), Rajapuri (plate 4h), Fazli (plate 4i), Totapuri (plate 4k), Jhamrukhiyo (plate 4l), Aamirpasand (plate 4n) and Gajariyo (plate 4o).

d. Floral whorls: Inflorescence had two types of flowers, bisexual (hermaphrodite) and male (staminate) flowers. The flowers are radially symmetrical, and usually have 5 petals. There is usually only 1 fertile stamen per flower; the 4 other stamens are sterile. The flower has a conspicuous 5- lobed disk between the petals and stamens.Based on the presence of staminodes and its number, flower appears to be an important character. The varieties can be grouped into two: flowers having well developed and poorly developed staminodes. In all the varieties, there were four staminodes and one well developed stamen. But in Desi, Langdo, Aambadi, Neelum and Gajariyo there were three staminodes, one medium sized stamen and one longer stamen. Hermaphrodite flowers were complete with the entire four accessory and essential whorls. Because of the presence of lateral style and staminodes, flower appeared to be zygomorphic. Staminodes are present on the anterior side. Mango varieties differ markedly in regard to the relative size of the stamens and pistil and their orientation on the flower disc. In all the varieties, stamen was longer than the

pistil, but in Alphonso, Kesar, Totapuri and Langda, the pistil and stamens are found to be of equal length in some flowers. The relative position of the stamens and pistil may be parallel or oblique to each other. All the varieties showed oblique position except Alphonso, Totapuri and Rajapuri which showed parallel situation. The ovary of all the varieties are round in shaped. Lateral style arises towards anterior side.

Based on the density of flower, varieties Cowasji, Pairi, Rucchado, Kesar, Neelam, Badshahpasand, Dudhpendo, Alphonso, Jahangir, Totapuri and Jhamrukhiyo were categorized into a densely flowered group. A large number of varieties including Batli, Jamadar, Mulgoa, Khodi, Jhumakhiya-2, Sopari, Langda, Aambadi, Desi, Rajapuri, Fazli, Kaju, Aamirpasand and Gajariyo were found with a medium density of flower in the inflorescence. While the laxly flowered group were observed in varieties Jhumakhiya-1, Sindoria, Goto, Ladvo and Asadiyo.

The ratio between bisexual and male flower showed variation. The ratio is mainly governed by the climatic conditions. In Cowasji (80:20), Sindoria (95:5), Jamadar (60:40), Asadiyo (60:40), Neelum (90:10), Dudhpendo (60:40), Fazli (60:40) and Gajariyo (60:40), the ratio of male to bisexual flower was more. In other varieties Batli (20:80), Jhumakhiya 1 (20:80), Pairi (30:70), Mulgoa (20:80), Rucchado (10:90), Ladvo (30:70), Jhumakhiya 2 (10:90), Sopari (20:80), Langdo (10:90), Aambadi (20:80), Badshahpasand (40:60), Desi (30:70), Rajapuri (40:60), Jahangir (20:80), Jhamrukhiyo (40:60), Kaju (10:90) and Amirpasand (20:80), had less male to female ratio. While the ratio was found equal (50:50) in Goto, Khodi, Kesar, Alphonso and Totapuri (table 4). Out of these varieties, late flowering ones Mulgoa, Langdo had more bisexual flower and Asadiyo, Neelam and Gajariyo had less bisexual flowers.

The size of the panicles is another character. Measurements of the length are taken for panicle. Length of panicle ranged between 12 cm to 30 cm. Jamadar had highest panicle length (38 cm), followed by Kesar (34.16 cm) while it was lowest in Batli (10.6 cm) (table 4).

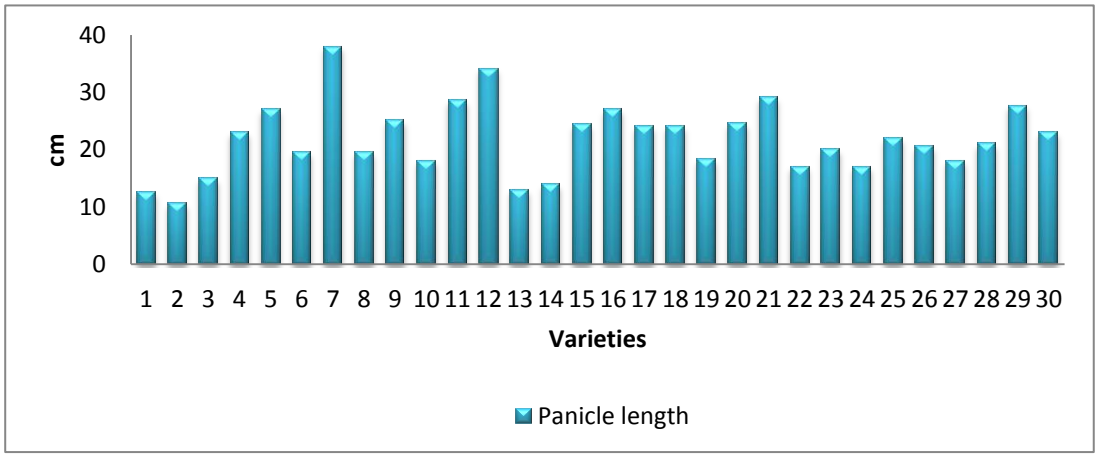


Figure 3: Variation in panicle length of *M indica* inflorescence.

iii) Fruit characters

The fruit is an irregular egg-shaped and slightly compressed fleshy drupe attached to the pendulous stalk. Conventional methods of recording fruit descriptions are employed in the present study. The fruit is held in its normal position. The observer faces the fruit in such a way to have the beak and concave surface to his left and the convex surface to his right. The stalk end is called the base and the opposite end, the apex. The left lobe which is generally larger in most varieties is designated as the left or the ventral shoulder and the opposite one as the right or the dorsal shoulder. The size and the nature of the stalk which attaches the fruit to the tree as well as the nature of insertion of the stalk to the fruit are given some prominence.

The stalk was either square found in Cowasji (plate 5a), Jhumakhiya 1(plate 5c), Pairi (plate 5e), Goto (plate 5f), Mulgoa (plate 5h), Kesar (plate 5l) Langdo (plate 5o), Asadiyo (plate 6b), Desi (plate 5e), Dudhpendo (plate 6f), Jahangir (plate 6j) and Kaju (plate 6m),or oblique in Batli (plate 5b), Sindoria (plate 5d), Jamadar (plate 5g), Rucchado (plate 5i), Ladvo (plate 5j), Khodi (plate 5k), Jhumakhiya 2 (plate 5m), Sopari(plate 5n), Aamabdi (plate 6a), Neelam (plate 6c), Badshahpasand (plate 6d),

Alphonso (plate 6g), Rajapuri (plate 6h), Fazli (plate 6i), Totapuri (plate 6k), Jhamrukhiyo (plate 6l), Aamirpasand (plate 6n) and Gajariyo (plate 6o). The depression that is often present near the point of attachment of the stalk of the fruit is commonly termed as the cavity. It can be extended as seen in Fazli (plate 6i)and Totapuri(plate 6k) , tapering in Langdo (plate 5o), rounded in Batli (plate 5b), Mulgoa (plate 5h), Neelam (plate 6c) and Rajapuri (plate 6h), obliquely rounded in Jhumakhiya 1 (plate 5c), Sindoria (plate 5d), Goto (plate 5f), Jamadar (plate 5g), Rucchado (plate 5i), Khodi (plate 5k), Kesar (plate 5l), Jhumakhiya 2 (plate 5m), Sopari (plate 5n), Badshahpasand (plate 6d), Alphonso (plate 6g) and Aamirpasand (plate 6n), flattened in Pairi (plate 5e), Asadiyo (plate 6b), Desi (plate 5e), Jahangir (plate 6j) and Kaju (plate 6m) or obliquely flattend in Ladvo (plate 5j), Aambadi (plate 6a), Jhamrukhiyo (plate 6l) and Gajariyo (plate 6o).The concavity which lies a little above the beak or 'Nak' is known as the sinus. Sinus was absent in varieties Cowasji (plate 5a), Jhumakhiya 1(plate 5c), Sindoria (plate 5d), Jamadar (plate 5g), Sopari (plate 5n), Langdo (plate 5o), Desi (plate 6e), Dudhpendo (plate 5f) and Alphonso (plate 6g). In other varieties it was slight, seen in Batli (plate5b), Goto (plate 5f), Mulgoa (plate 5h), Rucchado (plate 5i), Ladvo (plate 5j), Khodi (plate 5k), Kesar (plate 5l), Jhumakhiya 2 (plate 5m), Aambadi (plate 6a), Asadiyo (plate 5b) and Jahangir (plate 6j), shallow in Pairi (plate 5e), Neelam (plate 6c), Rajapuri (plate 6h), Fazli (plate 6i), Jhamrukhiyo (plate 6l) and Kaju (plate 6m) or deep in Totapuri (plate 6k), Aamirpsand (plate 6n) and Gajariyo (plate 6o). Beak was absent in Cowasji (plate 5a), Jhumakhiya 1(plate 5c), Sindoria (plate 5d), Jamadar (plate 5g), Mulgoa (plate 5h), Ladvo (plate 5j), Jhumakhiya 2 (plate 5m), Sopari (plate 5n), Langdo (plate 5o), Aambadi (plate 6a), Asadiyo (plate 6b), Neelam (plate 6c), Desi (plate 6e), Dudhpedno (plate 6f), Alphonso (plate 6g) and Jahangir (plate 6j).The distance from the point of attachment to the extremity at the distal end is termed as the length of the fruit. The maximum distance between the two shoulders is recorded as the major diameter.

Form of fruit is the most prominent varietal character and has been largely employed in the mango classification in the past. Symmetrical fruits are those which can be divided into two equal halves so as to have the shoulders almost equal and well balanced. When such an imaginary cut is not possible, the fruit is styled as asymmetrical. Other group consists of the variety having the shoulders are of a very unbalanced shape with one markedly higher than the other. The fruits of this group are classed as oblique shaped. Symmetrical shape was observed in Goto (plate 5f),Mulgoa (plate 5h), Langdo (plate 5o), Aambadi (plate 6a), Desi (plate 6e), asymmetrical shape was found in Cowasji (plate 5a), Pairi (plate 5e), Jamadar (plate 5g), Rucchado (plate 5i), Khodi (plate 5k), Kesar (plate 5l), Jhumakhiya 2 (plate 5m), Asadiyo (plate 6b), Dudhpendo (plate 6f), Rajapuri (plate 6h), Totapuri (plate 6k), Aamirpasand (plate 6n). Oblique type was seen in Batli (plate 5b), Sindoria (plate 5d), Ladvo (plate 5j), Sopari (plate 5n), Badshahpasand (plate 6d), Alphonso (plate 6g), Fazli (plate 6i) and Jhamrukhiyo (plate 6l) (table 4).

The most common fruit shapes in mango are roundish, ovate, oval, oblong, cordate, reniform, peento etc., with a number of shapes intermediate between these (table 4).

Quantitative characters like fruit weight (figure 4A), fruit length (figure 4B), fruit diameter (figure 4C), mesocarp thickness and stone thickness was taken for comparison among the varieties (table 4). The highest fruit weight was found in Jahangir (500 g) Rajapuri (440 g) and Cowasji (436.67 g) while other ranged between 300 g to 100 g. Lowest fruit weight was recorded in Sopari (48.33 g) and Khodi (64.44 g). The length of the fruit was found highest in Jahangir (17.35 cm) and Totapuri (16.35 cm) and lowest in Sopari (7 cm). The diameter of fruit was highest in Jahangir (30.05 cm) and Rajapuri (27.75 cm) while it was lowest in Jamadar (10.5 cm).Fruits of Jahangir were found to be the largest in size and weight. Thickness of mesocarp varied in all the varieties ranging from 5 to 15.5 cm. Highest mesocarp which is otherwise the pulp of the fruit was seen in Jahangir (15.5 cm) but very less pulp content was seen in Batli (5.1 cm), Sopari and Aambadi (5.2 cm).

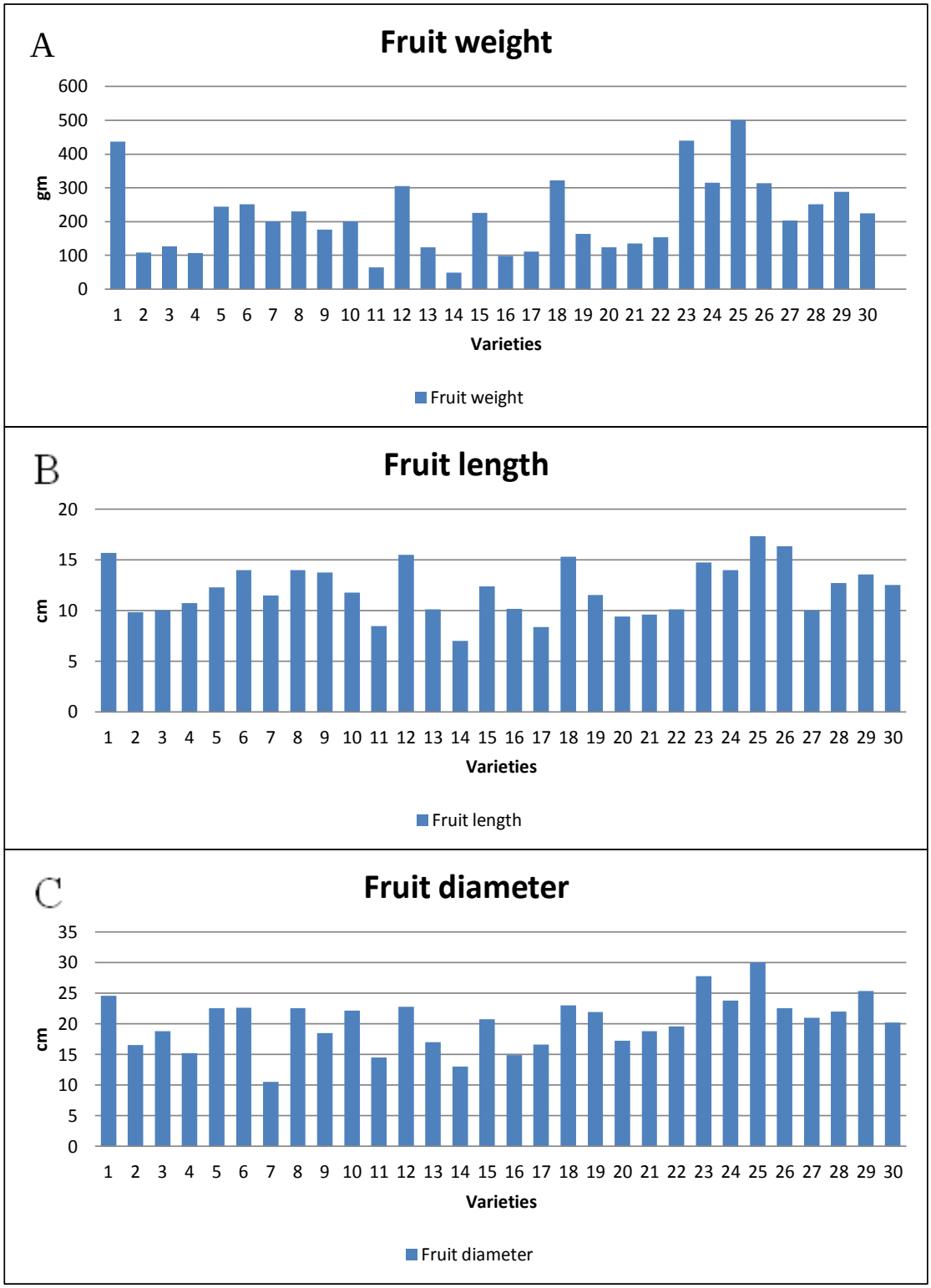


Figure 4: Variation in fruit weight (A), length (B)and diameter (C)in varieties of *M.indica*

Sr. no.	Varieties	Panicle length (cm)	Ratio (M: B)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit symmetry	Fruit shape	Mesocarp thickness (cm)	Stone thickness (cm)
1	Cowasji	12.5	80-20	436.67	15.7	24.6	Asymmetrical	Oblong	8.4	2.4
2	Desi Batli	10.6	20-80	108.33	9.83	16.5	Oblique	Roundish Oblique	5.1	2.2
3	Jhumakhiya-1	15	20-80	126.67	10	18.75	Asymmetrical	Oblong	7.5	1.9
4	Sindoria	23	95-5	106.66	10.75	15.2	Oblique	Oblong	7.0	1.9
5	Pairi	27	30-70	245	12.3	22.5	Asymmetrical	Ovate Reniform	9.6	2.5
6	Goto	19.6	50-50	251.11	14	22.6	Symmetrical	Oval	10	2.1
7	Jamadar	38	60-40	200	11.5	10.5	Asymmetrical	Ovate Oblong	4.3	1.8
8	Mulgoa	19.6	20-80	230	14	22.5	Symmetrical	Roundish	9.5	2.0
9	Rujhado	25	10-90	176.66	13.75	18.5	Asymmetrical	Oblong	7.1	2.0
10	Ladvo	18	30-70	200	11.75	22.11	Oblique	Cordate	9.8	2.2
11	Khodi	28.6	50-50	64.44	8.45	14.52	Asymmetrical	Oblong Oval	6.1	2.0
12	Kesar	34.16	50-50	305	15.5	22.8	Asymmetrical	Ovate Oblong	9.1	1.8
13	Jhumakhiya-2	13	10-90	123.33	10.1	17	Asymmetrical	Oblong	7.2	1.9
14	Sopari	14	20-80	48.33	7	13	Oblique	Ovate Oblique	5.2	2.0
15	Langdo	24.33	10-90	226.66	12.4	20.75	Symmetrical	Oblong	8.7	2.2
16	Aambadi	27	20-80	98.33	10.16	14.85	Symmetrical	Oblong	5.2	2.0

17	Asadiyo	24	60-40	111.66	8.36	16.6	Asymmetrical	Roundish	6.9	2.3
18	Neelam	24	90-10	321.66	15.33	23	Symmetrical	Oblong elliptic	10.1	2.1
19	Badshah-pasand	18.33	40-60	163.33	11.53	21.9	Oblique	Obliquely Oval	9.2	2.4
20	Desi	24.5	30-70	123.33	9.4	17.2	Symmetrical	Oval	7.4	2.2
21	Dudhpendo	29	60-40	135	9.6	18.8	Asymmetrical	Roundish	7.0	2.0
22	Alphonso	17	50-50	153.33	10.1	19.55	Oblique	Oval	8.2	1.8
23	Rajapuri	20	40-60	440	14.75	27.75	Asymmetrical	Ovate Oblong	13.1	2.1
24	Fazli	17	60-40	315.55	14	23.75	Oblique	Obliquely Oval	10.0	2.0
25	Jahangir	22	20-80	500	17.35	30.05	Asymmetrical	Ovate Roundish	15.5	2.3
26	Totapuri	20.5	50-50	313.33	16.35	22.5	Asymmetrical	Oblong Elliptic	9.6	1.8
27	Jhamrukhiyo	18	40-60	203.33	10	21	Oblique	Ovate Oblique	9.1	2.4
28	Kaju	21	10-90	251.66	12.7	22	Asymmetrical	Oval Reniform	8.8	2.0
29	Amir Pasand	27.6	20-80	288.88	13.55	25.35	Asymmetrical	Ovate Oblong	10.2	2.1
30	Gajariyo	23	60-40	225	12.5	20.2	Oblique	Obliquely Oval	8.1	2.0

Table 4. Quantitative characters of Inflorescence and Fruit. (M- male flower, B- bisexual flower)

Plate 1 - Morphological features of leaves.

a. Cowasji
b. Batli
c. Jhumakhiya 1
d. Sindoria
e. Pairi
f. Goto
g. Jamadar
h. Mulgoa
i. Rucchado
j. Ladvo
k. Khodi
l. Kesar
m. Jhumakhiya 2
n. Sopari
o. Langdo

Plate 2- Morphological features of leaves.

a. Aambadi
b. Asadiyo
c. Neelam
d. Badshahpasand
e. Desi
f. Dudhpendo
g. Alphonso
h. Rajapuri
i. Fazli
j. Jahangir
k. Totapuri
l. Jhamrukhiyo
m. Kaju
n. Amirpasand
o. Gajariyo

Plate 3- Morphological features of inflorescence

a. Cowasji
b. Batli
c. Jhumakhiya 1
d. Sindoria
e. Pairi
f. Goto
g. Jamadar
h. Mulgoa
i. Rucchado
j. Ladvo
k. Khodi
l. Kesar
m. Jhumakhiya 2
n. Sopari
o. Langdo

Plate 4- Morphological features of inflorescence

a. Aambadi
b. Asadiyo
c. Neelam
d. Badshahpasand
e. Desi
f. Dudhpendo
g. Alphonso
h. Rajapuri
i. Fazli
j. Jahangir
k. Totapuri
l. Jhamrukhiyo
m. Kaju
n. Amirpasand
o. Gajariyo

Plate 5- Morphological features of fruits

a. Cowasji
b. Batli
c. Jhumakhiya 1
d. Sindoria
e. Pairi
f. Goto
g. Jamadar
h. Mulgoa
i. Rucchado
j. Ladvo
k. Khodi
l. Kesar
m. Jhumakhiya 2
n. Sopari
o. Langdo

Plate 6- Morphological features of fruits.

a. Aambadi

b. Asadiyo

c. Neelam

d. Badshahpasand

e. Desi

f. Dudhpendo

g. Alphonso

h. Rajapuri

i. Fazli

j. Jahangir

k. Totapuri

l. Jhamrukhiyo

m. Kaju

n. Amirpasand

o. Gajariyo

DISCUSSION

Total number of varieties found in India is estimated to be around thousand. This large number of varieties has led to huge number of fancy names. The fact that the identification of the varieties by vegetative characters alone at the time of purchase is not possible. Therefore, it is necessary to describe and catalogue the existing varieties, so that the fruit grower is enabled to choose correctly the material suitable for his conditions (Gangolly *et al*. 1957). Vegetative characters are considered to be of great importance by many workers. Popenoe (1939) has described the leaf as lanceolate, commonly up to 30.5 cm in length, ridged, deep green, almost glossy, borne upon slender petioles, 2.5 to 10 cm long. According to Singh (1960), leaves are variable in shapes like oval-lanceolate, lanceolate, oblong, linear-oblong, ovate, obovate-lanceolate or roundish-oblong, apex ranges from acuminate to nearly rounded. In the present study, leaves were either ovate lanceolate, oval lanceolate or elliptic lanceolate shape. The apex was acute, acuminate or obtuse. The margin is usually entire, sometimes slightly undulated and wavy, rarely twisted or folded. Various studies showed variation in length and breadth from 12 to 45 cm and 2 to 12 cm, respectively, depending upon variety and growth. The present varieties had length ranging from 8.7 cm to 40 cm and breadth from 2.64 cm to 8.2 cm. Naik and Gangolly (1950) described 335 varieties of south Indian mangoes on the basis of characters like emerging leaves, colouration of panicle axis and laterals, size of the flowers, intensity of pubescence of panicle branches, nature of bracts and length of inflorescence. The inflorescence is pseudo-terminal, originating from a bud, together with the new leafy sprout; there are cultivars with lateral inflorescence. But no such lateral position of inflorescence was observed in the present varieties. The colour of the panicle may be yellowish-green, light green with crimson patches or with crimson

flush on branches. The shape of the inflorescence was conical or pyramidal, but was cylindrical in Rucchado and Kaju. The length of the main rachis between two consecutive secondary rachis of the panicle shortens leading to a clustered appearance of the panicle inflorescence in Jhumakhiya, so the inflorescence appeared to be clustered.

Mainly two types of flowers are observed in the mango inflorescence – male and bisexual, but sometimes neutral floweres are recorded. Neutral flowers were not recorded in the present varieties. Depending upon the variety, the number of flower in the panicle varies from 800 to 5000. The percentage of perfect flower varies between 0.74 in Rumani and 69.8 in Langra (Singh 1954). Here the variation in the ratio of perfect flowers was between 5 to 90. Varieties with longer panicle produced larger number of flowers, especially male flowers (Thimmaiah and Suman 1987).But such explanation cannot be made here, as the variety showing highest panicle length is not necessarily having more male flowers compared to the short panicle variety (table 4). Kesar and Totapuri varieties were having more panicle length but the ratio was found to be equal of male and female flowers. Also variety Pairi and Desi had only 30% male flower and Langdo had only 10% male flower as compared to the panicle length. The sex-ratio in different cultivars is greatly influenced by the environment. The percentage of hermaphrodite flowers in a panicle is subject to appreciable variation depending upon the early or late emergence of the panicle and the variety (Singh 1954). Ratio of perfect flower was highest (90%) in Rucchado, Jhumakhiya 2, Langdo and Kaju and male flowers was highes in Sindoria (95%) and Neelam (90%). Popenoe (1917) had reported that the percentage of perfect flowers varies from 2 to 70 accrding to the variety. Gandhi (1955) stated that flowering may start as early as November or during December in Andhar Pradesh and the south Konkan on the West

Coast of India. North India, mango flowers from February to March. He concluded that under milder climatic condition, mango may start flowering from December itself, whereas in extreme climatic conditions the flowering time is comparatively more precise and late. Burns and Prayag (1921) has reported mango flowering in Philippines during December-January. In the present study, the flowering occurs in November-December and bear mature fruits in March- April, but some varieties flower in January-February and some in March-April, which are considered as the medium and late flowering variety repectively. The fruit set in mango is a critical phenomenon as it is highly influence by the ratio of bisexual flowers, temperature, etc. More number of bisexual flowers was noticed in the panicles of medium and late flushes in Dashehari (Singh 1966).

Cultivar like Neelum, Baneshan, Allumpur, Janardhan sand and Willard when grown under north Indian conditions have significantly lower proportion of perfect flower than under south Indian conditions (Singh *et al*. 1965). Some workers have suggested that trees exposed to low temperatures during inflorescence development have reduced number of bisexual flowers (Singh *et al*. 1965, Singh and Dhillon 1987). Position of panicle also played a major role in the proportion of bisexual flower. The inner portions of the tree had higher ratio of bisexual flowers than the panicles located on the peripheral side. The terminal position of the panicle had more amounts of bisexual flowers. Thus it can be concluded that higher temperatures support the emergence of bisexual flowers and the late flowering variety have more number of bisexual flower and fruit set.

The position of the fertile stamen and pistil may be either parallel or oblique to each other (Naik and Gangolly 1950).Juliano and Cuevas (1932) reported three fertile stamens, in cultivar Pico. As many as ten stamens, which occur in other members of

the genus, may also occasionally be found in the form of primordia only (Maheshwari 1934). Present study also had some varieties Desi, Langdo, Aambadi, Neelam and Gajariyo with three staminodes and two fertile stamens. The present study showed that the androecium consists of stamens and staminodes, altogether five in number, of which usually one, or rarely two, was fertile and the rest are sterile. All the stamens are inserted on the inner margin of the disc.The developmental morphology of mango fruit greatly depends upon the variety. No generalization can be made for growth in size, weight, shoulder, etc. However, with increase in size, the fruit attains the shape typical to the variety. Fruit studies include colour, shape and size. Fruit shapes seen were oblong, roundish oblique, ovate reniform, oval and cordate. Cordate was a distinct shape observed in Ladvo. Depending upon the shape of the variety, the names have been coined by the growers, oval in Goto, ovate oblique in Sopari, roundish in Dudhpendo, oblong elliptic in Totapuri, and oval reniform in Kaju.

Chemical parameters were included in other chapter. Cheema and Dani (1934), on the basis of external colour and growth, defined four maturity stages during fruit development. Singh *et al*. (1937) also classified the life of mango fruit into four stages: juvenile stage up to 21 days after fertilization representing rapic cellular growth; adolescent stage between 21 and 49 days; climacteric stage upto 77 days when it attained repiration climacteric and the fruit started ripening; the last stage was senescent stage when the respiration dropped and the fruit became edible ripe with the characteristic aroma and taste. Classification proposed by Singh *et al*. (1937) was followed for the present study. Singh and Singh (1956) incorporated characters like fruit shape and beak, venation on the stones, colour of the panicles, leaf apex and folding of leaves. According to Hulme (1971), the shape varies from round to ovate-oblong and the skin color from green through yellow to red. Fruit weight ranged from

48 g to 500 g with the maximum being in Jahangir and minimum in Sopari. Cultivated fruits weigh upto 3 lb (Chia *et al*. 1988). Studies suggest, the fruit weight increase during ripening and decrease after (Mannan *et al*. 2003, Kundachikar *et al*. 2001).

The pulp content of the fruit was measured by thickness of mesocarp. It ranged from 5.1 to 15.5 cm.The most common varieties Alphonso, Kesar and Rajapuri had 8.2 cm, 9.1 cm and 13.1 cm respectively.almost in all the varieties, the shape of a mature fruit was oblong to roundish with green to yellow color. One of the largest trees known is that from Chandigarh (India), with a trunk of 3.5 m in diameter, limbs of 75 cm diameter, the crown spreading over 2250 m^2 with an annual production of about 16000 fruits in peak years at the age more than 100 years old (Singh 1960).

In leaf nature of folding, breadth of leaf, length of leaf, pulvinus base, petiole and node. For inflorescence, characters like inflorescence occurring in clusters or singly colour of peduncle, length of peduncle, ratio of male to female flower and density of flowe has been primarily considered. Ratio in inflorescence is most important as it is responsible for fruit setting. The more number of bisexual flowers more will be the chances of fruit formation. Characters like fruit colour having a red blush on the shoulder as seen in Alphonso were considered. It enhances the appealing quality of the fruit and one of the reasons of the mentioned variety to be so popular. Various fruit shape were recorded, some were very distinct like cordate, reniform and round. Three different artificial synoptic keys have been formulated on the basis of the morphological characters of leaf, inflorescence and fruit.

Key I to the identification of thirty different varieties of *M.indica* based on leaf characters

1. **Nature of leaf folding – fla**t- Cowasji, Jamadar, Rucchado, Aambadi, Asadiyo, Neelam, Badshahpasand, Dudhpendo, Alphonso, Rajapuri, Fazli, Totapuri, Kaju, Gajariyo

1.1 **Crinkling of leaf- twisted**- Jamadar, Neelam, Totapuri, Rajapuri

1.1.1 Leaf length- 23 cm

Petiole length- 1.6 cm

Angle between leaf and shoot- 35-40----------**Totapuri**

1.1.2 Leaf length- 18 cm

Petiole length- 1.84 cm

Angle between leaf and shoot- 30-47----------**Rajapuri**

1.1.3 Leaf length- 16 cm

Petiole length- 2.3 cm

Angle between leaf and shoot- 45-58 ----------**Jamadar**

1.1.4 Leaf length- 13 cm

Petiole length- 0.8 cm

Angle between leaf and shoot- 25-40----------**Neelam**

1.2 **Crinkling of leaf- wavy**- Aambadi, Badshahpasand, Asadiyo

1.2.1 Leaf apex- acute- Badshahpasand and Asadiyo

1.2.1.1 Leaf length- 22.3 cm

Petiole length- 5.4 cm ----------**Asadiyo**

1.2.1.2 Leaf length- 16.7 cm

Petiole length- 2.4 cm ----------**Badshahpasand**

1.2.2 Leaf apex- obtuse----------**Aambadi**

1.3 **Crinkling of leaf- straight**

Leaf apex-acute- acuminate- Fazli, Kaju

1.3.1 Pulvinus base length- 0.78 cm

Anlge between leaf and shoot 25-45----------**Kaju**

1.3.2 Pulvinus base length- 1.5 cm

Anlge between leaf and shoot 45-50----------**Fazli**

1.4 **Crinkling of leaf- slightly wavy**- Rucchado, Dudhpedno, Gajariyo, Alphonso, Cowasji

1.4.1 Leaf apex- acuminate

Base- acute

Length- 21.5 cm

Indternode length- 0.36 cm----------**Alphonso**

1.4.2 Leaf apex- sub-acuminate

Base- acute- Rucchado, Dudhpedno

Length of leaf lamina- 39.9 cm

Angle between leaf and shoot- intermediate----------**Dudhpendo**

Length of leaf lamina- 8.7 cm

Angle between leaf and shoot- outheld----------**Rucchado**

1.4.3 Leaf apex- acute

Base-obtuse- Cowaji, Gajariyo

Length of lamina- 27 cm

Breadth of lamina- 7 cm

Angle between leaf and shoot- 25-40----------**Cowasji**

Length of lamina- 22 cm

Breadth of lamina- 5 cm

Angle between leaf and shoot- 30-70----------**Gajariyo**

2. **Nature of leaf folding- slightly folded**-Batli, Jhumakhiya 1, Sindoria, Pairi, Goto, Mulgoa, Ladvo, Kesar, Jhumkahiya 2, Sopari, Langdo, Desi, Jhamrukhiyo, Aamirpasand

2.1 **Crinkling of leaf- wavy**- Jhumakhiya 2, Sindoria, Mulgoa, langdo, Kesar, Sopari

2.1.1 Angle between leaf to shoot- intermediate

2.1.1.1Leaf apex- acute---------- **Jhumakhiya 2**

2.1.1.2 Leaf apex- acuminate – Kesar, Sopari

2.1.1.2.1 Leaf lamina length- 30.8 cm

Internode length- 1.15 cm----------**Kesar**

2.1.1.2.2 Leaf lamina length- 17.3 cm

Internode length- 0.35 cm----------**Sopari**

2.1.2 Angle between leaf to shoot- upheld

2.1.2.1 Leaf apex- sub-acuminate

Leaf shape- elliptic lanceolate----------**Sindoria**

2.1.2.2 Leaf apex- acute

Leaf shape- oval lanceolate----------**Langdo**

2.1.3 Angle between leaf to shoot- outheld----------**Mulgoa**

2.2 **Crinkling of leaf- crinkled**- Pairi, Goto, Jhumakhiya 1

2.2.1 Angle between leaf to shoot- intermediate

Leaf apex- acuminate----------**Jhumakhiya 1**

2.2.2 Angle between leaf to shoot- upheld- Pairi, Goto

Leaf apex- sub-acumiante

Leaf base-acute----------**Pairi**

Leaf apex- acute

Leaf base- obtuse----------**Goto**

2.3 **Crinkling of leaf- slightly wavy**- Desi, Aamirpasand, Jhamrukhiyo

2.3.1 Leaf apex- acuminate

Leaf base- obtuse----------**Desi**

2.3.2 Leaf apex- acute

Leaf base- acute- Jhamrukhiyo, Aamirpasand

2.3.2.1 Leaf lamina length- 23.4 cm

Internode length- 1.2 cm----------**Aamirpasand**

2.3.2.2 Leaf lamina length- 17.6 cm

Internode length- 0.36 cm

2.4 **Crinkling of leaf- twisted**- Batli, Ladvo

2.4.1 Angle between leaf to shoot- upheld

Leaf apex- acuminate

Leaf lamina length- 15.2----------**Batli**

2.4.2 Angle between leaf to shoot- intermediate

Leaf apex- sub-acuminate

Leaf lamina length- 13.9----------**Ladvo**

3 **Nature of leaf folding- strongly folded**- Khodi, Jahangir

3.1 **Crinkling of leaf- twisted**

Breadth of leaf lamina- 5.6 cm----------**Khodi**

3.2 **Crinkling of leaf- crinkled**

Breadth of leaf lamina- 8.2 cm----------**Jahangir**

Key II- Iidentification of thirty different varieties of *M.indica* based on inflorescence characters

1. **Inflorescence arising in clusters**-Jhumakhiya 1, Kesar, Rajapuri, Jahangir

1.1Peduncle reddish yellow

1.1.1Length of inflorescence (33-35 cm) ----------**Kesar**

1.2Peduncle green

1.2.1 Length of inflorescence (15 cm) ----------**Jhumakhiya 1**

1.2.2 Length of inflorescence (20-22 cm) ----------Rajapuri, Jahangir

1.2.2.1 Ratio of (M:B) flowers 40-60----------**Rajapuri**

1.2.2.2 Ratio of (M:B) flowers 20-80----------**Jahangir**

2.**Inflorescence arising solitary**- Cowsji, Batli,Sindoria, Pairi, Goto, Jamadar, Mulgoa, Rucchado, Ladvo, Khodi, Jhumakhiya 2, Sopari, Langdo, Aambadi, Asadiyo, Neelam, Badshahpasand, Desi, Dudhpedno, Alphonso, Fazli, Totapuri, Jhamrukhiyo, Kaju, Aamirpasand, Gajariyo

2.1 Length of inflorescence (10- 15 cm) – Cowasji, Batli, Jhumakhiya 2, Sopari

2.1.1Ratio of (M: B) flowers 80: 20 ----------**Cowasji**

2.1.2 Ratio of (M: B) flowers 20: 80 ----------Batli, Sopari

2.1.2.1 Colour of Peduncle – green----------**Batli**

2.1.2.2 Colour of Peduncle – red----------**Sopari**

2.1.3 Ratio of (M: B) flowers 10: 90----------**Jhumakhiya 2**

2.2 Length of inflorescence (17-25 cm)- Sindoria, Goto, Mulgoa, Rucchado, Ladvo, Langdo, Asadiyo, Neelam, Badshahpasand, Desi, Alphonso, Fazli,Totapuri, Jhamrukiyo, Kaju, Gajariyo

2.2.1 Ratio of (M: B) flowers 95: 5 ----------**Sindoria**

2.2.2 Ratio of (M: B) flowers 50: 50 ----------Goto, Alphonso, Totapuri

2.2.2.1 Colour of peduncle – green with red tinge----------**Goto**

2.2.2.2 Colour of peduncle – red----------**Alphonso**

2.2.2.3 Colour of peduncle – green----------**Totapuri**

2.2.3 Ratio of (M: B) flowers 20: 80 ---------- **Mulgoa**

2.2.4 Ratio of (M: B) flowers 10: 90 ----------Rucchado, Langdo, Kaju

2.2.4.1 Colour of peduncle – green ------Rucchado, Kaju

2.2.4.1.1 Densely flowered inflorescence---------- **Rucchado**

2.2.4.1.2 Medium flowered inflorescence ----------**Kaju**

2.2.4.2 Colour of peduncle – green with red tinge ----------**Langdo**

2.2.5 Ratio of (M: B) flowers 30: 70 ----------Ladvo, Desi

2.2.5.1 Colour of peduncle – green --------- Ladvo, Desi

2.2.5.1.1 Medium flowered inflorescence----------**Desi**

2.2.5.1.2 Laxly flowered inflorescence ----------**Ladvo**

2.2.6 Ratio of (M: B) flowers 60: 40 ----------Asadiyo, Fazli, Gajariyo

2.2.6.1 Colour of peduncle – red ----------Asadiyo, Fazli, and Gajariyo

2.2.6.1.1 Medium flowered inflorescence ------ Fazli, Gajariyo

2.2.6.1.1.1 Number of staminodes in flower- 4----------**Fazli**

2.2.6.1.1.2 Number of staminodes in flower- 3 ----------**Gajariyo**

2.2.6.1.2 Laxly flowered inflorescence ---------- **Asadiyo**

2.2.7 Ratio of (M: B) flowers 90: 10----------**Neelam**

2.2.8 Ratio of (M: B) flowers 40: 60 ---------- Badshahpasand, Jhamrukhiyo

2.2.8.1 Colour of peduncle – green ----------**Badshahpasand**

2.2.8.2 Colour of peduncle – red ----------**Jhamrukhiyo**

2.3 Length of inflorescence (27-29 cm) - Pairi, Khodi, Aambadi, Dudhpendo, Aamirpasand

2.3.1 Ratio of (M: B) flowers 30:70 ----------**Pairi**

2.3.2 Ratio of (M: B) flowers 20:80 ----------Aambadi, Aamirpasand

2.3.2.1 Colour of peduncle - red ---------- **Aambadi**

2.3.2.2 Colour of peduncle – green----------**Aamirpasand**

2.3.3 Ratio of (M: B) flowers 50:50 ----------**Khodi**

2.3.4 Ratio of (M: B) flowers 60:40 ----------**Dudhpendo**

2.4 Length of inflorescence – (38 cm) ----------**Jamadar**

Key III to the identification of thirty different varieties of *M.indica* based on fruit characters (stage III)

1. **Colour of the fruit- green with red blush on the shoulders** - Cowasji, Jhumakhiya 1, Pairi, Jamadar Alphonso

1.1 **Fruit shape- oblong**------Cowasji, Jhumakhiya 1

1.1.1 Fruit weight 437 g----------**Cowasji**

1.1.2 Fruit weight 127 g----------**Jhumakhiya 1**

1.2 **Fruit shape- ovate reniform** ----------**Pairi**

1.3 **Fruit shape- ovate oblong** ----------**Jamadar**

1.4 **Fruit shape- oval** ----------**Alphonso**

2.**Colour of the fruit- green**- Batli, Sindoria, Goto, Mulgoa, Rucchado, Ladvo, Khodi, Kesar, Jhumakhiya 2, Sopari, Langdo, Aambadi, Asadiyo, Neelam, Badshahpasand, Desi, Dudhpendo, Rajapuri, Fazli, Jahangir, Totapuri, Jhamrukhiyo, Kaju, Aamirpasand, Gajariyo

2.1 Fruit shape – roundish oblique ----------**Batli**

2.2 Fruit shape – oblong -------Sindoria, Rucchado, Jhumakhia 2, Langdo, Aambadi

2.2.1 Fruit weight 98 g----------**Aambadi**

2.2.2 Fruit weight 107 g ----------**Sindoria**

2.2.3 Fruit weight 123 g ----------**Jhumakhiya 2**

2.2.4 Fruit weight 177 g ---------- **Rucchado**

2.2.5 Fruit weight 227 g ----------**Langdo**

2.3 Fruit shape – oval -----Goto, Desi

2.3.1 Fruit weight 123 g ---------- **Desi**

2.3.2 Fruit weight 251g ---------- **Goto**

2.4 Fruit shape – roundish ----Mulgoa, Asadiyo, Dudhpendo

2.4.1 Fruit weight 112 g ---------- **Asadiyo**

2.4.2 Fruit weight 135 g ---------- **Dudhpedno**

2.4.3 Fruit weight 230 g ---------- **Mulgoa**

2.5 Fruit shape – cordate ---------- **Ladvo**

2.6 Fruit shape – oblong oval ---------- **Khodi**

2.7 Fruit shape – ovate oblong ----Kesar, Rajapuri, Aamirpasand

2.7.1 Fruit weight 289 g ---------- **Aamirpasand**

2.7.2 Fruit weight 305 g ----------**Kesar**

2.7.3 Fruit weight 440 g ----------**Rajapuri**

2.8 Fruit shape – obliquely ovate ----Sopari, Jhamrukhiyo

2.8.1 Fruit weight 48 g ----------**Sopari**

2.8.2 Fruit weight 203 g ----------**Jhamrukhiyo**

2.9 Fruit shape – oblong elliptic -----Neelam, Totapuri

2.9.1 Fruit weight 313-322 g

2.9.1.1 Fruit symmetry - Symmetrical ---------- **Neelam**

2.9.1.2 Fruit symmetry - Asymmetrical ---------- **Totapuri**

2.10 Fruit shape - obliquely oval ----- Badshahpasand, Fazli, Gajariyo

2.10.1 Fruit weight 163 g ---------- **Badshahpasand**

2.10.2 Fruit weight 225 g ---------- **Gajariyo**

2.10.3 Fruit weight 315 g ---------- **Fazli**

2.11 Fruit shape – roundish ovate ---------- **Jahangir**

2.12 Fruit shape – oval reniform ----------**Kaju**

MICROMORPHOLOGICAL STUDIES

i) Leaf micromorphology

Plates 7 and 8 represents anisocytic, tetracytic, staurocytic, hemiparacytic and cyclocytic type of stomata present in thirty different varieties of *M.indica* studied. Characteristic features of the stomata, trichome and epidermal cells of the different varieties are represented in table 5.

Stomata and Trichome

The following account is based on the epidermal characters of thirty varieties of *Mangifera indica* L. General descriptions are supplemented by measurement of the epidermal characters of the studied varieties which are presented in table 5.

Epidermal Cell shape and size: All the terminologies used to describe the stomata are given below: All the varieties in the genus show an absence of stomata on the adaxial surface of the leaf (plate 9 e-h) and they have stomata confined to the abaxial epidermis only (i.e.hypostomatic).

The abaxial epidermis cell shape can be categorized into two types- polygonal and rectangular. Epidermal cells on the adaxial surface are prominently undulated in all varieties. The epidermal cell size ranges from 29.76 µm x 14.5 µm to 11 µm x 11.50 µm (table 5).

Anticlinal cell wall patterns: The anticlinal walls may be straight (plate 9g), arcuate/ arched (Plate 9 f, h) or sinuate/ undulating (plate 9e). Anticlinal walls of epidermal cells on the adaxial surface in all the varieties were undulating (plate 7 and 8) with slight variations in the degree of undulation.

Anticlinal walls of varieties Jhumakhiya 1, Sindoria, Mulgoa, Rucchado, Langdo, Badshahpasand, Desi, and Gajariyo were highly undulating (plate 7c,d,h,i,j,o, 8d,e,o), whereas in Batli, Goto, Jamadar, Khodi, Asadiyo, Alphonso and Totapuri were having straight walls (plate 7b, f,g,k, 8b,g,k) and other varieties were having wavy walls.

Five common types of stomata were observed in all the varieties of *M. indica*. They are anisocytic, tetracytic, staurocytic, and cyclocytic.

Anisocytic type (Metcalfe and Chalk 1950): Stomata surrounded by three cells of which one is distinctly smaller than the other two; synonyms proposed for this type is cruciferous; unequal celled type.

Other than the common type of stomata found in dicot families some abnormal type of stomata were also observed in some varieties of studied species. They are as follows.

Tetracytic type (Metcalfe 1963): Stomata with two lateral and two polar subsidiaries. Cells making four subsidiary cells in all.

Staurocytic type (Van Cotthem 1970): Stomata surrounded by four (sometimes three or five) similar subsidiary cells with anticlinal walls arranged crosswise to the guard cells.

Cyclocytic type (Stace 1965): Stomata with four or more subsidiary cells forming a narrow ring around the guard cells.

Hemiparacytic type (Van Cotthem 1970): Stomata accompanied by a single subsidiary cell, parallel to the long axis of the pore.

Polar contiguity (Farooqui 1979a): Two contiguous stomata touch each other at the polar ends.

Synonyms pole to pole contiguity, superimposed stomata.

Lateral contiguity (Farooqui 1979a): When two or more adjacent stomata touch each other on the sides.

Synonym side to side contiguity; juxtaposed stomata.

Polar subsidiaries (Tomlinson 1974): When the subsidiaries are situated at the poles of the guard cells.

Synonym terminal subsidiaries.

Pole-to-side contiguity (Farooqui 1979b): When the pole of one stomata is contiguous to the side to the other stomata.

Some unusual stomata were observed in the study.Varieties Aambadi, Alphonso, Desi, Jahangir, Kesar, Ladvo, Neelum, Pairi, Sopari, Goto, Asadiyo, Jamrukhiya, Dudhpendo, recorded lateral contiguity (plate 10d,e). While Alphonso,

Desi, Jahangir, Kesar, Ladvo, Neelum, Goto, Asadiyo, Jamrukhiya, Kaju, Mulgoa, Dudhpendo (plate 10g) had pole to side contiguity.The tetracytic condition which is a common type observed in the monocot leaves were observed in (plate 10f), variety Mulgoa. The two polar subsidiary cells were comparatively larger in size compared to two lateral ones.

Also in variety Alphonso, Kaju and Desi unequal sized stomata were recorded (plate 10 b, c, h). In variety Sindoria, stomata showed a single guard cell (plate 10a). Some varieties like Aambadi, Pairi, Goto, and Dudhpendo showed hemiparacytic (Van Cotthem 1970) condition with a lateral contiguity where two or more adjacent stomata touch each other on the sides also called as side to side contiguity or juxtaposed (Farooqui 1979b) stomata. In varieties like Aambadi, Alphonso, Desi, Gajariyo, Jahangir, Kesar, Ladvo, Neelum, Pairi, Sopari, Jamrukhiya, Kaju, Mulgoa and Dudhpendo the lateral contiguity was seen between two and three subsidiaries, while in Goto, it was seen only between two subsidiaries and in variety Asadiyo, it was found between two, three and four subsidiaries. The other condition was a pole to side contiguity (when the pole of one stomata is contiguous to the side of the other stomata) which was common in all the varieties. In Desi variety, diagnostically the subsidiary cell surrounding the stomata was six in number which was not found in any of the other studied varieties.

Stomatal index (figure 5) was found highest in Kaju (39.38) and lowest in Rucchado (20). The size of guard cell was found highest in Langdo (24.25 µm x 18 µm) and lowest in Desi (7.75 µm x9 µm).

Glandular trichomes were present in all the varieties on the abaxial surface only. They occured occasionally and structurally appeared capitate with unicellular stalk and multicellular head, made of sixteen cells arranged in two rows of eight each (plate 9 a-d). Trichome index (figure 6) was found highest in Jhumakhiya 2 (3.4) and lowest in Sindoria (0.71). Adaxial surface in all the varieties are free of stomata and trichomes (plate 9 e-h).

Sr.no.	Variety	Leaf surface	Epidermal cell shape	Epidermal cell length (µm)	Epidermal cell breadth (µm)	Guard cell size		Stomatal index (%)	Trichome index (%)
						Length (µm)	Width (µm)		
1	Cowasji	Abaxial	Rectangular	15.75	15	15	3.1	23.83	1.23
2	Batli	Abaxial	Polygonal	20	15.5	22	11.5	25.5	1.08
3	Jhumakhiya 1	Abaxial	Polygonal	22.5	14	18	15	22.5	0.95
4	Sindoria	Abaxial	Polygonal	16.5	13.50	24.25	12.5	32.45	0.71
5	Pairi	Abaxial	Polygonal	11.75	12	19.25	22	27.46	1.3
6	Goto	Abaxial	Polygonal	16.75	12	10	8	27.44	1.2
7	Jamadar	Abaxial	Polygonal	17	14	19.5	11.5	23.79	1.92
8	Mulgoa	Abaxial	Polygonal	11.75	12.75	9.75	7.5	21.35	1.8
9	Rucchado	Abaxial	Polygonal	18.5	20	11.5	10	20	0.98
10	Ladvo	Abaxial	Rectangle	15.25	12	18.25	10	31.46	1.42
11	Khodi	Abaxial	Rectangle	21.5	15	19.25	14	24.5	1.44
12	Kesar	Abaxial	Polygonal	21	13	17.5	10	31.05	1.26
13	Jhum 2	Abaxial	Polygonal	12.75	13.5	19	12.75	26.04	3.4
14	Sopari	Abaxial	Polygonal	11.25	12.25	20.25	12.25	24.54	0.91
15	Langdo	Abaxial	Polygonal	15.25	13.5	24.25	18	26.5	1.90
16	Aambadi	Abaxial	Polygonal	16.25	18.50	10	8.25	20.66	1.04
17	Asadiyo	Abaxial	Polygonal	13	12.5	17.5	7.5	23.13	1.09
18	Neelam	Abaxial	Polygonal	18	10.25	19.25	22	36.20	1.96
19	Basdshahpasand	Abaxial	Polygonal	15.5	12.25	19.25	22.5	23.86	1.38
20	Desi	Abaxial	Polygonal	13	13.75	7.75	9	22.38	1.5
21	Dudhpendo	Abaxial	Polygonal	15	15	10	7.5	26.37	1.25
22	Alphonso	Abaxial	Rectangular	16.75	12.5	10	10	28.63	1.23
23	Rajapuri	Abaxial	Polygonal	29.76	14.5	12.5	11	23.5	1.11
24	Fazli	Abaxial	Polygonal	15.25	17.75	22.5	20	21.27	1.3
25	Jahangir	Abaxial	Polygonal	11	11.50	10	7.25	22.9	1.07
26	Totapuri	Abaxial	Polygonal	11.25	17.75	21.5	12.25	25.3	2.4
27	Jhamrukhiyo	Abaxial	Polygonal	16.5	17.25	20.75	12.25	39.34	1.05
28	Kaju	Abaxial	Polygonal	16	13	20.75	12.25	39.38	1.43
29	Aamir pasand	Abaxial	Polygonal	18.5	15	21	14.5	24.5	1.41
30	Gajariyo	Abaxial	Polygonal	15	11.05	10	6.5	29.87	1.6

Table 5: Stomata and trichome in *M.indica*

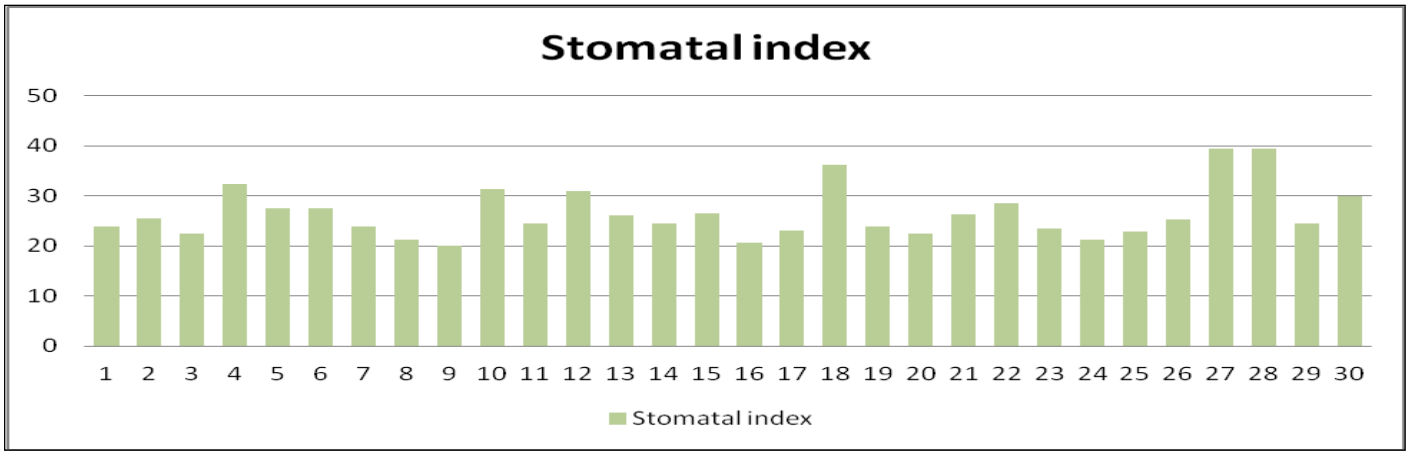


Figure 5: Stomatal index (%) in different varieties of *M.indica.*

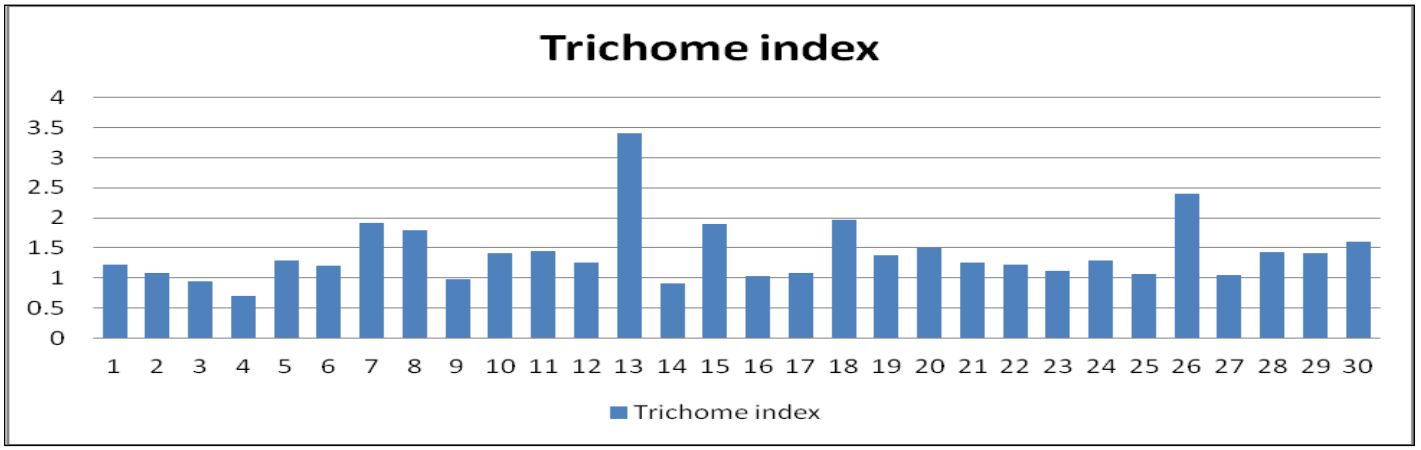


Figure 6: Trichome index (%) in different varieties of *M.indica*

Plate 7- Abaxial surface of leaf showing stomata

Magnification Bar: a to o - 10µm

a. Cowasji
b. Batli
c. Jhumakhiya 1
d. Sindoria
e. Pairi
f. Goto
g. Jamadar
h. Mulgoa
i. Rucchado
j. Ladvo
k. Khodi
l. Kesar
m. Jhumakhiya 2
n. Sopari
o. Langdo

Plate 8- Abaxial surface of leaf showing stomata

Magnification Bar: a to c, e-o - 10µm, d- 15µm

a. Aambadi
b. Asadiyo
c. Neelam
d. Badshahpasand
e. Desi
f. Dudhpendo
g. Alphonso
h. Rajapuri
i. Fazli
j. Jahangir
k. Totapuri
l. Jhamrukhiyo
m. Kaju
n. Amirpasand
o. Gajariyo

Plate 9- Adaxial surface of leaf showing trichome (a-d), epidermal cell (e-f)

Magnification Bar: a to d - 10µm, e to h- 5µm

a. Alphonso

b. Kesar

c. Sopari

d. Totapuri

e. Sindoria- epidermal cell- adaxial side

f. Mulgoa -epidermal cell- adaxial side

g. Alphonso- epidermal cell- adaxial side

h. Jhumakhiya 2 -epidermal cell- adaxial side

Plate 10- Abaxial surface of leaf showing unusual stomata

Magnification Bar: a to c, e, f, h - 10µm, d, g – 15 µm

a. Sindoria- Single guard cell

b. Desi- Unequal stomata

c. Kaju- Unequal stomata

d. Sopari- Common subsidiary cell

e. Kaju- Lateral contiguity

f. Mulgoa- Lateral contiguity

g. Kesar- Pole to side contiguity

h. Alphonso- Unequal stomata showing lateral contiguity

DISCUSSION

The present investigation has revealed the variation in patterns of the epidermal morphology of the genus *M. indica* L. Leaves are probably the most varied organs of the angiosperms. Irwine (1961), have reported the use of epidermal characters as identifiable aids of some families and genera and sometimes for the species. The leaves in *M.indica* are evergreen remaining on the tree for a longer time getting subjected to violent winds requires to have a rigid lateral interlocking of the whole epidermis. The upper epidermis has its outer wall cutinized while the lower epidermis is thin walled and the waviness adds to the rigidity of the entire cell and helps to prevent collapse when water is withdrawn. It is necessary for mechanical reasons that every epidermal cell should be firmly attached to its neighboring cells because it is constantly liable to be subjected to a variety of tensile stresses. Bending action of the wind produces tangential stresses mainly in the foliar epidermises.

Detailed study in stomata has been done for Angiosperm families such as Magnoliaceae (Paliwal and Bhandari 1962, Avita and Inamdar 1981), Piperaceae (Pant and Banerji 1965a) Celastraceae (Pant and Kidwai 1966) Ranunculaceae (Pant and Mehra 1963) Capparidaceae (Aleykutty and Inamdar 1978).

All the varieties studied were hypostomatic. Anisocytic, tetracytic, staurocytic, and cyclocytic type of stomata were seen on abaxial surface in all thirty varieties. Many workers have reported that stomata found in the leaves of a single plant can be of different types (Metcalfe and Chalk 1950, Webster 1956, 1958, Pant and Kidwai, 1964, Paliwal 1966, Pant and Baneerji 1965b, Inamdar, 1969a 1970, Barnova 1986, 1992). Aworinde (2009), Essiett (2004) on the same surface of an organ in a species. A combination of different types of stomata has also been observed on the abaxial surface of all the different varieties of *M.indica* studied.

The presence or absence of stomata on the epidermis of leaves is extremely useful in delineating taxa at species and generic levels. Anomocytic and anisocytic types are commonly observed in majority of taxa studied. Polocytic stomata were reported in *Tacca* by Ling (1981). According to Benecke (1892), presence of two or more subsidiary cells suggests that these cells help to preserve the guard cells from the injurious effects of the tensions that result form the shrinkage which leaves undergo through loss of water. The outer and inner walls of the subsidiary cells are thin walled and undergo deformation when the epidermis contracts and will thus protect the guard cells from compression to an extent.

Some of the unusual forms of stomata reported in the present study are single guard cell, guard cells of unequal size, adjacent stomata with common subsidiary cells and contiguous stomata. Stomata with a single guard cell would have been probably been developed by the meristemoid developing directly into a single guard cell without undergoing division to form the 2 guard cells or one of the guard cells would have degenerated before or after pore formation. This type of aberrant developments was noted along with the normal type of stomata. It has been reported in *Physalis minima* attributed partly due to extrinsic factors by Patel and Inamdar (1971) and confirmed by Carr and Carr (1990), *Acalphya* (Aniesua and Silas 2012).

In some studies, contiguous stomata are termed as stomatal clusters which are reported in a typical halophyte *Sonneratia alba* (Chen 1996). Yi Gan *et al*. (2010) suggest that the stomatal clustering is correlated with environmental signals and that it could serve as a new marker for environmental adaptation in terrestrial plants. Stomatal clustering has been reported in certain species of *Begonia*, *Bauhinia*, members of Crassulaceae, Sonneratiaceae and Moraceae (Payne 1970, Metcalfe and Chalk 1979, Albert and Sharma 2012, Hoover 1986, Chen 1996, Tang *et al*. 2002).

Yi Gan *et al*. (2010) have reported two main types of stomatal clusters, type A: clusters have 2 (or more) stomata place indirect contact without intervening epidermal cells between neighboring guard cells and type B: clusters are formed by groups of stomata that do not contact with each other (they are separated by subsidiary cells). All the varieties of *M.indica* showing stomatal clusters belong to type A stomatal clustering wherein 2-4 stomata are linearly clustered without epidermal cells intervening between them. Polarly as well as laterally contiguous stomata are also noted.

Yi Gan *et al*. (2010) have discussed that in non-contiguous stomatal clusters, overlaps of gaseous diffusion shell could reduce the total area of evaporation shells and water loss would be kept to a minimum, while on the other hand on the basis of the cellular hydraulic interaction theory (Mott *et al*. 1977, Mott *et al*. 1999) the normal regulation of stomata would be impaired due to large contiguous stomatal clusters. Direct contact of guard cells in contiguous stomatal clusters may cause cellular extrusion and impair normal stomatal function. So plants having larger number of stomatal cluster would be selected in a fluctuating environment. Additionally some abnormal environmental responsive gene expression (like ERECTA, MKK 4/5- MPK 3/6) may also be involved in this process. The same study reported an increase in stomatal density by 30% and 21 % in mild and severe drought stress.

Epidermal cell length of Aambadi, Alphonso, Goto and Jamrukhiya were almost same while the breadth of Aambadi was highest followed by Jamrukhiya. Breadths of Alphonso and Goto were almost similar. Now looking for the lowest values of length, varieties Jahangir, Pairi, Sopari and Mulgoa were close whereas breadth of these varieties was not so. The lowest values of breadth were seen in Neelum. In guard cell size, length was highest in Jamrukhiya, Kaju and Sopari while varieties Ladvo,

Neelum, Pairi were also close to them. The breadth was seen highest in Neelum and Pairi. Length and breadth of epidermal cells are a useful aid in distinguishing varieties (Kadiri 2005, 2006). The epidermis possess a number of important diagnostic characters that offer valuable clues for identification, like size, shape and orientation of stomata, guard cells and subsidiary cells, structural peculiarities of epidermal cell walls, distinctive or specialized form of trichomes (Dickison 2000). Boodle and Fritsh (1908) reported the significance of differences in the structure of the epidermis lies in the special shape of the cells or special structure of the cell walls in some *Cassia* species. As stated above, the varieties have 3 kinds of epidermal (anticlinal) cell wall patterns, and 2 types of epidermal cell shape.

Salisbury (1927) introduced the term stomatal index to express stomatal frequency independent of the size of the epidermal cells. The highest stomatal index was observed in Kaju and Jamrukhiya, while the lowest was seen in Aambadi. With the highest stomatal index of Kaju (39.38) and Jamrukhiya (39.34), guard cell length can be seen in correlation, 20.75 µm respectively. The other highest stomatal index is seen in Neelum (36.2) but here instead the guard cell breadth is in correlation 22 µm. Stomata studies can have great taxonomic significance in delimitation of different levels of taxa (Inamdar 1970, Kothari and Shah 1975). Stomatal density has been considered to be a useful diagnostic feature for distinguishing species (Noggle and Fritz 1976, Okeke 2004) Abdul Rahman *et al*. 2009 used this diagnostic feature to identify size different species of *Dioscorea*. Carlquist (1961) emphasized the contribution variation in stomatal size in delimiting species within a genus. According to Pataky (1969) stomata whose guard cells are less than 15 µm are called small and those in which guard cells are more than 38 µm are known as large. Among the 30 different varieties studied Goto, Aambadi, Dudhpendo, Alphonso, Jahangir and

Gajariyo are small with Desi being the smallest with 7 µm. The remaining varieties can be considered medium as the size ranges from 15 µm to 24 µm.Study by Metclafe and Chalk (1988), Beerling and Woodward (1997) and Abdul and Rahman and Oladele (2003) showed that large stomata resulted in low stomatal density while small stomata gave high stomatal density. No such correlation was observed between stomatal size and stomatal density in the present study (table 5). Stomatal density plays an important role in water use efficiency of plants thus, its numerical strength on the leaf surface is essential as reported by Wang *et al*. (2007) and Saadu *et al*. (2009). Spence (1987), Nejad and Von Meeterren (2005) and Royer (2001) showed that the geometry and resulting mechanical properties of small stomata enchanced the capacity of opening or maintain open pores with lower guard cell turgor pressures, relative to the turgor of the surrounding epidermal cells.

Four different morphological types of stomata namely; anisocytic, tetracytic, staurocytic, cyclocytic and polocytic have been recorded in all the 30 varieties of *M.indica*. Inamdar (1971) reported anomocytic, anisocytic and paracytic stomata in *F. debotrys*. Metcalfe and Chalk (1950), reported anomocytic stomata in Anacardiaceae were stated to be of anomocytic type, while Lin *et al*. (1984) reported an actinocytic type of stomata in *P. atlantica* leaves.

Stomatal frequency is one of the most widely used characters in taxonomy and pharmacognosy (Krishnamurthy and Sundaram 1970, Ogundipe *et al*. 2009, Kadri and Olowokudejo 2008). Ahmad (1964) has established the significance of stomatal frquency as taxonomical tool. This was an immense value in the present study. Main function attributed to stomata is regulation of water loss through transpiration and therefore stomatal frequency has direct relationship with drought tolerance in plants, apart from gas exchange. Lower stomatal frequency could be considered as an

adaptive feature towards preventing excess water loss through transpiration while higher stomatal frequency reflected poor tolerance to drought under field conditions (Mishra *et al*. 2011).

Stomatal index of variety Kaju (39.38) is the highest whereas variety Aambadi (20.22) is the lowest.The taxonomic significance of the variation in the stomatal index in the genus *Carya* was reported by Carpenter and Smith (1975). In this study the variation in the ratio of the average length and width of stomata which range from abaxial side, has been used to distinguish the species.

ii. Vein architecture

Venation pattern has been analyzed as per Hickey (1973). The architectural features of venation observed in the different varieties have been represented in plate 11, 12 and 13. Table 6 and 7 describes qualitative and quantitative features of vein architecture observed in the 30 different varieties.The Major venation pattern in all varieties was the pinnate type with a single primary vein (midvein) serving as the origin for the higher order venation. The size of the primary vein is determined by calculating the ratio between vein width to leaf width x 100%. It is called massive if more than 4 %, Stout, if between 2-4%, moderate, if between 1.25-2% and Weak, if below 1.25%. The highest value of primary vein size determined was in Kaju, followed by Totapuri and Badshahpasand. They were described as moderate while in all the other varieties it was weak. Here the secondary veins were not terminating at the margin but were found upturned and gradually diminishing apically inside the margin, connected to the superadjacent secondaries by a series of cross veins without forming prominent marginal loops and termed as Camptodromous. In minor venation, the highest order veins were identified up to 5 degrees. Marginal ultimate venation was fimbriate in all the varieties.

a. Primary veins

The course of the primary vein in all the varieties is straight i.e. lacking noticeable curvature or change in course and unbranched i.e. lacking ramifications of primary rank. The next veins are the secondaries, for which the angle of divergence is measured between the branch and the continuation of the source vein above the point of branching. The varieties showed acute, obtuse and right angles. In acute angle, it can be narrow, moderate or wide.

The terms descried are as follows:

i) Acute- angles less than 80˚

(a) Narrow < 45˚

(b) Moderate : 45-65˚

(c) Wide : 65-80˚

ii) Right angle or approximately so: 80-100˚

iii) Obtuse > 100˚

b. Secondary veins

Category of secondary vein in all the thirty variety is a cladodromous type in which the secondaries freely branch towards the margin. Course of secondary vein is either straight without noticeable deviation, recurved arching basally for a portion of its course, curved uniformly- arc smooth or gradually increasing in the degree of curvature or sinuous- repeated smooth changes in the direction of curvature. In varieties Batli, Jhumakhiya 1, Sindoria, Pairi, Jamadar, Rucchado, Ladvo, Kesar, Jhumakhiya 2, Sopari, Langdo, Aamabdi, Neelam Badshahpasand, Rajapuri, Jahangir, Jhamrukhiyo, Kaju, Aamirpasand and Gajariyo, the course was curved uniformly. It was found sinuous in Asadiyo and Totapuri, while in Dudhpendo, Alphonso and Fasli it was straight. Recurved course was found in Cowasji, Goto, Khodi, Desi, while in

Mulgoa, it was curved abruptly. All the secondary veins were unbranched except Totapuri, in which branches were with one or more secondary ramifications. Secondary vein spacing was either uniform as seen in Cowasji, Batli, Jhumakhiya 1, Pairi, Goto, Rucchado, Ladvo, Jhumakhiya 2, Sopari, Asadiyo, Neelam, Badshahpasand, Desi, Dudhpendo, Alphonso, Rajapuri, Jahangir, Jhamrukhiyo, Aamirpasand, Gajariyo or it can be seen increasing towards base observed in Sindoria, Jamadar, Mulgoa, Khodi, Kesar, Langdo, Fasli, Kaju and the vein spacing was seen to decrease towards base in Aambadi. Intermediate veins between second and third order veins, generally originating from the medial primary vein, interspersed among the secondary veins known as intersecondary veins. All the varieties showed composite intersecondary veins which are made up of coalesced tertiary vein segments for over 50 percent of its length. Intersecondary veins have a width and course similar to the secondaries, but they are usually thinner than the coastal secondaries and do not reach the margin. The present variety has either strong intersecondaries, which are prominently seen in varieties Cowasji, Khodi, Kesar, Sopari, Aambadi, Desi, Dudhpendo, Alphonso, Rajapuri or it had weak intersecondaries found in Batli, Jhumakhiya 1, Sindoria, Pairi, Goto, Jamadar, Mulgoa, Rucchado, Ladvo, Jhumakiya 2, Langdo, Asadiyo, Neelam, Badshahpasand, Fasli, Jahangir, Totapuri, Jhamrukhiyo, kaju, Aamirpasand, Gajariyo.

c. Tertiary veins

The pattern of veins is: i) ramified - tertiary veins branching into higher order without rejoining the secondary veins which include exmedial which means branching oriented towards the leaf margin or transverse, where branching is oriented across intercostals area ii) reticulate- tertiary veins anastomosing with other tertiary veins or with the secondary veins. It includes random reticulate in which the angles of

anastomoses vary or orthogonal reticulate where the angles of anastomoses predominantly at right angles. Ramified pattern was seen in Cowasji, Batli, Asadiyo, Badshahpasand, Desi, Alphonso, and Jhamrukhiyo. It was random reticulate in Jhumakhiya 1, Jamadar, Mulgoa, Ladvo, Khodi, Kesar, Aambadi, Neelam and Dudhpendo; while it was found orthogonal reticulate in Pairi, Goto, Rucchado, Jhumakhiya 2, Sopari, Langdo, Jahangir, Kaju and Gajariyo.

d. Higher order venation

Higher order venation includes quarteneries and quinterneries. The course of quaternery veins was either relatively randomly oriented or orthogonal. Varieties Cowasji, Jhumakhiya 1, Sindoria, Goto, Khodi, Jhumakhiya 2, Sopari, Aambadi, Neelam, Alphonso, Rajapuri, Fazli, Totapuri, Kaju, Aamirpasand and Gajariyo showed orthogonal course (plate 11a,c,d,f, 12a,c,d,f,h, 13b,c,d,f,h,i,j). Randomly oriented condition was seen in Batli, Pairi, Jamadar, Mulgoa, Rucchado, Ladvo, Kesar, Langdo, Asadiyo, Badshahpasand, Desi, Dudhpendo, Jahangir and Jhamrukhiyo (plate 11b,e,g,h,i, j, 12b,e,g,i,j, 13a,e,g).

Areolation is the smallest areas of the leaf tissues surrounded by veins which taken together form a contiguous field over most of the area of the leaf are called areoles and the appearance and characteristics of the areoles are termed areolation (Hickey 1973). The areoles are formed by all types of veins and the veins contribute to one or more sides of the areole. The shape may be triangular, quadrangular, pentagonal or polygonal (table 6). The development of areole was imperfect in Batli, Jhumakhiya 1, Pairi (plate 11e, arrow), Jamadar, Mulgoa (Plate 11h, arrow), Rucchado, Ladvo, Khodi, Kesar, Aambadi, Neelam, Badshahpasand, Desi, Rajapuri, Fazli, Jahangir and Gajariyo. Well developed areole was seen in Cowasji (plate 11a, arrow), Sindoria, Jhaumakhiya 2, Sopari, Langdo, Asadiyo, Dudhpendo, Alphonso, Totapuri,

Jhamrukhiyo and Aamirpasand while it was incomplete in Goto. Arrangement of areole was either oriented or random type. Veinlets are the ultimate veins of the leaf which occasionally cross the areoles to become connected distally are either simple or branched. Simple veinlets may be linear or curved. The branched ones may divide once or twice dichotomously. The vein lets were linear in all the studied varieties except in some Sindoria, Goto, Jhumakhiya 2, Asadiyo, Neelam, Desi, Rajapuri, Jahangir, Jhamrukhiyo and Gajariyo it was curved (plate 11d,f, 12c,g,h,j, 13c, e,g,j).

Number and size of areole, veinlet entering areoles, vein ending termination are given in table. Variation in the number of areole was noticed, ranging from 10 to 30, but highest number was observed in Cowasji (36) and lowest in Ladvo (5) (table 7). Large number of areole in the variety indicates smaller areole size per mm^2. This can be seen in Cowasji (plate 11a) and Mulgoa (plate 11h). Highest veinlets entering areoles were seen in Gajariyo (63) while lowest were found in Pairi (11). Number of vein termination was highest in Dudhpendo (113) and lowest in Cowasji (54). Marginal ultimate venation was found to be incomplete in Gajariyo while all the other varieties were with a fimbrial vein. As a great variation could beobserved in the pattern of venation, based on these features an identification key was prepared.

Cluster analysis for vein archietecture was done by SPSS 20, using average linkage method, represented in figure 7. Based on the vein analysis five groups are formed in the clusters.

Sr. no	Varieties	Primary veins	Secondary veins			Tertiary veins	Higher order venation	Veinlets branching	Areole		
		Size	Angle of divergence	Course	Intersecondary veins	Pattern	Course		Development	Arrangement	Shape
1	Cowasji	Weak	Acute-wide	Recurved	Composite	Ramified	Orthogonal	2 to 4 times	Well developed	Oriented	5,6,polygonal
2	Batli	Weak	Acute-moderate	Curved uniformly	Composite	Ramified	Relatively randomly oriented	2 to 3 times	Imperfect	Random	4,5,6, polygonal
3	Jhumakhiya 1	Weak	Acute-moderate	Curved uniformly	Composite	Random reticulate	Orthogonal	2,3 times	Imperfect	Random	4,5 ,polygonal
4	Sindoria	Weak	Acute-moderate	Curved uniformly	Composite	Ramified exmedial	Orthogonal	3 to 5 times	Well developed	Oriented	5,6,polygonal
5	Pairi	Weak	Acute-moderate	Curved uniformly	Composite	Reticulate orthogonal	Relatively randomly oriented	3,4 times	Imperfect	Random	5,6
6	Goto	Weak	Acute-wide	Recurved	Composite	Reticulate orthogonal	Orthogonal	3,4times	Incomplete	Random	5, polygonal
7	Jamadar	Weak	Right angle	Curved uniformly	Composite	Random reticulate	Relatively randomly oriented	4,5times	Imperfect	Random	5,6
8	Mulgoa	Weak	Right angle	Curved abruptly	Composite	Random reticulate	Relatively randomly oriented	2,3times	Imperfect	Random	5,6
9	Rucchado	Weak	Acute-moderate	Curved uniformly	Composite	Reticulate orthogonal	Relatively randomly oriented	3,4 times	Imperfect	Random	6, polygonal
10	Ladvo	Weak	Acute-moderate	Curved uniformly	Composite	Random reticulate	Relatively randomly oriented	3,4times	Imperfect	Random	5,6
11	Khodi	Weak	Acute-moderate	Recurved	Composite	Random reticulate	Orthogonal	2,3times	Imperfect	Random	4,5,6
12	Kesar	Weak	Right angle	Curved uniformly	Composite	Random reticulate	Relatively randomly oriented	2,3 times	Imperfect	Random	4,5
13	Jhumakhiya 2	Weak	Avute-moderate	Curved uniformly	Composite	Orthogonal reticulate	Orthogonal	2,3 times	Well developed	Oriented	5, 6
14	Sopari	Weak	Acute-narrow	Curved uniformly	Composite	Orthogonal reticulate	Orthogonal	3 to 5 times	Well developed	Oriented	6, polygonal
15	Langdo	Weak	Acute-narrow	Curved uniformly	Composite	Orthogonal reticulate	Relatively randomly oriented	3 to 5 ttimes	Well developed	Oriented	5, 6

16	Aambadi	Weak	Acute-narrow	Curved uniformly	Composite	Random reticulate	Orthogonal	2,3 times	Imperfect	Oriented	4,5, polgonal
17	Asadiyo	Weak	Obtuse	Sinuous	Composite	Ramified	Relatively randomly oriented	3 times	Well developed	Random	5,6, polygonal
18	Neelam	Weak	Right angle	Curved uniformly	Composite	Random reticulate	Orthogonal	3 to 4 times	Imperfect	Oriented	6, polygonal
19	Basdshah pasand	Moderate	Acute-narrow	Curved uniformly	Composite	Ramified	Relatively randomly oriented	3 times	Imperrfect	Oriented	5,6, polygonal
20	Desi	Weak	Acute-narrow	Recurved	Composite	Ramified	Relatively randomly oriented	3 to 4 times	Imperfect	Random	6 ,polygonal
21	Dudhpendo	Weak	Right angle	Straight	Composite	Random reticulate	Relatively randomly orietned	3 to 4 times	Well developed	Random	5, 6, polygonal
22	Alphonso	Weak	Right angle	Straight	Composite	Ramified	Orthogonal	3 times	Well developed	Random	3,4,5, 6, polygonal
23	Rajapuri	Weak	Acute-wide	Curved uniformly	Composite	Ramified	Orthogonal	3 to 4 times	Imperfect	Oriented	5, 6 polygonal
24	Fazli	Weak	Acute-wide	Straight	Composite	Transverse ramified	Orthogonal	3 to 4 times	Imperfect	Oriented	6, polygonal
25	Jahangir	Weak	Obtuse	Curved uniformly	Composite	Reticulate orthogonal	Relatively randomly oriented	3,4times	Imperfect	Oriented	4,5angled
26	Totapuri	Moderate	Right angle	Sinuous	Composite	Random reticulate	Orthogonal	3,4 times	Well developed	Random	5,6, polygonal
27	Jhamrukhiyo	Weak	Acute-wide	Curved uniformly	Composite	Ramified	Relatively randomly oriented	3 to 4 times	Well developed	Random	5, 6, polygonal
28	Kaju	Moderate	Acute-moderate	Curved uniformly	Composite	Reticulate orthogonal	Orthogonal	3,4times	Imperfect	Oriented	4,5
29	Aamir pasand	Weak	Acute-narrow	Curved uniformly	Composite	Random reticulate	Orthogonal	3 to 4 times	Well developed	Random	5, 6, polygonal
30	Gajariyo	Weak	Right angle	Curved uniformly	Composite	Reticulate orthogonal	Orthogonal	3,4 times	Imperfect	Oriented	4

Table 6: Qualititative vein architecture features in *Mangifera indica* L. (4- Quardrangular, 5- pentangular, 6 hexangular)

Sr.no.	Variety	No. of areoles per mm^2	Average size (µm) of areoles per mm^2	Veinlet entering areole per mm^2	Vein termination per mm^2
1	Cowasji	36	12	20	54
2	Batli	15	26.5	12	61
3	Jhumakhiya 1	13	25	15	62
4	Sindoria	9	22	15	84
5	Pairi	11	23.3	11	66
6	Goto	11	22.6	18	55
7	Jamadar	24	18	21.5	70
8	Mulgoa	31	14	24	64
9	Rucchado	8	28.6	13	86
10	Ladvo	5	48	12	98
11	Khodi	20	11	28	106
12	Kesar	28	21	16.5	102
13	Jhumakhiya 2	10	31	18	84
14	Sopari	17	28	26	90
15	Langdo	16	25.3	24	63
16	Aambadi	9	22	29	78
17	Asadiyo	12	23.6	32	81
18	Neelam	17	23.3	48	90
19	Basdshahpasand	18	21	41	92
20	Desi	13	27.6	36	110
21	Dudhpendo	17	20.3	42	113
22	Alphonso	19	18.6	56	95
23	Rajapuri	15	21.5	43	92
24	Fazli	18	26	58	98
25	Jahangir	14	22.5	43	102
26	Totapuri	19	18	56	96
27	Jhamrukhiyo	22	16.5	47	106
28	Kaju	18	22.5	60	98
29	Aamir pasand	21	20	55	89
30	Gajariyo	17	24	63	104

Table 7: Quantitative vein architecture features in *Mangifera indica* L.

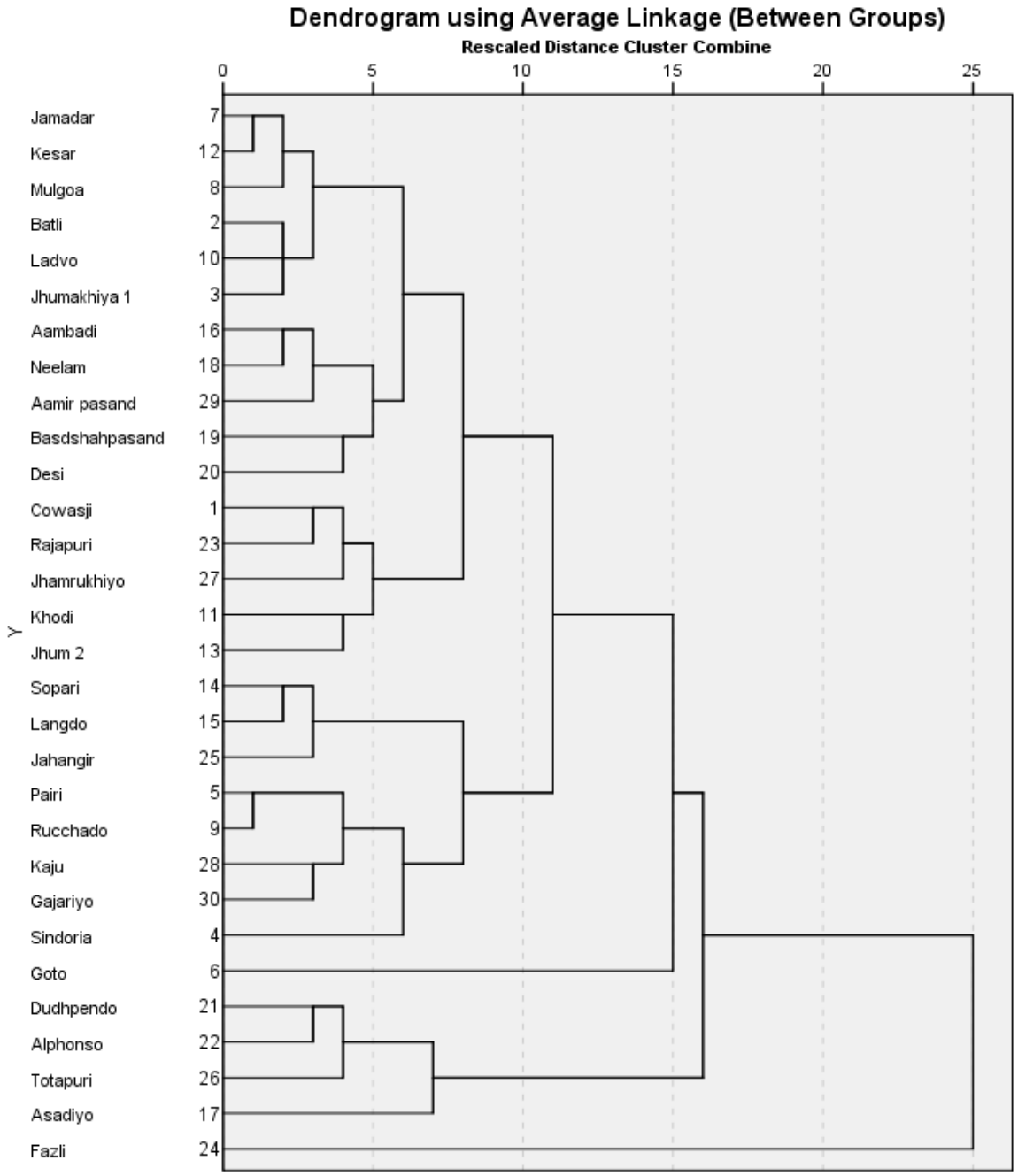


Figure 7: Dendrogram of vein archietecture.

Plate 11- Cleared leaf section showing venation patterns

Magnification Bar: a, c-f, i- j- 500µm, b, g, h- 200µm

a. Cowasji-well developed areole (arrow) with dichotomously branched veinlets

b. Batli- imperfect areole with dichotomously branched veinlets

c. Jhuamakhiya 1- imperfect areole with dichotomously branched veinlets

d. Sindoria- well developed areole with dichotomously branched veinlets

e. Pairi- imperfect areole (arrow) with dichotomously branched veinlets

f. Goto- incomplete areole with dichotomously branched veinlets

g. Jamadar- imperfect areole with dichotomously branched veinlets

h. Mulgoa- imperfect areole with dichotomously(arrow) branched vein lets

i. Rucchado - imperfect areole with dichotomously branched veinlets

j. Ladvo – imperfect areole with dichotomously branched vein lets

Plate 12- Cleared leaf section showing venation patterns

Magnification Bar: a, c-f, h, i, j- 500µm, b, g- 200µm

a. Khodi- imperfect areole with dichotomously branched veinlets

b. Kesar – imperfect areole with dichotomously branched vein lets.

c. Jhumakhiya 2 – well developed areole with dichotomously branched vein lets.

d. Sopari – well developed areole with dichotomously branched vein lets.

e. Langdo – well developed areole with dichotomously branched vein lets.

f. Aambadi – imperfect areole with dichotomously branched vein lets.

g. Asadiyo - well developed areole with dichotomously branched vein lets.

h. Neelam – imperfect areole with dichotomously branched vein lets.

i. Badshahpasand – imperfect areole with dichotomously branched vein lets.

j. Desi – imperfect areole with dichotomously branched vein lets.

Plate 13- Cleared leaf section showing vein pattern

Magnification Bar: a-d- j- 500µm, e-i- 200µm

a. Dudhpendo – well developed areole with dichotomously branched vein lets.

b. Alphonso - well developed areole with dichotomously branched vein lets.

c. Rajapuri – imperfect areole with dichotomously branched vein lets.

d. Fazli – imperfect areole with dichotomously branched vein lets.

e. Jahangir – imperfect areole with dichotomously branched vein lets.

f. Totapuri – well developed areole with dichotomously branched vein lets.

g. Jhamrukhiyo - well developed with dichotomously branched vein lets.

h. Kaju – imperfect areole with dichotomously branched vein lets.

i. Amirpasand – well developed with dichotomously branched vein lets.

j. Gajariyo – imperfect areole with dichotomously branched vein lets.

DISCUSSION

In angiosperm families leaf architectural studies have been done by many researchers Foster (1950, 1961, 1966, 1968, 1970), Hickey (1973), Melville (1976), Hickey and Wolfe (1975). Leaf architecture refers to the placement and form of various elements constituting the outward expression of leaf structure, including leaf shape, leaf size, marginal configuration, gland position and venation pattern (Hickey 1973). The leaf architecture has been the subject of several studies to resolve taxonomic and evolutionary relationships (Premoli 2008).Venation studies have been carried out by Banerjee and Deshpande (1973), Banerjee (1978) in certain members of Compositae. Macrofossils studies have shown that the leaf venation patterns can be extensively utilized in identifying fossil taxa in plaeobotany (Cleal 1981).

A study done on *Didymaea* suggests that the leaf architecture provides enough characters to diagnose species within the genus, which are useful for the identification of sterile samples (Trejo *et al*. 2009). According to Wagner and Lorence 1998, Davis and Rakotonasolo (2001) leaf architecture studies can contribute with important taxonomic characters at different hierarchical levels and should be routinely conducted. Owing to its importance for systematic classification, attention is paid largely to the architectural properties of leaf venation in both extinct and extant plant materials (Singh *et al*. 1976, Jain 1978, Inamdar and Murthy 1981, Mohan and Inamdar 1984, Annamani and Prabhakar 1991, Kohler 1993, Walther 1998).

Carlquist (1961) has stated that leaves have many features of potential taxonomic significance. One of them is the venation pattern (Foster 1961). Hickey (1973) has given the classification on vein archietecture, in which importance of major and minor venation patterns along with other features like shape, base, apex, texture, margin etc. has been specified. There is a distinct venation pattern in each plant. Venation can be

differentiated depending upon the number of size classes of primary, secondary and tertiary veins. According to Plymale and Wylie (1944), first, second and third categories form major and the ultimate veinlets constitute minor venation pattern. Primary vein of the leaf serves as the first point for the classification of the various vein orders. Primary vein, also known as midrib, is the thickest vein of the leaf. The present study reveals that the leaves in all the selected variety of *Mangifera indica* L. were simple with the cladodromous type of venation (Hickey and Wolfe 1975). The course of primary veins in all the varieties was straight and unbranched. In leaves with Cladodromous venation, usually there is a single primary vein serving as the origin for the higher order venation, secondary veins freely branching toward the margin (Hickey 1973).

Studies on Brassicaceae members showed craspedodromous (secondaries terminating at the margin) or pinnate-festooned bronchidodromous (secondaries joined together in a series of prominent arches) type of major venation pattern (Rao and Inamdar 1983). In family Acanthaceae, major venation patterns observed were pinnate craspedodromous in *Acanthus ilicifolius* and acrodromous (two or more secondaries running in convergent arches toward the leaf apex) in *Lepidagathis trinervis* (Chaudhari and Inamdar 1984). In *Coffea arabica,* simple with camptobrochidodromous (having one or more additional sets of loops outside of the amin brochidodromous loop) type of venation was found with composite intersecondary veins (Mishra *et al*. 2010). Inter secondary veins are observed in some varieties. They are intermediate in thickness between those of secondary and third order veins. They may be either simple as in sindoria, sopari, badshahpasand and totapuri or composite as in other varieties (table 6). According to Levin (1929), Gupta (1961) and Varghese (1969), the vein islet number is constant for a species while

Banerjee and Das (1972), Sehgal and Paliwal (1974) suggested that the vein ending and vein islet termination are highly variable. This variation can be seen in the present study, where in a large range is seen.

An important aspect of foliar architecture is the minor venation pattern. Some contrasting reports regarding the taxonomic utility of minor venation pattern are found in different plants. In present study, the course of higher order venation in some varieties is orthogonal or relatively randomly oriented.Tertiary veins arise from the secondaries, are relatively thinner than the secondary veins. The predominant angles of tertiary veins are acute. The pattern is randomly reticulate, orthogonal reticulate or ramified. The relationship of the tertiary veins to mid vein is oblique with three angles remaining approximately constant or at right angles or longitudinal approximately parallel.The next finer order of vein is known as quaternary vein. The course of orientation is either orthogonal or relatively randomly oriented. The veins originating from these and those of equal size form lower orders are the quinternaries. Both lower and higher order veins serve both mechanical and conducting functions of the leaf. Lower order veins provide for fast, long distance transport while higher order viens carry out local dispersion (Vogel 1994).

Marginal ultimate venation- the veins at the margins form two different patterns in different varieties. The major portion of the marginal ultimate venation is fimbriate, where higher veins orders fused into a vein running just inside of the margin (fimbrial vein). According to Ravindranath and Inamdar (1982), the characters such as number of secondaries, size of areoles, the number of vein endings entering into the areole vary from species to species and even within the same species. Variation in size of areoles was seen in the present study, where varieties Batli, Jhumakhiya 1, Rucchado, Sopari, Jhumakhiya 2, Desi, Fazli were above 25µm, while Cowasji, Mulgoa, Khodi

were below 15 µm and other varieties were in between. Also vein entering into areole were more in Gajariyo, Kaju, Aamirpasand, Fazli, Alphonso while it was less in Cowasji, Batli, Pairi, Rucchado, Ladvo.

According to many authors the vein islet number is more or less constant for a species and could be used as a specific character Levin 1929; Gupta 1961; Varghese 1969), while other reports suggests that the size of the areole, the number of vein endings and vein islet termination number are highly variable and can be used as reliable taxonomic criteria (Banerjee and Das 1972). Rao and Inamdar (1985) reported that there is no relationship between the size of areoles and the number of vein endings. According to Sehgal and Paliwal (1974) the number of vein endings inceases inrelation to the areole size in *Euphorbia*, but no such correlation was observed in the present study. On the basis of the characteristic features of the venation which includes the primary vein size, angle of divergence of secondary vein, course and pattern of the secondary, tertiary and higher order venation and the vein islets, a key to the identification of the 30 varieties has been prepared as presented below:

Key for the identification of variety based on vein architecture

1. Primary vein size- weak- Cowasji, Batli, Jhumakhiya 1, Sindoria, Pairi, Goto, Jamadar, Mulgoa, Rucchado, Ladvo, Khodi, Kesar, Jhumakhiya 2, Sopari, Langdo, Aambadi, Asadiyo, Neelam, Desi, Dudhpendo, Alphonso, Rajapuri, Fazli, Jahangir, Jhamrukhiyo, Aamirpasand, Gajariyo

1.1 Angle of divergence of secondary vein- acute wide- Cowasji, Goto, Rajapuri, Fazli, Jhamrukhiyo

1.1.1 Course of secondary vein- recurved- Cowasji, Goto

1.1.1.1 Patterns of tertiary vein- ramified----------**Cowasji**

1.1.1.2 Patterns of tertiary vein- reticulate orthogonal----------**Goto**

1.1.2 Course of secondary vein-curved uniformly- Rajapuri, Jhamrukhiyo

1.1.2.1 Higher order venation- orthogonal----------**Rajapuri**

1.1.2.2 Higher order venation- relatively randomly oriented------**Jhamrukhiyo**

1.1.3 Course of secondary vein- straight----------**Fazli**

1.2 Angle of divergence of secondary vein- acute moderate- Batli, Jhumakhiya 1, Sindoria, Pairi, Rucchado, Ladvo, Khodi, Jhumakhiya 2

1.2.1 Course of secondary vein- recurved----------**Khodi**

1.2.2 Course of secondary vein-curved uniformly- Batli, Jhumakhiya1, Sindoria, Pairi, Rucchado, Ladvo, Jhumakhiya 2

1.2.2.1 Patterns of tertiary vein- ramified----------**Batli**

1.2.2.2 Patterns of tertiary vein- random reticulate- Jhumakhiya 1, Ladvo

1.2.2.2.1 Higher order venation- orthogonal----------**Jhumakhiya 1**

1.2.2.2.2 Higher order venation- relatively randomly oriented----------**Ladvo**

1.2.2.3 Patterns of tertiary vein- ramified exmedial----------**Sindoria**

1.2.2.4 Patterns of tertiary vein- reticulate orthogonal- Pairi, Rucchado, Jhumakhiya 2

1.2.2.4.1 Higher order venation- orthogonal----------**Jhumakhiya 2**

1.2.2.4.2 Higher order venation- relatively randomly oriented- Pairi, Rucchado

1.2.2.4.2.1 Vein termination per mm^2- 66----------**Pairi**

1.2.2.4.2.2Vein termination per mm^2- 86----------**Rucchado**

1.3 Angle of divergence- right angle- Jamadar, Mulgoa, Kesar, Neelam, Dudhpendo, Alphonso, Gajariyo

1.3.1 Course of secondary vein- curved uniformly- Jamadar, Kesar, Neelam, Gajariyo

1.3.1.1 Pattern of tertiary vein- reticulate orthogonal----------**Gajariyo**

1.3.1.2 Patterrn of tertiary vien- random reticulate- Jamadar, kesar, Neelam

1.3.1.2.1 Course of higher order venation- orthogonal----------**Neelam**

1.3.1.2.1 Course of higher order venation- relatively randomly oriented- Jamadar, Kesar

1.31.2.2.3Veinlet branching- 4 to 5 times----------**Jamadar**

1.3.1.2.2.4 Veinlet branching- 2 to 3 times----------**Kesar**

1.3.2 Course of secondary vein- curved abruptly----------**Mulgoa**

1.3.3 Course of secondary vein- straight- Dudhpedno, Alphonso

1.3.3.1 Pattern of tertiary vein- random reticulate----------**Dudhpendo**

1.3.3.2 Pattern of tertiary vein- ramified----------**Alphonso**

1.4 Angle of divergence- acute narrow- Sopari, Langdo, Aambadi, Desi, Aamirpasand

1.4.1 Course of secondary vein- recurved----------**Desi**

1.4.3 Course of secondary vein- curved uniformly-Sopari, Langdo, Aambadi, Aamirpasand

1.4.3.1 Patterns of tertiary vein- orthogonal reticulate- Sopari, Langdo

1.4.3.1.1 Course of higher order venation- orthogonal----------**Sopari**

1.4.3.1.2 Course of higher order venation- relatively randomly oriented----------**--Langdo**

1.4.3.2 Patterns of tertiary vein- random reticulate- Aamabdi, Aamirpasand

1.4.3.2.1 Areole development- imperfect----------**Aambadi**

1.4.3.2.2 Areole development- well developed----------**Aamirpasand**

1.4.4 Course of secondary vein- sinuous----------**Asadiyo**

1.4.5 Course of secondary vein- curved uniformly----------**Jahangir**

2. Primary vein size- moderate- Badshahpasand, Totapuri, Kaju

2.1 Angle of divergence of secondary vein- acute narrow---------**Badshahpasand**
2.2 Angle of divergence of secondary vein- acute moderate----------**Kaju**
2.3 Angle of divergence of secondary vein- right angle----------**Totapuri**

ANATOMICAL STUDIES

Anatomical variations in the petiole and lamina of the 30 different varieties of *M.indica* were evaluated. The anatomical features are represented in table 8and 9.

iii) Petiole anatomy

Epidermis: Outline structure of the petiole was either circular abaxially and adaxially or roundish abaxially and planoconvex or highly convex adaxially and invaginated. Varieties Cowasji, Batli, Jhumakhiya 1, Pairi, Jamadar, Rucchado, Ladvo, Khodi, Kesar, Jhumakhiya 2, Sopari, Langdo, Aambadi, Neelam, Desi, Dudhpendo, Rajapuri, Fazli, Jahangir, Totapuri, Jhamrukhiyo, Kaju, and Gajariyo (plate 14a,b,c,e, 15a,c,d,e,f,16a,b,c,d,f, 17b,c,d,e,f, 18a,b,c,d,f) had a circular shape while in varieties Badshahpasand, Alphonso, Aamirpasand the shape was planoconvex (plate 17a, d, 18e) and in Sindoria, Goto, Rucchado, the condition was highly convex (plate 14d,f, 15c).

Epidermis was uniseriate and cuticularised in all the varieties. Epidermal cells were barrel shaped and radially elongated compared to the tangential wall; and they were compactly arranged without intercellular space. Epidermal cells were papillate and loosely arranged found in Jhumakhiya 2 and Rajapuri (plate 19m, 20h) while they were much elongated in varieties Jamadar and Jahangir (plate 19g, 20j). Epidermis was cuticularised with varying thickness in different varieties. Cuticle was very thick in Batli (13µm) and it was thinnest (5 µm) in Cowasji, Sindoria, Dudhpedno and Aambadi (figure 8).The extent of cutinization can be classified into three groups. (i) Cutinization was confined to the epidermal cell's surface as seen in Batli, Jhumakhiya

1, Pairi, Jamadar, Kesar, Sopari, Langdo, Aambadi, Fazli, Jahangir, Kaju and (plate 19b,c,e,g,l,n,o, 20a,i,j,m) (ii) Cuticle penetrates halfway radially between two epidermal cells as in Cowasji, Rucchado, Asadiyo, Badshahpasand, Desi, Dudhpendo, Alphonso, Rajapuri, Gajariyo, Neelam and Jhamrukhiyo (plate 19a,i, 20b,c,d,e,f,g,h,l,o,) (iii) Cutinization extends completely along the radial wall as in Sindoria, Goto, Mulgoa, Ladvo, Khodi, Jhumakhiya 2, Totapuri and Aamirpasand (plate 19d,f,h,j,k,m, 20k,n).

Hypodermis: Hypodermis was collenchymatous (3-4 layers) in all the varieties with some interrupted patches (4-5 cells) of stone cells, embedded in it. In variety Aambadi, Fazli, Dudhpendo, Jamadar, Kaju, Ladvo, Rajapuri, Rucchado, Sopari, the stone cells were in small patches of 3-5 cells. While in varieties Desi and Jahangir, a continuous layer of stone cells were found. In varieties Batli, Badshahpasand, Goto, Jhumakhiya 2, and Gajariyo, the layer of stone cells was discontinuous.

In Dudhpendo, Jahangir, Rucchado, Goto, Desi and Gajariyo, the stone cells were present immediately below epidermis; while in other varieties they were found at the end of the hypodermal layer.

Cortex: Below hypodermis the cortex was parenchymatous consisting of 5 to 7 layers. Some parenchyma cells were filled up with rhomboidal crystal (plate 20d).

Vasculature: The vascular tissue was in a prominent central arc. The vascular bundles were conjoint, collateral with 2-3 layers of sclerenchymatous cap. Just below sclerenchymatous cap, a resin canal is present. The number of resin canal in entire petiole section varied between 10 to 20, with highest diameter 117.09 µm in Jahangir and lowest 72.10 µm in Fazli. Quantitative studies including cross sectional area of the transverse section of petiole, cross section of vascular arc, distance between sides of arc, total visual count of vessel in main arc, radial multiple of vessel in main arc,

distance between two ends of arc and cross section area of vessel were measured by micrometry.

Cross sectional area in the petiole of Jahangir was highest followed by Rajapuri and Kaju. Lowest area was found in Badshahpasand followed by Ladvo (table 9). Cross sectional area of vascular arc was highest in Rajapuri followed by Jhumakhiya 2 while lowest was seen in Gajariyo followed by Ladvo (table 9). Distance between sides of the arc was more in Aambadi adaxially and abaxially and it was found less in Sindoria. Total visual count of vessels in main arc was maximum in Sindoria, followed by Rajapuri and Batli. Lesser vessel count and radial multiple of the vessel were observed in Fazli. While the maximum number of radial multiple of the vessel were found in Batli (figure 9). Raphides were present in all the varieties while sphaeraphides were found only in some varieties (table 8).

Statistical analysis was done by SPSS 20. Dendrogram (figure 10) was prepared using average linkage method. Five clusters were formed; the first cluster contains varieties Batli, Jamadar, Asadiyo, ladvo, Jhamrukhiyo and Badshahpasand. Except for Badshahpasand, all varieties have similarity in having cirlcular shape of petiole and arc shaped vascular bundle. The other cluster consists of Pairi, Amirpasand, Rajapuri and Neelum, having circular shaped petiole and arc shaped vascular bundle but both these groups can be seen at distance as cuticle thickness ranges from 6.8 to 13 µm, diameter of resin canal 84 to 106 µm for the first group while the later group had 8 to 12 µm cuticle thickness and 106 to 109 µm diameter of resin canal.

Sr.no	Variety	Thickness Cuticle (µm)	No. of resin canal (µm)	Diameter of resin canal (µm)	Crystal Sp	Crystal Rh	Shape of petiole on adaxial side	Vascular bundle shape
1	Cowasji	5	15	111.38	+	+	Circular	Circular
2	Batli	13	16	88.53	-	+	Circular	Arc-shaped
3	Jhumakhiya 1	6.24	18	88.53	+	+	Circular	Arc-shaped
4	Sindoria	5	13	122.80	-	+	Highly convex	Circular
5	Pairi	12	14	108.52	-	+	Circular	Arc-shaped
6	Goto	8.6	10	102.41	+	+	Highly convex	Broad-arc shaped
7	Jamadar	9.1	12	84.21	-	+	Circular	Arc-shaped
8	Mulgoa	8.3	14	100	+	+	Highly convex	Arc-shaped with 2 lobes
9	Rucchado	10	17	79.96	-	+	Circular	Arc-shaped
10	Ladvo	7	14	88.53	+	+	Circular	Circular
11	Khodi	10	15	119.95	+	+	Circular	Circular
12	Kesar	11.4	16	124.34			Circular	Arc-shaped with 2 lobes
13	Jhumakhiya 2	6.24	16	88.50	-	+	Circular	Arc-shaped with 2 lobes
14	Sopari	6.3	20	124.61	-	+	Circular	Arc-shaped with 2 lobes
15	Langdo	9.5	16	116.5	-	+	Circular	Circular
16	Aambadi	5.2	16	89.32	-	+	Circular	Arc-shaped with 2 lobes
17	Asadiyo	7	14	92.10	-	+	Circular	Arc-shaped
18	Neelam	8.4	18	106.34	-	+	Circular	Arc-shaped
19	Basdshahpasand	6	13	88.53	+	+	Planoconcex	Broad arc-shaped with 2 lobes
20	Desi	8	13	94.61	-	+	Circular	Circular
21	Dudhpendo	5	16	85.68	+	+	Circular	Circular
22	Alphonso	8.5	13	114.24	+	+	Planoconvex	Arc-shaped with 2 lobes
23	Rajapuri	10	14	109.21	-	+	Circular	Arc-shaped with 2 lobes
24	Fazli	8.6	14	72.10	-	+	Circular	Arc-shaped
25	Jahangir	7.5	14	117.09	-	+	Circular	Arc-shaped
26	Totapuri	7.4	14	102.38	-	+	Circular	Arc-shaped with 2 lobes
27	Jhamrukhiyo	6.8	12	88.20	-	+	Circular	Arc-shaped with 2 lobes
28	Kaju	9	12	96.74	-	+	Circular	Arc-shaped with 2 lobes
29	Aamir pasand	11.7	14	109.64	-	+	Planoconvex	Deep arc-shaped with 2 lobes
30	Gajariyo	12	16	97.28	+	+	Circular	Arc-shaped

Table 8: Anatomical features of the petiole in *Mangifera indica* L. (Sp- sphaeraphides, Rh- Rhomboidal, (+) present, (-) absent)

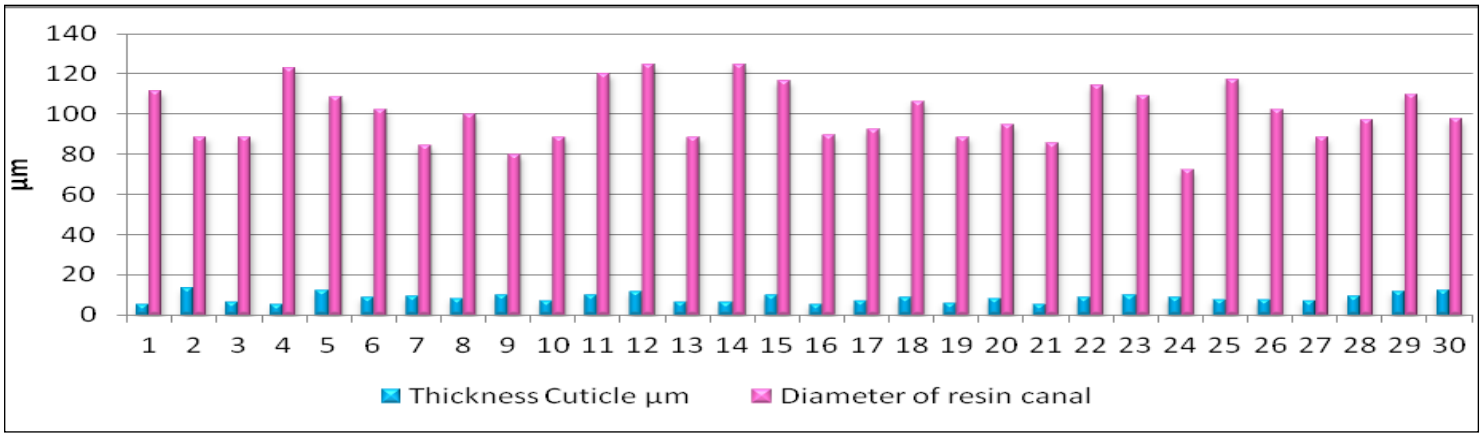


Figure 8: Quantitative features of cuticle and resin canal in the petiole of *M.indica*.

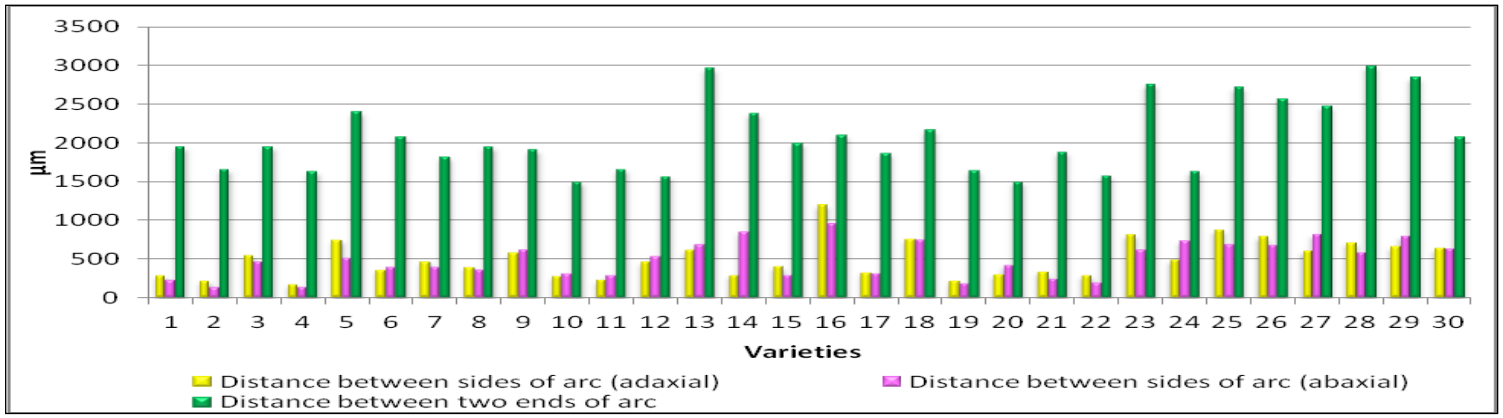


Figure 9: Quantitative features of vascularture in the petiole of *M.indica*.

Sr. no	Variety	Cross section area (µm)		Cross section of vascular arc(µm)		Distance between sides of arc (µm)		Total visual count vessel in main arc	Radial multiple of vessel in main arc	Distance between two ends of arc(µm)	Cross section area of vessel (µm)
		L	B	L	B	adaxial	abaxial				
1	Cowasji	2542±34.5	2440±16.3	2116±24.5	2090±19.43	280±26.6	230±28.67	218.6±10.15	61.5±1.4	1946±26.74	73.9x57.2
2	Batli	2146±25.03	1942±17.51	1810±54.3	1728±45	214±32.72	130±16.9	270.8±7.67	73.9±3.41	1658±42.63	53.0x50.6
3	Jhumakhiya 1	2670±150.3	2800±114.3	1902±38.2	1942±39.3	540±28.28	456±99.68	154.7±15.67	48.7±3.4	1948±36.5	41.2x46.6
4	Sindoria	1988±56.72	1894±37.7	1738±56.9	1710±21.3	170±27.08	132±19.32	362.3±12.3	57.2±2.14	1630±68.15	46x43
5	Pairi	3006±55.01	3618±106	1822±6.3	2538±43.6	740±47.14	508±37.94	194.8±10.62	63±5.6	2396±24.5	39.6x35.4
6	Goto	2812±13.9	2632±25.2	2660±77.1	2060±36.51	360±24.94	386±46.23	185.9±5.4	49.4±1.89	2072±61.24	79.3x55.1
7	Jamadar	2210±23.5	2950±21.6	1306±21.1	1714±16.4	464±22.7	386±21.18	133.1±2.02	45.6±0.84	1814±18.9	43.9x41.5
8	Mulgoa	2411±21	2321±24	1298±24	1804±15.2	395±24	358±27	146±8.1	51.3±12	1946±31.5	42.6x52.6
9	Rucchado	2762±59.9	2732±28.3	1530±27	2180±23	576±18.3	612±13.98	134±2.6	44.9±15.08	1908±96.2	35.75x34.84
10	Ladvo	1726±25.03	1976±8.4	1162±33.2	1552±44.4	272±31.5	310±21.6	134.6±4.6	47.1±3.9	1490±25.38	63.6x50.2
11	Khodi	2052±42.37	2050±82.3	1496±141.9	1538±127.6	222±44.67	288±40.22	172.1±9.80	35.5±1.35	1654±21.18	36.9x41.5
12	Kesar	2483±24	2684±75.2	1287±30	1796±17.6	455±15	526±31	174±8.6	43±3.6	1564±28	53.2x61.5
13	Jhumakhiya 2	2820±17.8	3514±18.9	2708±44.4	2140±28.2	612±19.32	684±35.02	179.3±1.8	69±42.7	2960±38.8	33.63x29.96
14	Sopari	3026±36.5	4246±50.8	1786±68.6	2898±99.5	290±21.6	844±63.10	165.6±8.6	55.5±2.9	2378±39.3	40.6x36.3
15	Langdo	2436±88.3	2690±27.08	1692±10.3	2162±17.51	396±137.5	288±26.9	209.8±4.04	56.6±3.4	1994±37.77	69.69x53.9
16	Aambadi	3288±34.2	3628±32.9	1592±45.4	2104±112.2	1194±13.4	948±56.7	114.5±4.4	41.8±1.8	2100±81.6	48.1x40.9
17	Asadiyo	2122±26	2126±24	1862±29	2480±24.2	325±12.3	311±17.5	115±5.2	51.3±2.5	1866±31	42.6x35.2
18	Neelam	3127±30	3566±28.3	1681±24	2350±26.3	754±11.8	742±22	158±13	45.1±10	2164±27	63x58.6
19	Badshahpasand	1706±9.6	1800±31.5	1460±60.40	1572±37.94	214±32.72	178±31.9	162.3±9.01	54.7±40	1640±40	52.41x38.48
20	Desi	1970±14.1	1954±13.4	1618±14.7	1606±9.6	292±13.98	412±10.32	117.3±1.05	35.6±0.51	1494±13.4	73.02x64.23
21	Dudhpendo	2436±84.74	2396±86.3	1926±32.7	2022±90.6	330±43.46	240±13.3	172.5±7.8	53.9±2.07	1878±28.98	73.9x61.8
22	Alphonso	1928±30.1	1958±22.01	1506±9.6	1524±1524	282±31.9	194±31.3	174.9±3.03	53.5±1.43	1572±63.3	73.9x46.6
23	Rajapuri	3562±91.6	3690±83	3128±99.8	3300±65.3	806±34.05	606±41.15	275.3±10.10	50.5±2.20	2752±35.52	51.2x34.2
24	Fazli	2112±88.04	2822±19.8	1530±39.1	1702±17.51	482±31.9	730±36.8	71.7±1.4	20.5±0.70	1630±36.8	49.6x54.2
25	Jahangir	3588±19.32	3218±23	2222±19.8	1576±27.9	870±10.54	680±13.3	210.5±0.84	60.8±2.14	2714±34	34.5x35.7
26	Totapuri	2531±24	2147±31	2140±20.3	2866±24	784±9.66	675±21.3	168.5±26	56.3±8.6	2568±23.2	58.2x49.3
27	Jhamrukhiyo	2981±32	3423±26	2011±21.4	2945±28.1	598±12.5	812±15.8	181.8±24.9	58.1±4.6	2471±15.9	71.6x66.5
28	Kaju	3532±61.9	4138±83.5	2150±80.1	2782±19.8	708±16.8	582±17.5	212.1±1.9	50.6±0.84	2986±16.4	38.17x37.26
29	Aamir pasand	3284±21	3822±28	2184±25.2	2654±31.3	657±11.8	791±21.8	134.6±6.8	56.5±1.2	2841±19.5	49.3x39.2
30	Gajariyo	2088±10.3	2230±14.1	952±19.3	1308±10.3	630±14.14	624±18.3	291.6±7.3	62.8±1.03	2072±10.32	54x46.8

Table 9: Quantitative anatomical features in the petiole of different varieties of *M. indica* (mean ± SD).

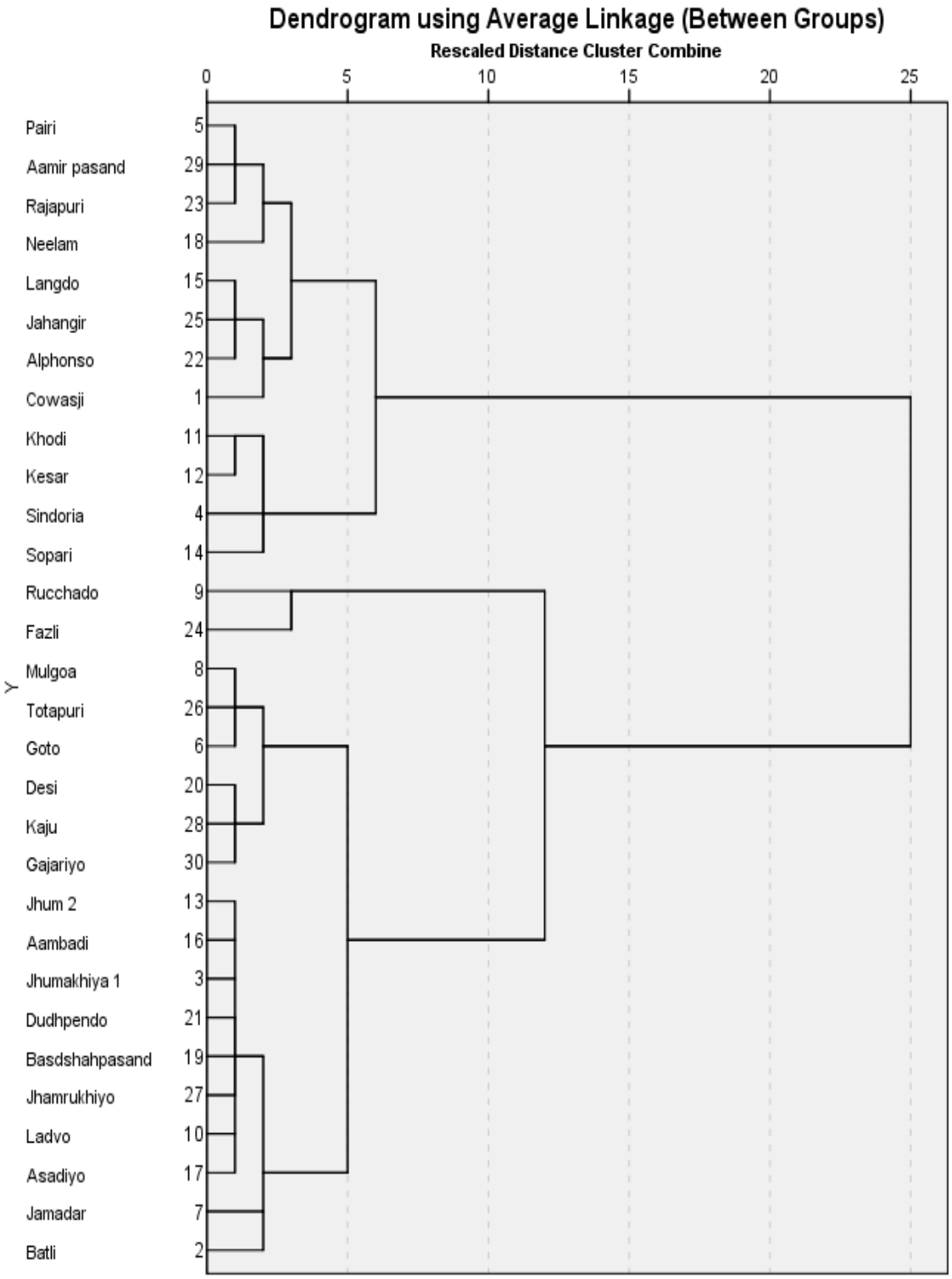


Figure 10: Dendrogram showing clusters of Petiole characters in thirty varieties of *M.indica*

iv) Leaf lamina anatomy

Transverse section of leaf had upper epidermis single layered made of barrel shaped cells. The epidermal cells were covered by a thick cuticle. The shape of epidermal cell and amount of cuticle deposition varied in the different varieties (table 10). Quantitative features included length of epidermal cell on abaxial and adaxial side (figure 11A), length of pallisade and spongy tissue and number of epithelial layer surrounding resin canals (table 10). Upper epidermis is homogenous. Lower epidermis is covered by cuticle. Trichome and stomata are seen along with the lower epidermal region. The length of epidermal cell varied from 10 µm in Goto to 25 µm in Desi, on adaxial side while on the abaxial side it varied from 15.5 µm in Alphonso to 6.9 µm in Sopari.

In the midrib region, next to epidermis, a layer of stone cells was observed. The layer was either continuous or interrupted by parenchyma cells. This region also showed presence of crystals, mainly consisting of two types- sphaeraphides and rhomboidal. All the varieties invariably showed presence of rhomboidal crystal except in Desi variety which showed absence of rhomboidal but presence of sphaeraphides. Some of the varieties showed presence of both (table 10).

This was followed by parenchymatous cells in the cortex. The pallisade tissue length was maximum in Jahangir (111.36 µm) while it was lowest in Fazli (24.13 µm) (figure 11B). Area occupied by spongy tissue in a transverse view between the upper and lower epidermis varied in the different varieties. It was maximum in Jamadar (199.92 µm) and least in Fazli (90.55 µm). Ratio of pallisade to spongy was always found lower in all the varieties. The pallisade tissue length was highest in Jahangir (111.36 µm) while it was lowest in Fazli (24.13 µm). Spongy tissue length was highest in Jamadar (199.92 µm) and lowest in Fazli (90.55 µm).Number of the pallisade layer varies; it may be one, two, or three. One layer of pallisade tissue is commonly seen in most of the varieties Cowasji, Batli, Pairi, Jhumakhiya 1, Goto,

Mulgoa, Rucchado, Ladvo, Khodi, Kesar, Langdo, Asadiyo, Badshahpasand, Desi, Totapuri, Jhamrukhiyo and Gajariyo (plate 21a,b,c,e,f, 22b,c,d,e,f, 23c,e, 24a,b, 25b,c,f). Two layers are seen in Sindoria, Jhumakhiya 2, Aambadi, Neelam, , Rajapuri, Fazli, Jahangir, Kaju, and Aamir pasand (plate 21d, 23a,d, 24c,e,f, 25d,e) while three layers were seen in Alphonso, Jamadar, Ladvo, Sopari and Dudhpendo distinctly differentiating them from the other varieties (plate 22a,23b,f, 24d, 25a). Length of the pallisade cells in 2 and 3 layered was different (plate 23f, arrow).

Vasculature comprises of 8-10 vascular bundles separated by parenchyma cells. A wide phloem region was found on the upperside of the vascular bundles. Resin canals of different size were found embedded in this region. The resin canals were surrounded by one or two layer of epithelial cells (table 10). Almost all the varieties had the resin canal surrounded by a single or two layer of epithelial layers varied from 3-4. The shape of the resin canals was round, oval or oblong. The xylem was formed of 3-5 rows of xylem vessels. The central pith region was made up of parenchyma cells. In the lamina region, next layer following epidermis was pallisade tissue. Followed by pallisade tissue is 8-12 layers of spongy tissue, with differences in the compactness of arrangement.In varieties Cowasji, Jhumakhiya 1, Sindoria, Pairi, Rucchado, Ladvo, Khodi, Langdo, Neelum, Badshahpasand, Desi, Jhamrukhiyo, Gajariyo, spongy tissue is compactly arranged (plate 21a,c,d,e, 22c,d,e, 23c,f, 24a,b, 25c,f) while in Batli, Goto, Jamadar, Mulgoa, Kesar,Jhumakhiya, Sopari, Aambadi, Asadiyo, Dudhpendo, Alphonso, Rajapuri, Fazli, Jahangir, Totapuri, Kaju, Aamirpasand the spongy tissue is loosely arranged with more intercellular spaces (plate 21b,f, 22a,b,f, 23a,b,d,e, 24c,d,e,f, 25a,b,d,e).

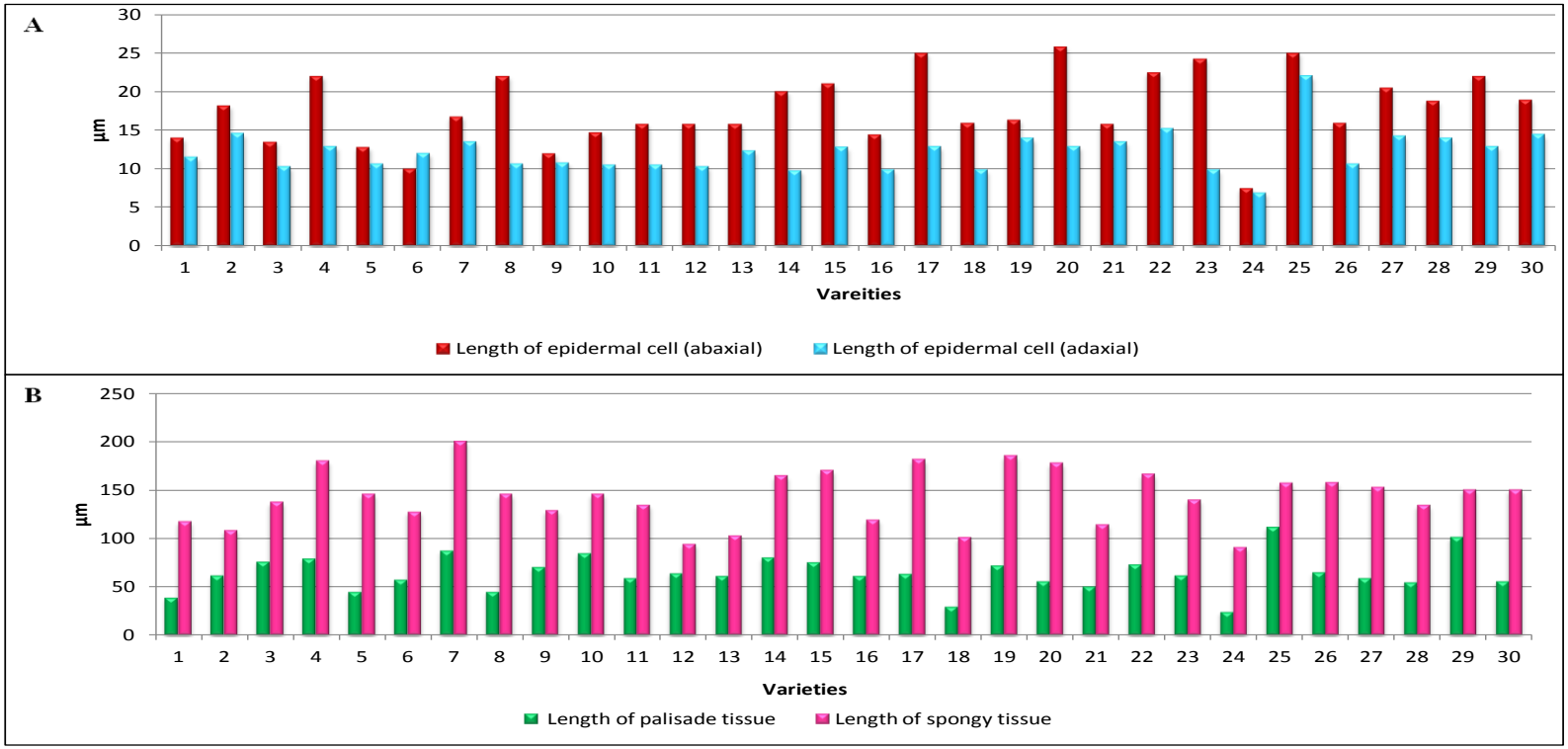


Figure 11: Quantitative characteristic feature of leaf lamina

Sr. no	Variety	Thick-ness of leaf (µm)	Length of epidermal cell (µm)		Length (µm)		Shape of resin canal	No. of Epithelial layer	Type of crystal
			a	b	Pallisade tissue	Spongy tissue			
1	Cowasji	181.14	14±1.29	11.5±1.29	38.55±6.90	117.09±13.12	oval and elongated	2	Rh
2	Batli	201.7	18.18±4.29	14.54±5.42	61.20±5.42	107.86±8.73	oblong	1-2	Rh
3	Jhumakhiya 1	236.01	13.5±2.93	10.25±0.79	75.68±6.90	137.08±20.42	round and oval	2	Rh
4	Sindoria	293.13	21.96±6.72	12.87±1.52	78.78±5.53	179.52±13.86	round, oblong	1	Rh, Sp
5	Pairi	180.2	12.8±1.52	10.60±1.75	42.42±11.06	114.38±5.17	round	1	Rh
6	Goto	206.21	10±0	12±1.05	57.12±0	127.09±28.12	round	3-4	Rh
7	Jamadar	317.228	16.75±2.06	13.5±2.93	87.108±12.5	199.92±13.46	round	1-2	Rh
8	Mulgoa	222.69	21.96±2.90	10.60±1.75	44.69±3081	145.44±6.55	round	1-1	Rh
9	Rucchado	231.2	12±2.58	10.75±1.21	69.97±8.11	128.52±26.07	round	1	Rh
10	Ladvo	262.31	14.7±2.49	10.5±1.05	84.25±18.37	145.6±9.03	round	2	Rh
11	Khodi	225.23	15.75±2.37	10.5±1.05	58.54±10.54	134.23±24.46	round and oval	2	Rh
12	Kesar	189.82	15.75±1.36	10.30±1.66	63.63±3.03	93.93±8.02	oblong	1	Rh
13	Jhumakhiya 2	195.86	15.8±2.51	12.3±0.86	61±5.2	102.6±18.66	round and oval	1	Rh, Sp
14	Sopari	276.44	20±1.67	9.75±1.42	79.96±12.04	164.22±44.78	round and oval	2	Rh
15	Langdo	277.36	21±1.54	12.8±1.23	75.1±4.8	170±20.3	round and oval	2	Rh, Sp
16	Aambadi	211.78	14.39±2.90	9.84±1.51	60.6±2.47	118.92±2.90	round, oblong	1	Rh
17	Asadiyo	276.17	24.99±5.17	12.87±1.52	62.87±2.90	181.04±5.17	oblong	1	Rh,Sp
18	Neelam	162.54	15.90±1.52	9.84±1.52	29.54±3.81	100.74±4.55	round, oblong	1-2	Rh
19	Bsdshahpasand	288.59	16.36±1.66	13.93±1.66	71.50±5.07	184.83±16.87	round	1-2	Rh
20	Desi	264.8	25.75±1.75	12.87±1.52	55.29±7.16	177.25±9.42	oblong	1-2	Sp
21	Dudhpendo	196.48	15.75±1.69	13.5±3.37	49.98±7.53	114.24±15.05	round	2-3	Rh
22	Alphonso	271.02	22.42±5.91	15.15±2.14	72.72±4.29	166.04±9.19	round, oblong	2-3	Rh, Sp
23	Rajapuri	232.99	24.24±2.47	9.84±1.52	61.35±1.51	139.38±25.35	oblong	1	Rh
24	Fazli	146.948	7.5±0.62	6.9±1.1	24.13±1.27	90.558±5.2	round and oval	2	Rh, Sp
25	Jahangir	300.41	25±2.90	21.97±1.51	111.35±6.72	156.80±5.17	round, oblong	1	Rh
26	Totapuri	254.2	15.90±1.52	10.60±1.75	64.38±14.71	157.56±6.55	oblong	1-2	Rh
27	Jhamrukhiyo	243.2	20.5±1.44	14.2±1.57	58.63±10.3	152.31±3.2	round	2	Rh, Sp
28	Kaju	220.72	18.78±3.32	13.93±1.66	54.54±5.67	133.92±9.68	oblong	2-3	Rh, Sp
29	Aamir pasand	133	21.96±2.90	12.87±1.52	100.74±8.34	149.98±3.03	round	1-2	Rh
30	Gajariyo	87.55	18.93±3.81	14.39±3.81	55.29±5.17	149.98±3.03	round	1-2	Rh

Table 10: Qualitative and quantitative internal features of lamina (mean ± SD). (a-adaxial, b- abaxial, Rh- rhomboidal, Sp- sphaeraphides)

Plate 14- Anatomical features in the petiole of *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Cowasji- circular outline of petiole

b. Batli- circular outline of petiole, arc shaped vasculare

c. Jhumakhiya 1- circular outline of petiole, arc-shaped vasculature

d. Sindoria- highly convex outline of petiole, circular vasculature

e. Pairi- circular outline of petiole, arc-shaped vasculature

f. Goto- highly convex outline of petiole , shape of vasculature arc-shaped in two lobes

Plate 15- Anatomical features in the petiole of *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Jamadar- circular outline of petiole, arc-shaped vasculatue

b. Mulgoa- highly convex outline of petiole, shape of vasculature arc-shaped in two lobes

c. Rucchado- circular outline of petiole, arc-shaped vasculatue

d. Ladvo- circular outline of petiole, circular shape of vasculature

e. Khodi- circular outline of petiole, circular shape of vasculature

f. Kesar- circular outline of petiole, shape of vasculature arc-shaped in two lobes

Plate 16- Anatomical features in the petiole of *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Jhumakhiya 2- circular outline of petiole, shape of vasculature arc-shaped in two lobes

b. Sopari- circular outline of petiole, shape of vasculature arc-shaped in two lobes

c. Langdo- circular outline of petiole, circular shape of vasculature

d. Aambadi- circular outline of petiole, shape of vasculature arc-shaped in two lobes

e. Asadiyo- circular outline of petiole, arc-shaped vasculature

f. Neelam- circular outline of petiole, arc-shaped vasculature

Plate 17- Anatomical features in the petiole of *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Badshahpasand- planoconvex outline of petiole is, shape of vasculature arc-shaped in two lobes

b. Desi- circular outline of petiole, circular shape of vasculature

c. Dudhpendo- circ`1`ular outline of petiole, shape of vasculature circular

d. Alphonso- planoconvex outline of petiole, shape of vasculature arc-shaped in two lobes

e. Rajapuri- circular outline of petiole, shape of vasculature arc-shaped in two lobes

f. Fazli- circular outline of petiole, arc-shaped vasculatue

Plate 18- Anatomical features in the petiole of *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Jahangir- circular outline of petiole, arc-shaped vasculature

b. Totapuri- circular outline of petiole, shape of vasculature arc-shaped in two lobes

c. Jhamrukhiyo- circular outline of petiole, shape of vasculature arc-shaped in two lobes

d. Kaju- circular outline of petiole, shape of vasculature arc-shaped in two lobes

e. Aamirpsand- planoconvex outline of petiole is, shape of vasculature deep arc-shaped in two lobes

f. Gajariyo- circular outline of petiole, arc-shaped vasculature

Plate 19- Epidermal and subepidermal regions of petiole in *M.indica* varieties.

Magnification bar: a, c, d,j,m,n- 2µm, b, e to I, k to o- 5µm

a. Cowasji –cuticle penetrates halfway radially between 2 epidermal cell
b. Batli- cuticle confined to epidermal cell's surface
c. Jhumakhiya 1- cuticle confined to epidermal cell's surface
d. Sindoria- cuticle penetrates completely between epidermal cell
e. Pairi- cuticle confined to epidermal cell's surface
f. Goto- cuticle penetrates completely between epidermal cell
g. Jamadar- elongated epidermal cells, cuticle confined to epidermal cell's surface
h. Mulgoa- cuticle penetrates completely between epidermal cell
i. Rucchado- cuticle penetrates halfway radially between 2 epidermal cell
j. Ladvo- cuticle penetrates completely between epidermal cell
k. Khodi- cuticle penetrates completely between epidermal cell
l. Kesar - cuticle confined to epidermal cell's surface
m. Jhumakhiya 2- loosely arranged epidermal cells
n. Sopari- cuticle confined to epidermal cell's surface
o. Langdo- cuticle confined to epidermal cell's surface

Plate 20- Epidermal and subepidermal regions of petiole in *M.indica* varieties.

Magnification bar: a, d, e, l - 2µm, b, c, f to k, m to o- 5µm

a. Aambadi- cuticle confined to epidermal cell's surface
b. Asadiyo- cuticle penetrates halfway radially between 2 epidermal cell
c. Neelum- cuticle penetrates halfway radially between 2 epidermal cell
d. Badshahpasand- cuticle penetrates halfway radially between 2 epidermal cell, rhomboidal crystal in cortex
e. Desi - cuticle penetrates halfway radially between 2 epidermal cell
f. Dudhpendo- cuticle penetrates halfway radially between 2 epidermal cell
g. Alphonso- cuticle penetrates halfway radially between 2 epidermal cell
h. Rajapuri- loosely arranged epidermal cell
i. Fazli- cuticle confined to epidermal cell's surface
j. Jahangir- elongated epidermal cell, cuticle confined to epidermal cell's surface
k. Totapuri- cuticle penetrates completely between epidermal cell
l. Jhamrukhiyo- cuticle penetrates halfway radially between 2 epidermal cell
m. Kaju- cuticle confined to epidermal cell's surface
n. Amirpasand - cuticle penetrates completely between epidermal cell
o. Gajariyo - cuticle penetrates halfway radially between 2 epidermal cell

Plate 21- Anatomical features of the leaf lamina in *M. indica*

Magnification bar: a to f – 10mm

a. Cowasji- single layered palisade, spongy layer cells compactly arranged

b. Batli- single layered pallisade, a continuous layer of spongy compact cells below pallisade (arrow).

c. Jhumakhiya 1- single layered pallisade, spongy layer compactly arranged

d. Sindoria- two layer pallisade (arrow), spongy layer compact

e. Pairi- single layered pallisade, spongy layer compact ,rhomboidal crystal near vascular bundle

f. Goto- single pallisade, spongy layer cells loosely arranged.

Plate 22 - Anatomical features of the leaf lamina in *M. indica*

Magnification bar: a to f – 10mm

a. Jamadar- 3 layers of pallisade with cells of different size, spongy layer cells compactly arranged

b. Mulgoa- single layered pallisade, spongy layer loosely arranged

c. Rucchado- single layered pallisade, spongy layer compactly arranged

d. Ladvo- single layered pallisade, spongy layer compactly arranged

e. Khodi- single layered pallisade, spongy layer compactly arranged

f. Kesar- single pallisade, spongy layer loosely arranged, crystal at the periphery of vascular bundle (arrow) and in spondy layer cells (arrowhead)

Plate 23- Anatomical features of the leaf lamina in *M. indica*

Magnification bar: a to f – 10mm

a. Jhumakhiya 2- 2 layer pallisade, spongy layer cells loosely arranged
b. Sopari- 3 compact layer of pallisade cells, continuous layer of spongy below pallisade (arrow)
c. Langdo- single pallisade, spongy compactly arranged
d. Aambadi- 2 layer pallisade cells, spongy cells loosely arranged
e. Asadiyo –single layered pallisade, spongy loosely arranged
f. Neelam- 3 layers loosely arranged pallisade of different length (arrow), spongy layer cells compactly arranged

Plate 24- Anatomical features of the leaf lamina in *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Badshahpasand- single layered pallisade, spongy layer cells compactly arranged

b. Desi- single layered pallisade, spongy layer cells compactly arranged

c. Dudhpendo- 2 pallisade layers, spongy layer cells loosely arranged

d. Alphonso- 3 pallisade layers, spongy layer cells compactly arranged

e. Rajapuri- 2 pallisade layer, spongy layer cells loosely arranged

f. Fazli- 2 pallisade layer, spongy layer cells loosely arranged

Plate 25- Anatomical features of the leaf lamina in *M. indica* varieties.

Magnification bar: a to f – 10mm

a. Jahangir- 3 pallisade layer, spongy layer cells loosely arranged

b. Totapuri- single pallisade layer, spongy layer cells loosely arranged

c. Jhamrukhiyo- single layered pallisade, spongy layer cells loosely arranged

d. Kaju- 2 pallisade layer, spongy layer cells loosely arranged

e. Aamirpsand- 2 pallisade layer, spongy layer cells loosely arranged

f. Gajariyo – single layered pallisade, spongy layer cells compactly arranged

DISCUSSION

The results obtained from the study showed that the anatomical characters can be used to separate varieties. Anatomical structures of the petiole and leaf lamina of 30 varieties of *M.indica* were examined and compared. The distinctive characters in petiole anatomy included shape of petiole cross-section, thickness of cuticle, number of resin canal, diameter of resin canal, presence or absence of crystal, cross section area of the petiole, cross section area of vascular arc, distance between sides of the arc, total visual count of vessel in main arc, radial multiple of vessel in main arc, distance between two ends of the arc and cross section area of vessel. Leaf lamina studies were carried out to observe the variation in epidermal cell shape and size, length of pallisade and spongy tissue, shape of resin canal, number of epithelial layer near resin canal and presence of crystal.

Anatomical characters are found useful in determining the relationship between different genera, families, orders and other taxonomic categories. Howard (1962, 1970), Schofield (1968), Dickson (1969,1980), Datta and Dasgupta (1979) have indicated the importance of nodal and petiolar anatomy in taxonomic treatments. Heneidak *et al*. (2007) examined 15 tree species of the Fabaceae family (Papilionoideae) and showed the importance of the shape of the petioles, the features of the epidermal cells, fibres, crystal types, secretion elements, hairs and the anatomy of the vascular bundles. Dorismilda *et al*. (2009) reported that petiole anatomy contains taxonomic information that can be used in systematic studies in tribe Hameliaceae and other Rubiaceae members.

Differences among the shape of the petiole were noticed in the studied varieties. Three types of shapes were common among the varieties; they were circular, seen in most of the varieties, highly convex in Sindoria, Goto, Mulgoa and Planoconvex in Badshahpasand, Alphonso, Aamirpasand. Olowokudejo (1987) studied the petiole shapes of 46 taxa belonging to the Cruciferae family and reported the differences in the petiole shapes. This study suggested that determining the difference in the anatomical characteristics of the petioles can be more useful way of taxonomic

classification. Essiest (2010) has divided the 3 genus on shapes and arrangement of vascular bundle in the petioles. Ogunrade and Saheed (2012) has described different species of *Citrus* according to the general outline of the median regions of petiole and they have also reported number of collenchyma cells layer to be diagonostic to some extent in the species. Shape of the vascular bundle was circular in Cowasji, Sindoria, Ladvo, Khodi, Langdo, Desi, Dudhpendo, arc-shaped in Batli, Jhumakhiya 1, Pairi, Jamadar, Rucchado, Asadiyo, Neelam, Fazli, Jahangir and Gajariyo, arc-shaped with 2 lobes in Mulgoa, Kesar, Jhumakhiya 2, Sopari, Aambadi, Alphonso, Rajapuri, Totapuri, Jhamrukhiyo and Kaju. Two very distinct shapes were seen in rest three varieties, broadly arc-shaped with 2 lobes in Goto, Badshahpasand and deep arc-shaped with 2 lobes in Aamirpasand. Oznur *et al*. (2011) studied petiole anatomy of 7 taxa belonging to Lamiaceae describing arrangement and number of vascular bundles in petiole, shape of vascular bundle, petiole shapes, presence of collenchyma and structure of epidermis.

The arrangement of the vascular system in the petiole and the midvein can be useful in the diagnosis of some plant species. Maksymowych *et al*. (1983) examined petiole anatomy of 26 herbaceous and ligneous taxa and showed that the vascular bundles were positioned differently in each and every different type. In Rubiaceae, Kocsis *et al*. (2004) examined anatomy of the petioles, structure of the vascular bundles and the hair types and showed importance of these characters in taxonomic classification. In recent years, anatomical characters have been used in taxonomy (Agbagwa and Ndukwu 2004, Kharazian 2007). The organization of the vascular system can be used in superior taxonomic levels in Rubiaceae (Martinez *et al*. 2009). The structure of petiole shows differences between genera and species. Thus, useful petiole anatomical charcters are determined in designating taxonomical structures of some species but in the present study, it has been used to identify the variety of same genus and speices.

No significant correlation could be noticed in the cross sectional area and number of resin canals or diameter of the resin canal except in Sopari which had the maximum width in cross sectional area (4246±50.8 µm) with the maximum number of resin

canals (13) and maximum diameter of the resin canals (122.80 µm). In Goto, the number of resin canals was found to be the least but the cross sectional area of the vessel element was found to be maximum (79.3x55.1 µm). The number of vessel elements in the main arc was found to be maximum in Sindoria (362.3±12.3 µm) but the size (cross section) of the vessel elements were least in Jhumakhiya 2 (33.63x29.96 µm) indication that though the number of vessel elements was large their size was small. The number of resin canals was less (13) but the diameter of it was more (122.80 µm) compared to that found in Jhumakhiya 2 which had more number of resin canals (16) of smaller diameter (88.50 µm).

Epidermal cell was papillate in Rajapuri, Jhumakhiya 2 while they were much elongated in varieties Jahangir and Jamadar was barrel shaped and radially elongated in all other varieties. Mavi *et al*. (2010) differentiated 2 species of *Hordeum*, mainly on the indumentation and epidermal cell wall properties. The cuticle penetration was important as it was used as one of the characters for differentiating the varieties. Varieties Batli, Jhumakhiya 1, Pairi, Jamadar, Sopari, Langdo, Aambadi, Fazli, Kaju Kesar and Jahangir had cuticle adhering to the epidermal cell, Cowasji, Rucchado, Badshahpasand, Desi, Dudhpendo, Alphonso, Rajapuri, Gajariyo, Asadiyo, Neelam and Jhamrukhiyo had cuticle penetrating halfway while Sindoria, Goto, Ladvo, Khodi, Jhumakhiya 2, Mulgoa, Totapuri and Aamirpasand had cuticle penetrating all along the epidermal cell. Thickness of cuticle ranged from 5 µm to 13 µm. According to Ashton (1992), differences in cuticle thickness between species appears related to both light and drought tolerance. So, it can be said that the varieties Batli, Pairi, Rucchado, Khodi, Kesar, Aamirpasand and Gajariyo which has thick cuticle are drought tolerant and varieties Cowasji, Jhumakhiya 1, Sindoria, Ladvo, Jhumakhiya 2, Sopari, Aambadi, Asadiyo, Badshahpasand, Dudhpedno, and Jhamrukhiyo having thin cuticle are drought intolerant.

Number of resin canal indicates more amount of secretion. Variety Sopari had more amount of resin canal as compared to other varieties while, the number was very less in Goto. Presence of crystal is also considered as an important crieteria. Crystals like

sphaeraphides and rhomboidal crystal were found in the hypodermal region. Varieties Cowasji, Jhumakhiya 1, Goto, Mulgoa, Ladvo, Khodi, Badshahpasand, Dudhpendo, Alphonso and Gajariyo had both the crystals present, while all the other varieties had only rhomboidal crystal present in hypodermal region. Dinc *et al*. (2008) found sphaerocrystal in the upper epidermal cell of leaf of *Teucrium* species. Lamina region also witnessed the presence of crystals in the upper epidermis, pallisade tissue and vascular bundle. Calcium oxalate crystals have been reported for defining the sub families, tribes and sub tribes of Rubiaceae (Arruda *et al*. 2010). Crystal distribution has been reported helpful in delimiting taxa by Metcalfe and Chalk (1950). Metcalfe (1983) has reported the significance of sphaerocrystal as it has restricted occurrence.

Around 4 to 12 resin canals were seen, categorized into big and small size and position in the midrib region. Mostly all the varieties had 2 big resin canals placed on either sides of the midrib vasculature within the lamina except for varieties Sindoria, Badshahpasand, Sopari and Gajariyo which had 3 or 4 big resin canals. Small resin canal ranged from 2 to 7 in number but varieties Sindoria, Desi and Rucchado had 9, 10 and13 small resin canals respectively. Variety Sopari was distinct in having 1 small resin canal in the centre of the midrib. The resin ducts are not located in the vascular bundles but are merely associated with them in a characteristic and constant manner. In the petiole of the studied varieties, each vascular bundle is accompanied by a resin duct in the cortical region and opposite to the phloem. Resin duct here probably has an ecological role of protection. The chemical nature of the resin provides chemical protection against small assailants which if penetrated into the interior will be effetively discouraged from attacking the conducting strands, the continuity of which is vital to the well being of the plant. Similarly presence of raphides and sphaeraphides perfoms an ecological function of providing mechanical protection against pathogens.

The amount of vessels was found maximum in varieties Sindoria, Rajapuri and Batli, while it was minimum in Fazli. Ozdemir and Senel (1999, 2001) showed importance in the amount of vascular bundles and its arrangement within the petiole in the *Salvia*

species. Data obtained was used for proper and easy identification and clarification of the taxonomic relationship of these species.

Mavi *et al*. (2010) found that the leaf blades vary between the taxa in both qualitative and quantitative value. In the present study, lamina region, variation was seen in the layer of pallisade tissue, where it was single in many, double in Batli, Jhumakhiya 1, Sindoria, Goto, Jhumakhiya 2, Aambadi, Neelam, Alphonso, Rajapuri, Fazli, Jahangir, Kaju and Aamirpasand while 3 layers were seen in Jamadar, Ladvo, Sopari and Dudhpendo. Salimpur *et al*. (2009) reported 3 species of *Geranium* with single layer of pallisade, 2 species with two layer pallisade and some with 3 to 6 layers of pallisade.

The leaves of all the varieties are dorsiventral with the upper epidermis underlain by one to three layered cylinders. Pallisade tissue was loose in texture having its radial walls separate form one another. This feature connected with the necessity for the presence of air spaces in immediate contact with the photosynthetic cells also indicates that each pallisade element has a tendency to become independent of its neighbouring cells, neither receiving raw food materials from the latter nor supplying them with synthetic products. They carry out the interchange of materials with its two extremities. Frequently in some varieties (plate 24e, 25b) a small group of pallisade cells converge at their lower ends so as to form a little fan shaped group resting upon a single underlying cell, the upper end of which is delated indicating them to be collecting cells, which receive the photosynthetic products from all the members of a group of pallisade cells and transmit them more or less directly to the main channels of translocation.

Based on the evaluated characters a synoptic key has been prepared to provide an ease to the identification of the studied thirty varieties.

1a. Outline circular abaxially and adaxially: Cowasji, Batli, Jhumakhiya 1, Pairi, Goto, Jamadar, Ladvo, Khodi, Kesar, Jhumakhiya 2, Sopari, Langdo, Aambadi, Asadiyo, Neelam, Desi, Dudhpendo, Rajapuri, Fasli, Jahangir, Totapuri, Jhamrukhiyo, Kaju,Gajariyo----------2

1b. Outline roundish abaxially and adaxially planoconvex and invaginated- Sindoria, Mulgoa, Rucchado, Badshahpasand, Alphonso, Aamirpasand----------21

2a. Outline smooth: Cowasji, Batli, Goto, Jamadar, Ladvo, Sopari, Asadiyo, Neelam, Desi, Dudhpendo, Rajapuri, Fazli, Dudhpendo, Jahangir, Totapuri, Kaju----------3

2b. Outline wavy: Jhumakhiya 1, Pairi, Khodi, Kesar, Jhumakhiya 2, Langdo, Aambadi, Jhamrukhiyo----------15

3a. Vascular bundles arranged to form a closed continuous crescentric cylinder: Cowasji, Goto, Neelam, Desi, Dudhpendo, Gajariyo----------4

3b. Vascular bundles widely spaced and arranged to form discontinuous crescentric cylinder- Batli, Jamadar, Ladvo, Sopari, Asadiyo, Rajapuri, Fazli, Jahangir, Totapuri, Kaju----------8

4a. Epidermal cells radially elongated compared to tangential wall---------- **Goto**

4b. Epidermal cells broad, cuticle penetrates halfway in between epidermal cell: Cowasji, Neelam, Desi, Dudhpendo, Gajariyo----------5

5a. Thickness of cuticle- 12 µm,Number of resin canal- 16---------- **Gajariyo**

5b. Thickness of cuticle- 5 µm- Cowasji, Dudhpendo----------6

5c. Thickness of cuticle- 8-9 µm- Desi, Neelam----------7

6a. Diameter of resin canal- 111.38 µm---------- **Cowasji**

6b. Diameter of resin canal- 85.68 µm---------- **Dudhpendo**

7a. Number of resin canal-13---------- **Desi**

7b. Number of resin canal- 18---------- **Neelam**

8a. Epidermal cells elongated---------- **Jahangir**

8b.Epidermal cells radially elongated compared to tangential wall- Batli, Ladvo, Sopari, Totapuri, Kaju, ----------9

9a. Cuticle penetrates halfway- Asadiyo, Rajapuri ----------10

9b. Cuticle penetrates all along the radial wall- Ladvo, Goto, Totapuri----------11

9c. Cuticle confined to the surface of epidermis- Batli, Sopari, Jamadar, Kaju, Fazli----------12

10a. Thickness of cuticle- 7 µm---------- **Asadiyo**

10b. Thickness of cuticle- 10 µm---------- **Rajapuri**

11a. Diameter of resin canal- 88.53 µm---------- **Ladvo**

11b. Diameter of resin canal- 102.38 µm---------- **Totapuri**

12a. Thickness of cuticle – 13µm, Number of resin canal – 16---------- **Batli**

12b. Thickness of cuticle- 6.3 µm, Number of resin canal- 20---------- **Sopari**

12c. Thickness of cuticle- 8-9 µm –Jamadar, Fazli, Kaju----------13

13a. Number of resin canal – 14, Diameter of resin canal- 72.10 µm ---------- **Fazli**

13b. Number of resin canal – 12- Jamadar, Kaju----------14

14a. Diameter of resin canal- 84.21 µm---------- **Jamadar**

14b. Diameter of resin canal- 96.74 µm---------- **Kaju**

15a. Cuticle penetrate halfway---------- **Jhamrukhiyo**

15b. Cuticle penetrates all along the radial wall- Khodi, Jhumakhiya 2----------16

15c. Cuticle confined to surface of epidermis- Jhumakhiya 1, Pairi, Kesar, Langdo, Aambadi----------17

16a. Thickness of cuticle 10 µm---------- **Khodi**

16b. Thickness of cuticle 6.24 µm----------**Jhumakhiya 2**

17a. Thickness of the cuticle- 5-7 µm- Jhumakhiya 1, Aambadi----------18

17b. Thickness of the cuticle- 9-12 µm- Pairi, Kesar, Langdo----------19

18a. Number of resin canal- 18, Sphaeraphides and rhomboidal crystal present and vascular bundle arc-shaped----------**Jhumakhiya 1**

18b. Number of resin canal- 16, rhomboidal crystal present and vascular bundle arc-shaped with two lobes----------**Aambadi**

19a. Number of resin canal 14, diameter of resin canal- 108.52 µm----------**Pairi**

19b. Number of resin canal 16----------20

20a. Diameter of resin canal – 124.34 µm, Shape of vascular bundle- arc-shaped with two lobes ---------- **Kesar**

20b. Diameter of resin canal- 116.5 µm, Shape of vascular bundle- circular -- **Langdo**

21a. Adaxial surface circular. Vascular bundles arranged in a closed crescentric arc and pith large- Sindoria, Mulgoa, Alphonso----------22

21b. Adaxial surface elongated. Vascular bundles elongated deeply and arranged in a closed crescentric arc, pith highly reduced- Rucchado, Badshahpasand, Aamirpasand----------24

22a. Adaxial surface convex and slightly flattened----------**Alphonso**

21b. Adaxial surface highly convex and cuticle deeply penetrated - Sindoria, Mulgoa----------23

23a. Thickness of cuticle- 5µm---------- **Sindoria**

23b. Thickness of cuticle- 8.3µm---------- **Mulgoa**

24a. Thickness of cuticle- 6 µm---------- **Badshahpasand**

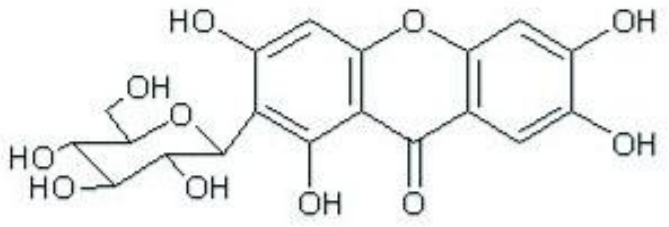
24b. Thickness of cuticle- 10-12 µm- Rucchado, Aamirpasand----------25

25a. Number of resin canal- 17 µm, diameter of resin canal 79.96 µm ------**Rucchado**

25b. Number of resin canal- 14 µm----------**Aamirpasand**

STUDY OF MANGIFERIN CONTENT IN LEAVES

Mango is rich source of biologically active compound, mangiferin that is a C-glycosyl xanthone structure. In the present study, mangiferin was isolated from leaves of thirty mango varieties cultivated in Gujarat (table 11). Mangiferin content ranged from 1.04 to 47.02 mg/g of leaf powder, where varieties Batli (1.04 mg/g) Aambadi (4.79 mg/g), Khodi (7.15 mg/g), had lesser values as compared to Ladvo (47.02 mg/g), Jahangir (38.71 mg/g) and Jhamrukhiyo (35.27 mg/g). Other varieties ranged in between, which is given in the table 11.



Mangiferin

Mangiferin is a phenolic compound and occurs in response to stress. It is abundant in mango leaves. Chromatograms of mangiferin in thirty varieties are given in figure 12-15. Maximum mangiferin was recorded in Ladvo (figure 13D) followed by Jahangir (figure 15C) and minimum amount was seen in Kaju (figure 15F) followed by Aambadi (figure 14B). Hierarchical cluster analysis was done using squared Euclidean distance, which is a measure of dissimilarity. Neelam, Amirpasand, Mulgoa and Totapuri were ranging between 21-23 mg/g of mangiferin. Rajapuri, Fazli, Langdo, Jhumakhiya 1, Goto, Khdoi were in a range of 6-10 mg/g. Jhumakhiya 2, Asadiyo, Jamadar, Desi, Gajariyo, Kesar, Dudhpendo, Cowasji, Pairi, Rucchado, Alphonso and Sindoria were found in the range 10-20 mg/g.

Sample	Variety	Mangiferin in mg/g of leaf powder
1	Cowasji	17.49
2	Batli	10.41
3	Jhumakhiya 1	9.50
4	Sindoria	18.9
5	Pairi	16.10
6	Goto	8.40
7	Jamadar	12.73
8	Mulgoa	21.88
9	Rucchado	16.24
10	Ladvo	47.02
11	Khodi	7.15
12	Kesar	16.75
13	Jhumakhiya2	13.05
14	Sopari	28.62
15	Langdo	10.20
16	Aambadi	4.79
17	Asadiyo	13.23
18	Neelam	21.57
19	Badshahpasand	30.09
20	Desi	12.36
21	Dudhpedno	16.92
22	Alphonso	15.48
23	Rajapuri	10.37
24	Fazli	10.53
25	Jahangir	38.71
26	Totapuri	22.16
27	Jhamrukhiyo	35.27
28	Kaju	3.46
29	Amirpasand	21.56
30	Gajariyo	14.18

Table 11: Mangiferin content extracted from leaves of thirty varieties of *M. indicia.*

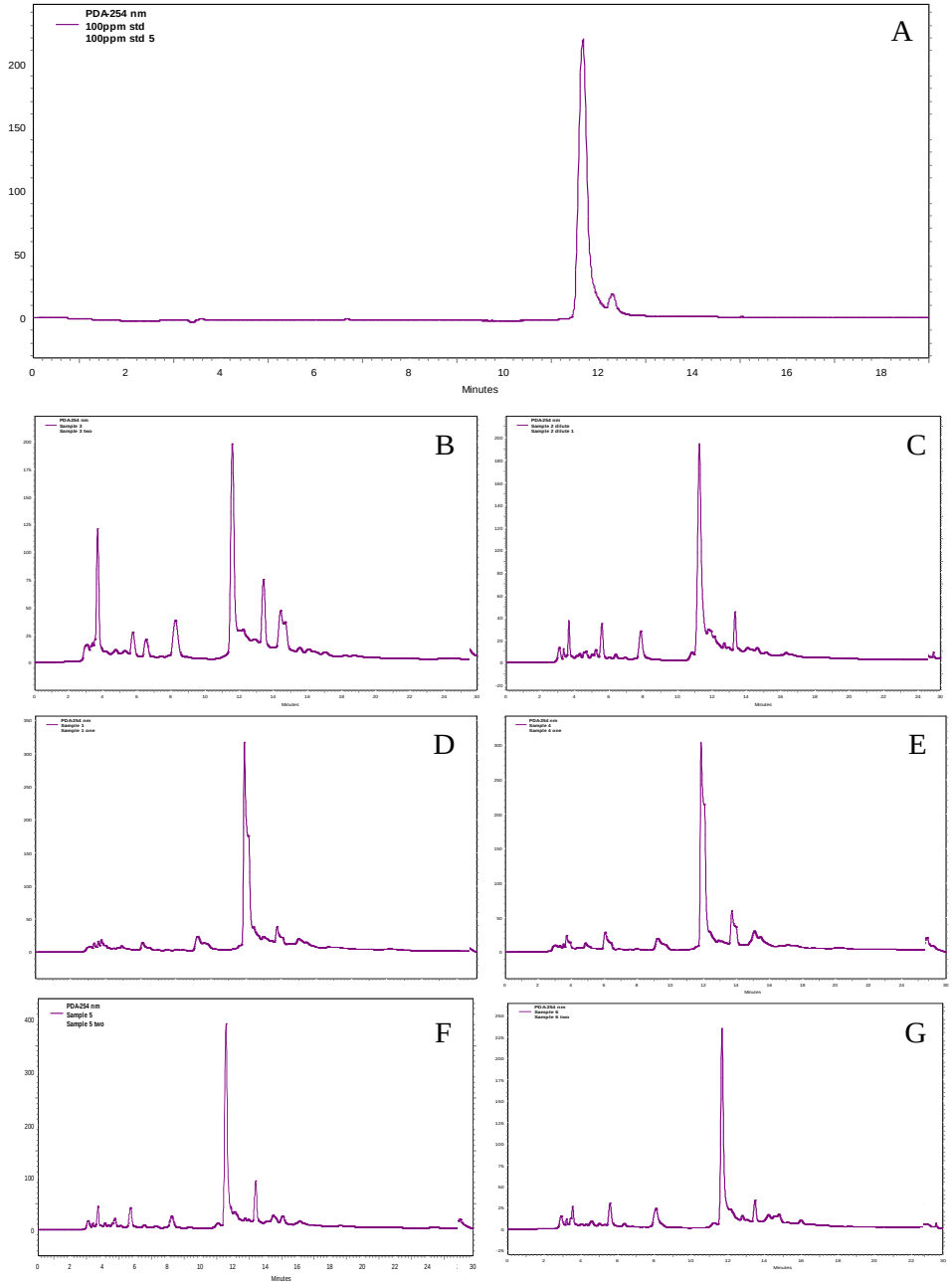


Figure 12: HPLC profile of purified Mangiferin. A-standard (100 ppm), B-sample 1, C- sample 2, D- sample 3, E- sample 4, F- sample 5, G- sample 6

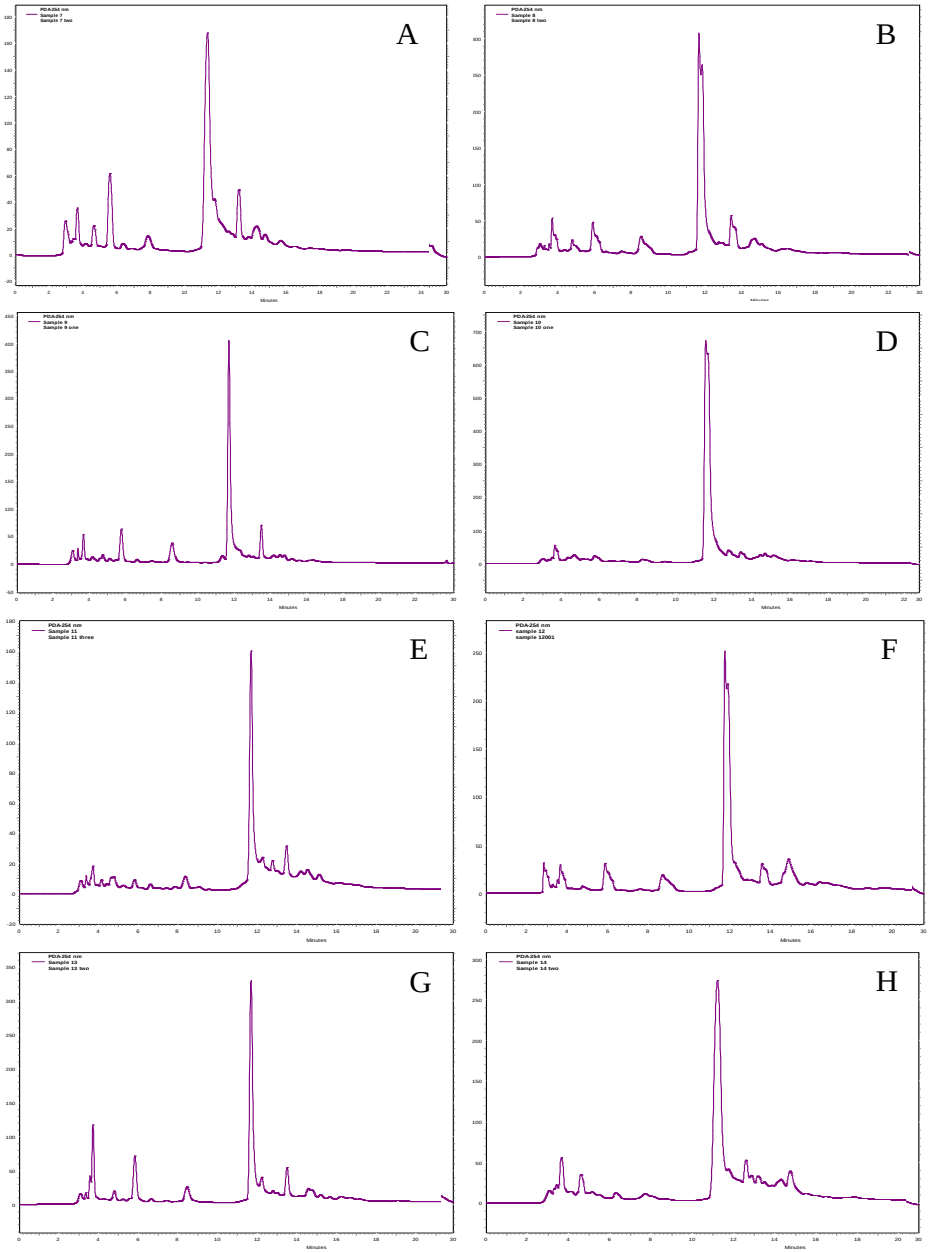


Figure 13: HPLC profile of purified Mangiferin. A-sample 7, B-sample 8, C- sample 9, D- sample10, E- sample 11, F- sample 12, G-sample 13, H-sample 14

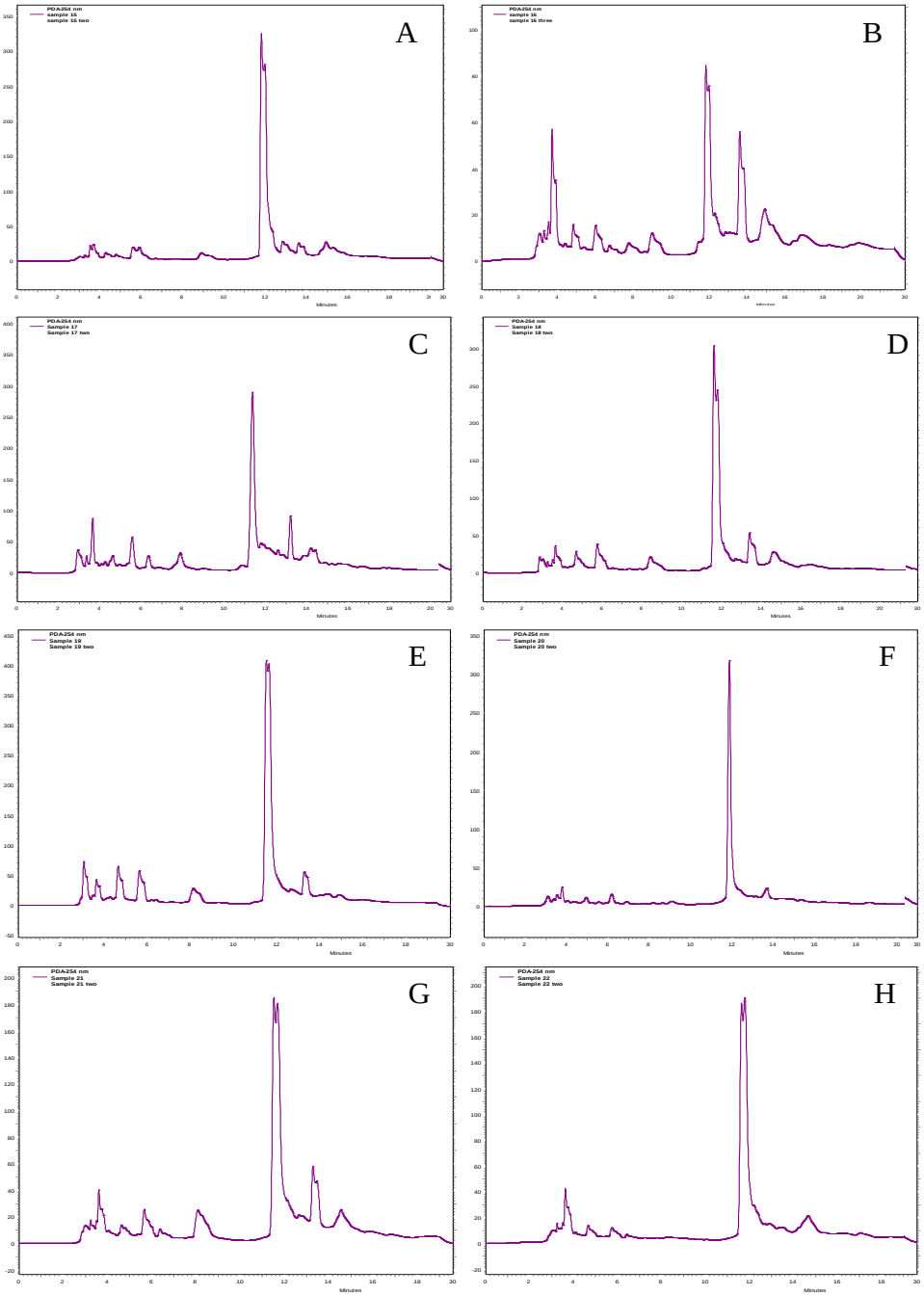


Figure 14: HPLC profile of purified Mangiferin. A-sample 15, B-sample 16, C- sample 17, D- sample18, E- sample 19, F- sample 20, G-sample 21, H- sample 22

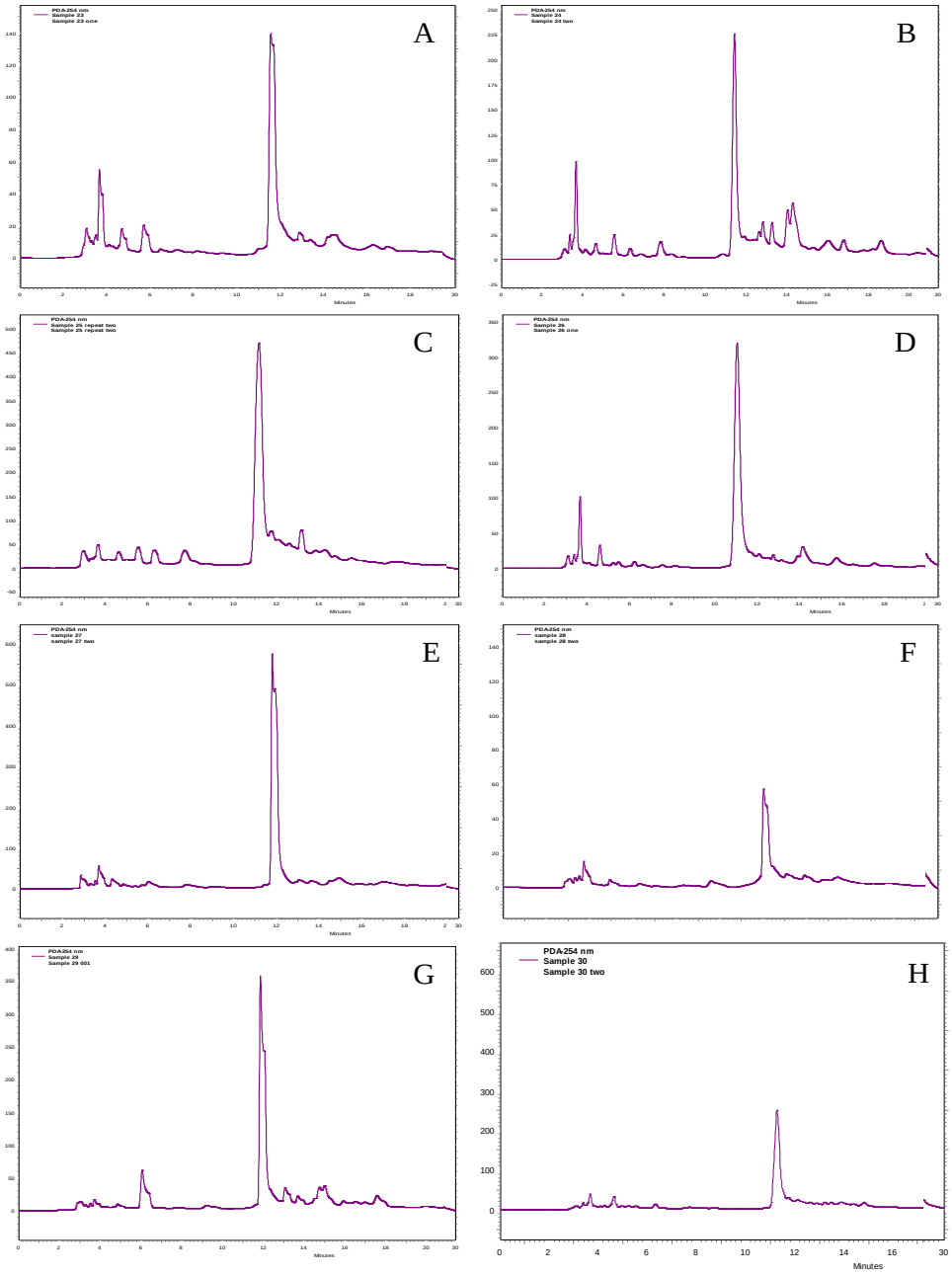


Figure 15: HPLC profile of purified Mangiferin. A-sample 23, B-sample 24, C- sample 25, D- sample 26, E- sample 27, F- sample 28, G-sample 29, H-sample 30

DISCUSSION

Mangiferin is a glycoside (2-β-D-glucopyranosyl-1, 3, 6, 7- tetrahydroxyxanthen-9-one), is the major component of mango leaves, fruit and bark. Studies have shown that mangiferin has anti-oxidant, anti-bacterial, anti-viral, immune regulation and anti-tumor activity (Yoshimi 2001, Peng 2004). Mangiferin and iso-mangiferin are usually found in the mango leaves as isomers. Mangiferin is well known to be found in various parts of M.indica. Content of mangiferin in stem bark is very high (71.4 g/ kg). In Cuba the stem bark aqueous decoction is used as a nutritional supplement (Selles *et al*. 2002) and the standard aqueos extract is available in pharmaceutical formulations under the brand name Vimang (Rivera *et al*. 2006). It is also reported that mango pulp, seed kernel and peel showed 4.4 mg/kg, 42 mg/kg and 1960 mg/kg respective mangiferin content. But mangiferin is also found in other plants like *Coffee, Salacia chinensis* and *Anemarrhena asphodeloides*. Augustyn *et al*. (2011) compared mangiferin content present in mango leaf of three cultivars with that of honeybush (*Cyclopia genistoides*), an indigenous south African herbal tea used for its antioxidant property and other health benefits. Their results indicate that mango leaves may have more health benefits than honeybush tea.

In a study by Muruganandan *et al*. (2005) mangiferin showed significant and consistent reduction in fasting blood glucose levels and improved the body weight loss at different intervals throughout the period of experiment indicating its potent

antidiabetic activity. Yoshikawa *et al*. (2001) found that mangiferin inhibits α glycosidase enzyme (sucrose, isomaltase, maltase). Mangiferin is a phenolic compound and occurs in response to stress. In *Coffee* plants, localization of mangiferin is found in association with the photosynthetic tissue and also found accumulated at early stages of fruit formation. Franklin *et al*. (2009) reported the presence of mangiferin for its antioxidant and antimicrobial properties along with plant defense against biotic stress. Study (Singh 2006) shows that level of mangiferin could be considered as a potential biochemical indicator for screening mango genotypes to malformation. In the present work also the aim was to determine the levels of mangiferin in the leaves of thirty different mango cultivars and to understand its health benefits. Thirty varieties of *M.indica* leaves showed a high peak of mangiferin content. Quantitative evaluation showed the mangiferin content to range between 4-47, with a maximum in Ladvo (47.2 mg/g) and minimum in Aambadi (4.79 mg/g). Dried mango leaves are used as a remedy for the treatment of relapse sickness in Tonger (Odyek *et al*. 2007), also in India (Scartezzini *et al*. 2000). Fresh leaves are also known to be used for treatment of diabetes.

The present study demonstrated thirty varieties cultivated in Junagadh orchard to be an interesting source of the pharmacologically active c-glycosyl xanthone mangiferin. It can be used as a health beverage especially because of its antioxidant properties.

SOIL ANALYSIS AND MINERAL NUTRIENT ANALYSIS IN LEAF

NUTRIENT ANALYSIS IN LEAF:

Mango flowering is predominantly influenced by the biochemical constituents present in the phase for the floral stimuli at bud break stage. The leaf nutrient status is the indication of the healthy status of vigor. The trees selected for the study was between 40-45 yrs of age. Foliar content variation of N, P, K, Cu, Zn, Mn and Fe was studied. The data regarding the status of macronutrients and micronutrients in mango leaves during the three different development phases of the tree is presented in table 12 and 13 and the graphical representation in given in figures 16-18.

The N, P and K concentration were different during the three different phases. All the three macronutrients show an increase in concentration when flowering stage is initiated after the vegetative phase. But after the flowering stage during the fruiting stage the concentration remains same or it reduces. Range of variations in macronutrients can be clearly seen in the 30 varieties. It varies from 0.8% to 1.5% in N, 0.04% to 0.2% in P and 0.1% to 0.9% in K. In 18 different varieties, N reduces after flowering. In 10 different varieties it increases from vegetative to flowering phase and then remains the same during fruiting stage. Only in Kesar variety the concentration of N remains same during the three different phases (table 12). Amount of Nitrogen (N) in vegetative phase was found maximum (1.5%) in Pairi, Jamadar and Alphonso, while it was recorded minimum (0.8%) in Sopari, Asadiyo and Jhamrukhiyo. During flowering stage it increase from 1.5% to 1.7% in Jamadar and Alphonso, while in Pairi it increased from 1.5% to 1.6%. In variety Sopari also it

showed increased level from 0.8% to 1%. At fruiting stage, Pairi showed huge decrease in N level while other varieties remained somewhat constant. Flowering phase had slightly more amount of NPK but showed a decreased level at fruiting phase which can be seen in the table 12 and figure 16A, B andC.

Maximum Phosphate (P), (0.2%) was present in Kesar and Rajapuri which are the most exploited varieties of Gujarat but Sindoria and Aambadi had minimum phosphate in vegetative phase. Phosphate levels remained constant in Kesar and Rajapuri variety in all the stages but the varieties which showed a minimum value in vegetative stage recorded increased level in flowering and fruiting stage. Potassium increased in 21 varieties at flowering stage and then reduces in fruiting. In Batli, Ladvo and Alphonso the concentration is same in vegetative and flowering phase and reduces in fruiting phase while in Jhumakhiya 2, Desi, Fazli and Totapuri the concentration increases and remains same in flowering and fruiting phase. In Mulgoa it is same in all three phases. Potassium (K) was maximum (0.9%) in Alphonso and minimum (0.1%) in Jhamrukhiyo 2. In flowering stage it remained almost constant but decreased from 0.9% to 0.7% in fruiting stage while Jhumakhiya 2 showed increased level from 0.1% to 0.4% in flowering stage which remained constant for fruiting stage (figure 16).

Concentration of the micronutrients Cu, Zn, Mn and Fe also varied during the three different phases (table 13). In all the 30 varieites studied the concentration of micronutrients Cu, Zn, Mn increased from vegetative phase to flowering phase. Further during fruiting phase the concentration remained same or reduced. Concentration of Fe in almost all the varieties remained same during all the 3 phases.

Copper (Cu) ranges between 16.5-81 µg/g (figure 17A). Many varieties showed increased Cu concentration like Cowasji (25.1 µg/g to 28 µg/g), Sindoria (32.6 µg/g to 39.5 µg/g), Goto (16.5 µg/g to 19.5 µg/g), Langdo (81 µg/g to 84 µg/g). But all these varieties showed a marked decrease in level at fruiting stage. For instance, Cowasji (28 µg/g to 25 µg/g), Batli (45 µg/g to 40 µg/g), Goto (29.5 µg/g to 15.5 µg/g) while in some varieties it remained constant. Maximum concentration was recorded in langdo whereas the minimum Cu concentration was observed in Goto variety. Cu concentration increased during flowering phase and remained same in the fruiting phase in varieties Langdo while in Aamirpasand the concentration was same during vegetative and flowering phase reducing during fruiting phase.

Zinc was maximum in Fazli (41.1 µg/g) and minimum in Totapuri (4.5 µg/g) (figure 17B). Similar to Cu, Zn concentration also increased in flowering stage and reduced during fruiting stage in almost all the varieties except Jhumakhiya 2 and Aambadi where during the vegetative and flowering phase the concentration is same and it reduces during fruiting stage.Manganese (Mn) was found maximum in Jhamrukhiyo 2 (84.2 µg/g) and minimum (11.8 µg/g) in Jhamrukhiyo (figure 18). In Cowasji, Jumakhiya 2, Aambadi, Jahangir, Jhamrukhiyo and Aamirpasand concentration of Mn is same during the vegetative and flowering stage, reducing during fruiting stage. In Aambadi and Totapuri the concentration remains almost same during the three different phases. Iron (Fe) was maximum in Sindoria and minimum in Fazli (figure 18B).

Sr. no.	Variety	Vegetative phase			Flowering phase			Fruiting phase		
		N	P	K	N	P	K	N	P	K
1	Cowasji	1.2	0.09	0.2	1.3	0.1	0.3	1.4	0.1	0.1
2	Batli	1.0	0.08	0.5	1.2	0.08	0.5	1.2	0.06	0.3
3	Jhumakhiya 1	0.9	0.1	0.3	1.1	0.1	0.4	1.1	0.09	0.2
4	Sindoria	1.4	0.04	0.2	1.5	0.06	0.5	1.4	0.06	0.3
5	Pairi	1.5	0.1	0.6	1.6	0.08	0.8	1.0	0.08	0.4
6	Goto	1.2	0.09	0.2	1.2	0.1	0.5	1.1	0.1	0.3
7	Jamadar	1.5	0.1	0.4	1.7	0.15	0.6	1.5	0.15	0.5
8	Mulgoa	1.3	0.06	0.3	1.5	0.08	0.3	1.4	0.08	0.3
9	Rucchado	0.9	0.05	0.3	1.0	0.08	0.4	1.0	0.08	0.2
10	Ladvo	1.1	0.1	0.2	1.3	0.12	0.2	1.2	0.12	0.1
11	Khodi	1.2	0.08	0.8	1.4	0.09	0.9	1.2	0.09	0.8
12	Kesar	1.4	0.2	0.5	1.4	0.2	0.9	1.1	0.2	0.4
13	Jhumakhiya 2	1.0	0.05	0.1	1.2	0.08	0.4	1.0	0.08	0.4
14	Sopari	0.8	0.08	0.4	1.0	0.09	0.6	1.0	0.09	0.4
15	Langdo	1.2	0.1	0.2	1.3	0.14	0.4	1.5	0.14	0.3
16	Aambadi	0.9	0.04	0.7	1.1	0.08	0.9	1.0	0.08	0.8
17	Asadiyo	0.8	0.05	0.2	1.0	0.09	0.5	1.0	0.09	0.3
18	Neelam	1.2	0.08	0.7	1.3	0.1	0.9	1.2	0.1	0.4
19	Basdshahpasand	0.9	0.06	0.4	1.0	0.08	0.5	1.0	0.08	0.4
20	Desi	1.1	0.08	0.2	1.4	0.1	0.4	1.2	0.1	0.4
21	Dudhpendo	1.0	0.06	0.6	1.2	0.09	0.8	1.1	0.09	0.6
22	Alphonso	1.5	0.1	0.9	1.7	0.1	0.9	1.6	0.1	0.7
23	Rajapuri	1.3	0.2	0.8	1.5	0.2	0.9	1.4	0.2	0.8
24	Fazli	0.9	0.09	0.4	1.1	0.12	0.6	1.1	0.12	0.6
25	Jahangir	0.9	0.05	0.5	1.0	0.08	0.8	1.0	0.08	0.7
26	Totapuri	1.2	0.1	0.6	1.3	0.1	0.8	1.2	0.1	0.8
27	Jhamrukhiyo	0.8	0.05	0.7	1.0	0.08	0.9	1.0	0.08	0.7
28	Kaju	1.0	0.1	0.6	1.2	0.15	0.8	1.1	0.15	0.6
29	Aamir pasand	0.8	0.06	0.2	1.0	0.08	0.4	1.0	0.08	0.4
30	Gajariyo	1.1	0.08	0.5	1.3	0.09	0.7	1.2	0.09	0.5

Table 12: Macronutrients in *M.indica* (% of dry matter).

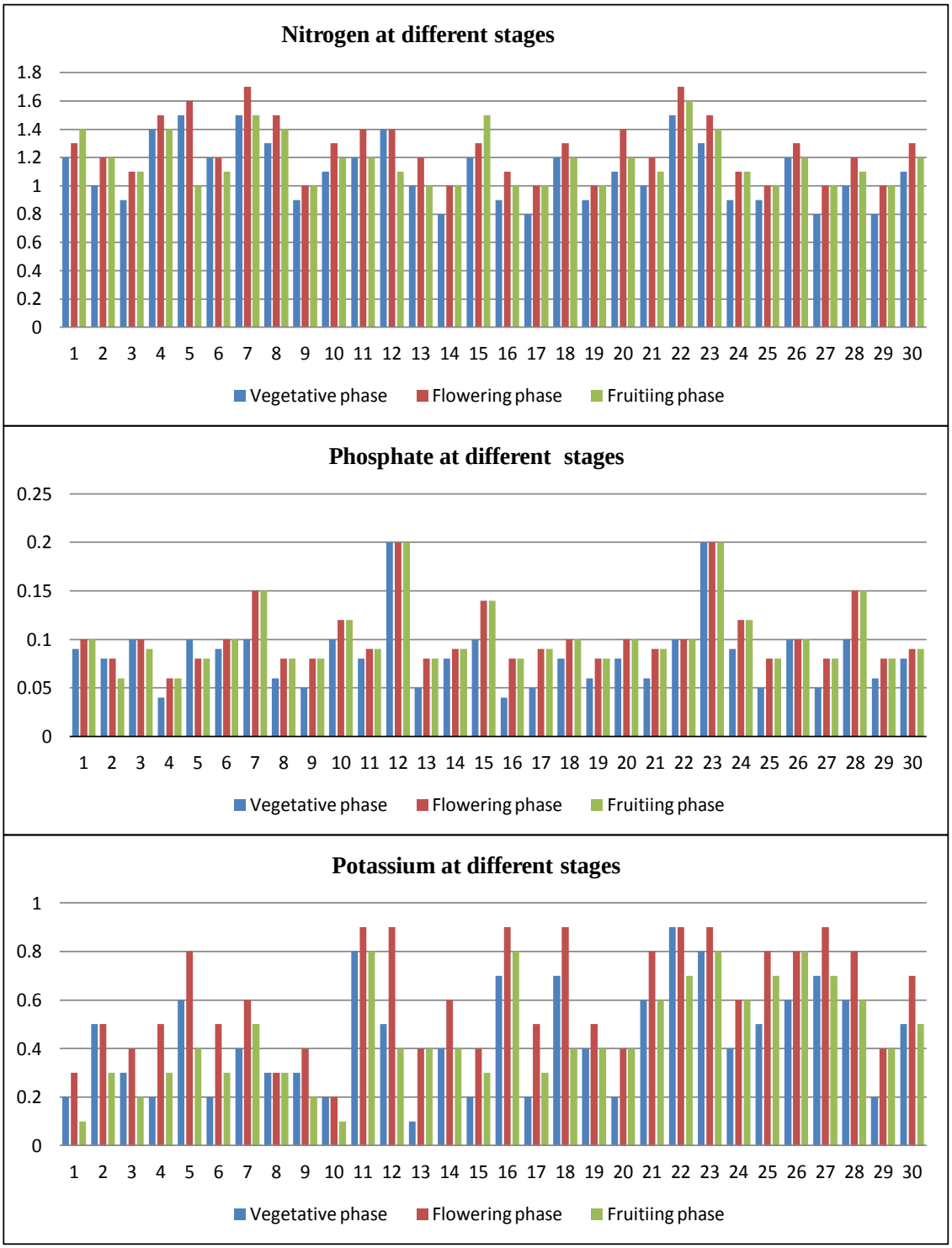


Figure 16: Amount of Nitrogen (A), Phosphate (B) & Potassium (C) in % of dry matter at different stages of *M.indica* in thirty varieties.

Sr. no.	Variety	Vegetative phase				Flowering phase				Fruiting phase			
		Cu	Zn	Mn	Fe	Cu	Zn	Mn	Fe	Cu	Zn	Mn	Fe
1	Cowasji	25.1	11.2	35.2	164.2	28	12	35.0	165	25.0	11.0	34.0	162.0
2	Batli	41.8	40.5	34.5	278.1	45	44	36	278.5	40.0	38.0	32.5	276.5
3	Jhumakhiya 1	26.5	8.1	23.6	366.2	30.2	9	25	365	24.5	7.5	23.0	362.0
4	Sindoria	32.6	11.2	45.8	521.6	39.5	13	46	520	32.0	11.0	44.0	510.0
5	Pairi	35.8	7.5	66.5	328.6	40	8.5	66.0	328.6	34.0	5.5	65.0	325.0
6	Goto	16.5	12.5	36.2	274.1	19.5	14	38	275	15.5	12.0	35.5	272.0
7	Jamadar	27.6	5.2	25.6	236.2	29.5	7	26	236.5	25.0	5.0	25.0	235.5
8	Mulgoa	33.2	23.5	42.5	244.5	36	25.5	44	246	30.5	20.5	41.5	240.5
9	Rucchado	22	28.5	32.8	428.3	25.5	30	35	428.5	21.0	22.5	31.5	428.0
10	Ladvo	53.5	11.1	52.1	220.2	58.2	12	54	221	52.0	11.5	50.5	215.0
11	Khodi	46.3	12.5	35.2	115.2	49.5	16	37	117	45.5	14.0	34.5	115.0
12	Kesar	52.5	10.4	21.2	234.6	55	12	23	236.2	50.5	10.5	21.0	230.0
13	Jhumakhiya 2	38.5	14.2	84.2	259.2	41.5	14.2	84.5	260	38.0	11.5	83.5	258.0
14	Sopari	38.2	25.1	50.0	162.3	46.5	27	50.7	164	37.0	25.0	49.5	161.5
15	Langdo	81.0	21.0	54	257.5	84	22	55	258	84.0	20.0	52.5	256.5
16	Aambadi	33.4	8.5	30.0	211.5	38.4	8.5	30.2	211.5	32.0	7.5	30.0	210.0
17	Asadiyo	51.2	10.6	44.8	324.0	58	14	46	326.2	50.5	10.0	44.0	320.0
18	Neelam	35.2	11.2	38.0	520.0	40	11.5	39	520.5	31.2	10.0	36.5	519.0
19	Basdshahpasand	32.0	12.5	42.9	275.5	41.2	15	46	276.5	30.0	12.0	42.0	265.0
20	Desi	39.1	8.0	26.4	241.7	45	10	28	242	36.1	7.5	24.0	240.0
21	Dudhpendo	27.2	14.0	45.2	166.2	32	14.5	45.5	166.5	26.2	13.5	44.5	164.5
22	Alphonso	21.3	9.2	35.0	112.3	26.5	11	36	114	20.2	9.0	35.0	111.5
23	Rajapuri	41.0	38.5	26.4	231.2	45	39.5	28	231.5	41.0	37.0	25.5	230.5
24	Fazli	43.1	41.1	28.5	89.0	46	43	30.5	90.0	41.5	40.0	26.5	89.0
25	Jahangir	42.2	8.7	35.7	225.5	45	9.0	36	226.5	40.5	8.0	35.0	223.5
26	Totapuri	35.8	4.5	20.2	219.5	37	6.5	20.5	220.0	35.0	3.0	20.0	215.5
27	Jhamrukhiyo	33.5	11.2	11.8	189.6	35	11.5	12	189.5	32.0	11.0	10.0	189.0
28	Kaju	30.0	12.3	12.6	264.0	31	15	14	266	30.0	12.0	12.2	262.0
29	Aamir pasand	36.5	11.1	53.1	429.0	36	12	53.5	429.5	35.0	10.5	52.5	427.5
30	Gajariyo	42.2	10.0	35.2	274.2	44	10.5	37	280	42.0	9.5	34.0	273.5

Table 13: Micronutrients in *M.indica* (µg/g) during the three different phenologiccal stages.

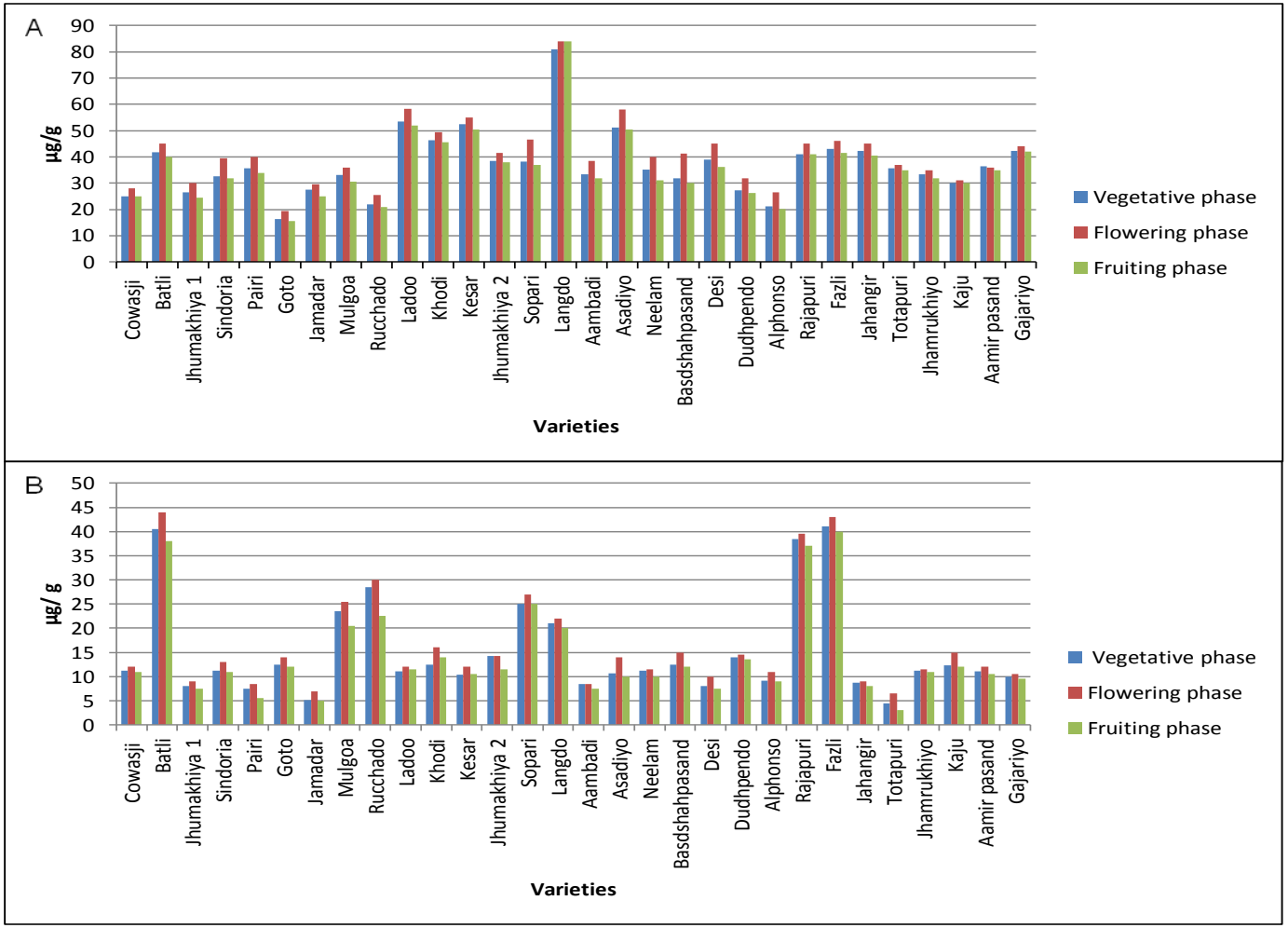


Figure 17: Cu (A) & Zn (B) concentration at different stage in *M.indica* in thirty varieties

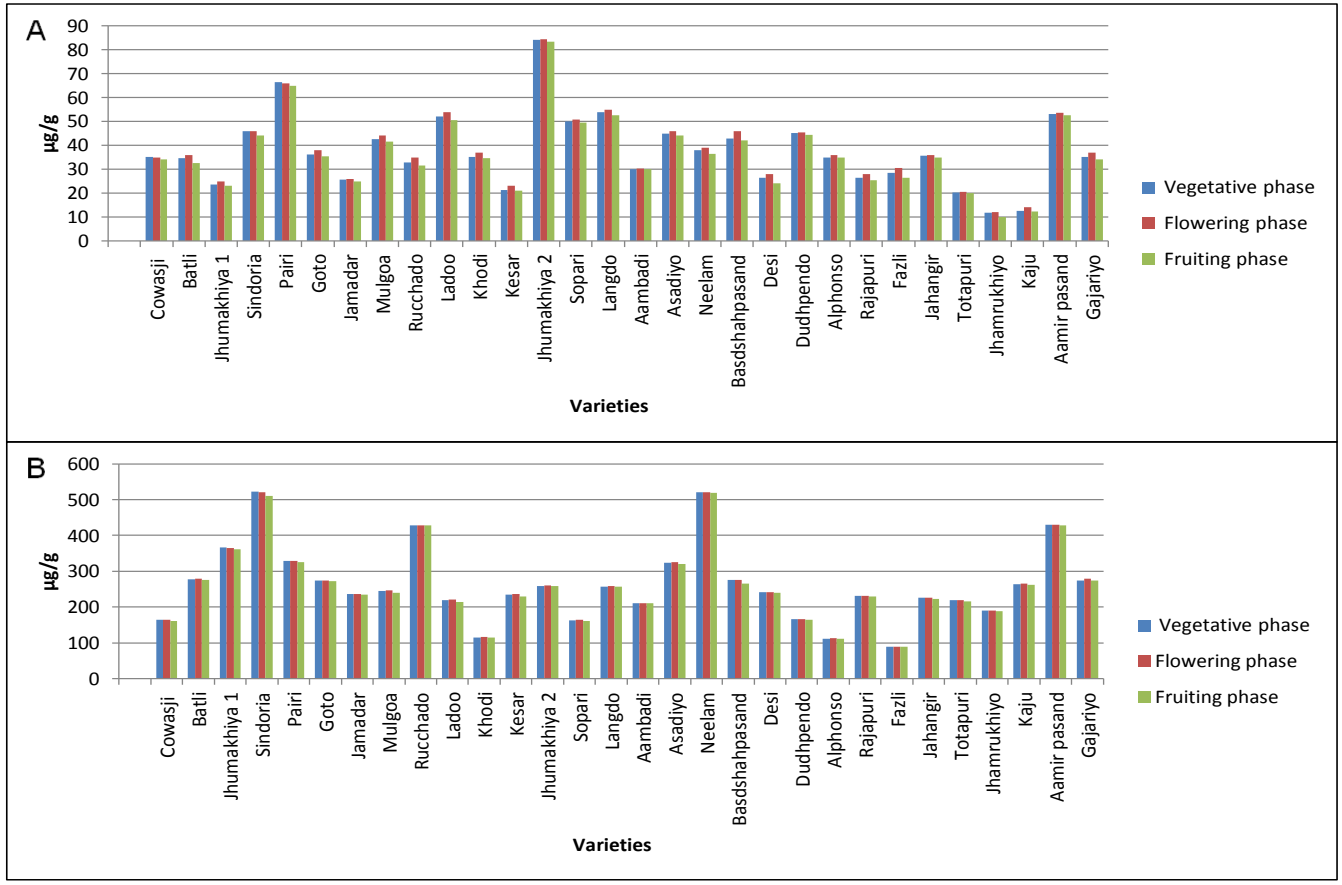


Figure 18: Mn (A) & Fe (B) concentration at different stage in *M.indica* in thirty varieties.

SOIL ANALYSIS:

Thirty different varieties studied for the comparison was collected from Junagadh, so the soil analysis was also done.The macronutrients and micronutrients present in the soil of Junagadh is given in table14. Macronutrients Nitrogen and Phosphorous were in medium amount while Potassium was found in very less amount in soil (table 14). Micronutrients in soil were more in amount than the normal ones. Macronutrient N was 0.74% in soil while in plant the range varied from 0.8% - 1.5 %. So it can be said that Sopari, Asadiyo, Jhamrukhiyo and Aamir pasand were having almost similar nitrogen content to that of soil. While other varieties were having comparatively higher amount of nitrogen. Phosphorous in soil was around 0.1%, while in plant the range was 0.04% -0.2% with many varieties having same concentration as in soil (table 14). Potassium was very high in soil around 2.5% , while in plant it ranged from 0.1% to 0.9% (table 14). When compared with the plant micronutrients, it was found that the micronutrients in soil were comparatively very less to the ones present in plant (table 13 and 14).

Macronutrients	
Nitrogen (OC %)	**0.74**
Phosphorous (P_2O_5, Kg/AC)	**4.00**
Potassium (K_2O, Kg/AC)	**100.0**
Micronutrients (ppm)	
(Copper)Cu	**3.92**
(Zinc) Zn	**2.06**
(Manganese) Mn	**12.96**
(Iron)Fe	**20.14**

Table 14: Soil analysis of Junagadh region.

DISCUSSION

Nutrient concentration of a plant changes depending on mineral mobility and mineral functions during growing period. The nutrients with high metabolic activity such as potassium, phosphorus or nitrogen moves easily from older tissue to newly growing part of the plant. Therefore, concentrations of highly phloem-mobile elements decrease throughout the leaf development. During the growing stage, as a rule, export of mineral nutrients increase and thus decrease in net concentration and decrease in amount per organ such as leaf (Beaufils 1973). During vegetative growth, mineral supply to roots is often either permanently insufficient or temporarily interrupted. Remobilization of mineral nutrients form leaves to growing parts is vitally important during reproductive growth, when seEds, fruits and storage organs are formed. Remobilization of highly phloem-mobile mineral nutrients can be lead to rapid decline in their concentration in the vegetative shoots (Wood *et al*. 1986, Mauk and Nooden 1992).

In a study conducted by Garz (1966), with pea plant, changes of some nutrients concentration were examined from flowering to harvesting and it was seen that nitrogen, phosphorus and potassium levels increased first and decreased continuously until ripening time.

Macronutrient and micronutrients are absorbed by the plants from the soil. Nitrogen is the constituent of protein, chlorophyll and nucleic acids which is obtained by the plant form air or soil. Phosphorus is the constituent of many proteins, coenczymes, nucleic aicd and metabolic substrates; important in energy transfer, obtained from soil and potassium which is involved in photosynthesis, carbohydrate tranlocation, protein synthesis, etc is also obtained from soil. The micronutrients obtained from soil are Cu, Fe, Mn, Mo, Ni, Zn, B.

Nitrogen is absorbed by the plants from the soil, in the form of nitrate or ammonium, phosphorous in the form of phosphare, potassium in the form of K^+ ions, Fe in the form of ferrous or ferric. Each nutrient cannot be taken up by plants in its elemental or non-charged from, but instead is taken up in an ionic or chaged form. All the nutrients move relatively easily from the root to the growing portion of the plant throught the xylem. Nutrient uptake is dependent on both the plant's ability to absorb a nutrient and the nutrient level at the root surface.

Copper is a component of plaxtocyanin which plays a role in protein and carbohydrates synthesis.Cu is essential in several plant enzyme systems involved in photosynthesis.Cu is part of the chloroplast protein plastocyanin, which forms part of the electron transport chain. Cu may have a role in the synthesis and/or stability of chlorophyll and other plant pigments.

Iron, constituent of cytochrome for respiration. It brings about chlorophyll formation and electron transport. Fe is essential in the heme enzyme system in plant metabolism (photosynthesis and respiration). The enzymes involved include catalase, peroxidase, cytochrome oxidase, and other cytochromes. Fe is part of protein ferredoxin and is required in nitrate and sulfate reductions.Fe is essential in the synthesis and maintenance of chlorophyll in plants and strongly associated with protein metabolism.

Manganese is an activator of enzymes in photosynthesis. Essential for chlorophyll formation. Mn is involved in the oxidation-reduction process in photosynthesis.

Zinc activator of enzymes in respiration. It has role in protein synthesis. Essential for auxin synthesis.Zn is required in the synthesis of tryptophan, which in turn is necessary for the formation of indole acetic acid in plants.

In the present study the main aim was to monitor the variation in concentration of the major mineral nutrients during the different phases of the growth. Compared to soil,

the plant nitrogen was more, however potassium was in a very high amount in soil while phosphorous could match with the soil nutrient status of plants. All the micronutrients in soil were very less compared to the nutrients in plants.

In a study conducted by Tahir *et al*. (2003) it was reported that bearing flushes had low level of nutrients at harvest time, which indicates maximum amount of nutrients were consumed in fruit setting and development. In the present study also all the 30 varieities show an increase in the level of micro and macronutrients during the transition from vegetative to flowering stage and then a decrease during fruiting stage indicating consumption during the fruit development.While non bearing flushes exhibited elevated levels of nutrients at harvest stage, which decreased gradually upto bud burst stage. Another study (Kumar *et al*. 2013) clearly indicated that all the biochemical constituents in leaves and shoot at vegetative stage, flowering stage and harvesting stage were significantly influenced by season and varieties. NPK level reached the highest level at pea stage and declined thereafter reaching the lowest level at full grown fruit stage (Pathak and Pandey 1977). NPK and Mn concentration increased as the leaf age decreased demonstrating the mobility of these elements. Leaf N, P, K, Mn and S concentrations were low during flowering and fruiting (Poncher *et al*. 1993). Soluble protein N contents of leaf, bark and wood were significantly higher during initiation and differentiation period (Sen *et al*. 1963). Same study reported low level of N and P at flowering stage in blooming than non-blooming flushes. In the present study, it has been found that the macro and micronutrients increase at flowering stage and decrease during fruiting. This indicated that maximum amount of

nutrients were consumed in fruit setting and development. Tahir *et al*. (2003) has found low level of nutrients at initial stages but after harvest as there is no sink, the level of nutrients increased.

In Cowasji and Langdo varieties, the nitrogen content increased at flowering phase and in fruiting also (table 13). While in Batli, Jhumakhiya 1, Rucchado, Sopari, Asadiyo, Badshahpasand, Fazli, Jahangir, Jhamrukhiyo and Amirpasand nitrogen level increased form vegetative to flowering phase but remained constant at fruiting phase (table 13). In varieties Sindoria, Pairi, Goto, Jamadar, Mulgoa, Ladvo, Khodi, Kesar, Jhumakhiya 2, Aambadi, Neelam, Desi, Dudhpendo, Alphonso, Rajapuri, Totapuri, Kaju, Gajariyo there was a rise in nitrogen level at flowering phase but it decreased at fruiting phase (table 13). Goto and Kesar showed constant nitrogen levels at flowering stage. Kumar *et al*. (2013) found that the nitrogen content of leaf at vegetative stage increased in flowering stage in Alphonso and Neelum, while it decreased in Mulgoa. At the commencement of fruiting season, the nitrogen content decreased in all the three varieties. Nitrogen content along with carbohydrates and starch reserves favoured flower bud differentiation. Nitrogen is a part of chlorophyll, it helps plant with rapid growth, increasing seed and fruit production and improving the quality of leaf and forage crops. According to Urban *et al*. (2004) changes in leaf nitrogen content may also result from depletion of leaf nitrogen by sinks such as developing flowers or furits.

Phosphate levels increased in Cowasji, Sindoria, Goto, Jamadar, Mulgo, Rucchado, Ladvo, Khodi, Jhumakhiya 2, Sopari, Langdo, Aambadi, Asadiyo, Neelam, Badshahpasand, Desi, Dudhpendo, Fazli, Jahangir, Jamrukhiyo, Kaju, Aamirpasand and Gajariyo at flowering stage. However it remained constant in Batli, Jhumakhiya 1, Kesar, Alphonso, Rajapuri, Totapuri while Pairi had decrease phosphate levels.

Fruiting phase was either having constant levels of phosphate seen in all the varieties with an exception of Batli and Jhumakhiya 1 where it decreased. Phosphorus is involved in the formation of all oils, sugars and starches. It encourages blooming and root growth.

Potassium levels increased in all the varieties except in Batli, Mulgoa, Ladvo and Alphonso, where it was constant with the vegetative phase. During fruiting phase potassium levels decreased in most of the varieties while it was constant in Jhumakhiya 2, Desi, Fazli, Totapuri and Aamirpasand. In variety Mulgoa, it was found to be constant in all the phases. Potassium is absorbed by plants in larger amounts than any other mineral element except nitrogen. Helping in building of protein and fruit quality.

Copper concentration in all the varieties rose form vegetative phase to flowering phase and decreased at fruiting phase in all the varieties except in Langdo, where it remained constant. Zinc concentration also increased in flowering phase except in Jhumakhiya 2 and Aambadi, where it remained constant. While at fruiting phase it decreased in all the varieties. Manganese concentration also increased in all the varieties except in Pairi, which showed constant values. Iron concentration raised in all the varieties except in Pairi, Amabadi and Jhamrukhiyo at flowering phase and reduced at fruiting phase.

As indicated by the other researchers, the present study also show similar results, where the macronutrients and micronutrients showed decrease in levels from flowering to fruiting stage. Concluding that the nutrients shift from leaves to fruits during its development. Also it can be said that the leaf vigor is important for a healthier fruit development.

COMPARATIVE STUDIES CONDUCTED ON THREE DIFFERENT VARIETIES FROM TWO DIFFERENT REGIONS

FRUIT

Fruit is a seed receptacle developed from an ovary. The wall of the fertilized ovary transforms during development into the pericarp of a mature fruit. Mango (*Mangifera indica*) is a simple fruit of drupaceous type, and contains a single large seed surrounded by fleshy mesocarp. The fruit provides essential mineral nutrients and vitamins. The quality of a fruit is influenced by variety and nutritional status and environmental conditions during growth of the parent plant. Fruits play a very important role in human nutrition, by providing an additional source of energy, necessary growth factors, carbohydrates, dietary fibers and antioxidants, essential for maintaining normal health. Fruits also contain a very high percentage of their fresh weight as water.

Mango is the most important fruit of India, with more than a thousand varieties known (Iyer 1991). Most of Indian varieties possess strong aroma and more intense peel coloration, characterized by attractive fragrance, delicious taste. Mango fruits have been used in every stage of growth, while the raw fruits are utilized for products like pickles, chutney or mango sauce, amchoor, the ripe ones are used in making pulp, juice, nectar, squash, mango leather, frozen and canned slices, jam, mango puree, mango cereal flakes, mango powder, mango toffee and mango fruit bars (Singh 1990).

Mangoes from two different famous mango growing regions of Gujarat viz Junagadh and Navsari were selected for developmental studies. Morphological, anatomical and biochemical studies at different stages of fruit development in three varieties Alphonso, Kesar and Rajapuri have been evaluated.

MORPHOLOGICAL STUDIES

Developmental changes of the ovary leading to the development of a mature fruit showed variations in all the three varieties.

Inflorescence has two types of flowers, bisexual (hermaphrodite) and male (staminate) flowers. The flowers are radially symmetrical and usually have 5 petals. There is usually only 1 fertile stamen per flower (plate 26a, arrowhead); the 4 other stamens morphologically appearing sterile. The flower has a conspicuous 5- lobed disc present between the petals and stamens. Based on the presence of staminodes and its number, flower appears to be an important character. All three varieties had four staminodes (plate 26) and one well developed stamen but in Junagadh (plate 26f, arrow) and Navsari Kesar (plate 26f, arrow, h), Junagadh and Navsari Rajapuri stamens could be categorized into three types; there was one long stamen, two medium sized stamens and two staminodes (plate 26 e-l). Hermaphrodite flowers were complete with all four accessory and essential whorls (plate 26 a, c, e, g, i, k). Because of the presence of lateral style and staminodes, flower appeared to be zygomorphic. Staminodes were present on the anterior side. Shape of the ovary varies with the variety which contributes to the shape of the fruit. Junagadh and Navsari Alphonso had a round ovary with a lateral straight style (plate 26a, arrow, c), Junagadh Kesar had ovate ovary shaped with lateral curved style (plate 26e) while Navsari Kesar had a round ovary with lateral straight style (plate 26g). Junagadh and Navsari Rajapuri had oval shaped ovary with lateral curved style (plate 26i, k). Male flowers are incomplete with a rudimentary ovary placed beneath the disc (plate 26b, d, f, h, j, l) and the flower appears actinomorphic as the style is not visible here because the ovary is rudimentary (plate 26h, arrow).

Fruit morphology is the most common way of identification of the variety. In both the regions the shape and fruit symmetry was similar. Shape of Alphonso is oval and the fruit symmetry is oblique (plate 27 a, b). Kesar (plate 27 c, d) and Rajapuri (plate 27 e, f) had ovate oblong shape and were asymmetrical. As per the categorization of (Gangolly *et al*. 1957) flesh of the fruit in Junagadh and Navsari Alphonso and Kesar was soft while in Junagadh and Navsari Rajapuri, the flesh was firm. Flavor of the fruit was delightful in Alphonso and Kesar and aromatic in Rajapuri of both regions.

Endocarp/ stone in the fruits from the two different regions were also compared. It showed variation in the density and location of fibers and pattern of the venation as observed on the surface of the endocarp. Stone was medium sized, oblong in Junagadh and Navsari Alphonso covered with dense, soft fibre located on the ventral edge (plate 28a, b, arrowhead) and short fibre on the rest of the surface. Veins on the stone were parallel and slightly depressed in Juangadh Alphonso while the veins were forked and prominently raised (plate 28b, arrow) in Navsari Alphonso. Stone in Junagadh and Navsari Kesar was medium, oblong covered with dense, short and soft fibre all over with parallel and slightly depressed veins (plate 28 and d). Stone was large and oblong oval in Junagadh and Navsari Rajapuri. Fibers were sparse, short, stiff all over but soft dense fibres were found on the ventral edge in Junagadh Rajapuri (plate 28 e, arrow) while in Navsari Rajapuri, stone was covered with sparse, short and soft fibres all over the endocarp surface. Veins were parallel and prominently raised (plate 28f, arrow) in the endocarp of both regions.

ANATOMICAL DEVELOPMENT OF FRUIT

Figure 19: Different regions of mango fruit

When an ovary develops into a fruit, the ovary wall becomes pericarp. The ovary wall at a very young stage of the opened flower can be differentiated into an outer and inner epidermal layer and a central parenchymatous region of 4 to 5 layers covering the ovary chamber. In between the parenchymatous tissue were embedded vascular bundles. Longitudinal view shows a single large vascular bundle on the basal side of the ovary wall and 5 to 6 lateral bundles smaller in size (plate 29a b,c, arrow). Main large vascular bundle along with the lateral small bundles arises from the vasculature of the peduncle. At very young stage of the ovary itself resin ducts are distinctly observed (plate 29 a-f, 30 a-e). Resin ducts are seen in large number towards outer and innermost parenchymatous region. With further enlargement of the fruit wall/

ovary wall resin ducts prominently appear in large numbers in the mid parenchymatous regions. During maturation the pericarp frequently undergoes an increase in the number of cells. Its ground tissue differentiated into parenchyma and sclerenchyma.

The pericarp of mature mango fruit can be differentiated into 3 layers- exocarp, mesocarp and endocarp (figure 19) which is very distinct just in 2 weeks after fertilization. The ovary of *Mangifera indica* L. is one-carpellate and encloses a single seed (plate 29 d-f, 30 c-e). Vascular system was found in various parts of the fruits. The different stages of the development of different parts of fruits have been represented in plate 31 to 36. Development of the endocarp has been discussed in detail separately.

The developmental studies are categorized into the exocarp, mesocarp and endocarp. The developmental changes taking place during the different stages and its comparison of the two different regions are described.

DEVELOPMENT OF EXOCARP

In a ripe fruit when the exocarp is removed it includes cuticle, epidermis, hypodermis and some part of mesocarp. The exocarp also designated as the epicarp of a mature fruit showed three distinct layers from outside to inside; cuticle, epidermis and hypodermis (plate 31 and 32). All the three varieties prominently showed presence of cuticle outside the epidermal layer but the thickness varied. Junagadh and Navsari Rajapuri had cuticle more thicker (31.25 and 33.5 µm) than that of Alphonso (19.6 and 16.2 µm) and Kesar (24.4 and 22.1 µm) respectively. Cuticle thickness was found maximum in Rajapuri (plate 31c and 32c) variety followed by Kesar (plate 31b and 32b) and Alphonso (plate 31a and 32 a) for both the region, Junagadh and Navsari.

Stage I: The exocarp develops from the outer epidermis and associated outer layers of ovary walls lying just below the outer epidermis. At the early stages, the outer epidermis cells are somewhat elongated radially and divide intensely in an anticlinal direction increasing in surface area. Parenchyma cells below the epidermis divide both anticlinally and periclinally developing into hypodermis (plate 31b). Epidermal layer in stage I showed variation in all the three varieties of Junagadh. The entire epidermal layer in Rajapuri was smooth and even. In Junagadh Kesar, the epidermal layer was undulating with an uneven surface and intermittently multicellular uniseriate trichomes composed of 3-5 cells emerged from the epidermal cells. Epidermal layer in Junagadh Alphonso was found to be undulated only at some sites and was completely free of trichomes. Thickness of exocarp was more in Rajapuri (135 µm).

Stage I: Stage I showed rapid growth in size. Epidermal cells in the exocarp region were elongated and compactly arranged in Alphonso and Kesar (plate31andb) while it appeared irregular and loosely arranged in Rajapuri (plate 31c) because of the thick cuticle impregnated between the epidermal cells. Hypodermis was made up of collenchyma cells formed from parenchyma which result after the division of epidermal cells. It has a protective function and takes part in formation of the skin. It is 5-6 layered in Alphonso, 6-7 in Kesar and 3-5 layer in Rajapuri. The hypodermal cells remain meristematic dividing anticlinally and periclinally (plate31andb). Number of hypodermal layer was more in Kesar (7-9) followed by Alphonso (5-6) and Rajapuri (3-5). Also the thickness of hypodermis was maximum in Kesar (72.5 µm). Sphaeraphides were seen in the hypodermal region of variety Rajapuri.

During development at the end of stage I, the outer tangential walls of the epidermis cells become more thickened and cutinized. In Navsari, stage (I) of Alphonso had an

undulated epidermis, while Kesar and Rajapuri showed smooth epidermis. The epidermal cell shape was similar as in Junagadh region, elongated and compactly arranged while in Rajapuri, irregular and loosely arranged. Thickness of exocarp was maximum in Rajapuri followed by Alphonso and Kesar (table 15).Hypodermis ranged from 7-9 layers which was recorded in Kesar. Also maximum thickness of hypodermis was observed in Kesar (71 µm).

Stage II: Also called as adolescent stage. The undulation of epidermis reduced in Junagadh Alphonso and Kesar but the thickness of the cuticle increased in all the three varieties. Cuticle thickness and exocarp thickness also reduced in this stage (table 15). Number of hypodermal layer was more or less constant as in stage I. But the hypodermis thickness showed variation with maximum observed in Alphonso (64.2 µm) and minimum in Rajapuri (36.1µm). In stage (II) of all varieties of Navsari, undulations were reduced and stomata were seen in the epidermis of Alphonso distinctly differentiating it from Junagadh region. As observed in Junagadh region, this region also showed reduced thickness of cuticle and exocarp region (table 15). Also thickness of hypodermis reduced in size at this stage with maximum in Kesar (55.2 µm).

Stage III: It is the climacteric stage which attains ripening with a maximum rate of respiration. Cuticle thickness increased at this stage (table 15) but the thickness in hypodermis and exocarp region decreased probably due to flattening of cells (table 15). However, number of hypodermal layer remained almost constant.

Stage IV: The cuticle thickness remained constant at this stage. Hypodermis and exocarp thickness recorded a minor reduction from stage III. In stage (IV) limited cell divisions were observed and lenticel cavity increased in size due to increased cell enlargement. Enlargement of the fruit led to increased tension on the cells above these

resin ducts and therefore caused the epidermis to rupture which left an opening on the fruit surface. The lenticels opened up and formed cup-like lenticels with a cuticle.

DEVELOPMENT OF MESOCARP

Mesocarp development initiates after endocarp differentiation and lignifications. In mature fruit the mesocarp also called as sarcocarp which constitutes the edible part of the fruit mainly consists of polygonal parenchyma cells closely aligned to one another with thin walls (plate 33 and 34). Cell walls were uniform in thickness. Mesocarp can be distinguished into two regions, outer and inner mesocarp. Inner mesocarp contains more starch as compared to the outer one.

Stage I: All the three varieties from the two different regions showed similar structure in stage I. The to be differentiated mesocarp region at this stage comprises of thin walled large rounded compactly arranged parenchyma cells. A differentiation of outer and inner mesocarp region could be identified at this stage. The outer mesocarp showed compactly arranged small densely stained parenchymatous cells while the inner mesocarp cells were differentiated by its larger size, lightly stained and compactly arranged cells (plate 33a-c, 34a-c). In the inner mesocarp region, initials of resin ducts and resin ducts were also observed. Resin duct initials were identified by group of densely stained cells (plate 33 a-c and 34 a, b). The initials arise by anticlinal and periclinal divisions of the parenchyma cells (plate 33 f, 34b).

The parenchymatous region between the outer and inner epidermal layer of the ovary wall differentiates into the mesocarp of the mature fruit which is parenchymatous with resin canals, vascular bundles and starch grains. Stage (I) shows the presence of parenchyma cells with the initials of resin duct formation in all varieties (plate 33a-c and 34 a-c). These initials were distinct from other parenchyma cell in having a dense cytoplasm (plate 33 d-f and 34 d-f).

Stage II: In both regions Junagadh and Navsari, stage (II) had vascular bundles, resin ducts, starch granules and tanniferous cells dispersed in the parenchyma. Resin duct initials and developed resin ducts especially in the inner mesocarp region and region near the endocarp were seen at this stage. The peripheral small-celled part of the pericarp was poor in or devoid of starch whereas towards inside larger cells follow with characteristically compound and simple starch grains (plate 35 d-i and 36 g-i). Starch granules were absent in epidermis (plate 35 a-c and 36 a-c) and outer 2 or 3 layers of hypodermal cells and were sparse in inner hypodermal cells (plate 35 d-f, 36 d-f). Starch increases from outer to inner cortex.

Stage III: Stage (III) showed more amounts of resin ducts and starch granules but soon after this stage the starch decreases due to hydrolysis (plate 35 j-l). Resin duct was found mainly located between hypodermal collenchymas and the vascular bundles. Number of ducts was seen to be coming out from the fruit base. The diameter of the ducts varied from 30µm but large duct had diameter up to 156µm.

Stage IV:

Resin duct remains constant as in stage III but the amount of starch decreases. Developed resin ducts with vascular bundles can be seen in plate 33 (n, o, p) and plate 34 (j, k, l).

DEVELOPMENT OF ENDOCARP

According to the classification made by Garcin (1891) endocarp is heterogenous type with sclereids and fibers contributing to its compostion.

During the fruit development the first subepidermal layerlying above the inner epidermis of ovary wall divides periclinally many times to give rise to the endocarp layer. The mature endocarp mainly consisted of fibers elongated in various directions,

forming a complex network of cells. Vascular bundles were also present in the endocarp. The inner epidermis transformed into sclereids with strongly thickened wavy walls (plate 29 k, arrow, m and 30 g-i).

Initial stage I have single layer endocarp which can be differentiated from the parenchyma cells in mesocarp by the thickening in the cells. The stony nature is seen in stage (III and IV), where the seEds develop totally giving it a tough texture. In Junagadh region, variety Alphonso and Kesar, stage I were seen with some signs of endocarp formation and many small resin ducts surrounding that region. Stage II showed three layers of sclereids forming the endocarp. In all the varieties in later stages (III and IV), the hardening of sclereids layer increases and the endocarp becomes stony (plate 29 j-m, 30 g-i).

As the fruit is derived from the carpels, its vascularization reflects that of the carpels. The vascular supply of the fruit is principally the same as in the ovary and becomes only extended during fruit development. Finer bundles are seen in the fleshy parts i.e. mesocarp regions.

The endocarp (or putamen) forming the stone (or pyrene) of the fruit develops as a hard stony and lignified layer (sclerocarp) which protects the seed. When fruit grows to about 4-5 cm in length fundamental changes occur, in the endocarp, the anticlinal multiplication (plate 29k, arrowhead) and cell expansion occurs in the daughter cells whose size and form with tendencies toward an isodiametric shape. Endocarp cells divide and daughter isodiametric cells become a meristematic inner epidermal tissue.

Endocarp is not stratified, consisting mainly of brachysclereids, fibers and vascular elements, develops from the inner epidermis and adjacent tissue of the young ovary wall including the procambium strands. During endocarp formation, periclinal cell

divisions were observed in the first subepidermal layer. When the fruit grows to about 4-5 cm in length fundamental changes occur, in the endocarp region, the anticlinal multiplication and cell expansion occurs in daughter cells whose size and form with tendencies towards an isodiametric shape. The result is an undulating pattern where crest corresponds to the largest cells and valleys to the smallest cell.

The fibers formed are of many layers interlacing each other arranged parallel to the long axis of the fruit .Sclereids of fruit arise through a belated sclerosis of ordinary parenchyma cells. Cell wall of fibers shows secondary thickenings and usually lignified. They develop secondary walls and may invade intercellular spaces. Transitional forms between sclereids and parenchyma cells were observed in stage I along the boundary between endocarp and mesocarp. The cells of endocarp are squarish in Junagadh Alphonso, Kesar, Rajapuri and Navsari Kesar, while Navsari Alphonso and Rajapuri had elongated cells. Thickening in the cell was more or less similar except for Navsari Kesar, where the cells were more thickened.

The endocarp is divided into a multiseriate outer layer of tangentially elongated fibers. Endocarp sclereids vary greatly in size and shape some are relatively short, other are 200 µm in length with a diameter of 10-12 µm. Longer ones are found in the inner portions and shorter forms appear to be more abundant. Transitional forms between sclereids and parenchyma cell often develop along the boundary between endocarp and mesocarp.

LENTICEL DEVELOPMENT

Epidermis characteristically shows presence of lenticel in stage I in all the varieties collected from both Junagadh and Navsari region and development of the lenticel varies in the different variety. Origin of lenticel appears to be either below the stomata or from the hypodermal layer.

Distribution of lenticel on fruit surface was throughout uniform in Junagadh and Navsari Alphonso and Kesar while in Rajapuri it was more on the stalk region compared to other region. In all the three varieties of mango from Junagadh lenticel develops beneath the cracks arising in the continuous epidermis caused by the inability of this layer to keep pace with the expanding inner tissue. Hypodermal cells intermittently below the epidermis become meristematic and undergo anticlinal and periclinal divison increasing the number of cells. Because of the rapid increase in the cells, the epidermal layer under tension cannot cope up with the increase in the cells thereby breaking. In Junagadh Alphonso, phellogen could also be distinctly observed (plate 31g, arrow). The hypodermal layer becomes meristematic dividing periclinally repeatedly forming the phellogen. Periclinal rows of cells can be distinctly observed. The loosely arranged cells towards the outerside of the phellogen breaks open the epidermis under pressure. Epidermis is characterized by lenticel development (plate 31d-f, 32a-c). Epidermis consisted of a single layer of cells undergoing active anticlinal cell division. A continued anticlinal cell division of epidermal cells resulted in the fruit surface with an undulating appearance.

In Navsari Alphonso, origin of lenticel takes place below the stomata (plate 32a, arrow). Anticlinal and periclinal divisions are observed in the cells of the substomatal chamber below the stomatal apparatus. Because of the increase in number of cells the stomata with further development of the fruit appears to be raised above the level of the epidermal cells. It appears to be protruded. Stomata are forced outwards by lenticel originating beneath them. The repeated division of substomatal cells forms groups of filling cells under the pressure of which the guard cells separate and the pores remain open leading to the formation of the lenticel opening (32d ,g). In Navsari

Alphonso with further development of the fruit below the stomata in the deep lying tissue of the pericarp where the lenticel formation takes place the cells appear to become meristematic, becoming very intense giving rise to 3-4 layers of anticlinal rows of several cells, becoming side by side with rows of very few cells where meristematic activity is slow. In plate 32g, the cell formed below the stomata are arranged in a very irregular manner and appears densely stained.

In all three varieties, a depression was seen just before initiation of lenticels development. The cells in hypodermal region were arranged in a crowded manner, pushing the epidermal cell outside and forming a space on the epidermis (plate 31 d-g and 32 d-f). In Junagadh Kesar and Rajapuri (plate 31 i, j), the space formed was witnessed by loosely arranged cells; however Alphonso (plate 31 h) had compactly arranged cells. Epidermal cell division nearly stopped but cell enlargement continued both in the epidermis and in sub epidermal cells.

RESIN DUCT DEVELOPMENT

Primary resin duct in all the varieties shows epithelial cells at various stages of secretion (plate 33 k-m, 34 j-l).The resin ducts originate from a group of cells which differ from the surrounding parenchyma cells in their dense protoplasm and their conspicuous nuclei; these cells increase in number by cell divisions. Resin duct run through the pedicel and branch into the receptacle. Some ducts end up in the receptacle, while others penetrate the sepals, petals and filaments. After anthesis, a lysigenous duct develops in the ovary wall. Resin ducts occur both in the exocarp and mesocarp region. Resin duct form lysigenously and at every stage of development, the epithelial cell disintegrate. The adjacent parenchyma cells become glandular forming the epithelial cells (plate 33 g-m, 34 g-i).

Initially during resin duct formation, there was disintegration of the single procambial row of cells. Space released by the disintegration of these cells becomes the duct. At the stage of differentiation of the epithelial cells the duct becomes narrow and irregular and appears bulged. Disintegration of epithelial cells also occurs during later stages where the crushed cells were seen in the epithelium (plate 33 g-j).The mature ducts which may reach a diameter of 150 µm and more are surrounded by a ring of numerous secretory cells. Junagadh region showed stage I with initiation of resin duct in Alphonso, Kesar, and Rajapuri while there were no starch grains observed at this stage. Stage II had the initiation of starch granules in all the varieties. Stage III had cells filled with starch granules (plate 33h, arrow) in Alphonso and Kesar but sparse amount was observed in Rajapuri (plate 35i). In stage IV, cell wall dissolution occurred and disappearance of starch granules was observed in all the varieties. Many types of crystals were observed in all the varieties like sphaeraphides, rhomboidal crystals and osteosclerids .Similar observations were recorded in Navsari region, where the starch appears at stage II, at stage III the cells of mesocarp were filled with starch grains and after the hydrolysis at stage IV, starch disappears (plate 36j, k, l). Increase in cell size and intercellular spaces are more rapid in mesocarp region. This process continues till maturity.

Stage and variety	Thickness of cuticle (µm)	No. of layers in hypodermis	Thickness of hypodermis (µm)	Thickness of exocarp (epidermis + hypodermis) (µm)
Stage I				
Junagadh Alphonso	19.6 ± 1.6	5-6	52.7	108
Junagadh Kesar	24.4 ± 1.2	7-9	72.5	111
Junagadh Rajapuri	31.25 ± 2.4	3-5	41.6	135
Navsari Alphonso	16.2 ± 0.9	5-7	66.3	110
Navsari Kesar	22.1 ± 1.2	7-9	71.0	102
Navsari Rajapuri	33.5 ± 2.4	3-5	42.3	126
Stage II				
Junagadh Alphonso	17.5 ± 1.6	5-6	64.2	92
Junagadh Kesar	23.2 ± 0.4	6-7	60.4	76
Junagadh Rajapuri	28.5 ± 0.9	3-4	36.1	102
Navsari Alphonso	14.8 ± 0.4	5-7	52.7	96
Navsari Kesar	20.9 ± 1.0	6-7	55.2	82
Navsari Rajapuri	30.2 ± 0.9	2-4	34.2	101
Stage III				
Junagadh Alphonso	19.0 ± 1.0	5-6	50.3	90
Junagadh Kesar	23.9 ± 1.1	6-7	58.6	74
Junagadh Rajapuri	30.0 ± 1.6	3-4	35.1	98
Navsari Alphonso	16.2 ± 0.4	5-6	68.4	95
Navsari Kesar	22.0 ± 1.4	4-6	51.6	82
Navsari Rajapuri	31.4 ± 1.1	2-4	38.0	100
Stage IV				
Junagadh Alphonso	18.4 ± 1.0	5-6	64.4	88
Junagadh Kesar	22.0 ± 1.4	6-7	66.2	74
Junagadh Rajapuri	32.4 ± 1.0	3-4	39.0	95
Navsari Alphonso	18.4 ± 0.6	4-6	55.2	92
Navsari Kesar	24.1 ± 0.9	3-6	46.8	80
Navsari Rajapuri	32.0 ± 1.1	2-4	34.6	100

Table 15: Anatomical features of exocarp in all the three varieties of mango fruit collected from Junagadh and Navsari

Plate 26- Flower of *M.indica.*

a. Junagadh Alphonso bisexual flower, ovary (arrow). Fertile stamen (arrowhead)
b. Junagadh Alphonso male flower
c. Navsari Alphonso bisexual flower
d. Navsari Alphonso male flower
e. Junagadh Kesar Bisexual flower
f. Junagadh Kesar male flower, staminodes (arrow)
g. Navsari Kesar Bisexual flower
h. Navsari Kesar Male flower, rudimentary ovary (arrow)
i. Junagadh Rajapuri bisexual flower
j. Junagadh Rajapuri male flower
k. Navsari Rajapuri bisexual flower
l. Navsari Rajapuri male flower

Plate 27- Fruit (stage III)

a. Junagadh Alphonso
b. Navsari Alphonso
c. Junagadh Kesar
d. Navsari Kesar
e. Junagadh Rajapuri
f. Navsari Rajapuri

Plate 28- Stone characteristics in *M.indica*

a. Junagadh Alphonso - stone fibres on the ventral edge
b. Navsari Alphonso - stone fibres on the ventral edge (arrowhead) and prominent forked veins (arrow)
c. Junagadh Kesar - stone fibres all over, vein depressed
d. Navsari Kesar - stone fibres all over, vein depressed
e. Junagadh Rajapuri- veins prominent
f. Navsari Rajapuri- veins prominent (arrow)

Plate 29 -Fruit development and endocarp region (Junagadh)
Magnification Bar: a to i - 10µm, j-m - 15µm

a. Ripened ovary initial stage in Alphonso
b. Ripened ovary initial stage in Kesar
c. Ripened ovary initial stage in Rajapuri
d. Endocarp and seed development in Alphonso
e. Endocarp and seed development in Kesar
f. Endocarp and seed development in Rajapuri
g. Developing seed in Alphonso
h. Developing seed and endocarp in Kesar
i. Developing seed and endocarp in Rajapuri
j. Endocarp in Alphonso
k. Endocarp in Alphonso (arrow), dividing subepidermal cell (arrowhead)
l. Endocarp in Kesar
m. Endocarp in Rajaprui

Plate 30- Fruit development and endocarp region (Navsari)

Magnification Bar: a to f- 10µm, g –i - 15µm

a. Ripened ovary initial stage in Alphonso
b. Ripened ovary initial stage in Kesar
c. Ripened ovary initial stage in Rajapuri
d. Endocarp and seed development in Alphonso
e. Endocarp and seed development in Kesar
f. Endocarp and seed development in Rajapuri
g. Endocarp in Alphonso
h. Endocarp in Kesar
i. Endocarp in Rajapuri

Plate 31 -Lenticels development (Junagadh)

Magnification Bar: a to m - 15µm

a. Exocarp layer in Alphonso
b. Exocarp layer in Kesar
c. Exocarp layer in Rajapuri
d. Formation of lenticel in Alphonso
e. Cork cells compactly arranged near exocarp in Kesar (arrowhead)
f. Formation of lenticels in Rajapuri (arrowhead)
g. Formation of lenticels in Alphonso (arrowhead)
h. Exocarp region developing a gap in Alphonso (arrowhead)
i. Exocarp region developing a gap in Kesar, cuticle seen
j. Exocarp region developing a gap in Rajapuri
k. Lenticel in Alphonso
l. Lenticel in Kesar
m. Lenticel in Rajapuri

Plate 32 -Lenticels development (Navsari)

Magnification Bar: a to m - 15µm

a. Exocarp layer in Alphonso, Stomata (arrow)
b. Exocarp layer in Kesar
c. Exocarp layer in Rajapuri, cork cells (arrow)
d. Formation of lenticel in Alphonso
e. Cork cells compactly arranged near exocarp in Kesar
f. Formation of lenticels in Rajapuri
g. Lenticels in Alphonso
h. Lenticel in Kesar
i. Lenticel in Rajapuri (arrow)

Plate 33 -Resin duct- (Junagadh)

Magnification Bar: a to p - 15µm

a. Exocarp and mesocarp layer with resin duct intials in Alphonso
b. Exocarp and mesocarp layer with resin duct intials in Kesar
c. Exocarp and mesocarp layer with resin duct intials in Rajapuri, sphaeraphides (arrow)
d. Resin duct initials in Alphonso
e. Resin duct initials and resin ducts in Kesar
f. Resin duct initials in Rajapuri (arrow)
g. Formation of resin duct besides vascular bundle in Alphonso
h. Formation of resin duct besides vascular bundle in Kesar, starch containing cells (arrow)
i. Resin duct initials in Rajapuri
j. Initials forming circular structure in Rajapuri
k. Developed resin duct in Alphonso
l. Developed resin duct in Kesar, cell lysis (arrow)
m. Developed resin duct in Rajapuri, epithelial layer (arrow)
n. Resin duct in Alphonso, vascular bundle (arrow)
o. Two resin duct in Kesar
p. Resin duct near vascular bundle in Rajapuri

Plate 34 -Resin duct- (Navsari)

Magnification Bar: a to l - 15µm

a. Exocarp and mesocarp layer with resin duct intials in Alphonso
b. Exocarp and mesocarp layer with resin duct intials in Kesar
c. Exocarp and mesocarp layer with resin duct intials in Rajapuri
d. Resin duct initials in Alphonso
e. Initials forming circular structures in Kesar
f. Resin duct initials in Rajapuri
g. Formation of resin duct in Alphonso
h. Formation of resin duct besides vascular bundle in Kesar
i. Resin duct initials forming space in Rajapuri
j. Resin duct near vascular bundle in Alphonso
k. Developed resin duct in Kesar
l. Two resin duct in Rajapuri, arrow pointing vascular bundle

Plate 35 -Starch grain- mesocarp region (Junagadh)

Magnification Bar: a to f- 10µm, g-i -12µm, m to n - 15µm

a. Alphonso- stage I
b. Kesar- stage I
c. Rajapuri- stage I
d. Starch grains in Alphonso- stage II
e. Starcg grains in Kesar- stage II
f. Starch grains in Rajapuri- stage II
g. Cells in mesocarp filled with starch grains- Alphonso- stage III
h. Cells in mesocarp filled with starch grains- Kesar- stage III
i. Cells in mesocarp filled with starch grains- Rajapuri- stage III
j. Alphonso- stage IV- less starch grains
k. Kesar- stage IV- less starch grains
l. Rajapuri- stage IV- less starch grains
m. Magnified view of starch grain in Alphonso
n. Magnified view of starch grain in Kesar
o. Magnified view of starch grain in Rajapuri

Plate 36 -Starch grain- mesocarp region, Navsari

Magnification Bar: a to i - 10µm, j-l – 12 µm

a. Alphonso- stage I
b. Kesar- stage I
c. Rajapuri- stage I
d. Starch grains in Alphonso- stage II
e. Starcg grains in Kesar- stage II
f. Starch grains in Rajapuri- stage II
g. Cells in mesocarp filled with starch grains- Alphonso- stage III
h. Cells in mesocarp filled with starch grains- Kesar- stage III
i. Cells in mesocarp filled with starch grains- Rajapuri- stage III
j. Alphonso- stage IV- starch grains
k. Kesar- stage IV- starch grains
l. Rajapuri- stage IV- starch grains

DISCUSSION

When an ovary develops into a fruit, the ovary wall (carpel wall) becomes the pericarp (in Greek peri, 'around', and carpos, 'fruit'). If the ovary wall matures into a pericarp with a conspicuous stony endocarp and a fleshy mesocarp, the fruit is called a drupe. The carpel forms the fundamental structure of the fruit and is regarded as a modified foliage leaf in which the lamina part prevails (Roth 1977). In *M.indica* the simplest and basic form of a carpel formed by conduplicately folded leaf with its margin more or less fused is found. As a true leaf, the carpel has a midrib bundle and two to three marginal bundles embedded in the parenchymatous tissue which further during the growth of the fruit develops into the mesocarp. The entire ovary wall of the fruit or the external part differentiates into a parenchymatic tissue whose cells retain their protoplast in the mature fruit. An immature fleshy fruit wall has firm texture, but it becomes softer as the fruit ripens. Chemical changes in the cell contents and in the structure of the walls are responsible for the softening (Reeve 1959). The cells may even become dissociated from each other.

The outer epidermis or exocarp is uniseriate in the initial stages of development of all the 3 varieties growing in the two different regions. With further development towards the maturity the uniseriate epidermis divides giving rise to a hypodermal layer composed of collenchymatous tissue of 3–9 layers. Depending upon the variety the numbers of layers differ, in Alphonso 5-6, in Kesar it is 6-9 and in Rajapuri 3-5 layers of hypodermis. The differentiation of the exocarp region from the mesocarp region is not prominent but the demarcation of the latter from the endocarp region is well defined. Pericarp has many functions like to protect the embryo from damage, so it develops hard layers, such as endocarp or sclerenchymatous rings or groups of stone cells or sometimes it may become sclerified entirely. Varietal differences in the

cuticle layer were reported in fruits of mango (Baker 1982). Variability in thickness of hypodermis was recorded for olive fruits (Mulas 1994). According to Paul *et al*. (2007), thickness of exocarp region declined with fruit maturity in variety Langra and Amrapali while Dusheri and Ramkela recorded no significant changes. The present study also shows a decreased level in the thickness of exocarp but Navsari Kesar recorded a minor increase.

The basic form and structure of the fruit is laid down in the early stages of cell division. Cell enlargement contributes to fruit enlargement and to changes in proportion. During the time of differentiation the final structure is reached and the different tissues are developed. The period of ripening mainly concerns changes in cell wall structure and cell contents; it may also affect the structure of the surface and forming of intercellular spaces.

Maturity at harvest is the most important factor that determines storage-life and final fruit quality. Immature fruits are more prone to mechanical damage and are of inferior flavor quality when ripe. Overripe fruits are likely to become soft. Fruits picked either too early or too late in their season are more susceptible to postharvest physiological disorders than frits picked at the proper maturity.

Initially the growth of the fruit is mainly due to cell division accompanied by cell enlargement together with an increase in the size and number of laticiferous canals in all three regions of the fruit. Once the cell division stops, other factors like cell enlargement and increase in size and number of laticiferous canals, add together to fruit growth. Increase in cell size and intercellular spaces are most rapid in the mesocarp region than other regions. The process continues till the fruit becomes mature (stage III) and reduces gradually in later stages (IV). Hardening of the endocarp region started 64 days after anthesis in Dashehari and slightly later in

Chausa and continues up to maturity. Singh and Paliwal (1980) observed that the reserve materials particularly starch grains started accumulating in the cells after they ceased to divide. Waste product accumulated in the fruit, only druses were located right from anthesis to maturity. Maturity is a stage of full development of the tissues of the fruit, only after which it will ripen normally. During ripening stage, the cell wall becomes thin, with increased intercellular spaces, loss of cell structure integrity appeared. The fruit loses firmness of the flesh and becomes soft to touch during ripening. Cell compactness was also lost during ripening. The ripening phenomenon is associated with loss of firmness, hydration of cell wall, changes in cell wall thickness, decrease in the structural integrity and increase in the intracellular spaces (Tucker and Grierson 1987). When fruit ripens, the pulp softens and the fruit acquires the desirable texture, which is an important attribute for its acceptability. Texture is one of the most important organoleptic characteristics of fruit that is altered during ripening. Textural softening in fleshy fruits is primarily due to cell wall modification (Jackman and Stanley 1995, Rizvi and Tong 1997, Van Buren 1979). At the cell wall, the extent of pectin and hemicelluloses dissolution and solubilization is generally related to the degree of textural softening and cell wall changes during ripening (Huber 1983, Waldron *et al*. 1997). Pectins belong to group of acidic polysaccharides, found in large amount in fruits and contribute in a substantial way to their texture (Brownleader *et al*. 1999, Voragen *et al*. 1995). Less information is available on mango cell wall changes during ripening. But according to Selvaraj and Kumar (1989), considerable differences occur between cultivars. Gowda and Huddar (2001), Selvaraj *et al*. (1989) observed major changes in mango fruits during ripening, which included reduction in fruit weight, firmness, acid content, starch, vitamin C with simultaneous increase in total soluble solids, pH, sugar/acid ratio and carotenoids.

Fruit weight was seen to reduce in variety. In the present study, cell compactness was more in Rajapuri, so it is firm and it was less in Alphonso and Kesar so the ripe fruit is soft.

During the growth of apple fruit, the cuticle on the epidermis increased in thickness (Tetley 1930, 1931). At the initial stages of development, stomata are seen which are further replaced by lenticels consisting mostly patches of suberized cells (Clements 1935). Sometimes lenticels arise beneath scars left by fallen trichomes and breaks in the skin (Krapf 1961).

Lenticels are macroscopic openings occurring also on the surface of mango fruit and responsible for gaseous exchange and transpiration. Lenticels originate from the ruptured stomata during fruit enlargement and growth, reaching their maximum size at full maturity of fruit. The mechanism of lenticels discoloration is poorly understood, but few studies revealed that it is due to several factors including cultivar differences, movement of air and water through lenticels and membrane damage and liberation of phenolics. Discoloration of mango lenticels is cultivar dependant (Du Plooy 2006). Ultrastructure studies of lenticels suggest physiological irritation responsible for the stimulus for development condition.

Lenticels differentiate from existing stomata that lose their function and protrude above the fruit surface as a result of rapid anticlinal cell divisions in the epidermis of the exocarp. Lenticels can be found on the surface of stems, old roots and several fruit types, including apples, pears, avocados and mangoes (Dietz *et al*. 1988). In the absence of stomata, lenticel takes over the vitally important process of gaseous exchange needed for photosynthesis, respiration and transpiration (Mauseth 1988). Cork cambium and cork cells seems absent in mango lenticels, which can be the most important reason for lenticels discoloration. The degree of lenticels discoloration may

vary in different mango cultivars. According to Dietz *et al*. (1988), mango fruit lenticels may develop from either pre-existing stomata, or from rupturing of the epidermis. Usually an active cork cambium gives rise to loosely packed cork cells, enabling gaseous exchange and preventing microbial infection of the plant organ. A radial cell division of cork cambium also enables expansion and elongation of the tissue surface. But mango fruit lenticels are unusual, lacking a cork cambium. Mango lenticels do not elongate and expand to cope with tissue growth. This leads to cell wall sheathing and cell collapse of sublenticellular cells. The studied varieties can be categorized into 2 groups on the basis of lenticel development 1) below stomata 2) below epidermis at restricted sites. Navsari Alphonso showed the first category where the lenticel develop below the stomata, while the other varieties Junagadh Alphonso, Kesar , Rajapuri, Navsari Kesar and Rajapuri had second category with the lenticel developing below the epidermis.

Most peculiar seen distinct growth periods are in stone fruits, such as peach, the apricot, the cherry etc. where the competition between pericarp and embryo development expresses itself in alternative growth cessation; during first period, the pericarp enlarges rapidly, while the embryo remains small; during the following period, the embryo develops and pericarp growth ceases, while in the third period growth processes are reversed again, when the fruit enters a new period of pericarp enlargement (Nitsch 1953).

Fruit does not grow continuously at the same degree. Three distinct growth periods are: cell division, cell enlargement and fruit ripening (Kobel 1954). Long (1943) considered in the last growth period, weakening of the cell wall as a result of hydrolysis, color change, increase in sucrose and rapid dehydration. Drupes with

restricted number of ovules and a strong endocarp are distinguished from other fruits by their discontinuous growth period. Lileland (1930) has described distinct periods in apricot fruit, period after anthesis; a rapid growth of the flesh can be observed which is suddenly slowed down in the second period, when the endocarp increases in weight, while resumption of the rapid growth rate of the flesh during the third period causes the slowdown of endocarp growth. The slow growth of the flesh during the second period may be ascribed to competition for food which is used for endocarp formation. Depending on the composing tissues Garcin (1891) made a distinction between homogenous and heterogenous endocarp formation. In all the three varieties of studied *M.inidca* the endocarp formation was heterogenous i.e. it consists of mixture of sclereids and fibers. Topographically, the endocarp originates from a mixture of inner epidermis and subepidermal layers. The subepidermal layer divides periclinally forming the endocarp layer. Further anticlinal multiplication and cell expansion occurs in daughter cells of the endocarp region.

Endocarp lignification occurs in a wide range of angiosperms, including both dry and fleshy fruits. This suggests that either it is an adaptive process that occurs through relatively simple evolutionary changes or that it represents an ancestral state in which case fruits with non-lignified endocarp would have lost this character (Dardick *et al.* 2010). The endocarp is the first part of the fruit that reaches its maximum size (Ragland 1934).

A similar phenomenon has been observed in other stone fruits such as the peach, the plum and the cherry (Tukey 1934, 1936). In *Prunus cerasus*, a rapid fruit

development follows fertilization, whereas development which continues up to maturity. Nitsch (1953) emphasizes that during a period I, the pericarp of stone fruits generally enlarges rapidly and that the integuments of the seed develop at the same time, whereas embryo remains small. Blake and Davidson (1936) has established four stages of pericarp development in peach. The cell division stage extends from fertilization to pit hardening; the cell wall thickening period represents the second stage during which cell walls of the flesh thickens and the pit hardens. In the third stage of development, called the pre-ripe stage, mainly cell enlargement takes place and cell walls decrease gradually in thickness. During this time, increase in fruit size becomes more apparent, while a slow decrease in flesh ripening stage extends only over a short period, when the fruit softens rapidly and becomes fully mature.

According to Gustafson (1926) growth and enlargement of a fruit does not proceed at a constant rate, but follows a sigmoid curve, characteristic of most growth processes. Growth usually starts slowly, then goes on with increasing speed to slow down again until it ceases entirely. Growth does not proceed continuously in stone fruits as described earlier but is divided into 3 clearly defined stages, a rapid pericarp increase in stage one, a slowing down of pericarp growth in stage 2, when pit and embryo are being formed, and a third period of pericarp growth resumption.

The texture of the flesh in berries and drupes especially of the cultivated forms, which is of the most important for quality and edibility of fruits changes very much during ripening. These alterations can be brought about by changes of cell wall structure and chemistry and by the formation of intercellular spaces or complete separation of the cell during the maceration process. During ripening pectic substance increases which

is responsible for fruit softening. The increase begins with the softening and reaches its maximum in the fully ripe fruit. According to Blake and Davidson (1943), Addoms *et al.* (1930), the decrease firmness characteristics of ripening peaches is directly correlated with a decrease in propectin, in cellulose and in thickness of the cell walls. The 'melting' characters of the flesh of ripe fruits is due to a low content of cellulose and propectin and to thin cell walls, some of which break actually, liberating the cell contents into the intercellular spaces and thus producing an increase juiciness of the flesh (Yashoda 2003).

Fruit endocarp lignification occurs in many angiosperms and plays a critical role in seed protection and dispersal. Studies on Peach have reported deposition of lignin in peach initiates near the blossom end within the endocarp layer. Quantitative polymerase chain reaction studies revealed that phenylpropanoid and lignin pathway genes were induced in the endocarp layer over a 10 day time period, while two lignin genes were co-regulated with falconoid pathway genes which were mesocarp and exocaro specific (Dardick *et al*. 2010). A stony endocarp composed of elongated curved sclereids, varying in orientation in different layers, occurs in the drupelets of raspberry (Reeve 1954).During endocarp formation; Reeve observed periclinal divisions in the involved in the derivatives are elongated more or less tangentially, whereas the elongation in the subjacent tissue is at right angles. This arrangement is lost in later stages and many sclereids lie in different directions.

Resin ducts are uniformly arranged in the shoots of all Anacardiaceae species (Engler 1896). In fruits they occur throughout the mango exocarp (Joel 1980). Present study confirmed the observations made by Joel and Fahn (1980), resin duct were usually closely associated with vascular bundles. But the resin ducts are not uniformly

arranged in the fruits of the various species of this family (Copeland 1961, Roth 1974, Grundwag 1976). The fruit of mango has secretory ducts which secrete a skin-irritant resin similar to that secreted by poison-ivy (Keil *et al*. 1946). Epithelial cells lining the resin duct are responsible for secretion of resin tinto the resin duct. The resin in the ducts is under high pressure and it is well-known that a fairly large amount of sap exudes from the pedicel after the fruit has been picked. Some investigation of this phenomenon revealed that sap had leaked out of a resin duct through the lenticels. Also the microscopic observation revealed a damaged resin duct just below the lenticels. The cell walls of the resin duct were also discoloured and appeared similar to the other discoloured lenticels. Resin ducts development initiates within the parenchyma cells of a very young ovary wall even before differentiation of the different tissues in the ovary wall leading to the formation of pericarp, mesocarp and endocarp. In all the three varieties the resin ducts develop lysigenously. During stage I with the differentiation of the exocarp and mesocarp resin ducts was prominent in outer and inner layers of mesocarp and with further development an irregular distribution of large number of resin ducts was noticed throughout the mesocarp. The diameter of resin duct ranged from 56x35 µm in Alphonso, 42x25 µm in Kesar and 35x23 µm in Rajapuri.

This resin mainly contains terpenes and some amount of phenols and protein-carbohydrate mucilage (Molisch 1931, Ulmert 1970). The resin of mango fruit consists of lipophilic and mucilaginous substances. Regarding the sites of resin synthesis, Joel and Fahn (1980) have suggested three interpretations: 1) each

organelle in which osmiophillic droplets occur is capable independently of synthesizing all the resin component. 2) There are several steps of resin synthesis, each by a specific organelle. 3) Different resin components are synthesized by different organelles.

Study by Joel (1981) shows that the duct systems of both the fruit and the stalk penetrate the transition zone, lies between the fruit base and its stalk. Fruit duct resin is under pressure and when the fruit is picked, resin is rapidly exuded from the ducts. Separation of the fruit duct system from the stalk duct system is reported for other members of the Anacardiaceae. Roth (1974) reported that the ducts of the pseudocarp of the cashew (*Anacardium occidntale*) are not connected with the cavities of the fruit. Many plants contain schizogenous ducts or cavities (Curtis and Lersten 1990, Turner *et al*. 1998) formed by the separation of cells from each other during development. The resulting cavity becomes bounded by a layer of secretory cells (sometimes called as epithelial cells). These cell secrete various substances into the cavities formed such as mucilage by certain members of the Malvaceae, Tiliaceae, Sterculiaceae and resin or gums as in many conifers as well as in several families of angiosperms (eg. Anacardiaceae).

BIOCHEMICAL STUDIES

The chemical composition of mango pulp varies with the location of cultivation, variety and stage of maturity. The major constituents of the pulp are water, carbohydrates, organic acids, fats, mineral pigments, tannins, vitamins and flavor compounds.

Development stages of mango fruit has been categorized into 4 growth phases. Stage I and stage II were the initial developmental stages of the fruit, where carbohydrates, proteins, enzymes, vitamins were in minimal amount. Stage III and stage IV showed the developmental changes from unripe to ripe fruit, during which lot of biochemical changes take place. Therefore only stage III and IV were considered for the present study.

The sugars and amino acids present in the pulp of Alphonso, Kesar and Rajapuri varieties of mangoes at various stages of ripeness were analyzed by HPTLC. Ripening was associated with loss of firmness and pulp acidity with increases in total sugars. Preliminary studies showed presence of only three sugars, which were identified as glucose, fructose and sucrose.

Plant cell walls are the major source of dietary fiber. Dietary fiber, sometimes called roughage is the indigestible portion of food derived from plants. Dietary fiber is categorized into two main components: (i) soluble fiber which dissolves in water. It is readily fermented in the colon into gases and physiologically active byproducts and can be viscous (ii) Insoluble fiber which does not dissolve in water. It can be metabolically inert and provide bulking, metabolically fermenting in the large intestine. Soluble fibers tend to slow the movement of food through the system.

Biochemical study showed variation in the concentration of the constituents in mangoes collected from the two different regions.

Stage III: The total sugar content in Kesar and Alphonso varieties did not show much difference but Rajapuri showed a major difference. Total sugar content was significantly low in Rajapuri of Junagadh and Navsari almost amounting to 2-3 times lesser than Kesar and Alphonso of Junagadh and Navsari (figure 20 A). Reducing sugars ranges from 1.30-2.48% and non-reducing from 1.02-1.35%. (table 16). Reducing sugars in Junagadh and Navsari Rajapuri was less (1.35 and 1.30% respectively) compared to Junagadh and Navsari Kesar and Alphonso. Starch content in Junagadh Alphonso was very low as compared to the other two varieties (figure 20 C). The pH value of the pulp was measured, where it was found that, Kesar and Alphonso had higher pH value in Junagadh region compared to Navsari while in Navsari Rajapuri and higher pH value than Junagadh (figure 20E). Acidity was measured in terms of citric acid. Junagadh Kesar had more acidity than that of Navsari Kesar while in Alphonso and Rajapuri were more acidic in Navsari region compared to Junagadh. Acidity content was maximum in Navsari Rajapuri (figure 20G). Protein content in Junagadh and Navsari Rajapuri was significantly less compared to Junagadh and Navsari Kesar and Alphonso in which the content was more or less same ranging between 0.81 to 0.89%. Moreover among the two regions compared the protein content was more in Jungadh Kesar and Alphonso in comparison to Navsari Kesar and Alphonso. But in Rajapuri, Navsari Rajapuri had higher protein content than Junagadh. Highest amount of total phenols was found in Navsari Kesar and lowest was found in Junagadh Rajapuri. Dietary fibers were distinctly more in Rajapuri and minimum in Navsari Kesar (table 16). Sugar acid ratio

ranged between 5.40 to 28, with a maximum ratio in Junagadh. Junagadh and Navsari Rajapuri were with minimum ratios.

Stage VI: Total sugar, starch and pH value increases in this stage while acidity decreases (table 16). Total sugar content was found almost similar in Kesar, while in Alphonso more amounts was found in Navsari and in Rajapuri more amount of total sugar was found in Junagadh region (figure 20B). Reducing sugars ranges from 2.18-2.47% and non-reducing sugars ranges form 1.92-2.30%. Starch was more in Alphonso at Navsari region (figure 20D). pH values were comparable, except for Alphonso where Junagadh region had more pH value than Navsari (figure 20F).Acidity was more in Rajapuri, but not much difference occurred between two regions (figure 20H). Total phenols were found to be increasing in stage IV except in Navsari Kesar, where it decreased from 31.12 µg/ml to 26.01 µg/ml. When compared among the region, it was found that Navsari Kesar had almost double amount of total phenols than Junagadh Kesar. Also in Navsari Alphonso and Rajapuri phenol content was more than Junagadh Alphonso and Rajapuri. Dietary fiber was more in stage 4; Junagadh Rajapuri had maximum value while Navsari Kesar had minimum value (table16). Sugar acid ratio ranged from 9.45 to 57.57, with a maximum in Navsari Alphonso (57.57) and Junagadh Alphonso (53.33). Junagadh Kesar and Rajapuri had more amounts 53.12 and 27.77 respectively than in Navsari Kesar (48.38) and Rajapuri (9.45).

Among the three different varieties of *M.indica*, variety Alphonso from Navsari showed maximum amount of starch content in both unripe and ripe stages (figure

20C, D). In the varieties from Junagadh, Kesar showed maximum starch content during the unripe stage while in the ripe stage both Kesar and Rajapuri showed same amount of starch content. Total sugar content was maximum in both unripe and ripe stage of Alphonso from Navsari. Total sugar content was significantly low in unripe and ripe fruit of both Junagadh and Navsari Rajapuri in comparison to the other two varieties (figure 20A, B). Ripe fruits of Junagadh and Navsari Alphonso had the highest reducing sugar content amongst all the other varieties. Junagadh Kesar showed equally same amount of reducing sugar content. Percent reducing sugar was found to be more than non-reducing sugars in all the ripe varieties except in Rajapuri. In Junagadh Rajapuri percent non-reducing sugar was more than reducing while in Navsari Rajapuri as found in other varieties the reducing sugar is more than non-reducing. Dietary fiber increased at ripe stage in all the varieties but Junagadh and Navsari Rajapuri have exceptionally more amount of it compared to Kesar and Alphonso. Over all there was increase in all the contents from an unripe to ripe stage of the fruit. pH of all the varieties from the two different region showed an increase from unripe to ripe stage but still in the acidic range (fig 20E, F). Percentage acidity reduces from unripe to ripe stage of the fruit (fig 20G, H). Sugar acid ratio increased at stage IV. It was found maximum in Navsari Alphonso followed by Junagadh Alphonso and was minimum in Rajapuri varieties (table 16).

Sr.no.	Varieties	Kesar		Alphonso		Rajapuri	
	Stage	Junagadh	Navsari	Junagadh	Navsari	Junagadh	Navsari
pH	III	3.58	2.91	2.99	2.71	3.17	4.09
	IV	5.96	5.81	5.57	4.93	4.02	3.83
Acidity (%)	III	0.50	0.48	0.52	0.58	0.69	0.74
	IV	0. 32	0.31	0.30	0.33	0.36	0. 34
Starch	III	1.11	1.40	0.90	1.58	1.02	1.24
	IV	1.3	1.45	1.12	1.81	1.33	1.43
Total sugar (%)	III	14	12	12	15	5	4
	IV	17	15	16	19	10	7
Reducing sugars (%)	III	2.33	2.15	2.48	2.36	1.35	1.30
	IV	2.45	2.26	2.42	2.47	2.18	2.32
Non-reducing sugars (%)	III	1.22	1.14	1.35	1.30	1.14	1.02
	IV	2.11	1.92	2.30	2.03	2.21	2.20
Proteins ($100g^{-1}$)	III	0.89	0.82	0.84	0.81	0.59	0.65
	IV	0.97	0.98	1.0	0.99	0.71	0.74
Total phenols (µg/ml)	III	15.81	31.12	11.60	18.55	11.37	15.24
	IV	17.59	26.01	16.76	21.59	9.76	14.13
Dietary fiber (%)	III	8.5	8.2	10	9.6	11.60	18.55
	IV	9.1	8.5	11.4	10	30.7	29.3
Sugar : acid ratio	III	28	25	23.07	25.86	7.24	5.40
	IV	53.12	48.38	53.33	57.57	27.77	9.45

Table 16: Physico-chemical attributes of the three different varieties of *M.indica* fruit at unripe and ripe stages collected from two different regions.

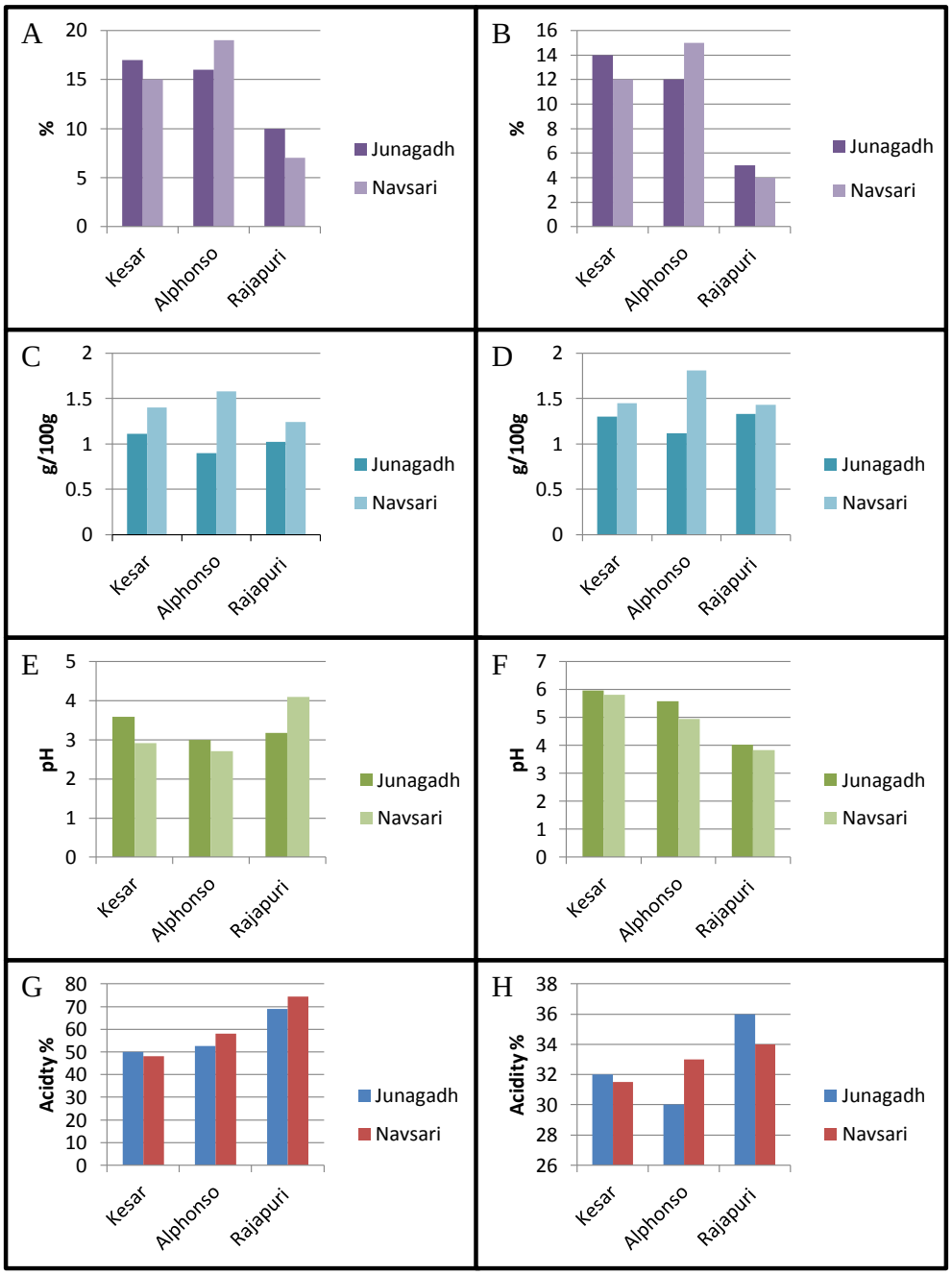


Figure 20: Graph showing concentration of total sugars(A & B), starch (C & D), pH (E & F)and acidity (G & H) during unripe and ripe stages of the three varieties of *M. indica* from the two different regions.

Enzymes

Enzymes amylase, catalase, invertase, peroxidase, cellulase, polygalacturonase, and pectinmethylesterase were analyzed in all the three varieties of unripe and ripe stages at both the regions Junagadh and Navsari.

Stage III: In all the three varieties from two different regions content of amylase exceed the other enzymes. Amylase was maximum in Junagadh Alphonso and minimum in Junagadh Rajapuri. Catalase was more in Navsari Kesar and less in Junagadh Alphonso and Navsari Rajapuri. Invertase was found maximum in Junagadh Kesar followed by Junagadh Rajapuri and Junagadh Alphonso and minimum was observed in Navsari Alphonso. Peroxidase was more in Navsari Alphonso and minimum was seen in Navsari Rajapuri. Cellulase was more in Junagadh and Navsari Kesar while it was minimum in Junagadh and Navsari Rajapuri. The maximum polygalacturonase was observed in Alphonso of Junagadh region (0.18) and minimum in Junagadh Kesar. Pectinmethyesterase (PME) at stage III was maximum in Junagadh Rajapuri and minimum was found in Navsari Rajapuri (table 17).

Stage IV: Table 17 shows high amounts of amylase, catalase and invertase in stage IV, which is the ripe stage. Amylase was seen to increase from 0.9 to 2.5 in Junagadh Rajapuri which was highest, while Navsari Alphonso did not show much difference in rise. Catalase was seen to be rising in Junagadh Alphonso from 0.5 to 1.5, which was highest and lowest amount was seen in 0.6 to 0.9. Rise of invertase enzymes was seen highest in Navsari Alphonso but levels were found similar in both stages in Junagadh

Rajapuri. Peroxidase also showed a rise in level where it was maximum in Junagadh Alphonso and minimum in Navsari Kesar. Cellulase showed increased level in this stage. Minimum was found in Junagadh and Navsari Rajapuri while maximum was found in Junagadh Kesar and Navsari Alphonso. In stage IV the maximum (0.24) polygalacturonase was seen in Navsari Kesar while minimum (0.20) was in Navsari Alphonso. It was found that it increases during ripening. PME found to be increasing in this stage with maximum in Junagadh Rajapuri while minimum was found in Navsari Alphonso.

Stage	**Varieties**	**Kesar**		**Alphonso**		**Rajapuri**	
	Regions	Junagadh	Navsari	Junagadh	Navsari	Junagadh	Navsari
III	**Amylase** (mg maltose/min/mg protein)	1.2	1.5	1.6	1.2	0.9	1.1
IV		2.8	2.4	2.3	2.1	2.5	2.6
III	**Catalase** (units/min/mg protein)	0.8	1.1	0.5	0.7	0.6	0.5
IV		1.2	1.5	1.5	1.4	0.9	1.2
III	**Invertase** (mg glucose/hr/mg protein)	0.7	0.5	0.5	0.4	0.6	0.5
IV		1.3	1.5	1.4	1.8	0.6	0.8
III	**Peroxidase** (units/min/mg protein)	0.25	0.22	0.26	0.30	0.24	0.21
IV		0.41	0.38	0.44	0.41	0.42	0.40
III	**Cellulase** (mg glucose/min/mg protein)	0.08	0.09	0.05	0.07	0.03	0.02
IV		0.25	0.22	0.21	0.24	0.08	0.09
III	**Polygalacturonase (PG)** (mg glucose/min/mg protein)	0.14	0.15	0.18	0.16	0.10	0.12
IV		0.22	0.24	0.22	0.20	0.13	0.13
III	**Pectinmethylesterase** (mg glucose/min/mg protein)	0.08	0.08	0.09	0.07	0.10	0.06
IV		0.05	0.06	0.05	0.03	0.08	0.04

Table 17: Enzymes in the fruit of *M. indica* at stage III (unripe) and stage IV (ripe).

HPTLC studies

i) Sugars

Earlier reports attribute mango to comprise major sugars fructose, glucose and sucrose (Chan and Kwok 1975) and trace amounts of mannoheptulose and sedoheptulose (Ogata *et al*. 1972). In the present study estimation was carried out only for the major sugars: glucose, fructose, sucrose and its variation in the 3 different varieties of 2 regions during their unripe and ripe stages.

Preliminary studies on sugars were done by paper chromatography. Sugars detected distinctly were spots of sucrose, glucose, fructose, maltose, xylose, and arabinose. The later two were in trace amount indicated by small faint spots. So the three major sugars (sucrose, glucose and fructose) were considered for further study. Different composition of mobile phase was tested and the desired separation with reproducible peak was achieved by using mobile phase. The spot for glucose, sucrose and fructose in sample was confirmed by comparing the R_f and spectra of the spot with that of the standard. The peak purity of these 3 sugars was assessed by comparing the spectra at three different levels.

Stage III: Sugar pattern in different varieties showed a great variation. Glucose ranged from 0.3 to 2.8 at unripe stage including both Junagadh and Navsari region. When compared region wise Junagadh Kesar had more amount (0.8 w/w%) than Navsari Kesar (0.4 w/w%). Navsari Alphonso (2.1 w/w %) had more glucose than Navsari Alphonso (2.8 w/w %) and Junagadh and Navsari Rajapuri were almost in same amounts i.e. (0.3 and 0.4 w/w% respectively, figure 21A). Among the three varieties Navsari Alphonso had exceptionally maximum concentration of glucose (table 18). Sucrose was found in range of 0.5 to 5.1 w/w%. Region wise comparison showed that sucrose was more in Navsari Kesar and Rajapuri with an exception of

Junagadh Alphonso, where it was more than Navsari Alphonso. Junagadh and Navsari Rajapuri had remarkably more amount of sucrose than other two varieties (figure 21A). Fructose ranged from 0.34 to 2.8 w/w%, where it was maximum in Navsari Rajapuri and minimum in Junagadh Alphonso. Fructose was seen in more amounts in all varieties belonging to Navsari region (table 18).

Stage IV: This stage was the ripening fruit stage where more conversion of sugars took place. Glucose was in a range between 2.4 to 10.1 w/w%, with a maximum amount in Navsari Alphonso (figure 21B). Glucose was found more in Navsari region in Alphonso and Rajapuri, while in Kesar it was more in Junagadh region. Junagadh Alphonso and Rajapuri had almost same amount (3.3 and 3.4 w/w% respectively) of glucose (table 18). Sucrose ranged from 3.2 to 9.5 w/w%. Junagadh Alphonso and Navsari Rajapuri had more glucose 9.5 and 9.1 w/w% respectively (table 18), while in Junagadh (3.3w/w %) and Navsari Kesar (3.2w/w %) it was comparatively less. Fructose was maximum 10.2 w/w% in Junagadh Rajapuri and minimum 3.0 w/w% in Navsari Alphonso (figure 21B). It increased several folds in ripe stage when compared with unripe stage (figure 21A, B). Contradicting to stage III, it was found that fructose was more in Junagadh region with the only exception of Navsari Kesar (figure 21B).

In Junagadh Kesar stage III, glucose was 0.8 w/w%, which increased three times at stage IV, 2.8w/w% (table 18). Navsari Kesar also showed similar result, stage III showed 0.4w/w% which increased 6 times showing the level to be 2.4 w/w% at stage IV (figure 21 A, B). Sucrose also showed same pattern in increasing from 1.2 (stage III) to 3.9 (stage IV) in Junagadh Kesar while in Navsari Kesar sucrose increased from 2.8 (stage III) to 3.2 (stage IV) w/w%. Fructose showed increase from 0.47 to

4.1 w/w% in Junagadh Kesar while in Navsari Kesar 0.7 to7.4w/w% (table 18). In Junagadh Alphonso glucose, at stage III was 2.1w/w% which increase to 3.3w/w% in stage IV while Navsari Alphonso was 2.8 w/w% at stage III and 10.1 w/w% at stage IV. Sucrose was 1.04 w/w% increased to 9.5 w/w% in Junagadh Alphonso which was about nine times more at stage IV but in Navsari Alphonso it increased from 0.5w/w% to 5 w/w% in stage IV. Fructose was 0.34 in stage III which raised to 4 at stage IV in Junagadh Alphonso. In Navsari Alphonso it showed a rise from 0.4 (stage III) to 3 (stage IV) w/w% of fructose (figure 21A, B). Junagadh Rajapuri had 0.3 w/w% glucose at stage III which rises to 3.4 stage IV. In Navsari Rajapuri it raised from 0.4 (stage III) to 3.9 (stage IV) w/w%. Sucrose was 3.5 w/w% in stage III which increased to 4.6w/w% at stage IV in Junagadh Rajapuri. In Navsari Rajapuri it was more as compared to Junagadh, 5.1 w/w% at stage III which showed raised level (9.1w/w %) in stage IV. Fructose was found 2.1 w/w% at stage III which rose to 10.2 w/w%, almost five times more at stage IV in Junagadh Rajapuri (figure 21A, B). While in Navsari Rajapuri it was 2.8 w/w% at stage III which rose to 3.4 w/w% at stage IV (table 18).

Among all three studied sugars, Navsari Kesar had more of fructose which was 7.4 w/w% at stage IV while in Junagadh Kesar it was 4.1 w/w% (figure 21). Maximum rise was also seen in fructose where it rose from 0.47 to 1.1 w/w% in Junagadh Kesar and 0.7 to 7.4 w/w% in Navsari Kesar. Minimum amount was of glucose (0.4 w/w %) in Navsari region (figure 21 A, B). Sucrose was more (2.8w/w %) at stage III in Navsari region but at stage IV, it was more (3.9w/w %) in Junagadh region.

Comparing Alphonso for sugars, glucose was highest (10.1 w/w %) at stage IV which is followed by sucrose (9.5 w/w %) at stage IV of Junagadh region. Fructose was more in Junagadh region (4 w/w %). Rajapuri had maximum amount of fructose (10.2 w/w %) at stage IV in Junagadh region followed by sucrose (9.1 w/w %) at stage IV in Navsari region. Glucose was more in Navsari region (3.9 w/w %) while in Junagadh it was 3.4 w/w% (figure 21 A, B).

Stage IV showed more amount of sugars as compared to stage III in both regions of all three varieties. As fruit ripens, the conversion of sugar from starch occurs by starch hydrolysis, which leads to sweetness in fruit at stage IV, therefore compared to stage III, more amount of sugars are found in stage IV. Navsari Alphonso showed maximum amount of glucose and other sugars, sucrose and fructose were found in more amounts in Navsari region. Kesar also showed increase with maximum rise in Fructose in both regions. Glucose and sucrose as well showed a rise from stage III to IV. Rajapuri had maximum fructose in Junagadh region, while other sugars can also be found to increasing (figure 21 A, B).

Amongst all the three sugars percent fructose was found to be maximum in Junagadh Rajapuri. Followed by this sucrose was found to be the second highest (9.1-9.5 %). Sample tracks with standard track for comparing peaks can be seen for all sugars (figure 22 A, B, C). Spectra compared can be seen in figure (figure 23), where the pattern were seen to be matching with the respective sugars, along with the graph for linearity.

	Varieties	Kesar (w/w %)		Alphonso (w/w%)		Rajapuri (w/w%)	
Sugars	**Regions**	**Junagadh**	**Navsari**	**Junagadh**	**Navsari**	**Junagadh**	**Navsari**
Glucose	**Stage III**	0.8	0.4	2.1	2.8	0.3	0.4
	Stage IV	2.8	2.4	3.3	10.1	3.4	3.9
Sucrose	**Stage III**	1.2	2.8	1.04	0.5	3.5	5.1
	Stage IV	3.9	3.2	9.5	5.0	4.6	9.1
Fructose	**Stage III**	0.47	0.7	0.34	0.4	2.1	2.8
	Stage IV	4.1	7.4	4.0	3.0	10.2	3.4

Table 18: Sugars in three varieties of *M.indica* of Navsari and Junagadh region during stage III (unripe) and stage IV (ripe).

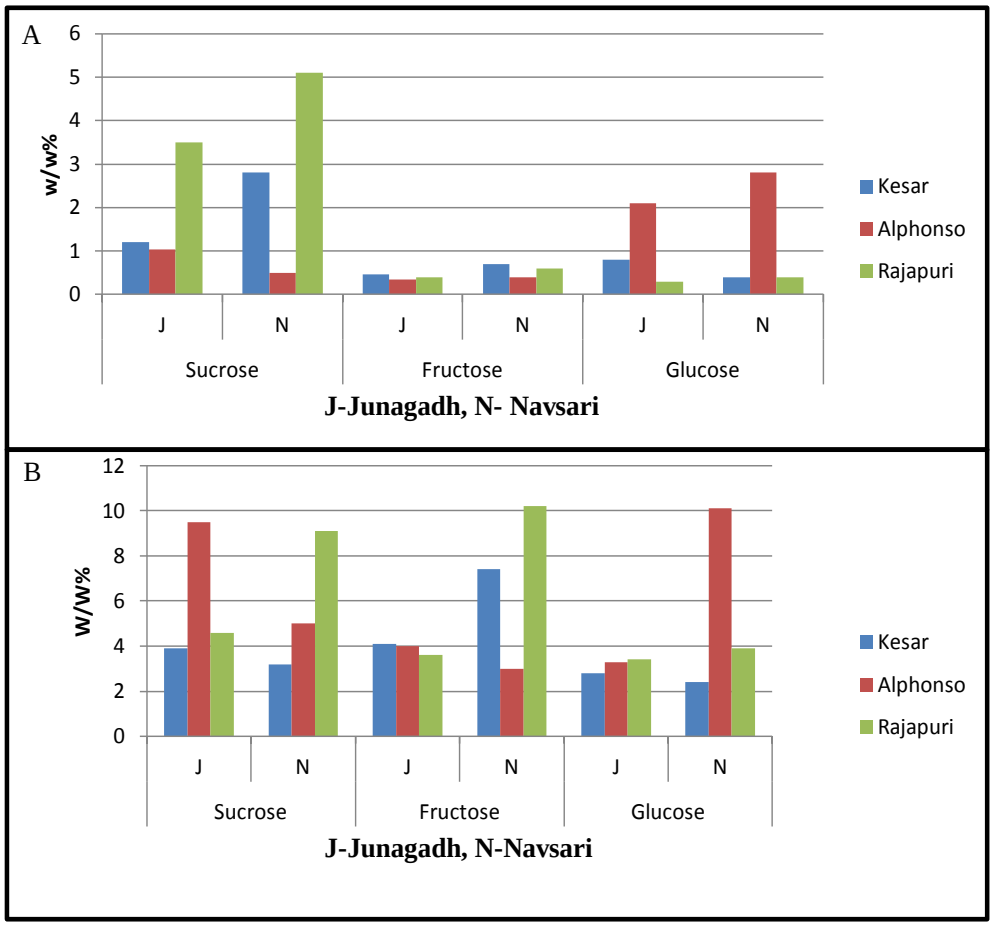


Figure 21: Sugars in three varieties of *M.indica* at (A)stage III & (B) IV in both regions.

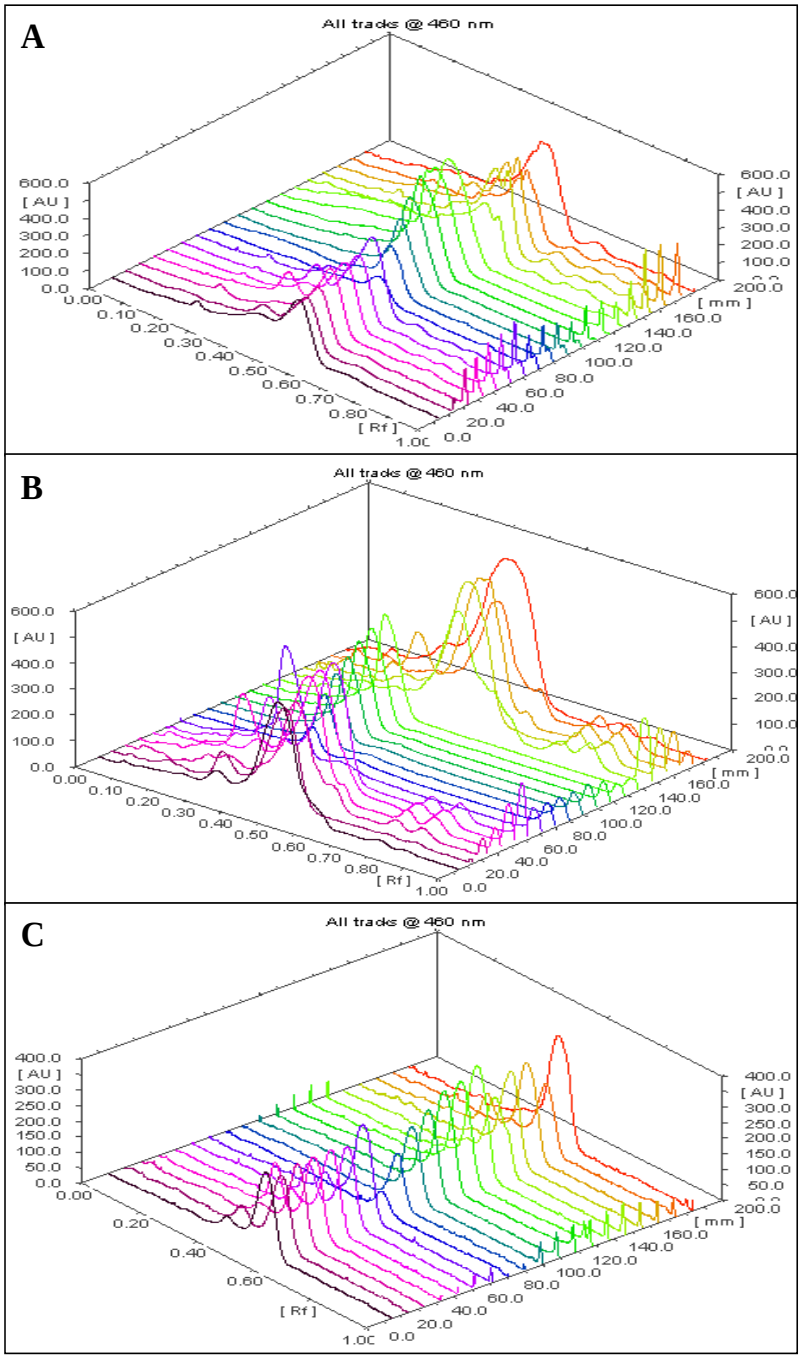


Figure 22: Glucose -3D (A), Sucrose -3D (B) & Fructose-3D (C) overlaid chromatogram of standard tracks and sample tracks at 460 nm.

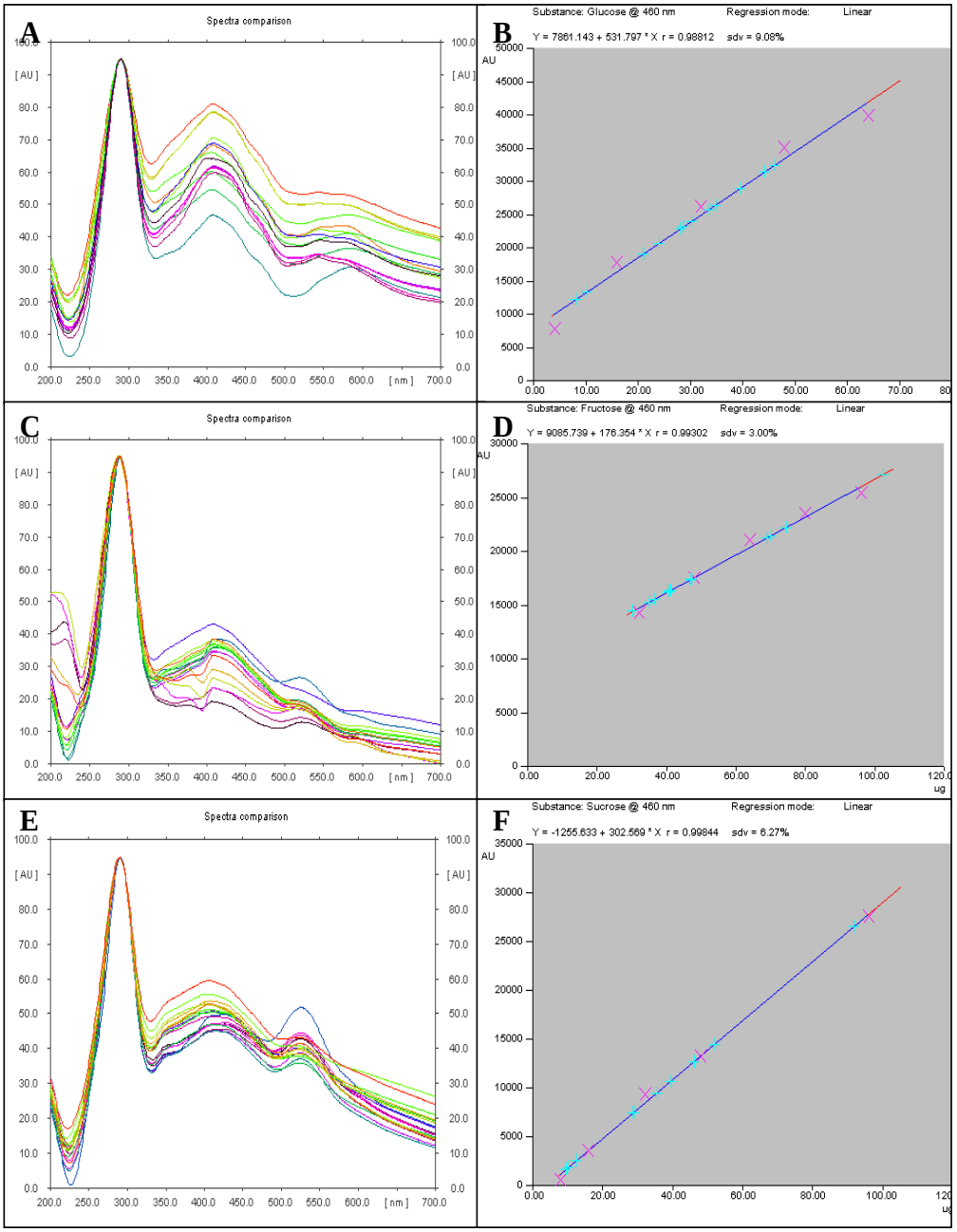


Figure 23: Spectral comparison of Glucose (A), Sucrose (C), Fructose (E) and linearity graph with samples & standards for Glucose (B), Sucrose (D), and Fructose (F).

ii) Amino acids

HPTLC analysis of Amino acids

17 aminoacids were analysed in three varieties namely Kesar, Alphonso and Rajapuri. Arginine, histidine, lysine, methionine, proline were not detected indicating the amino acid to be absent in both the stages or maybe they were below the detection limit. Alanine, asparagine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tyrosine, valine. Fruit pulp was calculated on dry weight basis. Standard tracks along with samples tracks can be seen in figure (24, 25 and 26). Also the spectral analysis done for all 17 amino acids along with linearity graph is represented in figure (27, 28, 29 and 30)

Stage III: Alanine content ranged from 0.002 to 0.008 w/w%. Junagadh Kesar had more alanine than Navsari Kesar while Navsari Alphonso and Rajapuri had more alanine than Junagadh variety. Maximum alanine was seen in Navsari Rajapuri and minimum in Junagadh Alphonso. Asparagine content ranged from 0.009 to 0.17 w/w% with maximum content in Junagadh Kesar followed by Junagadh Alphonso. In all three varieties, Junagadh region has more asparagine content than in Navsari. Aspartic acid was maximum in Navsari Rajapuri while it was not detected in Junagadh and Navsari Alphonso. Junagadh Kesar had more amount of Aspartic acid than in Navsari Kesar. Glutamic acid was maximum in Navsari Kesar and minimum in Junagadh Kesar and Rajapuri and Navsari Rajapuri which had same amount (0.0001 w/w%). Glycine was maximum in Navsari Alphonso followed by Navsari Rajapuri. In both varieties it was more in Navsari region but Junagadh Kesar had more glycine than of Navsari. Minimum glycine was found in Junagadh Alphonso (0.019 w/w %). Isoleucine ranged from 0.0001 to 0.010 w/w%. Maximum Isoleucine was seen in Junagadh Alphonso (0.010 w/w %). Isoleucine content was found more in Junagadh region except in Rajapuri where it was more in Navsari region. Leucine was maximum in Junagadh Kesar and Alphonso while it was minimum in Navsari Alphonso. Phenylalanine was maximum in Junagadh Rajapuri followed by Junagadh Kesar. In Navsari region phenylalanine was less in all the varieties. Serine was

maximum in Navsari Alphonso and Rajapuri while in Navsari region it was found comparatively less. Threonine was constant in all the varieties of both the regions. Tryptophan was more in Junagadh region and was maximum in Junagadh Alphonso. Varieties in Navsari region had less tryptophan with minimum amount in Navsari Alphonso. Tyrosine ranged from 0.005 to 0.057 w/w%. Maximum concentration was found in varieties from Junagadh region (table 19).

Stage IV: Alanine ranged from 0.002 to 0.008 w/w% (table 19) Maximum concentration of alanine was found in Navsari Rajapuri while minimum was found in Junagadh Kesar and Rajapuri. Asparagine ranged from 0.01 to 0.008. Aspartic acid ranged from 0.001 to 0.024 w/w% with maximum amount in Navsari Kesar, while it was below detection limit in Navsari Alphonso and Junagadh Rajapuri. Glutamic acid was comparatively in lower range than other amino acids, ranging from 0.00001 to 0.00925 w/w%. It increased at this stage (table 19). Glycine was maximum in Navsari Rajapuri and minimum in Navsari Alphonso. Isoleucine was highest in Junagadh Rajapuri and less in Navsari Kesar. Leucine was maximum in Junagadh Rajapuri and minimum in Navsari Kesar. Phenylalanine reduced at ripe stage with a maximum amount in Junagadh Rajapuri. Serine was found highest in Navsari Rajapuri but was less in Navsari Alphonso. Threonine was below detection limit in Navsari Alphonso and Rajapuri, while in other varieties it was found in same amount. Tryptophan was below detection limit in Navsari Kesar while it was maximum in Junagadh Rajapuri. Tyrosine was found maximum in Jungadh Rajapuri (table 19).

Alanine decreased at stage IV in Kesar of both regions, while it remains same in Alphonso (Junagadh) but showed rise in content in Alphonso (Navsari). Rajapuri (Navsari) was also with similar content at both stages but Rajapuri (Junagadh) decrease from stage III to stage IV. Asparagine decreased in stage IV in all three varieties. Aspartic acid was below the detection limit in Alphonso, both regions but was detected in Alphonso (Junagadh) at stage IV. Here also a decrease in level can be seen from stage III to IV. Glutamic acid was seen to be increase in Rajapuri (Junagadh) at stage IV. Glutamic acid was comparatively low than other amino acids

except Threonine, where the levels were constant for all the varieties and some were below detection limit also. Glycine increased in all the varieties. Maximum glycine was found in Rajapuri (Junagadh). Kesar and Alphonso showed decreased level of isoleucine in both regions while Rajapuri (Junagadh) had a small rise at stage IV. Like isoleucine, results were similar recording decrease in levels in varieties Kesar and Alphonso. But increased levels were found in Rajapuri (Junagadh). Phenylalanine decreased in all three varieties at stage IV in both regions. Serine increased at stage IV in all three varieties of both regions except for Alphonso (Navsari) where it got decreased. Threonine was almost similar in all the variety. It remained constant. Typtophan increased in Alphonso (Navsari) and Rajapuri while in others it decreased. Tyrosine was also seen to decrease in all varieties, both regions.

Alanine content in Junagadh Alphonso remained the same in both the stages while Navsari Alphonso showed a decrease in % of alanine. The percent reduction from stage III to stage IV was almost half fold. In case of Rajapuri, it was found to be reverse i.e. in Navsari Rajapuri the alanine content did not change in stage III and IV but in Junagadh Rajapuri there was a decline of alanine content when the unripe mangoes turned ripe. Asparagine content was more in stage III. Maximum content was found in Junagadh Kesar at stage III. In Junagadh Kesar it decreased more than three times at stage IV while in Navsari Kesar it was almost same in stage III and IV. Junagadh Alphonso showed decreased levels at stage IV but Navsari Alphonso showed very less difference at stage IV. Junagadh Rajapuri had 10 times more aspragine at stage III than at stage IV and in Navsari Rajapuri it was double the amount in stage III when compared to stage IV. Aspartic acid was not detected in Junagadh Alphonso stage III and Rajapuri stage III, Navsari Alphonso stage III and IV. In Junagadh Kesar it decreased two times at stage IV while in Navsari Kesar, it showed reverse effect where it rose many times from stage III to stage IV. In Junagadh Rajapuri it was not detected at stage IV while in Navsari there was increase in aspartic acid at stage IV.

Glutamic acid was maximum in Junagadh Rajapuri at stage 4 followed by Junagadh Alphonso. Glycine decreased from stage III to stage IV in Navsari Alphonso while in all others it got increase at stage IV. It was found maximum in Navsari Rajapuri stage IV followed by Navsari Rajapuri stage IV. Navsari Alphonso had more glycine content as compared to Junagadh Alphonso. Isoleucine decreased in all the varieties at stage IV in both regions. It was found more in Junagadh Kesar then in Navsari Kesar. In similar manner, it was found that the content was less in Navsari region fruit than to Junagadh. Leucine reduced in all the varieties except in Junagadh Rajapuri where it increased in stage IV. In Junagadh Kesar it was more in amount than in Navsari Kesar. Junagadh Alphonso had more content than of Navsari Alphonso. Same was observed for Rajapuri also. Phenylalanine was not detected in Navsari stage III. Maximum amount was observed in Junagadh Alphonso at stage III. In Kesar, it decreased in stage IV. Junagadh Alphonso decrease at stage IV. Junagadh Rajapuri and Navsari Rajapuri decrease at stage IV. Serine was maximum in Navsari Rajapuri at stage III and IV followed by Navsari Alphonso. In Navsari and Junagadh Kesar it raised form stage III to stage IV. Navasari and Junagadh Alphonso increased in stage IV also in Navsari and Junagadh it increase in stage IV. Threonine was constant in all the varieties and all stages. It was below detection limit in Navsari Alphonso and Navsari Rajapuri. Tryptophan decreased in Junagadh Kesar at stage IV but in Navsari Kesar it was not detected at stage IV. In Alphonso it decreased in Navsari and Junagadh regions at stage IV. Tyrosine also decreases at stage IV in all the varieties. It was found maximum in Junagadh Kesar at stage III followed by Junagadh Alphonso at stage III. It was minimum in Navsari Kesar at stage IV.

Histidine (figure 26A), lysine (figure 26B), methionine (figure 26C), proline (fig ure 26D) and Arginine (figure 26E) could not be detected. Probably because it was in a negligible amount in mango fruit and so below detection limit.

Sr. No.	Stage	Varieties	Alanine W/W%	Asparagine W/W%	Aspartic Acid W/W%	Glutamic Acid W/W%	Glycine W/W%	Isoleucine W/W%	Leucine W/W%	Phenylalanine W/W%	Serine W/W%	Threonine W/W%	Tryptophan W/W%	Tyrosine W/W%
1	Stage III	Kesar (J)	0.006	0.17	0.005	0.00001	0.041	0.005	0.004	0.0094	0.027	0.0001	0.0068	0.057
2		Kesar(N)	0.005	0.03	0.001	0.0084	0.031	0.002	0.001	0.0045	0.023	0.0001	0.0024	0.020
3	Stage IV	Kesar (J)	0.002	0.02	0.002	0.000019	0.190	0.001	0.0009	0.0028	0.175	0.0001	0.0013	0.014
4		Kesar N)	0.004	0.02	0.024	0.00001	0.199	0.0006	0.0005	0.0015	0.180	0.0001	---	0.004
5	Stage III	Alphonso (J)	0.004	0.10	---	0.00728	0.019	0.010	0.0045	0.0134	0.012	0.0001	0.0159	0.050
6		Alphonso (N)	0.007	0.009	---	0.0034	0.281	0.003	0.0002	---	0.200	0.0001	0.0002	0.005
7	Stage IV	Alphonso (J)	0.004	0.006	0.003	0.00001	0.173	0.002	0.0014	0.0042	0.153	0.0001	0.0025	0.021
8		Alphonso (N)	0.003	0.008	---	0.00001	0.148	0.001	0.0009	0.0022	0.122	---	0.0016	0.015
9	Stage III	Rajapuri (J)	0.006	0.10	0.002	0.00001	0.068	0.0001	0.0037	0.0096	0.052	0.0001	0.0052	0.063
10		Rajapuri (N)	0.008	0.06	0.0008	0.00001	0.240	0.0027	0.0031	0.0066	0.204	0.0001	0.0035	0.009
11	Stage IV	Rajapuri (J)	0.002	0.01	---	0.00925	0.211	0.003	0.0041	0.0090	0.192	0.0001	0.0069	0.033
12		Rajapuri (N)	0.008	0.03	0.001	0.00001	0.260	0.0024	0.0018	0.0049	0.206	----	0.0037	0.0060

Table 19: Amino acids in *M.indica* from two different regions (J- Junagadh, N-Navsari).

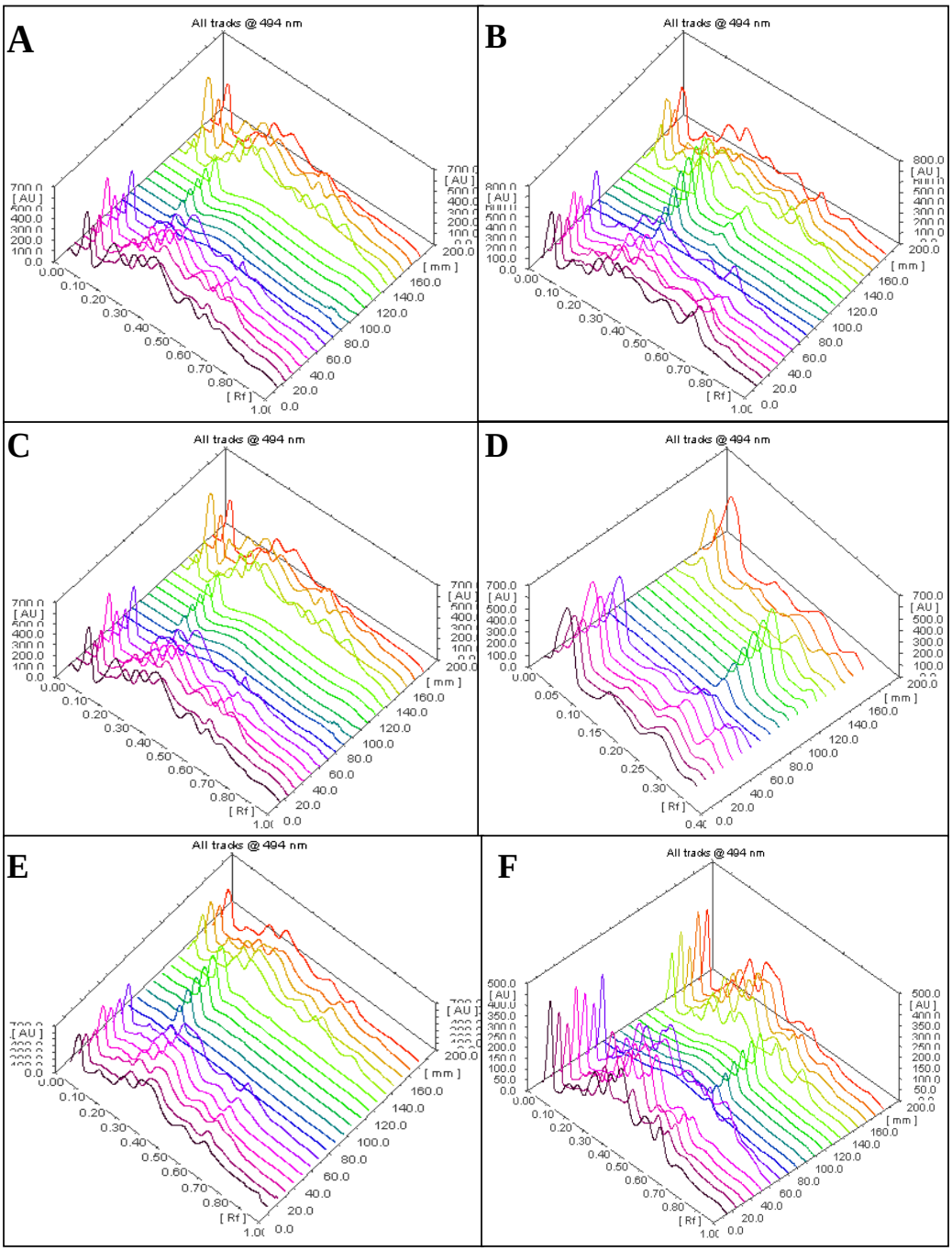


Figure 24: 3D overlaid chromatogram of standard tracks and sample tracks at 494 nm detecting different amino acid. Alanine(A), Asparagine (B), Aspartic aicd (C), Glutamic acid (D), Glycine (E), Isoleucine (F).

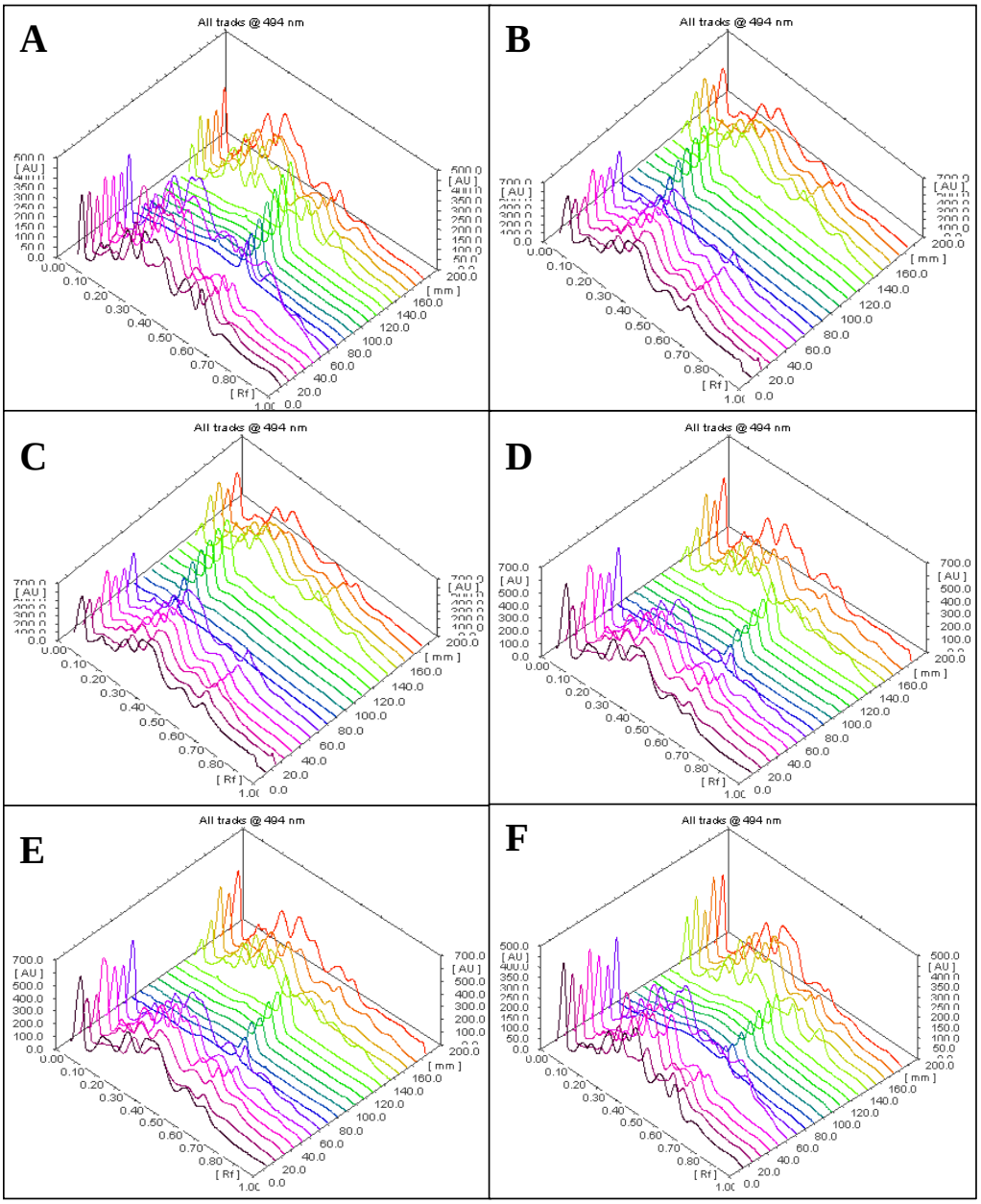


Figure: 25: 3D overlaid chromatogram of standard tracks and sample tracks at 404 nm detecting different amino acid. Leucine(A), Serine (B), Threonine (C), Tryptophan (D), Tyrosine (E), Phenylalanine(F).

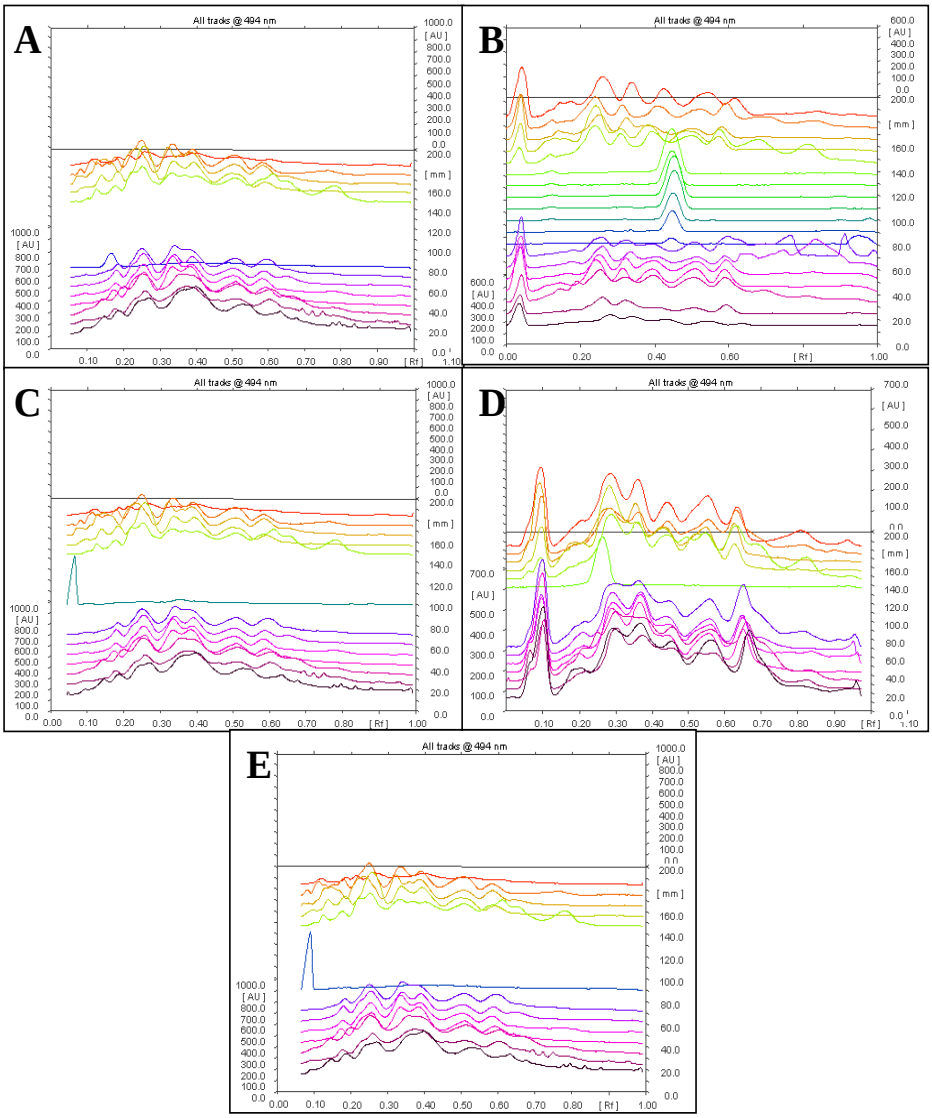


Figure 26: 3D overlaid chromatogram of standard tracks and sample tracks at 494 nm detecting different amino acid. Histidine (A), Lysine (B), Methionine (C), Proline (D), Arginine (E).

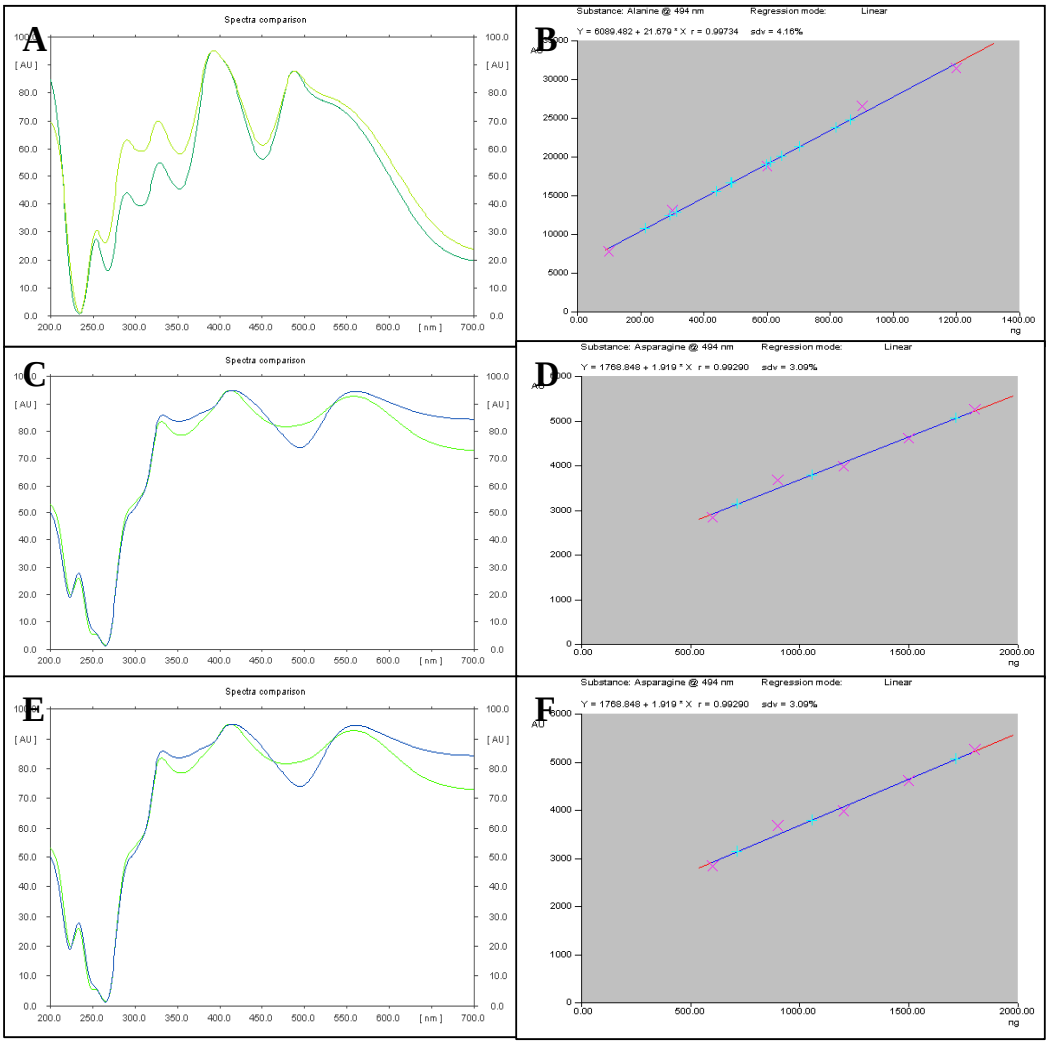


Figure 27: Spectral comparison of Alanine (A), Asparagine (C), Aspartic acid (E) and linearity graph with samples & standards for Alanine (B), Asparagine (D), and Aspartic acid (F).

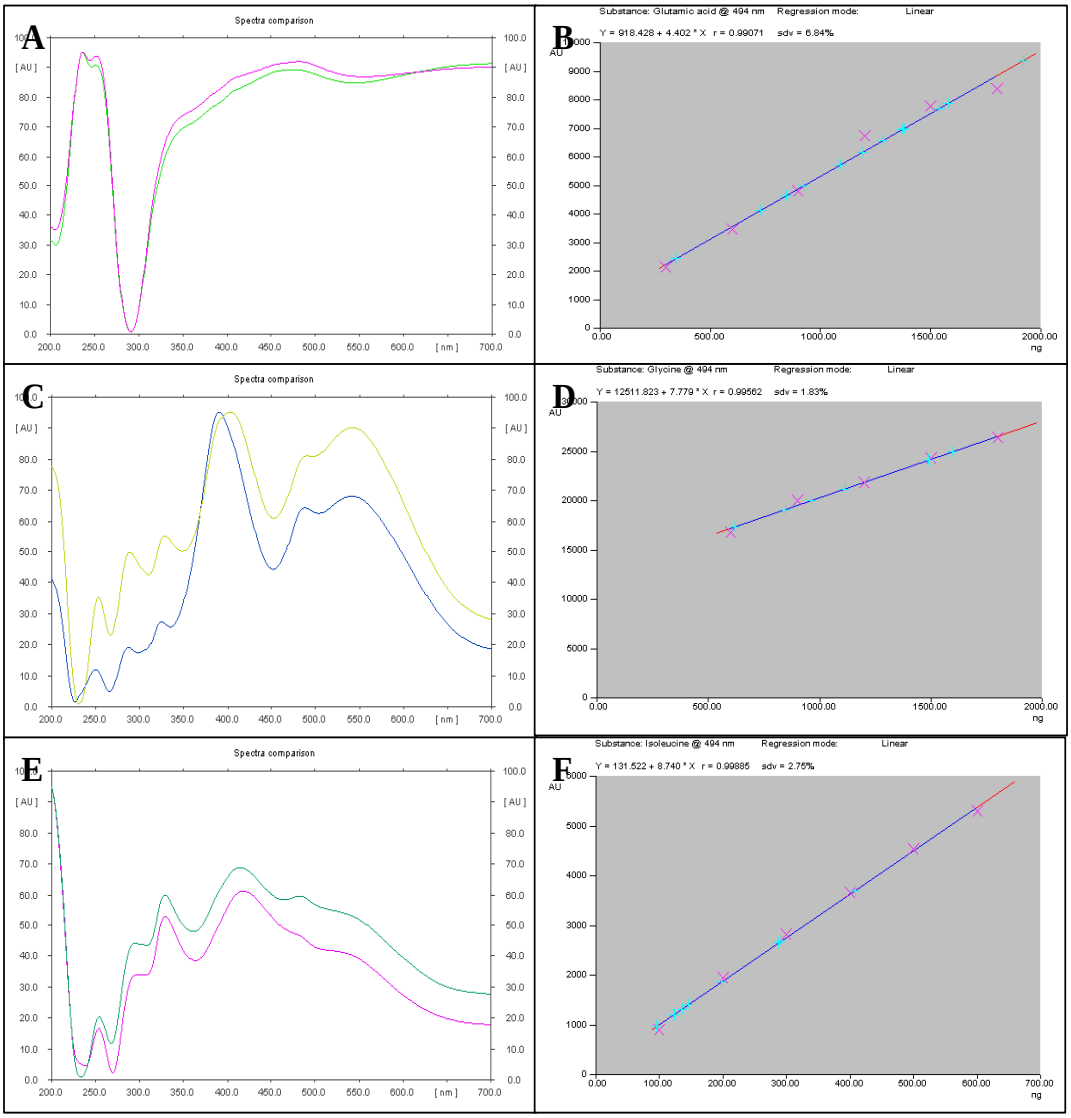


Figure 28: Spectral comparison of Glutamic acid (A), Glycine (C), Isoleucine (E) and linearity graph with samples & standards for Glutamic acid (B), Glycine (D), and Isoleucine(F).

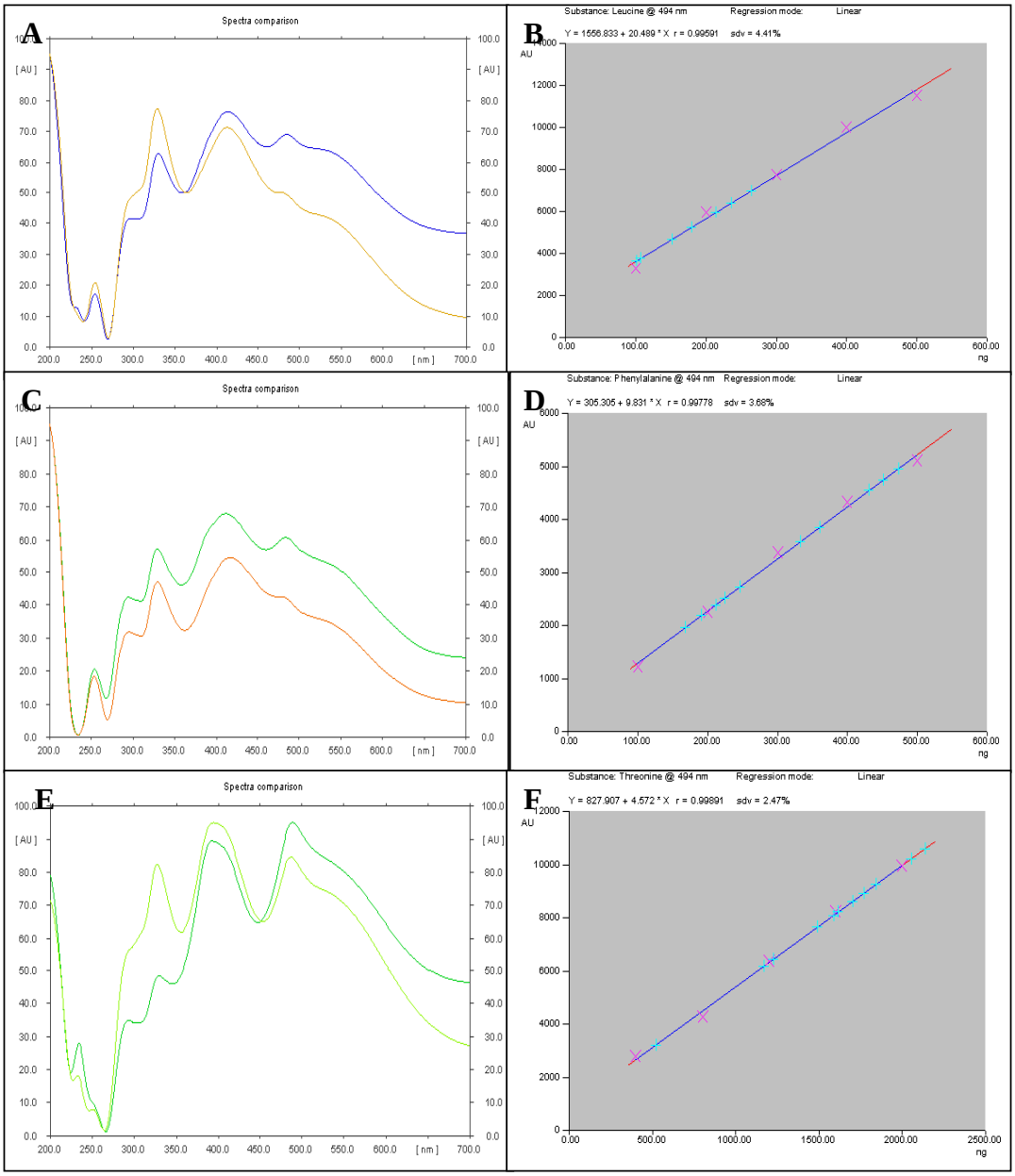


Figure 29: Spectral comparison of Leucine (A), Phenylalanine (C), Serine (E) and linearity graph with samples & standards for Leuicne (B), Phenylalanine (D), and Serine (F).

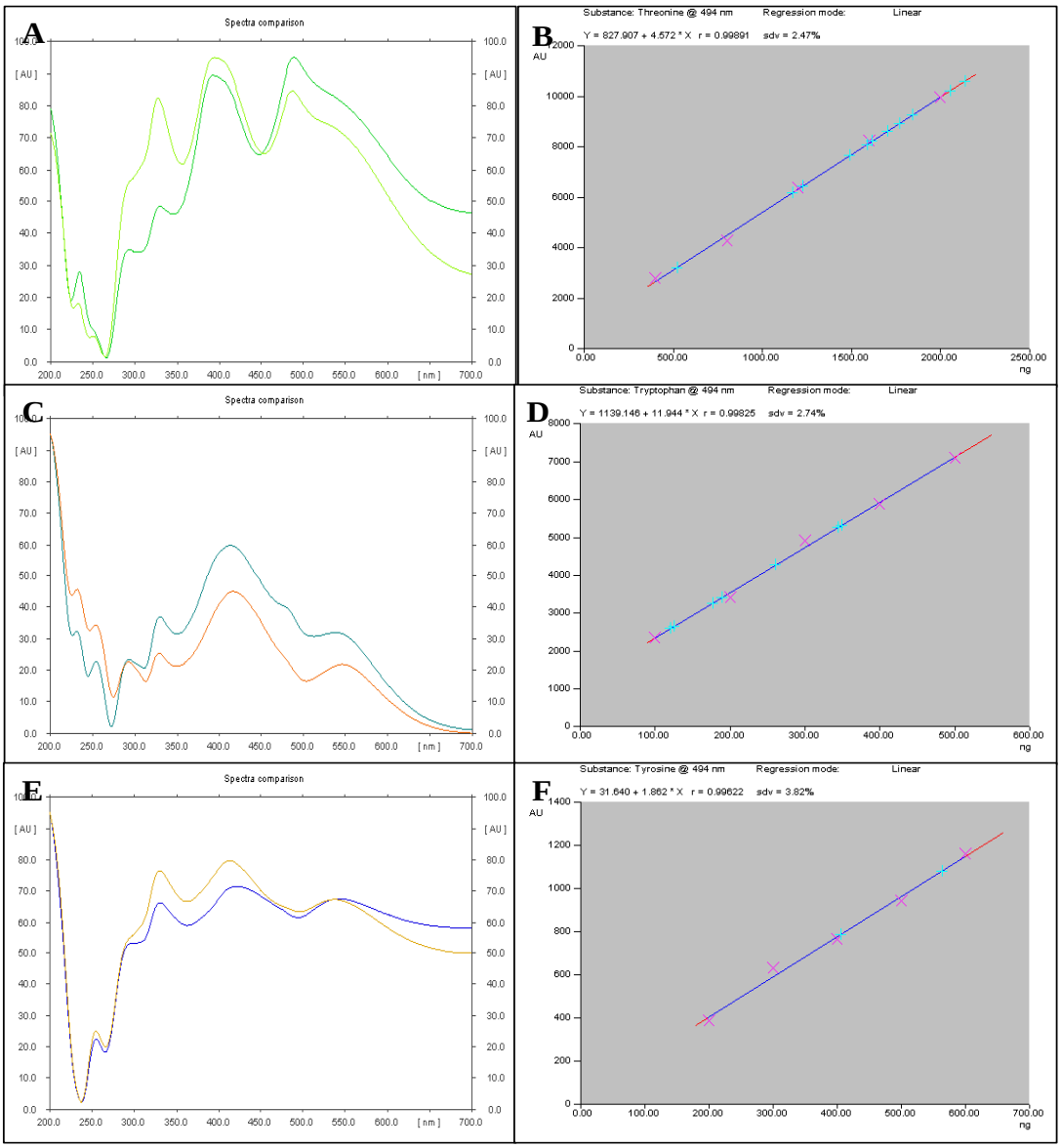


Figure 30: Spectral comparison of Threonine(A), Tryptophan(C), Tyrosine (E) and linearity graph with samples & standards for Threonine (B), Tryptophan (D), and Tyrosine (F).

HPTLC analysis of phenolic acids:

In order to identify whether phenolic acids were present in the two different stages of fruit development of the 3 varieties collected from the 2 different regions Junagadh and Navsari, HPTLC analysis of phenolic acids was done. Different compositions of mobile phase were tested and the desired separation of different phenolic compounds with symmetrical and reproducible peak was achieved by using the mobile phase of acetone: chloroform: n-butanol: glacial acetic acid: water (60:40:40:40:35, v/v/v/v/v). After post chromatographic derivatization with FBS, 4 major phenolic compounds were detected (figure 31, 32). The details are as follows; Compound I (R_f 0.75±0.02); compound II (R_f 0.64±0.02); compound III (R_f 0.36±0.02) and compound IV (R_f 0.21±0.02). Compound I was detected in third stage of Kesar (Junagadh) and fourth stage of Rajapuri(Junagadh and Navsari), Kesar(Junagadh) and Alphonso (Navsari) variety of *M. indica* (table 20). Third stage of Alphonso (Navsari), fourth stage of Rajapuri (Junagadh and Navsari) and Alphonso (Navsari) varieties showed the presence of compound II (table 20). All the varieties studied showed the presence of compound III and IV (table 20). The compounds (I, II, III and IV) detected in different varieties were confirmed as same compounds by R_f value and spectral analysis (figure 33 A, B, C, D, E, F, G ,H).

Peak area is directly proportional to the quantity of the compound; hence the mean peak area of the compound was used to find the variation in the quantity of these detected phenolic constituents among different varieties of *M. indica*.

Stage 3: Compound I was not detected in Navsari Alphonso (figure 31) while in Navsari Kesar it was having significant amount (table 20). Compound II was not detected in Navsari Kesar while it was present in Navsari Alphonso (figure 31). Compound III was found in both Navsari Alphonso and Kesar, but a slight higher

amount was found in Navsari Alphonso. Compound IV was found maximum in Navsari Kesar (table 20).

Stage 4: Compound I was found maximum in Navsari Alphonso and Junagadh Rajapuri while least amount was present in Junagadh Kesar (table 20). Compound II was found in higher quantities in Navsari Alphonso while it was not detected in Junagadh Kesar. Compound III was in similar amount as in stage III, where Junagadh Rajapuri had higher amount of phenolic acid. Compound IV was in higher amount in Junagadh and Navsari Rajapuri (table 20).

Comparatively fourth stage of Navsari Alphonso variety showed the least quantity of phenolic compounds. Significant higher quantities of phenolic compounds were found in the fourth stage of Navsari Rajapuri. All the other varieties stage III, Navsari Alphonso and Kesar and, stage IV Rajapuri and Kesar (Junagadh) showed moderate presence of these phenolic compounds. The variety Alphonso (Navsari) at stage 3 showed absence of compound I. Similarly, Kesar (Navsari) at stage III and Junagadh Kesar at stage IV also showed the absence of compound II. The absence of compounds in these varieties do not assure the absolute absence, may be the compounds are below the limit of detection at this concentration. StageIV Navsari Rajapuri showed significant quantity of phenolic compound I and II. Compound III and IV predominated in stageIV Junagadh Kesar and stage III, Navsari Alphonso varieties respectively. Of the studied varieties of *M. indica*, relatively Navsari Rajapuri showed higher phenolic content and Navsari Alphonso showed the least quantity of phenolic constituents.

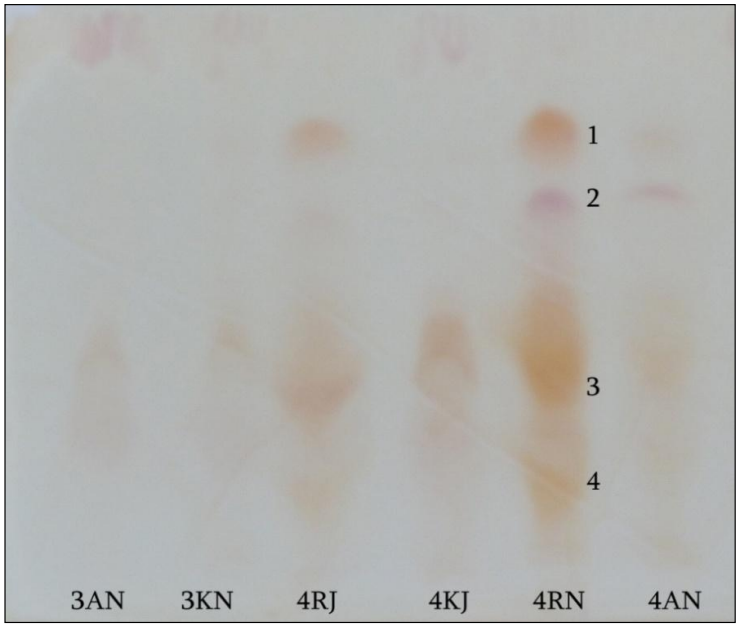


Figure 31: Photograph of the TLC plate showing 4 major phenolic compounds in different varieties of *M. indica*

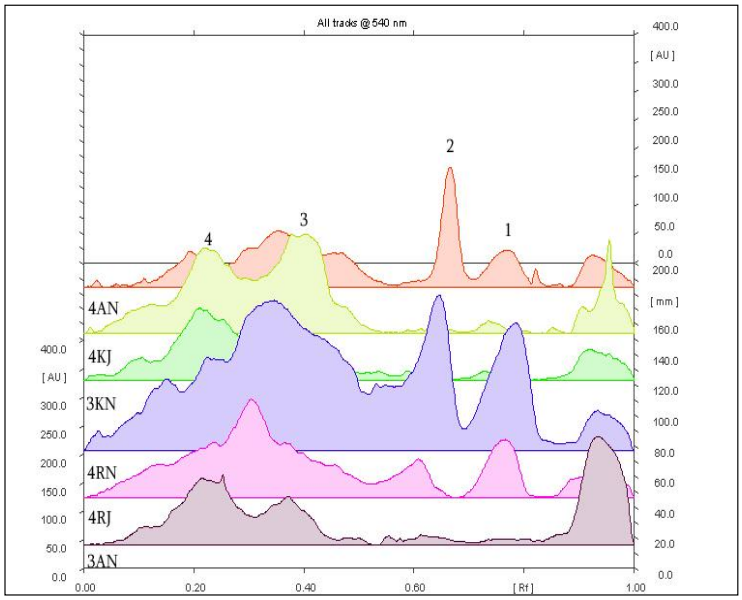


Figure 32: Densitogram of different varieties of *M. indica* showing four major phenolic compounds. 1-compound I; 2-compound II; 3-compound III, and 4-compound IV.

Varieties of *M. indica*	R_f	Peak area c1	R_f	Peak area c2	R_f	Peak area c3	R_f	Peak area c4
3AN	ND	ND	0.65	247.45±67.67	0.37	2626.1±324	0.21	7195.6±1041.6***
4RJ	0.76	6138.75±684.35***	0.62	2481.1±290.9	0.37	1262.55±1262.55	0.22	1700.75±113.55
4RN	0.77	11539.25±457.85***	0.65	11649.2±2063***	0.35	4049.15±4049.15***	0.22	815.3±85.7
3KN	0.74	695.6±224	ND	ND	0.36	1059.5±163.6	0.23	6040.85±358.75***
4KJ	0.74	573.35±92.05	ND	ND	0.37	7195.95±37.55***	0.21	4485.35±463.15
4AN	0.76	2349.35±328.25	0.66	5107.75±532.1	0.36	1798.95±257.25	0.20	1957.6±100.3

Table 20: Mean peak area from compound I, II, III and IV in different varieties of *M.indica.*

Values are expressed as Mean ± SEM; n=3; ***-p<0.001; ND-not detected.
(c1-compound I, c2- compound II, c3- compound III, c4- compound IV)
(3AN- Navsari Alphonso stage III, 4RJ- Junagadh Rajapuri stage IV, 4RN- Navsari Rajapuri stage IV, 3KN- Navsari Kesar stage III, 4KJ- Junagadh Kesar stage IV, 4AN- Navsari Alphonso stage IV)

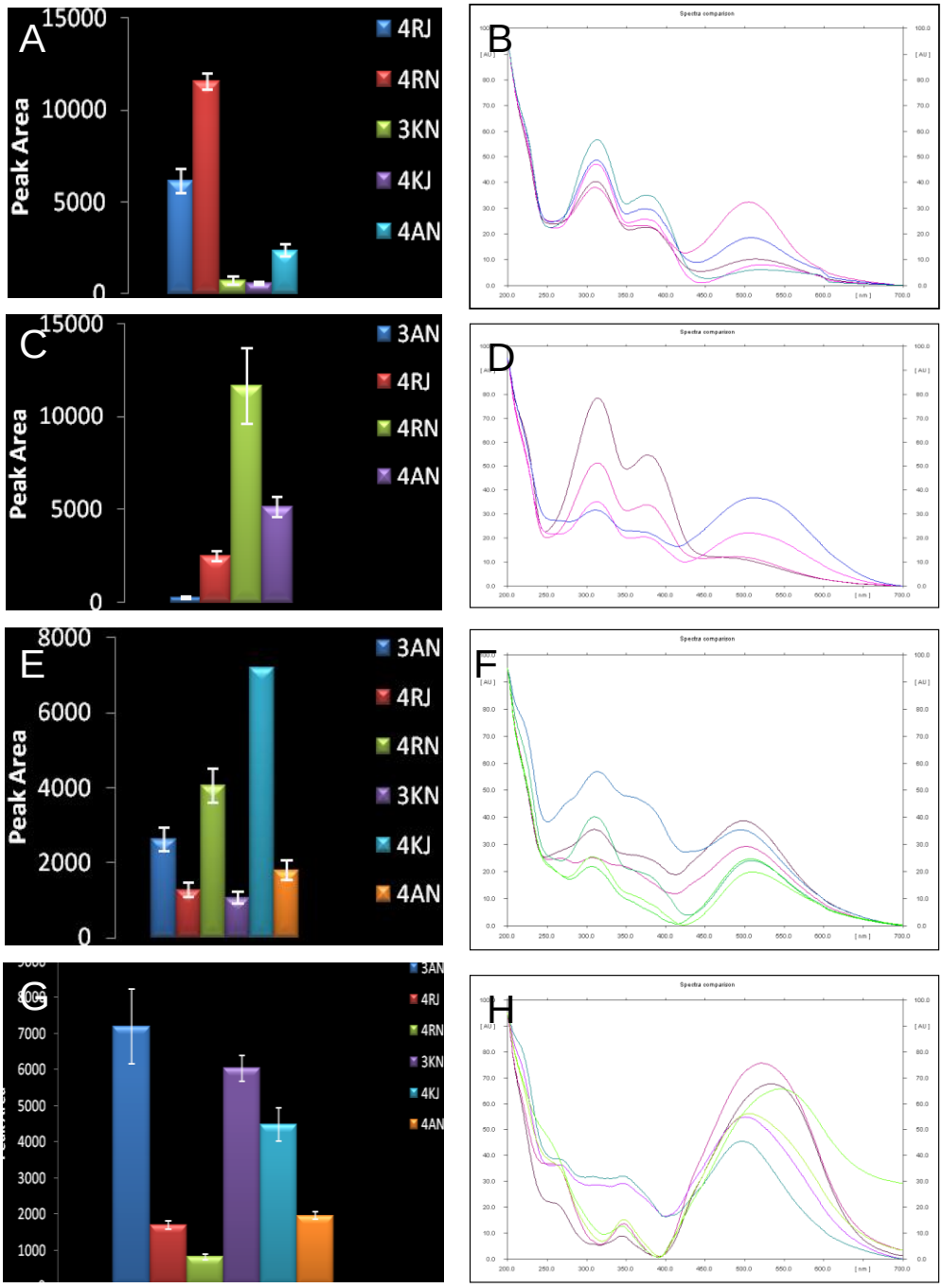


Figure 33: Comparison of mean peak area & Spectral comparison in 4RJ, 4RN, 3KN, 4KJ and 4AN varieties of *M. indica.* for compound I (A & B), compound II (C & D), compound III (E & F), compound IV (G & H) in different varieties of *M. indica*

DISCUSSION

Studies reported earlier (Watt and Merill 1963, Stafford 1984) precisely mentions mango to be an excellent source of vitamin A and C. Mango ranks equal or superior to Orange and Apricot which are generally considered to be above average in nutritional qualities. Comparison of sugar, starch, protein, total acidity of some fruits along with mango is presented in table 21.

Fruit	**Sugar**	**Starch (g)**	**Protein (g)**	**Total acidity**
Apple	5.7-16.1	0.3-0.4	0.-0.4	2.1-13.7
Cherry	9.5-11.9	0	1.2-1.3	2-10.3
Grape	11.5-17.6	0	0.4-1.4	3.5-9
Banana	15.1-22.4	3	1.1-2.7	2.9-9.1
Guava	-	-	0.1-1.5	7.7
Mango	0.2-6.9	-	0.43-1.2	1.3-6.9

Table 21: Comparison of sugar, starch, protein total acidity in different fruits.

(Adel and Barrett 2005)

The process of ripening involves the onset of a number of metabolic processes in which new proteins are synthesized and many enzymatic activities have started like rise in respiration (Calmers and Rowan 1971), malic enzyme (Frylinck *et al*. 1987), polygalacturonase activity (Hobson 1965) and ethylene production (Pratt *et al*. 1969). Regulation of these activities in order to slow down the ripening process with the help of chemicals or through environmental manipulation is studied by many workers. Malic enzyme which catalyzed the oxidative decarboxylation of L-malic to pyruvic acid occurs in the three-quarter-ripe and ripe stages and the activity pattern during ripening is similar in Alphonso, Dasheri, Fazli, Langra and Suvarnarekha (Selvaraj and Kumar 1994). The activity of malic enzyme increases during

ripening, reaching a maximum immediately after the climacteric peak, and then declines (Dubery *et al*. 1984).

The growth pattern of the mango appears to take the form of a simple, rather than double, sigmoid curve (Lakshminarayana 1970). During growth and maturation of mango, starch accumulation is the main chemical change in the pulp tissue (Leley *et al*. 1943, Quintana *et al*. 1984). In developing mango fruits, acidity increased at early growth phase, reached a peak and then declined gradually until harvest (Wardlaw and Leonard 1936). Present study had more amount of titrable acidity at stage 3 which is the unripe stage while it got decreased in stage 4, where the fruit was ripe.

Tandon and Kalra (1984) found that water soluble pectin showed a steep rise after 70 days, reaching a maximum at 101 days of mango fruit growth. Table 16 shows a decrease in titratble acidity as ripening progresses. This decline is most evident when fruits change from stage III to stage IV. Organic acids are important in relation to the fruits flavor and that influence perception of sweetness and the acidity reduction plays an important role in acid sugar balance. Acidity loss was shown by decreasing titrable acidity and increasing pH values. pH values were seen to be increasing in all varieties from unripe to ripe stages of fruit in present study. A large decrease in citric acid and a small reduction in malic acid were responsible for the loss of acidity. Shafique *et al.* (2006) reported pH of the mangoes ranged from 2.5 to 3.5, 2.7 to 4.2 and 4.2 to 5.4 for immature, mature and ripe mangoes respectively. Acidity of mangoes decreases with maturity due to the breakdown of starch into more sugars thereby lowering down the percentage of acidity of the fruits (Tandon and Kalra 1986). In the present study in all the three varieties of mango collected from the two different regions % acidity is found to reduce when the unripe mangoes become ripe. Starch content increased with advancement of maturity. The gradual decrease in acid content may be due to conversion of acids into sugars by some physiological and biological changes in the fruits.

The present findings agree with the results as reported by Robbani *et al.* (1996) and Shafique *et al.* (2006). In the present study, all the three varieties studied from two different regions, the total sugars increases with the advancement of unripe mango into ripe mango.Physiologically mature mangoes contain significant levels of organic acids but during ripening most of these are lost. The organic acids in the ripe Alphonso mangoes were citric (61%), malic (24%), succinic (10%) and uronic acid (5%).The rate of starch accumulation was rapid at the beginning of fruit growth and slowed down later but it continued to increase up to maturity.

Proteins are omnipresent in the living organisms with each cell containing a few hundred to many thousands of them. The structural proteins contribute to the form and stability of the cell and organisms, and the enzymes are responsible for the metabolism within. Mango fruit contains 0.5-1.0% protein on a fresh weight basis (Lakshminarayana 1980). In the present study, protein was found to increase in all ripe (stageIV) variety of both regions (table 16). It ranged from 0.59 -0.89 100/g in stage III while in stage IV it ranged from 0.71 to 1.0 100/g. In case of Dashehari mango variety, a decrease in the soluble protein content was observed up to 44 days after fruit set, which increase again until 96 days (Tandon and Kalra 1983). A Peruvian variety has a remarkably high content ranging from 1.57-5.42% of protein (Jain 1961). The skin of Java grown fruit contains 1-2% protein and the pulp 0.6-1%. Quantitative changes in soluble protein during fruit ripening have been repeatedly demonstrated (Matoo and Modi 1969). Abu-Goukh and Abu-Sarra (1993) reported that the total protein in pulp and peel of three mango cultivars increased up to the full ripe stage and then decrease at the over ripe stage. Pandey *et al*. (1974) detected 12 amino acids during fruit growth. At peak stage, only alanine, arginine, glycine, serine, and leucine- isoleucine were detected, while others were present in traces. At maturity their levels were predominant which decreased during ripening. In present study, HPTLC anaylsis was done for amino acid estimation. Amino acids arginine, histidine, lysine, methionine, proline were found in trace amount and so were

not detected. Others were alanine, asparagine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, serine, thronine, trptophan and tyrosine which were analysed for unripe and ripe mangoes. Amino acids are critical to life and have many functions in metabolism of secondary metabolites. Proteins are linear, large complex molecules, hetrogenous polymers genetically mandated with 20 different building blocks of all living organisms, which residues linked by covalent peptide bonds (-CO-NH) into the polypeptide chain Protein is essential for normal function, growth and maintenance of body tissues. They are utilized to form various cell structures, of which they are key components and they serve as source of energy.

Selvaraj *et al*. (1989) reported that total lipid in seven commercial cultivars ranged between 0.26 and 0.67% at harvest. The characteristic odor that appeared in the fruits during ripening is due to components of ester and carbonyl types. Some of the phenolic compounds identified in mango are gallic acid, indigallic acid, gallotannin, quercetin, isoquercetin, mangiferin and ellagic acid (El-Ansari *et al*.1969, Rhodes 1980). Probably these phenolic acids are present in the present varieties. Singh *et al*. (2004) reported tannic acid, gallic acid and caffeic acid in six important commercial mango cultivars (Deshi, Langra, Chausa, Mallika, Dashahari and Amrapali). In Compound I, R_f varies from 0.74-0.76, compound II R_f 0.62-0.65, compound III R_f 0.35-0.37, compound IV R_f 0.20-0.23. Comparison with R_f of standards reveals that compound I possibly was astragalin (0.70), compound II quercetin, compound III gallic acid, and compound IV coumarin. Gosh (1960) reported 36 mg of folic acid in 100g of green fruit and Gopalan *et al*. (1989) reported 0.08 mg of thiamin and riboflavin and 0.09 mg of niacin per 100g of ripe mangoes. Crude fiber remains more or less constant (Kalra *et al*. 1995).

Mango takes 6-14 days to ripen under ambient conditions, depending upon the variety and environmental conditions. The mango is a climacteric fruit and its period of ontogeny is characterized by a series of biochemical change initiated by the auto-catalytic production of ethylene and increase in respiration (Rhodes 1980). The principal change that occurred in

mango during ripening was the breakdown of starch to sugars (Kalra and Tandon 1983). The starch that has accumulated in the maturing fruit of mango is rapidly lost during ripening (Selvaraj *et al*.1989, Subramanyam *et al*. 1976) and this loss is evident in the chloroplast where the starch granules becomes progressively smaller as ripening proceEds. Starch granules completely disappear in the ripe fruit (Parikh *et al*. 1990, Medlicott *et al*. 1986), which usually contains negligible levels of starch (Morga *et al*. 1979, Fuchs *et al*. 1980). Starch hydrolysis in the ripening mango has been associated with amylase activity (Fuchs *et al*. 1980). The complete disappearance of starch may be attributed to an upsurge of amylase as ripening is completed. As a consequence of starch hydrolysis, total sugars increase during ripening, with glucose, fructose and sucrose constituting most of the monosaccharides (Selvaraj *et al*. 1989). Total sugar content is also an important parameter which can be used as a measure of quality for most of the fruits. In the present study, total sugar content of Junagadh and Navsari Rajapuri is the minimum compared to Alphonso and Kesar which had almost double concentration of total sugar content. It is well known that Kesar and Alphonso are quality mangoes and have a greater demand than Rajapuri. Because of its lesser total sugar content and more acidity percent which determines the sour taste these mangoes are more preferred for pickles. Just like total sugar content, sugar-acid ratio is also considered a measure of quality of fruit. Quality fruits normally should have lower sugar-acid ratio.

There is a continuous decrease in acidity of fruits during ripening (Krishnamurthy *et al*. 1971, Shashirekha and Patwardhan 1976, Selvaraj *et al*. 1989). The ripening phenomenon is associated with loss of firmness. It appears that pectin polymers became less tightly bound in the cell wall during ripening, and the cell wall loosening involved hydrolysis of galactose containing polysaccharides (Seymour *et al*. 1989). An increase in soluble and a decrease in insoluble proteins were reported during ripening of mango fruits (Tandon and Kalra 1983, Sharaf *et al*. 1989).

Ethylene synthesized in the fruit before the onset of climacteric activates the enzymes and inactivates the inhibitors present in unripe fruits (Matoo and Modi, 1969). It was observed that catalase and peroxides increased several fold in ripening mangoes (Matoo *et al*. 1968, Singh and Chundawat 1991). Amylase activity also reached a peak in fully ripe fruits and declined half life thereafter (Kalra and Tandon 1983).

Signs of ripening start with the changes in color, texture, degree of acidity, and aroma of the fruit that make it palatable. Colour changes in fruits are the result of transition of chloroplast into chormoplast rich in yellow or red carotenoid pigments and increase in water soluble anthocyanins. The reduction in the skin colour represents a change from green to yellow. As seen in climanteric fruits, mangoes harvested at maturity stages complete the ripening processes after harvest, showing profound changes in some attributes such as skin color and firmness. Also in ripening fruits it is very common scenario in which the decrease in acidity is associated with an increase in sugar content. Ripening involves a series of differentiation events. Pectin modifications alone may not be adequate to account for the observed differential softening rates; it may depend upon the decline in starch content.

The soluble sugars of the fruit pulp consisted mainly of glucose, fructose and sucrose (Tandon and Kalra 1983, Pandey *et al*. 1974). Jain (1961) reported the presence of glucose, fructose and maltose, in addition found xylose in ripening mangoes. The total sugar content of mangoes varies between 11.5 and 25% (fresh weight). The major sugars in Haden mangoes were determined by Chan and Kwok (1975) to be 20.6% fructose, 5.3% glucose and 74.1% sucrose. In the three varieties studied from Junagadh and Navsari also the major sugars present were identified as glucose, sucrose and fructose. But the concentration of sugar pattern varied in the three varieties. Glucose was maximum in Junagadh Alphonso while it was less in Navsari Kesar. Sucrose was more in Junagadh Alphonso while less in Navsari Kesar. Fructose was more in Junagadh Rajapuri while comparatively less was found in Navsari Alphonso. Glucose values ranged from 0.3% to 2.8 % in stage III which increased

to 2.4-10.1 %. In Navsari Alphonso the sugar content was maximum (2.8%) in the unripe stage which increased to 10% in a ripe stage. The glucose concentration was almost three fold more than its content in the other two varieties. Navsari Alphonso significantly different in the concentration of glucose from Junagadh region. Mango the king of fruits is considered one of the most favourite delicacies among other fruits. Because of its high glucose content it can be suggested that people who are hyperglycemic should not consume Navsari Alphonso. Concentration of fructose values ranges from 0.34 to 2.8% in the unripe stages of the three varieties which increases to a range between 3-10.2% in ripe stage. There is a marked increase in the fructose content in Junagadh Rajapuri, the percent of sweetness being almost similar to Navsari Alphonso. So it can be suggested that hyperglycemic people consume Junagadh Rajapuri instead of Alphonso. The concentration of sucrose ranges between 0.5-5.0% in unripe stages of the three varieties and increases to 3.2 -9.5% in the ripe stage. Junagadh Alphonso and Navsari Rajapuri shows maximum concentration of sucrose in the stage IV but in stage III, Navsari Rajapuri shows maximum concentration which was the highest value of concentration of sugar in the unripe stages of amongst all the varieties. The effect of cultivar, stage of maturity, postharvest treatments and storage conditions on sugar and acid levels in mango have been studied extensively (Gowda and Ramanjaneya 1994, Tandon and Kalra 1983, Kumar *et al*. 1992).

In the market consumer acceptance of a particular variety is markedly influenced by sweetness to acid balance and because fructose is sweeter than glucose, a fully ripe Rajapuri from Junagadh is a suitable healthy and economically important variety. Sugar acid ratio increased at stage IV in all the varieties. The ratio was minimum in Rajapuri, so the the flavor of this variety is not much appealing while it was comparatively higher in Alphonso and Kesar due to which these two varieties possess excellent flavor and aroma.

Ripe mango contains up to10-20% total sugars on a fresh weight basis, depending on the cultivar and the stages of ripeness. At the beginning of ripening, reducing sugars make up

most of the sugar content, while there are more non-reducing than reducing sugars in completely ripe fruit. Sucrose contributes 57% of the total sugar in ripe Keitt mangoes with fructose and glucose making up 28% and 15% respectively (Medlicott and Thompson 1985). Sucrose content increases during ripening as a result of starch hydrolysis form increased amylase activity (Mattoo and Mod 1969, Tandon and Karla 1983). Maximum catalytic activity of sucrose synthase is constant throughout the ripening period and contributes significantly to sucrose metabolism. The acitivities of neutral and acid invertase were very low in comparison with the other enzymes of sucrose synthesis. Invertase activity increases and later decreases during ripening.

The major textural changes resulting in the softening of the fruits are due to enzyme-mediated alteration in the structure and composition of cell wall, partial or complete solubilization of cell wall polysaccharide (pectins and celluloses, Tucker and Grierson 1987) and hydrolysis of starch and other storage polysaccharides (Selvaraj *et al*. 1989, Fuchs *et al*. 1980). Pectin degrading enzymes such as polygalacturonase, pectate lyase, and pectimethylesterase are implicated in ripening and textural softening of fruits such as tomato, banana and guava (Aina and Oladunjoye 1993, Elzoghlri 1994, Selvaraj and Kumar 1989). The loss of firmness, climacteric rise of respiration and ethylene evolution in ripening fruit was directly correlated with marked increase in cellulase activity (Pesis *et al*. 1978). Cellulase has been implicated in softening process in tomato (Hobson 1981). Cellulase activity was reported in several Indian mango cultivars.

Amylase is responsible for starch degradation and it is found in high amount during ripening. Catalse and invertase does the function of conversion of starch to sugars. Polygalacturonase (PGs) is an enzyme produced in plants which is involved in the ripening process. PGs degrade polygalacturonan present in the cell walls of plants by hydrolysis of the glycosidic bonds that link galacturonic acid residues. Polygalacturonan is a significant carbohydrate component of the pectin network that comprises plant cell walls. The activity of the

endogenous plant PGs work to soften and sweeten fruit during the ripening process. Softening during ripening fruits due to pectic enzymes, such as polygalacturonases is influenced by pectinmethylesterase (PME). PME is widely distributed in all higher plants and its function is related to cell wall extension during cell growth and fruit ripening by de-esterification of pectin to low ester pectin (McMillian and Perombelon 1995). PME can increase firmness in fruits and vegetables by deesterification of pectin and chelation of calcium as crosslinks between carboxyl groups of adjacent chains (Van Buren 1979). An increase in soluble but decrease in insoluble proteins was reported during ripening of mango fruits (Sharaf *et al*. 1989). Fruit ripening is controlled by ethylene, which is autocatalytically synthesized in small concentration prior to initiation of ripening, which triggers changes during ripening. During the process of maturation the fruit receives a regular supply of food material from the plant. When mature, the abscission or cork layer which forms at the stem end stops this inflow. The carbohydrates are degraded and sugars accumulate until the typical brix or sugar: acid ratio natural for the particular variety is established. Also typical flavor and the characteristic colour develop. According to a study conducted by (Yamaki and Kakiuchi 1979) PGs and cellulase activities in Japanese pear were mainly related to softening and β-galactosidases partially degrading the hemicellulase components were related to tissue breakdown at over-ripening.

It was found in the present study that cellulase which is responsible for cell wall degradation increased during ripening stage, at the same time polygalacturonase also increased but the PME activity decreased at ripening stage (table 17). PG in ‘Alphonso’ mango increased upto half-ripe stage and declined thereafter (Selvaraj and Kumar 1989). PG activity was barely detected in unripe fruits (Hobson 1981). While the present study showed PG in range of 0.14 to 0.16, which got increased at ripe stage to 0.20 to 0.24. Similar trend of increase in PG activity was reported in fruits like tomata, banana, papaya, pear, peach, kiwi, nectarine, mango and African mango (Crookes and Grierson 1983, Brownleader *et al*. 1999, Pathak and

Sanwal 1998, Selvaraj and Kumar 1989). Cellulase and amylase showed a steady increase in activity in all the varieties. Cellulase was minimum in Junagadh and Navsari Rajapuri variety, which remained firm on ripening while the other two varieties had more amount of cellulase and became soft on ripening. PME activity was maximum in Junagadh Rajapuri and minimum PME was seen in Navsari Alphonso. Polygalacturonase activity was low in unripe durian aril and increased markedly during ripening (Ketsa and Daengkanit 1999).

During the development of the fruit on the tree up to the climacteric stage, starch accumulation in the pulp is the main activity. According to Simao *et al*. (2008) starch content of unripe mango Keitt is seen to be converted to soluble sugars during the ripening. Also changes in physical aspects of starch degradation were observed. During maturity, the total solids increase until fruit ripens, after which they decrease. Non-reducing and total sugars increase gradually showing a fall near ripening, while reducing sugars remain more or less constant during development. Reducing sugars during ripening are several times higher than the non-reducing sugars (Mann *et al*. 1974).

During ripening, mangoes show a decrease in acidity and an increase in sugars (Tripathi 1980, Morga *et al*. 1979). The predominant acids are citric with lesser amount of succinic and malic and tartaric with small amount of citric (Shashirekha and Patwardan 1976).

Acidity of the fruit increases in the initial stages followed by a gradual decline at ripe stage (table 16). Total acidity varied from 0.13 to 0.71%. Jain *et al*. (1959) reported the presence of oxalic, citric, malic, succinic, pyruvic, adipic, galacturonic, glucuronic and mucic acids, together with two unidentified acids. Stahl (1935) noted the presence of tartaric acid. Citric acid is the major organic acid present in mango fruit. Vitamin C content which is high during the tender green stage decreases rapidly with the growth and development. Crude fibre remains constant.

The ripening process is concerned mainly with alterations in biochemical components already existing in the organ. Fruit ripening is a genetically programmed, highly co-ordinated and

irreversible phenomenon involving a series of physiological, biochemical and organoleptic changes that lead to the development of a soft, edible, ripe fruit with desirable quality. A spectrum of biochemical changes such as, increased respiration cholorophyll degradation, biosynthesis of carotenoids, anthocyanins, essential oils and flavor components, increased activity of cell wall degration enzymes, and a transitory increase in ethylene production are instrumental for these changes involved during fruit ripening (Brady 1987, Rhodes 1980).

The amino acid content of fruit is studied mainly because of the contribution to nutritional value. A quantitative difference in the amino acid content of grapes has been reported by a number of workers (Kliewer 1969, Callander 1974, Castor and Archer 1956). Drawert reported the use of determining amino acids as a measure of maturity of grapes which relates more probably to flavor and aroma of the grape. In the present study the chromatographic separation of amino acids fractions from juices of the three different varieties from Navsari and Junagadh indicated very little qualitative differences.

Glycine was the most abundant amino acid in stage IV of all the three varieties of *M.indica* from both Junagadh and Navsari and Threonine was the most negligible amino acid in all varieties at both stages. In stage III Kesar from Junagadh and Navsari showed maximum concentration of asparagine. In Junagadh Alphonso and Rajapuri also asparagine was found to be in maximum concentration but in Navsari Alphonso and Rajapuri showed concentration of glycine maximum. During ripening form stage III to stage IV the percent content of some amino acid showed decline in some varieties of a region while in other there was an increase. In Junagadh and Navsari Kesar glycine and serine increased in stage IV while all the other amino acids showed a decline in Navsari Alphonso. In Navsari Alphonso an increase in leucine, tryptophan and tyrosine is observed from stage III to IV, while in Junagadh Alphonso these three amino acid content declines from stage III to IV. Rajapuri from Junagadh and Navsari showed an increase of glycine, serine and tryptophan content from stage III to IV. Especially glycine content of Junagadh Rajapuri showed a marked increase.

Aspartic acid and tyrosine content of Navsari Rajapuri increased from stage III to IV while it showed a decline in Junagadh Rajapuri while Isoleucine and leucine increased from stage III to IV in Junagadh Rajapuri while declined in stage III to IV of Navsari Rajapuri. Taken together these results indicate that there is a great variation in the composition of amino acid at the two stages of ripening process. During ripening fruits intensify their flavor and tastes, changes in the composition of different compounds are expected and free amino acids are essential non volatile compounds involved in the overall taste of foods.

In all the chromatograms 11-12 amino acids were obtained and identified as alanine, asparagine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, serine, Threonine, tryptophan and tyrosine. Aspartic acid was found to be totally absent in Junagadh and Navsari Alphonso in stage III on maturity and ripening stage IV. Junagadh Alphonso showed 0.003% of aspartic acid while Navsari Alphonso continued to show an absence of aspartic acid. In Junagadh Rajapuri 0.002% aspartic acid was found present in stage III but stage IV showed complete absence of it. Similarly Navsari Alphonso showed absence of phenylalanine in stage III but showed negligible amount in stage IV. Amino acid Threonine present in stage III of Navsari Rajapuri and Navsari Alphonso (0.0001%) is completely absent in their ripe stage.

Ripe mango slices gave positive catalase reactions and no activity was found in green slices, a trace of catalase activity was found in a homogenate of green mangoes. Invertase activity in berries ranged from 0.145 to 1.771. Hawker (1969) reported the occurrence of invertase activity in grape berries and showed, using labeled sugars, that sucrose is both hydrolysed and synthesized within berries. About 500% increase in PG activity was recorded in tomato compared to about 300 and 250% increase in papaya and banana, respectively. These fruits, incidentally, recorded highest PG activity levels in ripe tissues ranging from about 6 in papaya to about 8 and 10 nkat g^{-1} FW in tomato and banana, respectively. The very rapid-

and slow-softening Beaumont guava and carambola, respectively, had comparable PG levels and registered a small 150% increase compared to their initial activity of about 1.4 nkat g^{-1} FW (Zainon *et al*. 2004).