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Tertiary Trisomics of Pearl Millet (*Pennisetum americanum* (L.) K. Schum): Its Cytomorphology, Fertility and Transmission

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Summary. Nineteen tertiary trisomics were isolated from some translocation heterozygotes and interchange trisomics of pearl millet. Cytological analysis of these trisomics indicates that chromosome association of trivalents, univalents and pentavalents were frequent in all the trisomics. But their ratio varied from one trisomic to the next. Other associations were relatively infrequent. The relative frequencies of 6 pentavalent configurations observed in different trisomics were studied and their probable association with mode of fertility and transmission rates have been discussed.

Key words: Tertiary trisomic – Pentavalents – Pearl millet – Transmission of trisomics

Introduction

As compared to reports available on primary trisomics of pearl millet (Pantulu 1967; Burton and Powell 1968; Gill et al. 1970; Nameeta 1973; Sai Kumar et al. in press), literature on tertiary trisomics is very limited. Recently, Venkateswarlu and Mani (1978) reported two tertiary trisomics in the progeny of triploid and interchange heterozygotes of pearl millet ($2n = 14$).

Tertiary trisomics could be of considerable importance in the genetics and breeding of pearl millet. The usefulness of tertiary trisomics in tomato for determining the position of centromere, orientation of linkage maps and arm location of markers has been amply demonstrated by Khush and Rick (1967). Ramage and Tuleen (1964) proposed the use of balanced tertiary trisomics for maintaining lines of lethal and sterile genes. The concept of hybrid barley production through the use of BTT was given by Ramage (1963, 1965) and a hybrid barley variety was released in the USA (Ramage and Wiebe 1969).

The present paper reports the cytomorphological behaviour, mode of fertility and transmission frequency of 19 tertiary trisomics of pearl millet.

Materials and Methods

Two hundred and ten seeds, obtained from an open pollinated interchange trisomic of pearl millet, were sown in pots during 1979. Out of 210 seeds, 118 seeds germinated and produced flowers. Of these, 37 plants were trisomic and the remaining disomic. Out of these 37 trisomics, 12 plants were tertiary trisomic in nature. Similarly, 7 more tertiary trisomics were isolated in the progeny of 5 different translocation heterozygotes of pearl millet. All these trisomics were tested over three seasons for their morphology, fertility and mode of transmission.

Material for cytological study was fixed in a freshly prepared Carnoy's fluid for 24 hours at room temperature and then stored in 70% alcohol until further study. Anthers were squashed in 2.0% acetocarmine and fresh preparations were used for study of various chromosome configurations and microphotography.

Results and Discussion

In the progeny of an interchange trisomic, random distribution of chromosomes may result in production of 3 kinds of trisomics i.e., interchange, tertiary and primary trisomics in the ratio of 1 : 1 : 1 in addition to normal disomics. Following this, when the 37 trisomics thus obtained in this study were cytologically analysed, 12 plants were tertiary trisomics, as already mentioned 11 plants were interchange trisomics and the remaining 14 plants were primary trisomics. This observed ratio of different trisomics was best fitted to the expected one ($\chi^2 = 0.38$, $p = 0.8$ to 0.9).

Morphology and Fertility

Data on some morphological characters and fertility of 18 of the 19 tertiary trisomics are presented in Table 1.

In general, trisomic plants were slender and weak, with short and narrow leaves and a small earhead having a high range of pollen and ovule sterility as compared to normal disomics. A considerable plant to

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Table 1. Some morphological characters and fertility in tertiary trisomics of pearl millet (*Pennisetum americanum*)

Trisomics No.	Parental source	Plant height (cm)	No. of tillers (no.)	Earhead length (cm)	Pollen fertility (%)	Ovule fertility (%)
TR-11	IT	114.0	14.0	12.0	65.2	70.0
TR-16	IT	82.0	37.0	13.2	25.1	30.0
TR-22	IT	97.0	8.0	12.8	80.7	80.0
TR-23	IT	74.0	3.0	11.5	95.3	90.0
TR-24	IT	74.0	16.0	10.0	58.1	20.0
TR-25	IT	63.5	3.0	14.0	55.4	60.0
TR-58	IT	96.0	3.0	7.7	23.3	80.0
TR-60	IT	47.0	2.0	7.5	6.5	40.0
TR-68	IT	51.0	3.0	8.8	38.9	50.0
TR-71	IT	85.0	18.0	9.0	21.2	30.0
TR-75	IT	72.5	3.0	11.5	6.8	10.0
TR-496	TH 1	77.0	30.0	11.0	11.5	90.0
TR-583	TH 2	96.5	4.0	8.5	37.0	50.0
TR-763	TH 3	77.2	11.0	12.2	41.1	50.0
TR-588	TH 4	106.5	7.0	11.0	70.6	80.0
TR-656	TH 4	76.0	8.0	10.0	2.9	5.0
TR-470	TH 4	93.8	7.0	11.0	12.6	10.0
TR-457	TH 5	94.0	4.0	14.5	15.6	20.0
Normal		131.5	9.0	13.5	95.0	95.0

Note: Data on TR-21 could not be taken. IT = Interchange trisomic; TH = Translocation heterozygote

plant variation was noticed within each trisomic class, with regard to plant height, number of tillers, leaf length and earhead length, which indicates the heterozygous nature of the parental sources of these trisomics.

In the trisomic set derived from interchange trisomic sources, the maximum pollen and ovule fertility was displayed by Tr-23 (95.3% and 90.0%), followed by Tr-22 (80.7% and 80.0%), Tr-58 (23.3% and 80.0%) and Tr-12 (65.2% and 70.0%) in descending order of magnitude. The remaining 7 trisomics showed low degrees of pollen and ovule fertility (Table 1). Consequently, based on these fertility traits and certain morphological characters, the 11 trisomics obtained in this class were classified into two groups. The first group consisted of Tr-12, 22, 23 and 58 while the other group consisted of Tr-16, 24, 25, 60, 68, 71 and 75. Two (groups) are expected in the progeny of an interchange trisomics due to involvement of two types of tertiary chromosomes in each interchange trisomic stock.

Similar plant to plant variation for different attributes has also been noticed in the trisomic stocks derived from interchange heterozygote of pearl millet (Table 1). The maximum ovule fertility was recorded in Tr-496 (90.0%) followed by Tr-588 (80.0%) whereas the lowest fertility was seen in Tr-656.

Cytology

Though morphological data of different trisomics do not have much correspondence with their cytological behaviour and hence grouping of trisomics on morphological basis is of little use in characterization of tertiary trisomics, several kinds of associations were observed at diakinesis and MI. There were univalents, trivalents, pentavalents, chains of four $+1^{I}$, 3^{I} etc. along with certain bivalents. But a complete absence of $\odot_4 + 1^{I}$ in meiotic cells confirms the tertiary trisomic nature of these stocks. Frequency of each type of associations and configurations observed in different trisomics is given in Table 2. A number of tertiary trisomics such as Tr-656, 470, 23, 24, 68 and 71 were associated with a high frequency of cells containing $6^{II} + 1^{III}$ (Fig. 7). The remaining trisomics showed an association of $7^{II} + 1^{I}$/cell (Fig. 8) in a higher percentages of PMC's. In most cases a chain of four $+1^{I}$ could be observed but in a very low frequency.

Other types of configurations were relatively infrequent. A considerable percentage of cells were seen with different configurations of pentavalents in many stocks of tertiary trisomics.

In this study, the average percentage of trivalents, univalents and pentavalents calculated over trisomics was 37.6%, 34.5% and 24.5% respectively. There was not much variation in mean chiasma frequency between trisomics and disomics. However, the average number of chiasmata/cell ranged between 13.94 to 14.89 in tertiary trisomics of pearl millet. Relatively more number of chiasmata were recorded in those trisomics where trivalents and dumble pentavalents were highly frequent.

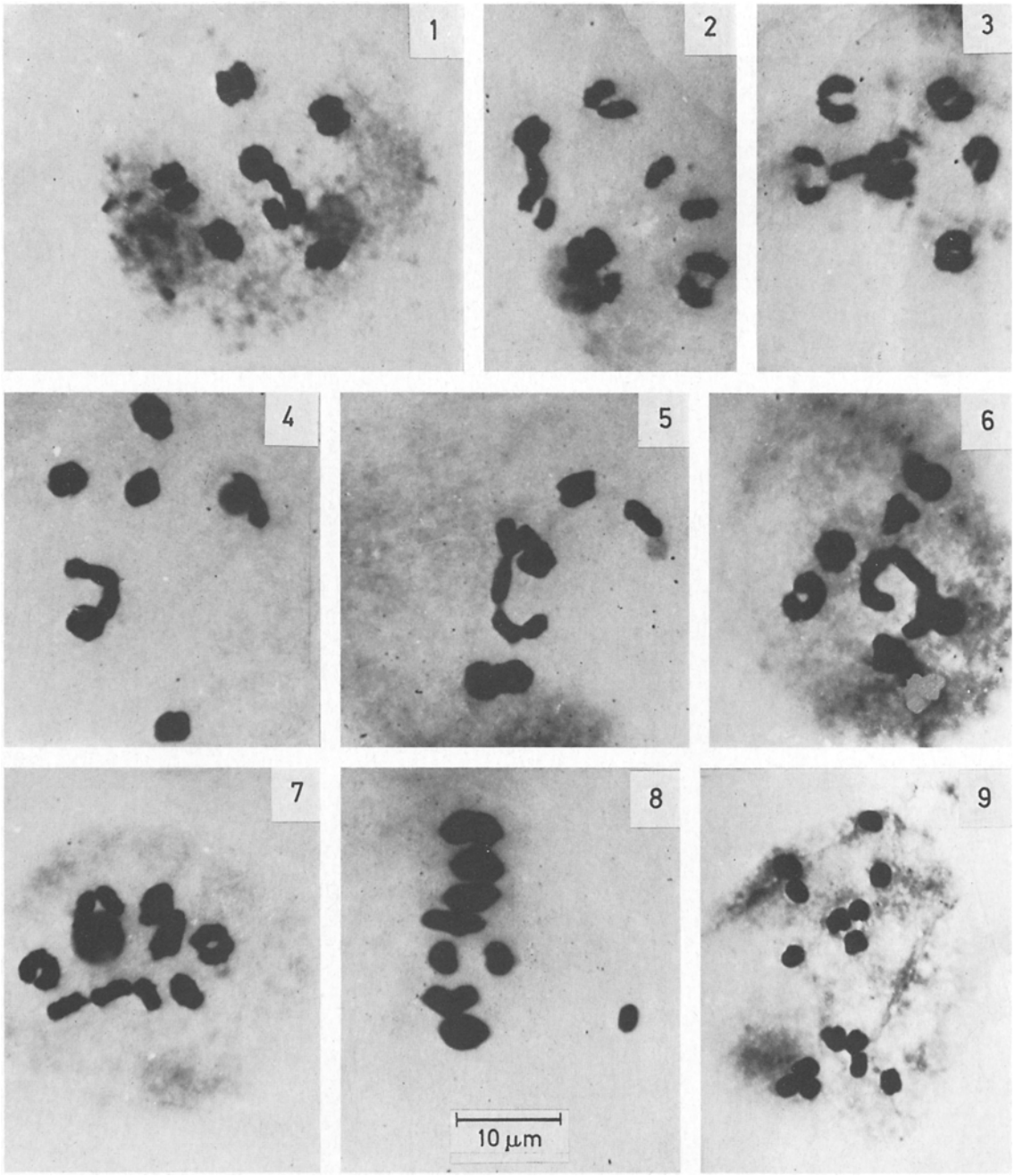
Table 2. Frequency of different chromosome associations in meiosis in different tertiary trisomics of pearl millet (*Pennisetum americanum*). Frequency in per cent

Trisomic No.	No. of cells analysed	$6^{II}+1^{III}$	$7^{II}+1^{I}$	$6^{II}+3^{I}$	$5^{II}+1^{V}$	$5^{II}+1^{IV^a}+1^{I}$
TR-12	1258	35.8	39.0	1.0	23.1	1.3
TR-16	962	30.1	40.5	0.7	27.0	1.6
TR-21	745	34.9	38.9	1.1	22.6	2.6
TR-22	830	34.9	49.3	1.4	12.5	1.7
TR-23	1140	40.4	34.2	1.2	22.1	2.1
TR-24	1874	41.1	32.6	0.7	23.7	1.9
TR-25	1249	38.4	43.2	0.6	16.3	1.4
TR-58	1172	33.3	22.2	0.9	42.3	1.4
TR-60	849	25.9	43.6	0.9	25.7	3.9
TR-68	893	44.8	38.1	0.8	13.9	2.5
TR-71	214	47.7	25.2	3.7	18.7	4.7
TR-75	511	31.3	39.1	2.2	23.5	3.9
TR-496	919	37.0	25.1	3.7	31.0	3.2
TR-583	834	33.6	21.6	1.2	42.0	1.7
TR-763	1702	23.5	32.3	0.6	42.8	0.8
TR-588	890	31.5	36.0	0.9	29.7	2.0
TR-656	613	57.5	13.5	1.8	25.0	2.2
TR-470	1806	53.7	32.7	–	12.2	1.4
TR-457	1912	38.7	48.1	0.3	11.4	1.5
Average percentage		37.6	34.5	1.3	24.5	2.2

[a] Only chain

Table 3. Frequency of different configurations of pentavalents in meiosis in different tertiary trisomics of pearl millet (*Pennisetum americanum*). Frequency in per cent

Trisomic No.	No. of cells analysed	5 chromosomes associations					
		Variants of dumble shape			Frying pan shape	Y-shape	Chain shape
		O—O	>—O	>—<	O- - -	>- - -	- - - - -
TR-12	290	–	1.4	2.1	9.3	5.2	82.1
TR-16	260	3.5	2.7	1.5	24.6	13.1	54.6
TR-21	168	0.6	2.4	1.8	19.1	14.3	61.9
TR-22	104	–	1.9	1.9	8.7	8.7	78.9
TR-23	252	0.8	1.2	2.8	11.1	6.4	77.8
TR-24	444	2.7	1.8	0.9	19.8	9.0	65.8
TR-25	204	–	1.0	1.0	6.9	17.2	74.0
TR-58	496	–	0.4	0.8	6.1	8.5	84.3
TR-60	218	1.8	3.7	2.8	22.9	20.6	48.2
TR-68	124	–	1.6	1.6	18.6	28.2	50.0
TR-71	40	5.0	2.5	2.5	22.5	20.0	47.5
TR-75	120	8.3	5.0	3.3	20.8	19.2	43.3
TR-496	46	–	–	–	16.5	16.4	67.2
TR-583	350	–	0.6	2.3	6.9	8.0	82.3
TR-763	728	–	0.3	0.8	3.4	4.1	91.4
TR-588	264	–	0.8	0.8	3.8	7.6	87.1
TR-656	85	30.6	14.6	10.2	17.2	15.4	12.5
TR-470	220	17.7	8.2	5.9	25.0	19.1	24.1
TR-457	218	3.7	1.8	2.8	13.8	20.6	57.3
Total	4631	74.7	51.7	45.7	276.8	261.5	1190.2
Average	244	3.9	2.7	2.4	14.6	13.8	62.6



Figs. 1–9. Different configurations of pentavalents in meiosis in tertiary trisomics of pearl millet (*Pennisetum americanum*). **1** Diakinesis $5^{II}+1^{V}$, dumble shaped (with both closed ends); **2** Diakinesis $5^{II}+1^{V}$, dumble shaped (with one closed end); **3** Diakinesis $5^{II}+1^{V}$, dumble shaped (with both opened ends); **4** Diakinesis $5^{II}+1^{V}$, pan shaped; **5** Diakinesis $5^{II}+1^{V}$, y-shaped; **6** Diakinesis $5^{II}+1^{V}$, chain shaped (attached to nucleolus); **7** Diakinesis $6^{II}+1^{III}$; **8** Metaphase I $7^{II}+1^{I}$; **9** Anaphase I 8 – 7 separation of chromosomes

Types of Pentavalent Configurations

Table 3 summarizes the observation on frequency of pentavalent variants found in different stocks of tertiary trisomics.

The presence of the specific number and position of the chiasmata and size of chromosomes involved are the deciding factors for a particular shape of pentavalents in tertiary trisomics (Khush 1973). The particular shapes of pentavalents like dumble, 2 modified forms of dumble (Figs. 2, 3) pan-, y- and chain-shapes have been observed in variable frequencies in different stocks of tertiary trisomics.

Among the pentavalent configurations, chain quinquevalent was highly frequent in most of the tertiary stocks ranging from 12.5% in Tr-656 to 91.4% noted in Tr-763. Contrarily among different variants of dumbles, the frequency of closed bivalent dumble (Fig. 1) ranged from zero per cent in many trisomics to 30.6% observed in Tr-656. Two other modified forms of dumbles (Figs. 2, 3) were noted of 1.1% in Tr-763 to a maximum of 24.8% in Tr-656.

Pan shaped pentavalents (Fig. 4) were highly frequent in Tr-470 (25.0%) followed by Tr-16 (24.6%) and Tr-60 (22.9%). Its lowest frequency was noted in Tr-763. Similarly, the y-shaped pentavalents varied from 4.1% to 28.2% (Table 3). The overall average frequency of pentavalents ranged between 2.4% for open bivalent dumble to 62.6% for chain shaped configurations. The maximum total frequency for dumble (Fig. 1) and its modified shapes (Figs. 2, 3) was noted in Tr-656 (55.4%) followed by Tr-470 (31.8%). In contrast, Tr-588 had the minimum frequency of dumbles (1.5%) and a very high frequency of chain quinquevalents (87.1%). All three stocks (Tr-656, 470 and 588) were derived from a single translocation heterozygote. These associations, thus, indicate that in the former two stocks, the extra chromosome was the longer one. The effect of extra chromosome length may be revealed in terms of pollen and ovule sterility since both trisomics also displayed a high degree of pollen (87.0 to 93.0%) and ovule sterility (90.0 to 95.0%). But the opposite is true with Tr-588 (Table 1). Venkateswarlu and Mani (1978) reported one tertiary trisomic (Tr-11) plant forming chain quinquevalents in only 20.0% of PMC's of pearl millet. They also noted a considerable difference with regard to morphological behaviour, pollen fertility and seed setting percentage.

There was a regular distribution of 8 : 7 (Fig. 9) at AI, with 9 : 6 found in rare PMCs.

Transmission

An observation on the rate of transmission and types of progeny produced in different tertiary trisomics when tested over three seasons revealed that there was a considerable variation from one season to other with regard to ratios obtained between normal disomics, primary and tertiary trisomics of pearl millet (Table 4). When data of different stocks over seasons were pooled, it was found that progeny consisted of 91.6% disomics, 5.5% primary trisomics and 2.9% plants with tertiary trisomics. Because of a greater possibility of adjacent segregation noted in chain, pan- and y-shaped pentavalents, the proportion of primary trisomics should be greater in the progeny of tertiary trisomics whereas alternate segregation and a high frequency of

Table 4. Average transmission of trisomics in progeny of different tertiary trisomics

Types of progeny	No. of trisomics				%
	Season 1	Season 2	Season 3	Total	
Primary trisomics	82	26	104	212	5.5
Tertiary trisomics	31	21	56	108	2.9
Disomics	1250	366	1885	3501	91.6
Total	1363	413	2045		

tertiary trisomics are commonly seen in those trisomics which are associated with dumble pentavalents (Khush 1973). Therefore, trisomic 496 produced a higher number of primary trisomics (12.4%) while tertiary trisomics were relatively more frequent (6.1%) in the progeny of trisomic 656 even though the transmission rate of extra chromosomes varied from plant to plant and season to season for the same trisomic. This may be related to sampling variation. Liang (1979) also noted a considerable variation in transmission rate from plant to plant in six sorghum trisomics.

In the progeny of most of the tertiary stocks, the frequency of primary trisomics was greater than that of tertiary trisomics. The possible explanation for this may be attributed to the frequent occurrence of adjacent segregation found in many stocks of pearl millet. Furthermore, it may be noted that the sum of the average frequencies of chain, pan- and y-shaped pentavalents constituted about 90% of the total pentavalent configurations observed in this study (Table 3). Hence the relative frequencies of pentavalent shapes seem to contribute significantly to the mode of transmission in tertiary trisomics of pearl millet. In addition, the different shapes and orientation of trivalents are generally such that both kinds of chromosome segregations (adjacent and alternate) can be almost equally frequent whereas univalents oftenly move to either pole (Fig. 8) and form tertiary gametes. This is in contrast to the behaviour of tomato tertiary trisomics especially 5L.7S, wherein the univalent remains unpaired in 56.1% of PMC's and is lost (Gill 1978).

Lastly, if the relative proportion of univalents, trivalents and pentavalents and their AI orientations are considered, there is a greater possibility of production of tertiary gametes than that of primary gametes. But tertiary chromosomes appear to cause more genetic imbalance which eventually leads to reduced gametic fertility and poor transmission of the same chromosome. Ramage (1965) reports a typical progeny of balanced tertiary trisomics in barley which consti-

tuted about 30.0% balanced tertiary trisomics and 70.0% diploids with less than 1.0% primary trisomics.

Relation of Pentavalent Configurations with Fertility and Transmission Rate

From this and other studies in tomato (Khush and Rick 1967), it is obvious that trisomics involving extra chromosome with two long arms tend to form a high percentage of dumble shaped pentavalents. In contrast, chain shaped configurations are highly frequent in those trisomics associated with a relatively smaller interchange chromosome. For example, dumble shaped pentavalents are almost missing in fertile trisomics-12, 22, 23, 58, 496, 583, 763 and 588 but a higher percentage of PMC's contained chain quinquevalents. On the other hand some trisomics, like Tr-656, 470, 71, 75, 457 and 16, have shown a high frequency of dumbles at the expense of reduced number of chain quinquevalents, and are also associated with low fertility of pollen and poor seed setting.

In general, the correlation of ovule fertility with the frequency of chain pentavalent is positive and highly significant ($r=0.78$, $p<0.001$) whereas it is negative and highly significant with dumble shaped configurations ($r=-0.66$, $p<0.01$). But there was no correlation between univalent frequency and ovule fertility ($r=0.07$). The involvement of a longer extra chromosome in dumble forming tertiary trisomics is the probable cause of reduced fertility and poor rate of transmission. Though no rule can be generalized regarding the relation of pentavalent shapes with transmission rate in pearl millet unless the relative length of extra chromosome involved in different trisomics is known, the negative association of chromosome length with viability of gametes and transmission rate has been reported in trisomics of tomato. The lowest transmission rate of an extra chromosome was reported in those trisomics which involved a longer chromosome of the set (Lesley 1932; Rick and Barton 1954; Khush 1973). Secondly, it is interesting to note that all the trisomics studied in this case had transmission through the female from trisomic × disomic crosses; no trisomic could be isolated in the progeny of the reciprocal cross (disomic × trisomic).

Study of the transmission of an extra chromosome in tertiary trisomics could be useful in both applied and theoretical studies. For example, one of the essential requirements for use of trisomics and genetic male sterility in hybrid production is a low transmission rate of the extra chromosome carrying the male fertile allele through the pollen (Ramage 1965). This report indicates that there is no transmission of extra tertiary chromosome through the male. Since cytoplasmic male sterility has been used exclusively in pearl millet hybrid production and only one type of cytoplasm (Tift-23 A) is being used so far, an alternative approach to using genetic male sterility to produce hybrid should be considered.

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