

**STUDIES ON GROWTH AND
DEVELOPMENT OF POMEGRANATE
(*Punica granatum* L.) FRUIT AND SEED
WITH SPECIAL REFERENCE TO HARD
AND SOFT SEEDEDNESS**

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**DIVISION OF HORTICULTURE
UNIVERSITY OF AGRICULTURAL SCIENCES**

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B. PRASANNA KUMAR

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CERTIFICATE

THIS IS TO CERTIFY that the thesis entitled
"STUDIES ON GROWTH AND DEVELOPMENT OF POMEGRANATE (Punica granatum
L.) FRUIT AND SEED WITH SPECIAL REFERENCE TO HARD AND SOFT
SEEDEDNESS." submitted in partial fulfilment of the
requirements for the degree of DOCTOR OF PHILOSOPHY in
HORTICULTURE to the University of Agricultural Sciences,
G.K.V.K., Bangalore is a record of bonafide research work
carried out by Mr. B. PRASANNA KUMAR under my guidance and
supervision and that no part of the thesis has been submitted
for the award of any other degree, diploma, associateship,
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INTRODUCTION

INTRODUCTION

Pomegranate (Punica granatum L.) belongs to the botanical family Punicaceae and is an important fruit of semi-arid and arid regions of India. The wide adaptability, hardy nature, low maintenance cost, steady and high yields, fine table and therapeutic values, better keeping quality and possibilities to throw the plants into rest period when irrigation potential is low are some of the qualities which make the fruit ideally suitable for semi-arid and arid regions.

Pomegranate is an ancient fruit, commonly known as 'Anar', 'Dalim' and 'Metulum' and also known as Malum Punicum (apple of Carthage). It was cultivated in Israel for more than five thousand years. The wild or semi-wild pomegranate still exists in the north of Syria, in Gilead and on Mount Carmel. According to Vavilov, the pomegranate originated in the near East. DeCandolle ascribed it to Iran and vicinity, whence its spread to the shores of the Mediterranean (Asaph Goor, 1967). Pomegranate is thought to be indigenous to the region of Iran where it was first cultivated in about 2000 B.C. (Evreinoff, 1949).

The pomegranate cultivation is of considerable importance in the Mediterranean countries of Spain, Morocco, Egypt, Afghanistan, Arabia and Baluchistan. It is also grown to some extent in Burma, China, Japan and California. The area under this crop

in India is about 1200 hectares and more than 800 hectares are in Maharashtra alone (Patil and Wavhal, 1980). Ahmednagar, Poona and Satara are important districts of Maharashtra growing this crop. It is also grown on large scale around Dholka in Gujarat. It is cultivated in small pockets in Almora, Aligarh, Tehri Garhwal, Meerut and Farrukhabad in Uttar Pradesh; Uthukuli, Michael Patti, Vellodu and Dindigal in Tamil Nadu; Pemukonda and Madakasira in Andhra Pradesh; and Kolar, Tumkur, Bangalore and Mysore districts of Karnataka.

The best quality fruit can only be grown in areas of cool winters and hot and dry summers. It thrives best in places with a hot dry summer provided irrigation is available. It can also adopt to a wide range of climatic conditions. It can be grown from the plains to an elevation of about 1800 meters. The tree can withstand frost but is injured by temperatures below 12°F. Pomegranate comes up well on different types of soil but heavy loam soil is favourable for profitable production of high quality fruits. It is more tolerant to alkali conditions of soil than most fruit trees.

There are many types and varieties of pomegranate grown in India including wild acidic 'anardana' and ornamental types. The fruit colour varies from green, pale yellow to purple. The colour of juicy pulp (aril) varies from almost colourless to blood red. The edible varieties can be broadly divided into

two groups namely, soft seeded or so called seedless (Bedana) and hard seeded. Dholka of Gujarat, Bassein Seedless of Karnataka and Paper Shell of South India are important established soft seeded varieties. The important soft seeded selections of recent origin are Ganesh (Cheema et al., 1954), CO.1 (Khader et al., 1982) Yercaud-1 (Sayed et al., 1985) and Jyothi (GKVK-1). The soft seeded varieties are preferred over hard seeded varieties. Some important hard seeded varieties are Alandi, Muskat Red, Vellodu, Jodhpur Red and Jodhpur White. Muskat is a medium soft variety from Maharashtra.

The ancient Egyptians made juice from the pomegranate which they called 'Schedou' as well as wine. The rind was considered to be having wormicidal effect against intestinal worms. The flowers were crushed to make a red dye for colouring fabrics and the peel yielded a yellow colour for dyeing leather (Asaph Goor, 1967). Pomegranate is used largely as a dessert fruit. The juice makes a delicious drink and a good variety gives about 40.1 percent juice (Siddappa, 1943). The fruits vary in size, shape and colour. The seeds of wild acidic types are dried and commercially marketed as 'Anardana' which is widely used as condiment. Analysis of the edible portion (68 percent) of pomegranate fruit is as follows: moisture 78.0, protein 1.6, Fat 0.1, fibre 5.1, other carbo-hydrates 14.5, and mineral matter 0.7%; calcium 10, magnesium 12, oxalic acid 14, phosphorus 70.0, iron 0.3, sodium 0.9, potassium 133.0, copper 0.2, sulphur

12.0, chlorine 2.0, carotene 0, thiamine 0.06, riboflavin 0.10, nicotinic acid 0.30, and vitamin C, 14 mg/100 g. The fruit rind constitutes one of the important tanning material as well as the source of dye which gives yellowish brown to khaki shades and has been used for dyeing wool and silk. The flowers yield a light red dye said to have been used in India for dyeing clothes. They contain the pigment pelargonidin 3-5, diglucoside.

Commercial growing of a large number of varieties and types in different parts of India and development of improved soft seeded selections like Ganesh, Jyothi (GKVK-1), Yercaud-1 and CO-1 have not in any way reduced the quantum of import of fresh pomegranate fruits from Afghanistan and Baluchistan. According to Singh (1969) pomegranate fruits constituted 20 per cent of fresh fruits imported into India. Fresh fruits of Kabul Bedana and Kandhari are imported from Afghanistan in large quantities and flood main markets of North India from August to November.

According to Purohit (1985) Kabul Bedana variety imported from Afghanistan has the softest seed and there is no comparable soft seeded variety available in India. Further, amongst hard seeded varieties fruits of Kandhari variety imported from Afghanistan yield the best quality juice. Both Kabul Bedana and Kandhari varieties are deciduous. They flower and fruit sparsely and produce highly acidic fruits when grown in India (Purohit, 1982). Attempts to introduce and acclimatize other deciduous varieties

from Afghanistan, Iran and USSR failed totally for the same reasons. Therefore it has become necessary to improve the indigenous evergreen varieties by selection and hybridization. Lack of lignification of the seed testa is reported as the main cause for soft seededness in pomegranate (Anonymous, 1969). However, no information is available on the nature, time and degree of lignification in soft seeded and hard seeded pomegranate varieties in relation to growth, development and maturity of fruit. Such basic studies will help in understanding lignification process in hard seeded varieties and arresting lignification of seed testa by application of growth regulators at proper time. Also, such studies will be useful in working out the inheritance of soft seededness in pomegranate. Therefore, the present investigations were undertaken with the following broad objectives.

1. To study the growth and development of fruits and seeds of soft seeded and hard seeded varieties from fruit set to maturity.
2. To study the anatomical/histological differences in time and degree of lignification in seeds of both the types in relation to growth and development of fruit.
3. To quantify the differences between soft seeded (so called seedless) and hard seeded varieties.
4. To study seasonal changes in fruit quality.
5. To study changes in macro and micronutrients during growth and development of fruits.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Pomegranate cultivation has attained commercial importance during last decade especially with exploitation and popularisation of soft seeded variety 'Ganesh' in Maharashtra. Systematic research on various basic and applied aspects of this fruit started only recently. Therefore, literature relevant to the present investigations in pomegranate and other fruits is briefly reviewed here.

1. Fruit Growth and Development

1.1 Pattern of fruit growth

Leopold (1964) stated that the growth of the fruit involves the enlargement of the ovary and the associated parts and that the growth phenomena were mainly non-polar or scarcely polar type. Fruits exhibit mainly two types of growth patterns. Some fruits like apple, strawberry and melons exhibit 'Sigmoid' type of growth whereas fruits like grapes, peaches, apricots, cherries and plums exhibit 'Double sigmoid' pattern of fruit growth.

The studies conducted by Connors (1919), Blake (1925) in species of *Prunus*, Tukey (1935) in sweet cherry and sour cherry and Lilleland (1931) in apricot, showed three characteristic growth periods in the developing fruits designated as Period I, a period of rapid enlargement beginning about the time of full bloom. Period II, a mid-season period of retarded development varying in duration as correlated with the class of fruit

and the season of fruit ripening; and Period III, a second period of rapid enlargement extending to fruit ripening. Crane (1964) reported that the period I of the growth curve of 'Double sigmoid' fruit involved the enlargement of the ovary with the exception of the embryo and endosperm, the growth of which took place during the period II of fruit growth. During period III the expansion of the mesocarp took place which lead fruits to maturity.

According to Zielinski (1955) pomegranate is a special horticultural fruit called 'hyp' and the individual fruits inside are little drupes. Shulman et al. (1984) studied pomegranate fruit development and maturation in coastal plain and in the Bet Shean valley of Israel and reported that the growth curve of the pomegranate fruit from both climatic regions showed a 'single sigmoid' pattern thus suggesting that the pomegranate is a non-climacteric fruit.

Dash (1983) reported that in pomegranate variety selection GKVK-1, fruit took 126 to 130 days to mature from fruit set and also reported single sigmoid growth curve pattern.

Josan et al. (1979) reported that after fruit set in pomegranate there was a rapid and continuous growth for 1 - 1½ months, this was followed by a period of slow development for another 25 days. Thereafter the fruit growth remained more or less the same. It was also observed that maximum fruit growth

was observed during the second fortnight after fruit set.

Kennard (1955) reported that in Paheri mango all parts exhibited a sigmoid type of curve when growth data such as length, diameter, fresh weight and volume were plotted against time.

Patil (1983) reported that the growth pattern in the lemon fruit is 'sigmoid' and this growth pattern could be divided into 3 distinct periods based on the rate of growth. The stage I being stage of rapid growth from first to fifth fortnight, stage II being stage of steady growth from fifth to eleventh fortnight and stage III being stage of decline growth from eleventh to seventeenth fortnight.

In apple the curve for increase of fruit fresh weight shows a smooth sigmoid form (Denne, 1960). Further, he distinguished 3 phases in growth rate. An initial slow increase in fruitlet weight for 6-12 days from pollination, a rapid exponential increase in fruitlet weight for 3 weeks and a declining rate of growth until harvest. The seeds appear to reach their maximum width well before the other parts of the fruits.

Ryugo (1962) studied the development of peaches from fruit set to maturity especially with regard to the physiological significance of the endocarp in the course of the fruit development. He observed continuous growth of the endocarp during the suspended lag phase of fruit growth. He proposed the theory of competition

for nutrients during lignification process leading to suspended growth phase in peaches. However, there has been no satisfactory theory so far which explains the periodicity of fruit growth in fruits exhibiting double sigmoid pattern of growth. Recently some theories have been put forth which attempt to explain the phenomenon of lag phase on the basis of hormonal levels (Rao, 1973).

Nitsch et al. (1960) observed the peak level of auxins at the end of stage I, while Coombe (1960) observed it during stage II of berry development in case of grapes which exhibit double sigmoid pattern of fruit growth. Coombe (1960) attributed the growth of berry in stage I to auxins and that of stage III to the osmotic attraction of water. Bertrand and Weaver (1972) observed low levels of auxins in stage II and III of grapes, as such the berry growth during these periods could not be correlated to auxins.

Iwahori et al. (1966) observed a high gibberellin activity in both seeded and seedless Tokey grapes at the early fruit set stage, The seedless variety maintained a higher level than the seeded variety. The gibberellin activity decreased more rapidly in seedless than in seeded and the activity was no longer detectable in stage II of both the varieties. This lead them to assume that the lag phase may be a consequence of low gibberellin level.

1.2 Morphological changes during development

1.2.1 Changes in fresh weight, volume and specific gravity of fruits

Shulman et al. (1984) concluded during a study of pomegranate fruit development in Israel, that the fruit grows continuously from fruit set until after the commercial harvest time. The pattern of fruit growth showed a single sigmoid curve for the variety Mule's head whereas in the variety Wonderful, the growth was more linear. In coastal plain the average fruits weighed 250 g in Mule's head variety whereas it was 350 g in Wonderful variety. In Bet Shean valley the average fruit weight of Mule's head was 350 g and that of Wonderful was 400 g. The smaller size of fruits in coastal plain was probably due to the cooler weather. Even the seeds grew continuously and they contributed about half the fruits weight. The seeds reached 0.4 - 0.5 g when fruit was well ripened while the stone weight was 0.03 - 0.06 g and variety Shami had the smallest seeds.

Arie et al. (1984) showed 2 phases of growth in pomegranate fruits. A rapid phase until mid June (fruit set April 27th) and thereafter a gradual phase until harvest. It was concluded that there was a constant rate of fresh weight increase throughout the growing season.

Pandey et al. (1975) while studying biochemical changes in the developing mango fruit reported that the fresh weight, volume, length and breadth of the whole fruit continued to increase until

the stage of maturity. The development of fruit was slow from pea stage to marble (3.68 g) but maximum was during stage of half grown to the stage of full maturity (140 g).

Gulhane and Gupta (1974) reported that the growth of guava fruits as judged by its weight and volume showed a steady increase during the entire period of its development until a fortnight prior to full maturity. This increase in weight and volume has been mainly contributed by the increase in moisture percentage and T.S.S. contents in fruits.

Tripathy and Gangwar (1971) observed in guava that the specific gravity gradually decreased with the advancement of maturity, indicating a slight decrease in the solid matter content of the fruits. Fruits having more than 1 specific gravity were hard in texture, green in colour and less than 1 were soft and light yellow in colour. This has been a good index for maturity.

Lakshminarayana and Subramanyam (1966) studied the changes in fresh weight of fruits from fruit set to maturity in Calcutta round variety of Sapota under Karnataka conditions and reported that the weight increased gradually in the initial stages followed by a rapid increase from 5th month till 7½ months and finally the increase was gradual upto maturity. The authors concluded that the fruits exhibited sigmoid type of growth when fresh weight increase was plotted against time.

1.2.2 Changes in length and breadth of fruit

Dash (1983) has observed in all the 3 crop seasons, the length and diameter of fruit increased continuously from set to maturity. In all the stages the diameter of fruit was more than the length. The highest increase in length and diameter was during second fortnight and sixth fortnight. Thereafter, the rate of increase decreased continuously till maturity.

Bal and Singh (1978) reported that in ber cv. Umran the size of the fruit increased with the advancement of the season. The growth was more active during the first 6 weeks and the last 12 weeks and also the increase in length was faster than the diameter during the first phase whereas in the 3rd phase the fruit increased in diameter than in the length.

Bhuva et al. (1978) reported that in Kalipatti variety of sapota increase in fruit length was faster whereas increase in diameter continued to be slow resulting in slightly oblong fruits. Singh (1951) measured the length and diameter of the developing sapota fruits. He found somewhat rapid growth in the initial stages as well as in the final stages of fruit growth. He also noted a higher increase in diameter than length in the initial stages (first 16 weeks of fruit growth) followed by a reverse trend for four weeks but with lower rates of growth. Lakshminarayana and Subramanyam (1966) working on Calcutta round variety of sapota reported that the length and breadth of fruits increased continuously from fruit set to maturity.

1.2.3 Changes in the fruit colour

According to Shulman et al. (1984) in pomegranate cultivar Mule's head and Wonderful of Israel, fruit colour developed gradually and it served as a criterion for picking. The stage at which 70-90% of the skin is red usually corresponds with a TSS/acid ratio suitable for commercial picking. Some cultivars like Malissi do not develop any red colour in the skin, so it has very limited market appeal because of its unattractive greenish colour (Goor and Liberman 1956) the pomegranate rind may be thick or thin, the colour varying from pale yellow to crimson according to varieties (Anonymous, 1969).

According to Patil et al. (1977) the variety Ganesh which will be greenish when young, will turn to reddish green when fully matured and they will have rough appearance on the skin due to presence of black dots.

Malhotra et al. (1983) while studying many varieties of pomegranate revealed that the fruit colour was either pink or rose with varying intensities and all the shades belonged to the standard red colour. Even the seeds of all cultivars attained same colours.

1.2.4 Shape of fruits

Several types of pomegranates are cultivated in India. They are distinguished by the shape of the fruit, the colour and thickness of rind and the taste and colour of the seeds. The fruits are round, oblate or obovate in shape and vary in diameter

from 8-12 cm (Anonymous, 1969). According to Malhotra et al. (1983) the shape of pomegranate varieties they studied in Punjab were either round or globose.

2. Chemical changes during fruit development

2.1 Changes in the moisture content

Translocation of water into fruits is greatly influenced by the external environmental conditions as well as the internal physiological status of the plant (Wozlowski, 1973). Climate influence the fruit enlargement by altering the plant water relation. Translocation of osmotically active solutes into the fruits is determined by the water content of the tissue as the tissue water content can alter the sap concentration. Lakshminarayana and Subramanyam (1966) studied the changes in the dry matter content of the developing 'Calcutta round' sapota fruits. Their study revealed that the moisture content of the fruit was low in the initial stages of fruit growth and increased thereafter before finally declining as the fruit approached maturity.

2.2 Changes in sugars

Shulman et al. (1984) reported that in 4 varieties of pomegranate the sugar content of the juice measured as TSS increased gradually during fruit development. At the beginning of harvest in mid August the TSS of Hale's Red variety was 11-14% and reached 14-15% later in September. Variety Wonderful reached 15-14% TSS in mid August and 15-16% by the end of September. Herd (1955) reported that the sugars in pomegranate juice were glucose and fructose.

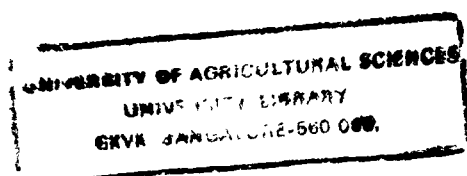
Lee et al. (1974) reported in pomegranate that the main sugars present in matured fruits were glucose and fructose and they are present in equal amounts in young fruit, and by 125 days after flowering only glucose remained.

Malhotra et al. (1983) studied different pomegranate cultivars in Punjab and reported that, all the varieties studied were higher with reducing sugars. They constituted about 90% of total sugars and non-reducing sugars were less than 1%. High reducing sugars may be attributed to be a genetic characteristic of this fruit.

Arie et al. (1984) reported that pomegranate fruits reached horticultural maturity for commercial harvest when the soluble solids content attained a fairly constant level of 15% and at this time the fruits appeared to be ripe in terms of quality.

Dash (1933) reported that in all stages of development of pomegranate fruit TSS increased continuously and the highest was 14.5° brix. The reducing sugars increased from 0.099 to 2.55% whereas total sugar increased from 2.713 to 10.265%.

In mango fruit Pandey et al. (1974) reported that the concentration of reducing sugar reached a peak at maturity (3.76 %/fruit). The level of non reducing sugars remained lower than that of reducing sugars till maturity.



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Gulhane and Gupta (1974) reported that TSS and total sugars in the guava fruits increased gradually during the entire period of fruit development. The increase being rapid between 75-135 days of growth.

Sachan et al. (1969) while studying influence of weather on chemical composition of guava variety Allahabad Safeda revealed that sugars were higher in winter season guava (9.57%) than rainy season guava (8.51%).

Sulladmath (1975) reported that in Kalipatti variety of sapota the sugar accumulation in the fruits was slow and steady during stage I of fruit growth. The rate of accumulation was slowed down during lag phase, but it was markedly accelerated after the termination of lag phase. Stage III of fruit growth was the most important stage for sugar accumulation since 80% of the total sugars observed at maturity accumulated during this stage. The reducing sugars increased rapidly during this stage and their proportion accounted for more than two thirds of the total sugars. The data expressed in terms of absolute amounts per fruit revealed a better trend of sugar accumulation than the one expressed on percentage basis.

2.3 Changes in acidity

Shulman et al. (1984) studied pomegranate fruit development and maturation and reported that the acid content of the juice decreased with maturation. Acidity in Mule's head was very low (0.5%) even at early stages of fruit development. In Wonderful

variety the acidity was very high in June and then decreased considerably until September. However, even at full maturation in September acidity did not drop below 1.5% in the coastal plain and below 0.9% in the Bet Shean valley. The predominant acids/ⁱⁿpomegranate juice are malic and citric acids (Nerd, 1965).

Dash (1983) reported that ascorbic acid increased continuously with advancement of maturity. The highest was reached in January-March crop i.e., 8.1 mg/100 g and lowest in October crop i.e., 5.8 mg/100 g.

Malhotra et al. (1983) observed acidity ranging from 0.49% to 2.30% as citric acid in pomegranate cultivars of Punjab.

Lee et al. (1974) reported that in pomegranate citric acid was the major organic acid in the fruits at 125 days with succinic, tartaric, fumaric and malic acids also present.

2.4 Changes in minerals

The role of minerals in fruit development has received scant attention. Bollard (1970) considers fruits as the physiological sinks in which the movement of the assimilates is preferential. There are several reports regarding source sink relationship between vegetative and reproductive structures like fruits. Cannel and Huxley (1969) have observed that in coffee the fruiting had a considerable bearing on the nutritional status of the plant mainly because the berries acted as 'physiological sinks'. Murneek (1926) concluded that the movement of the constituents

into fruits is directional. This translocation is regulated by plant hormones such as Kinetin (Mothes et al. 1959), auxins (David and Wareing, 1965) and cytokinin (Muller and Leopold, 1966).

Bal and Singh (1978) reported in ber, that phosphorus percent was less at 30 days after fruit set and it remained almost constant till 105 days, then a gradual decrease was noticed. A gradual decreased trend was also seen in calcium content upto 150 days, then it remained constant till ripening. The Iron content also remained constant in the beginning. After 120 days the maximum value of 1.52% was recorded, at later stages it decreased to 1.05%.

Litchi fruit growth and compositional changes during fruit development was studied by Paull et al. (1984) and they concluded that mineral uptake into the fruit paralleled fruit growth and potassium being the major cation. The Calcium content was 0.45 mg/g dry weight at 32 days after anthesis and it raised to 1.79 mg/g during 94 days after anthesis. Potassium was 2.13 mg/g at 32 days and the content was reduced to 1.44 mg/g at 94th day. The Magnesium content from 1.09 mg raised to 1.27 mg after 94 days from anthesis.

Lakshminarayan and Subramanyam (1966) observed that the total nitrogen content of developing sapota fruits decreased gradually from fruit set to 3½ months followed by a stationary

phase till 5½ months and finally there was a rapid drop till complete maturity of fruits.

Gardner et al. (1952) observed that the nitrogen content expressed as percentage on dry weight basis decreased gradually while the total absolute nitrogen content increased in developing apple fruits. The phosphorus content of fruits increased as the fruits developed and the content of the spurs decreased coincidentally, indicating the mobilization and translocation of phosphorus into the fruits.

Rao (1973) observed that in case of Pusa seedless grapes, the movement of ^{32}P in berries was hampered during the lag phase of berry development but was restored after the termination of lag phase suggesting that phosphorus movement may be a factor in fruit development.

Sulladmath (1975) observed in Kalipatti sapota that nitrogen content was high in the initial stages of fruit growth but continuously declined subsequently till maturity. The phosphorus content was very high during first fortnight but rapidly declined and reached a level of 0.241 mg/g dry weight at the end of first sub-phase of stage I of fruit development. There after the level remained rather stationary. The potassium content did not show any marked changes upto 8th fortnight of fruit growth. The potassium content, however, increased rapidly to 19.47 mg/g dry weight during 9th fortnight and steadily decreased till maturity.

The calcium content was very high 8.96 mg/g dry weight in the initial stages and declined to a level of 4.88 mg/g dry weight during 9th fortnight. The content increased again rapidly during the 11th fortnight and decreased to 3.26 mg/g dry weight during 13th fortnight. Then the level was stationary. Magnesium was high during 3rd fortnight (5.540 mg/g dry weight) but decreased rapidly during subsequent fortnight and rose to highest level of 6.864 mg/g dry weight during 9th fortnight of fruit growth. The content again continuously declined to reach a level of 1.950 mg/g dry weight at maturity.

Role of seeds in fruit development

Seeds are known to exert dominant influence on the development of fruit and it is evidenced by several correlation worked out between seed and fruit growth parameters. Luckwill (1953) reported that the development of the seed as well as the fruit in case of apple followed the same pattern.

Singh (1951) found a uniformly high correlation between fruit weight, seed weight, ratio of length to transverse diameter and sugar content in sapota fruit.

Cameron et al. (1960) observed that in case of Valencia oranges, the large fruits were associated with numerous seeds. Lange and Vincent (1972) confirmed the correlation between seed number and fruit weight in citrus fruits by comparing the fruits with different number of seeds. In case of grapes the berry weight was correlated with the seed number (Olmo, 1946).

Lignification in fruits

Kennard (1955) while working on development of the fruit, seed and embryo of the Paheri mango reported that endocarp lignification occurred during a period of three weeks, beginning about 10 weeks after fertilization, the husk, seed and embryo remained constant in size after husk hardening occurred, but the fleshy part of the fruit continued to enlarge slowly until maturity.

Anonymous (1969) stated that there are seeded and soft-seeded or so called seedless varieties in pomegranate. The seed coat varies in hardness, some of the softer seeded types being known as seedless (Bedana). Lack of lignification of the testa (outer integument) is the main cause of soft seededness (so called seedlessness) in pomegranate. The fleshy outgrowth called 'aril' of the seed testa constitutes the edible juicy portion in pomegranate fruit.

Causes of seedlessness in fruits

Seedlessness in fruits may be caused by parthenocarpy or stenospermocarpy. When the ovary of a flower is capable of developing itself into an edible fruit without pollination and fertilization, the phenomenon is called parthenocarpy. Parthenocarpic fruits are seedless. There are 2 types of parthenocarpy; i) Vegetative, ii) Stimulative.

In vegetative parthenocarpy the development of the ovary into an edible fruit is completely independent of pollination as

well as fertilization. This is seen in banana. Here, from the very beginning there is very high auxin content in the ovary, which takes care of its further development into an edible fruit. Stimulative parthenocarpy is seen in pineapple and certain cultivars of citrus. Here ovary receives its initial stimulus for its development into a fruit by way of pollination which is not followed by fertilization.

In certain fruit crops like seedless grapes, the ovary of the flower receives the initial stimulus of pollination followed by the fertilization of the ovules, however the fruits that are ultimately produced will be seedless. In this case there is abortion of the embryo that is produced as a result of fertilization. This phenomena is termed as 'Stenospermocarpy'.

As already explained above, the soft-seededness (so called seedlessness) of pomegranate is not due to parthenocarpy or stenospermocarpy but due to lack of lignification (Anonymous, 1969) The seeds of so called seedless pomegranate are fully viable and germinate without any difficulty.

Certain fruit crops like citrus will produce seedless fruits. Good number of them are proved to be triploids which results in various degree of male and female sterility which makes the parthenocarpic development of fruits possible.

In crops like tomato under low temperature conditions there will be production of parthenocarpic fruits, because of the

prevention of pollination and fertilization. In crops like cucumber which are grown under short day conditions followed by low night temperature conditions there will be production of large number of seedless parthenocarpic fruits.

Crane et al. (1961) tried gibberellin to induce parthenocarpy in J.H. Hale peach. GA induced parthenocarpic fruit set ranging from 53.8 to 69.3% and all the parthenocarpic fruits were elongated, smaller in diameter, and also there was no button fruits on GA sprayed bunches which otherwise would have produced button fruits in Hale peaches.

Stembridge and Gambrell (1970) studied effect of gibberellin and parthenocarpy on the shape and maturation of peaches. They reported that GA has caused some fruits to develop parthenocarpically and such fruits were elongated in shape and somewhat smaller than non-parthenocarpic fruits. The GA treated fruits were smaller and more elongated than the control fruits.

Effect of cytokinins and gibberellins on shape of Delicious apple fruits was studied by Williams and Stahly (1969). The study indicated that the chemicals affected fruit shape by increasing the length to diameter ratio of the fruits. Cytokinin caused fruits to be longer with prominent well developed calyx lobes. GA caused fruits to be longer but did not appreciably affected the calyx lobes.

Saavedra (1979) observed gibberellic acid significantly enhanced fruit set and stimulated growth of seedless fruit, in *annona cherimola*. Gibberellin induced severe cracks on fruit before harvest. Sunderarajan, et al. (1968) studied the effect of plant growth regulators on custard apple, and reported that GA consistently increased the fruit set by 45-70% and retention of fruits. The number of seeds were less in GA and NAA treated fruits.

Quantitative methods for measuring lignification of seed testa in pomegranate

Soft seeded types of pomegranate popularly but incorrectly called as seedless or Bedana, do not have lignification of seed testa. Till recently soft seededness was determined only after eating, but Purohit (1985) devised quantitative methods to measure the degree of softness in pomegranate. The mean thickness of seed testa, density of the whole seed, density of the seed testa alone and weight of the seed testa as percentage of whole seed weight were used to measure the degree of soft-seededness. On the basis of the 4 seed parameters and eating tests 'Bedana', 'Bassein Seedless', 'Ganesh', and 'Dholka' varieties of pomegranate are soft-seeded. Muskat variety is medium soft seeded while Alandi, Kandari and Kabul Yellow are hard seeded. This study indicated that a pomegranate variety can be called soft-seeded if the mean thickness of its seed testa is less than 0.5 mm, the density of the seed testa alone is less than 0.4 g/ml and the weight of the seed testa is less than 50% of the weight of the whole seed.

MATERIAL AND METHODS

MATERIALS AND METHODS

The present investigations were conducted at the Horticultural Experiment Station, Hessaraghatta of the Indian Institute of Horticultural Research, Bangalore during 1982, 1983 and 1984.

Description of the Experimental Block

The experimental Station of Indian Institute of Horticultural Research is located about 25 kms away from Bangalore City at 12.68°N latitude and 77.38°E longitude. It is in the elevated plane at an altitude of 863 meters above MSL. The experimental block has red sandy loam soil. The area receives rains both from South-West and North-East monsoons, amounting to about 900 mm annually. The climate is warm and slightly humid. The meteorological data for the years 1982, 1983 and 1984 are given in appendix - I. The mean monthly minimum temperature ranged from 15.5°C to 23.0°C and the maximum from 26.1°C to 34.6°C. The mean monthly relative humidity at 17.30 hours ranged from 29.3 to 70.4% and at 8.30 hours ranged from 64.0 to 90.0%. The wettest months were May, June, September, October while the most humid period was January, February, June, July. The monthly sunshine hours were high from December to May.

Details of Experimental Material

Five uniform plants each of four varieties viz., Bassein Seedless, Ganesh, Alandi and Kabul Yellow were selected from

varietal collection block. The plants were five year old and received uniform cultural practices including irrigation, manuring and plant protection. The soft seeded Bassein Seedless variety is grown commercially in Karnataka state. The hard seeded variety Alandi was once grown extensively around Poona, Maharashtra but it has been replaced by a soft seeded selection 'Ganesh' from Alandi (Cheema et al., 1954) the hard seeded variety Kabul Yellow is not grown commercially but has characteristic yellow coloured flowers and fruit colour.

Details of Experiment

The present investigations consisted of following major experiments.

- I. Growth and development of fruits and seeds in soft and hard seeded varieties.
- II. Anatomical and histochemical differences in time, nature and degree of lignification of seeds of two types of varieties.
- III. Quantifying differences between hard and soft seeded varieties.
- IV. Seasonal changes in fruit quality.
- V. Seasonal changes in macro and micronutrient contents of fruits.

I. Fruit growth and development

Eventhough pomegranate flowers thrice in an year, the number of flowers during January-February (Ambebahar) flowering flush is maximum and is considered as main flowering season under Hessara-ghatta conditions. The flowering lasts for 40 to 45 days during

this season. In each variety 150 perfect hermaphrodite flowers characterised by prominently bulged ovary were selected and tagged soon after fruit set on selected plants. The imperfect hermaphrodite flowers around the tagged flowers were removed.

Method of fruit sampling

First sampling of the fruit was done 10 days after fruit set (i.e. on 25th January, 1982, 7th February 1983 and 19th January, 1984). Subsequent samplings were done on 2, 30, 40, 50, 60, 80, 100 and 120 days after fruit set. At each sampling, 10 fruits were removed from each of the 4 varieties and data were recorded fruit wise on following characters and the mean values for sample of 10 fruits were calculated.

Details of observations recorded

(1) Fresh fruit weight (g)

By using electric balance fresh weight of each fruit was taken at every sampling as soon as the fruits were brought to the laboratory.

(2) Length and diameter of the fruit (cm)

The length of the fruit from the stalk end to the apex and the diameter at the equatorial plane was measured with the help of vernier callipers.

(3) Fresh weight (g) of rind and seeds (Aril + Seed)

The grains were removed carefully from the rind and the

weights of rind and grains were recorded separately by using electric balance.

(4) Moisture content (%) of the fruit or dry matter content

The cut fruits along with seed, pulp, rind were dried in hot air oven at 70°C till constant weights were obtained and the moisture content (%) was calculated.

(5) Specific gravity of fruit (g/ml)

The volume of fruit was determined by water displacement method, using a measuring cylinder and the specific gravity was calculated.

(6) Thickness of rind (cm)

Rind thickness was determined after cutting the fruit at the equatorial plane by using vernier callipers.

(7) 100 seed weight (g) of fresh and dry seeds

100 seeds were selected randomly and weights were taken on an electric balance. The same seeds were kept for drying in an hot air oven (70°C) and then dry weights were taken.

II. Anatomical and Histochemical Differences in seeds of soft and hard seeded varieties

Since degree of lignification of seed testa causes soft and hard seededness in pomegranate, the two types of varieties may differ in the time, nature and rate of lignification of seed

testa. There may also be histochemical differences. The anatomical differences were studied from seed sections and histochemical tests were conducted on the tissue - sections to localise the insoluble polysaccharides, DNA, RNA and proteins in fresh seed material. Details regarding collection of seeds, procedures for preparing seeds for sectioning, microtomy, fixing and staining of sections are described below.

2.1 Fixing of materials

The methods of histology and histochemistry have a common origin and a core of similar procedures, but differ in their staining techniques. In the present studies the following customary histological and the histochemical staining procedures, mainly chosen from Jensen's (1962) "Botanical histochemistry" were used.

2.2 Fixation and dehydration

The seeds extracted from every sampling at the interval of 10, 20, 30, 40, 50, 60, 80, 100 and 120 days were killed and fixed in FAA fixative (Formalin 5 parts + acetic acid 5 parts + ethyl 90 parts of alcohol). The seeds were fixed for 24 hours in this fixative. They were later treated in ascending grades of alcohol (50, 60, 70, 80, 90 and 100%), leaving the sample for 3 hours in each of the alcohol grades and then subjected to dehydration using absolute alcohol-butanol grades of 3:1, 1:1 and 1:3 proportions and finally treated with pure butanol twice.

2.3 Tissue infiltration and embedding

From the medium of pure butanol, the materials were transferred to small vials and the chips of paraffin wax were added successively until the medium reached a saturation point at the room temperature and later under the table lamp (40 watts). Finally, the materials were given changes with the molten pure paraffin in the oven at 60°C, thus replacing the last traces of butanol with paraffin. The materials were then embedded in paraffin employing paper boat method.

2.4 Microtoming

Serial microtome sections of 7 micron thickness were obtained using Erma rotary microtome. Care was taken to maintain constant thickness of the sections to obtain as far as possible uniform sections. When the ribbon was formed while microtoming it was laid on a clean black paper.

2.5 Affixing the sections to slides

Gelatin (0.2%) with a little quantity of potassium dichromate was used as an adhesive. Then the paraffin sections were cut into shorter units and arranged on the slides with adhesive. The slides were warmed for expansion and stretching of the ribbon. The excess adhesive was drained off and slides were dried before deparaffinising. Xylol was used to deparaffinise the sections. After a few minutes the slides were passed through the solution of xylol and butanol (1:1), pure butanol and then absolute alcohol

successively. The use of butanol ensures complete elimination of xylol which, when present even in small traces, creates a foggy-film on the tissue sections and prevents them from proper hydration and staining. The sections were hydrated passing through alcohol (down grade) series.

2.6 Histochemical staining

Hydrated sections were subjected to the histochemical staining, then dehydrated and mounted in DPX. Histochemical assessment was made on the fixed materials for insoluble polysaccharides, nucleic acids and proteins. Following are the details of the histochemical procedures which are described under each substance and adopted in the present investigation. The staining details are listed in Table 1.

TABLE-1

<u>Metabolite</u>	<u>Tests (From Jensen, 1962)</u>	<u>Indication</u>
Insoluble Polysaccharides	Periodic Acid-schiff's (PAS) test. (Hotchkiss, 1948)	Magenta colour
Proteins	Mercuric bromophenol blue method (Mazia, Brewer and Alfert, 1953)	Deep blue
DNA and RNA	Toluidine blue (Feder and O' Brien, 1968)	DNA-green RNA-Blue or violet.

2.6.1 Insoluble polysaccharides

Insoluble polysaccharides were assessed employing periodic acid -Schiff's (PAS) method.

Periodic Acid Schiff's (PAS) Method

Earlier, PAS method was used in histology only for the demonstration of mucin (McManus, 1946). Later, Hotchkiss (1948) elaborated the method and adopted the technique to demonstrate a variety of polysaccharides in plant tissues.

In PAS method, tissue sections were treated with a mild oxidative agent namely, periodic acid (HIO₄). During oxidation carbon -chains of polysaccharides containing 1 - 2- glycol groups were broken and the broken ends were oxidised to aldehyde groups. Such aldehydes react with leucobasic fuchsin (n - sulfinic acid) and produce bright pink colour-complexes.

Procedures: The following steps were adopted:

- (a) The paraffin embedded materials were sectioned at 7 micron thickness and mounted on slides using gelatin adhesive
- (b) The sections after deparaffinising in xylol, were hydrated through alcohol grades
- (c) Placed in oxidative reagent (prepared by dissolving 0.3 g of periodic acid in a mixture of 10 ml of 0.2 M sodium acetate and 30 ml of water) for 15 minutes
- (d) washed in running water for 10 minutes and stained with Schiff's reagent.

Preparation of Schiff's reagent: One gram of basic fuchsin was dissolved in 100 ml of 0.15 N HCl shaking the mixture at frequent intervals for 3 hours. Afterwards 2.0 g of potassium metabisulfite was added to the mixture, agitated and left overnight. Later, 500 mg of animal charcoal was added to it. The mixture was shaken and filtered immediately. Generally, the filtrate is colourless, but rarely shows straw-yellow colour. This stain-leucobasic fuchsin, was tested by adding a few drops of it to a few ml of formalin taken separately in a test tube. The immediate appearance of the magenta colour testifies to the needed quality of stain, while the delayed appearance of this colour-complex suggests otherwise indicating that the stain is not usable. The reagent was preserved in a refrigerator for a prolonged use.

Staining procedure:

(e) The tissue sections were rinsed in water and treated with bleach which was prepared by adding 5 ml of 1N HCl and 5 ml of 10 per cent potassium metabisulfite to 90 ml distilled water.

(f) The sections were washed in running water for 10 minutes, and

(g) dehydrated using alcohol -butanol grades, finally cleared in xylol and mounted in DPX (Distereene 80- resin - Dibutyl phthalate - plasticiser - Xylol - solvent).

2.5.2 Total proteins

The method used to identify protein is mercuric bromophenol

blue. This is adopted to recognise only the insoluble proteins as all the soluble ones are lost during fixation.

Mercuric bromophenol blue test (Mazia et al., 1953):

The original chemical procedure for the localization of proteins was modified by Mazia et al. (1953). The technique is an excellent one for the demonstration of proteins which are present even in smaller quantities. In this reaction basic proteins bind the bromophenol blue dye even when mercury is absent, while the other proteins bind the dye coupling thorough mercury. Thus, in the mercuric bromophenol blue method an excellent correlation exists **between** the amount of protein present and the amount of dye bound.

Procedure :

- (a) The sections were deparaffinised and brought to the absolute alcohol.
- (b) These were immersed in mercuric bromophenol blue stain for 15 minutes (the staining solution was prepared by dissolving 10 g of mercuric chloride and 100 mg of bromophenol blue power in 100 ml of absolute alcohol)
- (c) Superficial stain was removed by treating the sections with 0.5% acetic acid, and
- (d) immersed again in tap water which produces the final blue colour at the sites of proteins.
- (e) The sections were dehydrated in a n-butanol series, cleared in xylol and mounted in DPX. The proteins stain deep blue.

Toluidine blue method for DNA and RNA

- (a) Sections were deparaffinised and hydrated.
- (b) The sections were kept in 0.1% toluidine blue solution in water for 2 - 3 minutes.
- (c) The slides were washed in running water.
- (d) dehydrated in butanol, cleared in xylol and mounted in DPX. DNA containing sites appeared green.
RNA containing sites appeared blue or violet.

III. Quantifying Differences between Hard and Soft seeded Varieties

After the sectioning and staining the slides were photomicrographed for further study. Measurements of different layers of seed were made using ocular micrometer and tabulated for further presentation.

IV. Changes in Fruit Quality

The freshly extracted juice was subjected to following quality analysis.

1. Total soluble solids

The total soluble solids of the juice in ten fruit samples were recorded separately using the 'Erma' hand refractometer and the same was expressed in o brix.

2. Titratable acidity

10 ml juice sample was taken and diluted with distilled water and titrated against 0.1 N sodium hydroxide solution using

a few drops of 1.0% phenolphthalein as an indicator. The acidity was expressed as citric acid equivalents in 100 ml of juice (g %).

3. Reducing and non-reducing sugars

The reducing, non-reducing and total sugars were estimated by Sheffer - Somogyi's method (A.O.A.C., 1980) and the results were expressed as g/100 ml juice.

V. changes in Macro and Micro-nutrient contents of Fruits

The seeds and fruit rind was analysed for the following chemical constituents and expressed on dry weight basis.

1. Nitrogen
2. Phosphorus
3. Potassium
4. Calcium
5. Magnesium
6. Micronutrients (a) Iron, (b) Zinc, (c) Manganese
(d) Copper.

Nitrogen estimation: The nitrogen was determined by micro kjeldahl method (A.O.A.C., 1970).

Phosphorus, potassium, calcium and magnesium estimation: The extraction was done by wet ashing method as described by Chapman and Pratt (1961). This extract was used for the estimation of phosphorus, potassium, calcium and magnesium and micronutrients. Micronutrients were estimated by using Atomic absorption electro photometer.

RESULTS

RESULTS

The results of the investigations are presented below:

I. Pattern of fruit growth

1. Fresh weight of the fruits

The observations on the changes in the fresh weight of fruits upto maturity (120 days) were recorded at 10, 20, 30, 40, 50, 60, 80, 100 and 120 days interval from fruit set. The data were recorded on the fruits set in the ambe bahar (January-February flowering) for three years (1982, 1983 and 1984) and pooled mean figures for changes in fresh fruit weight are presented in Table 1 & 2 and Figure 1. The data were recorded for four varieties i.e., Ganesh, Bassein Seedless, Alandi, and Kabul Yellow. The pomegranate fruit grows continuously from fruit set until harvest time. The pattern of fruit growth in the four cultivars shows a 'Simple sigmoid curve' almost approaching a linear relationship. In Ganesh and Bassein Seedless there was appreciable decrease in growth rate and relative growth rate of fruit during 30 to 40 days, 50 to 60 days and 80 to 100 days. The fruits had made very fast growth during three distinct periods, i.e., between 20 to 30 days, 40 to 50 days and 60 to 80 days, thus periods of slow growth rate alternated with periods of fast growth rate. The data on growth rate revealed that the maximum growth rate in terms of increase in fresh fruit weight was observed around fruit maturity. In Alandi cultivar, fruit growth rate and relative growth rate

Table-1 Fruit growth, growth rate and relative growth rate of developing Pomegranate fruits Cv. Ganesh and Bassein Seedless (mean values over Three years)

Days after fruit set	GANESH				BASSEIN SEEDLESS			
	Fresh Fruit Weight (g)	Growth Rate/ Interval	Relative Growth rate (%)	Fresh Fruit weight (g)	Growth Rate/ Interval	Relative Growth rate (%)		
0	3.483	0.000	4.422	4.218	0.000	4.219		
10	15.206	11.723	7.506	14.351	10.133	4.944		
20	35.102	19.396	11.552	26.226	11.375	10.296		
30	65.721	30.619	5.057	50.956	24.730	5.465		
40	79.126	13.405	14.041	50.279	8.323	13.726		
50	116.344	37.218	6.538	92.247	32.968	8.707		
60	133.673	17.329	21.479	113.161	20.914	18.894		
80	190.604	56.931	5.041	153.540	45.379	7.598		
100	203.967	13.363	23.045	176.790	18.250	26.389		
120	265.050	61.083	-	240.170	63.380	-		

Table-2 Fruit growth, growth rate and relative growth rate of developing Pomegranate fruits Cv. Alandi and Iabul Yellow (Mean values over Three years)

Days after fruit set	ALANDI				IABUL YELLOW			
	Fresh fruit weight (g)	Growth rate/ Interval	Relative Growth rate (%)	Fresh fruit weight (g)	Growth rate/ Interval	Relative Growth rate (%)		
0	5.583	0.000	3.940	3.250	0.000	2.760		
10	15.131	9.548	6.686	8.453	5.203	6.324		
20	31.333	16.202	13.241	20.371	11.921	6.156		
30	63.421	32.088	7.109	31.975	11.604	8.583		
40	80.648	17.227	4.515	48.153	16.178	5.455		
50	91.590	10.942	15.697	58.433	10.282	11.326		
60	129.628	38.038	16.423	79.781	21.348	20.779		
80	169.425	39.797	10.450	118.946	39.165	13.555		
100	194.750	25.325	19.632	144.495	25.549	23.234		
120	242.323	47.573	-	188.475	43.980	-		

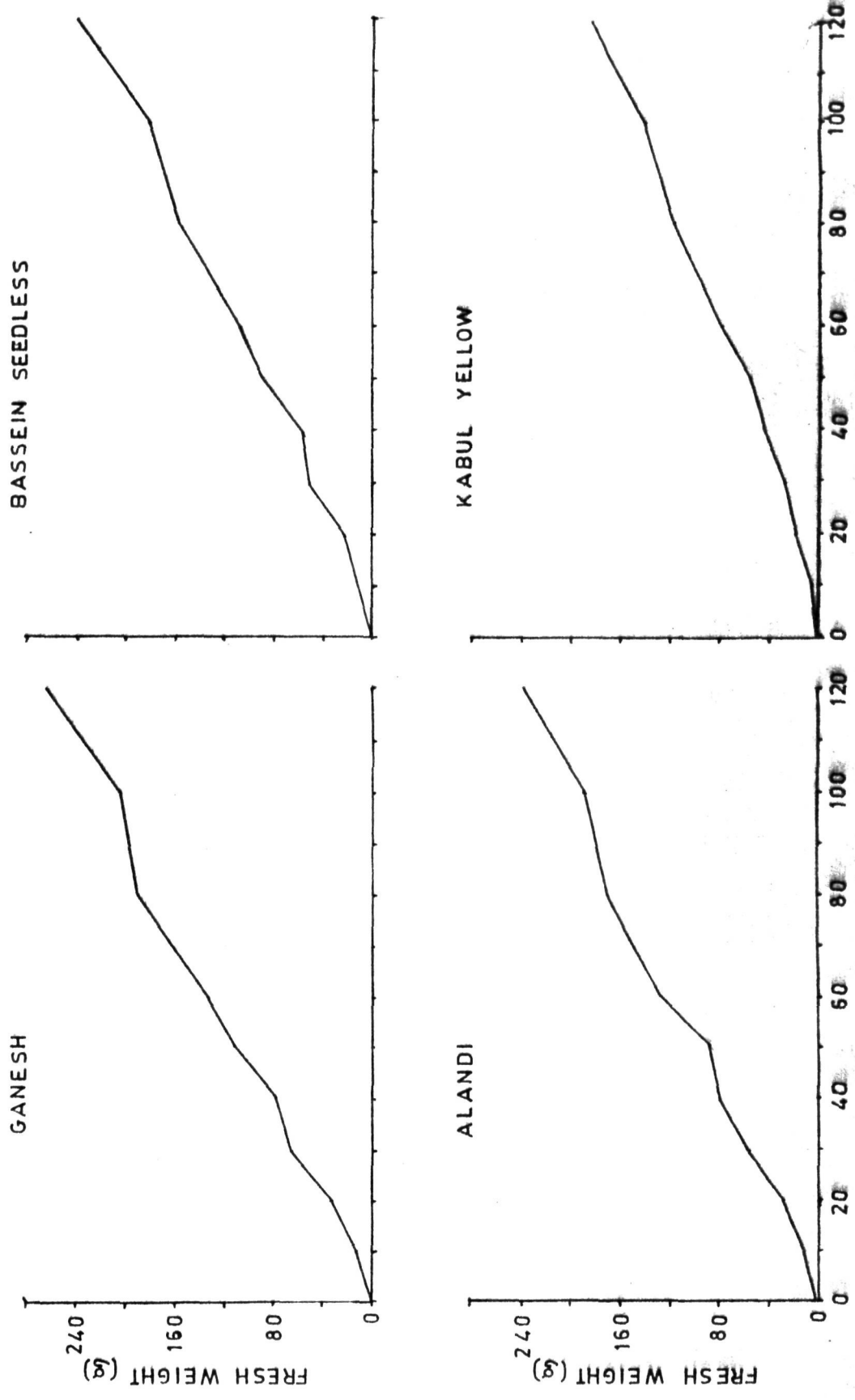


Fig. 1. CHANGES IN FRUIT WEIGHT (FRESH) IN DEVELOPING POMEGRANATE FRUIT

was low during 30 to 50 and 80 to 100 days while in Kabul Yellow it was low during 40 to 60 and 80 to 100 days. The slow growth periods alternate^{nat} with periods of fast growth rate. The fruits of Alandi had made very fast growth during 20 to 30 days, 50 to 80 days and 100 to 120 days whereas in Kabul Yellow the fast growth rate was observed during 50 to 80 days and 100 to 120 days. All the four cultivars exhibited very fast growth around maturity. At maturity (i.e., 120 days) the fresh fruit weights in the four varieties were, Ganesh 265.050 g, Bassein Seedless 240.170 g, Alandi 242.323 g and Kabul Yellow 183.475 g.

2. Length and breadth of the fruit

The data on the changes in the linear dimensions of the fruit namely, length and breadth are presented in Table 5 and figure 2. The length and breadth of fruit increased continuously from fruit set to maturity however, the increase in length was faster as compared to breadth during the early period of fruit growth. This growth pattern was observed in Ganesh between 0 to 40 days, 0 to 80 days in Bassein Seedless and 0 to 20 days both in Alandi and Kabul Yellow. In the later period of fruit growth increase in breadth was more pronounced than in length. Greater increase in breadth towards fruit maturity resulted in round fruits.

3. Length/Breadth ratio

The fruits of Ganesh, Alandi and Kabul Yellow had higher length/breadth ratio during the initial stages of fruit growth.

Table-3 Changes in Length, Breadth, Length/Breadth Ratio in developing fruits of four pomegranate varieties (Mean values after three years)

Days after fruit set	GANESI			BASSEIN SEEDLESS			ALANDI			KAPUL YELION		
	Length (cm)	Breadth (cm)	L/B ratio	Length (cm)	Breadth (cm)	L/B ratio	Length (cm)	Breadth (cm)	L/B ratio	Length (cm)	Breadth (cm)	L/B ratio
0	1.80	1.47	1.22	1.57	1.40	1.12	2.10	1.62	1.29	1.64	1.45	1.13
10	3.10	2.73	1.13	2.74	2.63	1.04	2.56	2.23	1.14	2.54	2.24	1.13
20	4.27	3.89	1.10	3.92	3.43	1.11	3.68	3.66	1.06	3.67	3.78	0.97
30	5.05	4.75	1.06	4.67	4.43	1.04	4.76	4.86	0.98	3.82	3.87	0.98
40	5.25	5.22	1.00	5.00	4.87	1.06	5.12	5.42	0.94	4.38	4.42	0.99
50	6.11	6.22	0.98	5.39	5.26	1.02	5.55	5.53	0.95	4.64	4.25	0.97
60	6.62	6.86	0.96	5.78	5.74	1.00	5.92	6.32	0.93	4.76	5.28	0.90
80	6.67	7.05	0.97	6.91	6.71	1.02	6.47	6.96	0.93	6.06	6.30	0.96
100	7.04	7.22	0.97	6.32	6.83	0.92	6.61	7.25	0.93	6.07	6.65	0.91
120	7.30	7.45	0.97	7.32	7.77	0.94	7.09	7.95	0.89	6.82	7.72	0.94

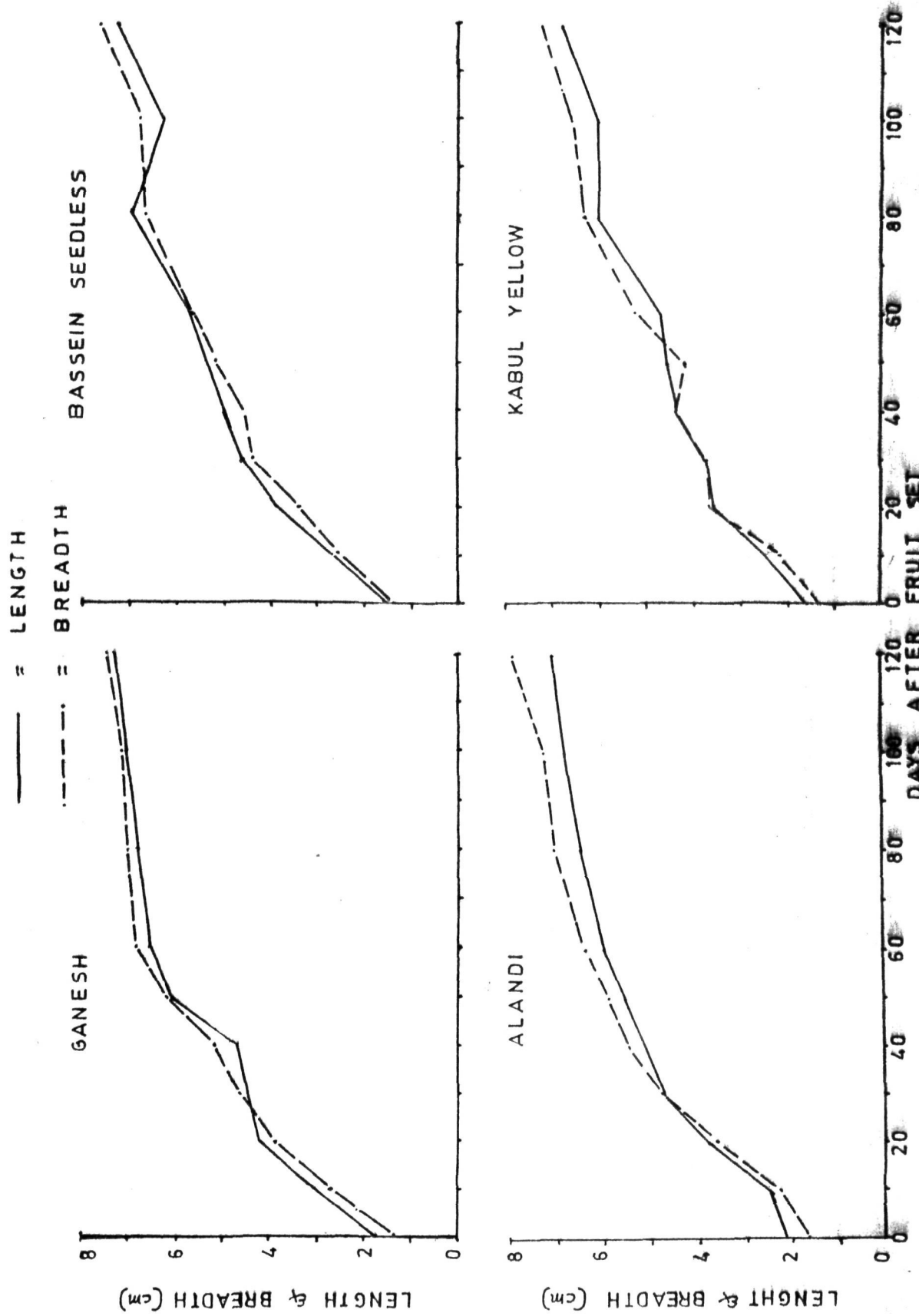


Fig. 2. CHANGES IN LENGTH AND BREADTH OF DEVELOPING POMEGRANATE FRUIT

The ratio gradually decreased towards maturity as a result of greater increase in breadth. Thus, the fruits in the initial stages were oblong ovate and subsequently they became round, globular or globose in shape. In the variety Ganesh the length/breadth ratio was 1.22 at fruit set and it decreased to 0.97 at maturity. In variety Bassein Seedless the length/breadth ratio remained around 1 upto 80 days indicating that increase in length and breadth was of similar magnitude during this period and the fruits were almost round. The ratio decreased to 0.94 at maturity. In Alandi variety the length/breadth ratio was 1.29 at fruit set which decreased to 0.89 at maturity while in Kabul Yellow the ratio decreased from 1.13 at fruit set to 0.94 at maturity.

4. Fresh weight of rind and seeds

The data on fresh weights of rind, seeds (i.e., all seeds covered with fleshy edible arils) and rind weight/seed weight ratio are presented in Table 4 and figure 3. The weight of rind and seeds increased continuously till maturity. During first 20 days of growth the rind weight was more as compared to seeds in all the four varieties. During 20 to 40 days, the weights of rind and seeds were roughly balanced. From 40 days till maturity, the weight of seeds was more than the rind. During most of the stages of fruit development the seeds constituted about half of the fruit weight. (The fresh fruit weights are given in Table 1 and 2).

Table-4 Changes in Rind weight, seed weight, seed weight/Rind weight ratio in Developing fruits of four pomegranate varieties (Mean values over three years)

Days after fruit set	GANESH			BASSEIN SEEDLESS			AIANDI			KAPUL YELLOW		
	Rind weight (g)	Total seed weight (g)	Seed weight/Rind weight ratio	Rind weight (g)	Total seed weight (g)	Seed weight/Rind weight ratio	Rind weight (g)	Total seed weight (g)	Seed weight/Rind weight ratio	Rind weight (g)	Total seed weight (g)	Seed weight/Rind weight ratio
0	3.20	0.21	0.08	4.03	0.18	0.04	5.44	0.14	0.02	3.11	0.13	0.04
10	13.05	2.24	0.17	12.04	2.31	0.19	12.40	2.67	0.21	7.38	1.05	0.14
20	25.82	10.18	0.39	18.95	7.27	0.38	22.96	8.37	0.36	11.00	0.10	0.82
30	36.31	20.38	0.51	24.02	25.93	1.12	35.80	27.62	0.77	13.41	12.56	0.64
40	30.7	40.32	1.03	20.57	30.70	1.07	35.14	45.51	1.29	20.25	1.1	0.79
50	55.92	60.35	1.08	39.06	52.00	1.31	41.22	50.37	1.02	28.00	30.11	1.08
60	59.55	74.08	1.24	40.50	60.67	1.72	55.71	75.32	1.41	34.12	45.65	1.33
80	69.52	120.32	1.73	56.53	102.04	1.80	66.50	102.43	1.54	47.17	71.78	1.52
100	95.30	118.16	1.39	65.36	106.43	1.62	79.51	115.24	1.45	40.14	94.85	1.93
120	107.33	157.72	1.47	95.66	145.51	1.52	5.31	140.98	1.54	67.33	121.14	1.79

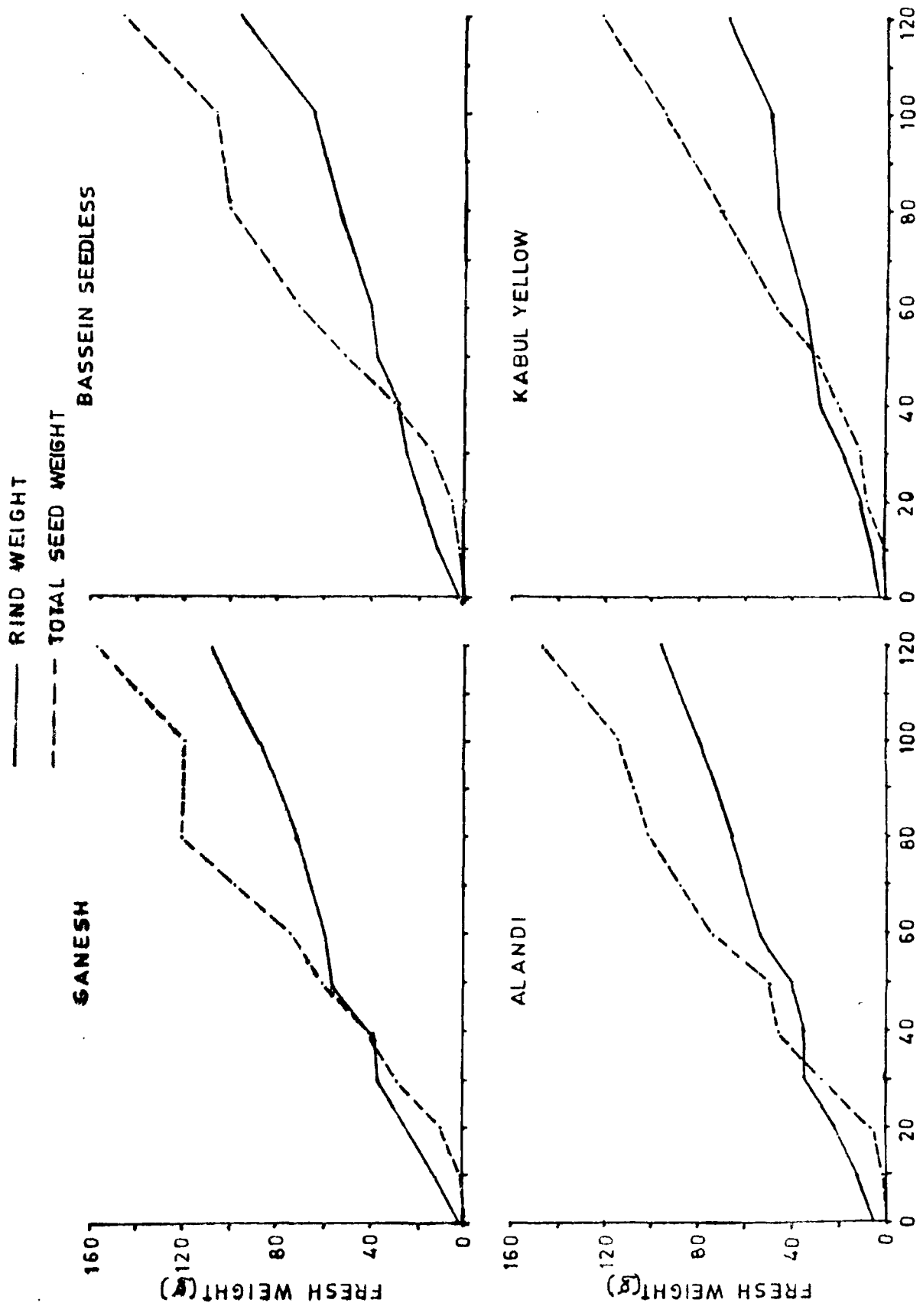


Fig. 3. CHANGES IN RIND WEIGHT (FRESH) TOTAL SEED WEIGHT (FRESH) IN DEVELOPING POMEGRANATE FRUIT

5. Seed weight to rind weight ratio

The data on seed weight/rind weight ratio (Table 4 and figure 4) revealed that the ratio increased gradually from fruit set up to 80 days and then it declined slight upto maturity (120 days). In Ganesh the ratio was 0.08 at fruit set and continued to be below 1 till 50 days and gradually increased to 1.47 at maturity. In Bassein Seedless the ratio was 0.04 at fruit set and 1.52 at maturity. In Alandi it was 0.02 at fruit set and 1.54 at 120 days. In Kabul Yellow the ratio was 0.04 at fruit set remained below 1 till 40 days and finally reached 1.79 at maturity stage. Although both seed weight and rind weight increased continuously from fruit set to fruit maturity, initially the newly set fruit consisted mainly of rind portion hence the ratio values were low. As the growth and lignification of seeds picked up, the ratio crossed the value of 1 around 40 days, after 40 days seed growth overtook rind growth, and thereafter the ratio kept on increasing and again around maturity i.e., at 100 and 120 days there was slight decline in the ratio. The data further revealed that total seed weight was highest i.e., 152.72 g in Ganesh followed by 146.98 g in Alandi, 145.51 g in Bassein Seedless and 121.14 g in Kabul Yellow. A comparison of fresh fruit weight (Table 1 and 2) and total seed weight at maturity (Table 4) revealed that fruit and seed weights were roughly correlated.

6. Dry matter content of fruit and seeds

The data on dry matter content (oven dry basis) are presented in Table 5 and illustrated in figure 5. The data showed that the dry matter content in case of fruits increased continuously till

Table-5 Changes in dry matter content of fruits and seed (%) (oven dried basis) in developing fruits of pomelo and varieties

Days after fruit set	GAMES		BASSIL SEEDLESS		ALADI		KABI YELLOW	
	Fruits (g)	Seed (%)	Fruits (g)	Seed (%)	Fruits (%)	Seed (g)	Fruits (g)	Seed (%)
10	31.03	6.40	30.87	5.72	25.71	4.95	2.17	6.10
20	33.16	10.80	32.08	11.40	22.59	10.70	31.07	12.12
30	33.39	12.36	36.64	11.95	30.58	13.09	35.55	14.15
40	42.19	13.06	35.91	12.73	32.37	13.53	37.82	15.72
50	38.29	15.15	37.66	15.70	33.90	14.63	41.13	20.25
60	35.47	16.94	34.84	16.48	29.93	15.33	33.31	22.09
80	34.55	17.09	30.71	18.14	28.51	15.82	38.27	22.64
100	28.78	17.76	23.59	19.07	26.94	16.73	37.58	23.14
120	26.70	17.91	23.23	20.09	23.01	17.64	35.71	21.72

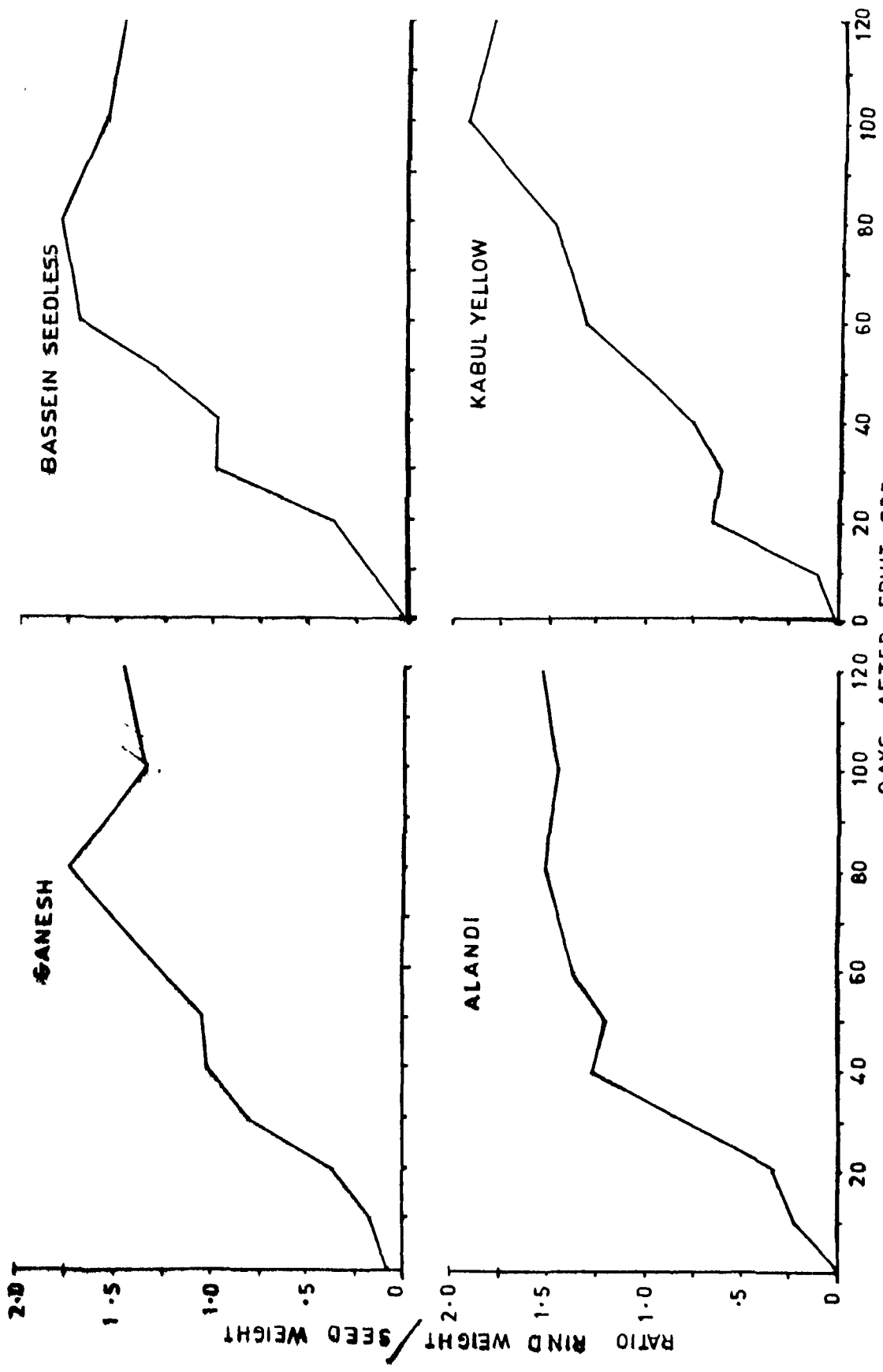


FIG. 4. CHANGES IN SEED WEIGHT / RIND WEIGHT RATIO IN DEVELOPING POMEGRANATE FRUIT

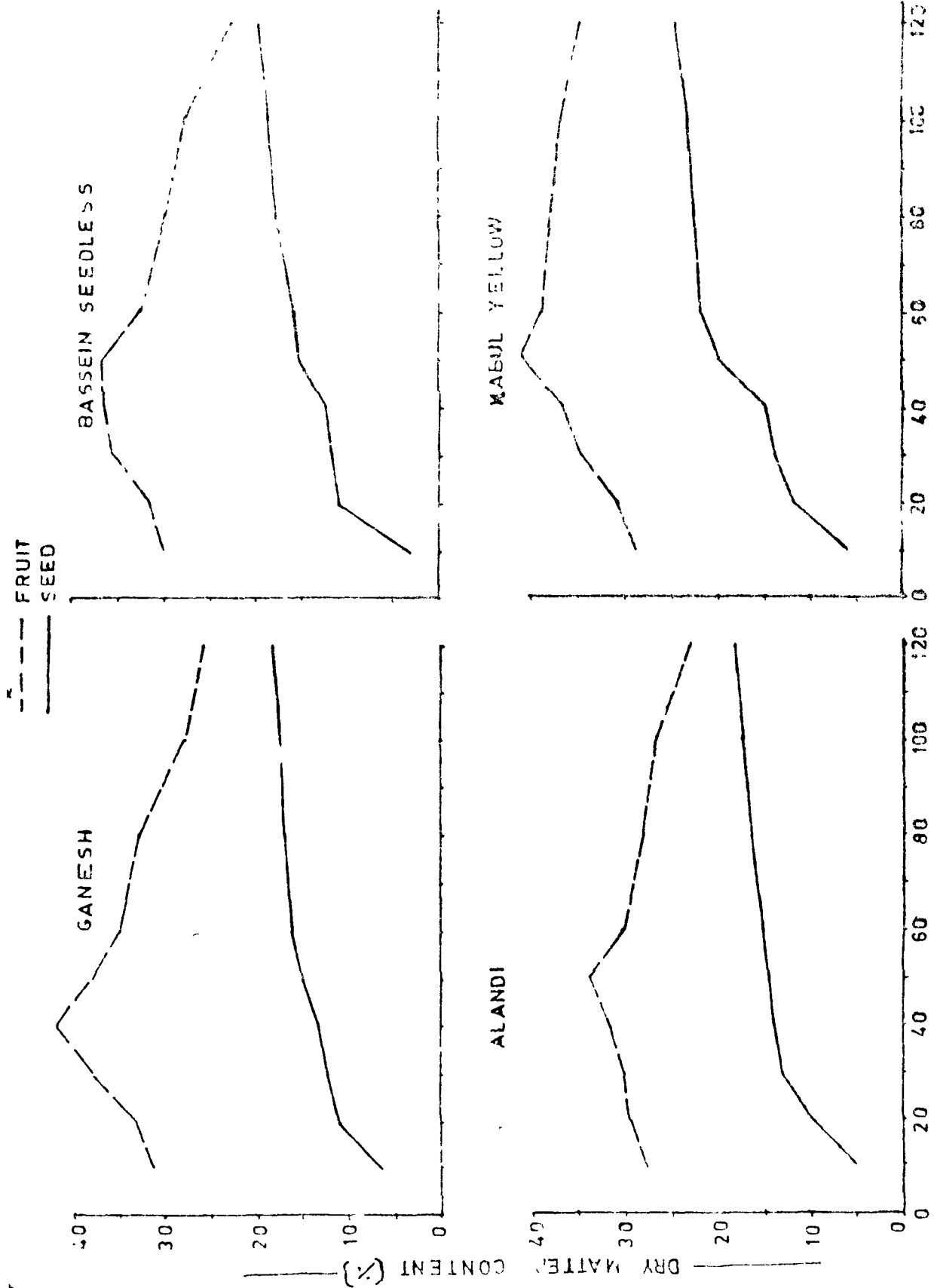


FIG. 5. CHANGES IN DRY MATTER CONTENT OF FRUIT & SEEDS IN DEVELOPING POMEGRANATE FRUITS

about 50 days in all the four cultivars and then declined gradually towards maturity, as a result of formation of juice in the fleshy and oil portion of seeds. However, in case of seeds the dry matter content increased continuously from fruit set till maturity. The present dry matter content of Ganesh fruits slowly increased from 41.03% at 10 days to 42.10% at 40 days and thereafter it declined to 26.70% level at maturity stage. In Bassein Seedless the dry matter content of fruit increased from 30.87% at 10 days to 33.66% at 50 days and then slowly decreased to 23.23% at maturity. In Alandi it increased from 23.71% at 10 days to 33.90% at 50th day and came down to 23.01% at 120 days. In Kabul Yellow the dry matter content at 10, 50 and 120 days were 29.17%, 41.15% and 35.01% respectively. This variety had highest dry matter content at maturity.

The dry matter content of seed in Ganesh slowly increased from 6.40% (at 10 days) to 17.91% at maturity. In Bassein Seedless the dry matter content was 4.72% at 10 days, incidentally which was the least when compared to other varieties, then it raised slowly to 20.09% at maturity stage. In Alandi it was 4.95% and 17.64% at 10 days and maturity stage respectively. Kabul Yellow recorded 6.10% of dry matter content during 10 days of growth which slowly increased to 24.72% at maturity. Out of all the four varieties studied Kabul Yellow recorded highest (24.72%) dry matter content at maturity stage.

7. Specific gravity of fruits

The data on specific gravity of fruits are presented in Table 6. It was higher in early stages of fruit development in all the four varieties, and then gradually showed a declining trend from fruit set to maturity. In Ganesh variety the specific gravity was more than 1 upto 30 days of fruit growth and then started declining towards maturity reaching 0.902. In Bassein Seedless the specific gravity showed a fall from 1.120 to 0.900. It was more than 1 upto 40 days of fruit growth. Alandi variety showed a similar trend as Ganesh. Variety Kabul Yellow had the maximum specific gravity till 80 days of fruit growth. It was more than 1 till that time, then only it came down to 0.965 at maturity. In general the pomegranate fruits had less than 1 specific gravity when they approached maturity and ripening stage.

8. Thickness of Rind

The data on rind thickness are presented in Table 7. The data revealed that the thickness was maximum at 20 days and it declined towards maturity, Ganesh and Bassein seedless had maximum thickness of 0.52 cm and 0.50 cm respectively, at 20 days and at that stage Alandi and Kabul Yellow had 0.45 cm and 0.43 cm rind thickness respectively. At maturity (120 days) Ganesh, Bassein Seedless, Alandi and Kabul Yellow had 0.34 cm, 0.30 cm, 0.30 cm and 0.33 cm rind thickness respectively.

Table-6. Specific gravity of fruits of Four Pomegranate varieties

Days after fruit set	Specific gravity (g/ml)			
	Ganesh	Bassein Seedless	Alandi	Kabul Yellow
0	0.750	0.800	0.990	0.690
10	1.114	1.122	1.049	1.092
20	1.086	1.126	1.018	1.093
30	1.024	1.080	0.908	1.024
40	0.996	1.016	0.987	1.024
50	0.971	0.983	0.946	0.981
60	0.957	0.971	0.945	1.089
80	0.921	0.967	0.920	1.047
100	0.908	0.902	0.921	0.968
120	0.902	0.900	0.901	0.965

Table-7. Rind Thickness in developing fruits of pomegranate (cm)

Days after fruit set	Rind thickness (cm)			
	Ganesh	Bassein Seedless	Alandi	Kabul Yellow
10	0.36	0.44	0.42	0.40
20	0.52	0.50	0.45	0.43
30	0.40	0.32	0.32	0.37
40	0.40	0.30	0.37	0.35
50	0.40	0.32	0.32	0.35
60	0.40	0.30	0.35	0.33
80	0.35	0.32	0.30	0.32
100	0.34	0.30	0.30	0.30
120	0.34	0.30	0.30	0.30

9. Hundred seed weight (Fresh and Dry)

The data on 100 seed weight (both fresh and oven dry) are presented in Table 8 and figure 6. The data showed that the fresh as well as dry seed weight continuously increased from fruit set till maturity in all the four varieties studied. In the variety Ganesh the fresh and dry seed weights at 10 days after fruit set were 0.113 g and 0.023 g respectively. In Bassein Seedless the fresh and dry weights were 0.130 g and 0.022 g respectively. Variety Alandi had 0.119 g fresh weight whereas dry weight was 0.014 g. In Kabul Yellow the fresh and dry weights were 0.131 and 0.018 g respectively.

At maturity, stage Ganesh variety recorded maximum fresh weight of 33.883 g for 100 seeds because of large aril and more juice content, the dry weight being 3.272 g. Bassein Seedless registered 26.571 g fresh weight and 3.900 g dry weight at maturity. Alandi variety had 23.649 g fresh weight and 4.133 g dry weight, whereas Kabul Yellow had 22.167 g fresh weight and 4.550 g dry weight.

II. Histological Studies

Histological changes observed in the integuments during seed development of pomegranate varieties Ganesh, Bassein Seedless, Alandi and Kabul Yellow were as follows and the micrometric observations at different intervals are presented in Table 9.

Table-8. Changes in hundred seed weight (fresh and dry) in developing fruits of pomegranate varieties

Days after fruit set	Ganesh		Rassein Seedless		Alandi		Kabul Yellow	
	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)
10	0.113	0.023	0.130	0.023	0.119	0.014	0.131	0.018
20	1.250	0.148	1.037	0.138	1.127	0.152	1.397	0.186
30	3.837	0.451	3.805	0.435	3.729	0.450	5.198	0.403
40	6.685	0.769	6.629	0.791	5.646	0.716	4.522	0.724
50	9.101	1.438	8.641	1.391	8.889	1.310	6.721	1.312
60	10.092	1.727	10.760	1.878	13.049	2.203	8.575	1.638
80	19.195	3.450	15.166	2.790	16.723	2.409	15.548	3.458
100	23.884	3.177	20.567	3.757	21.803	3.456	18.305	3.996
120	33.883	3.372	26.571	3.000	23.649	4.183	22.167	4.500

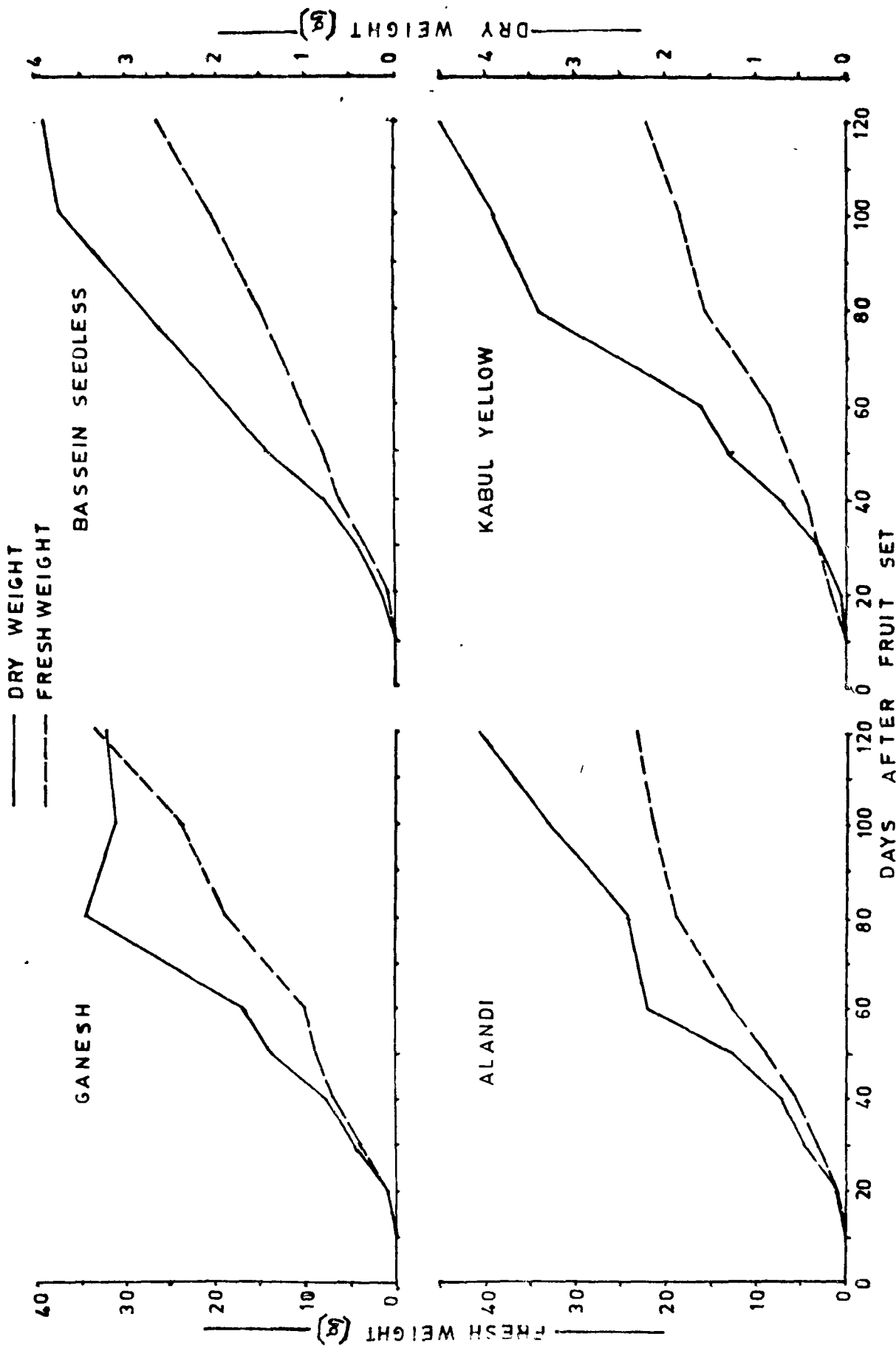


Fig. 6. CHANGES IN 100 SEED WEIGHT FRESH AND DRY IN DEVELOPING FRUITS OF FOUR POMEGRANTE VARIETIES

After fruit set i.e., 10 days after anthesis, the seed size of soft seeded varieties Ganesh and Bassein Seedless were $16.0 \times 7.68 \mu\text{m}$ and $16.96 \times 7.20 \mu\text{m}$, respectively. At the same time the seed size of hard seeded varieties Alandi and Kabul Yellow were $20.88 \times 8.48 \mu\text{m}$ and $22.40 \times 13.60 \mu\text{m}$, respectively. It is observed that the seed size in hard seeded varieties was more than that in soft seeded varieties.

During this stage the outer integument at chalazal region (Fig 21 & 24) had a thickness of 0.48 to $0.51 \mu\text{m}$ in all the varieties except Alandi which had $0.72 \mu\text{m}$. The cell size in all the varieties was almost the same (i.e., $0.12 \times 0.24 \mu\text{m}$). At lateral region of the seed the thickness of outer integument in Ganesh (Figs 20 and 23) and Bassein Seedless was 0.32 and $0.36 \mu\text{m}$ whereas in Alandi and Kabul Yellow it was 0.48 and $0.40 \mu\text{m}$ respectively but the cell size was almost equal in all the varieties studied. At micropylar end (Fig. 19 and 22) the thickness of outer integument in Ganesh and Bassein Seedless was 0.80 and $0.56 \mu\text{m}$ respectively, whereas it was 0.36 and $0.40 \mu\text{m}$ in Alandi and Kabul Yellow, respectively. In general the cells at outer integument were elongated with thickened cell walls.

A perusal at inner integument revealed that at chalazal region its thickness was $2.0 \mu\text{m}$ in Ganesh, $2.16 \mu\text{m}$ in Bassein Seedless, $2.0 \mu\text{m}$ in Alandi and $1.52 \mu\text{m}$ in Kabul Yellow (Fig. 26). At lateral region the thickness was $0.32 \mu\text{m}$ in Ganesh, $1.12 \mu\text{m}$ in Bassein Seedless, $0.98 \mu\text{m}$ in Alandi and $0.44 \mu\text{m}$ in Kabul

PLATE I

Figs: 19-24: Sections of 10 day old seed of Canesh (soft seeded variety) tested with periodic acid Schiff's. (PAS) reagent showing inclusions.

Fig : 19: Micropylar region of the seed. Note the wide portion of inner integument. X 100

Fig: 20: Lateral region of the seed showing different regions. Note the narrowness of inner integument. X 100.

Fig : 21: Chalazal region of the seed showing outer integument and inner integuments, without any starch deposition. The cells are small. X 100.

Fig : 22: Micropylar region of the seed x 400

Fig : 23: Lateral region of the seed x 400

Fig : 24: Chalazal region of the seed x 400

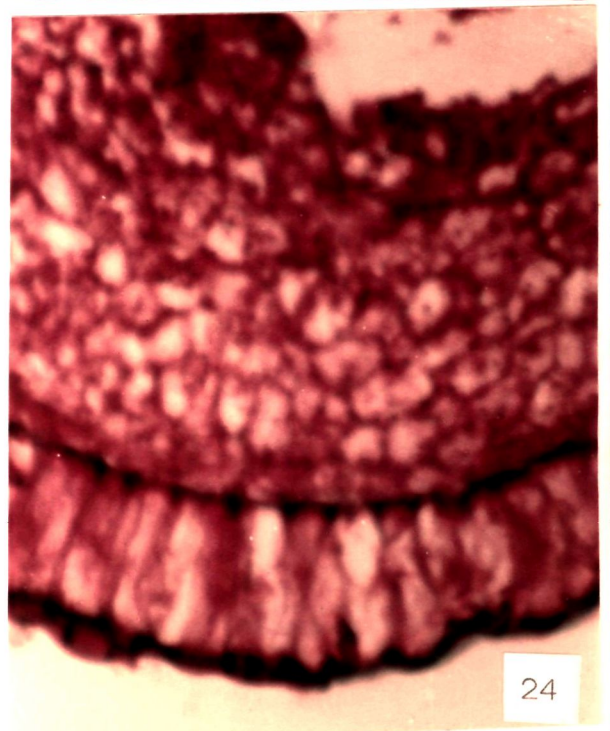
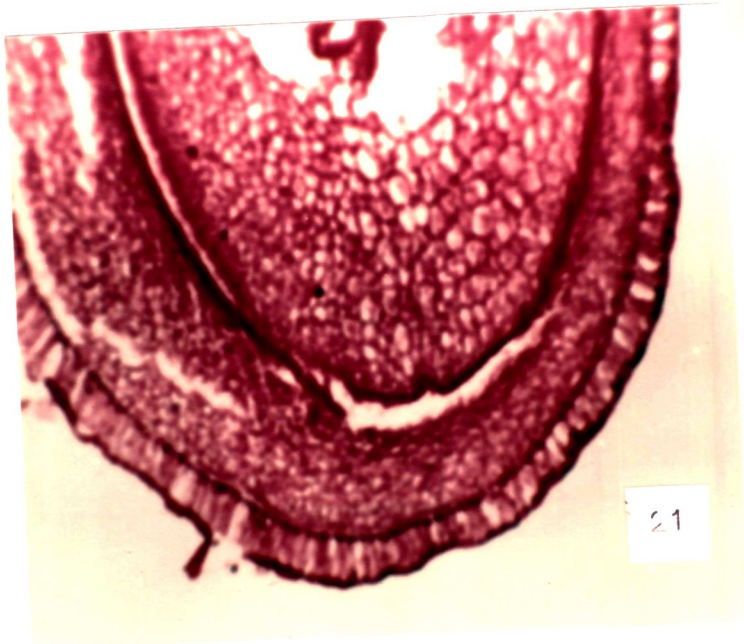
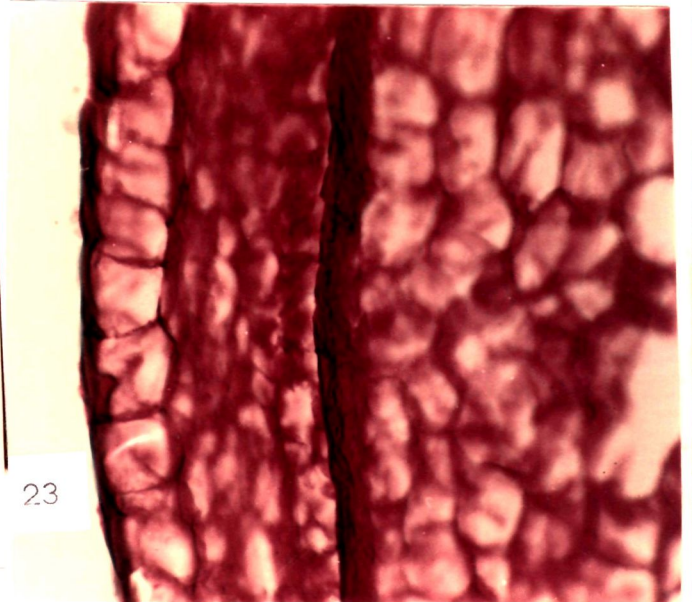
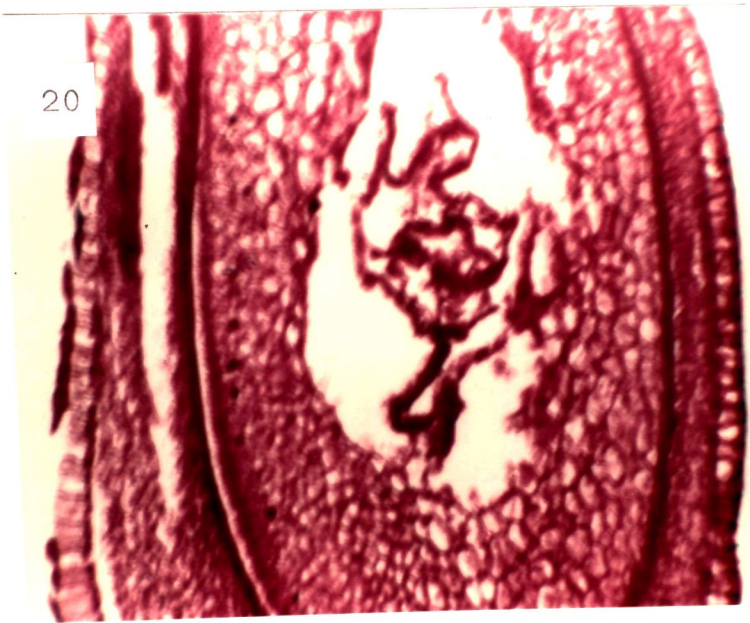
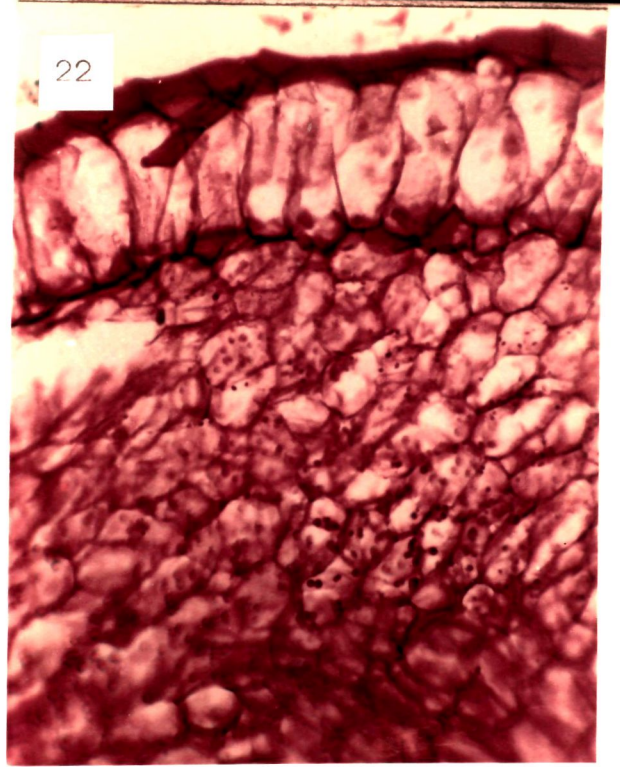
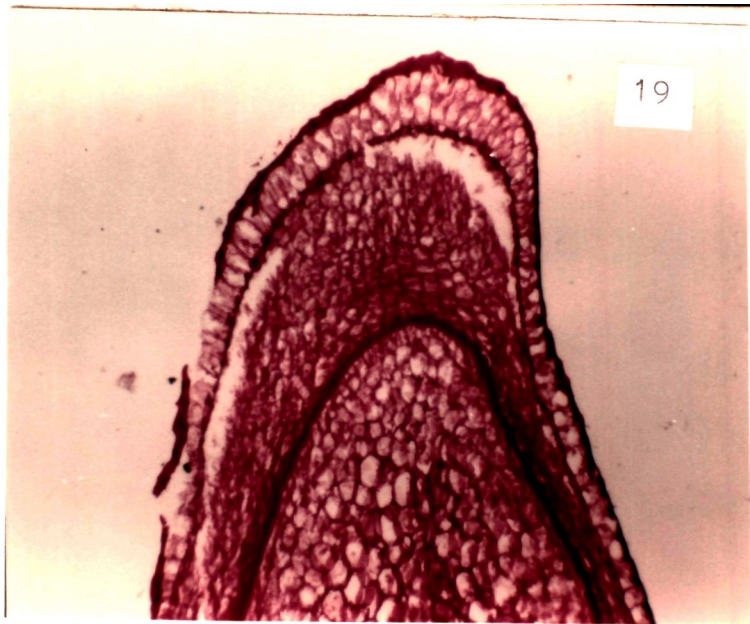


Table-9. Histological changes in integuments during seed development of pomegranate varieties (in μm)

Days after fruit set	Side size		OUTER INTEGUMENT									INNER INTEGUMENT					
			Chalazal			Lateral			Micropylar			Chalazal		Lateral		Micropylar	
	L	W	T	L	W	T	L	W	T	L	W	T	D	T	D	T	D
	0	16.00	7.68	0.48	0.40	0.12	0.32	0.20	0.16	0.80	0.28	0.18	2.00	0.12	0.32	0.20	2.40
0	18.96	7.20	0.44	0.32	0.16	0.36	0.30	0.20	0.56	0.44	0.16	2.16	0.12	0.12	0.12	1.52	0.12
0	20.88	6.48	0.72	0.48	0.12	0.48	0.44	0.20	0.36	0.24	0.20	2.00	0.16	0.98	0.12	4.10	0.16
0	22.40	13.60	0.52	0.48	0.20	0.40	0.35	0.24	0.40	0.32	0.24	1.52	0.16	0.44	0.20	4.56	0.16
20	21.28	10.68	1.08	0.72	0.20	0.74	0.42	0.20	1.04	0.52	0.20	4.80	0.16	1.24	0.32	3.04	0.22
20	38.40	14.72	1.06	0.56	0.16	0.56	0.56	0.22	1.30	0.28	0.16	4.16	0.25	1.84	0.20	4.20	0.28
20	33.44	19.20	1.02	0.92	0.20	0.96	0.72	0.25	1.26	0.80	0.16	3.20	0.24	0.28	0.20	4.84	0.24
20	26.40	15.68	0.80	0.72	0.19	0.64	0.76	0.28	1.48	0.28	0.24	3.68	0.40	3.10	0.32	5.20	0.24
40	55.20	17.28	1.20	0.80	0.80	1.20	0.80	1.00	1.60	1.60	0.64	5.36	0.30	4.80	0.35	4.80	0.28
40	59.20	22.40	2.80	1.00	0.60	1.12	0.80	0.60	1.52	0.20	0.48	5.76	0.32	4.10	0.34	5.20	0.28
40	44.80	21.60	2.16	2.00	0.75	2.00	1.40	0.32	1.76	1.90	0.48	4.18	0.40	4.65	0.40	4.20	0.20
40	38.40	24.48	1.72	0.80	0.56	1.52	0.88	0.72	1.80	0.40	0.48	6.32	0.40	3.60	0.38	6.40	0.25
80	73.60	30.08	6.08	5.80	0.80	1.60	1.40	0.60	4.48	3.80	0.75	6.08	0.46	6.80	0.40	8.38	0.45
80	68.08	25.60	5.20	5.00	0.60	1.88	1.60	0.64	3.20	3.00	0.60	6.80	0.32	6.20	0.45	9.40	0.40
80	60.32	28.30	5.76	4.60	0.80	2.24	1.75	0.70	2.60	2.15	0.80	5.25	0.40	4.85	0.35	7.20	0.35
80	54.14	28.60	2.60	2.00	0.85	2.40	1.95	0.68	2.12	1.95	0.75	6.40	0.50	4.64	0.40	7.45	0.30
120	81.28	36.80	6.80	6.40	0.80	1.70	1.50	0.60	6.35	5.95	0.80	9.28	0.55	7.88	0.50	9.60	0.50
120	85.60	38.40	6.60	5.80	0.95	4.80	4.55	0.45	4.40	3.60	0.75	7.48	0.45	6.40	0.45	9.28	0.45
120	82.40	43.20	6.40	6.10	0.85	3.25	3.00	0.40	3.65	3.10	0.50	6.85	0.80	6.00	0.95	7.90	0.65
120	68.00	40.00	3.80	3.50	0.70	3.10	2.95	0.45	3.00	2.80	0.45	7.80	0.75	7.00	0.70	8.00	0.70

L = Length, W = Width, T = Thickness, D = Diameter

Yellow. At micropylar region the thickness was $2.4 \mu\text{m}$ in Ganesh, $1.52 \mu\text{m}$ in Bassein Seedless, $4.10 \mu\text{m}$ in Alandi and $4.56 \mu\text{m}$ in Kabul Yellow variety (Fig 25). The thickness of outer integument at micropylar region was maximum in Alandi and Kabul Yellow which are hard seeded varieties in general. The cell size was almost the same in all the three regions ranging from 0.12 to $0.16 \mu\text{m}$.

On 20th day after fruit set the soft seeded varieties Ganesh and Bassein Seedless had a length and width of 21.28 and $10.63 \mu\text{m}$, and 36.40 and $14.72 \mu\text{m}$ respectively, while in hard seeded varieties Alandi and Kabul Yellow the length was 33.44 and $19.20 \mu\text{m}$ and the breadth was 27.40 and $15.68 \mu\text{m}$ respectively. At this stage near chalazal core the outer integument thickness of Ganesh (Fig 29) was $1.08 \mu\text{m}$, that of Bassein Seedless was 1.06 , that of Alandi was $1.02 \mu\text{m}$ whereas that of Kabul Yellow (Fig 30) was $0.08 \mu\text{m}$. The individual cell size was also different in the said varieties i.e., the length was $0.72 \mu\text{m}$ in Ganesh, $0.56 \mu\text{m}$ in Bassein Seedless, 0.92 in Alandi and $0.72 \mu\text{m}$ in Kabul Yellow, but the breadth was almost similar in all the varieties ($0.20 \mu\text{m}$).

Outer integument thickness at lateral regions was 0.74 , 0.56 , 0.96 and $0.64 \mu\text{m}$ respectively, in Ganesh (Fig 28), Bassein Seedless, Alandi and Kabul Yellow while the cell size ranged from 0.42 to $0.76 \mu\text{m}$ in length and 0.20 to $0.28 \mu\text{m}$ in breadth.

PLATE II

Figs. 25 to 26: Sections of 10 day old seed of Kabul Yellow (hard seeded variety) tested with PAS method.

Figs. 25: Micropylar region showing rich accumulation of starch granules in the inner integument. X 400.

Fig. 26: Chalazal region of the seed showing outer integument and part of inner integument. Note the presence of starch granules in the inner integument. X 400.

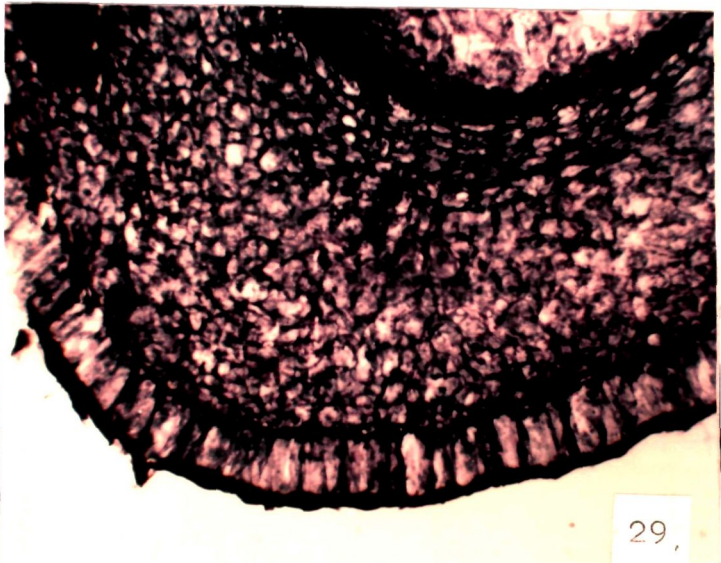
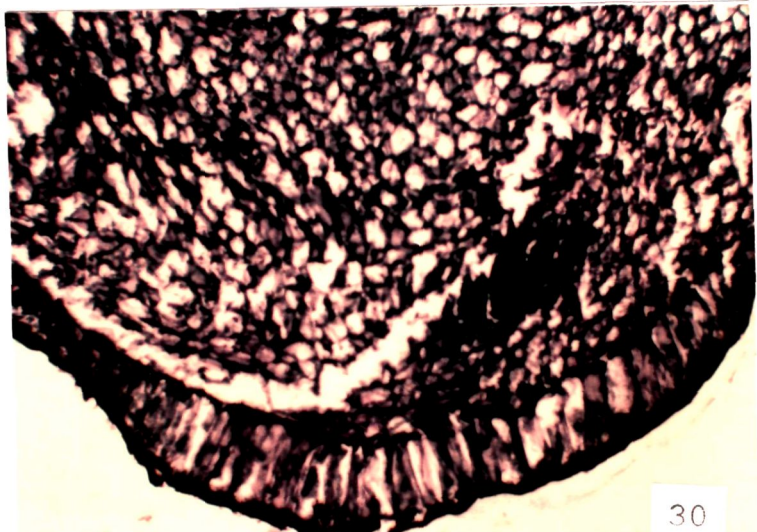
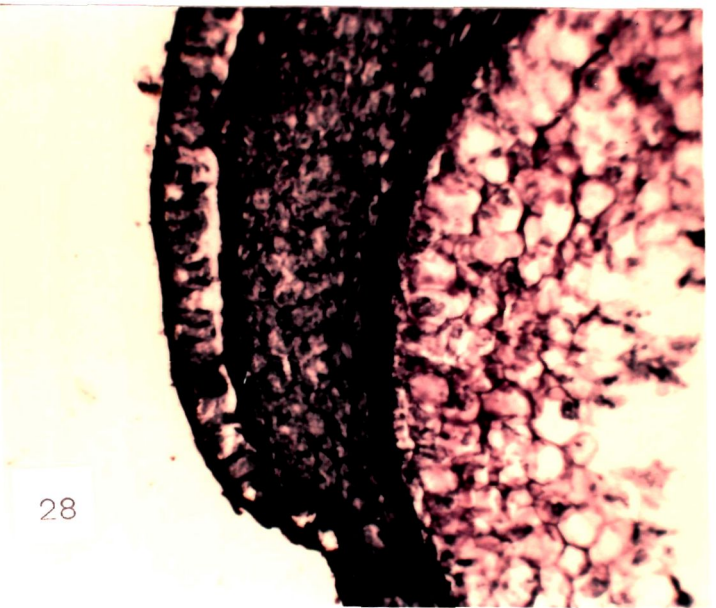
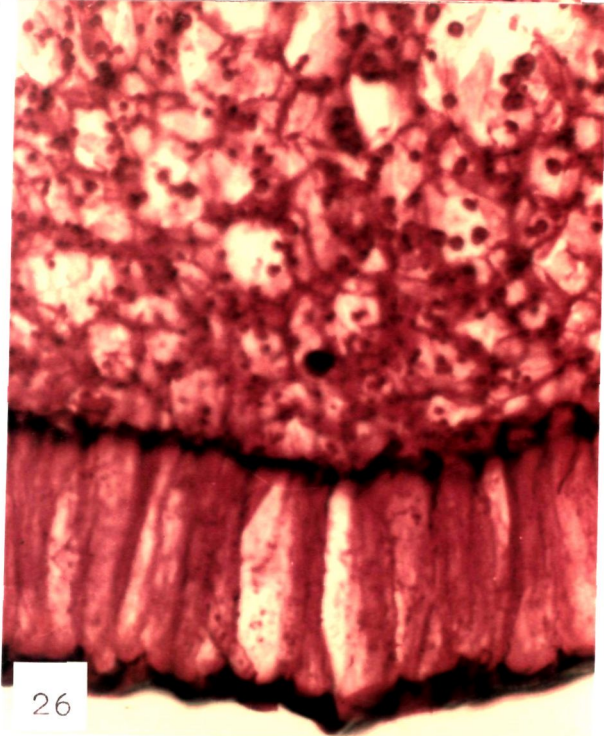
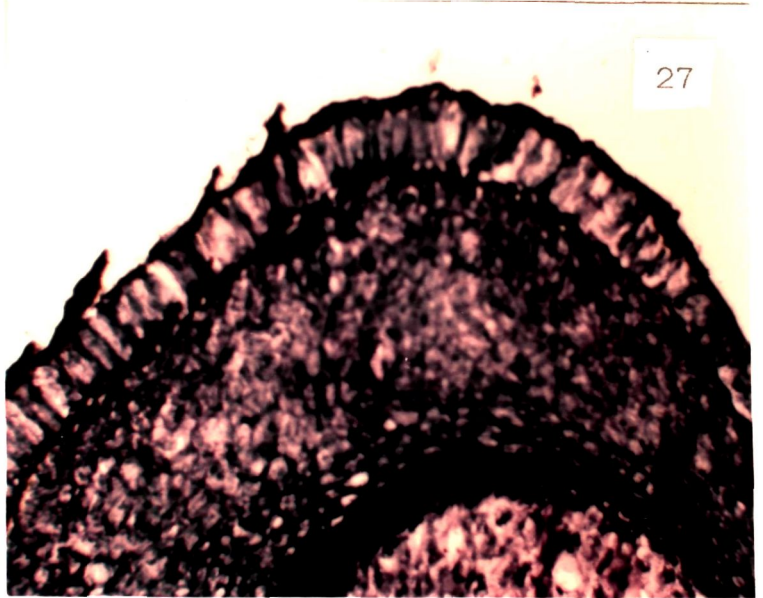
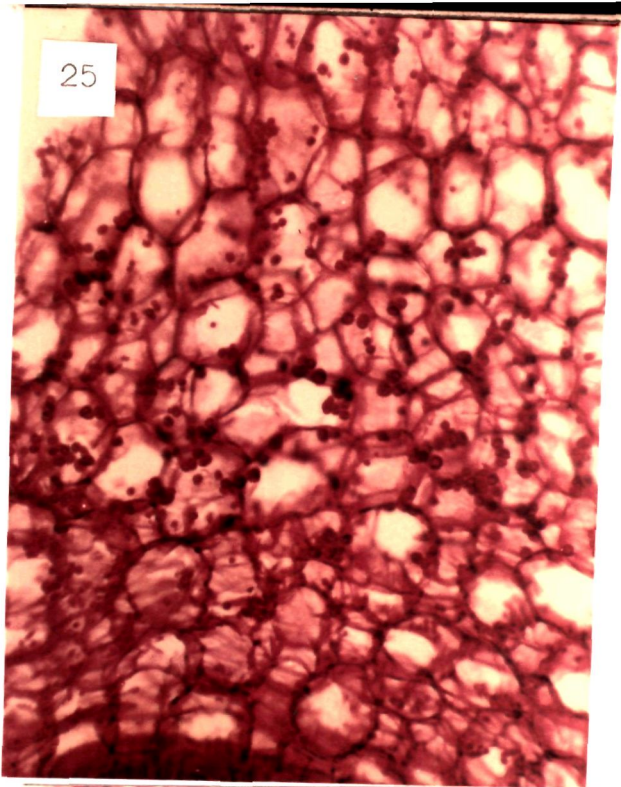
Figs. 27 to 29: Sections of 20 day old seeds of Ganesh (soft seeded variety) tested with Taluidine blue method (TB) showing different parts of integuments.

Fig. 27: Micropylar region showing the integuments. Note the wider inner integument at this region. X 150.

Fig. 28: Lateral region showing single layer of outer integumental cell with smaller portion of inner integument. X 150.

Fig. 29: Chalazal region of the seed showing outer and inner integuments. X 150.

Fig. 30: Section of 20 day old seed of Kabul Yellow (hard seeded variety) tested with T.B. method showing outer and inner integuments at Chalazal region. Note the wide inner integument with many layers of cells X 150.



At micropylar region the thickness of outer integument was almost the same in all the four varieties; Ganesh (Fig 27) had 1.04 μm , Bassein Seedless had 1.30 μm , Alandi had 1.26 μm , whereas Kabul Yellow had 1.48 μm . The individual cells differed in length, while Ganesh had 0.52 μm , Bassein Seedless had 0.28 μm , Alandi had 0.80 μm and Kabul Yellow had 0.28 μm but the breadth was almost same in all the varieties (it was 0.16 to 0.24 μm).

The inner integument at chalazal region showed that the variety Ganesh had 4.80 μm thickness, Bassein Seedless had 4.16 μm thickness, Alandi had 2.20 μm and Kabul Yellow had 3.68 μm thickness. The cell diameter was 0.16 μm in Ganesh, 0.25 μm in Bassein Seedless, 0.24 μm in Alandi and 0.40 μm in Kabul Yellow. In Ganesh variety the cells in inner integumental layer had medium sized cells with 18 to 20 layers of cells. The cells towards inner layer of inner integument were little bigger than the cells at both the ends. In Bassein Seedless at chalazal region the number of layers of cells ranged from 20 to 22. The cells were a little bigger in size and the size was same all along (thickness 4.16 μm and cell diameter 0.25 μm). At lateral region there were 10 to 12 layers of cells (thickness 1.34 μm and diameter 0.20 μm) and at micropylar region there were 20 to 25 layers of cells (thickness 4.20 μm and diameter 0.28 μm).

In Alandi variety at chalazal region the inner integument had 15 to 16 layers of cells (thickness 3.20 μm and cell size 0.24 μm). The outer 2 to 3 layers had smaller cells but towards

middle the cells were bigger and again towards interior proximal to the embryo sac they were smaller in size with slightly thickened walls and placed closer to each other.

At lateral region there were 10 to 12 layers of cells with small size (thickness 1.28 μm and 0.20 μm diameter), while at micropylar region the cell layers were 22 to 25 and placed closely with thick cell walls (the thickness was 4.84 μm and cell diameter was 0.24 μm).

In Kabul Yellow variety, the thickness of inner integument at chalazal region was 3.68 μm and the cells had 0.40 μm diameter.

At this region there were 10 to 12 layers of closely placed smaller cells with thickened cell walls. At the middle region of integument the cells were not closely arranged but were placed wide apart. At lateral regions the thickness of inner integument was 3.10 μm and the cell size was 0.32 μm in diameter. At micropylar region the thickness of inner integument was 5.20 μm with cells having 0.24 μm diameter.

On 40th day after fruit growth the seeds of Ganesh variety had 55.20 μm length and 17.23 μm breadth, Bassein Seedless variety had 59.20 μm length and 22.40 μm breadth. Between the hard seeded varieties Alandi had 44.30 μm length and 21.60 μm breadth while Kabul Yellow had 33.40 μm length and 24.43 μm breadth.

The outer integument in Ganesh (Fig 31 to 33) had a single layer of cells. Its thickness at chalazal region was $1.20 \mu\text{m}$ and the cell breadth was $0.30 \mu\text{m}$. At lateral region the thickness was $1.20 \mu\text{m}$ with a single layer of cells of smaller size with a diameter of $0.80 \mu\text{m}$. At micropylar region the thickness was $1.60 \mu\text{m}$ with cells having a breadth of $0.84 \mu\text{m}$.

The outer integument in Bassein Seedless (Fig 34 to 35) at chalazal region had a thickness of $2.80 \mu\text{m}$ with single layer of cells having $1.00 \mu\text{m}$ length and $0.60 \mu\text{m}$ breadth. At lateral region its thickness was $1.12 \mu\text{m}$ with 1 to 2 layers of cells having $0.80 \mu\text{m}$ length and $0.60 \mu\text{m}$ breadth. At micropylar region the thickness of this layer was $1.52 \mu\text{m}$ with 1 to 2 layers of cells and their size being smaller i.e., $0.20 \times 0.48 \mu\text{m}$.

In the variety Alandi (Fig 36 to 38) the outer integument was single layered throughout. At chalazal zone it had $2.16 \mu\text{m}$ thickness and the cells had $0.75 \mu\text{m}$ breadth. The thickness at lateral and micropylar region was $2.00 \mu\text{m}$ with the cell breadth of $0.40 \mu\text{m}$.

In Kabul Yellow variety (Fig 39 to 43) it had a single row of outer integumental cells at chalazal region and the thickness was $1.72 \mu\text{m}$. The cells had the size of $0.80 \times 0.56 \mu\text{m}$. The lateral zone thickness was $1.52 \mu\text{m}$ and the rows were having single layered cells. The cell size being $0.80 \times 0.70 \mu\text{m}$. The micropylar region had maximum thickness of $1.08 \mu\text{m}$ with single layered cells having cell size of $0.40 \times 0.48 \mu\text{m}$.

PLATE III

Figs. 31-33: Sections of 40 day old seed of Ganesh (soft seeded variety) tested with PAS method showing both outer and inner integuments.

Fig. 31: Micropylar region showing both the integuments. X 100.

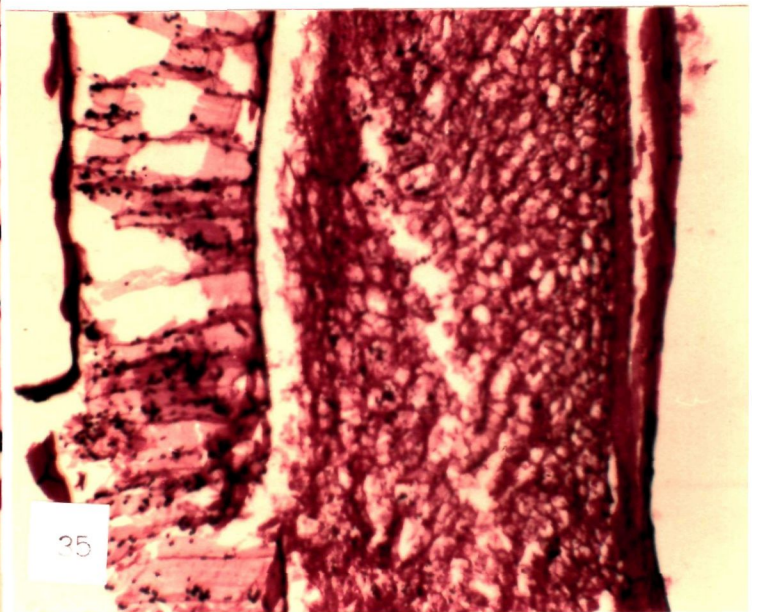
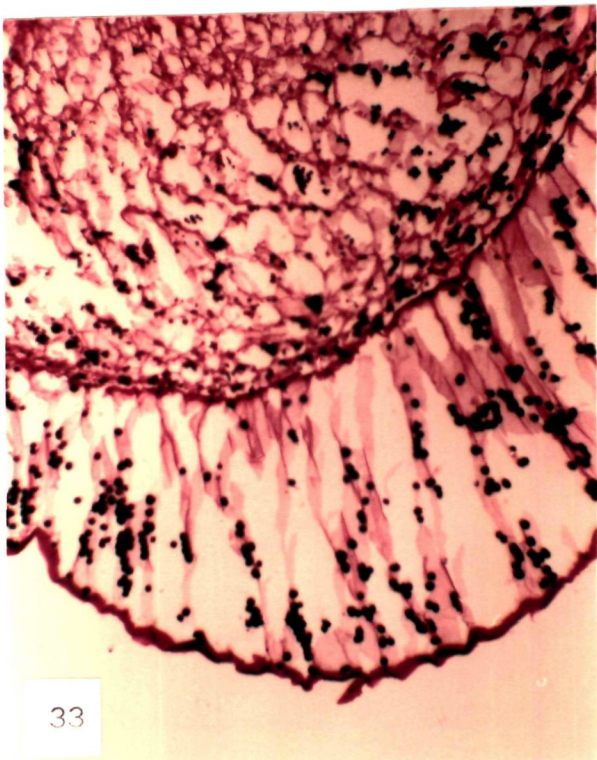
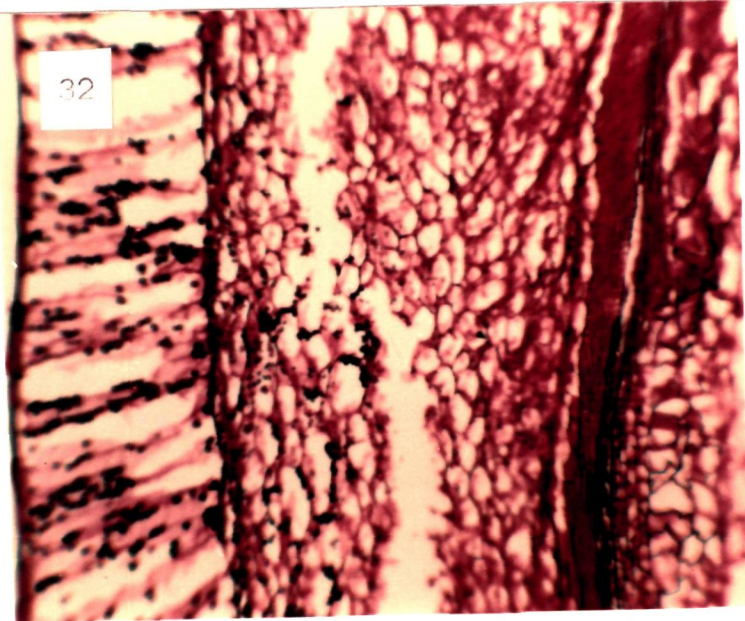
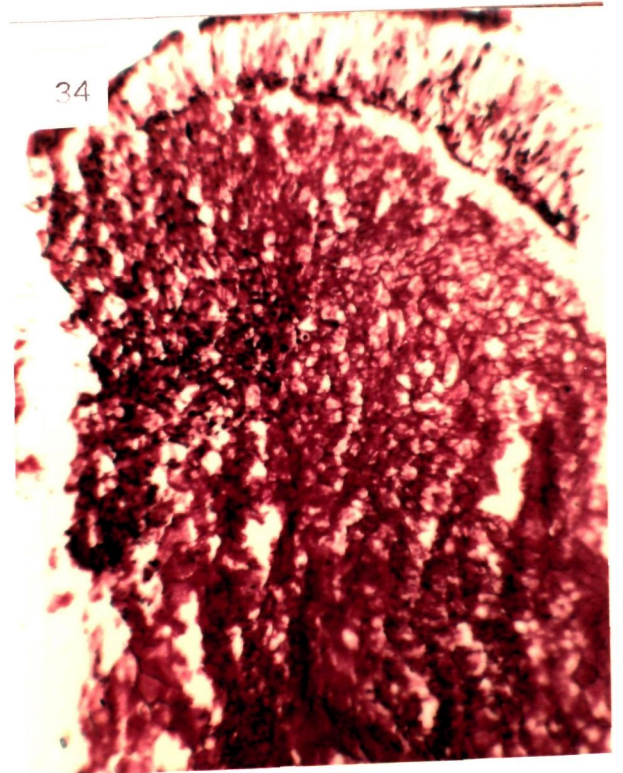
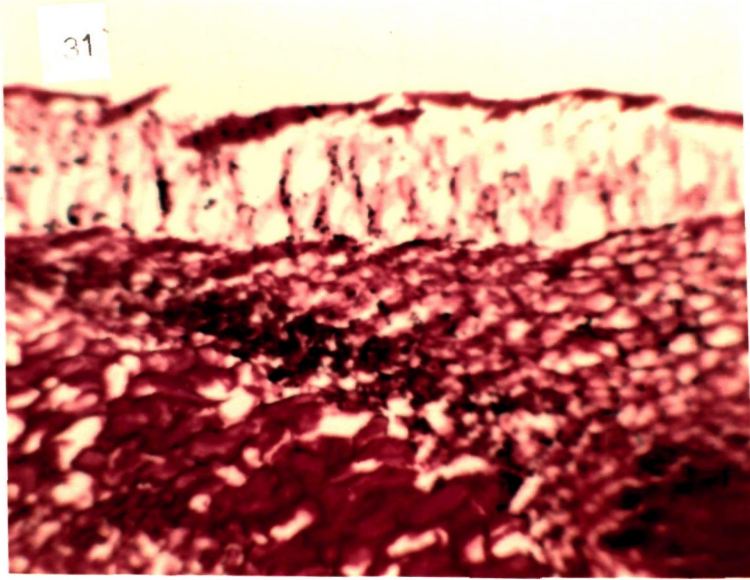
Fig. 32: Lateral region showing the outer and inner integuments. Starch granules are more in outer integument. X 100.

Fig. 33: Chalazal region showing the enlarged outer integument with a single cell layered elongated cells. Note the large quantity of starch accumulation. X 100.

Figs. 34-35: Sections of 40 day old seeds of Bassein Seedless (soft seeded variety) tested with PAS method showing the integuments.

Fig. 34: Micropylar region with both integuments. Note the large portion of inner integument with smaller cells. X 100.

Fig. 35: Lateral region showing outer and inner integuments. Note outer integument having rich starch accumulation, whereas only the peripheral layers of inner integuments have a little starch. The inner integumental cells are small. X 100.



During 40 days of seed growth the inner integument showed many changes. In the variety Ganesh the thickness of inner integument was $5.56 \mu\text{m}$ at chalazal region with 20 layers of cells. The cells of the peripheral layers were broader, while those of the middle region were smaller. The inner most 8-10 layers were highly lignified and their cells had $0.30 \mu\text{m}$ diameter. At lateral zone the thickness was $4.80 \mu\text{m}$ with 16 to 18 layers of cells. The cell diameter was $0.35 \mu\text{m}$. At micropylar region the thickness of this layer was $4.80 \mu\text{m}$ with 15 to 18 layers of cells with cell diameter of $0.28 \mu\text{m}$.

In Bassein Seedless variety the thickness of inner integument at chalazal region was $5.76 \mu\text{m}$ (cell diameter $0.32 \mu\text{m}$) consisting of 20 to 22 layers. At lateral region the layers were 20 to 25 with a thickness of $4.10 \mu\text{m}$ (cell diameter $0.34 \mu\text{m}$). At micropylar region the thickness of the layer was $5.20 \mu\text{m}$ having 25 to 28 layers with cell size of $0.28 \mu\text{m}$ diameter. The cells were thicker showing lignification. The lignification was more towards lateral and micropylar regions.

In Alandi variety the thickness at chalazal, lateral and micropylar regions was 4.12 , 4.65 and $4.20 \mu\text{m}$, respectively. The number of layers being 18 at chalazal region (cell diameter $0.40 \mu\text{m}$), 20 to 23 at lateral region (cell diameter $0.40 \mu\text{m}$), 25 to 28 at micropylar region (cell diameter $0.20 \mu\text{m}$). At chalazal region the cells were medium sized and heavily lignified.

PLATE IV

Figs. 36-38: Sections of 40 day old seed of Alandi (hard seeded variety) tested with PAS method showing both the integuments.

Fig. 36: Micropylar region showing narrow outer integument and wide inner integument. Note less starch granules in this region. X 100.

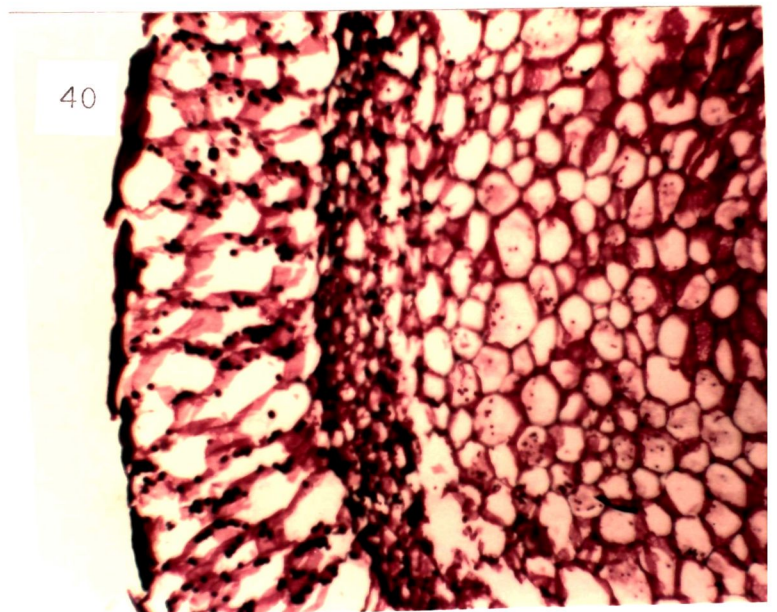
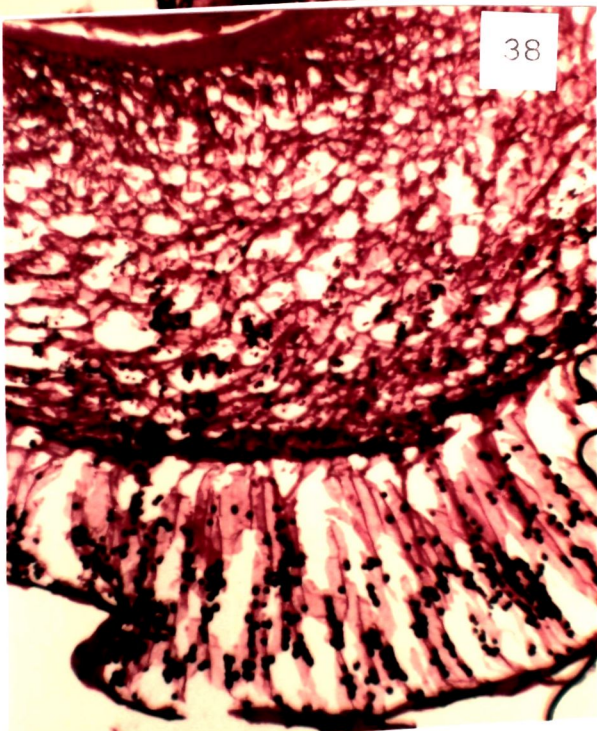
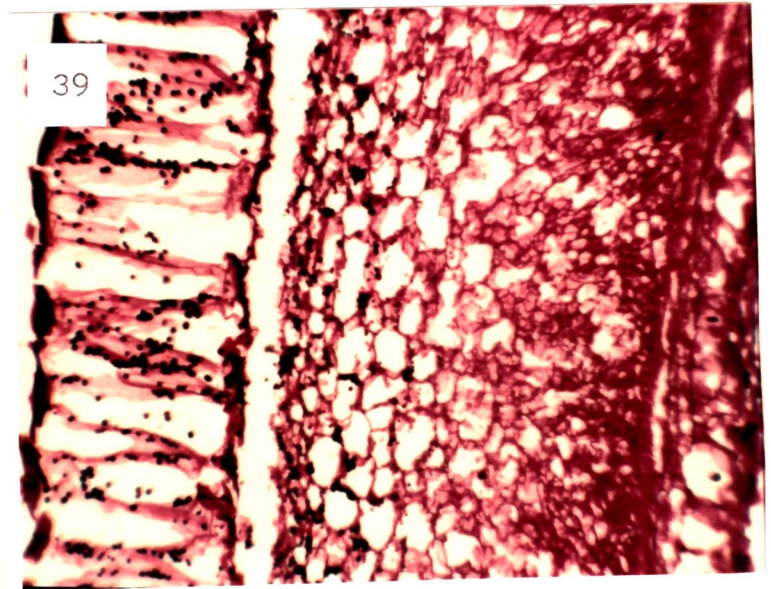
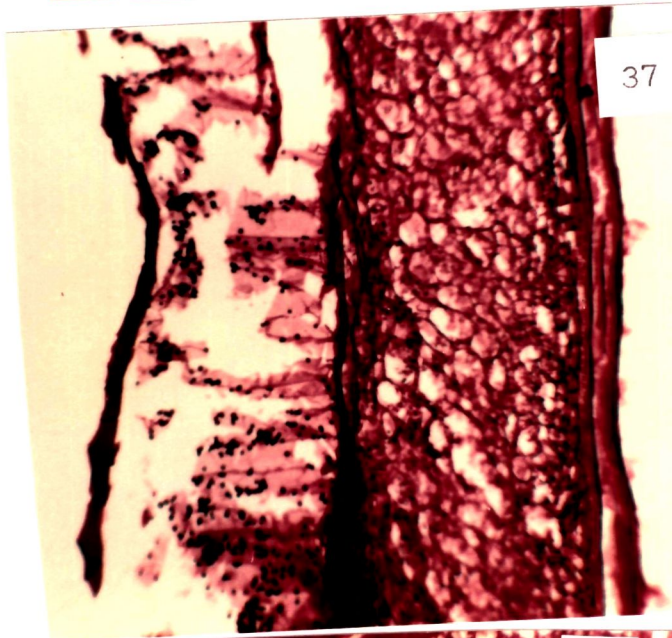
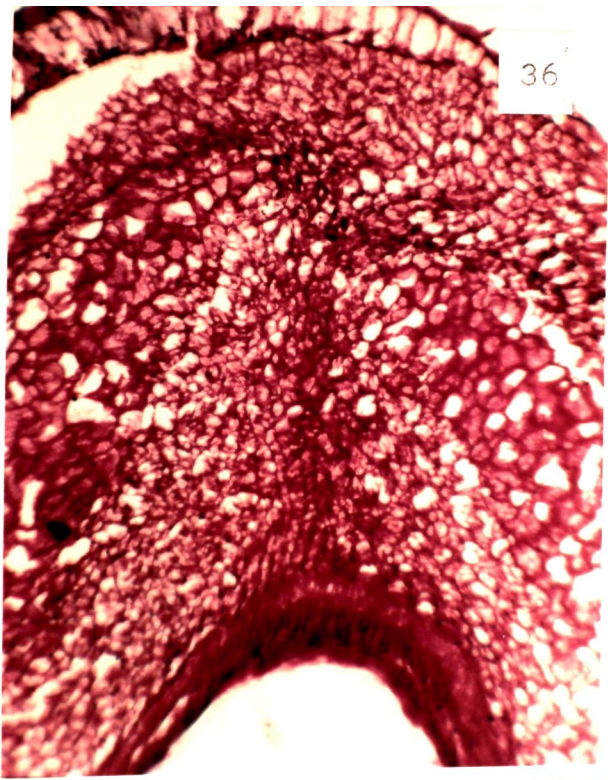
Fig. 37: Lateral regions showing both the integuments. X 100.

Fig. 38: Chalazal region with both the integuments. Note the starch granules in large numbers in the outer integument and also a few contiguous layers of the inner integument. X 100.

Figs. 39-40: Sections of 40 day old seed of Kabul Yellow (Hard seeded variety) tested with PAS showing integumental details.

Fig. 39: Lateral region with both the integuments. Starch grains are seen in outer integument and a few layers of inner integument. Note the initiation of lignification in the inner most layers of inner integuments. X 100

Fig. 40: Chalazal region with both the integuments. The outer integument is with starch grains and a few contiguous layers of inner integument also contains large amounts of starch grains. Note the initiation of lignification in the innermost layers of inner integument. X 100.



Lignification was highly demarcated especially in 8 to 10 layers of inner integument proximal to embryo sac.

In the variety Kabul Yellow the inner integument at chalazal zone was $6.32 \mu\text{m}$ thick and had 13 to 20 rows of cells having a cell diameter of $0.40 \mu\text{m}$. The first 2 to 3 rows of cells from outside were elongated with thick cell walls, the thickness due to lignification increased gradually in the inner layers proceeding from periphery to the interior. Maximum layers were lignified in this variety. At lateral zone the thickness was $3.80 \mu\text{m}$ with a cell size of $0.38 \mu\text{m}$ diameter and at micropylar zone the thickness was $6.40 \mu\text{m}$ with cell size of $0.25 \mu\text{m}$ diameter.

At 30 days of seed growth the seed in Ganesh variety was $73.50 \mu\text{m}$ long and $30.08 \mu\text{m}$ wide, in Bassein Seedless it was $68.08 \mu\text{m}$ long and $25.50 \mu\text{m}$ wide, in Aladi it was $60.32 \mu\text{m}$ long and $23.30 \mu\text{m}$ wide while in Kabul Yellow the seed had $54.14 \mu\text{m}$ length and $28.60 \mu\text{m}$ width.

The outer integument thickness in Ganesh was $6.08 \mu\text{m}$ with a single layer of longitudinal cells at chalazal region, with cell size $5.80 \mu\text{m}$ length and $0.30 \mu\text{m}$ width. At lateral region the thickness of the integument was $1.50 \mu\text{m}$ with cell size of $1.40 \mu\text{m}$ length and $0.60 \mu\text{m}$ width. At micropylar region the thickness was $4.43 \mu\text{m}$ and cell size was $3.30 \mu\text{m}$ length and $0.75 \mu\text{m}$ width. In general the cells were rectangular throughout the integument.

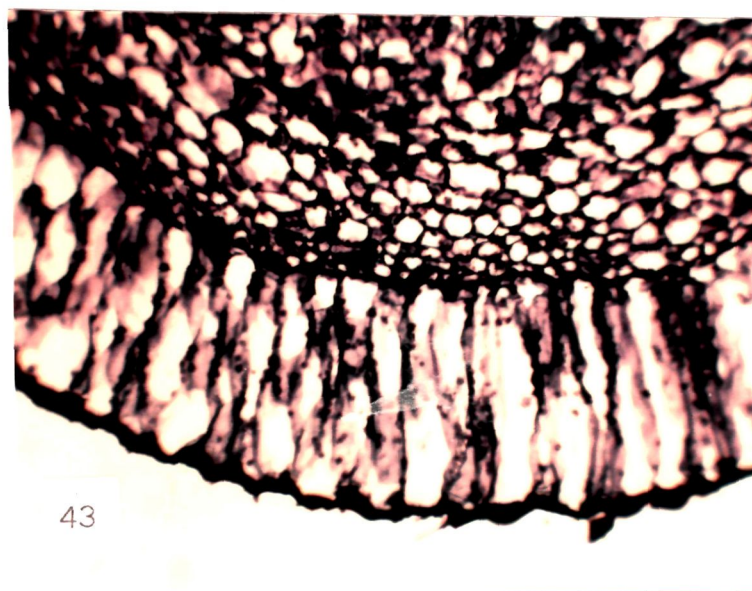
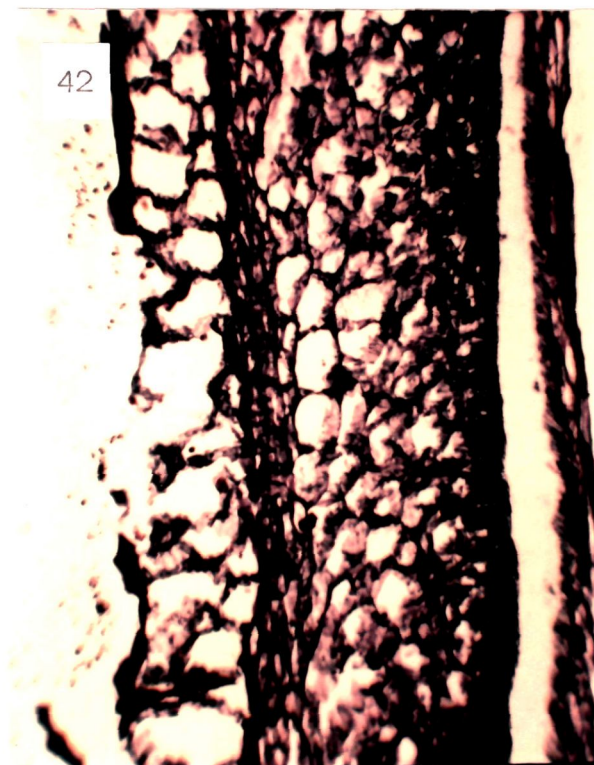
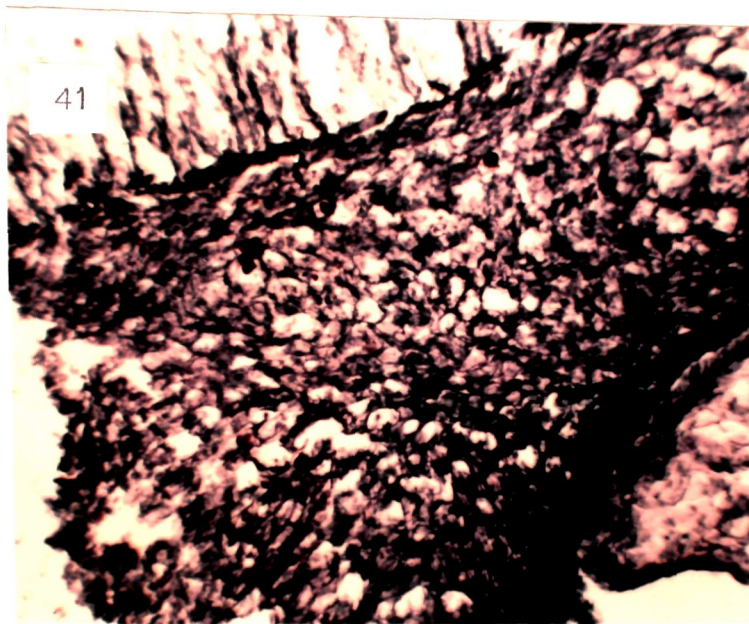
PLATE V

Figs. 41-43: Sections of 40 day old seed of Kabul Yellow (Hard seeded variety) tested with T.B. method showing integuments at different regions.

Fig. 41: Micropylar region of the seed showing part of outer integument and inner integument. X 150.

Fig. 42: Lateral region of the seed showing both the integuments. Note the narrowness of both the integuments and intensive lignification in the innermost few layers of inner integument. X 150.

Fig. 43: Chalazal region of the seed showing outer integument and part of inner integument. Note the intensive lignification in most of the cells of inner integument. X 150.



In Bassein Seedless variety at chalazal region the outer integument had a single layer of cells with the thickness of 5.20 μm and cells were 5.60 μm long and 0.60 μm wide. The cell arrangement was irregular. At lateral region the thickness of the integument was 1.88 μm and the cell size was 1.60 μm in length and 0.64 μm in width. At micropylar region the thickness was 3.20 μm and cell size was 3.00 μm in length and 0.60 μm in width. Here also a single layer of cells was observed.

In the variety Alandi (Fig. 47 and 48) at chalazal zone the outer integument had 5.76 μm thickness and cells had 4.60 μm length and 0.30 μm wide. At lateral region the thickness was 2.24 μm and cell size was 1.75 μm in length and 0.70 μm in width and at micropylar region the thickness of the integument was 2.60 μm and cell size was 2.15 μm in length and 0.80 μm in width. The outer integument was made up of a single layer of cells throughout in all the places.

The variety Kabul Yellow had a single layer of outer integumental cells at chalazal region and the thickness was 2.60 μm . The cells were of the size of 2.00 μm in length and 0.85 μm in width. At lateral region the thickness of integument was 2.40 μm with cell size of 1.95 μm in length and 0.68 μm in width and at micropylar region the thickness was 1.12 μm with cell size of 1.95 μm in length and 0.75 μm in width.

PLATE VI

Figs. 44-46: Sections of 60 day old seeds of Ganesh (soft seeded variety) tested with T.B. method showing larger portions of inner integument.

Fig. 44: Inner integument at micropylar region showing complete lignification in many of its cells. X 150.

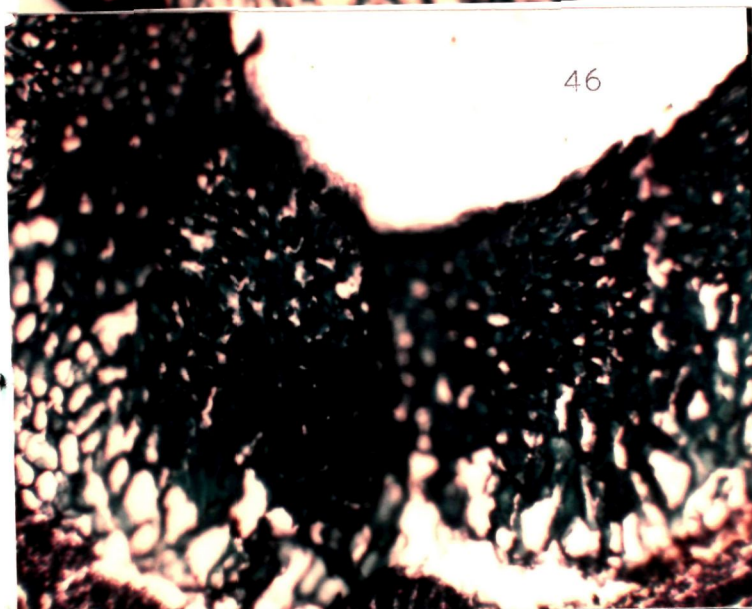
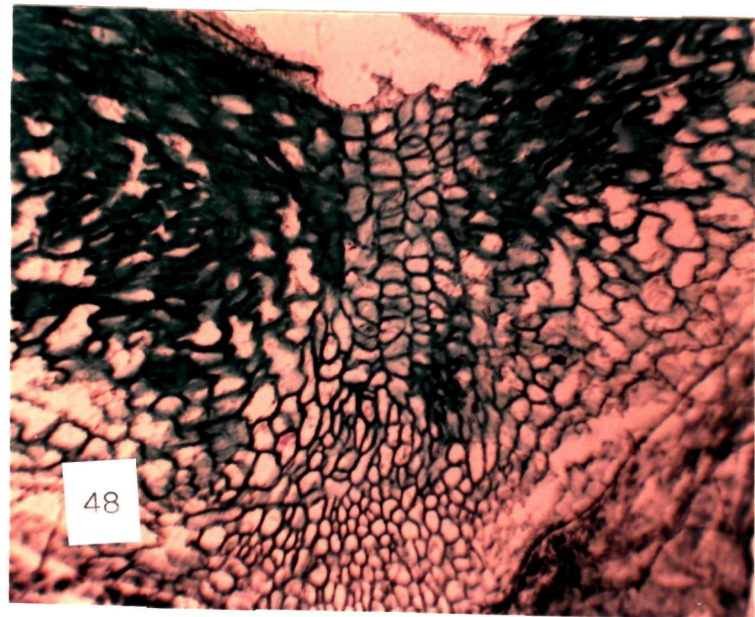
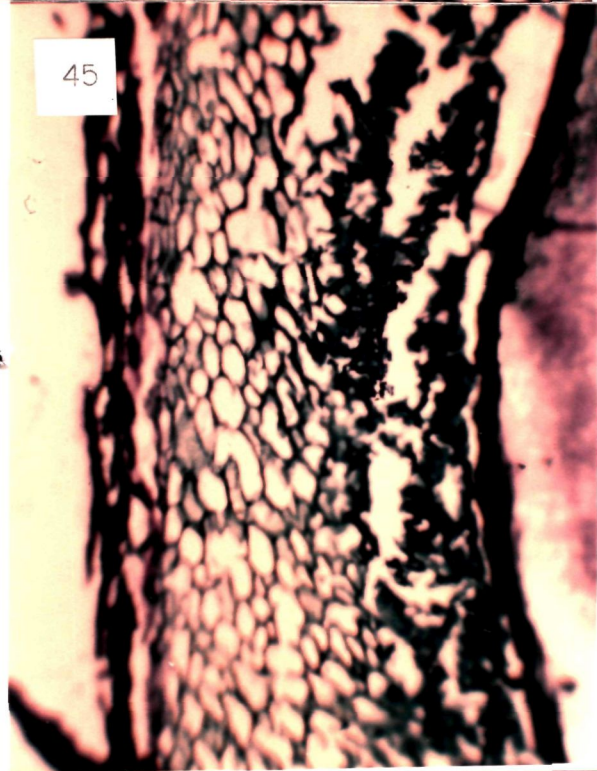
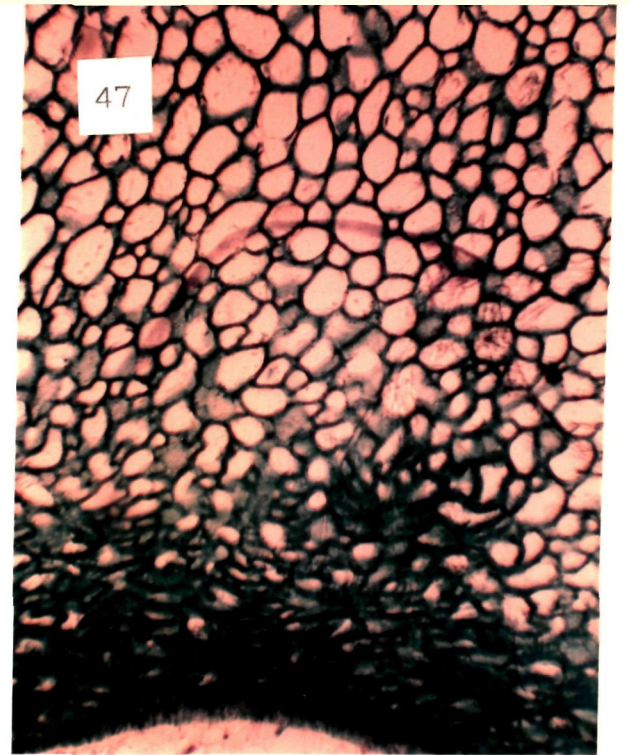
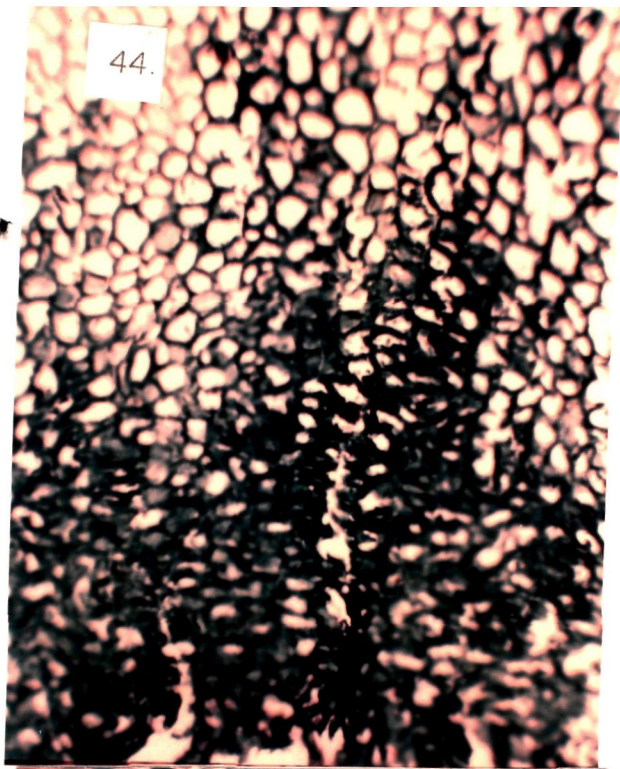
Fig. 45: Lateral region showing inner integument. Note the heavy lignification of the inner layers of the inner integument. X 150.

Fig. 46: Chalazal region of the seed showing lignification in most of its inner cell layers. X 150.

Figs. 47-48: Sections of 80 day old seed of Alandi (hard seeded variety) tested with T.B. method showing inner integument.

Fig. 47: Micropylar region of inner integument. Note the lignification of the inner layers and also the initiation of lignification in other layers too. X 150.

Fig. 48: Chalazal region showing the intensive lignification in almost all the cells of the inner layers. X 150.



At 80 days after fruit set the changes in the inner integument of seed showed that in the variety Ganesh the thickness of inner integument was $5.08 \mu\text{m}$ at chalazal region with 15 to 20 layers of cells. The outer 5 to 6 rows of cells were smaller, the next 5 to 6 rows were broader. Then the remaining rows had thick walled cells and lignified. The cell size was $0.46 \mu\text{m}$ in diameter. At lateral end the thickness of integument was $6.80 \mu\text{m}$ and the cell diameter was $0.40 \mu\text{m}$. The layers of cells were 15 to 20 and cells at outer layer were thin walled and small and remaining layers had thick cell walls and lignified with deeply stained cells. At micropylar region also the cells were with thick cell wall and deeply stained with 20 to 25 layers of cells. Here again 6 to 8 layers were thin walled followed by thick walled and highly lignified cells. The thickness was $8.38 \mu\text{m}$ and the cell size was $0.45 \mu\text{m}$ in diameter.

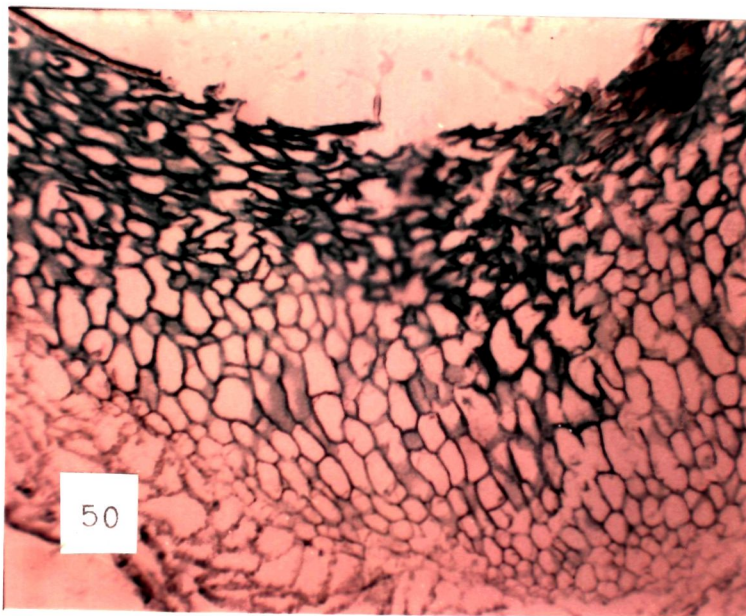
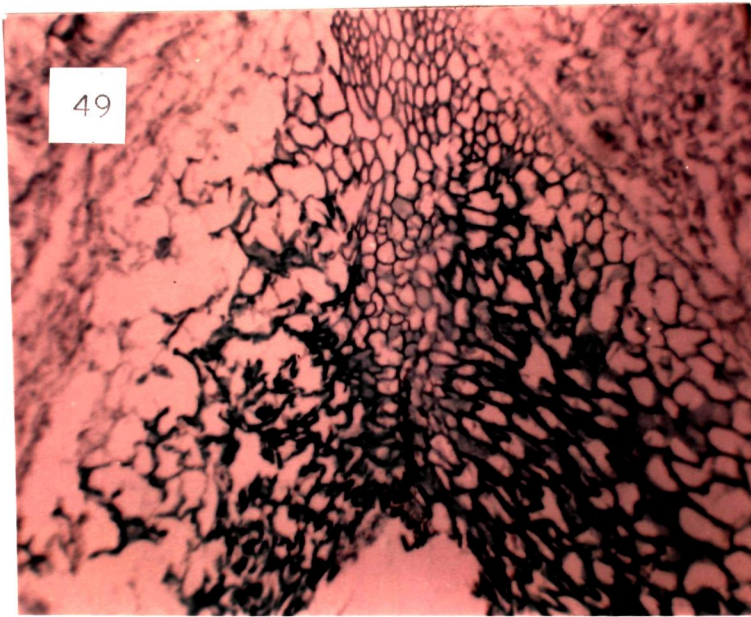
In Bassein Seedless the thickness of inner integument at chalazal region was $6.80 \mu\text{m}$ and cell size was $0.32 \mu\text{m}$ in diameter. There were 20 to 22 layers of cells. The outer 3 to 5 layers consisted of smaller cells. The next 5 to 6 layers had broader cells. The remaining layers had thick walled lignified cells. At lateral zone there were 16 to 18 layers of cells. The thickness was $6.20 \mu\text{m}$ and average cell size was $0.45 \mu\text{m}$ in diameter. The first 2 to 3 layers had thin walled narrow cells while the rest were highly lignified. At micropylar region

PLATE VII

Figs. 49-50: Sections of 100 day old seed of Ganesh (soft seeded variety) tested with T.B. method showing inner integument.

Fig. 49: Micropylar region of the seed showing inner integument. Note the lignification of most of the cell layers. x 150.

Fig. 50: Chalazal region of the seed showing inner integument. Note the poor lignification of cells at the inner layers of cells. X 150.



there were 25 to 26 layers of cells with thickness of 9.40 μm and average diameter of cells was 0.40 μm . The cells were smaller and poorly lignified in this region.

In Alandi variety the thickness of inner integument at chalazal region was 5.25 μm , at lateral zone 4.85 μm and at micropylar region 7.20 μm . There were 15 to 13 layers of cells at chalazal region with average cell diameter of 0.40 μm . There were 15 layers of cells at the lateral region with an average cell diameter of 0.35 μm . At micropylar region there were 20 to 25 layers of cells with average cell diameter of 0.35 μm . At chalazal region first 4 to 6 layers had broader cells with thin walls. The other layers had cells with heavy lignification. The lateral and micropylar regions were histologically similar to the chalazal region.

In the variety Kabul Yellow the chalazal region was 6.40 μm thick and the average cell diameter was 0.50 μm . At lateral zone the thickness was 4.64 μm and average cell diameter was 0.40 μm . The micropylar zone was 7.45 μm thick and average cell diameter was 0.30 μm . In this variety there was no clear cut demarcation of cell layers at any region. Almost all the cells/^{were} heavily lignified in this zone. Some cells had very thick walls with broader lumen.

At 120 days after fruit set which coincided with harvesting, the seed size in Gan sh variety was 91.28 μm in length and 36.80 μm in width. In Bassein Seedless it was 85.60 μm in

length and 38.40 μm in width. In Alandi it was 82.40 μm in length and 43.20 μm in width. In Kabul Yellow the length was 66.00 μm and the width was 40.00 μm .

The outer integument thickness in Ganesh variety (Fig. 51 to 56 and 57 to 59) at chalazal zone was 6.80 μm with a single layer of elongated cells. The cell size was 6.40 μm in length and 0.80 μm in width. At lateral zone there were 1 to 2 layers of cells with a thickness of 1.70 μm and the cell size was 1.50 μm in length and 0.60 μm in width. At micropylar zone there was a single layer of cells and the thickness was 6.35 μm and cell size was 5.95 μm in length and 0.80 μm in width.

In Bassein Seeless at chalazal region the outer integument was single layered and thickness was 6.60 μm . The cells were 5.80 μm long and 0.95 μm wide. At lateral zone also it was a single celled layer, with a thickness of 4.80 μm and the cell size was 4.55 μm in length and 0.45 μm in width. At micropylar region too the structure was similar with a thickness of 4.40 μm and cell size of 3.60 μm length and 0.75 μm width..

In the variety Alandi the outer integument at chalazal region was single layered with a thickness of 6.40 μm . The cells were 6.10 μm long and 0.85 μm wide. At lateral region the thickness was 3.25 μm and cells were 3.00 μm long and 0.40 μm wide. The cell layers were not very distinct at chalazal as well as micropylar regions. The thickness was 3.65 μm and cell size was 3.10 μm in length and 0.50 μm in width.

PLATE VIII

Figs. 51-56: Sections of 120 day old seeds of Ganesh (soft seeded variety) tested with PAS method showing the integuments.

Fig. 51: Micropylar region of the seed showing both the integuments x 80.

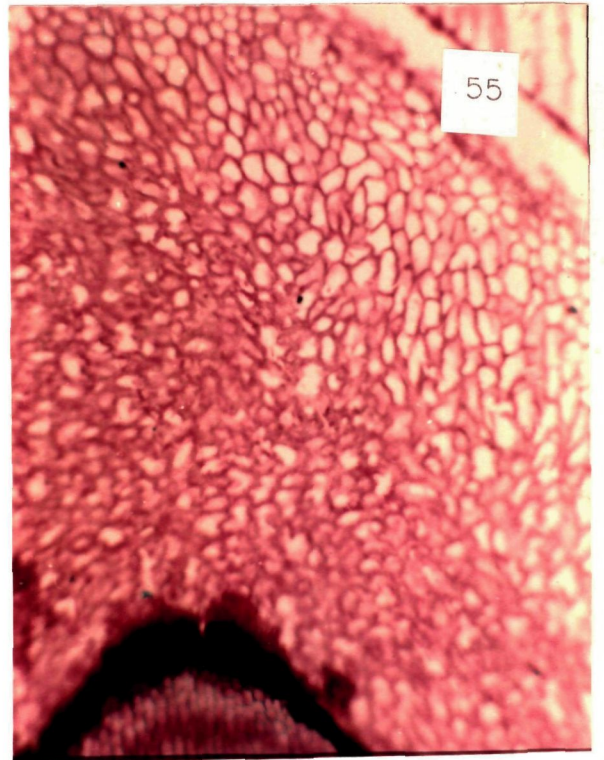
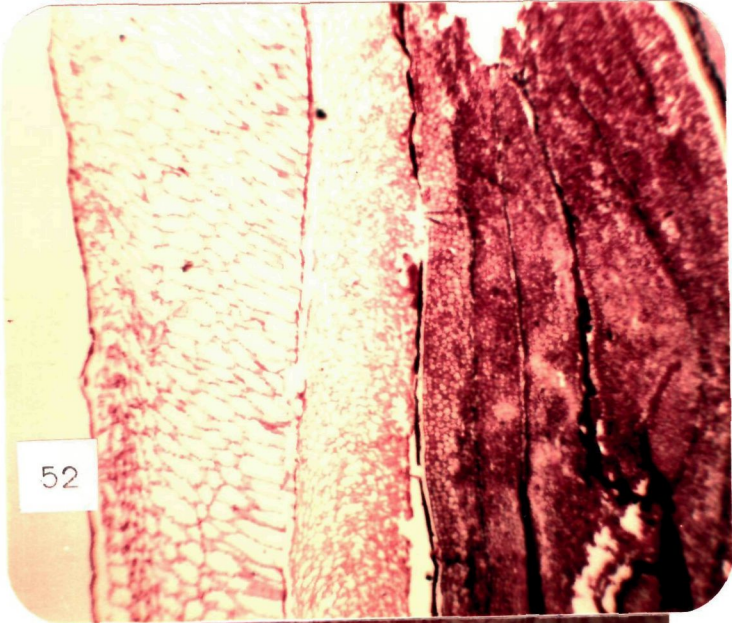
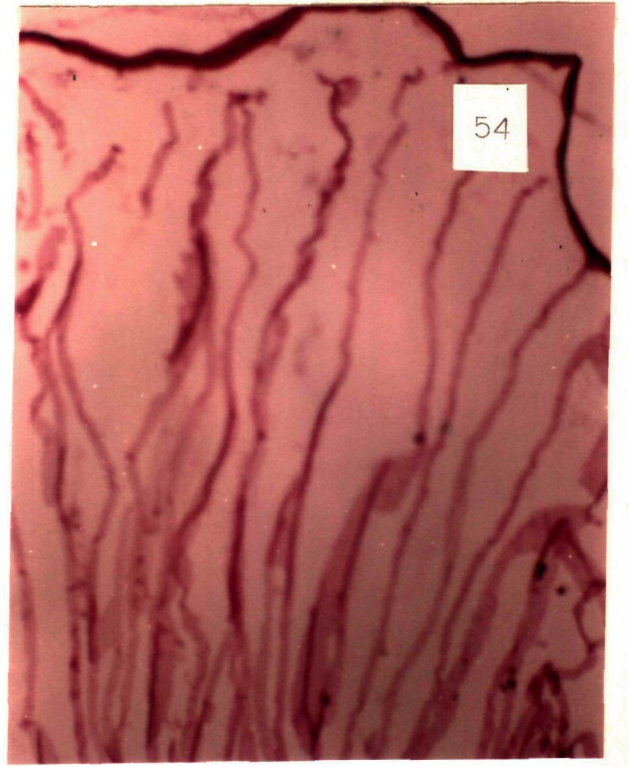
Fig. 52: Lateral region of the seed showing the integuments. Note the larger portion of outer integument with elongated single layered cells X 100.

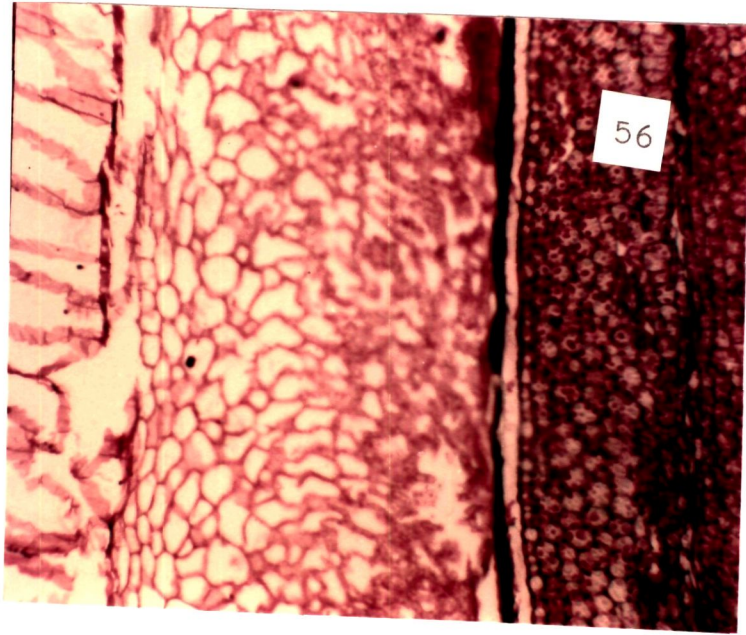
Fig. 53: Chalazal region of the seed showing maximum portion of outer integument. Note the enormously elongated cells of the layer lacking starch grains. X 100.

Fig. 54: A part of outer integument with enlarged cells lacking starch grains X 400.

Fig. 55: Micropylar region of the seed showing only inner integument with its poorly lignified cells. X 400.

Fig. 56: Lateral region of the seed showing most of inner integument with its cells poorly lignified. X 400.





In the variety Rabul Yellow (Fig 60 to 61) the thickness of outer integument at chalazal region was $3.80 \mu\text{m}$ and cell size was $3.50 \mu\text{m}$ in length and $0.70 \mu\text{m}$ in width. At lateral region there were no distinct layers. The thickness of this zone was $3.10 \mu\text{m}$ and the cell size was $2.95 \mu\text{m}$ in length and $0.45 \mu\text{m}$ in width. At micropylar region the thickness was $3.00 \mu\text{m}$ and the cell size was $2.80 \mu\text{m}$ in length and $0.45 \mu\text{m}$ in width.

At 120 days after fruit set the inner integument of seed in the variety Ganesh at chalazal region was having a thickness of $2.28 \mu\text{m}$ and average cell diameter was $0.55 \mu\text{m}$. The number of cell layers were 18 to 20 and the outer 4 to 5 layers were bigger in size and thin walled and the rest of the layers had highly lignified thick walled cells. At lateral region the thickness of integument was $7.88 \mu\text{m}$ and average cell diameter was $0.50 \mu\text{m}$. In this zone there were 15 to 17 layers of cells and the first 4 to 5 layers of cells were small in size and thin walled while the rest of the layers were lignified and thick cell walled. At micropylar zone there were about 20 layers of cells of small size. The thickness was $9.60 \mu\text{m}$ and the average cell diameter was $0.50 \mu\text{m}$.

In the variety Bassein Seedless at chalazal region there were about 20 layers of cells, in the inner integument out of which the outer 1 to 2 layers had very thin walled cells and the remaining layers had highly lignified thick walled cells. The thickness of this layer was $7.48 \mu\text{m}$ and the average cell diameter

PLATE IX

Figs. 57-59: Sections of 120 day old seeds of Ganesh (soft seeded variety) tested with TB method showing integuments at different regions.

Figs. 57: Micropylar region of the seed showing poorly lignified inner integument and part of outer integument .
X. 150.

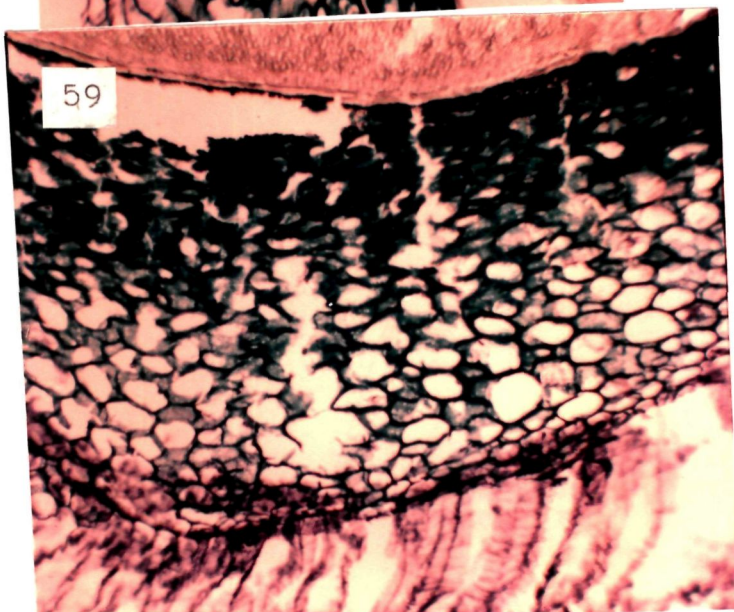
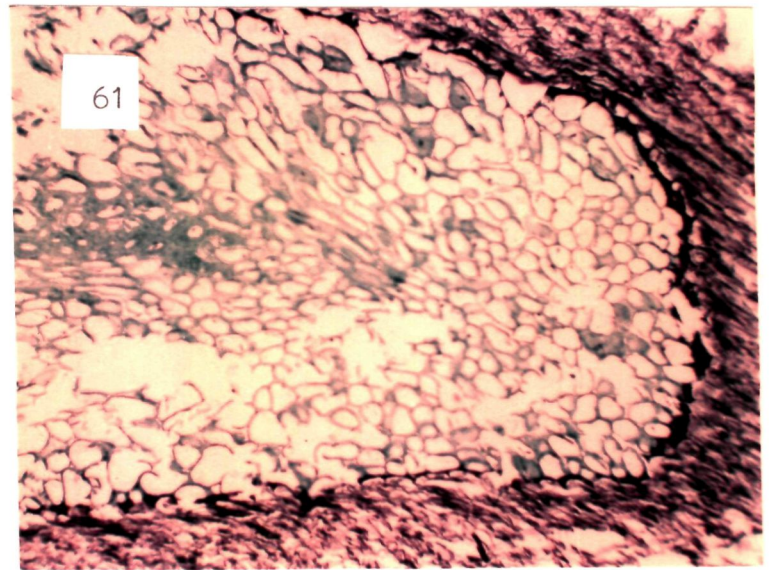
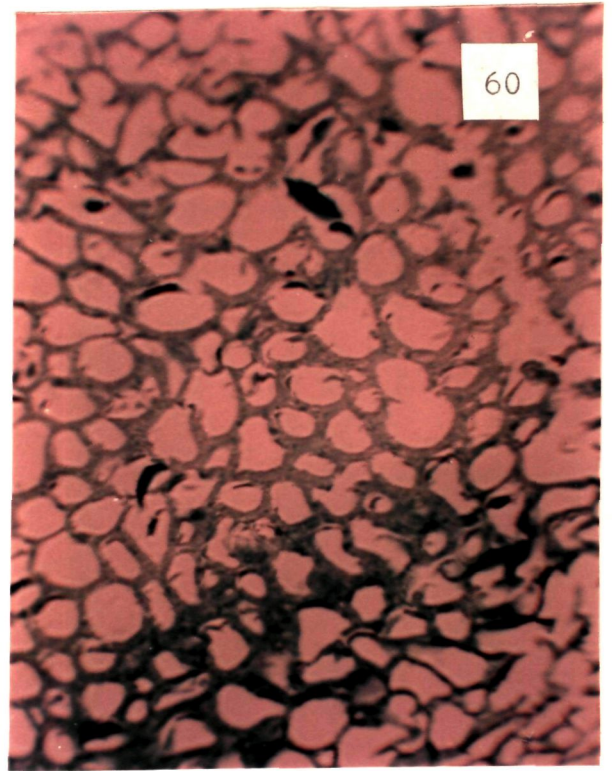
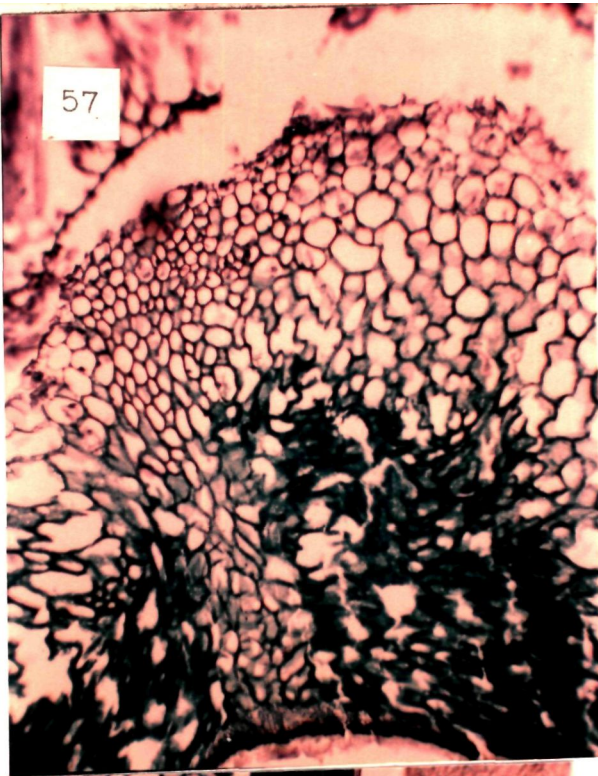
Fig. 58: Lateral region of the seed showing inner integument. Note the lignification of the inner layers of the inner integument X 150.

Fig. 59: Chalazal region of the seed showing lignified inner integument and part of outer integument. X 150.

Figs. 60-61: Sections of 120 day old seeds of Kabul Yellow (hard seeded variety) tested with T.B. method showing inner integument.

Fig. 60: Micropylar region of the seed showing lignification of inner integumentary cells. X 150.

Fig. 61: Chalazal region of the seed showing lignification of inner integumentary cells. X 150.



was 0.45 μm . The lateral zone had about 15 layers of cells. It had a thickness of 6.40 μm and the average cell diameter was 0.45 μm . The outer 8 to 10 layers had thin walled cells and the rest had highly lignified cells. At micropylar region it had 20 to 25 layers of cells and the first 10 to 12 layers were with thin walls and the remaining were highly lignified and thick celled. The thickness was 9.28 μm and the average cell size was 0.45 μm in diameter.

In the variety Alandi and Kabul Yellow there was no distinction of cell layers. The lignification occurred (Fig. 62 to 65) just after the outer 1 to 2 layers which were thin walled but the remaining layers had highly lignified cells. The cells were circular in outline with radiating pits and broader lumen. In Alandi variety at the chalazal region the thickness of the inner integument was 6.85 μm and the average cell diameter was 0.80 μm . At lateral zone the thickness was 6.00 μm and the cell diameter was 0.75 μm . At micropylar region the thickness was 7.90 μm and the average cell diameter was 0.65 μm .

In Kabul Yellow variety at chalazal region the thickness of inner integument was 7.80 μm and the average cell diameter was 0.75 μm . At lateral region the thickness was 7.00 μm and the cell diameter was 0.70 μm . At micropylar region the thickness was 8.00 μm and the average cell diameter was 0.70 μm .

PLATE X

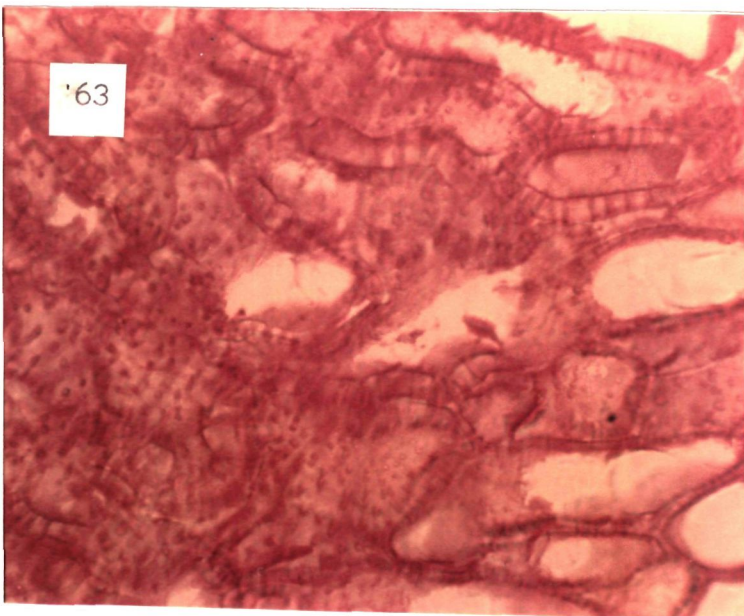
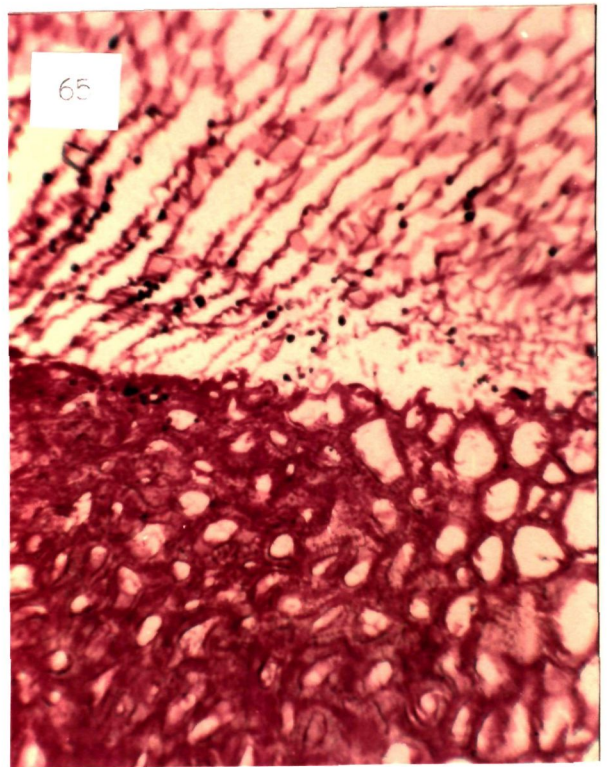
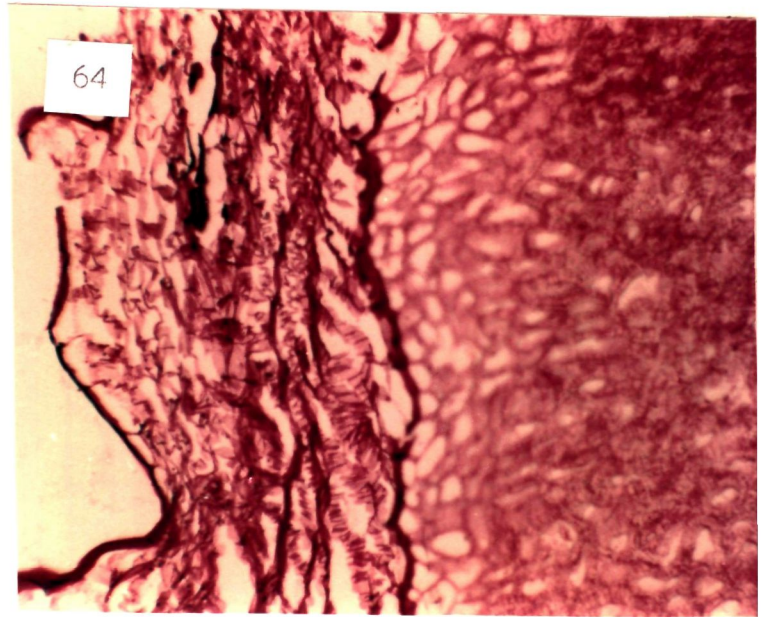
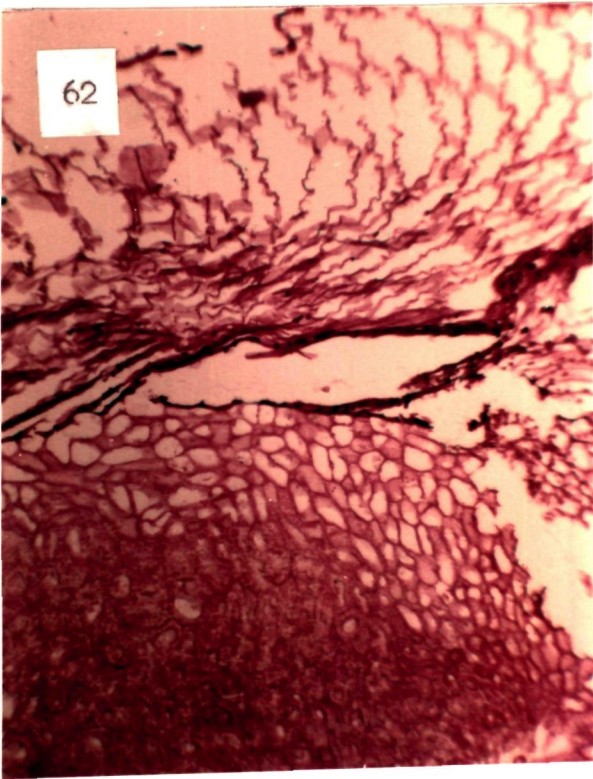
Figs. 62-65: Sections of 120 day old seeds of Kabul Yellow (Hard Seeded variety) tested with PAS method showing the integuments.

Fig. 62: Micropylar region showing both the integuments. Note the intensive lignification of inner integumentary cells X 100.

Fig. 63: Enlarged inner integumentary cells showing heavy lignification. X 400.

Fig. 64: Lateral region showing both the integuments. Note the heavy lignification of inner integumentary cells X 100.

Fig. 65: Chalazal region of the seed showing both the outer and inner integuments. Note the presence of few starch grains in the outer integument and also note the intensive lignification of all the cells in the inner integument . X 100.



III. Histochemical studies

Histochemical changes observed in the integuments during seed development of pomegranate varieties Ganesh, Bassein Seedless, Alandi and Kabul Yellow were as follows.

As far as changes in proteins and ribonucleic acids are concerned it was observed that, there was not much difference between the varieties studied. In all the four varieties studied the sugar content was less and there was large amount of starch accumulation and this accumulation was more intense in outer integuments and only few layers of inner integuments just after outer integuments were having starch grains. As the seed was growing the starch accumulation started reducing showing that the starch has started converting into sugar and this accumulation started early in soft seeded varieties and in these varieties it was clear from 80 days onwards that there was no starch grains at all and also the cells at outer integument were elongated with more sugar contents in their juice.

IV. changes in fruit quality

1. Total soluble solids

The data on TSS are presented in Table 10 and Figure 7. The sugar content of the juice measured as TSS increased gradually during fruit development and it increased continuously with advancement of maturity. In the variety Ganesh the TSS at 30 days of fruit growth was 3.75° Brix and it reached 8.20° Brix, later when

Table-10. Changes in T.S.S. Acidity and Brix/acid Ratio in developing pomegranate fruits

Days after fruit set	Ganesh			Passein Seedless			Alandi			Kabul Yellow		
	T.S.S. (oB)	Acidity (%)	B/A ratio	Acidity (%)	T.S.S. (oB)	B/A ratio	Acidity (%)	T.S.S. (oB)	B/A ratio	Acidity (%)	T.S.S. (oB)	B/A ratio
30	3.7	1.20	3.125	1.21	3.5	2.892	1.25	4.0	3.200	1.23	3.3	2.57
40	4.0	0.86	4.651	1.06	4.4	4.150	0.97	4.8	4.948	1.30	4.0	3.07
50	4.4	0.84	5.273	0.89	6.0	6.741	0.86	5.7	6.627	1.46	5.4	3.69
60	5.1	0.80	7.687	0.88	7.3	8.295	0.81	6.4	7.901	1.23	5.3	4.53
80	6.8	0.82	8.292	0.88	7.6	8.636	0.76	7.8	10.263	1.24	6.4	5.16
100	7.0	0.72	9.722	0.86	8.6	10.000	0.76	8.2	10.789	0.90	7.0	7.77
120	8.2	0.66	12.424	0.78	9.8	12.564	0.68	8.4	12.352	0.76	7.0	9.21

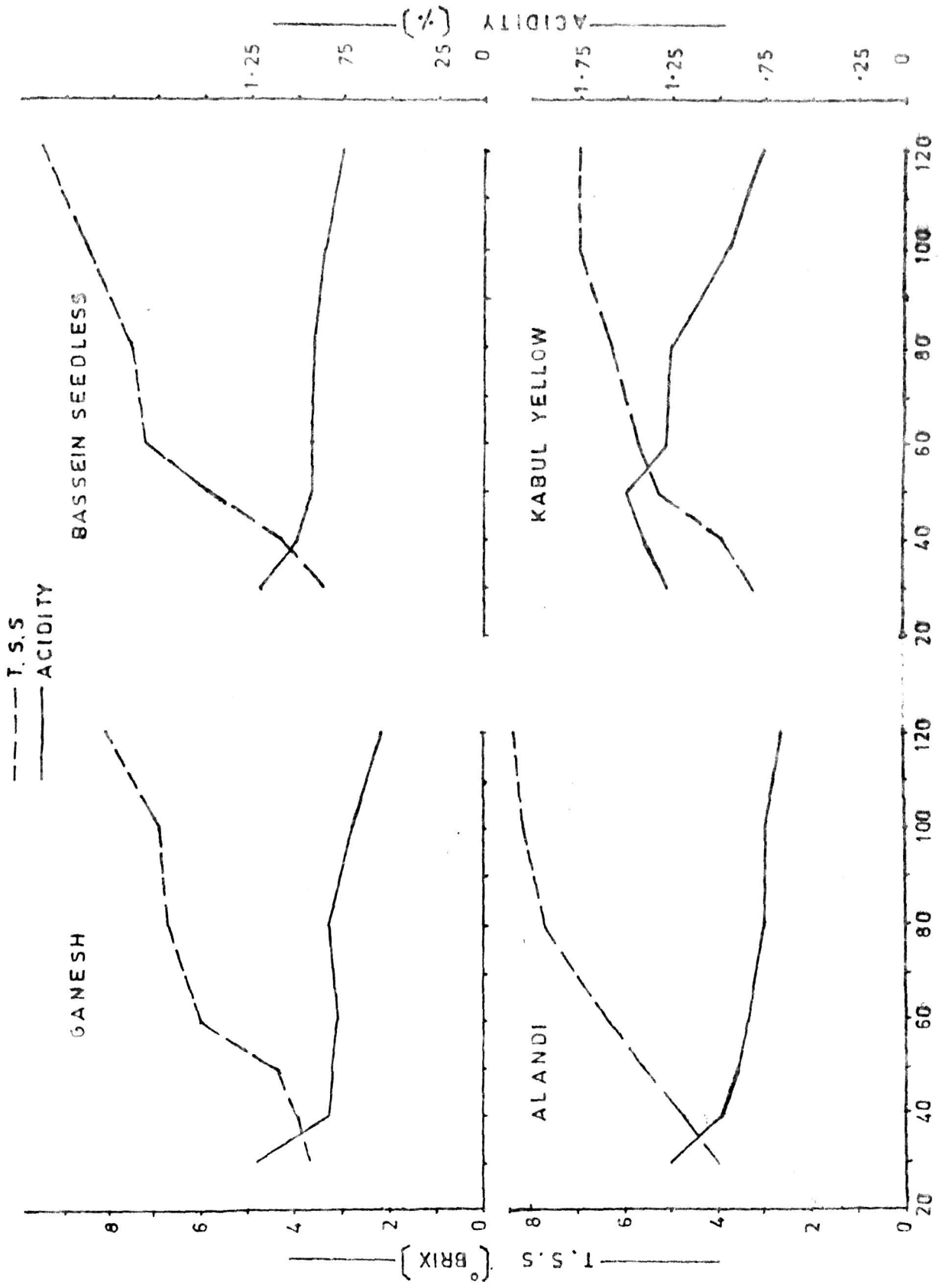


Fig. 7. CHANGES IN T. S. S. AND ACIDITY IN DEVELOPING POMEGRANATE FRUITS

the harvesting time was approached (120 days of growth). The variety Bassein Seedless had 3.50° Brix TSS at 30 days of fruit growth and reached 9.60° Brix at maturity. Incidentally out of all the four varieties studied Bassein Seedless variety recorded highest TSS of 9.8° Brix at maturity. The variety Alandi started with 4.0° Brix TSS at 30 days and reached 8.4° Brix at maturity and Kabul Yellow variety had 3.3° Brix TSS at 30 days growth and reached 7.0° Brix TSS at maturity which was incidentally the lowest TSS recorded when compared to other varieties.

2. Titratable Acidity

The data on titratable acidity and Brix/Acid ratio are presented in Table 10 and Fig. 7 and 8 respectively. The acid content of the juice which expressed as gram % of citric acid decreased with maturation of fruit. In Ganesh variety the acidity was 1.20% at 30 days and it decreased to 0.66% at full maturity (120 days). In the variety Bassein Seedless the acidity which was 1.21% at 30 days dropped down to 0.78%. In Alandi also the acidity dropped down from 1.25% at 30 days to 0.68% at maturity, but in Kabul Yellow the acidity which was 1.28% at 30 days did not drop below 1.24% till 80 days of fruit growth, and it came down only during maturity stage to 0.76%.

The Brix/acid ratio also increased as the fruit was growing from fruit set to maturity. In varieties Ganesh, Bassein Seedless and Alandi the ratio increased from approximately 3% at 30 days to 12% at maturity. But in the variety Kabul Yellow the

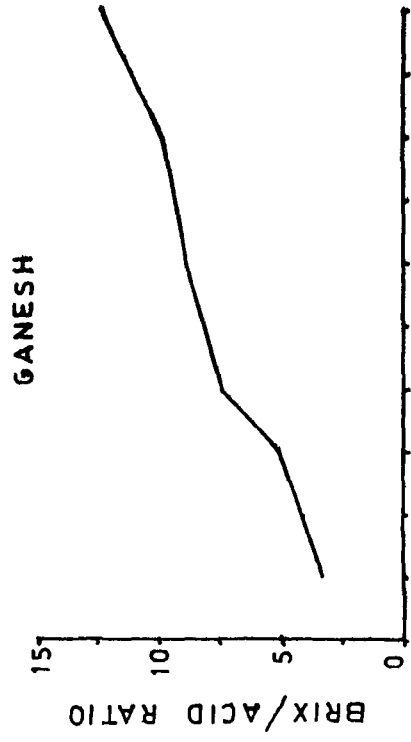
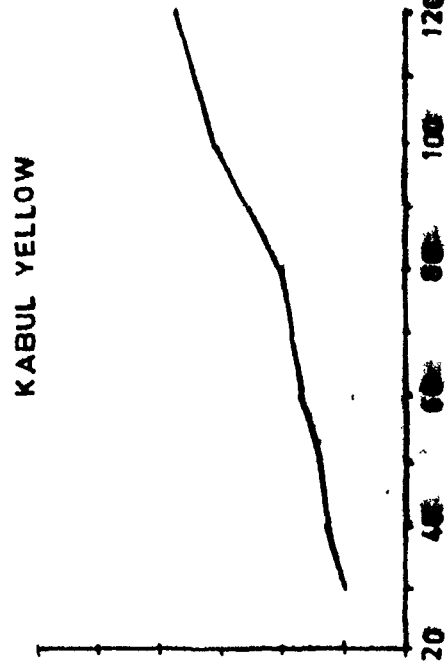
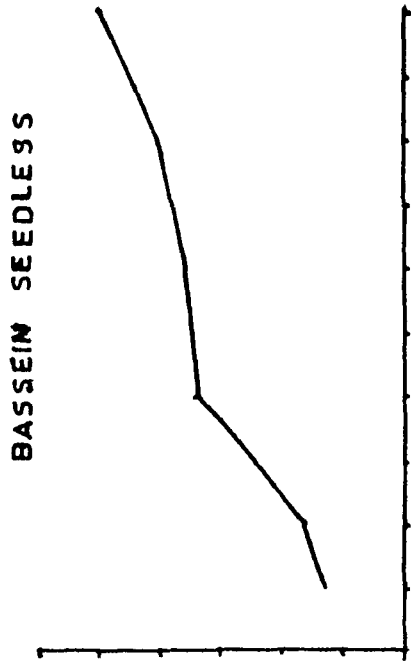


FIG. 8. CHANGES IN BRIX/ACID RATIO IN DEVELOPING POMEGRANATE FRUIT

ratio which was 2.573% at 30 days increased to 9.210% at 120 days of fruit growth. The increase in Brix/acid ratio and decreasing trend of titratable acidity could be used as criteria for fixing maturity standards in pomegranate fruits. In the three commercial pomegranate varieties Ganesh, Bassein Seedless and Alandi when the fruit reaches titratable acidity and Brix/acid ratio of around 0.6% and 12% respectively the fruits are ready for harvest.

3. Sugars

The data on the changes in reducing and total sugars are presented in Table 11 and Fig. 9. Both reducing and total sugars continued to increase during the development of fruits. The total sugars eventhough continued to increase with advancement of maturity the increase was rapid during 100 to 120 days. In the variety Ganesh the reducing sugars increased from 0.121 g/100 ml at 30 days of fruit growth to 0.469/100 ml at 120 days while the total sugars raised from 0.358 g/100 ml to 2.292 g/100 ml. In the variety Bassein Seedless the reducing sugars increased from 0.165 to 0.474 and total sugars increased from 0.305 to 1.800. In this variety the total sugar was the least when compared to other varieties. In Alandi the reducing and total sugars were 0.131 and 0.310 respectively at 30 days while at 120 days the reducing and total sugars were 0.423 and 2.260 respectively.

In Kabul Yellow the reducing and total sugars were 0.126 and 0.296 at 30 days respectively while they were 0.544 and

Table-11. Changes in reducing and total sugars in developing pomegranate fruits

Days after fruit set	Ganesh		Jassein Seedless		Alandi		Kabul Yellow	
	Reduced (g/100 ml juice)	Total (g/100 ml juice)	Reduced (g/100 ml juice)	Total (g/100 ml juice)	Reduced (g/100 ml juice)	Total (g/100 ml juice)	Reduced (g/100 ml juice)	Total (g/100 ml juice)
30	0.121	0.358	0.165	0.305	0.131	0.310	0.126	0.296
40	0.208	0.674	0.199	0.597	0.193	0.403	0.223	0.366
50	0.252	0.662	0.245	0.657	0.196	0.631	0.249	0.533
60	0.252	0.697	0.284	0.727	0.231	0.650	0.240	0.650
80	0.316	0.866	0.310	0.755	0.281	0.699	0.337	0.797
100	0.368	0.954	0.421	0.850	0.310	0.751	0.365	0.868
120	0.419	2.292	0.474	1.800	0.423	2.260	0.544	2.259

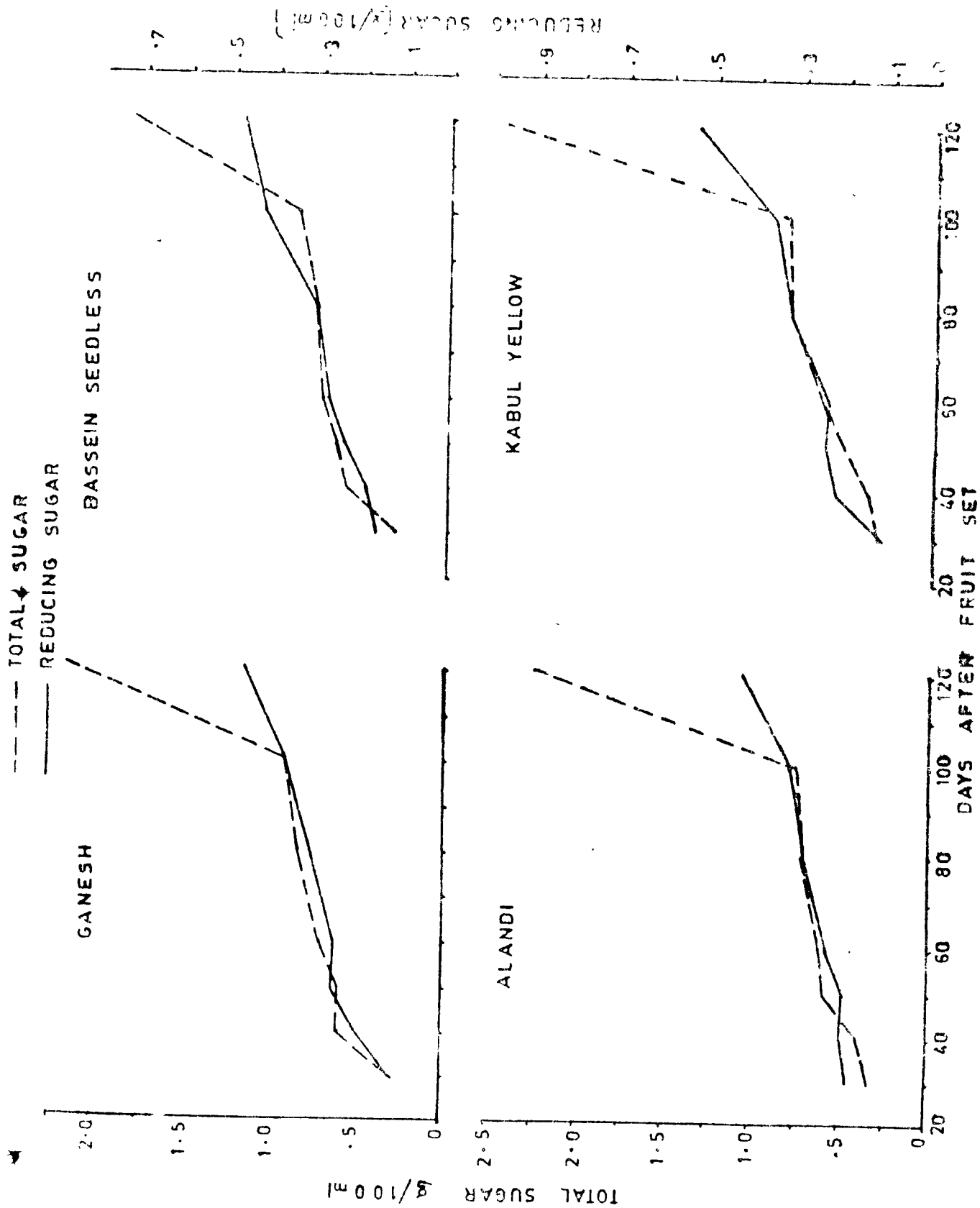


FIG. 9. CHANGES IN REDUCING AND TOTAL SUGAR IN DEVELOPING POMEGRANATE FRUITS

2.459 at 120 days. The maximum total sugars were in Ganesh 2.292 followed by Alandi 2.250, Tabul Yellow 2.259 and Bassein Seedless 1.800.

V. Seasonal changes in Macro and Micronutrient contents of Fruits and Seeds

A. Macronutrients

The data on changes in the mineral constituents of fruits and seeds are presented in Table 12 & 13 and Fig. 10, 11 and 12.

1. Nitrogen:

Nitrogen content (Fig. 10) in fruits was high at 10 days of fruit growth but declined thereafter continuously till maturity (120 days). Ganesh had 1.792% nitrogen at 10 days after fruit set and it decreased continuously till 80 days and then there was a little raise in nitrogen content from 100 days finally reaching 1.344% at 120 days. The nitrogen content of seeds also showed similar trend as fruits i.e, nitrogen was high in the initial stages of seed growth. At 20 days after fruit set it was 3.640% which declined to 1.792% at maturity (120 days after fruit set).

In Bassein Seedless variety, the nitrogen content of fruit was 1.524% at 10 days and went on decreasing till 60 days and again increased slightly to reach 1.176% at maturity of fruit. The seeds of Bassein Seedless had 3.416% nitrogen at 20 days which declined till 100 days only. There was increase in the nitrogen content between 100 to 120 days. At 120 days the nitrogen content of seed was 1.792%.

Table-12. Change in macro-nutrients of four pomarinate varieties (in fruits)

Days after fruit set	Nitrogen (%)			Phosphorus (%)			Potassium (%)					
	Ganesh Seedless	Alandi Yellow	Kabul Yellow	Ganesh Seedless	Bassein Seedless	Alandi Yellow	Ganesh Seedless	Bassein Seedless	Alandi Yellow			
10	1.702	1.624	1.702	1.568	0.194	0.175	0.213	0.171	1.702	1.680	1.805	1.470
20	1.568	1.344	1.456	1.232	0.100	0.131	0.153	0.171	1.512	1.512	1.518	1.502
30	1.298	1.176	1.344	1.232	0.106	0.100	0.120	0.156	1.302	1.302	1.412	1.176
40	1.230	1.008	1.064	1.120	0.031	0.106	0.120	0.100	1.213	1.113	1.344	1.134
50	1.120	1.008	1.068	1.064	0.075	0.100	0.125	0.100	1.176	1.302	1.252	1.134
60	1.120	1.064	1.076	1.064	0.059	0.075	0.094	0.053	0.543	1.008	1.250	0.452
80	1.064	1.120	1.054	1.164	0.050	0.063	0.075	0.030	1.302	0.840	0.152	1.176
100	1.176	1.176	1.043	1.024	0.106	0.081	0.084	0.088	1.554	1.384	0.914	1.680
120	1.344	1.176	1.021	1.032	0.100	0.144	0.106	0.088	1.882	1.722	1.853	1.696

Table-13. Changes in macro-nutrients of four pomegranate varieties (in seeds)

Days after fruit set	Nitrogen (%)			Phosphorus (%)			Potassium (%)					
	Ganesh Seedless	Alandi Yellow	Kabul Yellow	Ganesh Seedless	Bassein Seedless	Alandi Yellow	Ganesh Seedless	Bassein Seedless	Alandi Yellow			
20	3.540	3.416	3.135	3.024	0.613	0.625	0.600	0.663	3.024	3.102	3.192	2.604
30	3.528	2.600	2.962	2.958	0.420	0.550	0.563	0.538	2.688	2.520	2.520	2.352
40	2.856	2.856	2.928	2.632	0.400	0.438	0.400	0.50	1.754	2.016	1.596	1.512
50	2.296	2.408	2.296	2.576	0.288	0.373	0.420	0.325	1.596	1.428	1.754	1.512
60	2.016	2.128	1.904	1.512	0.200	0.315	0.213	0.263	2.150	2.550	2.300	2.150
80	1.648	1.904	1.904	1.792	0.138	0.125	0.100	0.100	2.450	2.400	2.400	2.150
100	1.805	1.008	1.450	1.344	0.182	0.153	0.265	0.213	2.412	2.400	2.400	2.134
120	1.792	1.792	1.500	1.456	0.200	0.288	0.215	0.245	3.024	2.552	2.448	2.680

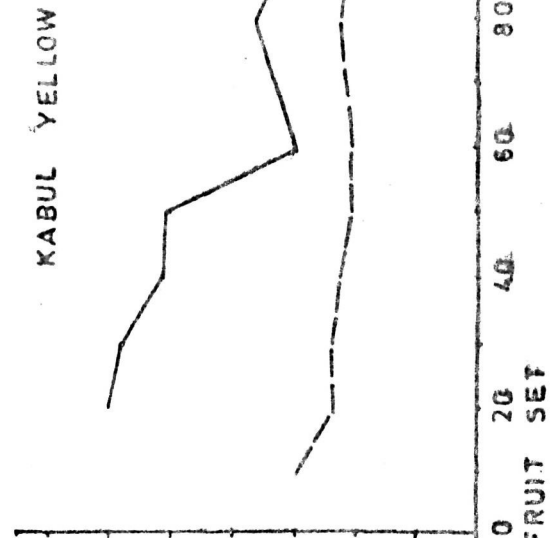
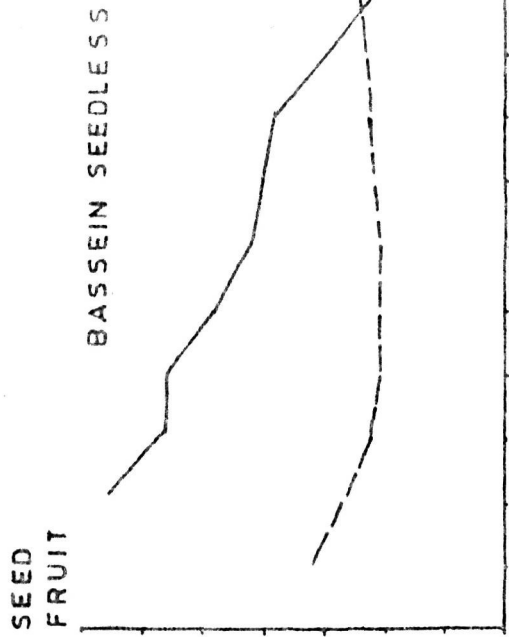
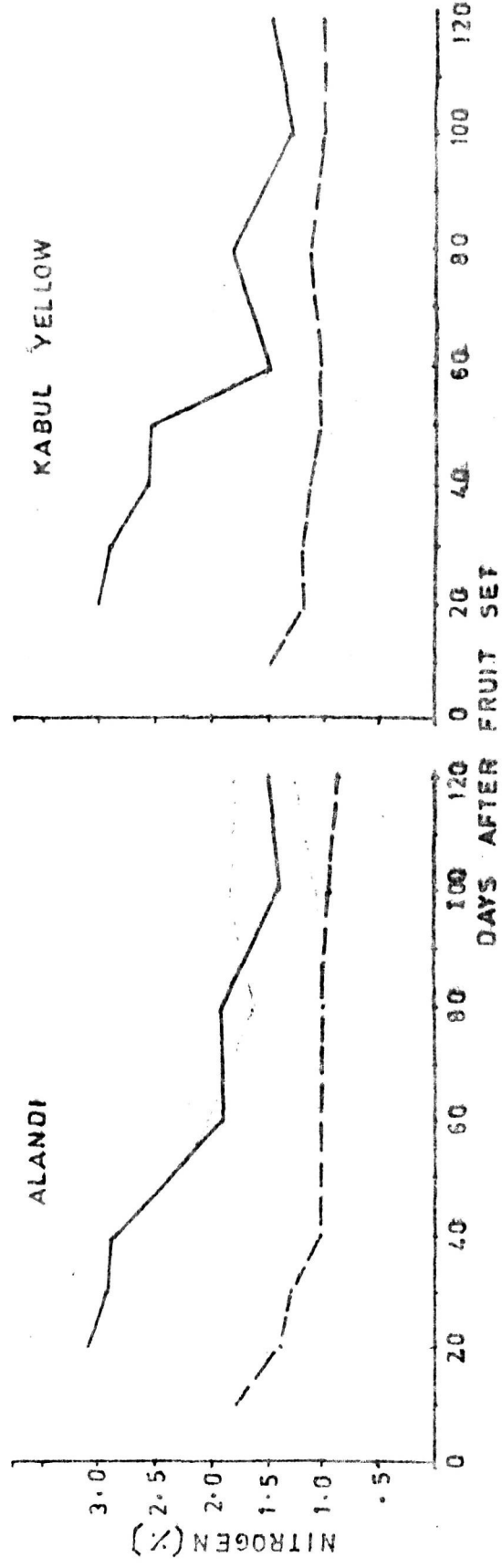
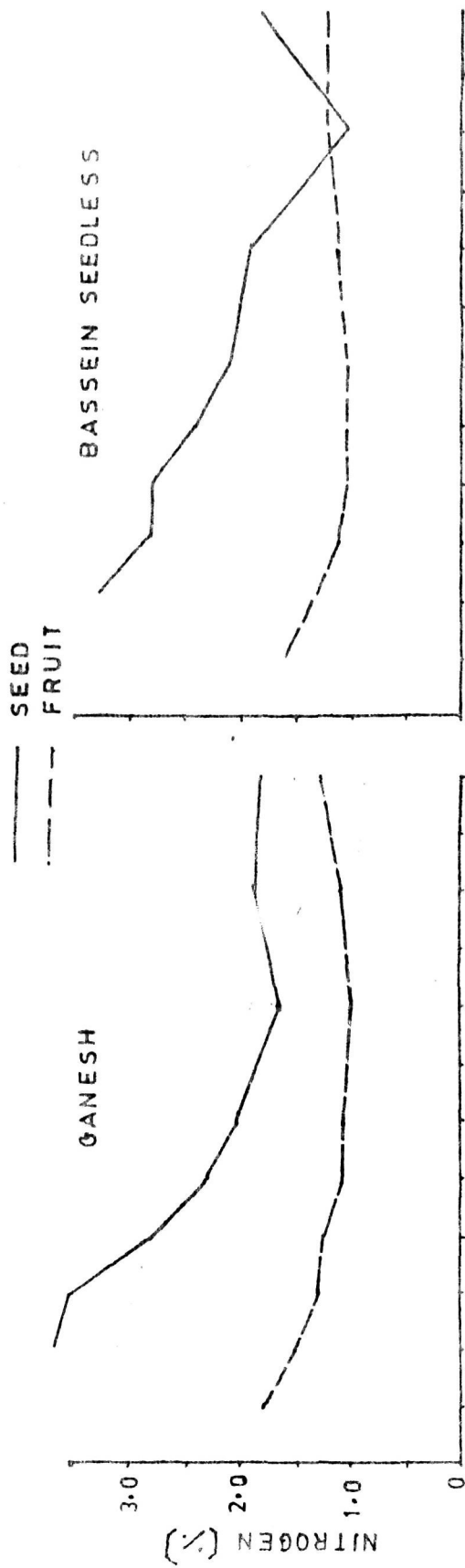


FIG. 10 CHANGES IN NITROGEN CONTENT OF DEVELOPING POMEGRANATE FRUIT

TABLE 10

The fruits of Alandi variety had 1.792% nitrogen at 10 days after fruit set and it declined continuously without any increase till 120 days finally reaching 1.021% nitrogen at maturity. The nitrogen content of seeds also exhibited similar trend. The nitrogen content at 20 days after fruit set was 3.136% and it reached 1.500% at maturity.

In Kabul Yellow variety the nitrogen content of fruits which was 1.568% at 10 days, declined to 1.052% at maturity. Similar trend was seen in nitrogen content of seeds also. It was 3.024% at 20 days and declined continuously till 120 days finally reaching 1.456% at maturity.

In conclusion the nitrogen content of both fruits and seeds was maximum immediately after fruit set (10 days) and second high was reached around maturity (120 days).

2. Phosphorus:

Phosphorus content (Fig. 11) was maximum at 10 days and then it gradually declined upto 80 days and again rose towards fruit maturity.

In Ganesh variety the phosphorus content was 0.194% at 10 days after fruit set, declined till 80 days (0.050%) increased slightly from 80 to 120 days and finally reached 0.100% at maturity. In seeds of Ganesh phosphorus content which was 0.613% at 20 days declined till 80 days (0.125%). A slight increase in phosphorus

— SEED
 - - - FRUIT

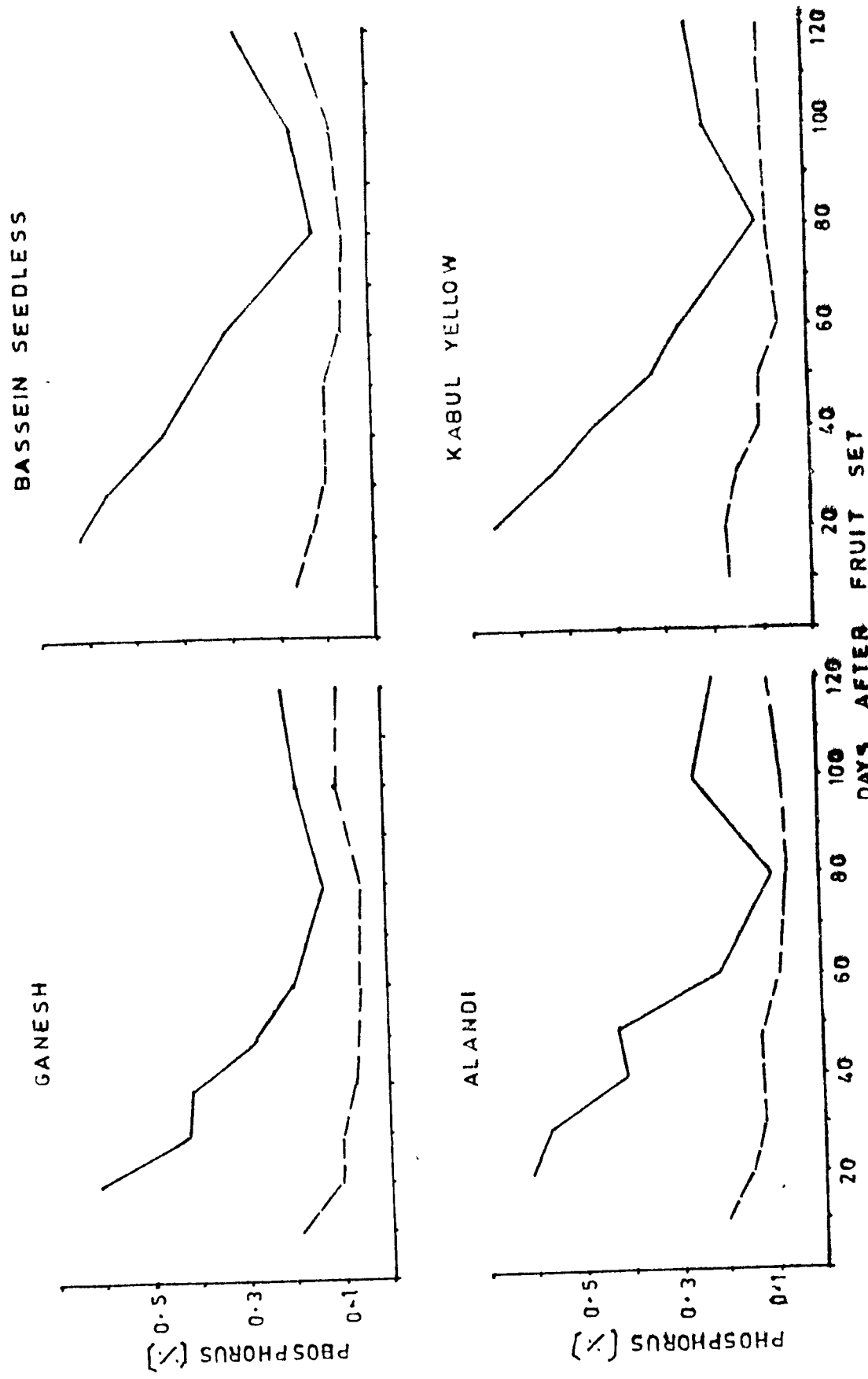


Fig. 11. CHANGES IN PHOSPHORUS CONTENT OF DEVELOPING POMEGRANATE FRUIT

content was seen between 80 to 120 days. The phosphorus content of seeds was 0.200% at 120 days.

The Bassein Seedless variety also showed similar trend as Ganesh. The phosphorus content at 10 days after fruit set was 0.175% it declined till 30 days (0.063%) and then a slight raise in phosphorus content was seen between 80 to 120 days, finally reaching 0.144% phosphorus at maturity. In seeds phosphorus content was 0.625% at 20 days after fruit set and it declined till 80 days (0.125%) and again raised a little during 80 to 120 days. At 120 days the phosphorus content in seed was 0.288% which was highest as compared to all the varieties studied.

In Kabul Yellow fruit the phosphorus content was 0.171% at 10 days after fruit set. It decreased and finally reached 0.088% at maturity. In this variety the phosphorus content of fruits continuously declined from fruit set to maturity. The phosphorus content of seeds at 20 days after fruit set was 0.663%. It decreased till 80 days (0.100%) and again slightly increased to 0.245% at 120 days.

In conclusion, the phosphorus content of both fruits and seeds was highest immediately after fruit set and a second high phosphorus content was seen at maturity.

3. Potassium:

The potassium content (Fig. 12) of fruits was high at 10 days but decreased thereafter and reached maximum at 120 days.

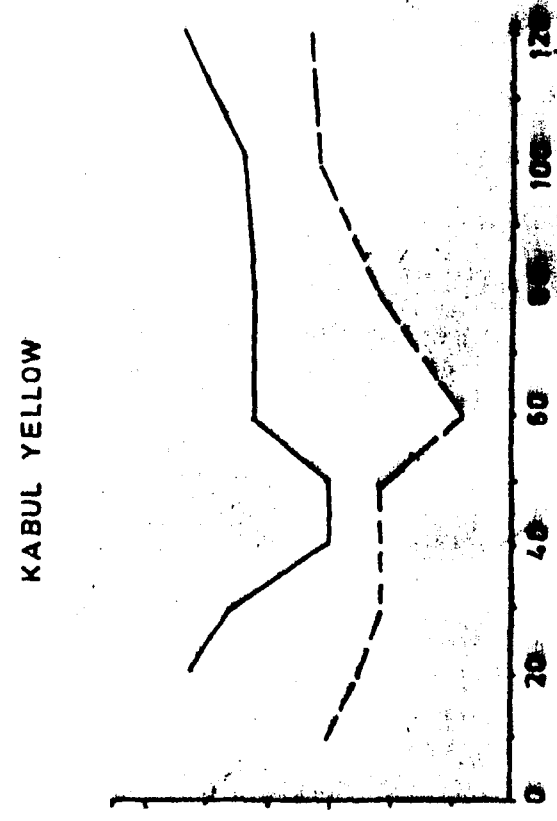
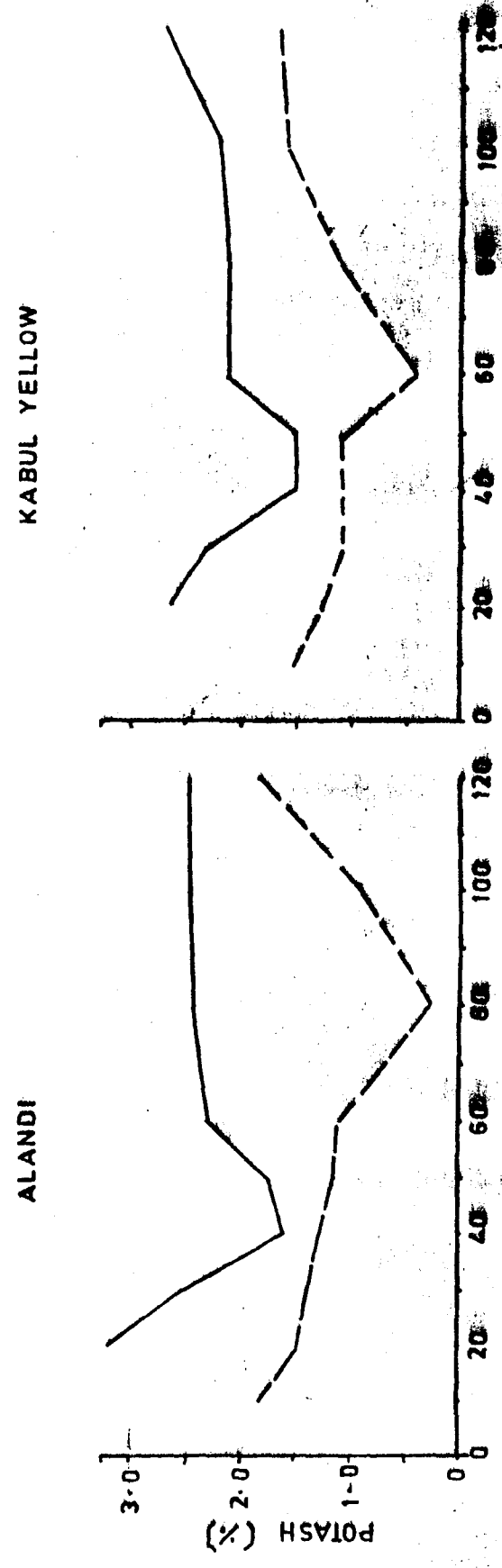
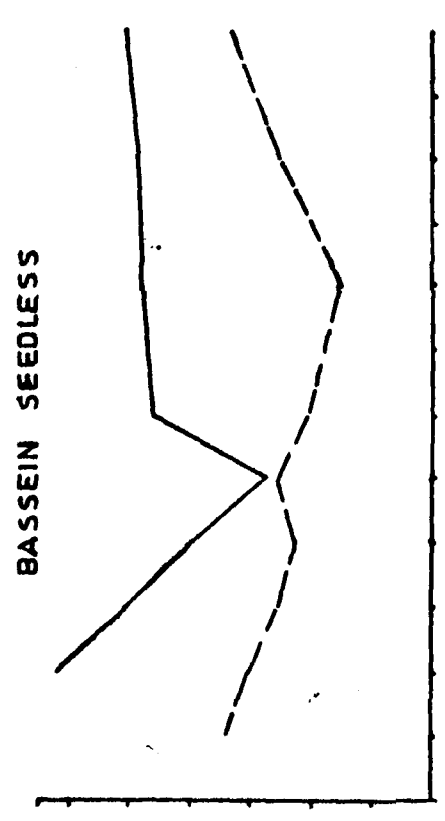
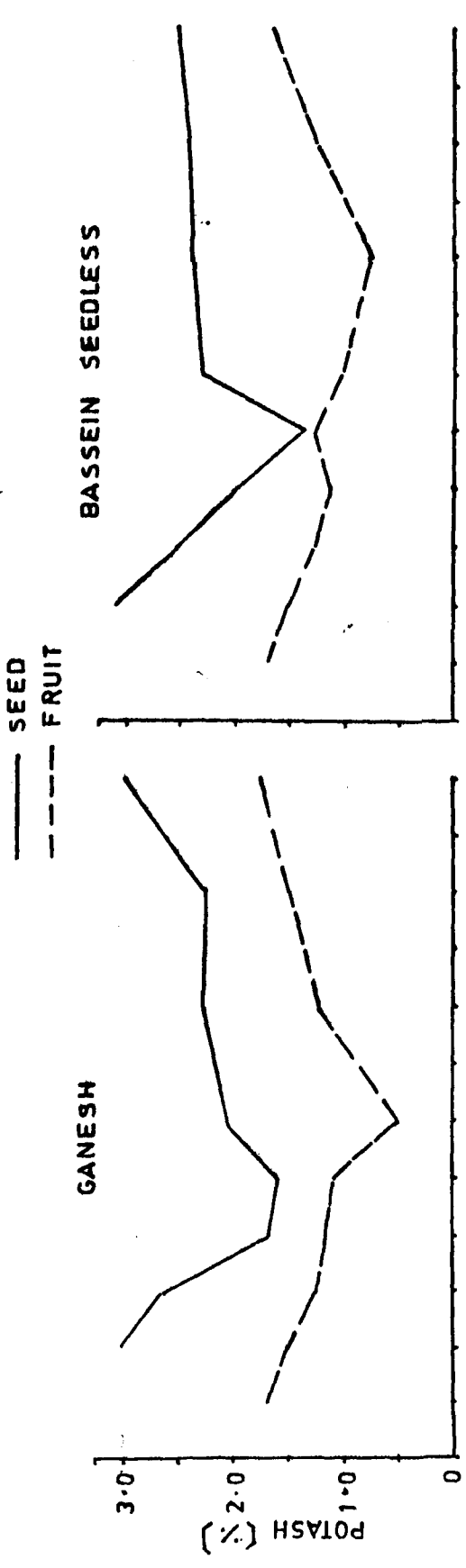


FIG. 12. CHANGES IN POTASH CONTENT OF DEVELOPING POMEGRANATE FRUIT

In Ganesh the potassium content was 1.722% at 10 days after fruit set, it slowly declined till 60 days (0.546%) and again increased sharply towards maturity. At 120 days the potassium content was 1.882%. In seeds the potassium content was maximum (3.024%) at 20 days declined to 1.596% at 50 days, and again increased sharply towards maturity, and reached 3.024% at 120 days after fruit set.

In Bassein Seedless similar trend was observed. The potassium content of fruits was 1.680% at 10 days and it slowly decreased to 0.840% at 30 days and at 120 days it again increased. At 120 days the potassium content was 1.722%. In seeds the potassium content at 20 days was 3.102%, it slowly decreased to 1.428% at 50 days and again increased sharply towards maturity (2.552%).

In Alandi variety the fruits had 1.806% potassium at 10 days after fruit set and it decreased to 0.462% at 80 days and increased slowly to reach 1.858% at 120 days after fruit set. In seeds of Alandi the potassium content at 20 days was 3.192%, at 40 days the contents decreased to 1.596% and increased steadily till 120 days and at maturity the potassium content was 2.448%.

In Kabul Yellow variety the potassium content in fruits was 1.470% at 10 days after fruit set, the content slowly decreased to 0.462% at 60 days and again slowly increased towards maturity. At 120 days the potassium content was 1.596%. The seeds of Kabul Yellow variety had 2.501% potassium at 20 days after fruit set,

it decreased to 1.512% at 50 day, and thereafter increased continuously till maturity reaching 2.680% at 120 days after fruit set.

In general the potassium content of fruits was high and highest at 10 days and 120 days respectively. The seed contained very high potassium both immediately after fruit set (i.e., 20 days) and at maturity (i.e., 120 days).

B. Micronutrients

The data on the changes in the micronutrient contents of four developing pomegranate fruits and seeds are presented in Table 14, 15, 16 and Figs. 15 to 18. The contents are in ppm.

In general, the micronutrient contents of fruits were high, initially after fruit set, then they declined in the intermediate stages and finally reached maximum values at maturity. However, the seeds had maximum micronutrients immediately after fruit set (i.e., 20 days) and the contents slowly decreased towards maturity.

1. Calcium:

The calcium content in fruits was more in the initial stages of fruit growth (fig. 13) in Ganesh the content was 32.5 ppm, in Bassein Seedless it was 48.0 ppm, in Alandi it was 46.5 ppm and in Kabul Yellow it was 44.0 ppm. The calcium content increased as the fruit was growing. The calcium content was decreased between 10 to 20 days and 50 to 60 days period in all the varieties except Kabul Yellow. In Kabul Yellow there was decrease in

Table-14. Changes in micro-nutrients of four pomegranate varieties, both in fruits and seeds

Days after fruit set	CALCIUM (in ppm)				MAGNESIUM (in ppm)			
	Fruits		Seeds		Fruits		Seeds	
	Bas- Alandi	Kabul Yellow	Bas- Alandi	Kabul Yellow	Bas- Alandi	Kabul Yellow	Bas- Alandi	Kabul Yellow
	Seed- less	Seed- less	Seed- less	Seed- less	Seed- less	Seed- less	Seed- less	Seed- less
10	52.5	48.0	46.5	44.0	24.5	25.0	23.5	24.5
20	27.5	34.0	37.5	41.0	37.0	51.0	33.0	59.0
30	41.5	57.5	56.0	40.0	47.0	47.0	51.0	29.0
40	47.5	60.0	62.5	55.0	62.0	52.0	45.0	50.0
50	43.5	77.5	69.0	51.0	67.5	62.5	56.0	33.5
60	46.5	42.5	51.0	42.0	33.5	12.0	17.5	16.0
80	51.0	47.5	70.0	57.0	19.0	20.5	16.0	20.0
100	54.0	17.0	57.0	43.0	13.0	21.0	13.0	13.0
120	55.0	1.0	57.0	57.0	17.0	16.5	13.5	13.5
					24.5	25.0	23.5	24.5
					13.5	12.0	16.0	11.5
					13.0	15.5	20.5	13.5
					10.5	18.5	13.5	13.0
					7.0	20.5	17.0	9.0
					11.5	10.5	13.5	8.0
					10.5	15.0	17.0	21.0
					43.0	33.0	41.5	42.0
					74.0	41.0	42.0	43.0
					27.0	27.0	25.5	25.5
					11.0	11.0	1.0	20.5
					20.5	20.5	20.5	21.0

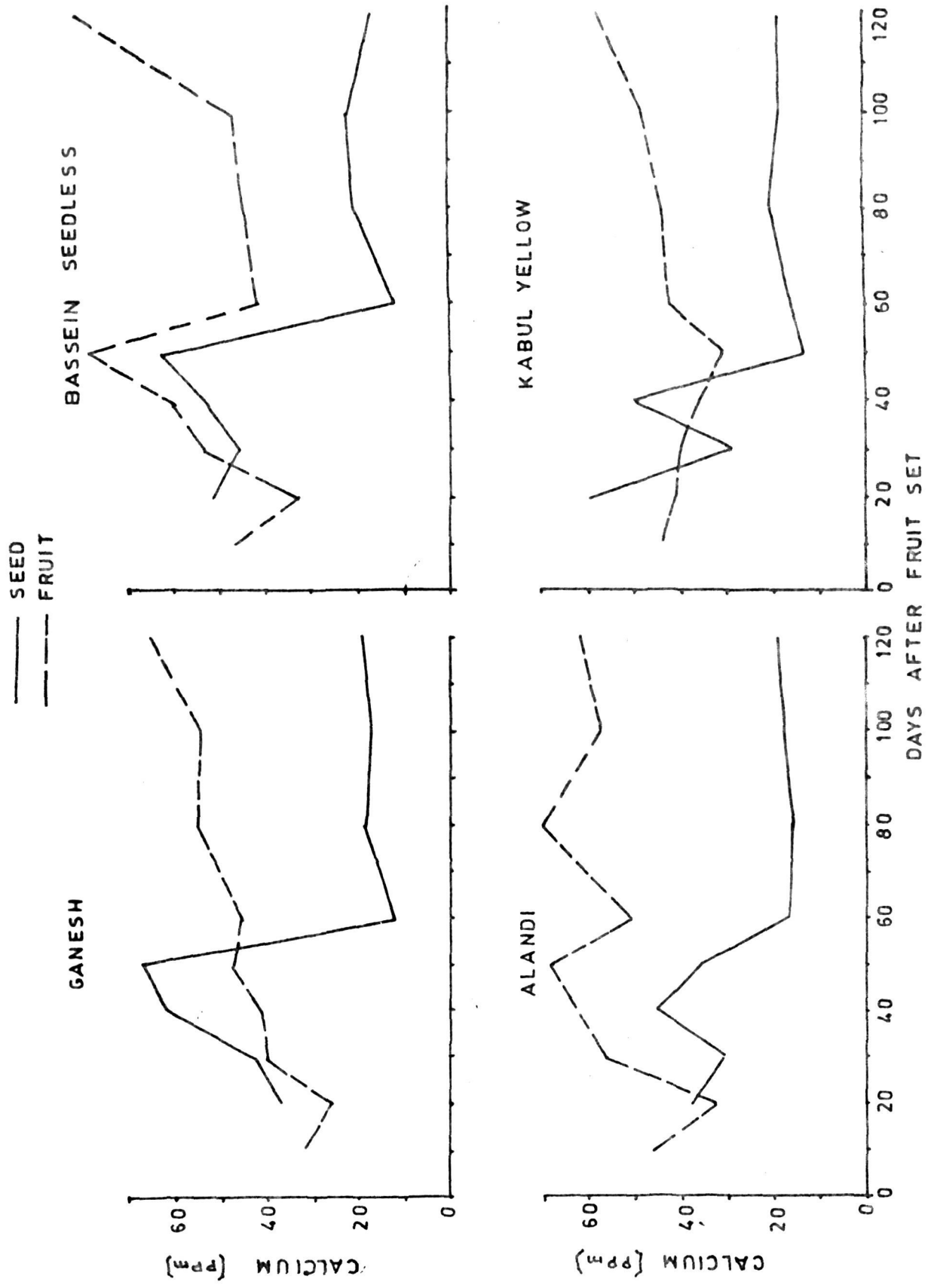


Fig.13. CHANGES IN CALCIUM CONTENT OF DEVELOPING POMEGRANATE FRUIT

calcium content till 50 days and then slowly it increased till maturity. At maturity, the calcium contents of different varieties were highest. In Ganesh variety 65.0 ppm, Bassein Seedless 61.0 ppm, Alandi 67.5 ppm and Kabul Yellow 57.0 ppm.

In the seeds the calcium content was maximum at fruit set, it declined during 20 to 30 days and again increased during 30 to 40 days in Alandi and Kabul Yellow. In Ganesh and Bassein Seedless the increase was during 30 to 50 days. Then the contents decreased slightly and then more or less remained constant till maturity. The calcium content of seeds at 20 days after fruit set in Ganesh variety was 37.0 ppm, in Bassein Seedless it was 51.0 ppm, in Alandi it was 33.0 ppm and in Kabul Yellow it was 59.0 ppm. The contents increased to 67.5 ppm in Ganesh and 52.5 ppm in Bassein Seedless at 50 days after fruit set. In Alandi the contents were 45.0 ppm and in Kabul Yellow the content was 50.0 ppm at 40 days after fruit set. At 120 days after fruit set the calcium content in seeds of different varieties were: Ganesh 19.0 ppm, Bassein Seedless 16.5 ppm, Alandi 19.5 ppm and Kabul Yellow 13.5 ppm.

2. Magnesium:

The magnesium content in the fruits (Fig. 14) of different varieties was more during 10 days after fruit set. It was 24.5 ppm in Ganesh, 27.0 ppm in Bassein Seedless, 27.5 ppm in Alandi and 24.5 ppm in Kabul Yellow. The contents declined till 60 days in all the varieties. In Ganesh it declined to 7.0 ppm (at 50

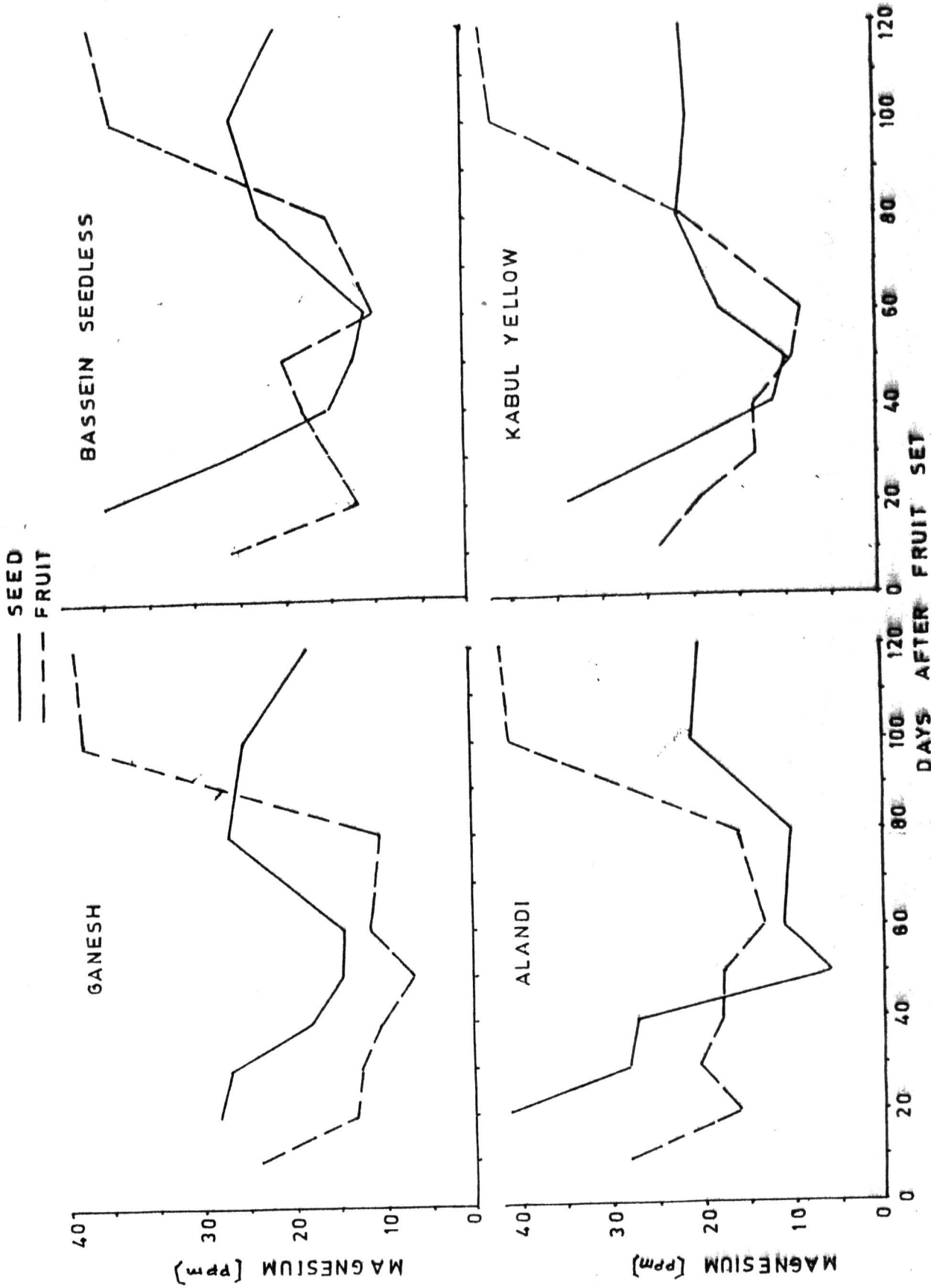


FIG. 14. CHANGES IN MAGNESIUM CONTENT OF DEVELOPING POMEGRANATE FRUIT

days), in Bassein Seedless it declined to 10.5 ppm, in Alandi to 13.5 ppm, and in Kabul Yellow to 8.0 ppm. The magnesium contents again increased to reach 44.0 ppm in Ganesh, 41.0 ppm in Bassein Seedless, 42.0 ppm in Alandi and 45.0 ppm in Kabul Yellow at maturity.

In the seeds the magnesium content was maximum at fruit set when compared to fruits in all the four varieties. The magnesium content at 20 days after fruit set in Ganesh was 28.0 ppm, in Bassein Seedless 40.0 ppm, in Alandi 41.0 ppm and in Kabul Yellow it was 34.0 ppm. The magnesium content almost became half at maturity. The magnesium content slowly decreased as the seeds were growing. The decrease was till 60 days and again it increased during 60 to 120 days. At 120 days Ganesh variety had 13.0 ppm, Bassein Seedless had 20.5 ppm, Alandi had 20.0 ppm and Kabul Yellow had 21.0 ppm.

3. Iron:

The iron content in fruits was more at 10 days after fruit set (Fig. 15) in all the four varieties. Variety Ganesh had 104.0 ppm, Bassein Seedless and Alandi had 132.5 ppm and Kabul Yellow had 90.0 ppm. The contents decreased as the fruit was growing till 30 days after fruit set and then increased steadily till harvest. At 120 days after fruit set the varieties had maximum amounts of iron. The variety Ganesh had 130.0 ppm, Bassein Seedless had 214.0 ppm, Alandi had 136.0 ppm, and Kabul Yellow had 154.0 ppm.

Table-15. Changes in micronutrients of developing four pomegranate varieties (in fruits)

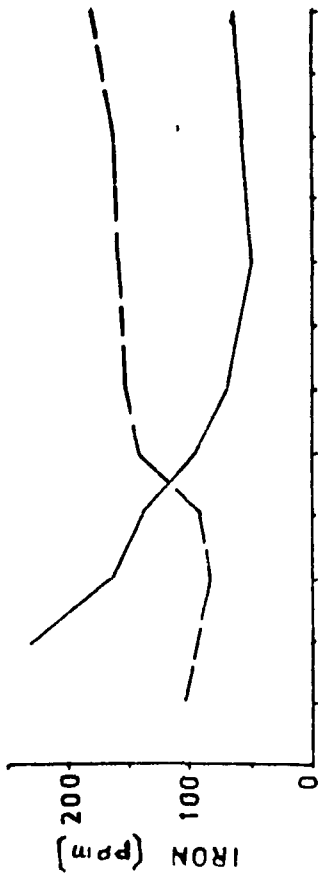
Days after fruit set	IRON (ppm)		MANGANESE (ppm)		ZINC (ppm)		COPPER (ppm)							
	Ganesh Bas- Seed- less	Alandi Kabul Yellow	Ganesh Bas- Seed- less	Alandi Kabul Yellow	Ganesh Bas- Seed- less	Alandi Kabul Yellow	Ganesh Bas- Seed- less	Alandi Kabul Yellow						
10	104.0	132.5	14.0	25.5	20.5	15.5	54.0	55.5	71.0	41.5	19.0	17.1	18.5	16.0
20	98.5	74.0	95.5	77.5	12.5	14.5	9.5	12.0	43.0	65.0	39.0	12.5	14.5	10.5
30	84.5	65.5	65.0	62.5	13.5	11.0	11.5	12.0	38.0	48.0	53.5	32.5	15.0	16.0
40	89.5	77.0	67.5	73.0	12.5	17.5	13.0	10.5	39.0	48.5	34.5	39.5	15.5	16.0
50	145.1	77.5	75.5	94.5	12.0	20.5	9.5	10.5	53.0	53.5	31.0	51.5	15.5	17.0
60	155.5	97.5	72.0	147.0	12.0	17.5	15.0	11.5	33.5	37.5	35.5	50.0	13.0	17.5
80	161.5	144.2	127.5	152.5	14.0	53.0	17.5	51.0	26.5	49.5	30.5	54.0	12.5	15.5
100	155.0	161.0	162.0	160.5	33.0	33.0	36.4	42.0	82.0	82.0	60.5	73.0	23.0	22.5
120	160.0	214.0	196.0	154.0	40.0	40.0	43.0	45.0	86.0	94.0	72.0	90.0	25.0	23.0

Table-16. Changes in microminerals of developing four pomegranate varieties (in seeds)

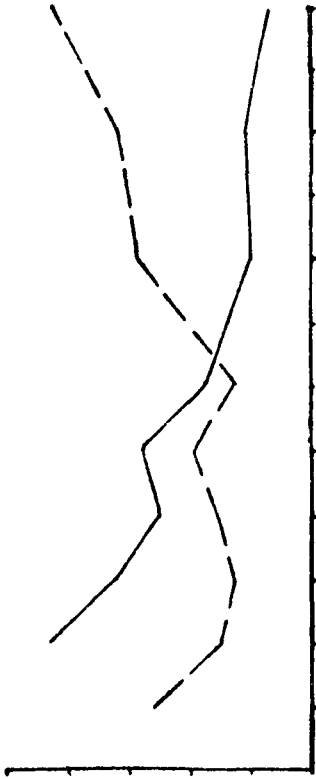
VARIETY	IRON (ppm)		MANGANESE (ppm)		ZINC (ppm)		COPPER (ppm)							
	Bas-sein	Alandi	Bas-sein	Alandi	Bas-sein	Alandi	Bas-sein	Alandi						
229.0	215.0	209.0	207.0	35.0	37.0	37.0	75.0	69.0	79.0	75.0	18.0	18.0	21.0	24.0
171.0	162.0	165.0	158.0	39.0	35.0	33.0	65.0	33.0	54.5	58.0	30.0	18.0	20.0	27.0
143.5	127.0	156.0	75.0	15.5	9.0	21.0	50.5	31.0	40.0	39.5	29.5	12.0	20.0	26.5
97.5	142.5	84.5	75.0	12.5	14.5	6.0	23.5	32.0	26.0	50.0	9.0	14.5	17.0	10.0
70.5	114.5	64.0	55.5	8.0	7.0	6.5	23.0	13.0	24.0	26.5	10.0	7.5	11.5	9.0
53.0	50.5	52.5	56.0	15.0	12.5	7.5	31.5	39.5	19.5	29.5	13.0	12.5	9.5	15.5
58.0	55.0	50.0	65.0	16.5	13.5	7.0	36.5	51.5	22.0	40.0	13.0	15.0	10.0	18.0
62.0	42.5	43.5	61.5	12.0	11.5	8.5	49.5	58.5	30.5	48.5	19.0	18.0	12.5	21.5

— SEED
 - - - FRUIT

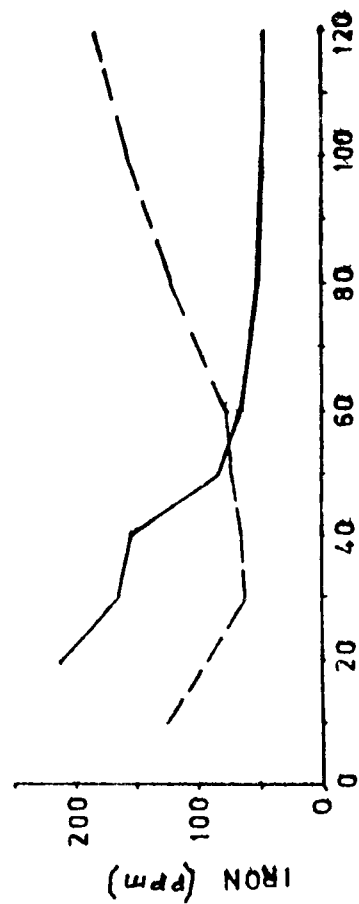
GANESH



BASSEIN SEEDLESS



ALANDI



KABUL YELLOW

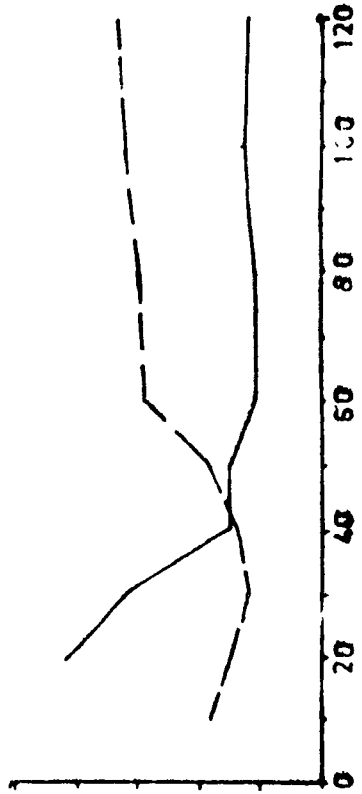


Fig. 15. CHANGES IN IRON CONTENT OF DEVELOPING POMEGRANATE FRUIT

The iron contents in seeds was maximum at fruit set. It was almost double the amount when compared to fruits. At 20 days after fruit set the iron contents of seeds in Ganesh was 229.0 ppm, in Bassein Seedless it was 216.0 ppm in Alandi and Kabul Yellow it was 209.0 ppm. The contents slowly decreased as the seed was growing and finally at 120 days after fruit set the iron content in all the four varieties were: Ganesh 63.0 ppm, Bassein Seedless 42.5 ppm, Alandi 48.5 ppm, and Kabul Yellow 61.5 ppm.

4. Manganese:

At fruit set the manganese content of fruit was more in all the four varieties (Fig. 16) Ganesh variety had 14.0 ppm, Bassein Seedless had 25.5 ppm, Alandi had 20.5 ppm and Kabul Yellow had 15.5 ppm. The manganese content declined after fruit set till 50 days and at that period the manganese content of Ganesh variety was 20.5 ppm, Bassein Seedless 20.5 ppm, Alandi 9.5 ppm and Kabul Yellow 10.5 ppm, and then there was a steady raise in manganese content till maturity. At maturity the variety Ganesh and Bassein Seedless had 40.0 ppm, Alandi had 43.0 ppm and Kabul Yellow had 45.0 ppm.

The manganese content of seed was similar to iron. The contents was highest at fruit set and it decreased continuously till maturity. At 20 days after fruit set the manganese content of Ganesh was 35.0 ppm. In Bassein Seedless and Alandi it was 37.0 ppm and in Kabul Yellow it was 35.0 ppm. The manganese

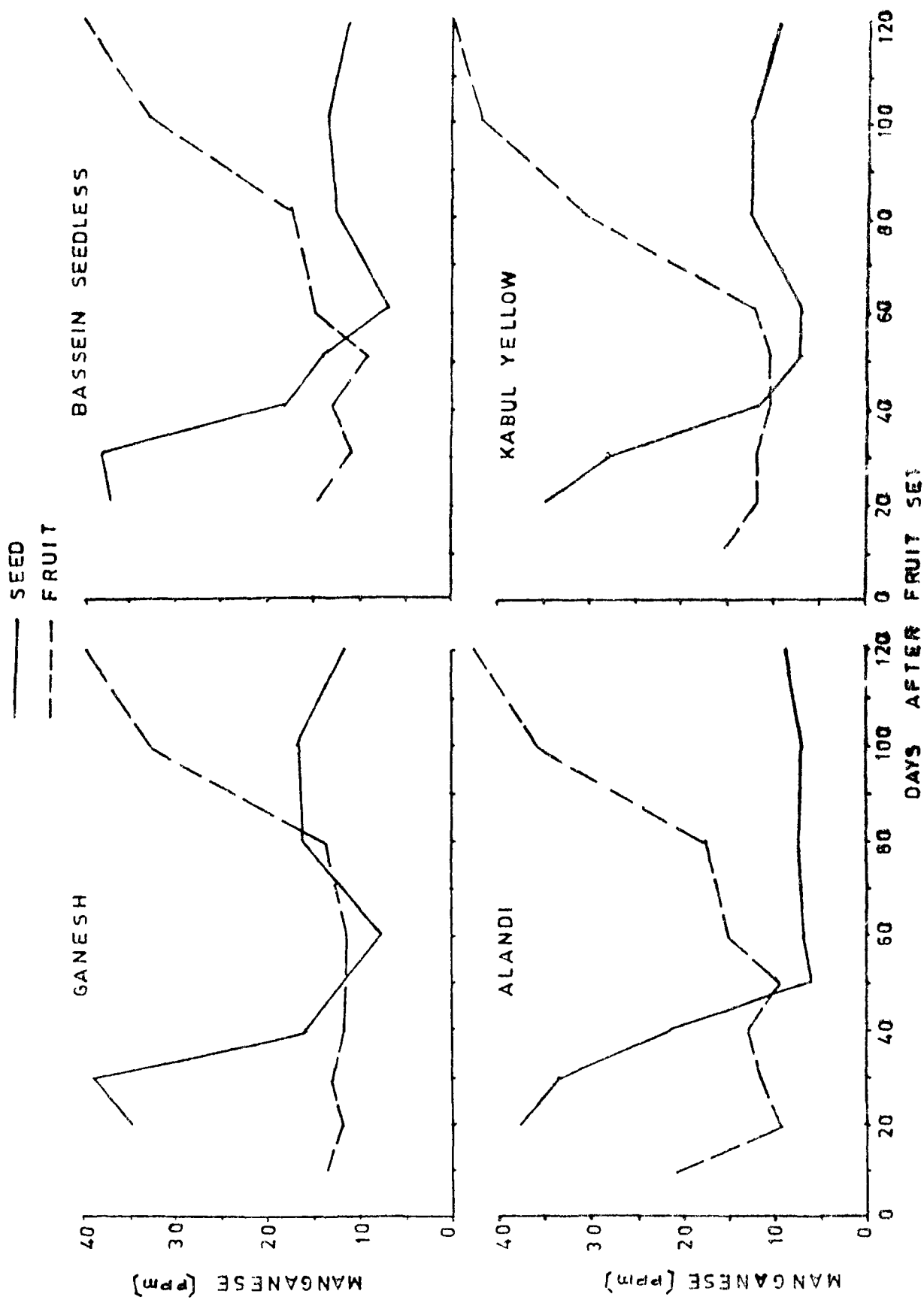


Fig.16. CHANGES IN MANGANESE CONTENT OF DEVELOPING POMEGRANATE FRUIT

content at harvest (120 days) in all the four varieties were: Ganesh 12.0 ppm, Bassein Seedless 11.5 ppm, Alandi 8.5 ppm and Kabul Yellow 3.5 ppm.

5. Zinc:

The amount of zinc both in fruit as well as in seed was similar to other micro-elements (Fig.17). In the fruit the amount of zinc was more at fruit set and it decreased till 60 days and increased again towards maturity. At 10 days after fruit set the amount of zinc was 54.0 ppm in Ganesh, 55.5 ppm in Bassein Seedless, 71.0 ppm in Alandi and 41.5 ppm in Kabul Yellow. The amount decreased to 33.5 ppm, In Ganesh, 37.5 ppm, in Bassein Seedless, 35.5 ppm in Alandi and 30.0 ppm in Kabul Yellow at 60 days and at 120 days after fruit set Ganesh had 85.0 ppm of zinc, Bassein Seedless had 94.0 ppm, Alandi had 72.0 ppm and Kabul Yellow had 90.0 ppm zinc.

The amount of zinc in seed was maximum at fruit set stage. The amount at 20 days after fruit set in Ganesh was 75.0 ppm, in Bassein Seedless it was 69.0 ppm, in Alandi it was 79.0 ppm and in Kabul Yellow it was 75.0 ppm. The amount of zinc declined continuously as the seed was developing. But only during 120 days after fruit set a slight increase in zinc content was seen. The amount of zinc at 120 days after fruit set in Ganesh was 47.5 ppm, in Bassein Seedless it was 53.5 ppm, in Alandi it was 34.5 ppm, and in Kabul Yellow it was 47.5 ppm.

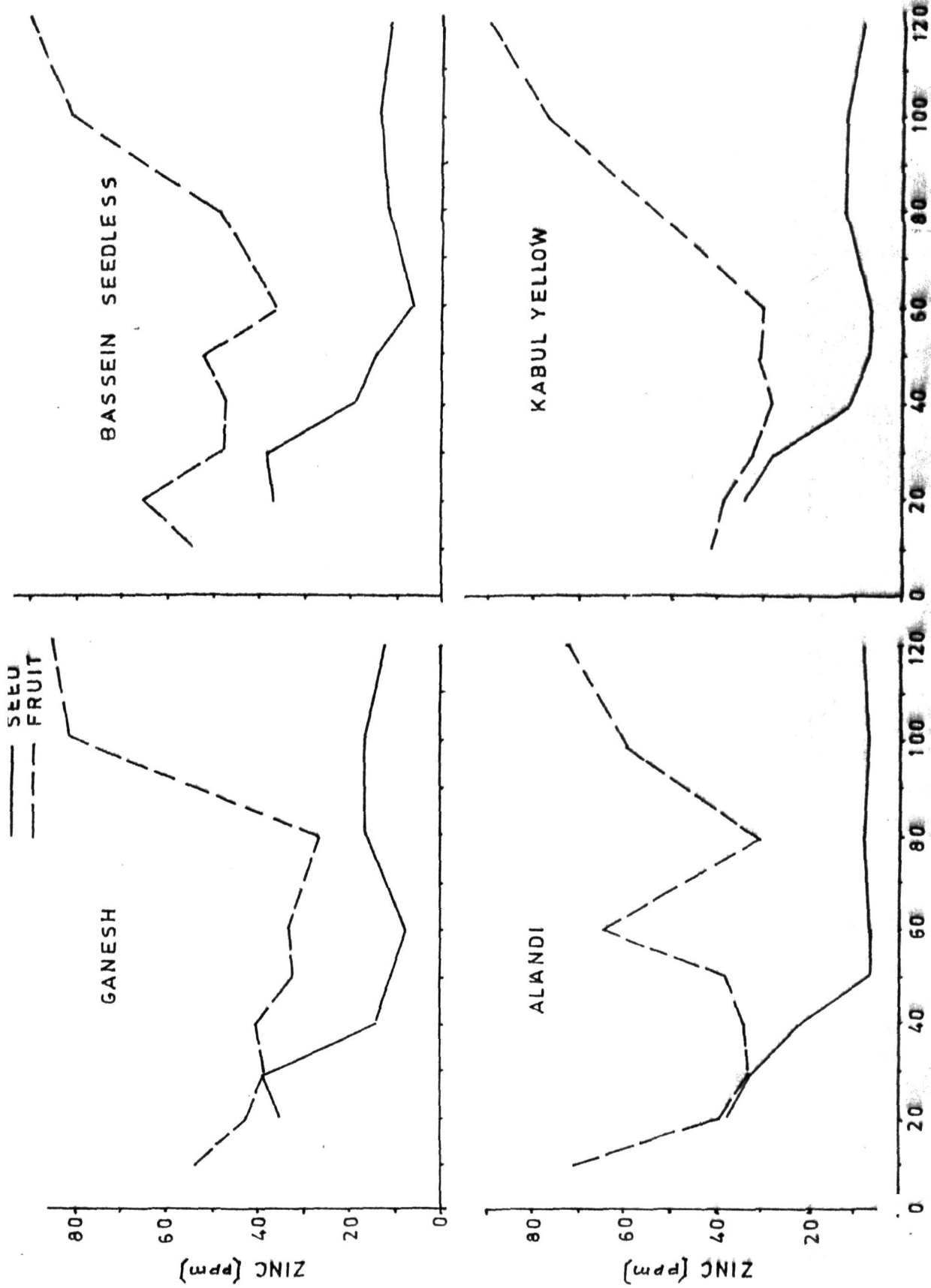


Fig.17. CHANGES IN ZINC CONTENT OF DEVELOPING POMEGRANATE FRUIT

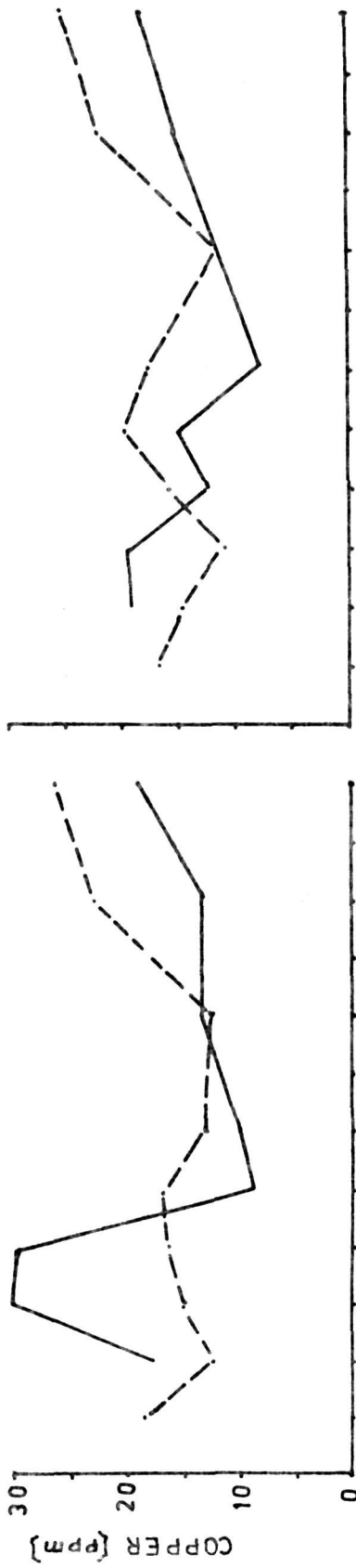
6. Copper:

Copper content in fruits (Fig. 10) was high at 10 days after fruit set, but declined thereafter continuously till 60 days. At 10 days, Ganesh had 19.0 ppm, Bassein Seedless had 17.1 ppm, Alandi had 18.5 ppm and Kabul Yellow had 16.0 ppm. The copper contents came down to 12.5 ppm in Ganesh, 12.0 ppm in Bassein Seedless, at 60 days and 14.0 ppm in Alandi and 11.5 ppm in Kabul Yellow at 60 days. The copper content again increased and reached to maximum at 120 days after fruit set. Copper content at 120 days in the four varieties were: Ganesh 26.0 ppm, Bassein Seedless 25.0 ppm, Alandi 23.0 ppm, Kabul Yellow 20.0 ppm.

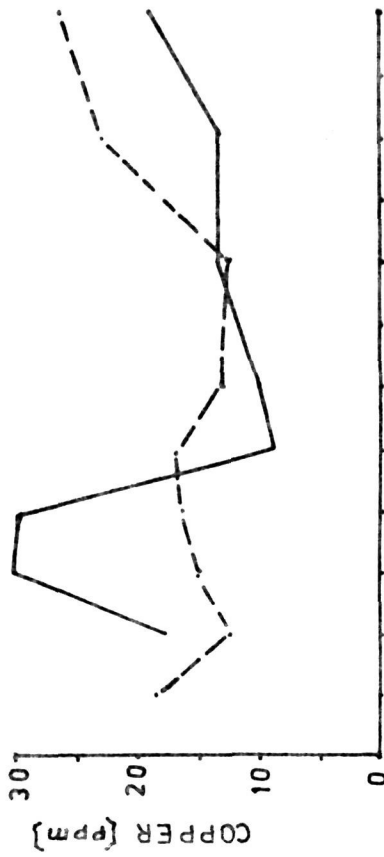
In the seeds the copper content was maximum at fruit set. In Alandi and Kabul Yellow the copper content was more and comparably it was less in Ganesh and Bassein Seedless. In Ganesh and Kabul Yellow the amount of copper slowly increased till 40 days after fruit set. In Bassein Seedless and Alandi the amount of copper remained almost constant till 40 days and then on the amount came down till harvest. In varieties Ganesh and Kabul Yellow the copper content declined after 40 days and it continued till maturity. At 20 days after fruit set the Ganesh variety had 18.0 ppm and it was 19.0 ppm at 120 days after fruit set. In Bassein Seedless at 20 days the copper content was 18.0 ppm and it was same at 120 days also. In Alandi it was 21.0 ppm at 20 days and it came down to 12.5 ppm at 120 days. In Kabul Yellow it was 24.0 ppm at 20 days and 21.5 ppm at 120 days after fruit set.

— SEEDS
 - - - FRUITS

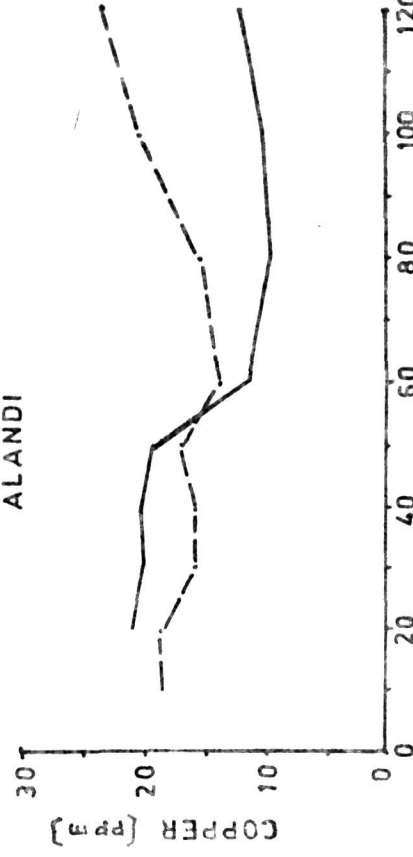
BASSEIN SEEDLESS



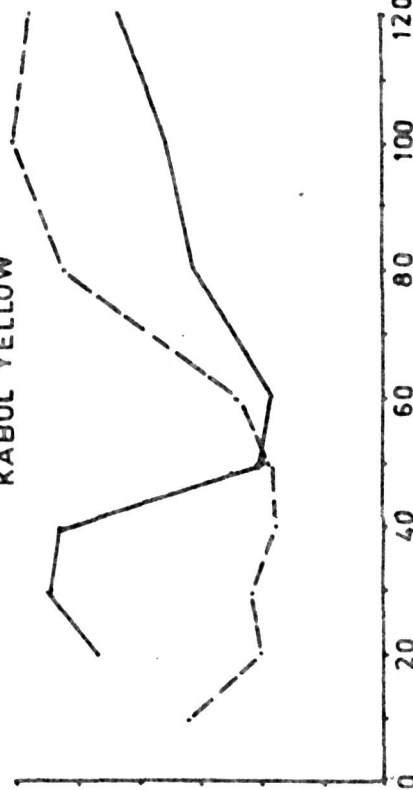
GANESH



ALANDI



KABUL YELLOW



DAYS AFTER FRUIT SET

Fig. 18. CHANGES IN THE COPPER CONTENT OF DEVELOPING POMEGRANATE FRUIT

Effect of Gibberellic acid on pomegranate fruits

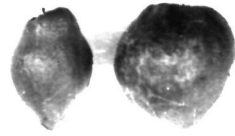
A preliminary experiment was taken up to see whether applied GA can induce seedlessness in hard seeded pomegranate variety Calnagar. Gibberellic acid was tried at 500, 750 and 1000 ppm concentration along with a control. The Gibberellic acid solution was taken in a beaker and the emasculated flowers were dipped in the solution for $\frac{1}{2}$ a minute. After $1\frac{1}{2}$ months it was seen that Gibberellic acid did not stimulate seedlessness in fruits. The treated fruits did not grow to a normal size but their shape was disfigured. The fruits turned into elongated ones while untreated fruits remained roundish (Fig. 66) and also the GA treated fruits developed beautiful reddish colour within $1\frac{1}{2}$ months after the treatment. At that stage when fruits were cut it was observed that the seeds have not developed at all (Fig. 67) but in control there was normal development of seeds. It can be concluded that in pomegranate gibberellic acid could not induce seedlessness and also pollination and fertilization is necessary for normal fruit development. Williams and Stahly (1969) tried cytokinin and gibberellin to see their effect on shape of delicious apple fruits. They observed that GA caused fruits to be longer and cytokinin also caused fruits to be longer with well developed calyx lobes. In peaches Stembriore and Cambrell (1970) observed that gibberellic acid induced parthenocarpic fruits. GA treated fruits were somewhat smaller and more elongated than control fruits.

PLATE XI

Fig. 66: Gibberellic acid treated fruits 45 days after the treatment. Note the small sized and irregularly shaped fruits of GA treatment.

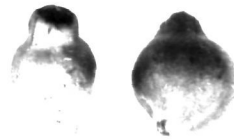
Fig. 67: Cross section of GA treated fruits after 45 days of treatment. Note the normal seed development in control fruits.

66



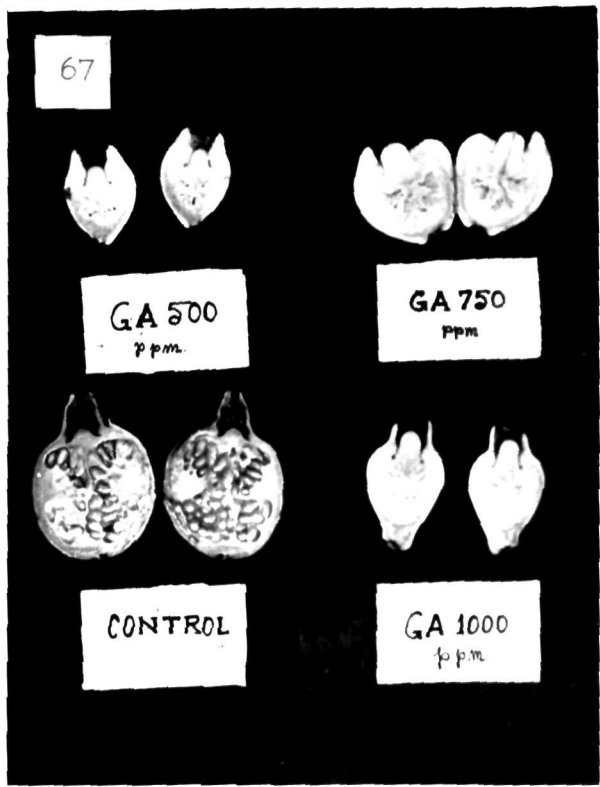
GA 500
ppm

GA 750
ppm

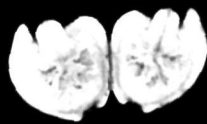
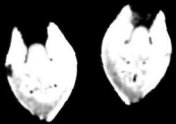


CONTROL

GA 1000
ppm

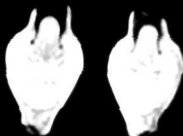
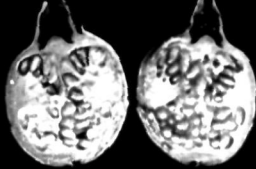


67



GA 500
ppm

GA 750
ppm



CONTROL

GA 1000
ppm

Crane et al. (1961) also found in F.F. Hale peach, that gibberellic acid treated fruits were parthenocarpic and were elongated and smaller in diameter.

DISCUSSION

DISCUSSION

I. Pattern of fruit growth

1. Fresh weight of the fruits

The fresh weight of fruits in all the four pomegranate cultivars continued to increase from fruit set till maturity. The pomegranate fruits required about 120 days to reach the stage of harvest maturity. These results are in confirmation with the studies of Shulman et al. (1984) Dash (1983) and Josan et al. (1979).

The pattern of fruit growth showed a simple sigmoid curve almost approaching a linear relationship. Shulman et al. (1984) reported that fruits of pomegranate variety Male's Head had simple sigmoid growth curve whereas the variety Wonderful made a linear growth. The results of the present study are in confirmation with those of Shulman et al. (1984) and Dash (1983).

In the present study during the period extending from fruit set to maturity there were periods of high growth rate alternating with slow growth and finally fruits reached maximum growth towards maturity. Similar results were reported by Dash (1983) in variety Jyothi, where the fruit weight increased greatly during the second fortnight and again during sixth fortnight with decreased weights during other periods. Josan et al. (1979) also observed similar trend in pomegranate under Punjab conditions. After fruit set they observed a rapid and continuous growth upto 35 to 40 days

followed by a period of slow development for another 25 days and there after the fruit size remained more or less the same. Arie et al. (1984) observed a constant rate of fresh weight increase in fruits of Wonderful variety of pomegranate throughout the growing season. According to Bollard (1970) the increase in fruit weight can be attributed to an increase in both the cell size and inter cellular spaces in the flesh, which resulted in the maximum possible accumulation of food substances. Ingrid Roth (1977) is of the opinion that fruit development is frequently initiated by cell division which is later followed by cell enlargement. The period of cell division may be short and eventually restricted to parenthesis time, while cell enlargement is always of greatest importance. Some fruits enlarge 200 to 300 times of their original size and cell volume increases to the same extent. Westwood et al. (1967) are of the opinion that the combined growth resulting from cell division, cell enlargement and air space formation results in a general sigmoidal curve when fruit volume or weight is plotted as a function of time. In the present investigations on pomegranate a continuous increase in fresh fruit weight from fruit set to maturity was the result of both cell division and cell enlargement.

2. Length and breadth of the fruit

The length and breadth of fruits increased continuously from fruit set to maturity. The increase in length was faster as compared to breadth during the early period of fruit growth, while

increase in breadth was more pronounced during the later part of fruit growth. The results are in agreement with the studies of Dash (1983) in pomegranate variety Jyothi (GAVK-1), where the length and diameter of the fruit increased continuously from fruit set to maturity. Arie *et al.* (1984) observed similar results in Wonderful variety of pomegranate. Inber Bal and Singh (1976) also observed that the increase in length was faster than increase in diameter during the first phase, (first six weeks after fruit set) whereas in the third phase the fruit increased in diameter than in the length. In Kagzi lime Hittalmani and Rao (1976) observed that, the length and diameter increased continuously but there were fluctuations with regard to rate of increase and finally concluded that the length was slightly more than the breadth throughout the growth period.

3. Length/Breadth Ratio

The differential length and breadth growth rates affected the length/breadth ratio of fruit during its development. In the fruits of Ganesh the length/breadth ratio was 1.22 at fruit set and it decreased to 0.97 at maturity. In variety Bassein Seedless the length/breadth ratio remained around 1 upto 80 days indicating that increase in length and breadth was of similar magnitude during this period and the fruits were almost round. The ratio decreased to 0.94 at maturity. In Alandi variety the length/breadth ratio was 1.29 at fruit set which decreased to 0.89 at maturity, while in Kabul Yellow the ratio decreased from 1.13 at fruit set to 0.94 at maturity.

The fruits of Ganesh, Alandi and Jabul Yellow had higher length/breadth ratio during the initial stages of fruit growth, and the ratio gradually decreased towards maturity as a result of or later increase in breadth. Thus it can be concluded that the fruits in the initial stages were oblong-ovate and subsequently they became round, globular or globose in shape.

4. Fresh Weight of rind and seeds

The weight of rind and seeds increased continuously till maturity (Table 4 fig 3). During most stages of fruit development the seeds accounted for about 50 per cent of the total weight of the fruit. The weight of rind and seeds were more or less equal in 40 day old fruits and thereafter the seed weight was higher than the rind weight, apparently due to enlargement of arils and accumulation of juice in arils.

Shulman et al. (1984) also observed that the seeds constituted about half of the fruit's weight during most stages of fruit development. Further, they reported that the seeds grew continuously during fruit development. At the beginning of fruit development the seed consisted mainly of a soft stone. Later the stone stopped growing and hardened while the juicy tissue (aril) continued to grow. They concluded that the pomegranate seeds resemble the development of drupe type fruits; however, it differs in that the drupe usually follows a double sigmoid growth curve (Lilleland, 1951; Lilleland and Newstone, 1954; Coombe, 1976), whereas pomegranate seeds grow continuously.

Data on seed weight obtained in the present study also showed that seeds grew continuously. Similar results were reported by Dash (1983) and Joan et al. (1979).

5. Seed weight/Rind weight ratio

In general it was observed (Table 4 and Fig 4) that the seed weight to rind weight ratio increased gradually from fruit set upto 80 days and then it declined slight upto maturity (120 days). Although both seed weight and rind weight increased continuously from fruit set to fruit maturity, initially the newly set fruit consided mainly of rind portion hence the ratio values were low. As the growth and lignification of seeds picked up, the ratio crossed the value of one around 40 days thereby indicating that at this stage the weight of seeds and rind were approximately equal. After 40 days seed growth overtook rind growth, and therefore the ratio kept on increasing. Around maturity (i.e., at 100 and 120 days) there was slight decline in the ratio probably because of setting in of ageing process in rind and loss of moisture from rind.

It has been reported (Cheema et al., 1954) that sudden and abrupt change as soil moisture availability around maturity causes cracking of pomegranate fruits. Present studies indicate that abrupt and steep increase in fresh seed weight (probably due to influx of moisture in seed aril) may cause cracking of rind as ageing process has already started in rind.

6. Dry matter content of fruit and seeds

The percent dry matter content of Ganesh fruits slowly increased from 31.05% at 10 days to 42.19% at 40 days and thereafter it declined to 26.70% level at maturity stage. In Bassein Seedless the dry matter content of fruit increased from 30.87% at 10 days to 37.55% at 50 days and then slowly decreased to 23.23% at maturity. In Alandi it increased from 23.71% at 10 days to 33.10% at 50th day and came down to 23.01% at 120 days. In Kabur Yellow the dry matter content at 10, 50 and 120 days were 29.17%, 41.18% and 35.91% respectively.

It can be concluded from the above results that the percent dry matter content of fruits increased continuously till about 50 days of fruit growth and thereafter it declined gradually towards maturity. The initial increase in dry matter content upto 50 days was possibly due to cell division. The dry matter content decreased after 50 days probably as a result of influx of moisture for cell enlargement and formation of juice in arils of seeds. These findings confirm the results of Shulman *et al.* (1984) who reported that the juice content was less than 25% of total fruit weight around 50 days of fruit growth. As the fruits matured the juice content continued to increase upto harvest time. In their study the juice content reached 40 to 45% of the total weight at maturity.

This pattern of change in dry matter content of fruit was also observed by Sulladmath (1975) in sapota fruits and by Hittalmani and Rao (1976) in Kagzi lime fruits.

In case of seeds the dry matter content increased continuously from fruit set till maturity. In Ganesh the dry matter slowly increased from 5.40% at 10 days to 17.91% at maturity. In Bassein Seedless the dry matter content was 3.72% at 10 days of fruit growth, it slowly raised to 20.09% at maturity stage. In Alandi it was 4.95% and 17.54% at 10 days and maturity stage respectively. Kabul Yellow recorded 6.10% dry matter content during 10 days of growth which slowly increased to 24.72% at maturity. The continuous increase in dry weight of seeds was due to higher rate of growth of seeds and also due to lignification of seeds.

7. Specific gravity of fruits

The specific gravity was more than unity (Table 6) in early stages of fruit development, in all the four varieties and then it gradually declined below 1 towards maturity. In Ganesh variety the specific gravity was more than 1 upto 30 days of fruit growth and then started declining towards maturity reaching 0.902. In Bassein Seedless the specific gravity showed a fall from 1.120 to 0.900, it was more than 1 upto 40 days of fruit growth. Alandi variety showed a similar trend like Ganesh. The variety Kabul Yellow had the maximum specific gravity till 80 days of fruit growth. It was more than 1 till that time. Then only it came down to 0.965 at maturity. In general the pomegranate fruits had less than 1 specific gravity when they approached maturity and ripening stage.

Bal and Singh (1973a) observed similar results in Ler variety Umran. The fall in specific gravity was rapid during 60 days after fruit set, then with a little change and again a rapid decrease was seen. Similar observations were reported in apple, peach and pear by Westwood (1962). Hittalmani and Rao (1976) also showed a declining trend in specific gravity in case of Kagzi lime. Roy and Singh (1980) observed in Iael fruit a high specific gravity in the initial stages and then it fell gradually for some time and increase was again noticed and remained constant. The increase in specific gravity with development in Bael was mainly due to increase in dry matter content of the fruit, but the fall during ripening after harvest was mainly due to loss in weight of fruits.

8. Thickness of rind

Thickness of rind was maximum at 20 days of growth and it declined towards maturity. Dash (1983) reported in Jyothi variety (GKVK-1) of pomegranate that rind thickness decreased continuously with advancement of fruit growth till maturity. Hittalmani and Rao (1976) reported in Kagzi lime that the rind thickness was maximum during fourth fortnight of fruit growth and it declined at maturity.

9. Hundred seed weight (fresh and dry)

The fresh as well as dry seed weights per 100 seeds (seed stone + aril) showed continuous increase from fruit set to maturity

in all the four varieties studied. Shulman et al. (1934) also reported that the seeds grow continuously during fruit development. Variety Ganesh recorded maximum fresh weight per 100 seeds because the aril portion covering the seed was more fleshy and contained more juice. Kabul Yellow recorded highest dry 100 seed weight and the seeds were hardest due to maximum lignification of seed testa.

II. & III. Histological and histochemical studies

The main parts of the seed are embryo, endosperm and seed coat. In pomegranate the seed coat i.e., the integuments are the edible portion, especially the outer integument which is also called aril in pomegranate. As the ovule develops into seed the integuments mature into seed-coats. During the transformation into seed-coats the integuments undergo significant histological changes.

After fruit set, 10 days after anthesis the seed size of soft seeded varieties Ganesh and Bassein Seedless was less than hard seeded varieties Alandi and Kabul Yellow. But as the seeds grew the seed size of soft seeded varieties increased and the hard seeded varieties especially Kabul Yellow recorded lesser size. During 120 days after fruit set Kabul Yellow recorded very less size when compared to other varieties. This is only because soft seeded varieties will have broader outer integument which is the edible portion in pomegranate and this portion will be less in hard seeded varieties, thereby the seed size will be lesser

The outer integument in pomegranate consists of a single layer of thin walled cells throughout the seed irrespective of specific zones and in this layer the size of the individual cells goes on increasing as the seed was growing. This is happening because of cell enlargement and also filling up of these cells with juice. And it was seen that maximum juice content was observed after 120 days of fruit growth. It was also observed that at initial stages there were plenty of starch granules inside the cells, and as the seeds were growing the starch granules started disappearing and finally the cells were without any starch which was converted into sugars as the fruit was growing.

The inner integument in the beginning, soon after fruit set was with a few layers of small sized cells. The thickness of this layer was maximum at micropylar region followed by chalazal region and the least was at lateral region. As the seeds started growing the thickness of inner integument started increasing with more layers of cells and the average number of cell layers at chalazal region was 15 to 20, at lateral region 13 to 15 and 20 to 25 layers at micropylar region. Lignification of cells in these regions also started early and more intensively in hard seeded varieties than in soft seeded varieties and the maximum number of lignified cells were found in hard seeded varieties only.

At 120 days after fruit set the inner integument of soft seeded varieties Ganesh and Bassein Seedless had first 3 to 4 layers with thin walled bigger cells and the rest of the layers were highly lignified whereas in hard seeded varieties Alandi and Abul Yellow there was no distinction of cell layers. The lignification started just after 1 to 2 layers.

IV. Seasonal changes in fruit quality

1. Total soluble solids (T.S.S.)

T.S.S. increased gradually during fruit development. The increase in T.S.S. from 30 to 120 days respectively in four varieties were Ganesh 3.75 to 8.2; Bassein Seedless 3.5 to 9.8; Alandi 4.0 to 8.4; and Abul Yellow 3.3 to 7.0. Shulman et al. (1984) also reported that the sugar content of the juice measured as TSS increased gradually during fruit development of two pomegranate varieties in Israel. Dash (1983) observed 14.5° Brix in Jyothi variety of pomegranate under GKVK conditions and Shulman et al. (1984) observed 14 to 15% in Mule's Head variety and 13 to 14% in the variety Wonderful in Israel. The higher TSS according to these authors was due to more advanced ripening with prevailing higher temperatures in the valley. Khader et al. (1982) recorded 11.9% TSS in CO.1.pomegranate variety. Sayed et al. (1985) also recorded 9.1 to 15.2% TSS in the variety Yercaud-1 which is a recent release from Tamil Nadu Agricultural University. Arie et al. (1984) reported that in Wonderful variety of pomegranate the TSS was 15% when the fruit appeared to be ripe in terms of quality under Californian conditions.

In the present investigation the TSS was less when compared to other places and varieties. According to Nitsch (1955) in fruit growth studies it will not be possible to follow the chemical changes in the same fruit, but it is necessary to sample periodically a certain number of comparable fruits, and the fruit development also spreads over a considerable period of time during which the weather often changes. Climatic conditions are known to exert a profound influence upon the chemical composition of fruits (water, sugars, ascorbic acid and other organic acids, etc.).

2. Titratable acidity

The titratable acidity of pomegranate juice decreased with maturity. The titratable acidity in the four varieties varied from 0.6 to 0.78. Similar results were reported by Shulman et al. (1984) in two varieties of pomegranate at two places in Israel. They found marked differences in acidity of juice between two places which may be explained by the advanced ripening in warmer valley as well as by the characteristically low level of acid production at high temperatures seen in many fruit species (Chandler, 1957; Kliwer, 1971). Nerd (1965) and Ulrich (1970) showed that the predominant acids in pomegranate juice are malic and citric acids. Decrease in titratable acidity with fruit maturity was also reported by Dash (1983) and Arie et al. (1984) in pomegranate. The results of increased P/T/A ratio with advance in the fruit maturity is in agreement with the results of Salunkhe et al. (1963) in peaches and apricots.

3. Sugars

Reducing and total sugars increased continuously during fruit development and particularly during ripening stage this increase was maximum. It appears that the metabolic activity towards glucose synthesis was more pronounced in pomegranate fruits particularly at maturity stage. Similar observations were reported by Dash (1935), Aric et al. (1934) in pomegranate. Nerd (1965) showed that the sugars in the juice of pomegranate were glucose and fructose.

V. Seasonal changes in Macro and Micro-nutrient contents of fruit and seeds

A. Macronutrients

1. Nitrogen

Nitrogen content in fruits was maximum in the initial stages of fruit growth, but continuously declined till maturity. In fruits the nitrogen content was more in Ganesh and Bassein Seedless at 120 days after fruit set as compared to fruits of Alandi and Kabul Yellow. Similar observations were recorded in sapota fruits by Sulladmath (1975), Lakshminarayana and Subramanyam (1966). They reported a gradual decline in nitrogen till 40 days of fruit growth and then followed by a stationary phase and then rapid decline till maturity. Similar trend in the fluctuation of nitrogen content of fruits was also observed in the present investigations.

Nitrogen content in seeds was more in all the varieties when compared to nitrogen content of fruits. The nitrogen content

declined as the seeds were growing. Fruits are characteristically low in proteins as well as in total nitrogen, compared to seeds, leaves and some other plant parts and tissues (Hansen; 1970).

2. Phosphorus

The phosphorus content of fruits was maximum during initial stages of fruit growth in all the four varieties. The amount rapidly and continuously declined till 80 days after fruit growth and finally the phosphorus contents increased slightly between 80 to 120 days. In case of seeds also the phosphorus content which was maximum at fruit set continued to decline till 80 days and then increased slowly up to 120 days.

In sapota fruits (Sulladmath; 1975) it was observed that the phosphorus content was maximum initially but declined during later period and again increased rapidly as the fruit entered maturity stage. Similar trend was observed in the present investigations also. The phosphorus contents in the initial stages of fruit growth was maximum i.e., 0.194% in Ganesh, 0.175% in Bassein Seedless, 0.213% in Alandi and 0.171% in Kabul Yellow. Then on the amount declined and remained almost constant till 80 days and from 80 to 120 days again there was a slight increase in the content of phosphorus. The amount of phosphorus at 120 days after fruit set was 0.100% in Ganesh 0.144% in Bassein Seedless, 0.106% in Alandi and 0.08% in Kabul Yellow, respectively.

Pull et al. (1964) also reported in litchi that phosphorus content in fruit which was maximum in the beginning of fruit growth, started declining after 60 days and then this amount was almost constant till fruit maturity.

5. Potassium

In all the four varieties of pomelo rate studied the potassium content of fruits was more at 10 days after fruit set. It was 1.722% in Ganesh, 1.690% in Bassein Seedless, 1.806% in Alandi and 1.470% in Kabul Yellow. The contents however started declining slowly as the fruit growth was in progress. This decreasing trend was observed till 60 days in Ganesh and Kabul Yellow and till 80 days in Bassein Seedless and Alandi, then on the contents increased rapidly and finally reached maximum at 120 days. The potassium content of different varieties at 120 days were: Ganesh 1.832%, Bassein Seedless 1.722%, Alandi 1.868% and Kabul Yellow 1.696%.

In litchi fruits also similar observations were recorded by Pull et al. (1964). In their studies it was seen that potassium content was maximum at 30 days after anthesis and it decreased as the fruit was growing till 80 days and again the potassium content went up as the fruit approached maturity and potassium uptake ceased when the fruit attained full maturity.

The rise in sugar content was observed to be closely related to potassium. The potassium is known to be involved in carbohydrate metabolism. In case of potato, pyruvic kinase is known to be activated by potassium (Hason and McElroy, 1965). Thus the direct correlation between potassium content and sugar during

maturity may be attributed to its role in carbohydrate metabolism.

In seeds also potassium content was maximum during 20 days after fruit set and it started declining till 50 days and then again increased slowly till maturity. However, the final potassium content in mature seeds, were less than the amount of potassium which was present at the beginning of fruit growth, in varieties Bassein Seedless and Alandi but the potassium content was almost equal to the amount of potash which was in the beginning of fruit growth in Ganesh and Kabul Yellow varieties.

B. Micronutrients

1. Calcium

The calcium content of fruits in the initial stages of fruit development was higher and then it declined during 20th day and again increased till 50th day and then on the calcium contents continuously increased till maturity. Similar result was observed by Sulladmath (1975) in sapota variety Kalipatti. He observed high calcium content at initial stages of fruit growth, at 9th fortnight the contents declined, again the contents increased rapidly during 11th fortnight and thereafter the content was stationery till 17th fortnight and finally it continuously decreased at maturity. Similar fluctuations in calcium content was observed by Paul et al. (1984) in litchi fruits.

It is believed that calcium is immobile in phloem tissues and that, once delivered to an organ in the transpiration stream this element tends to remain there (Bollard and Butler, 1966). It has already been noted that calcium is present in mature fruits at a level which is frequently much lower than the level of this element in leaves. Similar trend was seen in seed also. Seeds contain a maximum calcium content after fruit set and it was more till 50 days and then declined slightly and remained more or less constant till maturity.

2. Magnesium

The magnesium content in all the four varieties was more during fruit set. It declined slowly till 60 days from thereon it increased continuously till maturity stage. It was 44.0 ppm in Ganesh, 41.0 ppm in Bassein Seedless, 42.0 ppm in Alandi and 43.0 ppm in Kabul Yellow variety.

Similar results were observed by Paull et al. (1984) in litchi fruits. 32 days after anthesis the magnesium content was more (1.09 mg/g dry weight) and it decreased till 60 days and again increased slowly till 80 days and then on the contents rapidly increased till harvest and reached 1.27 mg/g dry weight, at 94 days after anthesis.

In the seeds the magnesium content was maximum at fruit set, the contents were more than the contents of fruits. At maturity the magnesium content became almost half.

3. Iron

The iron content was more at fruit set in all the varieties and it slowly declined till 50 days after fruit set and the contents increased steadily till harvest. At 120 days after fruit set the varieties had maximum amount of iron. Ganesh variety had 180.00 ppm, Paschim Seel had 171.0 ppm, Al di had 165.0 ppm and Mahal Bellow had 164.0 ppm. The results are similar to those reported by Lal and Sircar (1970). They observed in ber that the percentage of iron was more at fruit set and then it declined at 30 days and again increased and remained almost constant. In the beginning and 120 days after fruit set the iron content was maximum i.e., 1.62% then on a gradual fall in the value was observed and at last date of picking only 1.005% of iron was recorded.

Similar observations were made by Sastry (1970) in sapota fruits. He reported that these changes in the amount of mineral elements were due to their involvement in cellular metabolism, chlorophyll biosynthesis and synthesis of enzymes.

The iron content in seed was maximum at fruit set. The contents slowly decreased as the seed was growing and finally at 120 days the contents were almost $\frac{1}{4}$ of the amount which was present at fruit set.

4. Manganese

At fruit set the manganese content was more in all the four varieties. The contents declined till 50 days and again

there was a steady raise till maturity. At maturity the manganese content of fruits were; Ganesh and Bassein Seedless 40.0 ppm, Alandi 43.0 ppm and Kabul Yellow 45.0 ppm.

In seeds it was similar to iron. The manganese contents were highest at fruit set and decreased continuously till maturity. At 120 days after fruit set the manganese contents of seed was: Ganesh 12.0 ppm, Bassein Seedless 11.5 ppm, Aland 8.5 ppm, and Kabul Yellow 9.5 ppm.

5. Zinc

In the fruits the amount of zinc was more at fruit set and it decreased till 60 days and increased again towards maturity. At 10 days after fruit set the amount of zinc was 54.0 ppm in Ganesh, 55.5 ppm in Bassein Seedless, 71.0 ppm in Alandi and 41.5 ppm in Kabul Yellow. At 120 days after fruit set, the amount of zinc was 86.0 ppm in Ganesh, 94.0 ppm in Bassein Seedless, 72.0 ppm in Alandi and 90.0 ppm in Kabul Yellow.

The seeds had maximum zinc content at fruit set and it declined continuously till harvest.

6. Copper

Copper contents in fruits was high at fruit set. It was 19.0 ppm in Ganesh, 17.1 ppm in Bassein Seedless, 18.5 ppm in Alandi and 16.0 ppm in Kabul Yellow. The content declined in the middle and again increased between 60 to 120 days. At 120 days the copper content in four varieties were: Ganesh 26.0 ppm,

Bassein Seedless 25.0 ppm, Alandi 23.0 ppm and Kabul Yellow 20.0 ppm.

In the seeds the copper content was maximum at fruit set and then the amount declined till harvest. At 120 days the copper content of seeds were : Ganesh 19.0 ppm, Bassein Seedless 18.0 ppm, Alandi 12.0 ppm and Kabul Yellow 21.5 ppm.

The changes in zinc and copper content of the developing fleshy edible fruits was first attempted in the study. In the literature information about the changes in the above trace elements during fruit development is not available. Hence no attempt is made to compare these results with other workers.

SUMMARY

SUMMARY

Pomegranate is an important arid and semi-arid fruit which has received very scant attention. There are soft seeded, semi hard seeded and hard seeded varieties in pomegranate. Few improved selections of soft seeded varieties have been released recently. No information is available on the nature, time of initiation and degree of lignification of seed testa which causes hard/soft seededness in pomegranate. Differences, if any, in growth and development of fruit and seed between soft and hard seeded varieties are also not known. Therefore, the present investigations were carried out during 1982, 1983 and 1984 to study,

1. The growth and development of fruits and seeds of soft seeded and hard seeded varieties from fruit set to maturity.
2. The anatomical/histological differences in time and degree of lignification in seeds of both the types in relation to growth and development of fruit.
3. To quantify the differences between soft seeded and hard seeded varieties.
4. To study seasonal changes in fruit quality.
5. To study changes in macro-and micro-nutrients during growth and development of fruits.

The salient findings are presented below:

I. Pattern of fruit growth:

1. Fresh weight of fruits and growth pattern:

The pomegranate fruit grows continuously, from fruit set to harvest with periods of slow growth rate alternated with periods of fast growth rate. The pattern of fruit growth followed a "Simple sigmoid curve" almost approaching a linear relationship.

2. Length and breadth of the fruit:

Length and breadth of fruit increased continuously from fruit set to maturity. The length to breadth ratio was higher during the initial stages of fruit growth but it decreased towards maturity resulting in round fruits.

3. Fresh weight of rind and seeds:

The fresh weight of rind and seeds increased continuously from fruit set till maturity. The seed weight to rind weight ratio increased gradually from fruit set upto 80 days of fruit growth and then declined slightly towards maturity. During most of the stages of fruit development the seeds constituted roughly half of the fruits weight.

4. Dry matter content of fruit and seeds:

The dry matter content of fruits increased continuously till 50 days of fruit growth and then gradually declined towards maturity. This was due to formation of juice in the fleshy

aril portion of seeds. In case of seeds the dry matter content increased continuously from fruit set till maturity.

5. Specific gravity of fruits:

Specific gravity was higher in early stages of fruit development and then gradually declined. At maturity the pomegranate fruits had less than 1 specific gravity.

6. Thickness of rind:

Thickness of rind was maximum at 20 days after fruit set and it declined towards maturity. In general there was no marked difference between soft and hard seeded varieties.

7. Hundred seed weight:

The fresh as well as dry seed weights continuously increased from fruit set till maturity in all the four varieties studied. At maturity Ganesh variety had maximum 100 seed fresh weight followed by Barsein Seedless, Alandi and Kabul Yellow.

In general, there were no marked differences between soft and hard seeded pomegranate varieties with regard to growth and development of fruit and seed.

II. Histological studies

Ten days after fruit set the seed size of soft seeded varieties Ganesh and Barsein Seedless was less as compared to hard seeded varieties Alandi and Kabul Yellow. This trend was reversed with time and growth of fruit. At 120 days after fruit set the seed size (including the aril portion) in soft seeded varieties

was more as compared to hard seeded varieties.

A perusal of some interments revealed that the outer interment was a single layered one with elongated cells which were having thick cell walls as the seed was growing. During initial stages of seed growth the inner integuments in general had 4-5 layers of small cells at chalazal region, 2-3 layers at lateral region and 6-8 layers at micropylar region. As the seed matured the number of layers as well as size of cells of inner interment started increasing, and inner integumental cells started lignifying after 40 days of seed growth. However, lignification was more pronounced in hard seeded varieties. During last days of seed growth both in soft and hard seeded varieties all the cells were heavily lignified except 1-2 layers at the proximal end, and the lignification was more intense in hard seeded varieties.

III. Histochemical changes

As far as protein and nucleic acids are concerned there was not much difference between hard seeded and soft seeded varieties. Little difference was seen in insoluble polysaccharides. In all the four varieties studied it was seen that in the beginning of seed growth the outer interment as well as few proximal layers of inner interment were having large amounts of starch grains. The starch grains started disappearing as the seeds developed and at maturity the starch grains completely disappeared, showing that they were converted into sugars.

IV. Seasonal changes in fruit quality

1. Total soluble solids:

The TSS increased rapidly during fruit development and with advancement of maturity. Bassein Seedless recorded highest TSS and Jabul Yellow variety recorded least.

2. Titratable acidity:

The acid content decreased with maturation of fruit. The pectic/acid ratio also increased as the fruit matured.

3. Sugars:

The pattern of accumulation of sugars was commensurate with that of fruit growth. The sugar accumulation in the fruit was slow and steady during the beginning of fruit growth. Both reducing and total sugars continued to increase during the development of fruit and this increase was very rapid during 100 to 120 day's after fruit set just before harvest. Maximum sugars were recorded in Jabul Yellow followed by Ganesn, Alandi and Bassein Seedless.

V. Changes in mineral constituents:

The nitrogen and phosphorus content of both fruits and seeds was maximum and richest immediately after fruit set (10 days after fruit set) and second high nitrogen and phosphorus content was seen at maturity.

The potassium content of fruits was highest at 10 days and 120 days after fruit set. The seeds contained very high potassium content both immediately after fruit set and also at maturity.

The micronutrient contents i.e., calcium, magnesium, iron, manganese, zinc and copper contents of fruits were high initially after fruit set, then they declined in the intermediate stages and finally reached maximum levels at maturity. However the seeds had maximum micronutrients immediately after fruit set and the contents slowly decreased towards maturity.

REFERENCES

REFERENCES

- Anonymous, 1969. The wealth of India. Raw materials, CSIR
New Delhi, Vol. 8: 317-324.
- A.O.A.C., 1970. Official methods of analysis of the 'Association of official analytical chemists. Washington. 11th Ed.
- _____, 1970, Official methods of analysis of the 'Association of analytical chemists', Washington: 515-516, 13th Ed.
- Arie Ruth Len, Segal, N. and Sylvia Guelfet Reich. 1984. The maturation and ripening of the 'Wonderful' pomegranate. J. Amer. Soc. Hort. Science. 109(6) : 898-902.
- Asaph Goor. 1967. The history of pomegranate in the Holy land. Economic Botany. 21: 215-230.
- Bal, J.S. and Singh P. 1978. a Developmental physiology of ber (Ziziphus mauritiana lam) var. Umran. 1. Physical changes. Indian Food Packer. 32(3) : 59-61.
- _____. 1978. b Developmental Physiology of ber (Ziziphus mauritiana lam) Var. Umran. 2. major chemical changes with reference to total soluble solids, acidity, pH, sugars and starch. Indian food Packer. 32(3) : 62-65.
- _____. 1978. c Developmental Physiology of ber (Ziziphus mauritiana lam) Var. Umran. 3. Minor chemical changes with reference to total Phenolics, ascorbic acid (vit. C) and minerals. Indian food Packer. 32(3): 66-69.

- Bertrand, D.E. and Weaver, R.S. 1972. Effect of exogenous gibberellin application on the endogenous hormone content and development of black Corinth grape. *Vitis.*, 10: 292-97.
- Bhuva, H.P. and Chundawat, B.S. 1978. Flowering and fruit growth in sapota (Acharas sapota L.) Cv. Kalipatti. *udyanika*, Rajasthan. Hort. Society 2(1/2): 54-55.
- Blake, M.A. 1925. Growth of the fruit of the Elberta Peach from blossom bud to maturity. *Proc. Amer. Soc. Hort. Sci.* 22: 29-35.
- Bollard, E.G. 1970. The Physiology and nutrition of developing Fruits. Vol. 1. Ed. Hulme, A.C., Academic Press, London. 387-420.
- Bollard E.G. and Butler., G.W. 1966. Mineral Nutrition of Plants. *A. Rev. PL. Physiol* 17: 77-112.
- Cameron, J.W., Cole, D.Jr. and Naver, E.M. 1960. Fruit size in relation to seed number in Valencia orange and some other citrus varieties. *Proc. Amer. Soc. Hort. Science.* 76: 170-80.
- Cannel, M.G.R., and Huxley, P.A. 1969. Seasonal differences in the pattern of assimilate movement in the branches of Coffea arabica Linn. *Ann. appl. Biol.*, 64: 345-57.

- Chandler, W.H. 1957. The Pomegranate in: Deciduous orchards.
Lea and Febiger. Philadelphia, : 86-95.
- Chapman, H.D. and Pratt, P.F. 1961. Methods of analysis for soils,
Plants and waters. Univ. California, Div. Agric. Sciences.
- Cheema, G.S. Bhat, S.S. and Naik, K.C. 1954. Commercial fruits of
India. Mcmillan and co. Ltd., Madras.
- Connors, C.H. 1919. Growth of fruits of peach. New Jersey Agr.
Exp. Sta. Ann. Report. 40: 82-89.
- Coombe, B.G. 1960. Relationships of growth and development to
changes in sugars, auxins and gibberellins in fruits of
seeded and seedless varieties of Vitis vinifera. Pl. Physiol.,
35: 241-59.
- _____, 1976. The development of fleshy fruits. Annual Review
of Plant Physiol. 27: 207-28.
- Greene, J.C. 1964. Growth substances in fruit setting and develop-
ment. Ann. Rev. Pl. Physiol., 15: 303-32.
- _____, Rebeiz, C.A. and Campbell, R.C. 1961. Gibberellin
induced Parthenocarpy in the J.H. Hale Peach and the portable
cause of button production. Proc. Amer. Soc. Hort. Science.
73: 111-118.

- Dash, Dilip Kumar. 1983. Growth Dynamics, Flowering and fruit development in Pomegranate (Punica granatum L.) selection GNVK-1 and its Propagation by cuttings. Unpublished thesis submitted to the University of Agric. Sciences, Bangalore.
- Davies, C.R., and Wareing, P.F. 1965. Auxin directed Transport of radio Phosphorus in stems. *Planta* (Berl)., 65: 135-56.
- Denne, H.P. 1960. The growth of apple fruitlets and the effect of early Thinning on fruit development. *Ann. Bot.* 24: 397-406.
- *Evreinoff. 1949. The pomegranate fruits d'outre Mer, 4: 161-70.
- Feder, N and O'Brien, T.P. 1968. Plant microtechnique some principles and new methods. *Amer. J. Bot.* 55: 123-142.
- Gardner, V.R., Bradford, F.C. and Hooker, H.D. 1952. The fundamentals of fruit production., McGraw Hill Book Company, Inc. U.S.A.
- Goor, A. and Liberman, J. 1956. The Pomegranate. Bulletin of the ministry of Agriculture. Israel (Hebrew, with English Summary).
- Gulhane, A.R. and Gupta, P.K. 1974. Physico chemical changes in developing Lucknow-49 Guava fruits, Haryana. *J. Hort. Sc.* 3(3/4): 134-39.

- Hansen, Elmer. 1970. The biochemistry of fruits and their products, edited by A.C. Hill, Vol. 1, chap. 6 on Proteins: 147-158 Academic Press, London and New York.
- Hayes, W.B. 1960. Fruit growing in India. Utobistan, Allahabad, India.
- Hittalmani, S.V. and Rao, G.M. 1976. Studies on changes in physical parameters of the developing Kagzi lime (Citrus aurantifolia, S.) fruit. South Indian horticulture, 24(4): 122-26.
- Hotchkiss, R.D., 1943. Microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch Biochem. 16: 131-141.
- Ingrid Roth. 1977. Fruits of Angiosperms. Encyclopedia of plant Anatomy. GEBRÜDER BORNTRAEGER-BERLIN-STUTTGART.
- Iwahori, S., Weaver, R.J. and Pool, R.M. 1966, Gibberellin like activity in berries of seeded and seedless Tokey grapes. Pl. Physiol, 43: 333-37.
- Josan, J.S., Jawanda, J.S, Uppal, D.K. 1979. Studies on floral biology of pomegranate. III. Mode of Pollination, fruit development and fruit cracking. The Punjab Hort. Journal. 19(334): 131-138.

- Kenward, W.C. 1955. Development of the fruit, seed and embryo of the paheri mango. Bot. Gaz., 117: 28-52.
- Khader Abdul Md., J.P.M., Kulasekaran., N. and Muthuswami., S. 1962. Co-1. Ponecrinate- A soft seeded selection. South Indian Horticulture 30(4): 298.
- Hilwer, W.M. 1971. Effect of day temperature and light intensity on concentration of malic and tartaric acids in Vitis vinifera L. grapes. J. Amer. Soc. Hort. Science., 96: 372-7.
- Kozlowski, T.T. 1973. Ed. Water deficits and plant growth. Vol. III. Plant responses and control of water balance, Academic press, New York, U.S.A.
- Lakshminarayana, S. and Subramanyam, H. 1966. Physical, chemical and Physiological changes in sapota fruit (Achras sapota linn) during development and ripening. J. Ed. Sci. and technol, 3: 1-4.

- Lee, S. J., and V. J. ... 1971. A preliminary study of the effect of seed content on fruit growth. *Ann. Entomol. Soc. Amer.*, 4(1): 15-17.
- Lee, S. J., Kim, M. S., and Kim, S. D. 1971. Studies on changes in the concentration of respiratory rate during maturation (2) changes in sugar, organic acid, amino acids and the respiration rate. *J. Korean. Soc. Hort. Sci.*, 15(1): 57-64.
- Leopold, A. C. 1964. Plant growth and development. McGraw-Hill publication in Biological Sciences: 270-81.
- Lilleland, O. 1951. Growth study of the apricot fruit, *Proc. Amer. Soc. Hort. Sci.* 27: 237-245.
- _____, and Lewstone, I. 1934. A growth study of the cherry fruit. *Proc. Amer. Soc. Hort. Sci.* 32: 291-4.

- Luckwill, W.C. 1953. Studies on fruit development in relation to hormone production by seeds in apple fruits. *J. Hort. Sci.*, 31: 14-24.
- Malhotra, V.S., Khajuria, H.N. and Jawanda, J.S. 1953. Studies on physico-chemical characteristics of Pomegranate cultivars. I Physical characters and II chemical characteristics. *The Punjab. Hort. Journal.* 23(3&4): 153-161.
- Mazia, D., Brewer, P.A. and Alfert, M. 1953. The cytochemical staining and measurement of proteins with mercuric bromophenol blue. *Biol. Bull.* 104: 57-67.
- McManus, J.F.A. 1946. The histological demonstration of mucin after periodic acid. *Nature.* 158: 202.
- *Moes, K., Engelbrecht, L. and Kulayeva, O. 1959. über die wirkung des Kinetins auf stickstoffverteilung and Eiweibsynthese in isoljerten Blättern, *Flora (Jena)*., 147: 445-64.
- Muller, K. and Leopold, A.C. 1966. Correlative aging and Transport of ^{32}P in corn under the influence of Kinetin. *Plant (Berl)*., 68: 167-85.
- Murneek, A.E. 1926. Effects of correlation between vegetative and reproductive functions in Tomato (*Lycopersicon esculentum* mill) *Pl. Physiol.* 1: 2-56.
- Nason, A. and McElroy, W.D. 1963. In *plant Physiology*' (F.C. Steward), Volume 3: 451-456, Academic Press, New York.

- *Nerd, A. 1965. Anthocyanins in Pomegranate Juice, M.Sc. Thesis. The Hebrew university, Rehovot, (Hebrew).
- Nitsch, J.C. 1955. Physiology of fruit growth. Ann. Rev. of plant, Physiol. 4: 199-236.
- Nitsch, J.C., Pratt, Nitsch. C. and Shaulis, W.J. 1960. Natural growth substances in concord and concord seedless grapes in relation to berry development. Amer. J. Bot., 47: 566-76.
- Olmo, H.D. 1946. Correlation between seed and berry development in some seeded varieties of Vitis vinifera. Proc. Amer. Soc. Hort. Sci., 48: 291-97.
- Panday, R.M., Rao, M.M. and Singh, R.N. 1973. Biochemical changes in the developing mango fruit. Cv. Dashehari. Progressive Hort. 5(4): 47-59.
- Patil Shamee Kumar. 1983. Studies on growth and fruiting in limoneira lisbon and seville lemons (Citrus limon (Linn) Burmann) unpublished M.Sc (Hort) Thesis submitted to university of Agricultural Sciences, Bangalore.
- Patil, A.V., Rane, D.A and Sanghavi, K.U. 1977. Studies on growth habit of some Russian Pomegranate varieties under Rahuri conditions. Agril. and Agroindustries Journal. 10(6): 9-10.
- _____ and Wavhal, K.N. 1980. Improvement of fruit crops in Maharashtra, Punjab J. Hort. 20(1&2): 2-7.

- Paul Robert, E. Nancy Jung chem and jay deputy. 1984. Litchi growth and compositional changes during fruit development. J. Amer. Soc. Hort. Sci. 109(6): 817-821.
- Purohit, A.G.. 1982. Quantitative methods for estimation of soft seededness of pomegranate (Punica granatum L.) Journal of Maharashtra Agriculture Universities. 11(1): 115.
- _____. 1985. Soft seededness in Pomegranate, Indian Journal of Agricultural Sciences. 55(5): 367-368.
- Rao, M.M. 1973. Studies on the Physiological and biochemical factors associated with development, ripening and senescence of pusa seedless grapes (Vitis vinifera L.) Ph.D. Thesis , I.A.R.I., New Delhi.
- Roy, S.K. And Singh, R.N. 1980. Studies on changes during development and ripening of Bael fruit. Punjab Hort. Journal. 20(3&4); 190-197.
- Ryugo, K. 1962. Accumulation of lignin and the concurrent changes in the apparent density of the cell wall in the peach endocarp. Pro. Amer. Soc. Hort. Sci., 80: 197-203.
- Saavedra, E. 1979. Set and growth of Annona cherimola mill. Fruit obtained by hand pollination and chemical treatments. J. Amer. Soc. Hort. Sci. 104(5): 668-673.
- Sachan, B.P., Pandey, D. and Shanker, G. 1969. Influence of weather^{on} chemical composition of guava fruits var. Allahabad safeda. Punjab Hort. 9: 119.

- Salunkhe, D.K., Deshpande, P.B. and Do. J.Y. 1968. Effects of maturity and storage on physical and biochemical changes in peach and apricot fruits. *J. Hort. Sci.* 43: 255-42.
- Sastry, M.V. 1970. Biochemical studies in the physiology of sapota part III - Minor chemical changes. *Indian food Packer*, 24(5): 20-25.
- Sayed, S., Seemanthini Ramadoss, Nanjan, K., and Muthuswami. 1985. YCD-1 pomegranate. *South Indian Hort.* 33(1): 452.
- Shulman, Y., Fainberstein, L. and Iavee, S. 1934. Pomegranate fruit development and maturation. *J. Hort. Sci.* 59(2): 265-274.
- Siddappa, G.S. 1943. Pomegranate juice. *Ind. Fmg.* 4: 196-8.
- Singh, M.P. 1951. Preliminary growth rate studies of chikoo fruit. *Indian J. Hort.* 8(3):17-21.
- Singh, Ranjeet, 1969. *Fruits*. National Book Trust, India. New Delhi.
- Stembridge, G.E. and Gambrell, C.E. Jr. 1970. Comparative effect of gibberellin and parthenocarpy on the shape and maturation of peaches. *Hort. Science.* 5: 156-58.
- Sulladmth Vijay Kumar. 1975. Studies on fruit growth and development in sapota (*Achras sapota*. L.) Var. Kalipatti. M.Sc(Agri) Thesis, University of Agricultural Sciences, Dharwad.

- Sunderarajan, K.C., Shanmugavelu and Vethuswamy, S. 1968.
Effect of plant growth regulators on custard apple. South
Indian Hort. 16: 63-64.
- Tripathy, R.S., and Ganewar, B.M. 1971. Biochemical changes
as indices of maturity in Guava (Psidium guajava L.)
Progressive Hort. 3: 17.
- Tukey, H.B. 1933. Embryo abortion in early ripening varieties
of Prunus avium. Bot. Gaz. 44: 433-468.
- _____, 1935. Growth of the embryo, seed and pericarp
of the sour cherry (Prunus cerasus) in relation to season
of its ripening. Proc. Amer. Soc. Hort. Sci. 31: 125-144.
- Ulrich, R. 1970. Organic acids in, The biochemistry of fruits
and their products (Hulme, A.C., Ed.) Academic Press, New
York, London, 89-113.
- Westwood, M.N., Batjer, L.P. and Billingsley, H.P. 1967. Cell
size, cell number and fruit density of apples as related
to fruit size, position in cluster and thinning methods.
Proc. Amer. Soc. Hort. Sci. 91: 51-62.
- Williams, M.W., and Stahly, E.A. 1969. Effect of Cytokinins and
Gibberellin on shape of delicious apple fruits. J. Amer.
Soc. Hort. Sci. 94: 17-18.
- Zielinski, Q.B. 1955. Modern systematic Pomology, W.M.C. Brown
Co. Iowa. pp. 296.

* Originals not seen.

APPENDICES

METEOROLOGICAL DATA FOR 1952

Month	Temperature (C°)		Relative Humidity (%)		Rain fall (mm)	Sunshine hours for the whole month
	Mean Minimum	Mean maximum	At 05.00 hrs	At 17.30hrs		
January	14.8	26.9	62.9	43.7	-	283.7
February	17.1	30.7	65.0	29.0	-	262.5
March	20.4	33.6	65.8	51.9	1.1	276.8
April	22.2	34.6	70.0	33.0	16.1	247.1
May	21.9	35.4	75.4	44.5	100.2	223.2
June	24.1	33.5	87.0	63.0	144.1	100.9
July	19.6	28.5	84.0	61.0	46.9	103.7
August	19.6	28.1	84.0	55.0	81.0	120.8
September	17.3	29.1	85.1	55.0	206.5	180.9
October	19.6	29.5	79.0	55.0	139.4	217.8
November	13.4	27.0	84.0	65.0	23.9	172.1
December	15.6	26.4	84.0	49.5	1.2	251.5

METEOROLOGICAL DATA FOR 1983

Month	Temperature (C ^o)		Relative humidity (%)		Rain fall (mm)	Sunshine hours for the whole month
	Mean minimum	Mean maximum	At 05.30 hrs	at 17.30hrs		
January	24.8	29.2	78.0	30.0	0.0	267.6
February	19.0	32.3	70.0	28.0	0.0	240.8
March	21.1	34.5	59.0	22.0	0.0	234.4
April	22.7	35.7	62.0	23.0	0.2	272.0
May	22.0	34.8	75.0	38.0	106.2	252.4
June	20.9	30.1	82.0	55.0	218.4	153.0
July	20.2	28.9	86.0	64.0	64.2	119.9
August	20.0	27.4	92.0	79.0	178.5	92.0
September	19.6	26.7	94.0	77.0	279.8	107.0
October	19.2	23.1	82.0	69.0	55.7	136.5
November	16.3	27.8	70.0	47.0	2.9	244.9
December	17.2	25.4	87.0	65.0	67.8	145.7

INTERNATIONAL DATA FOR 1964

Month	Temperature (C°)		relative humidity (%)		rain fall (mm)	sunshine hours for the whole month
	Mean maximum	Mean minimum	At 8.30hrs	At 17.30hrs		
January	25.7	16.7	34	50	0.7	224.6
February	27.5	17.7	81	50	35.4	133.4
March	30.3	13.7	67	37	91.7	255.7
April	33.0	21.4	74	34	42.7	262.0
May	34.4	22.1	76	41	60.2	275.2
June	23.8	11.7	36	64	25.3	118.1
July	27.5	12.4	39	67	146.0	70.7
August	23.0	19.2	87	59	45.0	144.2
September	28.5	19.2	34	58	243.6	177.8
October	27.5	19.0	30	58	144.8	150.5
November	27.0	17.4	80	54	17.0	190.0
December	27.5	15.3	77	45	10.9	242.2

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