METABOLITES IN RELATION TO VARIETAL IDENTIFICATION AND RELATIONSHIP IN APPLE

THESIS
SUBMITTED TO THE UNIVERSITY OF JAMMU
FOR AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY

1993

ROHINI DEVI
DEPARTMENT OF BIOSCIENCES
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ROHINI DEVI
DEPARTMENT OF BIOSCIENCES
UNIVERSITY OF JAMMU
JAMMU-180 004
INDIA

Dedicated
to
my brother
Dr. Rajeshwar Chib

Head
Department of Bio-Sciences
University of Jammu
New Campus
Jammu-180004 (India)
Phone : 46330

No. JU / Bio-Sc. / /

Dated... 29-9-1993

Certificate

Certified that :

(i) the thesis entitled, **"Metabolites in relation to varietal identification and relationship in apple"** embodies the work done by **Rohini Devi** under our supervision for the period required under statutes,

(ii) the candidate has put in the attendance in the department for the period required,

(iii) the thesis being submitted for the degree of Ph.D. by **Rohini Devi** has not been submitted for any other degree and is worthy of consideration for the award of Ph.D. degree of the University of Jammu, and

(iv) the conduct of the research scholar remained satisfactory during the period of research.

29/9/93

(Prof.Shashi Kant)
Head of the Deptt.
Professor & Head
Deptt. of Biosciences
University of Jammu
JAMMU.

(Prof.A.K. Koul)
Supervisor

(Prof.M.C. Sharma)
Supervisor

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Rohini

(ROHINI DEVI)

I. INTRODUCTION

Apple is economically the most important rosaceous fruit crop of the temperate regions. There is almost a consensus that its primary centre of origin lies within the region that includes Asia minor, the caucasus, Soviet Central Asia and the Western Himalayas (Wilcox, 1962). Romans are believed to have spread apple throughout Europe including Great Britain wherefrom it found its way to North America and other

parts of the world. It was introduced to India by the early trader settlers from Europe and North America. By the middle of 18th century it became well established in the hilly areas of North India as a commercial fruit crop.

Presently the major apple producing countries of the world are United States of America, Australia, Japan, U.K., Turkey, West Germany and France. The total annual production of this fruit is estimated to be around 20 MT (Bailey, 1963). At present about 6,500 horticultural forms of the fruit are under cultivation all over the world (Wealth of India, Vol. VI).

In India apple cultivation dates back to the middle of 18th century (Wealth of India, Vol. VI). However, it is only recently that significant increase has been achieved in the area under cultivation of this crop in the hilly regions of the North India. Over the years, the crop has become commercially important in temperate parts of Jammu and Kashmir, H.P. and hilly parts of Uttar Pradesh. More than 40,000 acres of land is under cultivation of temperate fruits in the country of which major part is under apple cultivation.

The valley of Kashmir represents the richest

repository of apple germplasm in the country. The varieties that constitute the germplasm are introductions from U.S.A., U.K. and other European countries. During the period of their cultivation in the valley some of these varieties have accumulated variability through gene and chromosome mutations, bud mutations and repeated inter-breeding. Once produced, the altered forms have perpetuated vegetatively, and came to be recognised as new varieties.

Today, the valley hosts 285 known and several unknown varieties (Fougat, 1984; Raina, 1989). Exploration, evaluation and conservation of this valuable germplasm is the need of the hour. A few varieties, such as Ambri, which is considered an aristocrat among apples, is almost at the brink of extinction on account of environmental stress, pests and parasites and popularization of high yielding qualitatively superior varieties. So far, no standard procedures have been adopted to describe and delimit varieties. Varietal identification at places is therefore, faulty and confusing. Sometimes, identification in the field and the market becomes extremely difficult. Many times, inferior varieties are sold under the trade names of superior ones.

In our laboratory, germplasm assessment of apples cultivated in Kashmir valley was initiated in 1980. About 84 varieties have been identified and assessed for foliar, floral and fruit characteristics, floral biology, breeding system, chromosome number and meiotic system (Fougat, 1984; Koul et al., 1984; Singh and Wafai, 1984; Singh et al., 1984, 1985, 1987; Raina, 1989). It emanates from these studies that most of these varieties are diploid, some are triploid and a few tetraploid. By and large, diploid apples have been found better in quality, than the polyploids. During the present investigation 15 popular varieties have been evaluated for several biochemical parameters in order to determine how far the conclusions drawn on the basis of cytological and morphological characters are upheld by these findings.

The objectives identified for the present investigation were :

1. To evaluate the apple varieties for foliar flavonoids and amino acids.
2. To estimate sugar content in the fresh and stored fruits of these varieties.
3. To find how far metabolite constitution can help in working out interrelationships among the varieties assessed.
4. To evaluate the impact of storage on the quantity and quality of sugars within the pomes.

II. REVIEW OF LITERATURE

Cultivated apples were initially placed in genus Pyrus, under the specific name P. malus (Linnaeus, 1735). Seventeen years later, Miller (1752) separated out apples from this genus and assigned them to new genus named Malus Mill. The separation was justified on grounds of morphological characters; in apple carpels are adnate upto the level of locular apex, styles are connate at the base, fruit has marked depressions at

both ends and grit cells are absent from the flesh. Today, the genus has a strength of 25 species including Malus pumila (Chittenden, 1951) which includes cultivated apples.

II.1 Varietal identification :

Duhamel du Monceau (1768) was the first to describe 39 varieties of apple recognized on grounds of flower size and shape, petal dimensions and colour, pose of corolla and relative length of the style and stamens. Since then, many more varieties have been recognized from time to time during the 19th century (Poiteau and Turpin, 1807-1835; Poiteau, 1836-1846; Mas, 1865-1872, 1872-1884; Lauche, 1882-1883). During the present century notable contributions in this direction have been made by Hatton (1919), Bünyard (1927), Pearl (1932, 1933), Taylor (1936), Synge (1949), Smock and Neubert (1950), Bacharach (1973), Decourtye et al. (1974), Goldrie et al. (1974), Way (1975, 1979), Johnson and Atkinson (1976), Ballard (1977), Rapillard (1977), Herregods (1979), Mcalpin (1979), Dadlani et al. (1980), Williams et al. (1981), Lezec and Lespinasse (1982), Cummis et al. (1983) and Guo and Guo (1984).

In India, apples received little attention

from researchers till recently. During the sixties, Thapar (1960, 1967) initiated the work by describing fruit morphology of 10 varieties. Subsequently, Hathwala (1966) described apples of Kulu valley and raised as many as 75 varieties. Similarly, Motiyal (1968) described 15 varieties from Kashmir valley and Srivastava (1975) and Seth et al. (1981) described the varieties under cultivation in Uttar Pradesh. Farooqi and Zutshi (1978) described fruit morphology of 9 varieties growing in Kashmir. Fougat (1984) undertook detailed investigation of the phenotype of trees and the morphology of leaves and fruits of 70 commonly cultivated varieties of apple growing in different parts of Kashmir valley. Same year, Koul et al. (1984) put on record the enormous diversity in foliar, floral and fruit characteristics of varieties from the valley. Singh and Wafai (1984) compared two varieties, namely Hazratbali-I and Hazratbali-II for floral and fruit characteristics. Singh et al. (1985) described six more varieties which are under cultivation in the valley. On the basis of studies on fruit morphology, Randhawa (1987) has described some new introductions to Himachal Pradesh, namely Tydeman's Early Worcestor, Skyline Supreme, Red Delicious, Red Chief and Liberty. Raina (1989) described in detail about 14 varieties from the valley; these represent additions to the previous list.

II.2 Cytology :

Cytological studies of over a thousand varieties of apple have been conducted so far. Significant contributions in this direction are those of Kobel (1926, 1927), Shoemaker (1926), Heilborn (1928, 1935), Crane and Lawrence (1929), Maney and Welter (1929), Nebel (1929a,b,c, 1933), Darlington and Moffett (1930), Sax (1931, 1932, 1933), Meidzyrzecki (1933), Roscoe (1934), Edgecombe (1937), Lincoln and McCann (1937), Hurel-Py (1938), Stankovie (1938), Wanscher (1939), Olden (1945), Einset and Imhofe (1947, 1949), Fukushima (1948), Einset and Lamb (1951), Gagnieu (1955), Batra et al. (1963), Einset and Pratt (1963), Hathwala (1966), Radionenko (1972), Bavtuto (1974), Choudhary and Mehra (1975), Lespinasse et al. (1976), Kalmykov (1978), Pratt et al. (1978), Romanova (1979), Sankina and Kornienko (1980), Fougat (1984), Koul et al. (1984), Singh et al. (1984) and Raina (1989). It emerges from these studies that the base number in apple is 17 and the species exists in diploid (2n = 34), triploid (2n = 3x = 51), tetraploid (2n = 4x = 68) and pentaploid (2n = 5x = 85) cytotypes.

Choudhary and Mehra (1975) recorded presence of B-chromosome in var. Kulu-Kenya and this represents

the first report of the occurrence of supernumerary chromosomes in Malus pumila.

Singh et al. (1984) undertook studies on pmc meiosis in 58 diploid varieties. Raina (1989) extended the work to 12 more. Meiosis is regular in most of the varieties. However, anomalies in the form of precocious or delayed disjunction of some bivalents, secondary associations, heteromorphic bivalents, chromosome lagging and fragmentation, formation of micronuclei and unequal segregation at anaphase have been observed in some varieties. These anomalies have been considered as evidence of the hybrid nature of these varieties.

II.2.1 Polyploidy :

In apple polyploidy ranges from triploidy to pentaploidy. Rybin (1927) was the first to report triploidy in apple. Subsequently, many other triploids were discovered by Kobel (1927), Heilborn (1928, 1935), Sax (1931, 1932), Meidzyrzecki (1933), Roscoe (1934), Caldes (1945), Einset (1945, 1952), Einset and Lamb (1951), Batra et al. (1963), Hathwala (1966), Solov'eva (1973), Ponomarenko (1977), Pratt et al. (1978), Koul et al. (1984), Singh and Wafai (1984) and Singh et al. (1985). The first reliable information on chromosome

pairing in triploid apples was furnished by Nebel (1929a) and Darlington and Moffett (1930). On the basis of chromosome pairing, Moffett (1931a,b) classified triploid apples and other triploid taxa of Pomoideae into two broad categories : (i) forms with complete chromosome pairing, and (ii) forms in which pairing is not complete. Heilborn (1935) observed almost complete pairing in triploid var. Ribston. Trivalent formation was also observed by Swanson and Gardner (1944). Lespinasse (1975) observed 17III, which is the maximum number possible, in a triploid variety cv. Reinette-du-Canada. Hathwala (1966) reported failure of chromosome pairing in cvs. Bennou's and King David, and formation of tri-, bi- and univalents in eight other triploid varieties.

Koul *et al*. (1984) and Singh and Wafai (1984) observed formation of 17III in 10.16% and 4% pmcs of cvs. Rikhal Saharanpuri and Hazratbali-II, both from Kashmir valley. Subsequently, Singh (1985) and Raina (1989) investigated details of pmc meiosis in 6 and 2 triploids, respectively. Collectively, these workers have reported a total of 12 triploid varieties from orchards of Kashmir valley. Tetra and pentaploidy are comparatively rare among cultivated apples. Nebel (1929a,b) reported var. Kola as a tetraploid having

2n = 4x = 68. Subsequently, Lincoln (1940), Einset (1945) and Ponomarenko (1977) recovered several other tetraploid varieties from different parts of the world. Lincoln and McCann (1936) described tetraploids in four species of Malus namely, Malus platycarpa, M. glaucescens, M. glabrata and M. coronaria. Fougat (1984) reported that 'Khatoon Trail', a variety quite popular in Kashmir valley, is a tetraploid. Ponomarenko (1977) recorded a pentaploid cytotype, 2n = 5x = 85, in M. sieboldii.

II.2.2 **Aneuploidy :**

Several earlier workers (Kobel, 1927; Nebel, 1930; Newcomer, 1943) have recorded an array of aneuploid counts, ranging between 38 and 50, in Malus pumila. True aneuploids were reported in seed progenies of several polyploid varieties. The aneuploid seedlings had counts ranging between 34 and 71 (Darlington and Moffett, 1930; Evreinoff, 1931; Moffett, 1931a; Dermen, 1936; Bergstrom, 1938; Einset, 1945; Olden, 1945; Lespinasse, 1975).

The impact of numerical changes in chromosomes vis-a-vis fruit characteristics has been evaluated

(Fougat, 1984; Raina, 1989). The general conclusion is that polyploidy does not confer advantage of size, colour or scent in all cases.

II.3 Analysis of metabolites :

Although, the potentiality of metabolites for use in classifying plants has been realized since long, it was only in the 19th century that attempts were made by chemists to survey and characterize the chemical constituents of plants more precisely. After Darwin's (1859) publication of, "The origin of species", taxonomists started attributing greater significance to the presence of same or similar chemical substances in related plants. Abbots (1886) remarked that, "evolution in plant life is best illustrated by chemical constituents of the plant form". In the later part of 19th century, more and more information accumulated on chemical constitution of plants. Greshoff (1909) coined the term "comparative phytochemistry" to highlight the connection between natural relationships of plants and their chemical composition.

One of the earliest methods used for detecting protein differences among different taxa made use of the

antibodies. This technique helped in the growth and development of the field of serology. A monumental work in this field is the publication of "stammbaum" a family tree of plant relationship (Mez and Zeigenspeck, 1926) based largely on results of serological tests. Significance of comparative chemistry in plant taxonomy was championed for many years by McNair (1935) who highlighted the usefulness of variation in fats, oils and alkaloids in phylogenetic studies. He emphasized that a classification based on chemical profile employed to supplement the classification evolved on morphological characters, is likely to lead to the development of truly phylogenetic classification of angiosperms. This view is supported by modern taxonomists as well. It is now an established fact that chemotaxonomy cannot substitute the existing systems of plant classification but can certainly help to improve them.

The utility of plant metabolites in phylogenetic studies, has been highlighted at all levels of taxonomic hierarchy (Bate-Smith, 1962a,b; Alston and Turner, 1963; Davis and Heywood, 1963; Swain, 1963; Hegnaur, 1964; Desborough and Peloquin, 1966; Boulter et al., 1967; Harborne, 1971a,b; Reddi and Phipps, 1972; Smith, 1976; Yamamoto and Plitmann, 1980). The major

findings in this field have been reviewed in following pages.

II.3.1 Amino acids :

Amino acids form the building block of proteins and are ubiquitous constituents of plant tissue. Discovery of their presence in plant tissues dates back to 1806 (cf. Smith, 1976), the year when asparagine was isolated first. Although protein amino acids were analysed first, but soon it became known that the non-protein amino acids are far more numerous; their number is estimated at two to three hundred (Fowden, 1962). The former type is widely distributed, but the latter show scanty occurrence among plants. The non-protein amino acids as a distinct group can be exploited in chemotaxonomy. They have received maximum attention in comparison to the other type because they are less susceptible to environmental changes.

Importance of amino acid analysis from point of view of systematics dates back to the middle of this century, when Reuter (1957) identified the main amino acids (Hauptamino-sauren) in some 50 families of angiosperms. Subsequently, the extensive studies

conducted by Bell (1962, 1971) on protein amino acids of legumes led to formulation of infra-generic classification of Lathyrus and Vicia.

Seneviratne and Fowden (1968) worked out the amino acid profiles of Acacia and concluded that the seed amino acids of taxa included in section Gummiferea were totally different from those present in other sections. On this criterion alone a species can be assigned to a particular section. On the other hand, Pettigrew and Watson (1975) analysed amino acids from seed globulins of Acacia species and noticed identical pattern among closely related species. Similar results have been obtained by Watson and Creaser (1975) in grains of cereals and leaves of several dicots.

Dunnill and Fowden (1965) have projected the significance of amino acids in elaborating taxonomic and phylogenetic relationships in Cucurbitaceae; the same has been done by Evans et al. (1977) in Acacia species; Boulter et al. (1978) in Compositae; Krishnamurthy and Subramanian (1978) in Leersia and Oryza and Subramanian (1978) in Begonia.

Amino acid profiles have also proved helpful in the identification and delimitation of sub-generic

categories. Various workers, namely Alston and Irwin (1961), Bell and Tirimanna (1963), Bhalla and Dakwale (1978), Mangotra (1981), Bhargava (1983), Gupta (1983), Singh (1984) and Kaur (1985) have employed amino acid profiles for delimiting species of Cassia, Indigofera, Crotalaria, Narcissus, Argemone and Daucus, respectively.

II.3.2 Flavonoids :

Flavonoids constitute the largest group of naturally occurring phenolic compounds. They have assumed considerable importance in taxonomic studies. For purposes of characterization and identification of individual taxa, foliar as well as floral flavonoids have been employed. Classification of plants into herbs and trees is accompanied by difference in their flavonoid pattern (Bate-Smith and Lerner, 1954).

Taxonomic utility of flavonoid spectra has been demonstrated in many plant genera, including Iris (Bate-Smith, 1958; Riley and Bryant, 1961), Lathyrus (Pecket, 1959), Aegilops (Kaltsikes and Dedio, 1970), Corydalis (Fahselt, 1972), Sophora (Markham, 1973), Annona (Dass et al., 1975), Dicentra (Giannasi and Chuang, 1976), Citrus (Dass et al., 1978), Periderida

(Gornall and Bohm, 1980), Plectocomia (Madulid, 1980), Crotalaria (Mangotra, 1981; Gupta, 1983), Narcissus (Bhargava, 1983), Argemone (Singh, 1984) and Daucus (Kaur, 1985). Harborne and his associates (Harborne, 1971; Harborne and Williams, 1972; Harborne and King, 1976) have extensively explored the flavonoids of family Umbelliferae and concluded that the three sub-families, namely Hydrocotyledeae, Saniculoideae and Apiodeae raised on grounds of morphology are also unique in flavonoid patterns. In family Limnanthaceae, Parker and Bohm (1979) have identified three phyletic levels on the basis of flavonoid profile. These results highlight the importance of phenolic compounds in identification and in elaborating phylogenetic relationships at generic, species and varietal levels.

II.3.3 Effect of cold storage on metabolites :

Among the temperate fruits in India, apple is the only one which is stored on large scale to meet the market demands during off season. The fruit comes to market from August to October and at room temperature, cannot be stored for long. In order to extend shelf life it is stored in cold storages at temperatures ranging between 2-4°C. Apart from temperature, some

other physical factors are also controlled for extending the shelf life of this otherwise perishable fruit. During storage, the chemical composition of fruit is altered. The changes are affected by temperature, air/ oxygen supply, humidity and chemical treatment of the fruit before or after harvesting.

Storage has been shown to affect the fruit weight; the weight is reduced during storage probably on account of loss of water and certain gases. Response of this kind to extended storage is not peculiar to apples, but has also been reported in peaches and persimon (Anzueto and Rizvi, 1985). In papaya, wrapping of fruit by shrink filen wraps delays softening of fruit during storage (Ben-Yehoshua, 1978). The same holds true for tomato (Ben-Yehoshua et al., 1983) and mango also (Miller et al., 1986). Paull and Chen (1989) have also observed that in papaya increase in the duration of fruit storage at 10°C lead to greater weight loss in the unwaxed and waxed fruit, but not in wrapped fruit.

Change in mineral content has been reported during storage. Low concentration of calcium at harvest time, makes the apple fruit susceptible to senescent breakdown during cold storage (Van Goor, 1971; Fukuda, 1972; Ratkowsky and Martin, 1974; Dewey et al., 1981).

Other storage disorders, such as formation of bitter pits, were highlighted by Van Lune and Van Goor (1979). The distribution of total quantity of Ca in the fruit is reported to change during storage (Bramlage et al., 1979; Rigney and Wills, 1981). This has been taken to reflect a change in calcium solubility, since most likely, the water soluble form would be mobile in the fruit tissue. Saks et al. (1990) studied fruit before and after cold storage and observed strong correlation between the incidence of senescent breakdown and the total and water soluble calcium content in the pulp of "Jonathan" apple. Bünemann and Ludders (1975) and Li et al. (1985) have related the ratio of Ca content to Mg and K with the occurrence of various storage disorders. On the other hand, Saks et al. (1990) have reported that the total amount of Ca, Mg and K in the pulp tissue of "Jonathan" apples remains constant during storage and subsequent shelf life. Decrease in water soluble calcium during storage was reported in "Cox's Orange Pippin" apples (Perring and Plocharski, 1975a). Li et al. (1985) reported that the correlation between juice and total calcium content in apples was good in mature but poor in over mature stored fruit.

III. MATERIAL AND METHODS

III.1 Material and its source :

Fifteen varieties of apple (Malus pumila Mill.) constitute the material on which the present investigation is based. The investigation deals with the study of morphology, foliar metabolites, sugars, starch, keeping quality and effect of storage on chemical architecture of the fruit. The studies were

undertaken during the four year period extending from 1988-1991. The material was collected from various orchards in Kashmir valley listed in Table 1.

Table 1. Varieties studied and their source.

S.No.	Varietal name	Orchard*
1.	Ambri	1
2.	American Trail	1
3.	Blood Red	2
4.	Chamura	3
5.	Dil Ruba	1
6.	Double Kesari	1
7.	Golden Delicious	4
8.	Gole Delicious	1
9.	Hazratbali-II	3
10.	Kagzi-Kharoo	1,3
11.	Kharoo	1
12.	Maharaji	1,3
13.	Razakwari	1
14.	Red Delicious	1
15.	Royal Delicious	5

*1 = Shahdad Orchards, Habak, Srinagar, Kashmir.

2 = Nehru Memorial Botanical Garden, Chesmashahi, Srinagar, Kashmir.

3 = Koul's Orchards, Sadarbal, Srinagar, Kashmir.

4 = Jyoti Pvt. Ltd. Orchards, Boulevard, Srinagar, Kashmir.

5 = Malik Orchard, Khaunmoh, Kashmir.

III.2 Morphology :

Morphological studies were restricted to fruit. The fruit morphology has been described after the procedure standardised by Fougat (1984). For each variety at least 10 fruits were taken.

III.3 Chemical analysis :

The test material has been analysed for ninhydrin positive compounds, flavonoids and carbohydrates - sugars and starch. Paper and thin layer chromatography has been employed for the separation of ninhydrin - positive compounds and flavonoids. Sugar and starch were estimated through spectrophotometry using Systronics make (106 type) spectrophotometer. With respect to these chemical parameters, a minimum of 2-4 replicates have been used for each variety. Details of the procedures followed for analysis of metabolites, enumerated above are presented in the following pages.

III.3.1 Preparation of reagents :

All reagents and solutions used in experimentation were prepared in glass distilled water. The glassware was cleaned with chromic acid. The

chemicals used for various preparations were mostly of the analytical grade; names of manufacturers/suppliers of these chemicals have been indicated at appropriate places.

(i) Preparation of reagent C :

It was prepared by mixing the following reagents as indicated below.

Copper reagent A : 25 g anhydrous sodium carbonate (Mol. wt. 155. 99; SD's, Boisar), 25 g potassium sodium tartrate (Mol. wt. 282.22; SD's, Boisar), 20 g sodium bicarbonate (Mol. wt. 84.01; SD's, Boisar) and 200 mg of anhydrous sodium sulphate (Mol. wt. 142.04; SD's, Boisar) were dissolved in dist. water and the final volume was brought to 1,000 ml.

Copper reagent B : 15 g copper sulphate (Mol. wt. 249.68; Loba Chemicals, Bombay) was dissolved in dist. water and 2-3 drops of conc. hydrochloric acid were added to it. Final volume of the mixture was raised to 100 ml by the addition of dist. water.

Reagent C is prepared by mixing together copper reagents A and B in the ratio of 25:1 by volume.

(ii) Nelson's Arseno-molybdate reagent :

The reagent is a mixture of the two following solutions :

Solution A : 25 g ammonium molybdate (Mol. wt. 1235.9; SD's, Boisar) dissolved in 450 ml dist. water and supplemented with 2 ml conc. hydrochloric acid.

Solution B : 3 g sodium arsenate (Mol. wt. 312.02; Loba Chemicals, Bombay) dissolved in 25 ml dist. water.

Solution A and B were mixed together and maintained at 37°C in an oven for 24 hours before use.

(iii) 30% Potassium hydroxide solution :

30 g potassium hydroxide (Mol. wt. 58.11; BDH, Bombay) dissolved in 100 ml dist. water.

(iv) 52% Perchloric acid :

84 ml perchloric acid (62%) added to 16 ml dist. water.

(v) Preparation of sulphuric acid solutions :

(a) 71.5% Sulphuric acid solution : 71.5 ml conc. sulphuric acid added to 28.5 ml of dist. water.

(b) 76% Sulphuric acid solution : 76 ml conc. sulphuric acid added to 24 ml dist. water.

(vi) Preparation of anthrone solutions :

(a) 0.1% Anthrone solution : 100 mg anthrone powder (Mol. wt. 194.23; SD's, Boisar) dissolved in 100 ml of 76% sulphuric acid.

(b) 0.15% Anthrone solution : 150 mg of anthrone powder dissolved in 100 ml 76% sulphuric acid.

(c) 0.2% Anthrone solution : 200 mg of anthrone powder dissolved in 100 ml of 71.5% sulphuric acid.

(vii) Preparation of standard solutions :

100 mg of glucose (Mol. wt. 180.16; Merck, Bombay), fructose (Mol. wt. 180.16, SISCO Chem, Bombay) and sucrose (Mol. wt. 342.30; SD's, Bombay) were dissolved separately in 1 litre of dist. water, each. Further dilutions of these stock solutions were obtained by adding requisite quantities of distilled water as and when required.

(viii) 0.4% Ninhydrin solution :

Ninhydrin solution was prepared by dissolving 400 mg ninhydrin powder (Loba Chemical, Bombay) in 100 ml n-butanol (BDH, Bombay).

(ix) 1% Sodium hydroxide solution :

The solution was prepared by dissolving 1 g of sodium hydroxide (BDH, Bombay) in 100 ml of methanol.

III.3.2 Methodology for analysis of metabolites :

(i) Ninhydrin-positive compounds :

Ranjan and Lallorya's (1960) method with modifications incorporated by Bhargava (1983) has been followed for separation of these compounds. Fresh leaves, 2 g by weight, were crushed into fine paste, in a glass pestle and mortar containing 15 ml of 80% boiling ethanol. The entire set up was then left undisturbed at room temperature for at least 48 hours. The supernatant was drained out in a clean glass tube and fresh instalment of 20 ml of 80% ethanol was added to the residue. The mixture was left undisturbed for sometime and then centrifuged in REMI T8 centrifuge machine, at 5000 rpm for 10 minutes, at room temperature. From the spun mixture, the supernatant was collected and pooled with that obtained earlier. Final volume of this pool was reduced to 1 ml through oven-drying at 60°C. The extract so obtained, was maintained in refrigerator for further use.

Separation, detection and scrutiny of ninhydrin positive compounds was carried out through bidimensional paper chromatography. Whatman No. 1 chromatography paper has been used all through as the stationery phase. Various steps involved in the technique are as follows :

Loading of extract :

100 µl of the test extract were used for loading a rectangular chromatography sheet (57x46 cm) whose one edge was marked in advance. A little of the extract was applied at a time, to a predetermined point near the marked edge of the sheet and immediately thereafter the spot was dried with the help of hot air blower to prevent spreading of the extract. Alternate application and drying was continued till the entire quantity of the extract (100 µl) was loaded in as small an area as possible.

Development in solvent system :

The loaded chromatography sheet was developed in two separate solvent systems, in descending direction. The first comprised of n-butanol, acetic acid and distilled water in a proportion of 12:3:5 by

volume and the second made up of four parts of pre-distilled phenol and one part distilled water. Developing was carried out at room temperature, by suspending the loaded sheet, in a chromatography chamber sealed internally with a coating of wax and saturated with the first solvent system. The process was initiated by immersing the marked edge of the loaded chromatography sheet into the trough containing the first solvent system. Care was exercised to avoid immersing the loaded spot itself. The whole set up was left undisturbed till such time as the descending solvent front reached the predetermined level (40 cm) at the opposite edge of the sheet. The sheet was removed, air dried and reintroduced into the chamber now containing the second solvent system. The second run was performed exactly as the first, but only after the sheet had been rotated through 90° at the loaded spot. At the completion of the second run (30 cm length), the sheet was removed from the chamber, air dried and processed for detection of ninhydrin - positive compounds.

Detection of ninhydrin-positive compound spots :

Developed chromatograms were sprayed with 0.4%

ninhydrin solution, air dried and then oven dried at 70°C for 15-20 minutes, the purple or reddish spots which thus, appeared on the chromatograms were marked out.

Observation and identification of ninhydrin-positive compounds :

Tracings of spots were made on individual chromatograms. For purposes of comparison, chromatograms of different taxa were super-imposed to evolve a composite chromatogram. While constructing the composite chromatogram minor variation in spot position was disregarded. Spots in the composite chromatogram were numbered through triangulation by subtending consecutive imaginary isosceles triangles, each triangle having the point at which the extract was loaded on the sheet as its right angle vertex. Spots, in each triangle, were numbered from left to right in a continuous ascending order till the entire sheet was mapped. Thereafter, the chromatograms of all the test varieties were matched with the composite chromatogram to determine the spot pattern in each variety. Spots which bore identical number in different chromatograms were taken to represent identical chemical species. The

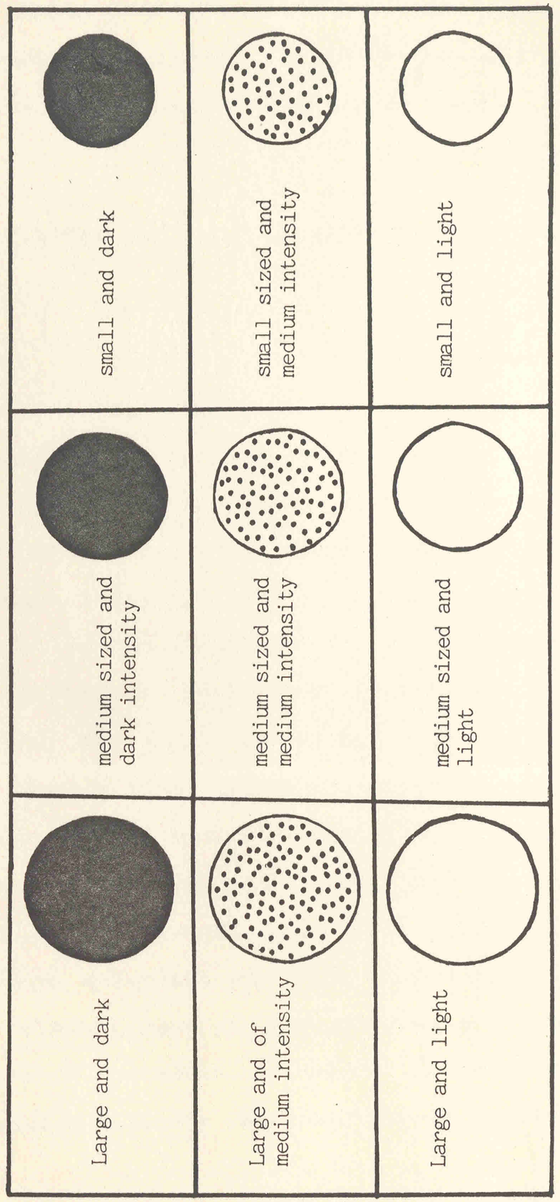


Fig. 1 Key to the nine types of spots identified in various aminograms on the basis of size and intensity of staining.

area under each individual spot was determined with the help of graph paper, and the intensity of their colouration was recorded for use in classifying them. The various categories thus raised are represented in Fig. 1. The spots with area of 80-250 mm^2 were designated as small, those with 251-550 mm^2 as medium sized and above 551 mm^2 as large.

(ii) Flavonoids :

Foliar flavonoids were extracted separately from 2 g of fresh leaves, fixed in 15 ml of 1% HCl in methanol. The material was left undisturbed at room temperature for at least four days. The supernatant was drained out in a separate, clean glass tube and a fresh instalment of 15 ml of 1% HCl in methanol was added to the leaves. Fixed material immersed in the fixative was crushed in a glass pestle and mortar. The mixture thus obtained was centrifuged at room temperature for 10 minutes at 5000 rpm. From the spun mixture, the supernatant was collected and pooled with that obtained earlier. Final volume of this pool was reduced to 1 ml, by placing it in an oven maintained at 60°C. The extract thus obtained, was processed for further use.

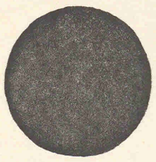
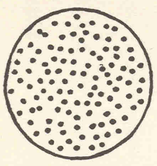
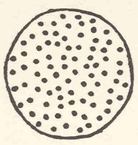
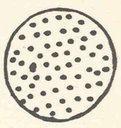
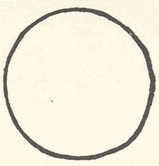
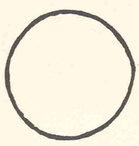
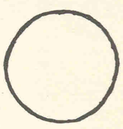
Large and dark 	medium sized and dark intensity 	small and dark 
Large and of medium intensity 	medium sized and medium intensity 	small sized and medium intensity 
Large and light 	medium sized and light 	small and light 

Fig.1 Key to the nine types of spots identified in various aminograms on the basis of size and intensity of staining.

area under each individual spot was determined with the help of graph paper, and the intensity of their colouration was recorded for use in classifying them. The various categories thus raised are represented in Fig. 1. The spots with area of 80-250 mm^2 were designated as small, those with 251-550 mm^2 as medium sized and above 551 mm^2 as large.

(ii) Flavonoids :

Foliar flavonoids were extracted separately from 2 g of fresh leaves, fixed in 15 ml of 1% HCl in methanol. The entire set up was left undisturbed at room temperature for at least four days. The supernatant was drained out in a separate, clean glass tube and a fresh instalment of 15 ml of 1% HCl in methanol was added to the leaves. Fixed material immersed in the fixative was crushed in a glass pestle and mortar. The mixture thus obtained was centrifuged at room temperature for 10 minutes at 5000 rpm. From the spun mixture, the supernatant was collected and pooled with that obtained earlier. Final volume of this pool was reduced to 1 ml, by placing it in an oven maintained at 60°C. The extract thus obtained, was processed for further use.

Separation of flavonoids has been achieved through thin layer chromatography (TLC) technique. For this purpose 0.6 mm thick cellulose layer, coated on glass plate has been used as the stationery phase, all through. The technique involved following steps.

Preparation of TLC plates :

20 g of cellulose powder (SD's, Bombay) was dissolved in 80 ml dist. water, and a homogenous slurry was obtained through continuous stirring. The slurry was spread uniformly over clean 20x20 cm glass plates with the help of a spreader so as to obtain a 0.6 mm thick layer. The glass plates were gently tapped at the base, from time to time, to prevent irregular surfacing. The plates were oven-dried over night at 40°C.

Loading of the extract :

30 µl of the test extract were loaded at a predetermined point in a corner of the TLC plate, by means of a micropipette. Immediately after application, the extract was dried with the help of hot air blower in order to keep the loaded spot as small as possible. Alternate application and drying of the extract was continued till the whole extract was loaded. A set of

four TLC plates for each test extract, was loaded for simultaneous development.

Development of TLC plates :

Loaded plates were developed at room temperature, in two separate solvent systems, in ascending direction. The first solvent system comprised 2% formic acid (BDH, Bombay) in water and the second one was a mixture of amyl alcohol, acetic acid and dist. water in the ratio of 10:6:5 by volume.

Developing was carried out inside an all glass chamber. Loaded TLC plates were so inserted into the chamber that they remained upright with their loaded edges just touching the first solvent. The whole set up was left undisturbed till the solvent front ascended to a predetermined level (16 cm from point of application of extract) on the plate. Thereafter, they were removed, air dried, rotated through 90° at the loaded spot and reintroduced into the chamber now containing the second solvent system. The second run was continued till the solvent front reached a distance of 17 cm from the point of application. Thereafter, the plates were removed and air dried.

Detection and scrutiny of spots :

All plates in a set were sprayed with methanolic solution of 1% sodium hydroxide. The plates treated thus, were scanned in a dark chamber under long wave ultra-violet light. This led to the appearance of yellow or bluish-white spots which were marked out and studied for their size and colour. Spot measurements were made from tracings of TLC plates. The tracings were super-imposed to obtain complete flavonoid profile. Later, individual profiles of different taxa were super-imposed to construct composite flavonoid profile for the total sample. The minor variations noticed during the preparation of composite chromatogram were disregarded. Numbering of spots on composite chromatogram was done as described for ninhydrin-positive compounds. Spots bearing same numbers were taken to represent the same flavonoid.

(iii) Carbohydrates

(a) Extraction :

Fruit pulp has been used for the extraction of sugar as well as starch. The actual procedure involved is as follows :

Sugars :

50 mg of apple flesh was reflexed in 5 ml of isopropanol, at 82°C, for 30 minutes. Later, it was washed with 5 ml of dist. water and the extract was collected in a beaker. Washing was repeated at least four times. To the extract (nearly 20 ml) was added 2 ml of lead acetate and the resulting solution was filtered. 1 ml of potassium oxalate was added to the filtrate and then it was centrifuged at 5000 rpm, for five minutes. The final volume of the extract was raised to 25 ml by adding dist. water.

Starch :

McCready et al.'s (1950) method with slight modification has been followed for extraction of starch. 5 g of the fruit flesh was reflexed in 20 ml of isopropanol at 82°C for 30 minutes. It was later repeatedly washed with dist. water. The sugar free residue thus obtained, was air dried. 100 mg of this dried material was taken in a beaker and 5 ml of distilled water and 6.5 ml of 52% perchloric acid were added to it. The contents were stirred continuously for 5 minutes on a magnetic stirrer.

The mixture was supplemented with 20 ml of

dist. water and then centrifuged at 5000 rpm for five minutes. The final volume of the mixture was brought to 50 ml with the addition of dist. water.

(b) Estimation :

Total free sugars :

These were estimated according to anthrone method of Yemn and Willes (1954). For this purpose 0.2 ml of the extract referred at page 34 was taken in test tubes and 5 ml of 0.2% anthrone solution in 71.5% H_2SO_4 was added to each. A set of three tubes representing three replicates for each test variety was employed. The whole process was performed by placing the test tubes in ice chilled water. The test tubes containing the extract and the anthrone mixture were heated for 8 minutes in a water bath. This resulted in the development of green colour. Optical density of the coloured solution was measured at 620 nm on a spectrophotometer (106 type, Systronics make) and recorded against the control which was prepared by substituting the test extract by an equal volume of dist. water. The quantity of total free sugars was estimated by comparing optical density of the treated sample with that of glucose solutions of various concentrations i.e., 10, 20, 40, 60, 80, 100 mg/l.

Reducing sugars :

For estimation of reducing sugars Nelson's (1944) method was followed with slight modifications. 1 ml of reagent C was aded to 1 ml of the extract (described at page 34). The mixture thus obtained (3 replicates for each variety) was boiled for 20 minutes inside a water bath and then cooled to room temperature. 1 ml of Nelson's molybdate reagent was added to it, followed by addition of 10 ml of dist. water. Optical density of this mixture was measured at 620 nm against a blank which was prepared by substituting equal amount of dist. water in place of test extract. The quantity of reducing sugars was estimated by comparing the optical density of the treated sample with that of glucose solutions of various concentrations (10 - 100 mg/l).

Sucrose :

Handel's (1968) method was employed for estimation of sucrose. 1 ml of extract (described at page 34) was taken in a test tube (3 replicates) and reduced to 0.1 ml through oven drying at 120°C. After bringing the test tube back to room temperature, 0.1 ml of 30% potassium hydroxide solution was added to it. The test tube containing the mixture was heated inside a

water bath for 12 minutes and then cooled immediately by placing it in ice cold water. 3 ml of 0.15% anthrone solution in 76% H_2SO_4 was added to the tube and the whole was incubated at 40°C for 10-15 minutes. Thereafter, optical density of the mixture was recorded at 620 nm in the same way as described earlier for other sugars. The optical density of the treated sample was compared with sucrose solution of concentrations varying between 10-100 mg/l.

Fructose :

Handel's (1968) procedure was adopted for estimation of this sugar also. 0.2 ml of the extract (described at page 34) in a test tube (3 replicates) was supplemented with 3 ml of 0.15% anthrone solution in 76% H_2SO_4. This procedure was carried out in ice cold water. Thereafter, the mixture was incubated at 40°C for 10 minutes and the test tube transferred to chilled water again. Optical density of the mixture was measured against the blank at 620 nm. The quantity of fructose was estimated by comparing optical density of the treated sample with that of the fructose solution of different concentration varying between 10-100 mg/l.

Starch :

0.2 ml of the test extract (described at page 34) was taken in a test tube (2 replicates for each variety) which was supplemented with 5 ml of 0.1% anthrone solution in 76% H_2SO_4. The mixture thus obtained was incubated in a hot water bath for 12 minutes, and then cooled to room temperature. Optical density of the coloured mixture was recorded against the blank at 630 nm. The quantity of starch was estimated by comparing the optical density of treated sample with that of glucose solution of various concentrations i.e. 10-100 mg/l and multiplying the equivalent by 0.90.

III.4 Post-harvest changes :

The changes in fruit during the storage period were monitored as below :

III.4.1 Rotting percentage :

Immediately after harvesting, at least 50 sorted and healthy fruits of each variety were placed in wooden boxes that were subjected to two temperature regimes viz. 2-4°C inside a cold storage room and 15-33°C at room temperature. Rotting percentage was

calculated at intervals of 30 days by applying the following formulae :

$$\frac{\text{Number of rotten fruits}}{\text{Total number of fruits stored}} \times 100$$

III.4.2 **Dry matter :**

50 g of the pulp of fresh as well as stored fruits of each variety (2 replicates each) was kept in an oven maintained at 60°C, till the weight of the sample became constant. Dry weight of the sample was calculated by following formula :

$$\frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Such observations were taken at regular intervals of 30 days.

III.5 **Statistical computation of the data :**

Data obtained for various parameters were processed statistically.

Paired affinity (similarity index), between any two taxa, was calculated after Alston and Turner

(1963) by using formula

$$\text{Similarity index} = \frac{\text{No. of spots common in A and B}}{\text{Total no. of spots in A and B}} \times 100$$

For calculations of correlation coefficient Pearson's formula was used

$$r = \frac{N\Sigma XY - (\Sigma X)(\Sigma Y)}{\sqrt{N\Sigma X^2 - (\Sigma X)^2}\ \sqrt{N\Sigma Y^2 - (\Sigma Y)^2}}$$

IV. OBSERVATIONS

The 15 cultivated varieties of apple (Malus pumila Mill.) from different orchards in Kashmir valley, that constitute the material for present investigation, include 10 sweet and 5 sub-acidic types. The latter group includes Chamura, Hazratbali-II, Maharaji, Kharoo and Kagzi-Kharoo. Cytologically, the sample includes 12 diploids (2n=34) and 3 triploids (2n=3x=51). The

Figs. 2 & 3 Fruits of cvs. Ambri and American Trail. Note the difference in fruit shape, size and colour.

triploids are, Double-Kesari, Hazratbali-II and Kharoo.

IV.1 Morphological studies

IV.1.1 Tree morphology :

Trees of most of the varieties studied are moderately vigorous; the crown is spreading, upright or semierect. The crown is round, wide or narrow; and loose or compact. The height and girth of the tree varies and so does the branching pattern. Branching starts 1 or 1.5 m above the ground in a tree but in some varieties the tree begins to branch only ½ m or in extreme cases only ¼ m above the ground level.

IV.1.2 Fruit morphology :

Ambri :

Fruit 6.5-7.0 x 6.4-6.8 cm, medium to large in size, oblong, lopsided, yellowish green in colour, usually red colour on the side exposed to sun (Fig. 2); skin smooth, tough, flesh white, soft fine grained, juicy, sweet and aromatic. Picking time, first week of September to end of the month.

Fig.4 A representative pome of cv. Blood Red, Note highly prominent lenticels on the skin.

Fig.5 Fruit of cv. Chamura with broad belly. Red blush on the cheeks is prominent.

American Trail :

Fruit 4.5-4.8 x 5.4-5.7 cm, oblate, regular, symmetric, uniform, occasionally compressed (Fig. 3), self-coloured in bright carmine, red with occasional islands of green or yellow particularly around the stem cavity; flesh white, quite firm, very juicy and sweet. Harvesting period last week of September - first week of October.

Blood Red :

Fruit 5.0-5.5 x 5.6-6.9 cm, oblate or spherical, occasionally angular, uniformly coloured, deep red at maturity (Fig. 4); skin covered with thin white powdery layer which gets easily rubbed off; flesh yellow, hard, juicy, sweet. Harvesting period last week of September - mid October.

Chamura :

Fruit 6.2-6.5 x 7.0-7.4 cm, ovoid or conical with a broad belly and abruptly narrow apex, regular with frequent blush of red on cheeks (Fig. 5); flesh cream coloured, juicy, hard, sub-acidic. Harvested during the last week of September.

Fig.6 A typical pome of cv. Dil Ruba with few lenticels.

Fig.7 A representative pome of cv. Double Kesari with uniformly distributed lenticels.

Figs. 8 & 9 Fruits of cvs. Golden Delicious and Gole Delicious. Note the golden colour and deep seated lenticels in the former and rounded shape of the latter.

Dil Ruba :

Fruit 5.8-6.6 x 6.4-7.0 cm, conical, uniformly red (Fig. 6); basin ridged; fruit flesh firm, cream coloured, juicy, sweet. Harvesting period August Ist-3rd week.

Double Kesari :

Fruit 5.6-6.6 x 6.7-7.2 cm, oblate, regular, frequently lopsided (Fig. 7), uniformly coloured in deep red at maturity, lenticels small, white, surrounded by light red rings; flesh greenish white, firm, juicy, sweet. Harvesting time, end of September or early October.

Golden Delicious :

Fruit 6.0-6.5 x 6.1-6.4 cm, ovoid or conical, occasionally nearly spherical, very regular, symmetric, uniformly coloured in golden yellow (Fig. 8); skin rough, spotted with raised, open, conspicuous cinnamon dots; flesh yellow, soft, very juicy, crisp, very sweet. Harvesting time third week of September to early October.

Figs. 10 & 11 Pomes of cv. Hazratbali-II and Kagzi-Kharoo; the latter bears raised russet dots on the surface.

Gole Delicious :

Fruit 4.2-6.0 x 6.0-6.7 cm, oblate to spherical, symmetric, angular, stripes of one or more shades of red against pale green background (Fig. 9); skin smooth, thin, clear, lenticels inconspicuous; flesh crisp, firm, cream coloured with greenish tinge, juicy and sweet. Harvesting time, mid or end of August.

Hazratbali-II :

Fruit 6.0-6.5 x 6.2-7.0 cm, spherical or nearly ovoid, regular, symmetric, uniform, striped or blushed in deep red against yellow background (Fig. 10); flesh pale yellow, firm, tough, juicy, sub-acidic. Harvesting period, July end or early August.

Kagzi-Kharoo :

Fruit 5.5-6.4 x 6.2-7.8 cm, oblate or spherical, occasionally ovoid, regular, symmetric, pale yellow with occasional blush of orange red on the exposed side (Fig. 11); skin rough on account of numerous dots; flesh white with yellow tinge, firm, hard, quite juicy, sub-acidic. Harvested during last week of August - mid September.

Fig.12 Fruit of cv. Kharoo. Note the rough, brown-coloured skin.

Fig.13 A pome of cv. Maharaji bearing red stripes that diverge from the basin towards apex. Note conspicuous lenticels.

Fig.14 A pome of cv. Razakwari. Note the elongated conical shape of the fruit.

Fig.15 A fruit of cv. Red Delicious. Note conical shape of the pome and red coloured stripes radiating from the basin towards the apex.

Kharoo :

Fruit 4.5-6.2 x 6.4-7.0 cm, spherical or oblate, regular, occasionally lopsided, yellow (Fig. 12); skin rough on account of numerous raised, open, brown, stellate, russet dots; flesh yellow, firm, tough, sub-acidic. Harvested towards the end of September or early October.

Maharaji :

Fruit 5.8-6.6 x 7.4-8.2 cm, oblate, regular in outline, symmetric, striped in red against yellow background (Fig. 13), uniformly coloured in bright red at maturity; skin smooth, lenticels small, white, depressed; flesh white, firm, little hard, very juicy, sub-acidic. Harvested from mid October to early November.

Razakwari :

Fruit 6.2-7.8 x 5.6-6.7 cm, oblong-conical, apical end forming typical beak, frequently compressed, lopsided, angular, striped or splashed with bright red against pale-yellow background (Fig. 14); skin clean; fruit flesh white, tender-crisp, juicy, sweet with fine

Fig.16 A representative fruit of cv. Royal Delicious having dark red skin with uniformly scatted lenticels.

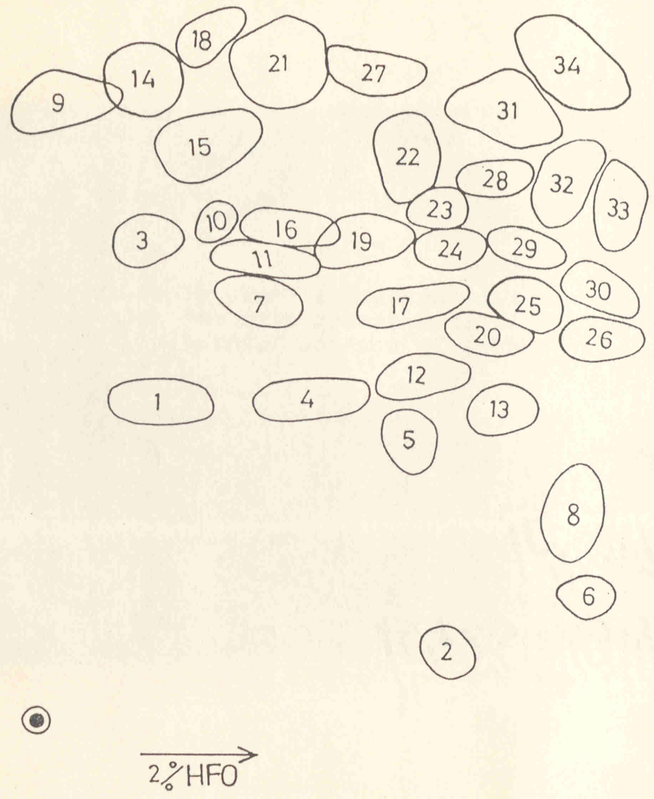


Fig.17 Reconstructed master chromatogram of the test sample showing 34 foliar flavonoid spots.

flavour. Harvesting period, end of July - mid August.

Red Delicious :

Fruit 6.4-7.8 x 6.3-8.0 cm, conical, striped in light red against pale yellow background, turning red coloured at maturity (Fig. 15); fruit flesh yellowish white, soft, crisp, juicy, very sweet with fine flavour. Harvested from 3rd week of September - first week of October.

Royal Delicious :

Fruit 6.2-7.9 x 6.5-8.2 cm, ovoid or conical; deep red coloured at maturity (Fig. 16); flesh white with yellowish tinge, sweet; fruit harvested from last week of August to mid September.

IV.2 Analysis of foliar-flavonoids

Foliar flavonoid pattern of the 15 varieties of apple analysed for the purpose have been presented in Table 2 and Figs. 17-32. Thirty four flavonoids have been located in these varieties; each flavonoid is represented by a specific spot. The latter have been

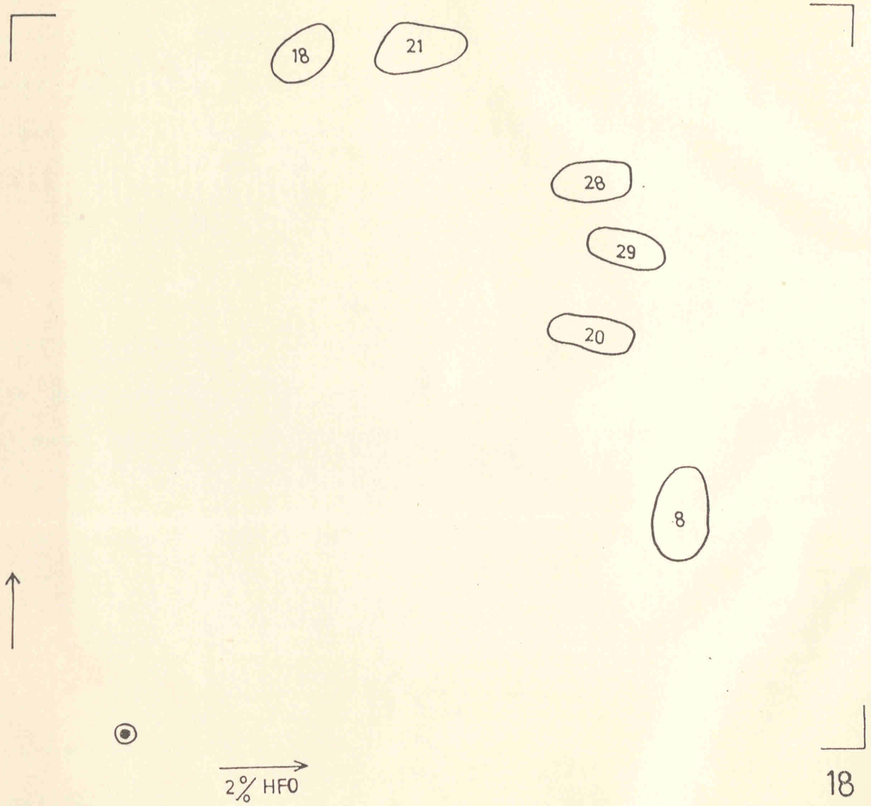
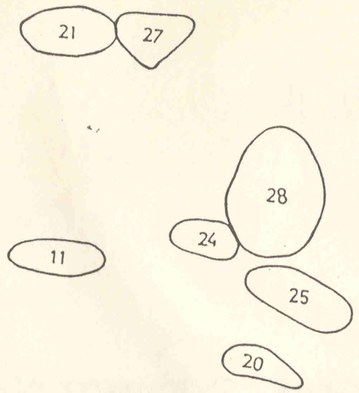


Fig.18 Chromatogram of cv. Ambri showing foliar flavonoid separation after various chemical treatments.

Soln.I - HFO - Formic acid in water

Soln.II - AMOH - Amyl alcohol; ACH - Acetic acid, H_2O - water.



10 AMOH
6 ACH
5 H_2O

2% HFO

Fig.19 Separation of foliar flavonoids of cv. American Trail after various chemical treatments.

characterised on the basis of respective size, fluorescence properties and position in the composite chromatogram. Fluorescence of spot was checked under UV light after bidirectionally run plates were sprayed with an enhancing chemical. Of the 34 spots located on the composite chromatogram only two appeared yellow, the rest are all bluish-white. The number of spots per variety varies and so does the spot size. The flavonoid pattern for the varieties analysed is described below :

Ambri :

Chromatograms of the variety have a maximum of six spots; nos. 8, 18, 20, 21, 28 and 29. Five of these are dark; no. 28 is of medium intensity. Four spots are medium sized, while nos. 20 and 29 are small(Fig.18).

American Trail :

Chromatogram of this variety has seven spots; nos. 11, 20, 21, 24, 25, 27 and 28 (Table 2, Fig. 19). It has three spots (nos. 20, 21 and 28) in common with var. Ambri but they vary in respect of size and intensity. Spot nos. 21, 24, 25, 27 and 28 are dark and nos. 11 and 20 are light. The spots also exhibit variation in size.

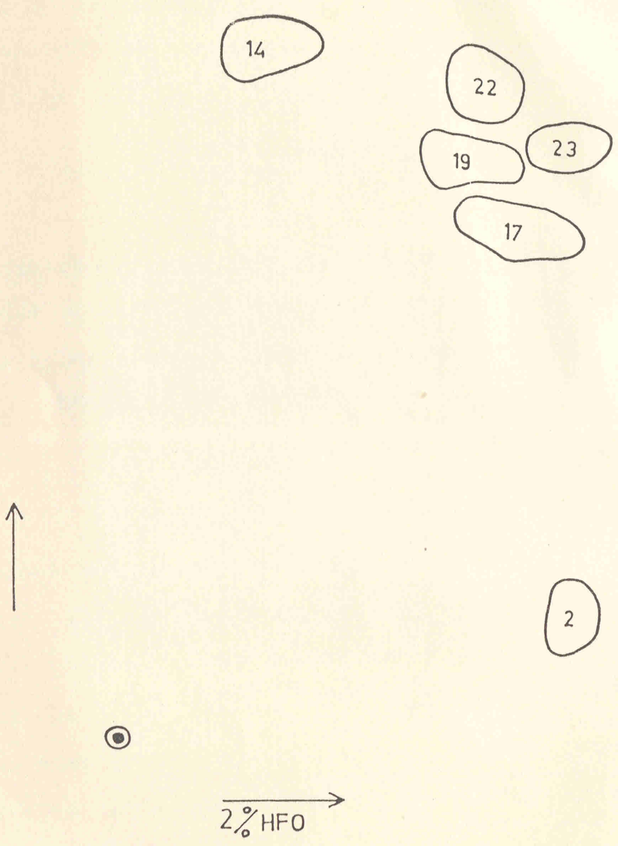


Fig.20 A bidimensional chromatogram of cv. Blood Red showing separation of foliar flavonoids after various chemical treatments.

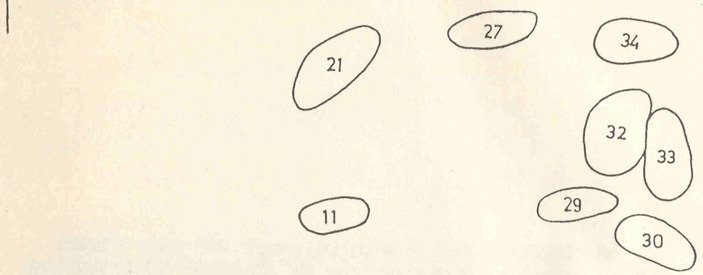
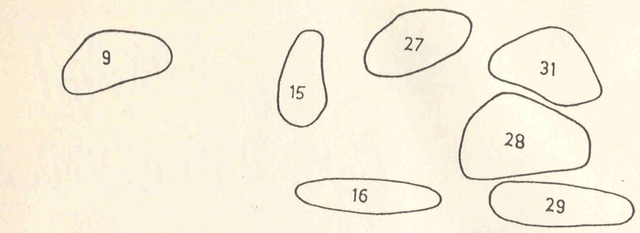


Fig.21 A chromatogram showing foliar flavonoid separation in cv. Chamura following chemical treatments.



10 AMOH
6 ACH
5 H_2O

2% HFO

Fig.22 Foliar flavonoid separation of cv. Dil Ruba after various chemical treatments.

Blood Red :

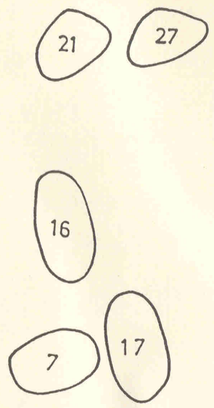
Fig. 20 bears spots of six flavonoids present in this variety. The flavonoids are represented by spot nos. 2, 14, 17, 19, 22 and 23. Five of these spots are medium sized and no. 17 is large. In respect of intensity of colouration, the 6 spots fall into three types; three spots (nos. 17, 19 and 22) are dark, two (nos. 14 and 23) are of medium intensity and one (no.2) is light.

Chamura :

Chromatogram of this variety shows a total of eight spots; nos. 11, 21, 27, 29, 30, 32, 33 and 34 (Table 2, Fig. 21). Spot no. 11 and 29 are light and small; no. 21 is light and medium sized; no. 33 is medium in size as well as intensity, nos. 27, 30, 32, 34 are dark coloured and medium sized. The flavonoid represented by spot no. 33 is unique to this variety.

Dil Ruba :

The variety carries a total of seven foliar flavonoids, which are represented by spot nos. 9, 15, 16, 27, 28, 29 and 31 (Fig. 22). Four of these spots



10 AMOH
6 ACH
5 H_2O

2

2% HFO

23

Fig.23 Chromatogram of cv. Double Kesari showing separated foliar flavonoid spots.

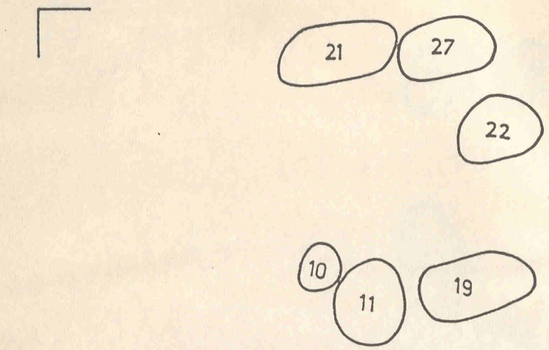
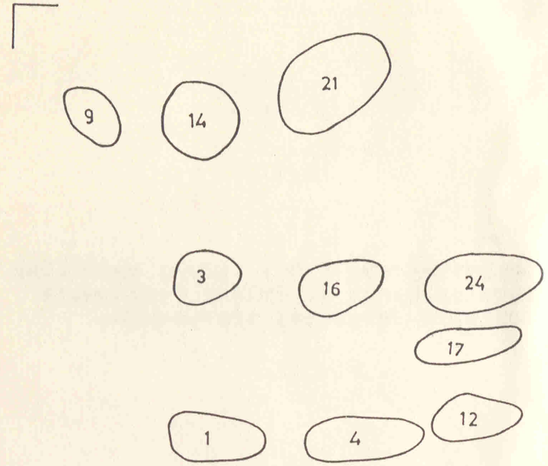


Fig.24 Chromatogram of cv. Golden Delicious showing spots of foliar flavonoids following chemical treatments.



25

(nos. 27, 28, 29 and 31) are dark and large sized, two (one medium and other large sized) are light and no. 15 is of medium size and colour intensity.

Double Kesari :

The pattern of foliar flavonoids in the triploid variety Double Kesari has been presented in Table 2 and Fig. 23. Chromatograms of the variety bear six spots, nos. 2,7, 16, 17, 21 and 27. Spot no. 2 is [illegible] light but both are medium sized; spot nos. 17, 21 and 27 are dark and medium or large sized.

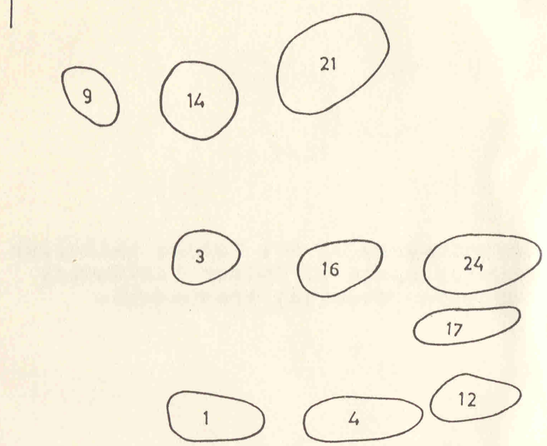
Fig.25 Chromatogram of cv. Gole Delicious showing foliar flavonoid separation.

Golden Delicious :

The chromatogram of this variety carries a total of six flavonoid spot nos. 10, 11, 19, 21, 22 and 27. These are either dark or of medium colour intensity. With respect to size, two spots are large, three medium sized and one small (Table 2 and Fig. 24).

Gole Delicious :

The flavonoid pattern of the variety is illustrated in Fig. 25. The ten spots detected in the



10 AMOH
6 ACH
5 H_2O

2% HFO

25

Fig.25 Chromatogram of cv. Gole Delicious, showing foliar flavonoid separation.

(nos. 27, 28, 29 and 31) are dark and large sized, two (one medium and other large sized) are light and no. 15 is of medium size and colour intensity.

Double Kesari :

The pattern of foliar flavonoids in the triploid variety Double Kesari has been presented in Table 2 and Fig. 23. Chromatograms of the variety bear six spots, nos. 2,7, 16, 17, 21 and 27. Spot no. 2 is light coloured and small sized, 7 and 16 are also light but both are medium sized; spot nos. 17, 21 and 27 are dark and medium or large sized.

Golden Delicious :

The chromatogram of this variety carries a total of six flavonoid spot nos. 10, 11, 19, 21, 22 and 27. These are either dark or of medium colour intensity. With respect to size, two spots are large, three medium sized and one small (Table 2 and Fig. 24).

Gole Delicious :

The flavonoid pattern of the variety is illustrated in Fig. 25. The ten spots detected in the

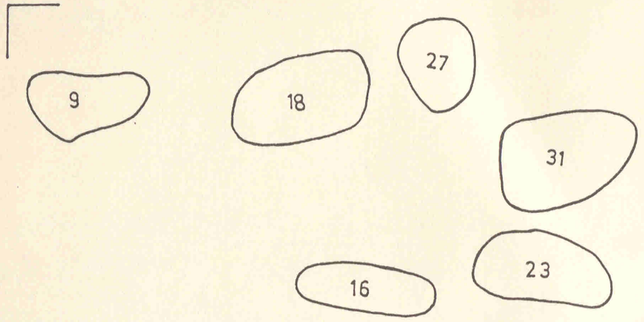


Fig.26 Chromatogram showing separation of foliar flavonoid in cv. Hazratbali-II.

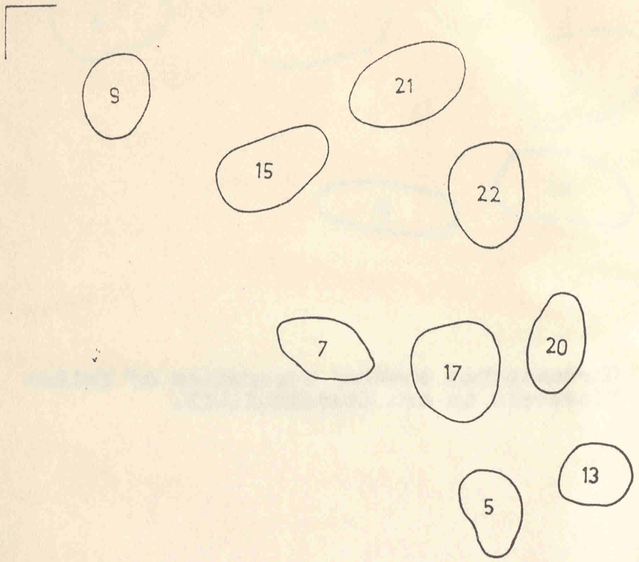


Fig.27 Bidimensional chromatogram of cv. Kagzi-Kharoo showing separation of foliar flavonoids after treatment, with detecting agents.

chromatograms of this variety are, nos. 1,3,4,9,12,14, 16,17,21 and 24. The flavonoids represented by spot nos. 1,3 and 4 are unique to this variety. It shares no. 14 with Blood Red, no. 16 with three other varieties and no. 17 with five varieties in the present sample (of 15). The spots exhibit variation both in size and colouration (Table 2).

Hazratbali-II :

The foliar flavonoid pattern of this triploid variety is illustrated by Fig. 26. The variety has six flavonoids represented by spot nos. 9, 16, 18, 23, 27 and 31. All the six spots are large sized but the intensity of their colour varies; three spots are dark (nos. 16, 23 and 31), one (no. 27) is of medium intensity and two (no. 9 and 18) are light.

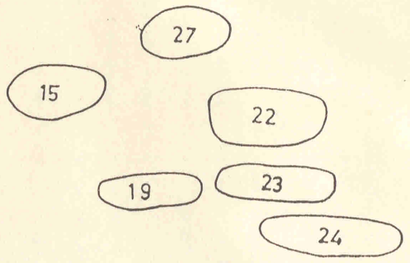
Kagzi-Kharoo :

The various spots detected in chromatogram of this variety are listed in Table 2 and depicted in Fig.27. The total number of spots detected is nine; nos. 5,7,9,13,15,17,20,21 and 22. From amongst these, the first four spots are light coloured and of medium size . Spot no. 15 is large and of medium intensity,

Table 2. Foliar flavonoid profiles of the 15 varieties of apple.

S. No.	Varietal name	Spot number and colour																																	Total no. spots/ variety
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	
		BW	BW	BW	BW	BW	BW	BW	BW	BW	Y	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	Y	BW	BW	BW	BW	BW	BW	BW	BW	BW	BW	
1.	Ambri	-	-	-	-	-	-	-	md	-	-	-	-	-	-	-	-	-	md	-	sd	md	-	-	-	-	-	-	mm	sd	-	-	-	-	-	6
2.	American Trail	-	-	-	-	-	-	-	-	-	-	ml	-	-	-	-	-	-	-	-	sl	md	-	-	md	ld	-	md	ld	-	-	-	-	-	-	7
3.	Blood Red	-	ml	-	-	-	-	-	-	-	-	-	-	-	mm	-	-	ld	-	md	-	-	md	mm	-	-	-	-	-	-	-	-	-	-	-	6
4.	Chamura	-	-	-	-	-	-	-	-	-	-	sl	-	-	-	-	-	-	-	-	ml	-	-	-	-	-	-	md	-	sl	md	-	md	mm	md	8
5.	Dil Ruba	-	-	-	-	-	-	-	-	ml	-	-	-	-	-	mm	ll	-	-	-	-	-	-	-	-	-	-	ld	ld	ld	-	ld	-	-	-	7
6.	Double Kesari	-	sl	-	-	-	-	ml	-	-	-	-	-	-	-	-	ml	ld	-	-	-	md	-	-	-	-	-	md	-	-	-	-	-	-	-	6
7.	Golden Delicious	-	-	-	-	-	-	-	-	-	sm	md	-	-	-	-	-	-	-	ld	-	lm	mm	-	-	-	-	md	-	-	-	-	-	-	-	6
8.	Gole Delicious	mm	-	ml	ld	-	-	-	-	sl	-	-	ll	-	lm	-	md	ml	-	-	-	ld	-	-	md	-	-	-	-	-	-	-	-	-	-	10
9.	Hazratbali-II	-	-	-	-	-	-	-	-	ll	-	-	-	-	-	-	ld	-	ll	-	-	-	-	ld	-	-	-	lm	-	-	-	ld	-	-	-	6
10.	Kagzi-Kharoo	-	-	-	-	ml	-	ml	-	ml	-	-	-	ml	-	lm	-	ld	-	-	md	md	lm	-	-	-	-	-	-	-	-	-	-	-	-	9
11.	Kharoo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mm	-	-	-	ml	-	-	md	md	md	-	-	mm	-	-	-	-	-	-	-	6
12.	Maharaji	-	-	-	-	-	sl	-	-	-	-	ml	-	-	lm	-	-	-	-	-	-	md	-	md	md	-	ld	-	md	-	mm	md	-	-		10
13.	Razakwari	-	-	-	-	-	-	ml	-	-	-	-	-	-	-	-	-	-	-	-	ml	-	-	-	-	md	md	-	mm	mm	-	ml	-	md		8
14.	Red Delicious	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ml	-	md	-	md	-	ml	ml	-	-	-	-	ml	-	-	-	-	-	-	-	6
15.	Royal Delicious	-	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ld	ml	-	md	ml	ml	-	mm	-	-	-	-	-	-	-	-	-	7

Abbreviations :- ld - large and dark; md - medium sized and dark intensity; sd - small and dark; lm - large and of medium intensity; mm = medium sized and medium intensity; sm - small sized and medium intensity; ll - large and light; ml - medium sized and light; sl - small and light; BW - bluish white; Y - Yellow.

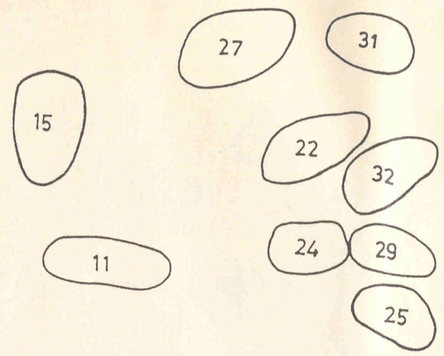


10 AMOH
6 ACH
5 H_2O

2% HFO

28

Fig.28 Chromatogram of cv. Kharoo showing separation of foliar flavonoids.



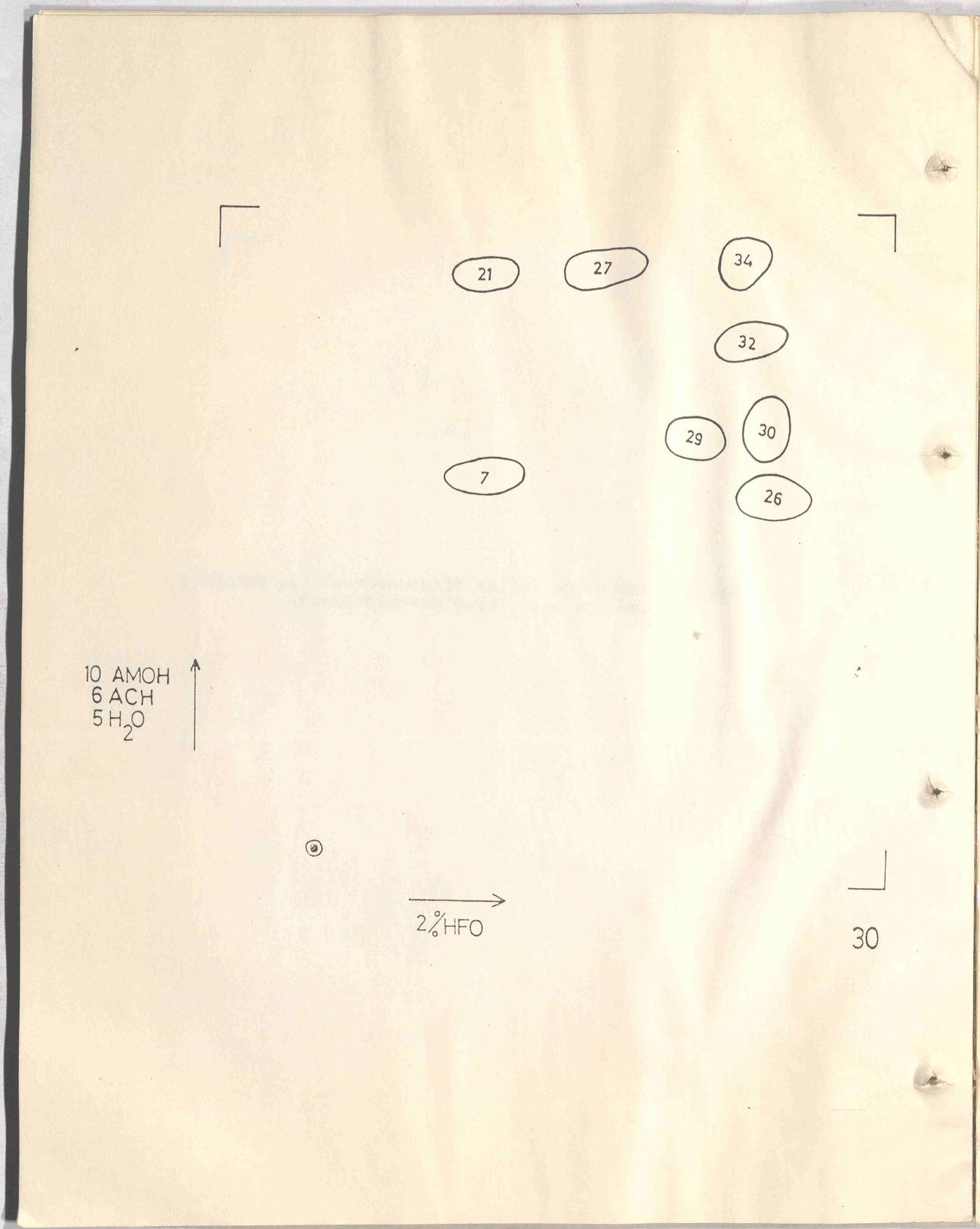
10 AMOH
6 ACH
5 H_2O

6

2% HFO

29

Fig.29 Separated foliar flavonoids of cv. Maharaji through thin layer chromatography.



no. 17 is large sized and dark coloured, no. 20 and 21 are dark and medium sized, no. 22 is large but of medium intensity.

Kharoo :

The triploid variety Kharoo has a total of six flavonoids represented in the chromatogram by spot nos. 15, 19, 22, 23, 24 and 27 (Fig. 28). From amongst these no. 19 is of light intensity and medium sized, nos. 22, 23, 24 are dark and medium; the remaining two spots nos. 15 and 27 are of medium intensity.

Fig.30 Tracings of bidimensional TLC plates of cv. Razakwari showing foliar flavonoid separation following treatment with detecting reagents.

Maharaji :

A total of ten spots have been detected on the chromatogram of this variety (Fig. 29). Spot no. 6 alone is unique to this variety. It shares the remaining 9 spots with 10 other varieties of the present sample. Variation has been noticed in the size and intensity of colouration of the various spots (Table 2).

Razakwari :

Chromatogram of variety Razakwari shows eight spots, nos. 7, 21, 26, 27, 29, 30, 32 and 34 (Fig. 30).

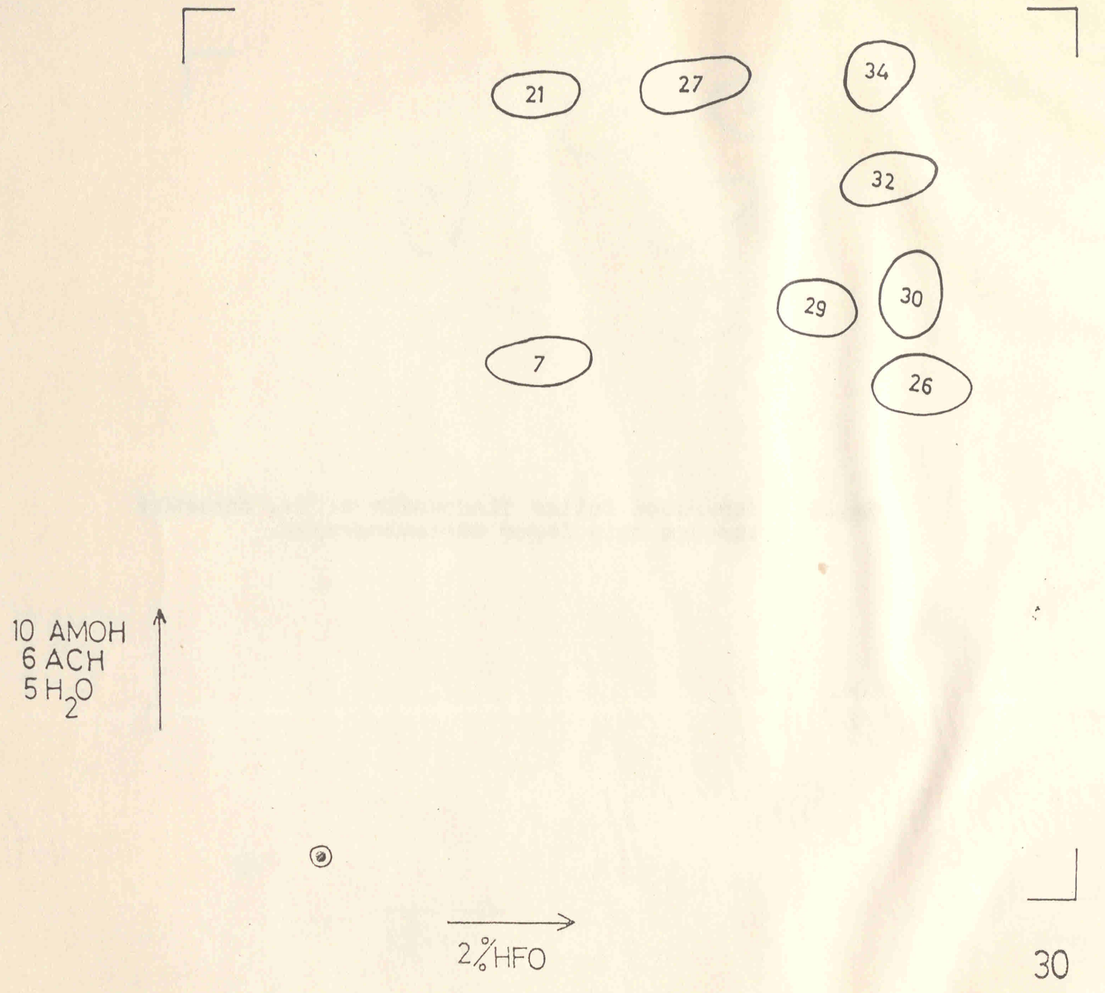


Fig.30 Tracings of bidimensional TLC plates of cv. Razakwari showing foliar flavonoid separation following treatment with detecting reagents.

no. 17 is large sized and dark coloured, no. 20 and 21 are dark and medium sized, no. 22 is large but of medium intensity.

Kharoo :

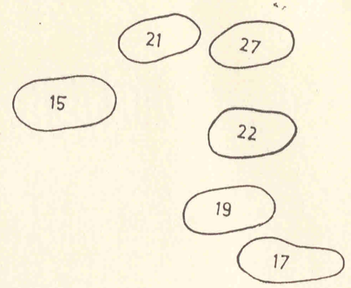
The triploid variety Kharoo has a total of six flavonoids represented in the chromatogram by spot nos. 15, 19, 22, 23, 24 and 27 (Fig. 28). From amongst these no. 19 is of light intensity and medium sized, nos. 22, 23, 24 are dark and medium; the remaining two spots nos. 15 and 27 are of medium size and intensity.

Maharaji :

A total of ten spots have been detected on the chromatogram of this variety (Fig. 29). Spot no. 6 alone is unique to this variety. It shares the remaining 9 spots with 10 other varieties of the present sample. Variation has been noticed in the size and intensity of colouration of the various spots (Table 2).

Razakwari :

Chromatogram of variety Razakwari shows eight spots, nos. 7, 21, 26, 27, 29, 30, 32 and 34 (Fig. 30).



10 AMOH
6 ACH
5 H_2O

2% HFO

Fig.31 Shows foliar flavonoid pattern in cv. Red Delicious.

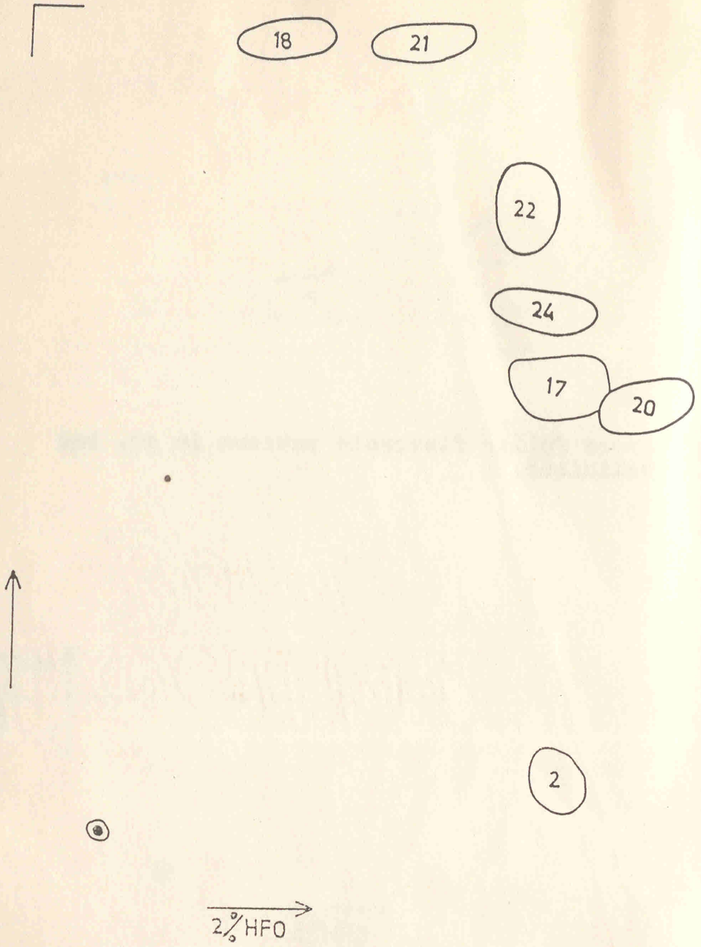


Fig.32 Tracings of TLC of cv. Royal Delicious, showing 7 foliar flavonoid spots.

All the spots are medium sized. They are however, variable with regard to the intensity of colour; spot nos. 7, 21 and 32 are light, nos. 29 and 30 are of medium intensity and nos. 26, 27 and 34 are dark. The flavonoid represented by spot no. 26 is typical to this variety.

Red Delicious :

Fig. 31 shows the six spots which have been detected in chromatograms of this variety. They bear nos. 15, 17, 19, 21, 22 and 27. Out of these spots the variety shares spot 19 with three and no. 21 with nine varieties. All spots are medium sized but they exhibit variation in the intensity of colour (Table 2).

Royal Delicious :

The total number of foliar flavonoids present in the variety is seven. They are represented by spot nos. 2, 17, 18, 20, 21, 22 and 24 (Fig. 32). Most of these spots are medium sized, spot no. 2 is small and no. 17 is large. Variation in the intensity of colouration in these spots is depicted in Table 2.

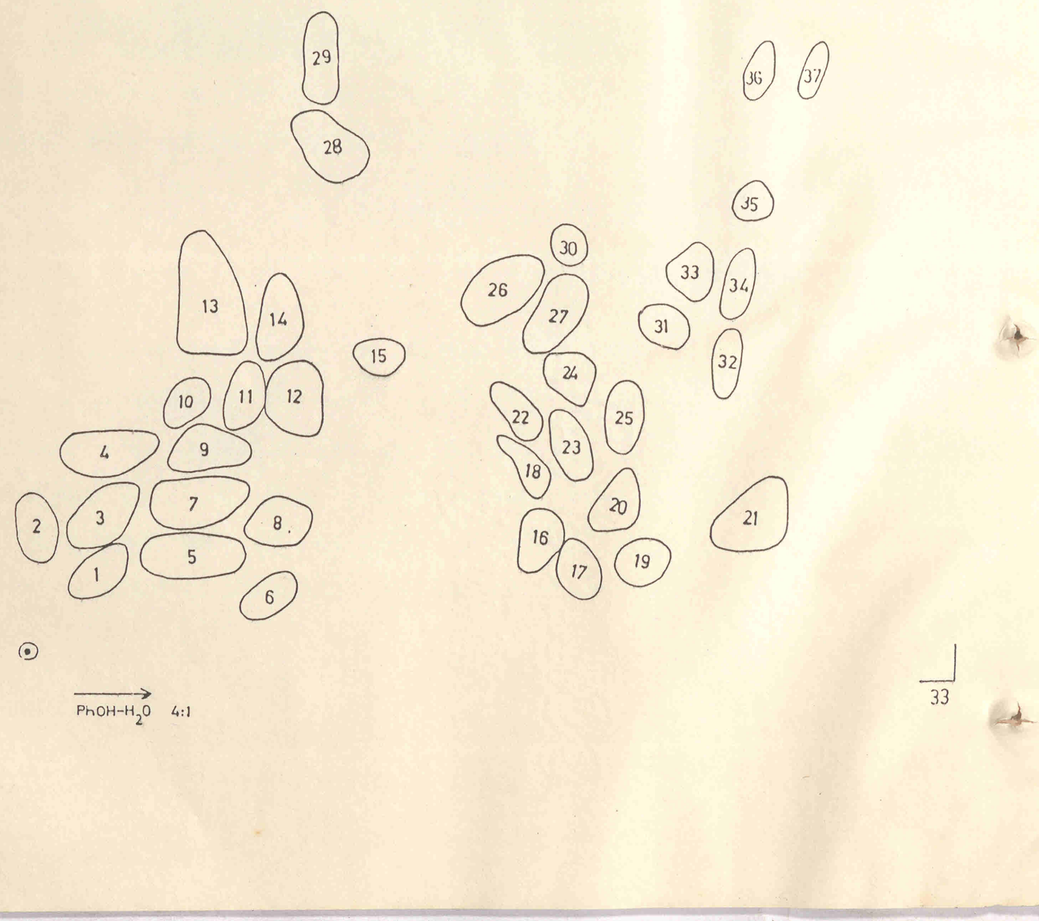
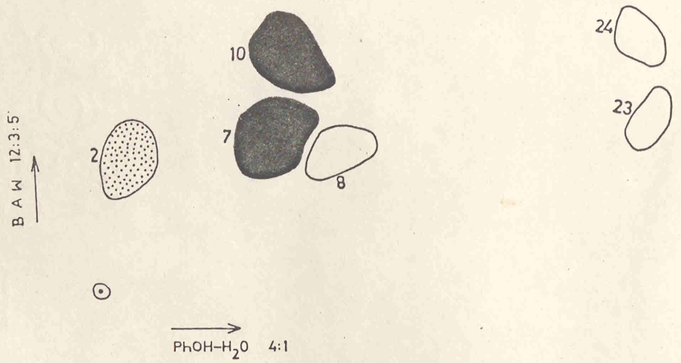


Fig.33 Reconstructed bidimensional aminogram of the test sample depicting a total 40 spots.

Fig.34 Aminogram of the cv. Ambri.

Soln.I - BAW - Butanol, acetic acid and water.

Soln.II - PhOH-H_2O - Phenol, water.



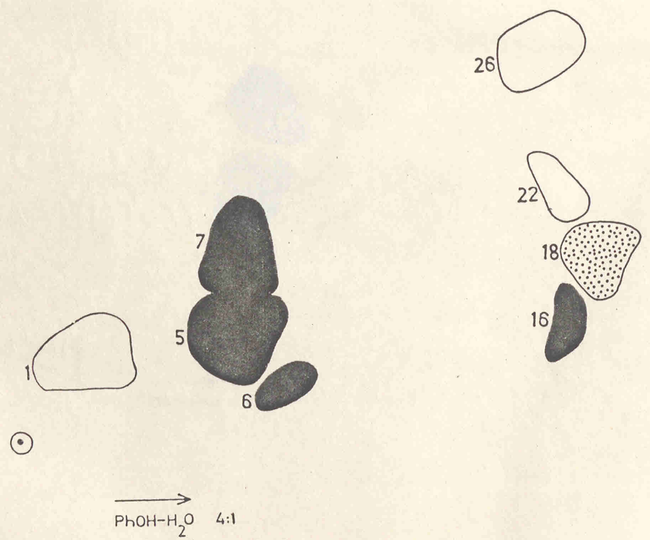


Fig.35 Chromatogram of cv. American Trail showing ninhydrin-positive compound profile.

IV.3 Analysis of ninhydrin-positive compounds

The 15 varieties screened for ninhydrin positive compounds bear a total of forty spots (Table 3, Fig. 33), each spot representing a different compound. Identification of spots has been made on the basis of their size, staining characteristics and location on composite chromatogram. The number of spots ranges between 6 and 12 per variety. Intervarietal differences also exist in the size and staining intensity of individual spots. The varietal profiles are described in the following pages.

Ambri :

Aminogram of this variety has six ninhydrin positive spots bearing nos. 2, 7, 8, 10, 23 and 24 (Table 3, Fig. 34). Five spots are purple; no. 23 alone is red. The six spots differ in the intensity of colour and in size.

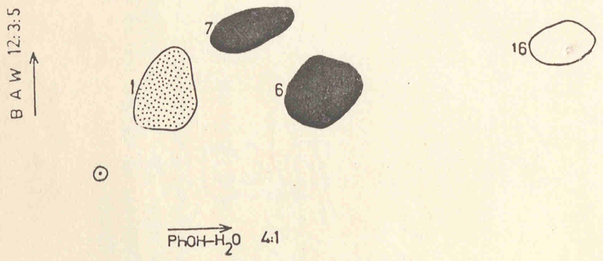
American Trail :

The spots detected in aminogram of this variety have been listed in Table 3 and illustrated in Fig. 35. The ninhydrin-positive compounds present in

Table 3. Ninhydrin-positive compound profiles of the 15 varieties of apple.

S. No.	Varietal name	Spot number and colour																																								Total no. of spots/ variety
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
		R	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	R	P	P	P	R	R	P	P	R	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
1.	Ambri	-	mm	-	-	-	-	ld	ml	-	ld	-	-	-	-	-	-	-	-	-	-	-	-	sl	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
2.	American Trail	ml	-	-	-	ld	sd	ld	-	-	-	-	-	-	-	-	sd	-	ml	-	-	-	sl	-	-	-	ml	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
3.	Blood Red	lm	-	-	-	-	ld	md	-	-	-	-	-	-	-	sm	ml	-	-	-	-	-	-	-	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
4.	Chamura	-	-	mm	-	-	-	-	md	-	-	mm	lm	-	-	-	-	-	-	-	sm	-	-	sl	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
5.	Dil Ruba	sm	-	-	-	md	-	-	mm	-	-	-	-	-	-	-	mm	-	sl	-	-	-	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
6.	Double Kesari	-	-	-	sm	-	-	-	-	-	ld	-	-	ld	sl	-	-	-	-	-	-	-	-	-	md	-	mm	sl	md	md	-	-	-	-	-	sl	-	-	-	sl	sl	12
7.	Golden Delicious	-	-	mm	-	-	-	-	ld	-	-	md	-	-	-	-	-	-	-	-	-	-	-	md	sm	-	-	-	-	-	-	-	sl	-	sl	-	-	-	-	-	-	7
8.	Gole Delicious	-	ld	-	-	-	-	mm	md	-	-	-	mm	mm	-	-	-	-	-	-	-	-	sd	sd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
9.	Hazratbali-II	-	-	ml	-	-	-	ld	-	md	-	-	-	-	-	-	sm	-	sl	-	-	-	-	-	-	-	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
10.	Kagzi Kharoo	lm	-	-	-	md	-	md	md	mm	sl	-	mm	-	-	-	-	-	-	-	ml	-	-	-	-	sm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
11.	Kharoo	-	-	-	ml	-	-	-	-	-	-	-	md	-	md	sl	-	-	-	-	-	-	-	-	md	-	-	mm	-	sl	sl	-	-	sl	-	-	-	-	-	-	-	9
12.	Maharaji	-	-	-	sl	-	-	-	-	-	-	ld	-	md	sl	-	-	-	-	-	-	-	sm	-	sl	-	-	ml	-	-	-	-	-	sl	-	-	-	-	-	-	-	8
13.	Razakwari	-	-	-	-	sl	ld	ld	-	-	-	-	-	-	-	-	sd	sd	-	ml	-	sl	-	mm	-	-	-	-	-	-	-	-	-	-	-	-	sl	sl	sl	-	-	11
14.	Red Delicious	md	-	-	-	-	-	ld	ld	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mm	-	mm	-	-	sl	-	-	-	-	-	-	-	-	-	-	-	-	6
15.	Royal, Delicious	-	ml	md	-	-	-	md	-	md	-	md	-	-	-	-	-	-	-	-	-	-	-	sl	sd	-	-	sl	-	-	-	-	-	-	-	-	-	-	-	-	-	8

Abbreviations :- ld - large and dark; md - medium and dark intensity; sd - small and dark; lm - large and of medium intensity; mm - medium sized and medium intensity; sm - small and of medium intensity; ll - large and light; ml - medium and light; sl - small and light.
R = Red; P = Purple.



36

Fig.36 Aminogram of cv. Blood Red after chemical treatments.

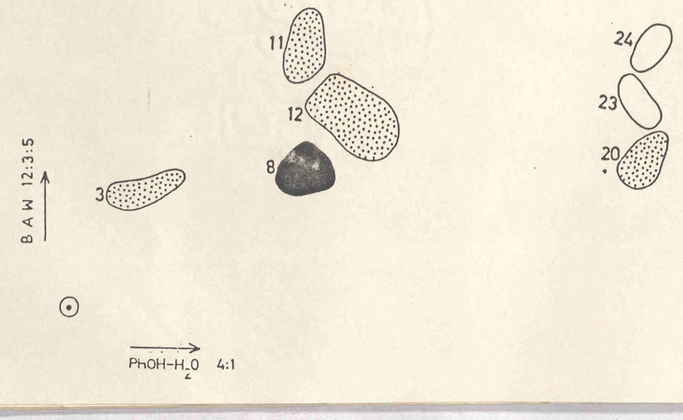


Fig.37 Bidimensional aminogram of cv. Chamura showing amino acid profile following treatment with detecting agents.

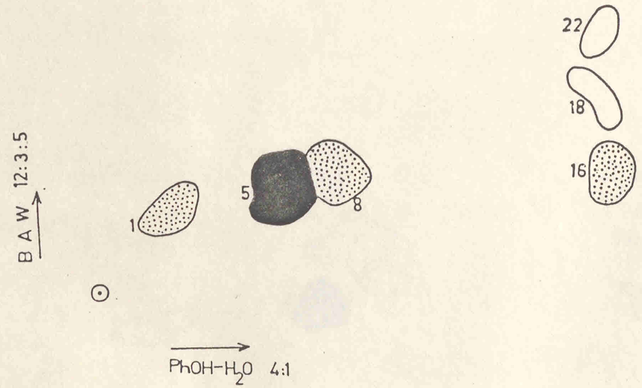


Fig.38 Aminogram of cv. Dil Ruba.

the variety are represented by spots 1,5,6,7,16,18,22 and 26. Amongst these nos. 5,6,7 and 16 are dark. The rest are light red. The spots vary a good deal in size.

Blood Red :

The chromatograms of this variety carry six spots, nos. 1,6,7,15,16 and 24. Spot 6 and 7 are dark purple, nos. 1 and 15 are medium intensity and nos. 16 and 24 are light purple in colour. The variety shares four spots bearing nos. 1,6,7 and 16 with American Trail (Table 3, Fig. 36) but they do not agree in size and colour intensity.

Chamura :

Fig. 37 which represents the aminogram of the variety has spot nos. 3,8,11,12,20,23 and 24. These spots exhibit variation in colour intensity and size. The variety shares three spots with Ambri (nos. 8, 23 and 24).

Dil Ruba :

The ninhydrin-positive spots identified in the variety are illustrated in Fig. 38. Six spots bearing nos. 1,5,8,16,18 and 22 characterise the variety. Out

Fig.39 Tracing of the paper chromatograph of cv. Double Kesari showing the amino acid profile.

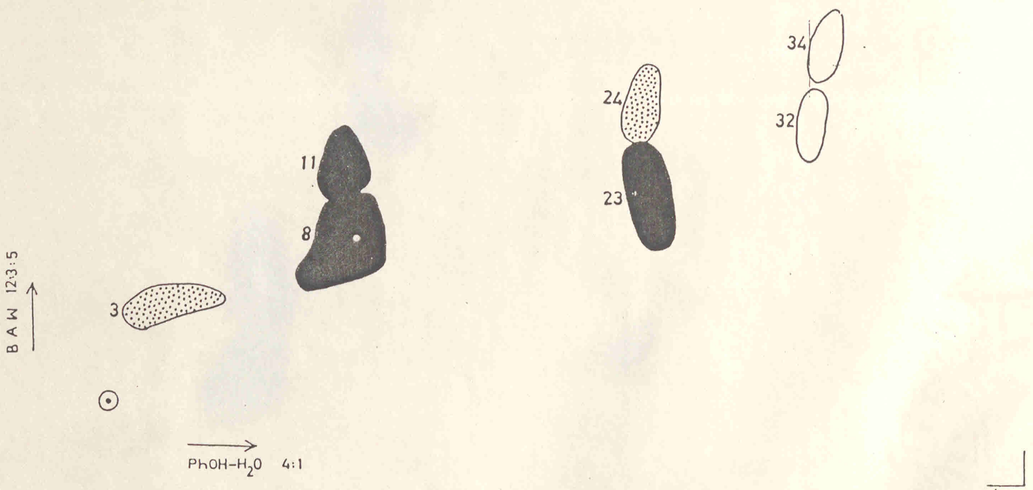


Fig.40 Aminogram of cv. Golden Delicious.

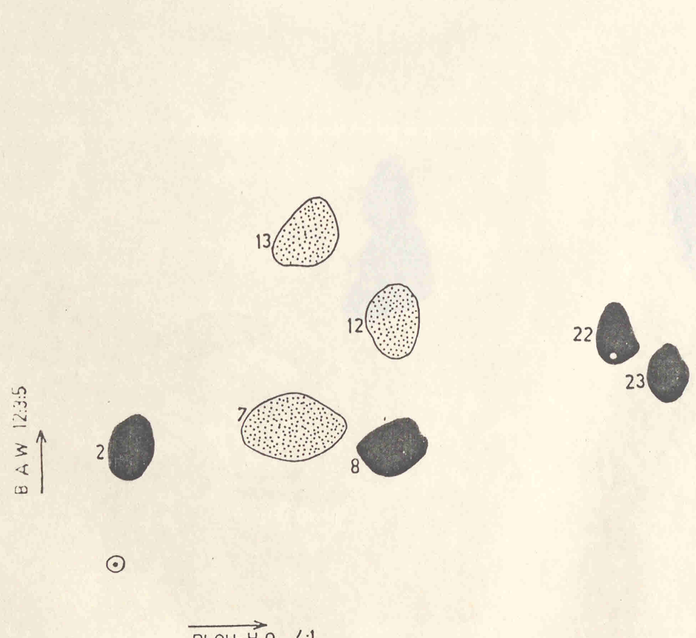


Fig.41 Amino acid profile of cv. Gole Delicious.

of these, three spots 1,8 and 16 are of medium intensity, two nos. 18 and 22 are light red , one no. 5 is dark purple. So far as the spot size is concerned, three are medium sized and three are small. The variety shares 5 spots (no. 1,5,16,18 and 22) with American Trail and three spots (nos. 1,5 and 8) with Kagzi-Kharoo.

Double Kesari :

The ninhydrin-positive spot profile of this variety is represented in Fig. 39. The variety has a total of twelve spots in its aminogram which exhibit variation in size, colour intensity and location. Spot nos. 35, 39 and 40 are characteristic of this variety.

Golden Delicious :

The variety has a total of seven spots, nos. 3, 8, 11, 23, 24, 32 and 34 (Fig. 40) of which only one spot is large and of dark intensity; spot nos. 3, 11 and 23 are medium sized and nos. 24, 32 and 34 are small. The spots are medium or light coloured and nos. 32 and 34 are unique to the variety.

Gole Delicious :

Fig. 41 shows the seven ninhydrin-positive

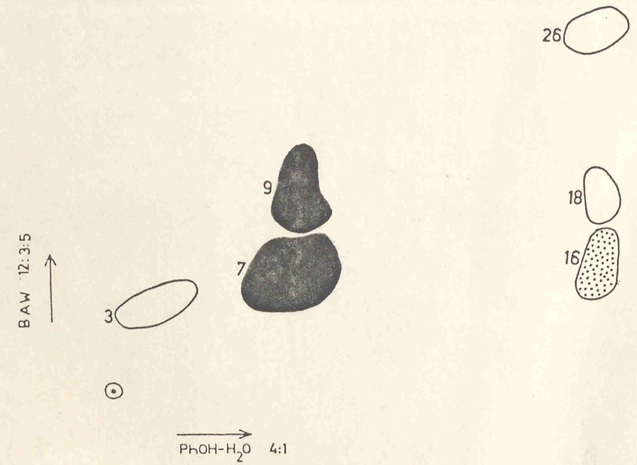
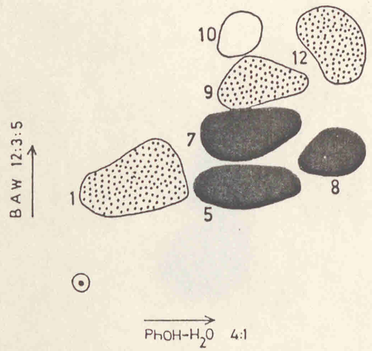


Fig.42 Aminogram of cv. Hazratbali-II following treatment with various chemicals.



43

Fig.43 Aminogram of cv. Kagzi-Kharoo.
Soln.I - BAW - Butanol, acetic acid and water.
Soln.II - PhOH-H_2O - Phenol - water.

spots detected in the aminogram of this variety. These bear nos. 2, 7, 8, 12, 13, 22 and 23. Three of these spots are of medium size and colour intensity, nos. 2 and 8 are large and medium sized respectively but both are dark purple in colour. Spot nos. 22 and 23 are small but dark coloured.

Hazratbali-II :

Fig. 42 depicts the ninhydrin-positive compound pattern of this variety. The variety bears a total of nine spots in its aminogram; they bear nos. 2, 3, 7, 9, 16, 18, 22, 23 and 26. Colour intensity and size of the spots exhibit variation; spot nos. 3 and 9 are medium sized, no. 7 is large and nos. 16, 18 and 26 are small. Spot nos. 7 and 9 are dark purple, no. 16 is medium purple and rest are light coloured.

Kagzi-Kharoo :

The variety bears a total of nine spots in its aminogram; they bear nos. 1, 5, 7, 8, 9, 10, 12, 20 and 25. Most of the spots are medium sized, nos. 10 and 25 are small and only one, no. 1 is large (Fig. 43).

spots detected in the aminogram of this variety. These bear nos. 2, 7, 8, 12, 13, 22 and 23. Three of these spots are of medium size and colour intensity, nos. 2 and 8 are large and medium sized respectively but both are dark purple in colour. Spot nos. 22 and 23 are small but dark coloured.

Hazratbali-II :

Fig. 42 depicts the ninhydrin-positive compound pattern of this variety. The variety bears a total of six spots i.e. nos. 3, 7, 9, 16, 18 and 26. Colour intensity and size of the spots exhibit variation; spot nos. 3 and 9 are medium sized, no. 7 is large and nos. 16, 18 and 26 are small. Spot nos. 7 and 9 are dark purple, no. 16 is medium purple and rest are light coloured.

Kagzi-Kharoo :

The variety bears a total of nine spots in its aminogram; they bear nos. 1, 5, 7, 8, 9, 10, 12, 20 and 25. Most of the spots are medium sized, nos. 10 and 25 are small and only one, no. 1 is large (Fig. 43).

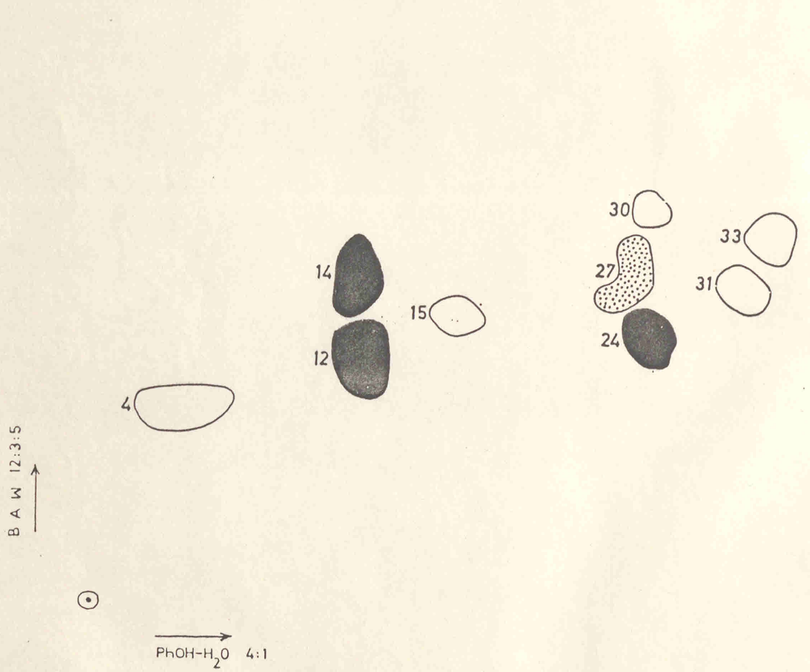


Fig.44 Chromatogram of cv. Kharoo showing amino acid separation.

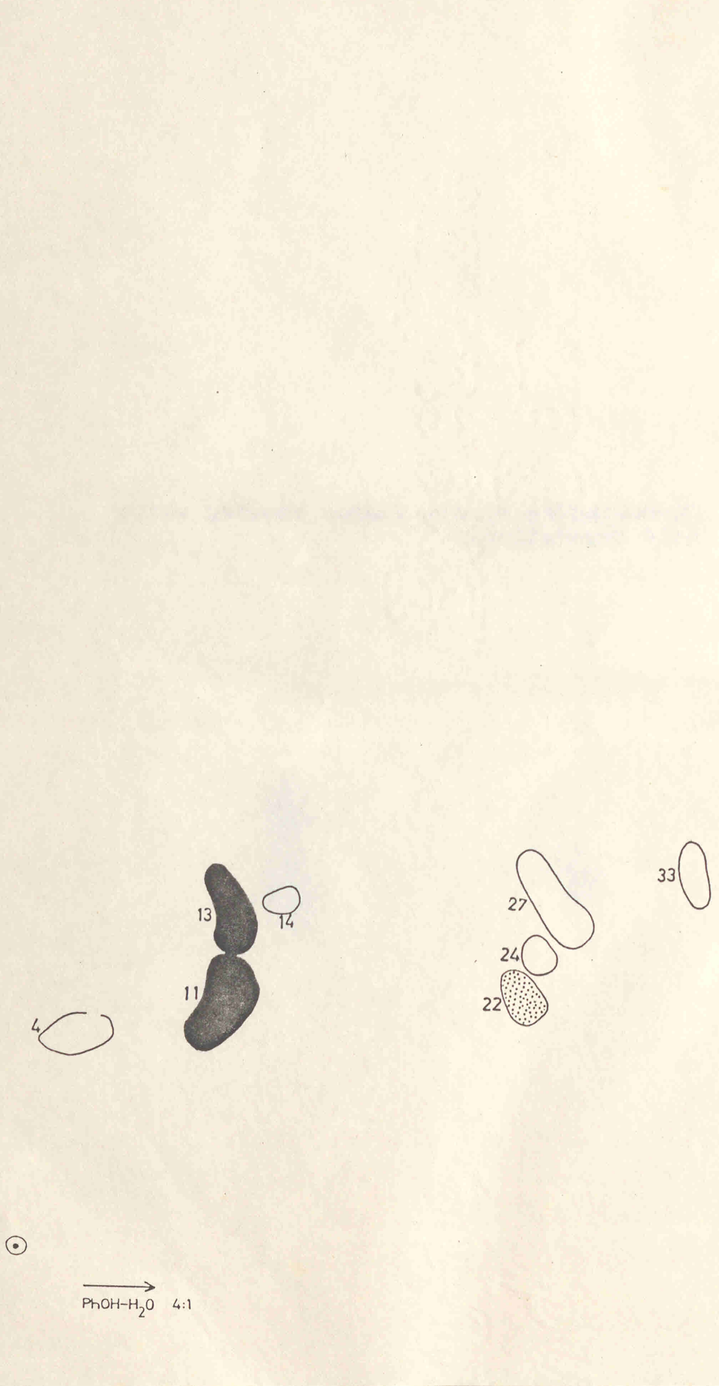


Fig.45 Chromatogram of cv. Maharaji showing amino acid compound profile.

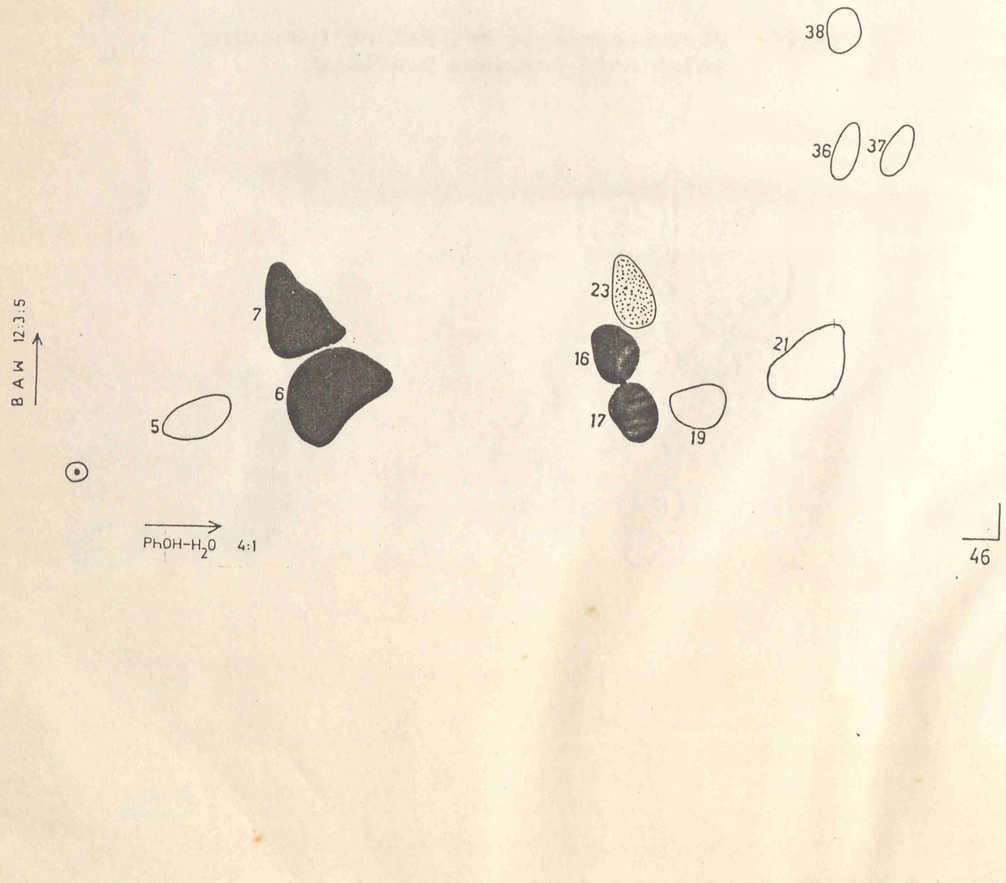


Fig.46 Aminogram of cv. Nazakwari.

Kharoo :

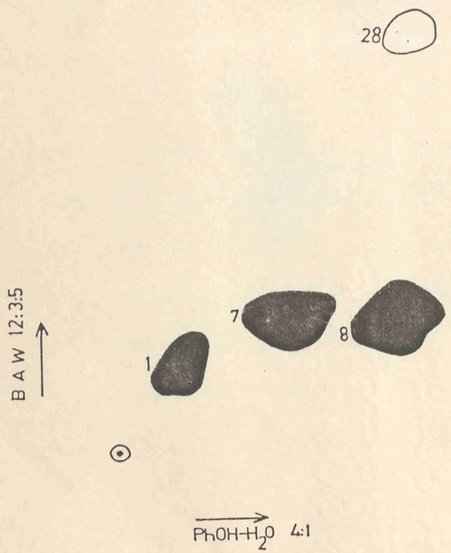
The spots detected in the aminogram of this variety listed in Table 3 and illustrated in Fig. 44 are nos. 4, 12, 14, 15, 24, 27, 29, 30 and 33. Out of these, five spots are medium sized and the rest are small; spot no. 30 is unique to this variety.

Maharaji :

The aminogram of this variety bears a total of eight spots (Fig. 45), nos. 4, 11, 13, 14, 22, 24, 27 and 33. Most of these spots are small, two (nos. 13, 27) are medium sized and one (no. 11) is large. Apart from size these spots also vary with regard to the intensity of colour.

Razakwari :

The ninhydrin-positive spot profile of this variety is illustrated in Fig. 46. The spots detected in the aminogram of this variety bear nos. 5, 6, 7, 16, 17, 19, 21, 23, 36, 37 and 38. It shares most of the spots with one variety or the other. However, spot nos. 17, 21, 36, 37 and 38 are unique to this variety. As in other varieties the size and colour intensity of individual spots varies.



47

Fig.47 Aminogram of cv. Red Delicious showing amino acid profile.

Fig.48 Aminogram of cv. Royal Delicious, after various chemical treatment.

Red Delicious :

The chromatogram of this variety carries six spots (Fig. 47), which bear nos. 1, 7, 8, 23, 25 and 28. The spots vary in colour and size.

Royal Delicious :

Aminograms of the variety bear eight spots, spot nos. 2, 3, 7, 9, 11 are medium sized while nos. 23, 24 and 27 are small (Fig. 48). The colour intensity of spots ranges from light to dark purple except no. 23 which is light red.

IV.4 Quantitative estimation of carbohydrates

Carbohydrates constitute a major constituent of apple fruit. Fruits of the 15 varieties listed at page 6 have been evaluated for various types of sugars immediately after they were picked and following storage at room temperature and at 2-4°C. The findings are detailed below :

IV.4.1 Reducing sugars :

The quantity of reducing sugars has been estimated in freshly picked fruits stored at room temperature and at 2-4°C (in cold storage). Evaluation

of the fruit immediately after they were picked from the trees has revealed that fruit of cv. Gole Delicious contain maximum quantity of reducing sugars (29.96 mg) followed by those of Red Delicious (22.81 mg) and Ambri (22.13 mg). Fruits of cv. Chamura contain the least amount i.e. 11.73 mg (Table 4).

After collecting data on the quantity of sugars in the freshly picked fruits, comparison was made with the quantity present in fruits stored at room temperature and in cold storage 2-4°C, for varying periods.

After storage for 30 days at room temperature fruits of cv. Ambri, were estimated to contain 39.71 mg of reducing sugar followed by cvs. Golden Delicious (38.51 mg) and Royal Delicious (34.40 mg). The quantity of reducing sugar is low in fruits of other varieties; the least (18.77 mg) being in cv. Kharoo. Fruits of only 4 of the 15 varieties constituting the sample could be evaluated after storage for 60 and 90 days; others could not stand storage for this long. Following 120 days of storage, fruits of Red Delicious and American Trail, were estimated to have 29.77 mg and 27.86 mg of reducing sugars respectively (Table 4).

Table 4. Reducing sugar content in fresh pomes and those stored at room temperature for varying periods.

S. No.	Name of variety	Reducing sugar content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	22.13	39.71	NA				
2.	American Trail	18.29	32.29	33.45	33.73	27.86	NA	
3.	Blood Red	16.42	27.33	NA				
4.	Chamura	11.73	NA					
5.	Dil Ruba	18.67	28.44	NA				
6.	Double Kesari	20.21	24.54	NA				
7.	Golden Delicious	19.55	38.51	NA				
8.	Gole Delicious	29.96	31.20	36.86	34.22	NA		
9.	Hazratbali-II	15.35	22.67	NA				
10.	Kagzi-Kharoo	13.78	20.71	NA				
11.	Kharoo	13.56	18.77	NA				
12.	Maharaji	17.20	26.37	27.67	22.22	NA		
13.	Razakwari	19.23	20.67	NA				
14.	Red Delicious	22.81	25.79	32.77	33.13	29.77	NA	
15.	Royal Delicious	15.70	34.40	NA				

*mg/g of dry weight

NA - Not available/perished during storage.

After storage for 30 days at 2-4°C, fruits of the varieties tested, registered increase in sugar content over that present in the freshly picked fruits. The maximum quantity was estimated in stored fruits of cv. Gole Delicious and the least in fruits of cv. Chamura. In the remaining 13 varieties the sugar content varied between the two extremes referred above. Changes in sugar content continue as the storage period extends. The quantity of reducing sugars in fruits of all varieties registers increase upto 4 months of storage at 2-4°C. Storage beyond this period has adverse effect on sugar content in fruits of cvs. Kagzi Kharoo, Kharoo, Razakwari and Royal Delicious. Table 5 summarises the data collected from stored fruits of different varieties at an interval of one month.

IV.4.2 Total free sugars :

Total free sugars have also been estimated from, freshly harvested fruits and those stored for varying periods at and below room temperature. From within the freshly picked sample, fruits of cv. Ambri were found to contain the maximum quantity i.e. 26.22 mg.

Table 5. Data on reducing sugar content of fresh and cold stored pomes of 15 varieties.

S. No.	Name of variety	Reducing sugar content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	22.13	23.01	26.31	27.81	30.27	30.68	NA
2.	American Trail	18.29	24.96	29.60	29.44	36.68	25.71	22.28
3.	Blood Red	16.42	18.49	18.79	18.69	19.65	27.26	34.89
4.	Chamura	11.73	14.63	16.96	18.69	20.66	22.14	NA
5.	Dil Ruba	18.67	22.86	33.55	33.90	36.67	22.12	19.61
6.	Double Kesari	20.21	21.53	21.57	24.33	26.26	23.18	16.79
7.	Golden Delicious	19.55	21.87	23.48	24.48	26.67	30.14	30.44
8.	Gole Delicious	29.96	36.78	38.03	38.90	39.69	30.05	21.23
9.	Hazratbali-II	15.35	17.15	18.84	20.27	21.08	NA	NA
10.	Kagzi-Kharoo	13.78	17.01	21.54	24.10	23.71	17.63	16.27
11.	Kharoo	13.56	16.98	20.23	22.00	21.62	16.25	13.87
12.	Maharaji	17.20	20.94	26.56	27.97	29.46	18.32	15.11
13.	Razakwari	19.23	23.55	23.98	24.35	20.38	25.18	NA
14.	Red Delicious	22.81	25.11	28.90	37.22	38.35	25.05	21.86
15.	Royal Delicious	15.70	20.98	22.62	24.29	23.01	32.24	34.13

*mg/g of dry weight

NA - Not available/perished during storage.

Fruits of only 14 varieties withstood 30 days storage at room temperature, those of cv. Chamura perish for which reason they could not be assessed for total free sugars. From the remaining 14 varieties, pomes of Royal Delicious contain highest sugar content (45.29 mg) followed by those of cv. Blood Red (37.87 mg). Least quantity of free sugars i.e. 18.5 mg exist in stored fruits of Dil Ruba. As the storage period is extended to 60 and 90 days, fruits of 10 more varieties perish; leaving these of only four varieties for evaluation. Out of these, cv. Maharaji has the highest quantity. Only two varieties, namely American Trail and Red Delicious withstood storage for 4 months; these fruits contained 44.15 mg and 36.02 mg of total free sugars respectively (Table 6).

The fruit placed in cold storage at a temperature of 2-4°C were analysed for free sugars at intervals of 30 days. At the end of first 30 days fruits of cvs. Gole Delicious, American Trail and Ambri were found to contain 36.78 32.94 and 32.60 mg of free sugars. Variety Kharoo, had the least quantity. 60 days storage in the cold store did not alter the profile of free sugars from what it was in fruits stored for 30 days. However, extended storage, for 90 days altered

Table 6. Total free sugar content in fresh pomes and those stored for varying periods at room temperature.

S. No.	Name of variety	Total free sugar content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	26.22	28.32	NA				
2.	American Trail	12.55	27.63	31.10	36.33	44.15	NA	
3.	Blood Red	18.51	37.87	NA				
4.	Chamura	22.52	NA	NA				
5.	Dil Ruba	14.72	18.57	NA				
6.	Double Kesari	19.36	26.41	NA				
7.	Golden Delicious	13.86	29.91	NA				
8.	Gole Delicious	10.28	29.05	31.95	31.98	NA		
9.	Hazratbali-II	9.08	29.11	NA				
10.	Kagzi-Kharoo	9.09	33.53	NA				
11.	Kharoo	9.52	20.99	NA				
12.	Maharaji	16.85	27.27	36.21	36.66	NA		
13.	Razakwari	11.21	22.05	NA				
14.	Red Delicious	17.74	29.01	30.99	33.88	36.02	NA	
15.	Royal Delicious	16.00	45.29	NA				

*mg/g of dry weight

NA - Not available.

the situation; cv. American Trail overtook Gole Delicious in sugar content; cvs. Red Delicious, Ambri and Dil Ruba following closely behind. Fruits of cv. Razakwari had the least sugar content. Following storage for 120 days, fruits of cv. Dil Ruba exceed those of other varieties in sugar content; fruits of American Trail and Red Delicious, occupy second and third positions. Prolonged storage for a period of 150 days results in reducing the sugar content. Thus, Royal Delicious fruit bore 33.28 mg and those of cv. Blood Red contained 17.48 mg. Fruits of only 11 varieties withstood storage of 180 days. Analysis of these varieties revealed highest sugar content (25.32 mg) in Golden Delicious followed by Royal Delicious. Variety Kharoo had the least quantity of free sugars (Table 7).

IV.4.3 Sucrose :

Data on the quantitative estimation of sucrose content, collected from fruits of 15 varieties, through spectrophotometry, are summarised in Tables 8,9. Perusal of these data reveals highest sucrose content (14.70 mg) in fruits of American Trail and least (4.69 mg) in Hazratbali-II.

Fruits of all the 15 varieties referred above

Table 7. Data on total free sugar content of fresh pomes and those stored for varying periods at 2-4°C.

S. No.	Name of variety	Total free sugar content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	26.22	32.60	33.20	33.55	33.24	25.13	NA
2.	American Trail	12.55	32.94	34.21	38.53	44.08	27.21	16.55
3.	Blood Red	18.51	19.65	21.07	22.04	27.77	17.48	18.95
4.	Chamura	22.52	22.86	25.45	27.44	26.69	24.64	NA
5.	Dil Ruba	14.72	27.50	27.68	33.29	46.45	26.96	17.83
6.	Double Kesari	19.36	27.96	25.81	27.41	28.80	21.59	14.82
7.	Golden Delicious	13.86	22.91	23.13	24.28	28.65	31.12	25.32
8.	Gole Delicious	10.28	36.78	38.03	37.46	37.19	25.10	20.54
9.	Hazratbali-II	9.08	14.79	18.25	21.44	20.53	NA	NA
10.	Kagzi-Kharoo	9.09	11.80	17.23	20.70	21.98	27.04	21.62
11.	Kharoo	9.52	11.04	13.49	18.16	23.72	26.71	12.33
12.	Maharaji	16.85	17.39	19.68	24.96	35.71	25.08	20.99
13.	Razakwari	11.21	12.37	14.38	15.84	15.46	18.42	NA
14.	Red Delicious	17.74	33.49	34.80	36.63	37.77	26.15	20.03
15.	Royal Delicious	16.00	18.91	19.72	20.33	21.70	33.28	22.84

*mg/g of dry weight

NA - Not available/perished during storage.

were stored at room temperature in order to compare the survival and study the impact on sucrose content. As in other experiments, so also in the present case, fruits of cv. Chamura could not survive storage even for a month. From among the remaining 14 varieties, stored fruits of cv. Golden Delicious have highest sucrose content (19.60 mg) followed by Gole Delicious (16.14 mg). The least sucrose content exists in sub-acidic fruits of Kharoo (Table 8). Longer storage, spanning 60 days, spoilt fruits of all varieties except four; sucrose content in fruits of these four is 17.20 mg in Gole Delicious, 14.37 mg in American Trail and 11.48 mg each in Maharaji and Red Delicious. Fruits of only four varieties, namely American Trail, Gole Delicious, Maharaji and Red Delicious survived 90 days storage.

When the fruits stored at 2-4°C for 30 days were assessed for sucrose content, pomes of Blood Red were found to contain 17.63 mg while those of American Trail, Kagzi Kharoo and Razakwari contained approximately 15 mg each. The sub-acidic fruits of Hazratbali-II have the least quantity i.e. 4.73 mg. The data collected from fruits stored for 60, 90 and 120 days reveal regular increase in sucrose content during storage, the quantum of increase varying from one variety to another (Table 9).

Table 8 Sucrose content in fresh pomes and those stored at room temperature for varying periods.

S. No.	Name of variety	Sucrose content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	14.30	13.23	NA				
2.	American Trail	14.70	13.97	14.37	16.14	4.41	NA	
3.	Blood Red	12.54	15.66	NA				
4.	Chamura	11.10	NA					
5.	Dil Ruba	10.05	11.61	NA				
6.	Double Kesari	11.10	13.06	NA				
7.	Golden Delicious	11.37	19.60	NA				
8.	Gole Delicious	11.62	16.14	17.20	20.17	NA		
9.	Hazratbali-II	4.69	11.75	NA				
10.	Kagzi-Kharoo	10.19	11.72	NA				
11.	Kharoo	8.08	9.14	NA				
12.	Maharaji	9.83	10.49	11.48	12.36	NA		
13.	Razakwari	13.14	13.81	NA				
14.	Red Delicious	10.35	11.13	11.43	12.80	10.80	NA	
15.	Royal Delicious	9.23	15.66	NA				

*mg/g of dry weight

NA - Not available/perished.

Table 9. Sucrose content in fresh pomes and those stored at 2-4°C for varying periods.

S. No.	Name of variety	Sucrose content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	14.30	14.38	16.29	17.23	17.56	5.55	NA
2.	American Trail	14.70	15.60	15.78	17.54	21.27	5.38	9.44
3.	Blood Red	12.54	17.63	17.93	18.41	18.60	10.80	8.84
4.	Chamura	11.10	12.19	14.54	16.28	16.64	12.45	NA
5.	Dil Ruba	10.05	10.93	15.30	15.41	23.22	12.72	7.13
6.	Double Kesari	11.10	11.46	11.97	17.01	17.28	5.68	2.76
7.	Golden Delicious	11.37	13.19	13.81	14.70	16.13	17.36	11.21
8.	Gole Delicious	11.62	12.26	13.79	14.76	21.10	4.24	4.45
9.	Hazratbali-II	4.69	4.73	5.88	7.05	11.50	NA	NA
10.	Kagzi-Kharoo	10.19	14.54	16.42	17.03	16.79	5.87	3.95
11.	Kharoo	8.08	11.89	12.97	17.40	8.37	6.45	3.08
12.	Maharaji	9.83	10.95	11.87	11.73	15.17	16.34	9.23
13.	Razakwari	13.14	15.09	15.58	15.55	14.29	2.09	NA
14.	Red Delicious	12.35	12.55	15.92	16.41	17.30	3.85	7.82
15.	Royal Delicious	9.23	9.75	12.76	15.81	15.16	16.76	7.70

*mg/g of dry weight

NA - Not available/perished.

Except fruits of Hazratbali-II, those of all others withstand storage for 150 days. From within the fruits stored for 5 months, Golden Delicious and Royal Delicious pomes which are both sweet contain maximum quantity of sucrose; Razakwari has the least i.e. 2.09 mg. During this period, the sucrose content suffers reduction in most of the varieties. Further storage is withstood by fruits of 11 varieties; in all these the sucrose content reduces further. The data have been listed in Table 9.

IV.4.4 Fructose :

In freshly harvested material, the amount of fructose is maximum in var. Blood Red (17.02 mg) followed by Ambri (15.30 mg). Two varieties, American Trail and Dil Ruba, contain almost the same quantity (14.7 mg). Fructose content in sub-acidic fruits of cv. Hazratbali-II is very low (5.32 mg) (Tables 10, 11).

The fruits picked from different varieties were stored at room temperature ranging between 15-33°C in order to assess changes caused to fructose content by storage. The studies conducted on fruits stored for 30 days reveals highest fructose content in cv. Royal

Table 10. Fructose content in fresh and apples stored at room temperature for varying periods.

S. No.	Name of variety	Fructose content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	15.32	31.09	NA				
2.	American Trail	14.79	15.52	17.89	23.06	27.04	NA	
3.	Blood Red	17.02	33.31	NA				
4.	Chamura	7.29	NA	NA	NA	NA		
5.	Dil Ruba	14.72	26.70	NA				
6.	Double Kesari	12.81	18.94	NA				
7.	Golden Delicious	7.46	36.79	NA				
8.	Gole Delicious	12.52	15.42	26.33	21.16	NA		
9.	Hazratbali-II	5.32	12.03	NA				
10.	Kagzi-Kharoo	8.26	20.44	NA				
11.	Kharoo	10.39	18.52	NA				
12.	Maharaji	10.88	12.88	35.04	15.08			
13.	Razakwari	8.01	19.16	NA				
14.	Red Delicious	12.35	21.39	22.35	15.31	23.53	NA	
15.	Royal Delicious	9.54	37.12	NA				

* mg/g of dry weight

NA - Not available/perished.

Table 11. Fructose content in fresh and cold stored fruits.

S. No.	Name of variety	Fructose content*						
		In fresh fruit	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	15.32	17.58	19.93	21.46	28.10	20.37	NA
2.	American Trail	14.79	22.53	25.32	25.06	33.60	28.11	27.96
3.	Blood Red	17.02	19.94	21.64	23.99	25.15	27.52	24.26
4.	Chamura	7.29	14.93	17.57	19.60	25.25	15.22	NA
5.	Dil Ruba	14.72	17.57	19.21	20.65	24.14	30.30	26.75
6.	Double Kesari	12.81	16.77	17.92	18.66	19.35	20.45	20.94
7.	Golden Delicious	7.46	18.40	19.33	20.17	24.04	21.29	26.92
8.	Gole Delicious	12.52	20.17	24.85	24.98	25.39	25.81	25.68
9.	Hazratbali-II	5.32	17.45	18.55	19.09	18.89	NA	NA
10.	Kagzi-Kharoo	8.26	13.44	19.65	25.94	25.44	16.93	18.37
11.	Kharoo	10.39	11.04	17.38	18.42	24.65	21.37	18.06
12.	Maharaji	10.88	14.49	17.18	21.35	22.91	31.28	20.15
13.	Razakwari	8.01	15.39	17.38	19.07	20.14	19.35	NA
14.	Red Delicious	12.35	15.84	23.30	25.20	27.10	28.63	31.58
15.	Royal Delicious	9.54	18.32	19.14	19.49	20.65	19.86	22.07

* mg/g of dry weight

NA - Not available/perished.

Delicious (37.12 mg) followed by Golden Delicious (36.79 mg), Blood Red (33.31 mg) and Ambri (31.09 mg). The sub-acidic fruits of cv. Maharaji contained 12.88 mg. On account of rotting, fruits of only four varieties remained available for estimation of fructose after 60 days of storage. From among these, fruits of Maharaji contain the highest fructose content (35.04 mg) followed by Gole Delicious (26.33 mg). Fructose content is least in var. American Trail (17.89 mg). Following storage for 90 days, American Trail contained 23.06 mg, Gole Delicious 21.16 mg and Red Delicious 15.31 mg of fructose (Table 10). The fruits of only two varieties, namely American Trail and Red Delicious withstood 120 days storage. Their fructose content was 27.04 mg and 23.53 mg respectively.

Following storage under low temperature conditions of 2-4°C, for 30 days, highest fructose content has been estimated in pomes of American Trail (22.53 mg) followed by those of Gole Delicious (20.17 mg). The least is in sub-acidic Kharoo (11.04 mg). Even after 60 days storage, varieties American Trail and Gole Delicious maintain the lead in fructose content; cv. Maharaji has the least quantity (Table 11). In general as the storage period prolongs, fructose content increases in almost all sweet fruits and one sub-acidic (Kagzi Kharoo) variety.

IV.4.5 Starch :

The estimation of starch content was undertaken from fruits of 15 cultivated varieties. The freshly picked pomes stored at room temperature (15-33°C) and 2-4°C in cold stores for varying length of time (Tables 12, 13).

In the freshly harvested material maximum quantity of starch (249.75 mg) exists in cvs. Kagzi-Kharoo and Red Delicious, followed by Kharoo (245.25 mg), Double Kesari (236.25 mg) and Gole Delicious (229.50 mg). Cv. Blood Red has the least quantity (103.5 mg). The actual quantity of starch in the various varieties screened for the purpose is listed in Table 12. When fruits of different varieties were stored in the laboratory at room temperature ranging between 15 to 33°C, lots of changes occurred in their starch content. From among the fruits, stored for 30 days, cv. Kharoo was found to contain the highest starch content of 207 mg followed by fruits of cvs. Double Kesari (198 mg), Kagzi-Kharoo (184.5 mg), Red Delicious (166.5 mg), Dil Ruba (132.75 mg) and Gole Delicious (121.5 mg). The comparison of the figures collected shows that least quantity of starch (54 mg) has been observed in stored pomes of cv. Royal Delicious. All other varieties fall in between so far as starch content is concerned. Fruit of only 4

Table 12. Starch content in fresh pomes and those stored at room temperature.

S. No.	Name of variety	Starch content*					
		In fresh fruits	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days
1.	Ambri	130.50	58.50	NA			
2.	American Trail	207.00	189.00	99.00	69.75	63.00	NA
3.	Blood Red	103.50	47.25	NA	NA		
4.	Chamura	157.50	NA				
5.	Dil Ruba	200.20	132.75	NA			
6.	Double Kesari	236.25	198.00	NA	NA		
7.	Golden Delicious	119.25	63.00	NA	NA		
8.	Gole Delicious	229.50	121.50	78.75	51.75	NA	
9.	Hazratbali-II	112.50	56.25	NA	NA		
10.	Kagzi Kharoo	249.75	184.50	NA			
11.	Kharoo	245.25	207.00	NA			
12.	Maharaji	166.50	110.25	92.25	87.75	NA	
13.	Razakwari	105.75	87.75	NA			
14.	Red Delicious	249.75	166.50	81.00	56.25	49.50	NA
15.	Royal Delicious	162.00	54.00	NA	NA		

*mg/g of dry weight.

NA - Not available/perished.

Table 13. Date on starch content of fresh and cold stored pomes of 15 varieties.

S. No.	Name of variety	Starch content*						
		In fresh fruits	After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	130.50	101.25	92.25	74.25	60.75	51.75	NA
2.	American Trail	207.00	171.00	101.25	94.50	90.00	76.50	54.00
3.	Blood Red	103.50	81.00	74.25	67.50	60.75	45.00	34.05
4.	Chamura	157.50	112.50	99.00	87.75	51.75	42.75	NA
5.	Dil Ruba	200.20	139.50	74.25	69.75	60.75	54.00	45.00
6.	Double Kesari	236.25	200.25	123.75	94.50	83.25	76.50	69.75
7.	Golden Delicious	119.25	94.50	76.50	56.25	47.25	40.50	31.27
8.	Gole Delicious	229.50	146.25	94.50	51.75	49.50	47.25	38.25
9.	Hazratbali-II	112.50	99.00	87.70	78.75	63.00	NA	NA
10.	Kagzi Kharoo	249.75	238.50	121.50	96.75	76.50	75.00	63.00
11.	Kharoo	245.25	175.50	112.50	96.75	78.75	67.50	26.00
12.	Maharaji	166.50	130.50	110.25	76.50	56.25	47.25	29.20
13.	Razakwari	105.75	94.50	83.25	76.50	63.00	24.75	NA
14.	Red Delicious	249.75	186.75	112.50	101.25	87.75	81.00	38.25
15.	Royal Delicious	162.00	123.75	103.50	76.50	56.25	29.25	27.12

*mg/g of dry weight.

NA - Not available/perished.

varieties withstood 60 days storage at room temperature; others perished. From among the surviving pomes, those of cv. American Trail contained maximum and cv. Gole Delicious the minimum content. In extended storage for 90 days pomes of two more varieties became a casuality : those of only two varieties survived. Out of the survivors, fruits of cv. Maharaji have highest starch content and those of Gole Delicious the least. Following 120 days storage, estimation was possible only in fruits of American Trail and Red Delicious which were found to contain 63 and 49.5 mg starch respectively (Table 12).

Fruits placed in cold stores at 2-4°C contain less starch as compared to the fresh fruit. In general, the starch content keeps on reducing as the storage period increases (Table 13). Following 30 days storage, reduction in starch content was maximum in fruits of Blood Red (81.00 mg) followed by those of Golden Delicious and Razakwari (94.50 mg) each; Kagzi-Kharoo and Double Kesari fruits suffered least change. In other varieties the reduction in starch content is of a magnitude varying between the two extremes referred above. During prolonged storage, the pattern of change in the starch content is not the same as in the first 30 days of storage.

Fruits of all varieties stand storage for five months at 2-4°C. Among the five months old pomes starch content in cv. Red Delicious is 81 mg, followed by those of cvs. American Trail, Double Kesari and Kagzi-Kharoo which contain 76.5 mg each. Razakwari contains the least quantity (24.75 mg). Fruits of only 11 varieties withstand 6 months storage; others perish. From among the survivors, pomes of Double Kesari have the highest starch content i.e. 69.75 mg, followed by those of Kagzi-Kharoo (63 mg), American Trail (54 mg) and Dil Ruba (45 mg). Fruits of Gole Delicious and Red Delicious have 38.25 mg each; Kharoo contains the least quantity (26 mg). Details are given in Table 13.

IV.5 Post-harvest changes :

IV.5.1 Shelf-life :

The shelf-life estimated in terms of rotting percentage, has been worked out in the varieties referred at page 6. The fruits were stored under two temperature regimes i.e. room temperature and cold storage. Rotting percentage was calculated separately for both sets of samples at intervals of 30 days.

a) Room temperature :

At room temperature, the loss is very high in

all varieties. Fruits of cv. Chamura suffer 100% rotting within 30 days of storage; those of cvs. Dil Ruba, Double Kesari, Kagzi-Kharoo and Kharoo perished in 60 days of storage. Pomes of some varieties namely, American Trail and Red Delicious could withstand longer storage (120 days) whereafter they perished (Table 14).

b) Cold storage :

Fruits of 15 varieties were placed inside cold store, under a temperature regime of 2-4°C immediately after they were picked. After 30 days storage at low temperature rotting percentage ranges from 8% in cvs. Blood Red and Royal Delicious to highest 30% in Chamura (Details are given in Table 15). The varietal performance varied, for example rotting recorded after 150 days in other varieties is to the tune of 100% in cv. Hazratbali-II, followed by 90% in Chamura, 84% in Razakwari, 82% in Kharoo, 70-80% in Kagzi-Kharoo and Ambri, 56% in Dil Ruba, 54% in Double Kesari and 48% in Blood Red and Royal Delicious. Only two varieties namely, American Trail and Red Delicious did not suffer any loss for storage period of 180 days.

Table 14. Rotting percentage in apples stored at room temperature.

S.No.	Name of Variety	Rotting percentage*				
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days
1.	Ambri	42%	88%	100%	NA	NA
2.	American Trail	9.5%	NL	NL	NL	NA
3.	Blood Red	26%	86%	100%	NA	NA
4.	Chamura	100%	NA	NA	NA	NA
5.	Dil Ruba	53.3%	100%	NA	NA	NA
6.	Double Kesari	46.6%	100%	NA	NA	NA
7.	Golden Delicious	14%	72%	100%	NA	NA
8.	Gole Delicious	12%	32%	60%	NA	NA
9.	Hazratbali-II	24%	80%	100%	NA	NA
10.	Kagzi-Kharoo	78%	100%	NA	NA	NA
11.	Kharoo	68%	100%	NA	NA	NA
12.	Maharaji	14%	32%	82%	100%	NA
13.	Razakwari	60%	90%	100%	NA	NA
14.	Red Delicious	4%	12%	NL	NL	NA
15.	Royal Delicious	30%	86%	100%	NA	NA

NA - Not available/perished
NL - No loss
] during storage.

Table 15. Rotting percentage in fresh and cold stored apples.

S. No.	Name of variety	Rotting percentage*					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	20%	32%	58%	70%	NA	NA
2.	American Trail	NL	NL	NL	NL	NL	NL
3.	Blood Red	8%	12%	28%	40%	48%	NL
4.	Chamura	30%	44%	52%	84%	90%	100%
5.	Dil Ruba	16%	26%	32%	46%	56%	64%
6.	Double Kesari	16%	24%	36%	42%	54%	64%
7.	Golden Delicious	NL	6%	14%	NL	NL	NL
8.	Gole Delicious	NL	6%	20%	NL	NL	NL
9.	Hazratbali-II	12%	32%	48%	88%	100%	NA
10.	Kagzi-Kharoo	22%	36%	42%	66%	70%	70%
11.	Kharoo	26%	38%	64%	72%	82%	90%
12.	Maharaji	NL	NL	18%	NL	26%	44%
13.	Razakwari	18%	28%	56%	80%	84%	100%
14.	Red Delicious	NL	NL	NL	NL	NL	NL
15.	Royal Delicious	8%	14%	36%	44%	48%	NL

NA - Not available/perished]
NL - No loss] during storage.

IV.5.2 Dry matter :

Dry matter was estimated from freshly picked fruits of all the 15 varieties, as per procedure described at page 39. Dry matter of stored fruit was estimated at intervals of 30 days, till such time the fruits developed symptoms of rotting.

50 g of the flesh of freshly picked fruit was dried at 60°C, for 15 days or till the time the weight did not change any more. The weight of dry matter left was compared. It emerged from those comparisons that the sub-acidic fruits of cv. Kagzi-Kharoo had maximum (9.070 g), dry matter followed in descending order by cv. Double Kesari (8.780 g) and Kharoo (8.660 g). Pomes of some sweet varieties, namely Gole Delicious, Dil Ruba and American Trail, leave minimum dry matter. The actual figures are listed in Tables 16, 17.

Dry matter of fruits stored at room temperature : During first 30 days of storage at ambient temperature, pomes of only cv. Chamura perished. Out of the remaining varieties, that withstand storage, maximum dry matter exists in pomes of cv. Razakwari (11.220 g), followed by those of Kharoo (10.120 g). In stored pomes of the

Table 16. Estimates of dry weight in fresh apples stored at room temperature.

S. No.	Name of variety	In fresh fruits	Pomes stored at room temperature for varying length of time					
			After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	7.340g	8.120g	12.870g	NA			
2.	American Trail	6.970g	8.050g	8.520g	8.670g	9.060g	NA	
3.	Blood Red	8.370g	8.780g	10.080g	NA			
4.	Chamura	7.880g	NA					
5.	Dil Ruba	6.960g	8.612g	NA				
6.	Double Kesari	8.780g	9.370g	NA				
7.	Golden Delicious	7.030g	7.270g	7.320g	NA			
8.	Gole Delicious	5.590g	6.970g	7.120g	8.180g	NA		
9.	Hazratbali-II	7.980g	8.930g	11.270g	NA			
10.	Kagzi Kharoo	9.070g	9.190g	NA				
11.	Kharoo	8.660g	10.120g	NA				
12.	Maharaji	7.120g	8.340g	8.490g	8.660g	NA		
13.	Razakwari	7.800g	11.220g	11.360g	NA			
14.	Red Delicious	7.890g	8.530g	9.840g	9.960g	10.410g	NA	
15.	Royal Delicious	7.120g	7.340g	7.900g	NA			

NA - Not available/perished.

Table 17. Dry weight estimates of fresh fruits of 15 varieties and that stored for varying periods at 2-4°C.

S. No.	Name of variety	In fresh fruits	Pomes stored for varying length of time					
			After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	7.340g	7.820g	7.980g	8.270g	9.250g	9.450g	NA
2.	American Trail	6.970g	7.210g	7.600g	7.980g	8.110g	8.360g	9.000g
3.	Blood Red	8.370g	8.650g	8.780g	8.960g	9.540g	9.720g	9.890g
4.	Chamura	7.880g	8.200g	8.250g	8.290g	8.710g	9.030g	NA
5.	Dil Ruba	6.960g	7.545g	7.675g	8.110g	8.180g	8.250g	8.410g
6.	Double Kesari	8.780g	8.940g	10.460g	10.580g	10.850g	11.000g	12.650g
7.	Golden Delicious	7.030g	7.200g	7.240g	7.310g	7.590g	7.630g	7.800g
8.	Gole Delicious	5.590g	6.320g	6.705g	6.940g	6.990g	7.070g	7.300g
9.	Hazratbali-II	7.980g	8.450g	8.490g	8.510g	9.130g	NA	NA
10.	Kagzi-Kharoo	9.070g	9.110g	9.285g	9.540g	10.120g	10.630g	10.750g
11.	Kharoo	8.660g	8.830g	9.635g	9.770g	10.750g	11.230g	11.350g
12.	Maharaji	7.120g	7.760g	8.000g	8.310g	8.400g	8.870g	8.930g
13.	Razakwari	7.800g	8.280g	8.340g	8.520g	10.670g	10.720g	NA
14.	Red Delicious	7.890g	8.360g	8.475g	8.530g	8.670g	9.080g	9.260g
15.	Royal Delicious	7.120g	8.460g	8.620g	8.850g	9.560g	9.690g	9.740g

NA - Not available/perished during storage.

remaining varieties the dry matter is more or less the same as for fresh pomes. Only 10 varieties survived storage at ambient temperature for 60 days. From among these, maximum dry matter exists in cvs. Ambri and Razakwari which have sweet fruits; varieties with sub-acidic fruits follow. The fruits of varieties those have longer shelf-life at room temperature bear the comparatively higher dry matter (Table 16).

Dry matter in fruits stored in cold store : In cold stored material, maximum dry matter was observed in sub-acidic fruits of cv. Kagzi-Kharoo; sweet fruits of cv. Gole Delicious had the least. Remaining varieties fall in between. As the storage period prolonged the dry matter registered increase in pomes of both sweet as well as sub-acidic varieties (Table 17). After 180 days of storage at low temperature, pomes of only 11 varieties survived and were available for assessment; fruits of the other varieties perished. In these samples also, pomes of cv. Gole Delicious have least dry matter. Maximum dry matter exists in cold stored fruits of cv. Double Kesari. In the sub-acidic group, pomes of cv. Kharoo have dry matter approximating 11.350 g, followed by 10.750 g in Kagzi-Kharoo and 8.930 g in Maharaji.

V. DISCUSSION

V.1 Taxonomic status of apple :

In the past, apples were lumped with pears and together they were referred to genus Pyrus. Although, the two fruits resemble in many features, they are distinct in several characters. In apple, styles are more or less connate at base, stone or grit cells are absent from fruit flesh and a distinct depression is

present at either end of the fruit. On account of these differences, apple was separated and referred to a new genus, named Malus. Several names were given to apple, from time to time, by different workers. These include Pyrus malus L. (Linnaeus, 1735; Hooker, 1878; Rendle, 1925; Rehder, 1954); P. malus L. var. pumila Bailey (Bailey, 1929); P. pumila Koch. (See Bailey, 1963) and Malus malus Brit. (See Bailey, 1963). Cultivated apples belong to Malus pumila Mill. (Miller, 1732). Species of Malus hybridise with one another but never with any species of genus Pyrus. This observation reflects the distinctness of the genera Malus and Pyrus.

Malus pumila is a variable species. Lot of confusion exists in literature regarding its precise nomenclature and taxonomic limits. Some workers have split Malus pumila into three taxa, namely M. sylvestris L., M. pumila Mill. and M. domestica Borkh. (Watkin, 1976). M. sylvestris is morphologically distinct; the trees are distinctly glabrous in contrast to the tomentose trees of M. pumila and M. domestica. The latter two taxa resemble in all characters except fruit size and quality.

Since M. domestica has been raised as distinct species on the basis of highly variable characters, its

validity has been challenged from time to time. Recently, Fougat (1984) expressed serious doubts about the status of this species and proposed its merger with M. pumila Mill. Even earlier, several workers namely Chittenden (1951), Rehder (1954) and Hathwala (1966) had opined that cultivated apples should be referred as M. pumila.

Some botanists consider M. domestica a putative parent of the cultivated apples. Others treat some variants of the species as hybrid derivatives of crosses involving M. sylvestris Linn. Mill., M. prunifolia (Willed.) Borkh and M. baccata.

A section of taxonomists refer all cultivated forms of apple to sub-sp. mitis (Wallrofh) of M. sylvestris (Linn.) Mill. The wild apples are, on the contrary, referred to sub. sp. sylvestris of M. sylvestris (Rehder, 1949; Chittenden, 1956; Bailey, 1949; Mansfield, 1959; Clapham et al., 1952). Of the many epithets given to cultivated apple, M. pumila Mill. enjoys widest acceptability.

V.1.1 Varietal nomenclature :

According to Fougat (1984) and Raina (1989)

some 285 varieties of apple are under cultivation in Kashmir valley. Of these, 139 are known by exotic names 56 by local names and 77 by both. The rest have not been identified definitely so far. Of the 15 varieties investigated now, 10 are known by Kashmiri names and the remaining 5 have English names. The varieties bearing English names are introductions from Europe or America where some of them are under cultivation even today. These varieties are, in all probability, recent introductions compared to the ones known exclusively by the local name. For instance, the Delicious group of apples, believed to represent recent introductions to the valley (Motiyal, 1968) have not been given any local names as yet. The varieties known exclusively by Kashmiri names could be indigenous in origin having evolved either as bud mutants or intervarietal hybrids. In apple, hybridization and bud mutations are the two major sources of varietal differentiation (Crane and Lawrence, 1934; Darrow, 1949; Einset, 1952; Dodds, 1958 and Watkins, 1976).

The nomenclature employed for varieties cultivated in the valley is, in many cases, misleading. Individual fancies of apple growers of the valley have played key role in coining varietal names (Kabu and

Rashid, 1978). The same variety is known by as many as 6 alternate names which accounts for the confusion prevailing in varietal nomenclature. Many a times, names of absolutely unrelated varieties, having different fruit characteristics, end up in the same suffix which confuses the buyer.

V.1.2 Classification of the varieties :

Malus is a large genus based on more than 6,500 varieties which, as pointed out earlier, owe their origin to hybridization, bud mutation and/or chromosome alternations (Crane and Lawrence, 1930; Darlington and Moffett, 1930; Darrow, 1949; Einset, 1952; Batra et al., 1963; Einset and Pratt, 1963). Enormity of number, calls for well thoughtout classification of the commercial varieties. Despite several attempts made in this direction, from time to time, no satisfactory key has been standardised so far.

Over a century ago, Hogg (1859) evolved a classification based on fruit shape, skin colour and harvesting period. He raised as many as 27 groups. The scheme suffered with the drawback of not taking care of many intermediate forms. Realizing this limitation, Hogg (1876) came out with a revised system based on

position of persistent calyx. The groups thus raised, were sub-divided on the basis of (i) fruit form, (ii) texture and colour of fruit skin, (iii) nature and flavour of flesh, (iv) shape of the eye, (v) thickness of fruit stalk etc. Almost half a century later, Bunyard (1920a) proposed a new system which he kept on revising from time to time (Bunyard, 1920b, 1927, 1934) with the aim of perfecting it. Bunyard's initial scheme was based on external morphology of fruit and harvesting period. Taylor (1936) also recognised the merit of such stable fruit characteristics as core, corelines, basin, eye, calyx tube etc. During the fifties and sixties of this century, the emphasis shifted to utilitarian characters. Accordingly, Smock and Neubert (1950) and Thapar (1960) took into account only such characters as skin colour and fruit shape, taste and size. Apart from utilitarian value, these features have the additional advantage of being easy to detect even by a layman. These merits notwithstanding, the schemes evolved only on the basis of these features suffer serious limitation; they serve only when the number of varieties to be compared and classified is limited. Srivastava (1975) tried to categorise some popular varieties of Himachal Pradesh on grounds of harvesting period, fruit size and the sugar-acid ratio. This and similar other

classifications which are based on one or a few characters, overlook the merit in employing correlated character combinations. Fougat (1984) made an attempt along this line to classify 70 varieties from Kashmir Valley. He characterised varieties on grounds of colour, shape, taste and size of the fruit. He assorted the varieties into four colour groups, namely red, yellow, green and saffron and referred 48, 10, 8 and 4 varieties respectively to them. The fruits within each colour group were conical, ovoid, spherical or oblate in shape and sweet, sub-acidic or acidic in taste. The sample investigated now, is conveniently sorted according to the criteria employed by Fougat (1984).

Pearl (1932, 1933), Bunyard (1934) and Brown (1940) have highlighted the usefulness of flowering period in categorising apples. Srivastava (1975) and Fougat (1984) have, however, expressed skepticism on grounds that flowering time is significantly influenced by such environmental factors as altitude, latitude, atmospheric temperature etc. Observations on the sample studied now support this conclusion.

Fruit shape seems to bear positive correlation with taste (Fougat, 1984). The conical apples are

mostly sweet and the oblate ones generally acidic. From within the sample, surveyed during present work, fruits of cvs. Razakwari, Red Delicious, Royal Delicious and Dil Ruba are conical in shape and sweet in taste while those of Kharoo, Kagzi-Kharoo and Maharaji are oblate and sub-acidic. As such, the correlation between fruit shape and taste as proposed earlier by Fougat (1984) stands even in case of the varieties constituting the test sample of present study.

V.2 Analysis of metabolites :

As pointed out earlier cultivated varieties of apple have overlapping morphology for which reason it is difficult to classify them and depict their relationship. Diverse keys have been proposed, but the confusion prevails. It is being realized increasingly that morphological characters are by themselves insufficient, therefore data on other aspects, such as metabolite constitution need to be raised to explore their usefulness (Loescheke and Stegmann, 1966; Smith, 1976; Harborne and Green, 1980). This proposition is based on the principle that, "evolution of plant life is best illustrated by the chemical constituents of the plant form" (Helen Abbots, 1986). The utility of plant

metabolites at all levels of taxonomic hierarchy has been proved by several workers (Bate-Smith, 1962; Desborough and Peloquin, 1966; Boulter et al., 1967; Reddi and Phipps, 1966; Davis and Heywood, 1973; Yamamto and Plitmann, 1980). In recognition of this fact, data have been collected on chemical constituents of different varieties of apple. Leaves as well as fruits have been employed to raise the necessary data on chemical architecture. Leaves have been used to analyse flavonoids and amino-acids.

V.2.1 Flavonoids :

Although restricted in distribution, flavonoids have gained great deal of popularity in chemosystematics; even unidentified flavonoids have come handy for unravelling species interrelationships (Harborne, 1971; Harborne and Williams, 1972; Harborne and King, 1976; Bhargava, 1983; Gupta, 1983; Mangotra, 1983; Singh, 1984; Kaur, 1985). The composite chromatogram of foliar flavonoids of the 15 varieties under reference bears a total of 34 spots, which are recognizable by their position, colour and size. Each spot represents a distinct flavonoid. The number of spots/flavonoids per variety ranges between 6-12. The

size and colour intensity of a spot representing a particular flavonoid exhibit lot of variation which is indicative of the difference in the quantity of that flavonoid in different taxa. Such variation has been reported in many plants including barley (Frost and Holen, 1972; Frost et al., 1975), some members of sub-family Pomoideae of Rosaceae (Challice, 1973), Perideridia (Gianassi and Chuang, 1976), Potentilla (Kohli and Denford, 1977), Boykinia (Gornall and Bohm, 1980), members of Oleaceae (Harborne and Green, 1980) and species of Crotalaria (Mangotra, 1981; Gupta, 1983) and Narcissus (Bhargava, 1983) etc.

Some varieties from the sample assessed presently possess variety specific flavonoids. A few are characterised by specific combinations of flavonoids. For instance, the combination of flavonoids represented by spot nos. 1,3,4 and 12 exists in cv. Gole Delicious, nos. 5 and 13 in Kagzi-Kharoo, no.6 in Maharaji, no.7 in Double Kesari, no. 8 in Ambri, no. 10 in Golden Delicious, no. 26 in Razakwari and no. 33 in Chamura. Table 18 reveals that no flavonoid is common to all the varieties screened for flavonoid constitution. The percentage occurrence of different spots varies between 6.6-66%.

V.2.2 Ninhydrin-positive compounds :

Ninhydrin-positive compounds represent a group of compounds considered useful in classifying some plant groups. Besides, the twenty essential amino-acids which all plant species must synthesize, another hundred are known of which different taxa bear different numbers. These are certainly not universally distributed throughout the plant kingdom, though a few occur very widely. They are restricted in distribution and it is this that makes them useful for exploitation in biosystematics. Some amino-acids are found in isolated members whereas others exist in closely-allied groups of plants. The amino-acid profiles are particularly helpful in identification and classification of sub-generic categories (Bell and Tirimanna, 1963; Ischiersch and Manelt, 1967). Alston and Irwin (1961), Bhalla and Dakwale (1978), Mangotra (1981), Gupta (1983) and Bhargava (1983) have employed amino-acids for delimiting species of Cassia, Indigofera, Crotalaria and Narcissus. Krishnamurthy and Subramanian (1978) have employed aminograms for exploring and evaluating interrelationships among the eight tribes of family Asteraceae.

The composite aminogram prepared by overlapping the individual aminograms of the 15 varieties screened, carries a total of 40 spots, each spot representing a different amino acid. The number of spots per variety and their details vary from one variety to another. The aminogram of cv. Double Kesari has 12 spots which is the highest number in any variety. On the contrary cvs. Ambri, Blood Red, Dil Ruba, Hazratbali-II, and Red Delicious have 6 spots each. None of the spots is common to all the 15 varieties tested. Spot no. 7 has highest representation, occurring in 60% taxa followed by spot nos. 24, 8 and 23 with 53.3, 46.6 and 46.6% representation respectively (Table 3). On the contrary, spot nos. 15, 17, 19, 20, 21, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 and 40 have very poor representation; these spots are present in one or at the most in two varieties.

Metabolites in relation to ploidy :

The sample of 15 varieties evaluated presently consists of two broad categories so far as chromosome number is concerned. One group consists of 12 diploid varieties and the second of three triploids, namely cvs. Double Kesari, Hazratbali-II and Kharoo. The triploid varieties resemble in having 6 flavonoid spots each in

their respective chromatograms. However, they differ with regard to the nature of the spots. Only spot no. 27 is shared by all the three varieties. Double Kesari and Hazratbali-II share spot no. 16 while Kharoo and Hazratbali-II share spot no. 23. Thus, despite similarity in chromosome number, the three varieties differ with regard to foliar flavonoid constitution. Their ninhydrin-positive compound profiles are also different. For instance cv. Double Kesari has 12 spots followed by Kharoo with 9 and Hazratbali-II has only 6. Double Kesari and Hazratbali-II share only 1 spot but Double Kesari and Kharoo share 4 spots.

Fougat (1984) has employed chromosome constitution and behaviour of chromosomes during meiosis to assess the nature of polyploidy of the three triploid varieties. He has concluded that cv. Hazratbali-II is an autotriploid whereas both Double Kesari and Kharoo are allotriploids, while it is not possible to comment on the nature of polyploidy of the three varieties on the basis of ninhydrin-positive compound profiles, it is however, quite evident that varieties Double Kesari and Kharoo are certainly more related to each other than either of them is to var. Hazratbali-II (Table 18). To this extent the inferences drawn on grounds of cytology

Table 18. Similarity index values (%) of 15 apple varieties computed on the basis of ninhydrin positive compound composition.

S. No.	Varietal Name	Ambri	American Trail	Blood Red	Chamura	Dil Ruba	Double Kesari	Golden Delicious	Gole Delicious	Hazratbali-II	Kagzi Kharoo	Kharoo	Maharaji	Razakwari	Red Delicious	Royal Delicious
1.	Ambri		7.6	20	30	7.6	20	30	44.4	7.6	25	7.1	7.6	14.2	33.3	40
2.	American Trail			27.2	-	55.5	5.2	-	15.3	40	21.4	-	6.6	26.6	7.6	6.6
3.	Blood Red				8.3	20	6.6	8.3	8.3	20	18.1	15.3	7.1	21.4	20	16.6
4.	Chamura					16.6	5.5	55.5	27.2	8.3	23	14.2	16.6	5.8	18.1	36.3
5.	Dil Ruba						-	8.3	30	20	25	-	7.6	13.3	20	-
6.	Double Kesari							5.5	5.5	5.8	5	23.5	33.3	-	5.8	11.1
7.	Golden Delicious								16.6	8.3	6.6	6.6	15.3	5.8	18.1	36.3
8.	Gole Delicious									8.3	23.01	6.6	15.3	12.5	30	25
9.	Hazratbali-II										15.3	-	-	13.3	9.09	27.2
10.	Kagzi Kharoo											-	5.8	11.1	36.3	13.3
11.	Kharoo												41.6	-	-	13.3
12.	Maharaji													-	-	23.07
13.	Razakwari														13.3	11.7
14.	Red Delicious															7.6
15.	Royal Delicious															

match with those drawn from the analysis of ninhydrin-positive compounds.

Although Double Kesari and Kharoo are both considered alloploids, they differ cytologically (Fougat, 1984; Koul et al., 1984; Singh and Wafai, 1984; Singh et al., 1984, 1985 and 1987). In the former, the number of univalents exceeds the number of bivalents per cell. Reverse is the true in the latter case. The frequency of trivalents is low in both the varieties. Studies on foliar flavonoids (Table 19) reveal that the affinity index between Double Kesari and Kharoo is only 9.09% while that between Hazratbali-II and Kharoo and Hazratbali-II and Double Kesari is 20%. This suggests that Double Kesari and Kharoo are phylogenetically more distant than they are with Hazratbali-II. The observation on ninhydrin-positive compounds and foliar flavonoid profiles of the two varieties suggest that different progenitors might be involved in the origin of the two allotriploid varieties.

The twelve diploid varieties share several flavonoids, but they differ with regard to the total flavonoid profile. Cvs. Gole Delicious and Maharaji possess 10 spots each, some others have only 6 each. Such variation has also been observed in species of Narcissus and Crotalaria (Bhargava, 1983; Gupta, 1983;

Table 19. Paired affinity values (%) of 15 apple varieties computed on the basis of foliar-flavonoid pattern.

S. No.	Varietal Name	Ambri	American Trail	Blood Red	Chamura	Dil Ruba	Double Kesari	Golden Delicious	Gole Delicious	Hazratbali-II	Kagzi Kharoo	Kharoo	Maharaji	Razakwari	Red Delicious	Royal Delicious
1.	Ambri		30	-	16.6	18.1	9.09	9.09	6.6	9.09	15.3	-	6.6	16.6	9.09	30
2.	American Trail			-	25	16.6	18.1	30	13.3	8.3	6.6	18.1	21.4	15.3	15.3	16.6
3.	Blood Red				-	-	20	20	14.2	9.09	15.3	33.3	7.1	-	33.3	30
4.	Chamura					15.3	16.6	27.2	5.8	7.6	6.2	7.6	28.5	60	16.6	7.1
5.	Dil Ruba						18.1	8.3	13.3	44.4	14.2	33.3	30.7	15.3	18.1	-
6.	Double Kesari							20	23.07	20	27.2	9.09	6.6	27.2	33.3	33.3
7.	Golden Delicious								6.6	9.09	15.3	33.3	23.07	8.3	50	18.1
8.	Gole Delicious									14.5	18.7	6.6	5.2	5.8	14.2	21.4
9.	Hazratbali-II										7.1	20	15.3	14.2	7.6	8.3
10.	Kagzi-Kharoo											7.1	11.7	12.5	33.3	30.7
11.	Kharoo												33.3	7.6	50	18.1
12.	Maharaji													20	23	13.3
13.	Razakwari														16.6	7.1
14.	Red Delicious															30
15.	Royal Delicious															

Mangotra, 1983). Among diploids, variety Chamura and Razakwari exhibit 60% similarity in flavonoid constitution although they vary a great deal in the taste of their fruit.

The variation in ninhydrin-positive spots is maximum in Razakwari which has 11 spots, followed by Kagzi-Kharoo, American Trail, Maharaji and Royal Delicious. Cvs. Ambri, Blood Red and Red Delicious have the least number of 6 spots each. Unique spots are present only in cvs. Double Kesari, Golden Delicious, Kharoo and Razakwari and these nos. are (17, 19, 21, 30, 31, 32, 34, 35, 36, 37, 38, 39 and 40). It emerges that cultivated apples exhibit enormous variability in ninhydrin-positive compound profiles which seems to have accompanied varietal divergence.

V.2.3 Carbohydrates :

Being ubiquitous carbohydrates enjoy little importance as tools in taxonomy. Percival (1961), Bhalla and Dakwale (1978), Mangotra (1983), Gupta (1983) and Bhargava (1983) who have evaluated different groups of angiosperms for sugars reiterate that carbohydrates are hardly of any consequence either for identification of individual taxa or assessment of phylogenetic

interrelationships. These observations notwithstanding, carbohydrates hold great potential in delimiting categories beyond the level of species and genera. Fungi, algae and some bryophytes have been distinguished, separated and classified on the basis of differences in the nature of the carbohydrates they contain. Thus, while starch is the chief storage carbohydrate in Marchantiales, fructans characterize the Jungermanniales (Quillet, 1956).

Reichert (1919), Blackman (1921), Hubbard (1948), Belval and deCugnac (1941), Williams et al. (1952) and Rix and Rast (1975) state that diversity in carbohydrate composition exists even among different groups of angiosperms and in certain cases it can fruitfully be exploited for classification. The study of carbohydrate profiles has helped to resolve, taxonomic dispute about Festuca gigantea (Belval and deCugnac, 1941), merger of Hippocrataceae and Celestraceae (Plovier, 1948), placement of Crotalaria verrucosa (Mangotra, 1981) etc. Carbohydrate profiles of species in Fritillaria are so variable that they find application in intrageneric classification.

The varieties of apple investigated presently

can be categorised into following four groups, on the basis of chromosome number and taste of fruit flesh :

(i)	Diploid with sweet pomes	: 9 varieties
(ii)	Triploid with sweet pomes	: 1 variety
(iii)	Diploid with sub-acidic fruits	: 3 varieties
(iv)	Triploid with sub-acidic fruits	: 2 varieties

Reducing sugars :

Reducing sugars include glucose and such other sugars as are capable of reducing oxidizing agents. When freshly picked pomes of 10 sweet varieties were compared for reducing sugar content, pomes of Gole Delicious were found richest and those of Royal Delicious found poorest. Of the 5 sub-acidic varieties assessed, fruits of diploid 'Maharaji' were estimated to contain 17.20 mg of reducing sugars. The reducing sugar content in sweet fruits is, in general, higher compared to sub-acidic fruits i.e. there is a strong correlation between taste of flesh and the quantity of reducing sugars.

Comparison of different varieties for total free sugars reveals that, fruit taste notwithstanding, diploid varieties are richer as compared to triploids.

The conclusion is, however, not categoric in view of the fact that some diploids, like cv. Kagzi-Kharoo, have lesser free sugars than triploid Double Kesari. On the whole, sweet varieties contain more free sugars than those bearing sub-acidic pomes.

With respect to sucrose and fructose content, sub-acidic varieties trail behind their sweet counterparts. Sweet fruits of cv. Blood Red contain highest quantity of sucrose, despite the fact that it is diploid. Obviously, polyploidy does not influence sucrose content in apple; some polyploids contain lesser quantity of sucrose than diploids.

Starch :

Among the various types of reserve polysaccharides in plants starch is the most abundant and important. It is widely distributed from lower to higher plants. Starch is typically present in storage organs.

In order to find out the impact of different temperature regimes on the starch content of 15 apple varieties, they were stored at room temperature (15-33°C) and cold storage (2-4°C).

An overall decrease in starch content was observed after the storage of 30 days at room temperature (Table 12). Sweet and diploid varieties suffered the maximum decrease e.g. Royal Delicious with 66.6%; Ambri with 55.1% and Blood Red with 54.3%. Details have been given in Table 20 .

Under cold storage conditions also diploid and sweet varieties suffered the maximum percentage decrease in starch content. The decreasing trend continues till the material survived under these conditions i.e. 150-180 days (Table 21).

Effect of storage on sugar content :

In order to assess the impact of storage on sugar content, fruits of different varieties were stored for varying periods under two temperature regimes viz. cold storage (2-4°C) and room temperature (15-33°C) and then analysed for different sugars. The findings have been discussed below :-

Reducing sugars :

During storage of fruits at 2-4°C the sugar content of pomes registers increase. The effect is not

Table 20. Percentage decrease in starch content of apple varieties stored at 15-33°C.

S. No.	Name of variety	%age decrease					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	55.1	-				
2.	American Trail	8.6	52.1	66.3	69.5	-	
3.	Blood Red	53.8	73.9	-			
4.	Chamura	-					
5.	Dil Ruba	33.6	-				
6.	Double Kesari	16.1	25.7	-			
7.	Golden Delicious	47.1	58.4	-			
8.	Gole Delicious	47.0	65.6	77.4	-		
9.	Hazratbali-II	50.0	60.0	-			
10.	Kagzi-Kharoo	26.1	-				
11.	Kharoo	15.5	-				
12.	Maharaji	33.7	44.5	47.2	54.0	-	
13.	Razakwari	17.0	-				
14.	Red Delicious	33.3	67.5	77.4	80.1	-	
15.	Royal Delicious	66.6	73.6	-			

Table 21. Percentage decrease in starch content of apple varieties stored in cold storage.

S. No.	Name of variety	%age decrease					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	22.4	29.9	43.1	53.4	60.3	-
2.	American Trail	17.3	51.0	54.3	56.5	63.0	73.9
3.	Blood Red	21.7	28.2	34.7	41.3	56.5	-
4.	Chamura	28.5	37.1	44.2	66.6	72.8	-
5.	Dil Ruba	30.3	62.9	65.1	69.6	73.0	77.5
6.	Double Kesari	15.2	47.6	60.0	64.7	67.6	70.4
7.	Golden Delicious	20.7	35.2	52.8	60.3	66.0	-
8.	Gole Delicious	58.8	77.4	78.4	79.4	83.2	-
9.	Hazratbali-II	22.0	30.0	44.0	-		
10.	Kagzi-Kharoo	51.3	61.2	69.3	74.7	-	
11.	Kharoo	54.1	60.3	67.8	74.0	85.3	-
12.	Maharaji	21.6	33.7	54.0	66.2	71.6	73.8
13.	Razakwari	10.6	21.2	27.6	40.3	76.5	-
14.	Red Delicious	25.2	54.9	59.4	64.8	67.5	84.6
15.	Royal Delicious	23.6	36.1	52.7	64.8	81.4	-

alike in all cases. In diploid sweet varieties the quantity of reducing sugars increases for some time and recedes thereafter. The period during which sugars register increase varies from one variety to another; 120 days for cvs. American Trail, Dil Ruba, Gole Delicious and Red Delicious, 150 days for cvs. Ambri and Razakwari and 180 days for Blood Red, Golden Delicious and Royal Delicious. Varietal difference exists with respect to percentage increase in reducing sugars. Among diploids pomes of Ambri register 13 fold increase in reducing sugar content from a mere 3.9% after 30 days to about 38.6% after 150 days of storage (Table 22). On the contrary, in cvs. American Trail and Dil Ruba the quantity of reducing sugars is merely doubled even after a storage period of 120 days (Table 5).

In diploid sub-acidic apples, the optimum period for which pomes stand storage is more or less alike; but in cv. Kagzi-Kharoo it does not exceed 90 days.

From among the three triploid varieties investigated presently, two, namely Hazratbali-II and Kharoo have sub-acidic fruits while the third, Double Kesari, has sweet pomes. Fruit taste notwithstanding,

Table 22. Percentage increase in reducing sugar content of apple varieties stored at 2-4°C.

S. No.	Name of variety	%age increase					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	3.9	18.8	25.6	36.7	38.6	-
2.	American Trail	36.4	45.4	60.96	100.5	40.5	21.8
3.	Blood Red	12.6	14.4	13.8	19.6	59.9	112.4
4.	Chamura	24.7	14.5	59.3	76.1	88.7	-
5.	Dil Ruba	22.4	79.7	81.5	96.4	18.4	5.03
6.	Double Kesari	6.5	6.7	20.3	29.9	14.6	-16.9
7.	Golden Delicious	0.6	20.1	24.9	36.4	54.0	55.7
8.	Gole Delicious	22.7	26.9	29.8	32.4	0.30	-29.1
9.	Hazratbali-II	11.7	22.7	32.0	37.3	-	
10.	Kagzi-Kharoo	23.4	56.3	74.90	72.0	27.9	18.0
11.	Kharoo	25.2	49.1	62.2	59.4	19.8	2.3
12.	Maharaji	21.7	54.4	62.6	71.2	6.5	-12.1
13.	Razakwari	22.4	24.7	26.6	5.98	30.9	-
14.	Red Delicious	10.0	26.7	63.1	68.1	9.8	-4.1
15.	Royal Delicious	33.6	44.0	54.7	46.5	105.3	117.3

the number of days during which reducing sugar level in all these varieties gets increased in cold storage matches; being 120 days in all cases.

Reducing sugar level of pomes increases even when fruits are stored at room temperature. However, storage at ambient temperature accelerates fruit rot. The ephemeral, sub-acidic fruits of diploid var. Chamura stand only 20-25 days storage at room temperature. On the contrary, pomes of cvs. Gole Delicious, Maharaji, American Trail and Royal Delicious stand storage for longer periods at room temperature. Fruits of these varieties register steady increase in the quantity of sugars upto periods varying between 60-90 days. All these varieties, except Maharaji, are diploid and sweet. Rest of the varieties could not stand even 60 days storage.

Total free sugars :

With regard to change in quantity of total free sugars following storage cv. Gole Delicious stands out distinct from the rest. It is the only variety in which free sugars attain peak within 60 days of storage (Table 23). In cvs. Golden Delicious, Kagzi-Kharoo, Kharoo and Royal

Table 23. Percentage increase in the amount of total free sugars of apple varieties stored at 2-4°C.

S. No.	Name of variety	%age increase					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	24.3	26.6	27.9	26.7	-4.1	-
2.	American Trail	162.4	172.5	207.0	251.2	116.8	31.8
3.	Blood Red	6.1	13.8	19.0	50.0	5.5	2.3
4.	Chamura	1.5	13.0	21.8	18.5	9.4	-
5.	Dil Ruba	86.8	88.8	126.1	215.5	83.1	21.1
6.	Double Kesari	44.4	33.3	39.9	48.7	11.5	-23.4
7.	Golden Delicious	65.2	66.8	75.1	106.7	124.5	82.6
8.	Gole Delicious	257.7	269.9	264.3	268.5	144.1	99.8
9.	Hazratbali-II	62.8	100.9	136.1	126.1	-	-
10.	Kagzi-Kharoo	29.8	59.7	127.7	141.8	197.4	137.8
11.	Kharoo	15.9	41.7	90.7	149.1	180.5	29.5
12.	Maharaji	5.3	19.2	51.2	116.4	52.0	27.2
13.	Razakwari	10.3	28.2	41.3	37.9	64.3	-
14.	Red Delicious	88.7	96.1	106.4	112.9	47.4	12.9
15.	Royal Delicious	18.1	23.2	27.0	35.6	108.0	42.7

Delicious the peak is reached within 150 days of storage. The remaining varieties fall inbetween these two extremes. The time taken for accumulation of free sugars during storage is independent of ploidy level. Thus, diploid Maharaji and triploid Hazratbali-II agree in attaining the peak for free sugar content within 120 and 90 days respectively.

Sucrose :

Storage also causes increase in the quantity of sucrose. In most of the sweet and diploid fruits sucrose content touched the peak after 120 days storage (Table 24). In cv. Razakwari maximum level was reached only within 60 days. Much longer period (150 days) was required in cvs. Golden Delicious and Royal Delicious.

In the diploid, sub-acidic group cv. Maharaji takes longest period (150 days) for accumulating maximum sucrose content followed by Chamura (120 days) and Kagzi-Kharoo (90 days). Although, the three varieties resemble in chromosome constitution and taste, yet they vary in their physiological response to storage.

Among triploids, taste of flesh has no bearing on physiological response of pomes to storage, at least

Table 24. Percentage increase in the amount of sucrose content in apple varieties stored at 2-4°C.

S. No.	Name of variety	%age increase					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	0.5	13.9	20.4	22.7	-61.2	-
2.	American Trail	6.1	7.3	19.3	44.7	-63.4	-35.8
3.	Blood Red	40.6	42.9	46.8	48.3	-13.8	-29.5
4.	Chamura	9.8	30.9	46.6	49.9	12.1	-
5.	Dil Ruba	8.7	52.2	53.3	131.0	26.5	-29.0
6.	Double Kesari	3.2	7.8	53.2	55.6	-48.8	-75.1
7.	Golden Delicious	15.6	20.9	28.6	40.9	51.5	- 1.3
8.	Gole Delicious	5.6	19.0	27.6	83.3	-64.9	-63.0
9.	Hazratbali-II	0.8	25.3	50.3	145.2	-	-
10.	Kagzi-Kharoo	42.7	61.1	67.1	64.7	-42.4	-61.2
11.	Kharoo	47.1	60.5	115.3	3.6	-20.17	-61.8
12.	Maharaji	11.4	20.7	19.3	54.3	66.2	- 6.1
13.	Razakwari	14.8	18.5	18.3	8.7	-84.0	-
14.	Red Delicious	1.6	28.9	32.8	40.0	-68.8	-36.6
15.	Royal Delicious	5.6	38.2	71.2	64.2	81.60	-16.5

with regard to accumulation of sugars. Thus, Double Kesari, having sweet pomes and Hazratbali-II bearing sub-acidic pomes take equal time (120 days) to accumulate maximum quantity of sugars.

Fructose :

Accumulation of fructose during storage does not display any relation with fruit taste or ploidy level. Most of the varieties show maximum fructose content after 120 days of storage (Table 25).

V.3 Post-harvest changes :

V.3.1 Shelf life :

Shelf-life is one of the most important attributes of a desert fruit like apple, it determines their commercial value. In order to evaluate shelf-life of the fruit of different varieties, pomes were stored under two temperature regimes - ambient temperatures ranging between 15-33°C and cold storage (2-4°C). Fruits of some varieties, namely American Trail and Red Delicious stand 90-120 days storage at room temperature. Surprisingly, on the other hand, sub-acidic fruits of Chamura are ephemeral with a shelf-life of only 30 days.

Table 25. Percentage increase in fructose content of 15 apple varieties stored in cold storage (2-4°C).

S. No.	Name of variety	%age increase					
		After 30 days	After 60 days	After 90 days	After 120 days	After 150 days	After 180 days
1.	Ambri	14.7	27.4	40.0	83.4	32.9	-
2.	American Trail	52.3	71.1	69.4	127.1	90.0	89.0
3.	Blood Red	17.1	29.0	39.8	47.7	61.6	42.5
4.	Chamura	104.8	136.7	168.8	246.3	108.7	-
5.	Dil Ruba	19.3	30.5	40.2	63.9	105.8	81.7
6.	Double Kesari	30.9	39.8	45.6	51.0	59.6	63.4
7.	Golden Delicious	61.1	99.5	98.4	102.7	106.1	105.1
8.	Gole Delicious	146.6	158.4	170.3	222.2	189.7	260.8
9.	Hazratbali-II	228.0	243.4	258.8	255.0	-	-
10.	Kagzi-Kharoo	62.7	137.8	214.0	207.9	104.9	122.2
11.	Kharoo	6.2	67.2	77.2	137.2	100.0	73.8
12.	Maharaji	33.1	57.9	96.2	110.5	187.5	-
13.	Razakwari	92.1	115.1	138.0	151.4	141.5	-
14.	Red Delicious	28.2	88.6	104.0	119.4	131.8	155.7
15.	Royal Delicious	92.0	98.1	104.2	116.4	105.4	-

Pomes of other varieties keep well upto a maximum 60 days. Pomes of only four varieties survive in storage beyond 90 days. Even among these, the percentage survival varies a great deal. While there is no loss in American Trail and Red Delicious over a period of 120 days, cv. Gole Delicious suffers 60% and Maharaji 82% loss.

Shelf-life of the fruit is extended under low temperature conditions. In cvs. American Trail and Red Delicious shelf-life is extended from a maximum of 120 to 180 days. Similarly, low temperatures prolong shelf-life of pomes of other varieties like Golden Delicious, Gole Delicious and Maharaji also. However, these varieties suffer a loss of 26% during the extended period of their shelf-life.

In terms of rotting percentage sub-acidic varieties suffer maximum loss in storage. The rotting percentage in cvs. Chamura, Kharoo and Kagzi-Kharoo is 44, 38 and 36% respectively. Rotting is comparatively lower in sweet fruits. During the 90 days of their storage at sub-zero temperature, sweet varieties, namely Ambri and Razakwari, suffer great loss. At least, 11 varieties survive 180 days storage. All of them, except

Maharaji, have sweet pomes. Maharaji is the only sub-acidic variety which stands long storage. Experiments on storage have revealed that the sub-acidic pomes perish more quickly both under room conditions as well as in cold storage than those having sweet pomes.

V.3.2 Dry matter :

As in other cases, so also in apple, weight loss during storage is caused by loss of water through transpiration (Kushman and Pope, 1972). Decay or internal breakdown reduces the weight further (Walter, 1989). During the present investigation, weight loss has been estimated in terms of dry matter. The percentage increase in dry matter during storage is affected by the duration of storage period and internal atmospheric condition of storage. Kushman and Deonier (1958) have reported that even soil temperature during the course of storage influences weight loss in sweet potatoes.

Cold storage also causes increase in dry matter. Maximum increase in dry matter has been calculated in cv. Gole Delicious and least in var. Kagzi-Kharoo. In order to determine whether changes in

the quantity of reducing sugars, fructose, sucrose and total free sugars have any impact on dry weight, Karl Pearson's coefficient of correlation was calculated between two parameters at a time. The 'r' values obtained are listed in Table 26 . It is evident from the table that strong positive correlation exists between dry mater and fructose content. Thus, as the quantity of fructose increases, dry weight of the fruit increases too. This correlation holds in 13 of the 15 varieties tested. However, the degree of correlation, 'r' value, ranges between 0.54-0.94. The two exceptions where no correlation exists between the two parameters are, cvs. Kagzi-Kharoo and Royal Delicious. Changes in reducing sugars, sucrose and total free sugars do not have any consistent effect on dry matter of stored fruit, as is brought out by Table 26.

Variety Hazratbali-II is typical because in this case dry matter changes during storage are associated with increase in the quantity of all the four sugars taken together, ('r' = 0.69-0.94). At the other extreme is cv. Royal Delicious whose dry matter changes are not associated with increase in the quantity of any of the four sugars.

Table 26. Coefficient of correlation among different parameters.

Varieties	Groups									
	1.2	1.3	1.4	1.5	2.3	2.4	2.5	3.4	3.5	4.5
1. Ambri	0.95	0.75	-0.41	0.09	0.80	-0.26	0.03	0.28	0.46	0.81
2. American Trail	0.22	0.91	-0.51	0.008	0.85	0.62	0.99	0.06	0.62	0.17
3. Blood Red	0.58	0.88	-0.46	0.12	0.41	-0.53	0.23	-0.15	0.14	0.68
4. Chamura	0.95	0.54	0.29	0.41	0.71	0.57	0.69	0.91	0.83	0.93
5. Dil Ruba	0.09	0.88	0.15	0.42	-0.03	0.40	0.52	0.05	0.22	0.68
6. Double Kesari	0.21	0.87	-0.44	-0.42	0.09	0.78	0.82	-0.27	-0.05	0.85
7. Golden Delicious	1.0	0.90	0.31	0.80	0.83	0.39	0.82	0.33	0.83	0.77
8. Gole Delicious	0.13	0.80	-0.18	0.43	0.18	0.84	0.75	-0.04	0.77	0.59
9. Hazratbali-II	0.86	0.69	0.94	0.74	0.82	0.82	0.97	0.47	0.90	0.67
10. Kagzi-Kharoo	0.02	0.33	-0.64	0.84	0.92	0.72	0.44	0.45	0.65	-0.26
11. Kharoo	0.02	0.80	-0.56	0.66	0.52	0.71	0.44	-0.15	0.84	-0.06
12. Maharaji	-0.02	0.85	0.43	0.51	0.10	0.39	0.52	0.8	0.62	0.71
13. Razakwari	0.14	0.69	-0.62	0.79	0.60	-0.33	0.61	-0.18	0.82	-0.63
14. Red Delicious	0.08	0.94	-0.56	-0.04	0.38	0.68	0.80	-0.28	0.13	0.66
15. Royal Delicious	0.12	0.10	0.05	0.10	0.25	0.14	0.85	0.31	0.50	0.55

Abbreviations :- 1. dry weight; 2. reducing sugars; 3. fructose; 4. sucrose; 5. total free sugars.

Another fact that has been highlighted by estimation of the co-efficient of correlation is the relationship between reducing sugars and total free sugars. Any change in the former is reflected by a similar change in the latter. Similarly, the amount of fructose and total free sugars also bears positive correlation in some varieties as is revealed by Table 26.

VI. SUMMARY AND CONCLUSIONS

The valley of Kashmir represents a rich repository of the germplasm of apple. The major sources that have contributed to this gene pool are Central Asia, U.S.A., U.K. and other European countries. After its introduction to the valley, from different parts of the world it has not only adapted to the ecoclimatic conditions of the valley, it has also

accumulated further variability through bud mutations, introgression and chromosome alterations. As a result, lot of diversity has accumulated and at present the valley hosts more than 285 known and several still unknown varieties.

The work contained in the thesis is based on 15 cultivated varieties. These were studied for prominent qualitative and quantitative characteristics, such as fruit shape, size, colour, surface texture and taste. Some of these varieties have been already assessed for floral biology, breeding system, chromosome number and meiotic system by Fougat (1984), Koul et al. (1984), Singh et al. (1984, 1985 & 1987) and Raina (1989).

Leaves of all the 15 varieties have been analysed for ninhydrin - positive compounds and flavonoids. Sugars and starch have been estimated from fruit. Paper and thin layer chromatography have been employed for separating out ninhydrin-positive compounds and flavonoids. Sugars and starch have been estimated through spectrophotometry, using a spectrophotometer of systronics make model 106. For standardising the profiles of these compounds, 2-4 replicates were run for

each variety. Cultivated varieties of apple have overlapping morphology which makes it difficult to classify them and explore their relationship. Many keys have been proposed but the confusion prevails, suggesting that morphologial characters are, by themselves insufficient. Therefore, data on other aspects such as metabolite constitution need to be obtained for use wherever necessary.

Leaves have been employed for analysis of flavonoids and amino acids. The composite chromatogram of foliar flavonoids of the 15 varieties bears 34 spots, which are recognizable by their position on the chromatogram, intensity of colour and size. Each spot represents a distinct flavonoid. The number of spots (flavonoids) per variety ranges between 6-12. Size and colour intensity of a spot representing a particular flavonoid, vary at the intervarietal level which is indicative of the difference in quantity of that flavonoid in different varieties. Nevertheless, a few varieties are characterised by specific combinations of flavonoids.

A total of 40 spots have been identified in the composite aminogram prepared from individual

aminograms of the varieties analysed. Each spot of the aminogram represents a distinct amino-acid. The number of spots and their details vary at the intervarietal level.

The 15 varieties evaluated consist of two broad categories, in so far as chromosome number is concerned. One group consists of 12 diploid and the other of three triploid varieties. Varieties in the latter category resemble in having 6 flavonoid spots each in their individual chromatograms. Similarity in number notwithstanding, the spots vary in their detail. Thus, despite similarity in chromosome count and number of flavonoids, the three varieties differ with regard to the quality of foliar flavonoids.

The ninhydrin-positive compound profiles of the three triploid varieties are also distinct. Thus, whereas cv. Double Kesari has 12 spots followed by Kharoo with 9, cv. Hazratbali-II has only 6. On the basis of ninhydrin positive compound profiles, it is not possible to comment on the nature of polyploidy of the three triploid varieties. However, the profiles suggest that varieties Double Kesari and Kharoo are more close to each other than either of them is to Hazratbali-II.

Neither flavonoid nor ninhydrin positive compound profiles show specificity for any variety. Most of the spots are common. The number of spots per variety varies. Regardless of their taste, vars. Chamura and Razakwari exhibit 60% similarity on the basis of flavonoid constitution.

For assessment of post-harvest changes and estimation of shelf-life, fruits of all varieties were stored in two batches. One batch of each variety was stored at room temperature and the other was placed in a cold store. The effect of storage was evaluated on fruit weight, rotting percentage and susceptibility to microbes. Observations were made at monthly intervals. The results indicate that weight loss and rotting are lower in cold storage than at room temperature. Maximum rotting, under both conditions, occurs in sub-acidic varieties. The shelf-life of sub-acidic fruits does not exceed a month or two. On the contrary, under similar conditions, the sweet varieties withstand storage for as long as 3-4 months. In fact, fruits of most of the sweet varieties suffered no rot even after six months storage at low temperature.

The effect of storage has been studied on sugar (reducing and non-reducing) and starch content. Estimations made at monthly intervals for a total period of 6 months indicate that storage enhances concentration of sugars and concomitantly reduces starch content.

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* Originals not seen.