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Fertility and Meiotic Chromosome Behaviour in Autotetraploid Pearl Millet

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Summary. Tetraploidy was induced in outbred pearl millet and selection for high and low seed set was started in the C_1 generation. Segregation in the C_3 generation was observed for fertility and also for meiotic features: per cent seed set in selfed earhead, chiasma frequency, chromosome association and chromosome distribution in pollen mother cells were all affected. However, variation in seed set was observed even between samples not differing in meiotic features. It is apparent that factors regulating seed set in autotetraploid pearl millet were genic as well as chromosomal.

A high frequency of univalents and trivalents was the main cause of sterility; quadrivalent misdisjunction was not a significant factor. As univalency decreased with increased chiasma formation, the gain was in the form of quadrivalents. However, individuals not differing in chiasma frequency did differ in chromosome association frequencies, indicating that the dependence of chromosome pairing behaviour on chiasmata was subject to genotypic influence.

Key words: Pearl millet – Autotetraploids – Tetraploid seed setting

Introduction

Tetraploids of pearl millet have been observed to have poor to variable seed set (Krishnaswamy et al. 1950; Raman et al. 1962; Gill et al. 1969; Jauhar 1970). Gill et al. (1969) and Jauhar (1970), though they had not consciously selected for fertility, saw a 'diploidizing trend' with fertility improvement; Hanna et al. (1976) also considered multivalents a reason for tetraploid infertility. The suggested inverse relationship between quadrivalents and seed set, though common, was not the rule for all tetraploid populations in some species. In rye and ryegrass tetraploids, where fertility improvement was accompanied by a bivalent increase in some populations, there were certain other populations where univalents not quadrivalents contributed to aneuploid gametes and sterility, while a bivalent increase was no condition for increased seed set (Hazarika and Rees 1967; Simonsen 1973; Hossain and Moore 1975).

Diploid pearl millet can be equated to rye in certain characteristics (Pantulu and Manga 1972; Rees and Jones 1977). Extended to the tetraploid level, this would mean that some tetraploid populations of pearl millet would show no trend towards diploidization. The present experiment begins with inducing tetraploidy in outbred diploid pearl millet for the purpose of studying possible segregation for seed setting and meiotic chromosome behaviour in tetraploid progenies by way of selection for high fertility over 2 generations.

Materials and Methods

Seeds from outbred IP 1475 diploid pearl millet, *Pennisetum typhoides* (Burm.) S. & H., were germinated in petri dishes and 3-day old seedlings of sufficient coleoptile length were collected. Coleoptile tips were cut, the stubs inserted through wire gauze and inverted into dishes of water and 0.075 per cent aqueous colchicine (25 seedlings each for controls and treatment). The roots were thus kept out of contact with colchicine and were kept moist with wet filter paper. Twenty-one hours later, all seedlings were washed in running water and planted in pots. For 2 weeks a daily spray of 50 ppm gibberellic acid was given.

Two of the treated plants were sectorial tetraploids but only one yielded seed. This plant was called C_0 and of its progeny (the C_1 generation), 6 plants were autotetraploids (the C_1 sample). The plants were numbered $C_1 \cdot 1$ to $C_1 \cdot 6$ in the order of decreasing seed set in selfed earheads (Fig. 1).

Three plants ($C_1 \cdot 1$, $C_1 \cdot 2$ and $C_1 \cdot 5$) of the C_1 generation were the progenitors of the experimental samples where inbreeding was continued up to the C_3 generation. These C_3

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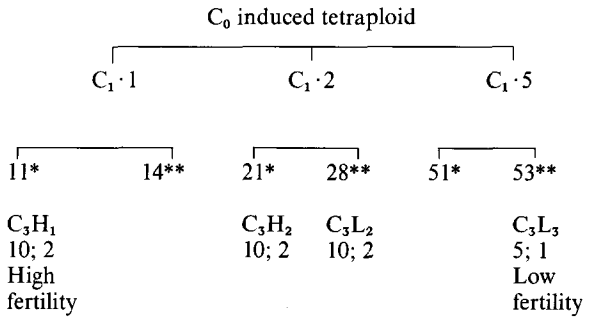


Fig. 1. Selection programme as represented by inbreeding sequence in tetraploids
*, ** among sibs of C_2 generation plants in each line one asterisk denotes the most fertile plant and two asterisks denote the least fertile plant
In the C_3 generation, the number of plants studied and the number of replications are given along with sample names

samples represented selection only for high or low seed set in selfed autotetraploids. One C_4 sample was studied which represented a selection of large seeds at the time of sowing and a selection of vigorous seedlings at the time of transplantation. This sample came from the most fertile C_3 plant in the C_3H_2 sample starting from $C_1 \cdot 2$. In the C_3 generation one other sample was studied which represented no selection. This line was maintained by collecting seed of autotetraploids from open pollinated earheads: the C_3 sample is called C_3OP. The parent line of C_3OP was not grown in isolation – the C_2OP plants were open to pollination from selected lines of the same generation.

Fertility in selfed earheads and chromosome pairing and distribution in the first division of pollen mother cells (PMC) were observed and recorded. All observations were from autotetraploids (4n = 28).

Fertility was recorded as per cent seed setting bisexual florets; 80 to 150 spikelets from each earhead were picked, the number of seeds counted and the percentage calculated. On average, a spikelet had 2 bisexual florets. Because some basal spikelets had 3 bisexual florets, spikelets for counting seed were sampled only above the 5 cm level from the base of an earhead.

Cytological preparations of PMC were made by acetocarmine squashes. Earheads were fixed in 1:3 acetic acid methanol mixture and stored in 70% methanol at low temperature. Chromosome associations were recorded in PMC at diakinesis, usually from 30 cells and not less than 15 cells per plant. Chromosome distribution was recorded in PMC at first anaphase (AI) from 50 cells per plant.

Results and Discussion

a) Selection Response and Lack of Bivalent Increase with Increased Seed Set

The source autotetraploids of the C_1 sample showed much variation in seed set and meiotic chromosome behaviour, and a similar case was found in the C_3OP sample. The mean values of some variables observed in these and 5 selected samples are presented in Table 1, along with the results of the paired 't' test of each of the derived samples with the source sample (*), and also of each derived sample with the unselected sample (†). The table also shows a comparison between the highest and the least fertile C_3 samples.

The C_3OP sample, representing no selection, was similar to the source sample but sharp differences arose in the selection experiment. The general pattern in the comparison of means was that the sample selected for high fertility did have the highest mean per cent seed set and that the low fertile sample contained the lowest seed set (Table 1). The major meiotic difference was that the source and unselected samples had much lower

Table 1. Measures of seed set and meiotic features in different tetraploid samples studied incorporating comparisons of each sample with source (*) and unselected (†) samples plus comparison of highest and least fertile samples

Observations	C_1 source	C_3 OP control	C_3H_1	Comparing H_1 to L_3	C_3L_3	C_3H_2	C_3L_2	C_4
Per cent seed set	27.50 ±5.46	30.83 ±6.64	44.20** ±5.12†	H > L***	12.20* ±2.01†	44.00** ±4.30	21.60 ±2.66	61.20*** ±2.13††
Mean frequency per cell of								
Chiasmata	23.00 ±0.30	23.63 ±0.53	23.67 ±0.44	H > L***	20.62*** ±0.76	23.44 ±0.40	22.48* ±0.23	25.36*** ±0.30
Quadrivalents	3.60 ±0.13	3.67 ±0.19	3.98* ±0.19	H > L**	3.05 ±0.35	3.88 ±0.21	3.40 ±0.09†	4.77*** ±0.12††
Bivalents	5.78 ±0.26	5.79 ±0.21	5.20* ±0.26†	H = L	5.22 ±0.33	5.78 ±31	6.14 ±5.19	3.85*** ±0.19†††
Trivalents	0.41 ±0.08	0.32 ±0.07	0.32 ±0.07	H < L***	1*** ±0.17†††	0.41 ±0.05	0.43 ±0.06	0.33 ±0.07
Univalents	0.79 ±0.12	1.06 ±0.33	0.73 ±0.11	H < L***	2.11** ±0.34†	0.88 ±0.1	0.83 ±0.08	0.44* ±0.07

H = high seed set; L = low seed set; OP = open-pollinated; Asterisks denote comparisons with source sample; crosses denote comparisons with control sample; no mark = P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001

quadrivalent formations than did the C_3H_1 sample. Furthermore, the C_3H_1 sample had the lowest bivalent frequency. Clearly, fertility improvement needed no increase in bivalent formation. Moreover, the least fertile C_3L_3 sample had the highest tri- and univalent frequencies.

The first anaphase (AI) data revealed a greater irregularity in the low fertile sample than in the unselected sample: C_3L_3 had a lower proportion of PMC with equal chromosome distribution ($P<0.05$) and a higher proportion of PMC with laggards ($P<0.001$). The pattern seems related to a reduced chiasma frequency in the C_3L_3 sample ($P<0.01$) in comparison to both the C_1 and C_3OP samples but is not related to any depletion of bivalents (despite fertility differences, the three samples have comparable bivalent frequencies).

The C_3H_1 and C_3L_3 samples did not differ with respect to their mean bivalent frequencies while the higher fertile sample C_3H_1 ($P<0.001$) had greater mean values of chiasmata ($P<0.001$), quadrivalents ($P<0.01$) and equal chromosome distribution at AI ($P<0.01$) as well as lower mean values of univalents and trivalents ($P<0.001$) and laggards in PMC at AI ($P<0.001$). Similar results were obtained in the comparison of a second high fertile sample with its corresponding low fertile sample ($C_3H_2 : C_3L_2$).

b) Seed Set and Meiotic Features – Regressions

Because chiasma formation is a critical factor in deciding the influence of meiotic features on tetraploid fertility (Hazarika and Rees 1967) the relevant regressions were examined (Figs. 2–4). The regression lines between samples were also tested for parallelism in pairs; no deviation from parallelism was found in any of the comparisons. This meant that the magnitude of change in a dependent character attributable to a given amount of change in a 'determinant' character was much the same in different samples over the ranges examined. While the rate of change was similar between samples, it was also true that a similar level of a 'determinant' character was associated with distinctly different levels of the determined character as was observed both in the relationship between fertility and meiosis and that of chromosome pairing and chiasmata. For example, C_3H_2 and C_1 samples hardly differed in meiotic features but differed markedly in seed set. Similarly, C_3L_3 had significantly lower chiasma frequency when compared to C_1 and C_3OP samples, but the 3 samples had much the same quadrivalent and bivalent frequencies. Therefore, pearl millet tetraploids are no exception to the well known generalization that 'the variation (in tetraploid seed set) is due to genic as distinct from chromosomal causes' (Hazarika and Rees 1967).

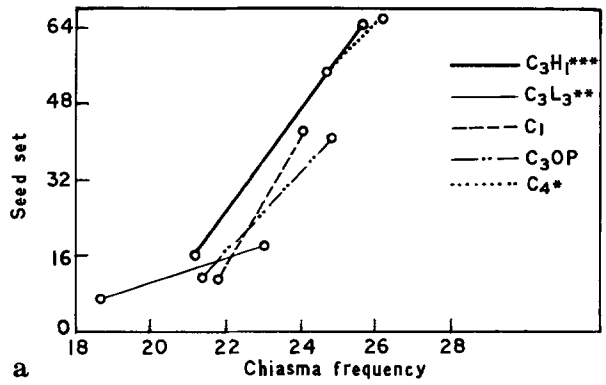
The increased seed set in derived samples over the source sample was accompanied by increased quadrivalent association but this was not to be anticipated from their correlation in the C_1 sample itself because, the only aspect of the seed set to meiosis relationship concerned univalency (Fig. 3). However, the results of the selection experiment would appear indicated when it is noted that in the C_1 sample itself the PMC population as a whole did bear out a significant positive relationship between quadrivalents and chiasmata and significant negative regressions of trivalents and univalents on chiasmata (Hossain and Moore 1975).

The pattern of regressions in source and control samples was similar for the most part but in the high and low fertile C_3H_1 and C_3L_3 samples the pattern differed. Seed setting and chiasmata showed strong association. A negative regression of seed set and cell variance of chiasmata was obtained in the C_3H_1 samples: this was in accordance with the view of Hossain and Moore (1975) that cell variance of chiasmata, being a measure of meiotic irregularity, should vary inversely with seed setting. The regression of seed set on the 14–14 distribution of chromosomes at AI (Fig. 2) was positive in the C_3H_1 samples ($P<0.001$) but insignificant in the C_3L_3 sample, whereas the regression of seed set on laggards at AI was insignificant in C_3H_1 but negative in the C_3L_3 ($P<0.05$) sample. In both samples, regression of the per cent seed set on the quadrivalent frequency was significantly positive while those on bivalent, trivalent and univalent frequencies were significantly negative (Fig. 3).

In general, the noted absence of a relationship between seed set and bivalents and the positive association of seed set to quadrivalents as well as the significant negative regression of seed set on trivalents and univalents were also seen in the C_3H_2 and C_3L_2 samples. In the present inbred autotetraploids of pearl millet, there is no trend towards diploidization. In this respect, note may be made of the breeding pattern in earlier reports. A process of diploidization was envisaged in the outbred tetraploids of pearl millet by Gill et al. (1969) and Jauhar (1970). The induced tetraploid from inbred BIL-4 diploids of Gill et al. showed a higher quadrivalent frequency than the induced tetraploid from T-55 diploids; the inbred BIL-4 originated from outbred T-55 diploids (Minocha et al. 1972).

To the extent that chromosome association frequencies are interdependent, the present tetraploids are similar to the rye tetraploids of Hazarika and Rees (1967). The dependence of chromosome association frequencies on chiasmata also reveals a similarity but the regular appearance of trivalents as well as quadrivalents of types other than chains and rings, are features distinguishing the present tetraploids from rye tetraploids.

Furthermore, numerical non-disjunction (NND) of quadrivalents was rare in rye tetraploids and metaphase I (MI) observations afforded a direct estimate of what Hazarika and Rees (1967) called a 'disjunction index'. It is relevant that most quadrivalents in rye were of the unbranched types and Hazarika and Rees remarked that in species with few and distally localized chiasmata quadrivalents are expected to



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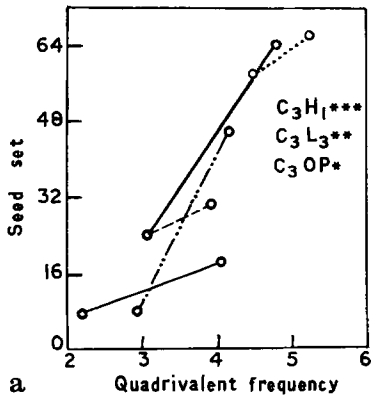

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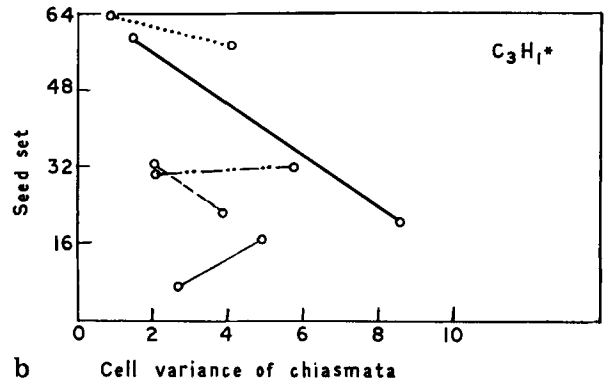

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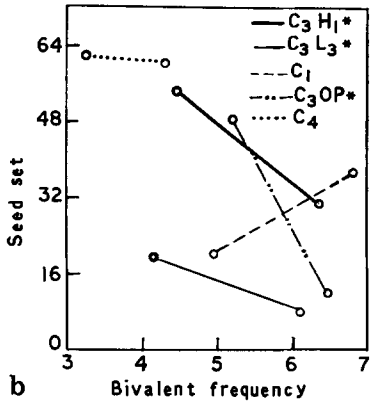

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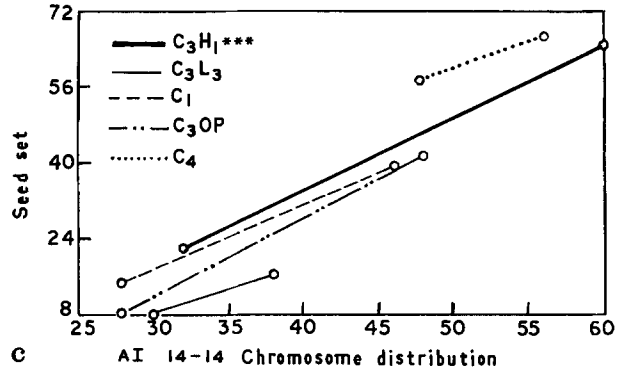

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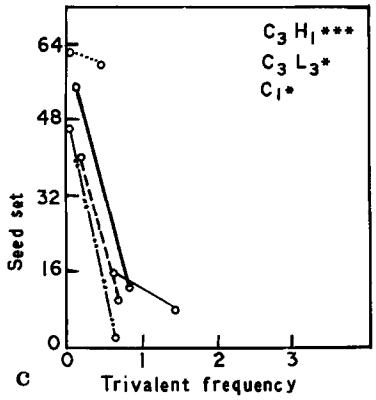

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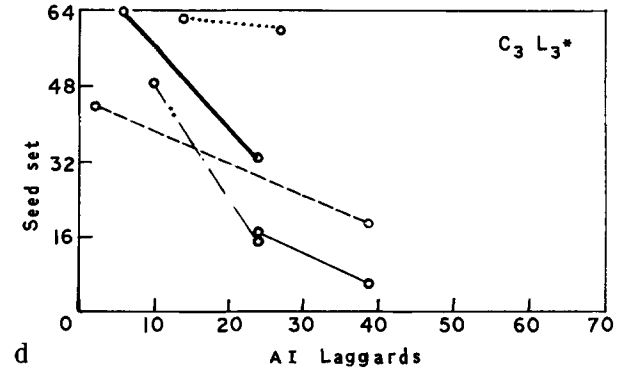

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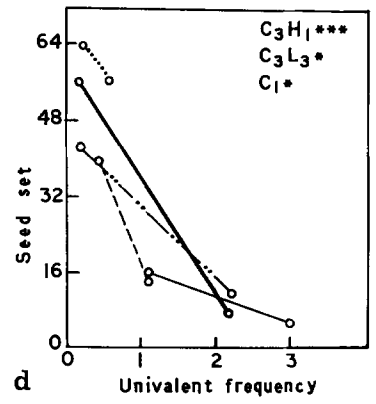

d

Fig. 2a–d

Fig. 3a–d.

Figs. 2a–d and 3a–d. 2a–d Relation of seed set to chiasmata (**a**, **b**) and to the distribution of chromosomes at AI (**c**, **d**). Astericks by sample names indicate significance levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **3a–d** Relation of seed set to chromosome associations. For significance levels, see legend of Fig. 2

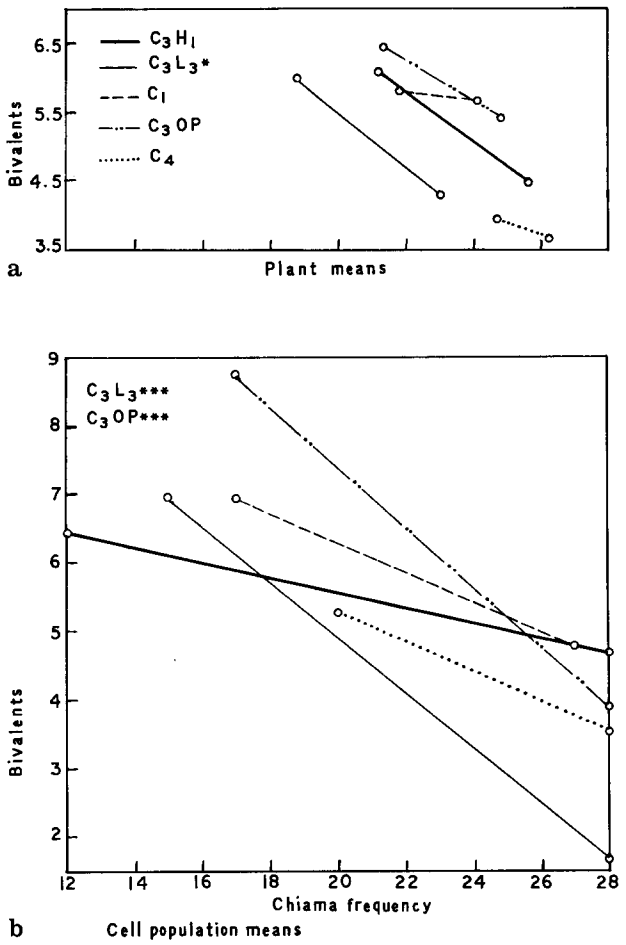

Fig. 4a and **b.** Relation of bivalents to chiasmata. For significance levels, see legend of Fig. 2

disjoin with respectable regularity. In fact, distal localization alongside optimum chiasma formation leaves little scope for trivalents, univalents and branched multivalent configurations (cf. p. 62 Rees and Jones 1977). Pearl millet is different. Although by the end of diakinesis, chiasmata are fully terminalized (Pantulu 1980), multivalents were often of the branched types. Types 8, 9, 10, 12, 13, 14, 15, 16 and 18 of Darlington (1937) were seen. These, as well as rings and chains of NND orientations, were often found at MI.

Although quantification of these observations was not attempted because of clumping on MI plates, there is little doubt that NND was greater in low fertile plants than in high fertile plants. The positive relation between seed set and quadrivalents was more marked in high seed set samples than in lower seed set samples.

That quadrivalents were not the chief reason for gametic aneuploidy is indicated from the consistent positive association between laggards at AI on one hand and trivalents and univalents on the other, as well as from the frequent positive association between quadrivalents and the 14–14 distribution of chromosomes at AI. Yet it is noted that laggards at AI did not always show a clear negative relationship to quadrivalents nor did quadrivalents always relate positively to the 14–14 distribution at AI. However, the AI data may not be read to imply a direct measure of eu/aneuploidy in gametes for two reasons

i) equal chromosome number in AI dyads may sometimes be just numerical and not qualitative – equality might arise from two compensating errors;

ii) where aneuploidy of AI dyads is the result of chromatid separation from a laggard from a single errant unit, say a quadrivalent, the consequent AII products (tetrads) have some chance of including euploid cells provided that the chromatids are not lost nor divide at AII. Loss of laggards or formation of micronuclei were rarely reported in pearl millet and in general a chromosome divides only once during meiosis. Apparently, AI data are of limited value particularly in the absence of MI data.

c) Chiasma Formation and Chromosome Pairing Pattern

The relationship between fertility and meiosis aside, cytological studies of tetraploids have shed some light on genetic control of chiasma formation at one or more levels:

1) mean chiasma frequency per cell

2) concentration of chiasmata to particular sets of homologues, and

3) restriction of chiasmata to separate pairs of chromosomes within a set of homologues (John and Henderson 1962; Timmis and Rees 1971; Verma 1977).

The present inbred tetraploids revealed segregation for chiasma frequencies (Table 1) as would indeed be expected on the basis of diploid studies (Pantulu and Manga 1972). Genotypic influence in the matter of chiasma distribution between and/or within homologous sets in these tetraploids is indicated on two counts.

i) Pairwise comparison of regressions of chromosome association frequencies on chiasma frequency revealed no deviation from parallelism. However, between plants not showing differences in chiasma formation, differences were noted with respect to chromosome association frequencies (in paired 't' tests between individuals within and between samples).

ii) While the regressions of chromosome association frequencies on chiasmata based on plant means were not significant, the same regressions based on cell population means (following Hossain and Moore 1975) were significant in some samples: C_3OP, C_3L_2 and C_4. Whereas the cell population analysis studies the covariation free from genotypic influence the other method does not. The disparate results mentioned (Fig. 4) might then be interpreted as reflecting some genotypic influence over chiasma distribution be-

Table 2. Results of chi-square tests comparing observed frequencies of quadrivalents and bivalents per cell against those expected on the basis of the John and Henderson model

Sample name	No. ofs cells scored[a]	Observed total number of quadrivalents and bivalents		Expected[b] number of quadrivalents and bivalents	Chi-squares D. F. = 1
C_1	71	IV	293	284	0.285^{ns}
		II	266		1.141^{ns}
C_3OP	98	IV	397	392	0.064^{ns}
		II	382		0.255^{ns}
C_3H_1	157	IV	666	628	2.299^{ns}
		II	522		9.198**
C_3L_3	39	IV	188	156	6.564*
		II	92		26.256**
C_3H_2	158	IV	686	632	4.614
		II	524		18.456**
C_3L_2	114	IV	458	456	0.009^{ns}
		II	452		0.035^{ns}
C_4	112	IV	534	448	16.509**
		II	276		66.056**

* $p < 0.05$; ** $p < 0.01$.

[a] Cells scored following Timmis and Rees (1971).

[b] Out of 28 chromosomes per cell, the nucleolar set of chromosomes was held out of consideration. Thus the expected frequency per cell of quadrivalents and bivalents is 4 per cell

tween and/or within homologue-quartets. From the evidence of two statistical tests, however, there was no indication of any specific regulatory mechanism.

Since chromosomes are not identifiable at diakinesis in pearl millet, the question of non-random distribution of chiasmata between homologue-quartets is examined indirectly following the method of McCollum (1958). Non-random chiasma allocation to different sets is indicated when there is no fit between the observed frequency distribution of cells with varying numbers of quadrivalents per cell (0, 1, 2, . . . 7 in pearl millet) and the frequency distribution expected on the basis of binomial expansion. A fit does not constitute proof or randomness. In the present population, 49 out of 51 plants showed good ($P < 0.05$) correspondence between observed and expected frequency distributions.

Timmis and Rees (1971) devised a treatment of the data for verification against the John and Henderson (1962) model for cases when chromosome identification is not possible. The model derives an expected equal ratio of quadrivalents and bivalents under certain conditions. Timmis and Rees (1971) found no fit to the 1 : 1 ratio in rye tetraploids and interpreted the excess of bivalents as arising from pachytene pairing restriction in at least one set of homologues which associated as two separate pairs.

One important consideration in the application of the model is that a given arm forms only one chiasma (such that possible partner exchanges are only in opposite arms for any chromosome). This is not so in all pearl millet chromosomes (two pairing blocks per arm are inferred from the appearance of branched multivalents Pantulu 1968; Srivastava and Balyan 1977) and the model appears unsuitable here because the probability of quadrivalent formation exceeds 0.67 in such sets. To this extent any excess bivalents arising in any of these or other sets through 'pachytene pairing restriction' will have to outnumber 'excess quadrivalents' before more than random bivalent association would become noticeable. Therefore more multivalents than expected overall could mean that unfailing partner exchanges in some sets may have masked the possible existence of 'pachytene pairing restriction' in other quartets. But then, fewer multivalents than expected overall can only point to the existence of a mechanism disallowing partner exchanges.

Results of chi-square tests (Table 2) against a 1 : 1 expectation of bivalents and multivalents in PMC with not more than one rod bivalent revealed occasional deviations only towards quadrivalent excess and not towards excess bivalents in the present samples. Though it seems that some control of chiasma distribution is indicated, its nature is unclear.

Acknowledgements

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