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Observations on Seedlessness, Fruit Development and Cytology of Varieties of Guava

by

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The cultivated guava commonly grown for its edible fruits, exists in varied forms and a number of varieties are known in cultivation (Hayes, 1957; Sham Singh *et al.*, 1963). Diploid, triploid and aneuploid forms of *Psidium guajava* L. have been reported (Atchinson, 1947; Kumar and Ranade, 1952; D'Cruz and Babu Rao, 1962; Majumdar and Singh, 1964), but cytological studies to reveal the relationship between morphological variation, sterility and polyploidy have been meagre. The investigations reported herein were directed towards an examination of these aspects in respect of the local country guavas and commercial varieties.

Materials and Methods: The materials utilised in the study conform to three species, *Psidium guajava* L., *P. Cujavillus* Burm. f. and *P. Cattleianum* Sabine. For cytological studies, the flower buds were fixed in Carnoy's fluid for 24 hours after which they were treated with 1:1 alcohol-conc. HCl. mixture and stored in 70% alcohol. Temporary acetocarmine smears were used for study of meiosis in P.M. cells.

Results and Discussion: *Psidium guajava* L. Thirty accessions of cultivated guavas collected from private gardens in the State were examined for their cytology and seed fertility. Among these eleven were found to be diploids and four triploids. The chromosome associations and levels of pollen and seed fertility were found to be similar to those observed in the accessions studied previously (Raman *et al.*, 1965).

One accession of guava with pyriform fruits originally described by Linnaeus as *P. pyriferum* (Bailey, 1937), was collected from a private garden locally. Although the tree was similar to other less-seeded varieties of the common guava, it differed in having pear-shaped fruits. The fruits were 9.5 cm in length and 19.4 cm in girth and the average number of seeds per fruit was 110. The study of meiosis in P.M. cell squashes revealed it to be a diploid (2n:22), 11 bivalents being formed at diakinesis and metaphase I. Certain abnormalities such as the presence of univalents at diakinesis and metaphase I were noted, due to the precocious disjunction of one to three bivalents. In 50 % of the P.M. cells examined at anaphase I, one to

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three delayed disjunction bridges were observed, but there were no abnormalities in the second division and normal tetrads were formed. The pollen fertility was high (90 %). The plant appears to be a structural hybrid. Such meiotic abnormalities have been recorded in a diploid 'seedless' variety by Seth (1959) but there was low fertility of pollen indicating possible hybrid origin of the material. Another accession called 'apple guava' was also found to be a diploid on examination of meiosis. At diakinesis, the association $10_{II} + 2_{I}$ was frequently met with. A rare chromosomal distribution as 14/8 was noticed at anaphase I.

The accession of guava, collected in the wild at yercaud had large glossy leaves and it flowered very profusely. The fruits were very small and highly seeded. Examination of P.M. cells revealed it to be a diploid (2n=22) with a regular meiosis but the high pollen sterility (60 %) was difficult to be accounted for, in terms of the meiotic behaviour observed. It may be that pollen fertility is differentially hampered due to genic influence. In common horticultural varieties studied earliar (Raman *et al.*, 1965) varying seed content had been observed in fruits and no clear relationship could be drawn between pollen sterility and seed formation in fruits.

The accessions of 'seeded', 'less-seeded' and 'seedless' guavas studied have been found to be distinct for the characteristics, size and shape of fruits, colour of flesh and seededness. It is evident that differences in chromosome number as well as genic dissimilarities contribute to these features. The 'seeded' and 'less-seeded' types are diploids while the 'seedless' ones are triploids. Triploidy has been found to confer enhanced vigour and seedlessness.

Comparison of characteristics of fruits in commercial varieties: For estimating the variation in edible content of fruits in commercial varieties of guava a comparison was made of different varieties (Vide Table I). The relative characteristics are given below: Diploids and triploids do not differ markedly in the entire fruit weight. The variety 'Anakapalle' appeared to be superior among the diploids and the varieties 'Bangalore', 'White Smooth' 'Red-fleshed', 'Benares' and 'Saharanpur seedless' were almost similar but lesser than 'Anakapalle' in this respect. In respect of fruit wall, triploids are significantly superior to diploids. Among the diploid varieties 'Anakapalle' appears to be the best. Triploids have significantly less number of seeds per fruit than diploids. The diploid variety 'White Smooth' had the highest number of seeds. Varieties 'Lucknow-49', 'Anakapalle' and 'Benares Round' had much lower seed set than other varieties. The diploid variety 'Benares Round' had the lowest weight of seeds and placenta, compared to the rest of the diploids. The triploid, 'Nagpur seedless' had much lower weight of seeds and placenta than the diploids.

TABLE 1. *Comparison of characteristics of fruits in commercial varieties of guava (Mean value)*

Variety	Weight of fruit (g)	Weight of fruit wall (g)	Weight of seeds and placenta (g)	Number of seeds per fruit	Weight of fruit. Wt. of seeds +placenta	Seed content percentage of ovules developing into seeds	Pollen sterility (%)
Diploid							
Bangalore	56.5	29.5	24.0	320	2.35		
White smooth	62.0	36.0	25.5	578	2.43	91.30	18.0
Red fleshed	63.0	35.0	28.0	328	2.25	67.17	28.1
Benares	56.5	34.0	22.0	297	2.57	78.50	7.0
Benares Round	43.5	29.0	14.5	161	3.00	62.04	15.0
Saharanpur seedless	59.0	36.0	22.5	258	2.62	65.10	17.6
Hofsi	49.0	29.0	21.0	257	2.33	71.10	9.2
Allahabad	58.0	36.0	21.5	278	2.70	92.20	13.0
Lucknow-49	53.0	27.0	26.5	206	2.00	55.20	14.0
Anakapalle	73.5	43.5	29.5	180	2.49	60.30	18.5
Triploid							
Nagpur seedless	52.0	47.0	5.0	15	10.4	6.77	46.6

The proportion of weight of fruit to weight of seeds and placenta in different varieties was worked out to provide a comparison of edible portion in the fruit. The triploid, as may be expected, is outstanding in this respect. Among the diploids, the varieties 'Benares Round' and 'Allahabad' are superior in having a greater edible portion. Yet, in having a larger fruit with lesser number of seeds than other diploid varieties, 'Anakapalle' will have a greater consumer appeal, though its proportion of fruit weight/seed + placenta weight is less.

Further observations on seed content and pollen sterility in the horticultural varieties revealed no clear relationship between the two features. 'Less-seeded diploids do set seeds but they are pollen fertile. Triploids show high infertility in pollen and abnormally low seed set; the degeneration of ovules was noticed from the 20th day after opening of flower and on an average only 4 % of the ovules developed into seeds. The size of the ovules is almost twice that in diploids and their number is greater in triploids. In case of less-seeded diploids, the percentage of ovules which developed into seeds ranged from 51 to 92 (Table 1 and 2).

TABLE 2. *Number, size and development of ovules in diploid and triploid guavas before and after anthesis and pollination (Mean values in mm)*

	Nagpur seedless (3n)			Red fleshed (2n)		
	No. of ovules	Length Mean/Range	Breadth Mean/Range	No. of ovules	Length Mean/Range	Breadth Mean/Range
On the day of flower opening	610	0.58/0.36 0.72	0.41/0.27–0.54	505	0.27/0.18–0.45	0.21/0.18 0.36
On the 5th day of flower opening	590	0.62/0.36–0.90	0.41/0.27–0.54	479	0.31/0.27–0.45	0.22/0.18–0.36
On the 10th day developing	567	0.75/0.54–0.90	0.44/0.36 0.54	466	0.53/0.36–0.72	0.38/0.27–0.54
Aborted	—	—	—		0.33/0.27–0.45	0.24/0.18–0.27
On the 20th day developing	132	1.37/0.99–1.71	1.06/0.99–1.35	394	0.79/0.54–0.99	0.66/0.54–0.90
Aborted	—	0.58/0.36–0.99	0.39/0.18–0.63		—	—
On the 30th day developing	40	3.91/2.50–4.80	3.10/2.00–3.80	367	1.13/0.90–1.53	0.87/0.63–1.17
Aborted	—	2.40/1.50–3.00	2.00/1.50–2.50		—	—
Mature fruit normal seeds	24	4.10/3.20–5.00	3.30/2.50–3.70	342	3.90/3.20 4.30	3.10/2.20–3.80
Aborted	—	3.10/2.50–3.50	2.50/1.70–3.00		—	—

To study the effect of pollination on fruit development flowers of triploids were emasculated and pollinated with pollen from diploids and flowers of diploids were bagged after emasculation with and without pollination. It was observed that stimulus of pollination was necessary for fruit set in both.

From the observations indicated above, three lines of improvenmet may be suggested : (i) utilisation of less-seeded diploid types in hybridisation. Superior commercial varieties which are less-seeded may be taken up as parents for hybridisation to select out progenies with reduced seed content and heavy production of better quality fruits, (ii) selection between and within triploid varieties. Variation in productivity and fruit type is met with in the triploids examined, pointing to the scope for selection between the types propagated. Emphasis should, therefore, be laid in multiplication programmes, on the choice of the tree from which propagating material is drawn. The lower productivity of the triploids in general, compared to the diploids, would suggest a more careful watch for occurrence of 'sports' with more profuse flowering and fruit setting. (iii) the development of autotetraploids can well be based on superior diploid stocks with the less-seeded nature. It could be

expected that the greater ovule sterility present in such forms may be enhanced and if suitable tetraploid shoots are realised they can be multiplied vegetatively.

P. cujavillus Burm. f. In the three accessions studied, the leaf size is significantly smaller (1.5–3.0" long and 0.5–1.0" broad) than the seeded country types of *P. guajava* L. The fruits are smaller, smooth in outline and are highly seeded. A small shrub yielding fruits rarely larger than a cherry, this is grown as an ornamental plant. The fruits (yellow with white flesh) are considered valuable in preparation of jams and jellies because of their acidity. The chromosome number of this species is also n=11 as in the case of the 'seeded' and 'less-seeded' varieties of guava. Chromosome behaviour at meiosis in P. M. cells was found to be normal. Eleven pairs, two of which were nucleolar were observed and disjunction at anaphase I was 11/11. The pollen diameter (mean of 14.4 μ with a range of 10.65 – 17.75 μ) was lesser compared to other horticultural varieties. The fruit wall was much less developed and the bulk of pulp was constituted by the placenta.

'Fruitless' triploids: An interesting case of guava plants grown in two coffee-estates at Yercaud flowering profusely and setting no fruits, was met with. Observations on meiosis of these plants showed them to be triploids having some deviation in meiotic behaviour from the normal 'seedless' triploids. Meiosis was irregular with univalents and trivalents being formed in addition to bivalents at diakinesis. The maximum chromosome association computed at metaphase I was $5_{III}+6_{II}+6_{I}$. The average association was $3.3_{III}+7.3_{II}+9_{I}$, the range being $1–5_{III}$, $4–11_{II}$ and $2–14_{I}$. Associations as $13_{II}+7_{I}$ and $11_{II}+11_{I}$ were also frequently observed. The separation of chromosomes at anaphase I was unequal. One to five lagging chromosomes were noticed besides division of 1–2 univalents. Groups of chromosomes were found away from the spindle. Bridges and spindle abnormalities were also often noted at anaphase II. In 13% of tetrads micronuclei were observed and 24% of the sporads had only triads of cells. The two 'fruitless' plants were more or less similar in their cytological behaviour. Pollen fertility ranged from 16 to 27%. It is interesting to record that the association of chromosomes in the 'fruitless' triploids is different from the highly seedless fruited triploid previously recorded (Raman *et al.*, 1965) in having a lower frequency of trivalents and with high pollen sterility.

The cause for non set of fruit appears to be due to a genic mechanism which would have originated by mutation, causing early drop of flowers.

Psidium cattleianum Sabine. (Cattley guava, Chinese guava or Strawberry guava) An ornamental small shrubby tree producing fruits which are small and palatable with an agreeable flavour like that of strawberry. The

fruit is considered excellent for making tarts, jelly or jam. Introduced from port Louis in 1908, the tree is a native of South America (Bailey, 1949). Flower buds of the strawberry guava bearing purplish red fruit were collected from a private garden at Yercaud. Cursory examination of meiosis in P.M. cells revealed it to be a high polyploid. At metaphase I, the frequent chromosome association noted was $1_{VI} + 7_{IV} + 3_{III} + 17_{II} + 11_{I}$. A compact metaphase plate was formed in a large number of cases. The nonsynchronisation of bivalents was also noted. Trivalents were also observed with a corresponding number of univalents. The scattered bivalents underwent delayed disjunction, lagged and consequently were eliminated. Late disjunction bridges at anaphase I and precocious disjunction at metaphase I were also observed. A maximum of 11 univalents was seen to undergo disjunction at telophase I. Chromosome extrusion at this stage also occurred. Inspite of the abnormalities, more than 50 per cent of the P.M. cells examined, revealed an equal distribution of 44/44 chromosomes at telophase I. Supernumerary spores or tetrad cells with micronuclei were rarely met with. Pollen fertility was 70 per cent, the mean diameter beirg 26.25μ with a range of 21.35μ. The pollen grains were larger than those of the diploid and triploid guavas.

Summary: A study of ten diploid varieties and one triploid of guava, with regard to the development of fruit wall and seeds, showed that in the latter there is a considerable increase in development of fruit wall and a reduction in number of seeds per fruit. The diploids showed variation in fruit wall development, and in development of placenta. It is evident that differrential development of tissues (ovary wall and placenta) can be attributed to a difference in female sterility is operative. In case of triploids which are highly seedless due to genic sterility super imposed on chromosome sterility. The stimulation in placenta development is retarded as fertilisation fails and there appears to result a greater development of the ovary wall. Examination of horticultural varieties of guava showed no relationship between seededness and pollen fertility. The pollen and ovule fertility appears to be differentially influenced by internal factors. An examination of the ovules at the time of flower opening in triploids and diploids indicated that the former has larger ovules in greater numbers. There was greater abortion of ovules in triploids compared to the diploids. Trials of artificial pollination in guava showed that in both triploids and diploids pollination is necessary for fruit set.

Three lines of improvement of varieties of guava can be indicated as i) hybridisation between less-seeded diploid types, ii) selection between and within triploid varieties and iii) development of autotetraploids of superior diploid varieties.

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Induced Autotetraploidy in *Zinnia linearis*

by

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Artificiat doubling of the chromosome number has been found to be a fruitful approach in breeding new types of ornamental plants. Its value in floriculture has been well recognized and attempts have been made to produce strains of sexually and asexually propagated ornamental plants with reasonable success (Emsweller and Ruttle, 1941; Bali and Tandon, 1956; Jain *et al.*, 1962). Tetraploid snapdragons, zinnias and marigolds have been introduced in garden practice (Elliott, 1958) and are favoured for their large flowers and longer flowering period and other attributes of value in floriculture. Reduced seed fertility in the tetraploids is a drawback to be reckoned with in sexually propagated ornamental annuals, which could be overcome by selection and other breeding techniques. Attempts were made to induce tetraploidy in *Zinnia linearis* and the observations are presented in this paper. This species is a common garden annual of the plains frequently grown for edging and for beds because of its short, compact stature, small leaves, profuse thin-branches and the mass of little, bright-yellow flowers. Two varieties are recognized viz., the yellow and white-flowered types (Bailey, 1957). Forms of this species with larger flowers would be of greater appeal to the floriculturist.

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