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Aneuploidy in Guava

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Abstract. Aneuploids have been identified cytomorphologically in progenies from triploid and diploid-triploid crosses. 30 trisomics, 2 double trisomics, 1 tetrasomic, and 2 higher aneuploids were obtained. Some of the aneuploids were found to be different from those reported earlier and higher aneuploids carried eight extra chromosomes. The plants with one extra chromosome occurred more frequently (67 %) than the other aneuploid types.

The changes in morphological traits such as a reduction in the amount of growth and the size of leaf etc. distinguished aneuploids from diploids. In the higher aneuploids the plant parts were highly exaggerated and pollen sterility was very high. The chromosomal counts in acetocarmine squashes confirmed the presence of extra chromosome(s). Aneuploids particularly trisomics were found to be promising and may lead to the production of commercially viable plants.

Aneuploidy is known for its application in plant breeding and fundamental genetical research in several crop species. The aneuploid of various sorts are obtainable in a plant species which can tolerate the presence of extra chromosomes at diploid level. Trisomics and other aneuploid types thus have been obtained and utilized in many plant species. The best source of obtaining different aneuploids is the progeny of triploid, aneuploids may, however, occasionally occur in the progeny of normal diploids as a result of aberrant meiosis.

The possibility of occurrence of aneuploidy and its usefulness in guava has been explored recently. The tolerance of extra chromosomes in a diploid guava ($2n = 22$) was shown for the first time by D'CRUZ and BABU RAO (1962). This was followed by the production of some aneuploid forms such as trisomics, tetrasomics, and higher aneuploids (MAJUMDER and MUKHERJEE 1970). These reports thus provide an evidence that aneuploidy combined with viability is possible in guava. Its useful exploitation, however, warrants more investigations. In order to gain all the attending advantages offered by

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aneuploidy, it is essential to isolate and identify different aneuploid forms.
In the present experiment some aneuploids were investigated cytomorphologically. The main purpose of this investigation was to create an array of different aneuploid types which may be utilized for further fundamental and applied research. For instance, they may be useful in identifying linkage groups, locating genes on particular chromosomes, and establishing chromosome addition lines. Furthermore, some aneuploids may be used directly as new cultivars or they may be used as one of the parents in breeding improved types.

Material and Methods

The morphological characters of guava (*Psidium guajava* L.) progenies raised from the open pollinated seeds of triploid and from the crosses between triploid and diploid (Seedless × Allahabad Safeda, Seedless × Lucknow-49) were studied during the winter and summer months of 1970. Most of the plants under study were four years old and the rest were two years old. The height of trees was measured and classified as very dwarf (less than 100 cm height), dwarf (100—150 cm height), medium (150—250 cm height), tall (250—400 cm height), and very tall (above 400 cm height). Trees were also classified according to their habit of growth as erect, semi-erect, and spreading on the basis of visual observations.

The growth rate was measured by marking the shoots and measuring at an interval of two months for one year. Five shoots were selected for the purpose at random in each tree. Shoots were measured from the point of mark up to growing point and diameter of shoots was taken simultaneously. The length between internodes was measured. An average rate of growth was calculated for a period of six months. The leaf samples consisting of ten leaves in each sample were taken at random from each tree, discarding diseased and abnormal specimen.

For cytological investigations buds were fixed at 9.30 A.M. to 11.00 A.M. in a modified Carnoy's fluid *i.e.* 6 parts of ethyle alcohol, 3 parts of acetic acid, and 1 part of chloroform to which few drops of saturated solution of ferric chloride had been added. The anthers were squashed the same day, after 4—5 h fixation. The slides were made permanent by sealing them with wax. They were then analyzed for meiotic study and were photographed.

Fertility of pollen grains was determined by squeezing out anthers without much mechanical pressure in a drop of 1 per cent acetocarmine after anthesis. All pollen grains which took up stains and had a regular outline were scored as fertile whereas empty unstained were scored as sterile. By this way the total number of fertile and sterile pollen grains were counted from ten fields of the microscope and percentage of fertility was calculated.

Analysis of variance was worked out for some of the morphological characters studied.

Results

Frequency of Aneuploids

The plants with one extra chromosome occurred more frequently than the plants with two and more than two extra chromosomes (Fig. 1). Of the total 45 plants studied, 10 diploids, 30 trisomics, 2 double trisomics, 1 tetrasomic, and 2 higher aneuploids carrying eight extra chromosomes were obtained.

Morphology of Aneuploids

All the aneuploids showed reduction in their vigour (Table 1) but it was more pronounced in plants which possessed one and two extra chromosomes. On the basis of their height, they were characterized as dwarf and very dwarf except higher aneuploids which were found to be vigrous and tall. A quantitative relationship among different morphological characters presented in Table 2 shows that in plants with two extra chromosomes, the growth rate was only 14.6 [cm per 6 months] as compared to 30.0 in trisomics and 37.5

in diploids. The growth rate in higher aneuploids and in plants with two extra chromosomes was more or less similar. The internode length decreased in plants with one and two extra chromosomes. There was a slight increase in the internode length of higher aneuploids. A reduction in leaf size (16.0 cm^2) took place in plants with one extra chromosome and in plants with two extra chromosomes (12.7 cm^2). There was, however, a gain in leaf size (46.4 cm^2) of higher aneuploids. An increase in leaf thickness was noted in trisomics and in higher aneuploids. Tetrasonics and double trisomics showed a decrease in the thickness of leaf.

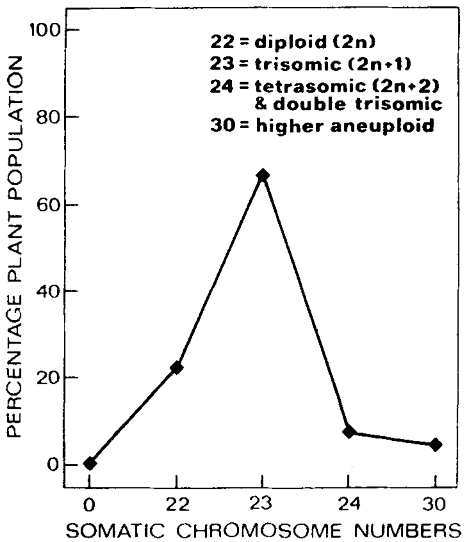

Fig. 1. Frequency of occurrence of different guava aneuploids in a population.
24 = tetrasomic (2n + 2) & double trisomic (2n + 1 + 1)
30 = higher aneuploid (2n + 8)

Pollen sterility was present invariably in all the aneuploids (Table 2). The plants with one and two extra chromosomes showed approximately the same pollen sterility. Higher aneuploids showed the highest pollen sterility (53.1 %).

Cytology of Aneuploids

Table 3 and Figs. 2 and 3 show the distribution of chromosomes at metaphase-I in aneuploids. The usual chromosomal configuration in trisomics were one univalent together with the normal number of bivalents ($11_{II} + 1_{I}$). The frequency of trivalents was very low. The chromosomal association at metaphase-I in plants with two extra chromosomes normally showed the

TABLE 1

DISTINGUISHING MORPHOLOGICAL FEATURES OF DIPLOID AND ANEUPLOID PLANTS

Morphological features	Plant types				
	diploid (2n)	trisomic (2n + 1)	double trisomic (2n + 1 + 1)	tetrasomic (2n + 2)	higher aneuploid (2n + 8)
Stature	tall	dwarf	very dwarf	very dwarf	tall
Habit	spreading	semi-erect	semi-erect	semi-erect	erect
Vigour	vigrous	less vigrous	stunted	stunted	vigrous

TABLE 2

RELATIONSHIP AMONG DIFFERENT MORPHOLOGICAL CHARACTERS OF ANEUPLOIDS AND DIPLOID

S. No.	Type of plant	Somatic chromosome number	Rate of growth [cm per 6 months]	Internode length [cm]	Leaf size [cm²]	Leaf thickness [μm]	Number of stomata/ unit area	Pollen sterility [%]
1.	Diploid	22	37.5	4.5	61.0	123.0	28.0	7.3
2.	Trisomic	23	30.0	3.8	36.0	173.5	25.0	24.7
3.	Tetrasomic or double trisomic	24	14.6	2.5	12.7	154.1	20.0	28.4
4.	Higher aneuploid	30	17.0	3.0	46.4	142.8	24.0	53.1
5.	S.E. ±	—	2.5	0.2	1.6	3.5	1.1	—
6.	C.D. at 5%	—	5.5	0.4	3.5	7.5	2.4	—

formation of one trivalent and one univalent in addition to the usual number of bivalents ($10_{II} + 1_{III} + 1_{I}$). Higher aneuploids formed higher number of multivalents but univalents also appeared occasionally.

The chiasma frequency was found to be correlated with the higher number of univalents per PMC (Table 3). The plants with two extra chromosomes were found to posses higher chiasma frequency per PMC than trisomics. In higher aneuploids the chiasma frequency was found to be still higher than the other aneuploid types.

The distribution of chromosomes at anaphase-I in aneuploids is presented in Table 4 and Figs, 4 and 5. In the trisomics 11—12 distribution of chromosomes was observed frequently. The 11—11 distribution with one laggard also occurred occasionally. The plants with two extra chromosomes usually showed 11—13 distribution of chromosomes at anaphase-I. In the higher aneuploids the distribution of chromosomes at anaphase-I was quite irregular but 16—14 distribution appeared more frequently.

Discussion

The plants obtained from open pollinated seeds of triploid and from crosses between triploid and diploid by MAJUMDER and MUKHERJEE (1970) offered

TABLE 3

FREQUENCY OF UNIVALENTS, TRIVALENTS, MULTIVALENTS, AND CHIASMA IN PLANTS WITH ONE AND MORE EXTRA CHROMOSOMES

Somatic number of chromosomes	Percentages			Chiasmata per PMC	Chiasma per chromosome
	Univalents	Trivalents	Multivalents		
23	72	28	—	17.56	0.90
24	47	47	6	21.66	0.93
30	65	25	10	27.50	0.92

material for the present study. The plants which were not identified earlier were subjected to cytomorphological investigation in this attempt. It resulted in the identification of different aneuploid types, some of which were not recorded earlier. In a total population of aneuploid plants, trisomics were

TABLE 4
ANAPHASE-I DISTRIBUTION OF CHROMOSOMES IN PLANTS WITH ONE AND MORE EXTRA CHROMOSOMES (10 cells observed).

Cell No.	Somatic Chromosome Numbers		
	23	24	30
1	11—12	11—13	17—13
2	11—12	11—13	15—15
3	11—12	11—13	16—14
4	11—12	12—12	18—12
5	11—11*	11—13	17—13
6	11—12	12—12	16—14
7	11—12	11—13	15—15
8	11—12	11—13	16—14
9	11—12	11—13	18—12
10	11—11*	11—12*	16—14

* With one laggard.

found to occur more frequently (67)% than any other forms). This is in agreement with the findings of VOGT and ROWE (1968), who found about 60 % trisomics in 75 % aneuploid progeny in the genus *Solanum*. A possible explanation about the preferential occurrence of the trisomics may be that the plants are able to tolerate the presence of one extra chromosome. The presence of more than one extra chromosomes appears to be deliterious, leading to only few viable plants.

The growth of the plants was affected adversely due to aneuploid condition. The plants were generally less vigrous and showed dwarfing habit (Table 1). In the plants with two extra chromosomes the growth rate was much slower and plants were very dwarf as compared to the other aneuploid types and diploids. The two higher aneuploids were, however, quite vigrous and tall. They almost behaved like triploid plants. These results are in conformity with the findings of MAJUMDER and MUKHERJEE (1970) and support the statement that aneuploids are in general less vigrous than normal plants (ALLARD 1960).

Among various morphological parts, leaves manifested greater degree of influence imposed by extra chromosomes. The leaf sine was reduced in trisomics and the reduction in leaf size was more severe in tetrasonic and double trisomics. On the contrary, there was an increase in the size of leaf of higher aneuploids. The differences in leaf size were also recorded by MAJUMDER and MUKHERJEE (1970) in guava aneuploids. NANDI (1937) and SACHAR *et al.* (1967) found smaller and deeply serrate leaves in jute trisomics.

All the above mentioned morphological abnormalities observed in guava aneuploids are expected because character expression is the matter of a balance between a large number of genes acting in different ways. The addition of extra chromosomes is believed to impair the genic balance and consequently result in the abnormalities of plants. Several investigators (BLAKESLEE 1922, SINNOTT *et al.* 1934, GOODSPEED and AVERY 1939, SAMPSON *et al.* 1961, and CHEN-CHICHANG and GRANT 1968) have used the theory (BRIDGES 1921) of genic balance to explain the morphological pecularities of trisomics and other aneuploid plants. ESEN and SOOST (1972) reported that viability and vigour of aneuploid plants undoubtedly depend on the number of extra or missing chromosomes as well as kinds of chromosomes that are extra or missing and the degree of ploidy at which they occur. Perhaps it may account for the differences observed in morphological characters of different aneuploids in the present study.

The study of chromosomes in all aneuploid plants was confined to the dividing pollen mother cells in anthers. This became inevitable in view of the nonavailability of root tip material. The cytological observations primarily involved the detection of extra chromosomes and the behaviour of extra chromosomes at various phases of meiotic division. The usual chromosomal configuration at metaphase-I in trisomics was a univalent and bivalents ($11_{II} + 1_{I}$). The tetrasomics formed 12 bivalents and thus distinguished from the double trisomics which formed univalent, trivalent and bivalents ($10_{II} +$ $+ 1_{III} + 1_{I}$). The chromosomal association in higher aneuploids was highly irregular, however, formation of a large number of univalents was a common feature. MAJUMDER (1968) in guava aneuploids and SACHAR *et al.* (1967) in jute trisomics found similar chromosomal configurations in metaphase-I. The occurrence of a large number of univalents may probably be due to asynaptic or desynaptic mechanisms.

The chiasma frequency was influenced by the presence of extra chromosomes. The plants with two extra chromosomes were found to possess higher chiasma frequency per PMC than trisomics. Higher aneuploids recorded still higher frequency. ARORA and KHOSHOO (1969) in moss verbena observed significant increase in the chiasma frequency, in all the trisomics in comparision to diploid plants. The increase in the chiasma frequency in aneuploid plants may be explained as due to the formation of trivalents and multivalents.

The distribution of chromosomes in anaphase-I in trisomics was observed to be 11—12. In the double trisomics and tetrasomics the distribution of chromosomes was found to be 11—13. The distribution of chromosomes in anaphase-I was not uniform in higher aneuploids (Table 4). In general in trisomics two of the three chromosomes go to one pole and the other goes to the opposite pole. The univalents are almost invariably included in either pole and rarely form micronuclei. It thus becomes clear that the distribution of chromosomes at anaphase-I in aneuploids depends on the number of extra chromosomes present.

It can be concluded that aneuploids of guava particularly trisomics obtained in the present study will make possible a thorough genetic analysis thereby contributing greatly to the accumulation of knowledge in guava improvement work. Since these aneuploids will not only be very useful tool for extending our knowledge about location of genes, determination of linkage groups, and development of chromosome addition lines but can be used directly as improved line also. The addition of chromosome to diploid number reduced the size and number of seeds. Aneuploids thus can be recommended as one of the important methods for overcoming the problem of seediness in guava and other tree fruits. Trisomics, double trisomics, and tetrasomics exhibited dwarfing habit. These may be used as dwarf rootstocks which are not yet known in guava.

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Figures at the end of the issue.

SHAFAAT MOHAMMED, Odd. zahradnické a ovocnářské technologie, Indický zemědělský výzkumný ústav, New Delhi, India: **Aneuploidie u gvajavy.** — Biol. Plant. **16** : 382—388, 1974.

Cytomorfologickým zjišťováním aneuploidů v potomstvu triploidů a z křížení triploidů a diploidů bylo získáno 30 trisomických rostlin, 2 dvojnásobní trisomici, 2 tetrasomici a 2 vyšší aneuploidi s 8 extrachromosony. Rostliny s jedním extrachromosomem se vyskytovaly častěji (67%) než ostatní aneuploidní typy.

U aneoploidnich rostlin dochází ke změnám morfologických znaků, redukci růstu a velikostí listů atd. se liší od diploidů. U vyšších aneuploidů s 8 extrachromosomy byly naopak rostliny i listy větší a pylová sterilita byla velmi vysoká. Acetokarmínové roztlaky potvrdily přítomnost extrachromosomů. Aneuploidů — zvláště trisomiků — může být využito k produkci komerčně důležitých rostlin.

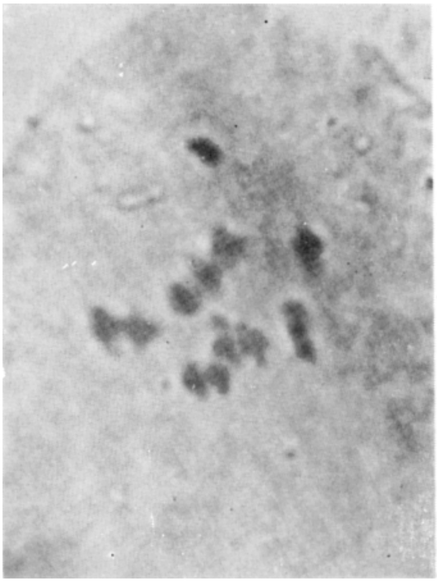

Fig. 2.

Fig. 3.

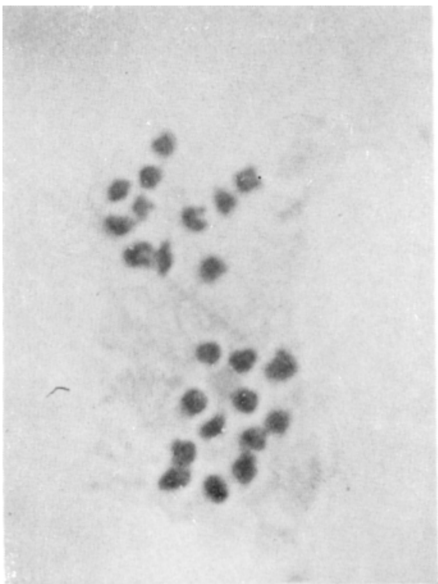

Fig. 4.

Fig. 5.

Figs. 2 and 3. Configuration of chromosome in meiotic metaphase-I of some of the guava aneuploids. — Fig. 2. Trisomic, $2n + 1 = 23$ ($11_{II} + 1_{I}$). × 2425. Fig. 3. Higher aneuploid, $2n + 8 = 30$ ($5_{III} + 5_{II} + 5_{I}$). × 1967.

Figs. 4 and 5. Distribution of chromosomes in meiotic anaphase-I of guava trisomics. — Fig .4. $2n + 1 = 23$ (11—12). × 2425. Fig. 5. $2n + 1 = 23$ (11—11, with one laggard). × 2425.