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Cytogenetical studies on autotriploid (2n × 4n) olitorius jute (Corchorus olitorius Linn. strain Chinsurah Green)

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With 24 figures

Introduction

Though artificial triploids in case of sugar beet (Beta vulgaris) have shown a remarkable improvement in root production, the utilization of triploids on a commercial scale involves many practical difficulties, such as low seed production and various technical procedures related to true triploid seedproduction. Despite this difficulties, commercial varieties including triploids are in successful cultivation in Germany, Northern Europe and Japan. Another very notable example of commercial utilization of autotriploids is the well-known triploid water melon. Besides this possible advantages, triploids are often utilized in obtaining specific cytological and genetical information, such as the nature of inter-homologous chromosome pairing and changes of some quantitative traits with the ploidy levels. With a view to get these information on these genetic variants, artificial triploids were raised in olitorius jute species (C. olitorius). Morphological, anatomical and cytogenetical studies of diploids and autotetraploids of the cultivated and the wild types were reported by the present author (1963). Some of these aspects on triploids are presented in this paper.

Material and Methods

Seeds from the population of autotriploids $(2 \text{ n} \times 4 \text{ n})$ olitorius jute were sown in the field. Suitable flower buds were fixed between 10 a.m. and 12 p.m. I.S.T. in acetic-alcohol (1:3) and kept for 24 hours. They were then washed in rectified spirit and stored in 70% alcohol. In order to prepare good well stained smears, it was found necessary to remove the mucilaginous substances from the flower buds. This is done by changing the materials in 70% alcohol every now and then. Temporary smears in acetocarmine and aceto-orcein were made for this study.

Soon after the flowering started, the plants having the same base diameter were harvested for studying the ultimate fibres. Sixth and seventh internodes were cut, bark removed and fixed in FAA. Bark was then thoroughly washed in tap water and finally in distilled water. A few bits of bark was boiled in 1% sodium hydroxide solution for three minutes and then washed in water. After this, the same is

warmed in a solution of bromine, which is prepared by adding 1 c.c. of bromine in 300 c.c. distilled water. Treatments were repeated alternately till maceration was complete, which could be evinced by the non-appearance of pink colour on the transfer of the material to 1% sodium hydroxide solution. Generally four to six repetitions were found to be sufficient to complete maceration. Fibres were then stained in 1% safranin. Length and breadth of 200 hundred ultimate fibres from several groups were measured in microns.

When the plants were at the young pod stage, the plant height was taken in inches and the base diameter was measured by vernier calliper in cms. at the second internode. Plant height and base diameter were found to be positively correlated characters with the yield of fibres in jute (cf. Ghose and Patel, 1945). Number of branches and number of pods were counted and recorded, when the plants stopped their growth and had most of the leaves. Length and breadth of the ripened pods were measured in cms. Length and breadth of leaves and guard cells were taken. Seeds were extracted from fully ripened pods and the number of full seeds were recorded pod-wise.

Observations

a) Morphological and anatomical studies

Height and base diameter of the plants, the number of branches and the number of pods and the size of ultimate fibres are important quantitative factors in determining the yield and quality of fibres in jute, besides having their influence in the production of seeds. Observations are summarized in Table 1.

It is evident from the following table 1 that on an average, plant height, number of branches, size of pods (Fig. 1) and ultimate fibres of autotriploids were found to be practically intermediate between diploids and autotetraploids; however, average base diameter of the autotriploids was slightly more than the autotetraploids. Setting of pods and full seeds per pod in 3 n material was poorer than that in 4 n material. Germination of seeds of 3 n material was recorded to be 19.9% as against 47.0% of the diploids and 23.8% of the tetraploids.

Table 1. Morphological and anatomical observations in diploid, autotriploid and autotetraploid cultivated jute species (Corchorus olitorius Linn. strain C. G.).

Material	Lea	ves	Sto	omata	Po	ds		Number of	Aver	age per plant	Average per fibre		
	Length in cm.	Breadth in cm.	Length in µ	Breadth in µ	Length in cm.	Breadth in cm.	Number of pods	full seeds per pod	Plant height in inches	Base diame- ter in cms.	Bran- ches	Length in μ	Breadth in μ
2 n C. G. 3 n C. G. 4 n C. G.	19.63 20.12 20.34	6.67 11.26 11.85	23.17 35.52 35.58	15.60 16.26 16.67	4.10 2.40 2.90	0.32 0.41 0.46	54.0 4.2 7.4	165.2 5.6 8.3	112.42 103.88 93.63	1.60 1.99 1.93		1989.00 2384.00 2618.00	21.95

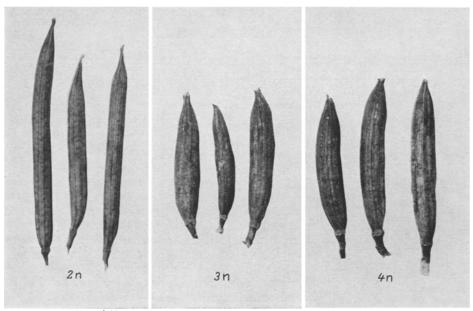


Fig. 1. Photo of Pods of 2 n, 3 n and 4 n jute (olitorius × Nat. Acze).

b) Cytological studies

A great variation in chromosome pairing was observed at diakinesis (Figs. 2-4). Mean number of various associations of chromosomes per p.m.c. in the autotriploids was found to be 2.48 univalents, 4.64 bivalents, 0.58 trivalents, 1.80 quadrivalents, 0.064 hexavalents and 0.064 fragments (vide Table 2). Quadrivalents were commonly noticed in the form of rings and chains. Lesser frequency of trivalents other than bivalents is probably due to the small sizes of chromosomes, which undergo block interference keeping pairing restricted between two homologous points and precluding the chance of 3rd chromosome to pair. The Y-shaped configuration of trivalents was common. Chromosome numbers of seven of 3 n plants were studied; it varied from 20 to 35. The female parent used in the cross previously showed normal meiosis of the diploid (2 n = 14) and occurrence, therefore, of different types of aneuploids in addition to expected triploid (3 n = 21) was naturally due to the functioning of the numerically unbalanced and unreduced gametes from the male parent-autotetraploid.

* range.

Most of the p.m.c.'s have one to three univalents at metaphase I-(Fig. 5). Laggards were commonly noticed during anaphase I and telophase I (Figs.6 to 12), occasionally bridges probably due to stickiness are also observed (Fig. 8). Fig. 6 shows irregular segregation of chromosomes at early AI. In Fig. 7 at late A I two late dividing bivalents are noted on the spindle. Fig. 8 shows at late A I many lagging and dividing chromatin bodies (chromosomal fragments) with cast out chromosomal fragments of different sizes in the cytoplasm. In telophase I

chromosomal bodies that fail to get included in either of the telophasic nuclei remain outside and may form micronuclei (Figs. 10 and 11). In Fig. 10 three chromatin bodies are seen attached to one another by deeply stained chromatin thread. In Fig. 11 one micronucleus has been formed by the side of a telophasic nucleus with a chromatin body lying out in the cytoplasm. Fig. 12 shows the unequal distribution of chromosomes. The analysis of 103 p.m.c.'s of the first anaphase division showed 23 with laggards, 20 with chromatin bodies lying in the cytoplasm and 3 with bridges due to stickiness. At times the lagging chromosomes are seen dividing during anaphase I; the ones that failed to get included in either of the telophasic nuclei formed micronuclei. Ultimate distribution of the chromosomes to two poles is very irregular.

At metaphase II the p.m.c.'s with the elimination of chromatin bodies lying in the cytoplasm is also commonly noted (Fig. 13). In Fig. 14 early A II a small dividing figure is noticed by the side of a spindle and a chromatin body is seen cast out in the cytoplasm. In Fig. 15 late A II chromatin

Table 2. Chromosome number and meiotic pairing in diploids, autotriploids and autotetraploids of C. olitorius strain C.G.

	Somatic	p.m.c.'s	Mean number of chromosome configurations											
Material	number	analysed	I	II	111	IV	_ v	VI	Fragments					
2 n C. G. 3 n C. G.	14 21	120 31	0 2.48 (0-5)*	7 4.64 (2-8)	o o.58 (o-3)	0 1.80 (0-3)	o	0 0.064 (0-1)	0 0.064 (0 1)					
4 n C. G.	28	43	$\begin{vmatrix} 2.34 \\ (0-8) \end{vmatrix}$	7.81 $(2-14)$	0.49 (0-2)	0-6)	0.14 $(0-2)$	0.07 (0-2)	0					

Table 3. Sporad analysis. Number of sporads with varying number of microspores with or without micronuclei.

Material	T		Di	ad			Triad				Γetrad			Pen	tad.	Heptad	Oc	Total	
	Monad		Micro	nuclei		М	icronuc]	lei		Miero	nuclei	Micro- nuclei	Micronuclei		No. of sporads analysed				
		0	1	3	8	0	1	2	0	1	2	3	4	0	1	1	0	1	anarysea
3 n C. G.	3 0.03 (%)	2 0.02	1 0.01	1 0.01	1 0.01	487 6.13	79 0.99	24 0.30	6510 82.03	622 7.83	120 1.51	12 0.15	1 0,01	48 0.60	4 0.05	1 0.01	4 0.05	1 0.01	7936

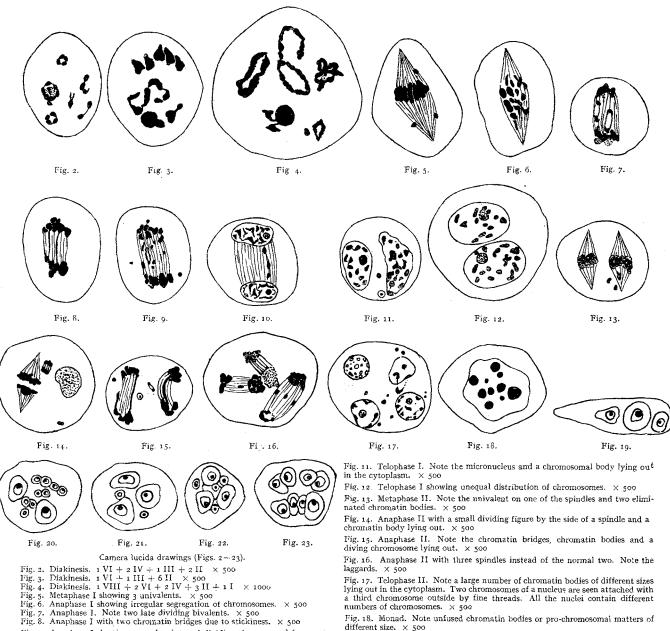


Fig. 9. Anaphase I showing many lagging and dividing chromosomal fragments and two chromosomal fragments of different sizes out in the cytoplasm. \times 500 Fig. 10. Telophase I. Note the chromosomal bodies that failed to get included in either of the telophasic nuclei. These three are attached to one another by deeply stained chromatic matter. \times 500

bridges are also noted in addition to chromatin bodies and a dividing chromosome lying out in the cytoplasm. In Fig. 16 three anaphasic spindles are noted with laggards in two of them. In Fig. 17 T II a large number of chromatin bodies of different sizes are seen scattered in the cytoplasm. Two chromosomes of a nucleus are seen attached with a third chromosome outside by fine threads. Careful examination reveals that all the telophasic nuclei contain different numbers of chromosomes. Number of p.m.c.'s observed with the chromatin bodies lying in the cytoplasm is much more (34.4%) in the second division of meiosis than in the first one (20.3%). These cast out chromatin bodies and chromosomes may form micronuclei.

A large number of sporads was analysed (vide Table 3) for the number of microspores and micronuclei in them. Monads to octads with or without micronuclei are observed (Figs. 18 to 23). One monad

Fig. 16. Anaphase II with three spindles instead of the normal two. Note the laggards. \times 500

Fig. 17. Telophase II. Note a large number of chromatin bodies of different sizes lying out in the cytoplasm. Two chromosomes of a nucleus are seen attached with a third chromosome outside by fine threads. All the nuclei contain different numbers of chromosomes. \times 500

Fig. 18. Monad. Note unfused chromatin bodies or pro-chromosomal matters of

different size. × 500
Fig. 19. Linear triad. × 500
Fig. 20. Diad with 9 micronuclei. × 500
Fig. 21. Triad with 2 micronuclei. × 500
Fig. 22. Tetrad with 3 micronuclei. × 500
Fig. 23. Octad with a micronucleus. × 500

(Fig. 18) with 10 chromatin bodies of different sizes is seen. It may be that in the resting stage of normal tetrads the pro-chromosomes cannot be distinctly visible due possibly to insufficient accumulation of nucleic acid charge on its surface in this species. But in the present case as possibly more than one nucleus is responsible for its origin, the prochromosomal matters are visible due to the fusion of a number of component parts. Though about 82% of the tetrads are normal looking i.e., with four microspores, size of microspores is found to vary in many of them. Occurrence of monads presumably due to the formation of restitution nuclei in the first division and its failure to divide in the second division of meiosis seems to be responsible for the production of pentaploid plants in the 2 n × 4 n population.

Percentage of stained grains in autotriploid olitorius (strain C. G.) is practically the same (a little over 63%) as that in autotetraploid *olitorius* (strain C. G.) (vide Table 4); however sizes of pollen grains vary much. Pollen germination in 4% sucrose agar gelatin shows 14.5% germination. Pollen appearance and germination are comparatively shown in Table 4.

Diameters of 200 stained grains are measured in microns at 12 x \times 44. Measurements are summarized in Table 5.

Table 4. Pollen appearance and germination.

Material	Stained	Non-Stained	Total No. of pollen grains	% Stained	% Non-Stained	% Germination
2 n C. G.	2117	39	2156	98.2	1.8	85.0
3 n C. G.	2836	1625	4461	63.5	36.5	14.5
4 n C. G.	747	415	1161	64.5	35.7	8.1

larger and broader than 2 n, the nature of relationship between the number of fibres and their size and the quality ratio are still to be worked out.

Though pod-setting and seed-setting is poorer, the 3 n material is not completely sterile as is usually found in many triploids (cf. Eigsti, 1957; Sen and Vidyabhusan, 1960). Unless a significant improvement is effected with reference to this character, its

utility becomes severely limited. A 3 n plant may produce n and $n \pm any$ number of chromosomes in gametes and 2 n and 2 n \pm any number of chromosomes in the somatic tissues. Triploid breeding has better future in case of vegetative propagation and in

Table 5. Frequency distribution of pollen grain diameters in diploid, autotriploid and autotetraploid. n=200Measurements in microns.

Material	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
2 n C. G. 3 n C. G. 4 n C. G.	1	1	2	7	6		8	18	20	12	16		18 8											3	0	- 2	o	1

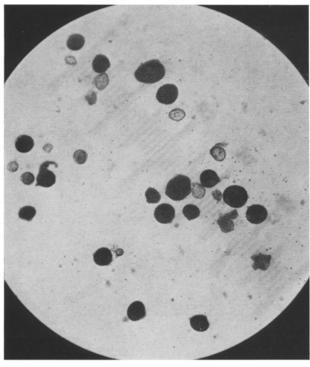


Fig. 24. Photo of different sizes of Pollen.

Discussion

Plant height in 3 n population of olitorius jute is less than 2 n but more than 4 n but base diameter is more than 2 n and 4 n. Though more branchy than 2 n, greater base diameter in 3 n may be advantageously utilized in jute breeding (cf. Ghose and Patel, 1945). Growth is slower in autotriploid jute, though in triploid ash and poplar, Elliot (1958) found them to grow twice as faster as the diploids. Lamm (1944) observed 3 n rye plant more vigorous morphologically.

Pod setting in 3 n is much less than in 4 n. 3 n pod length is also less than that of 4 n but in breadth it is more than 2 n but less than 4 n. Size of the ultimate fibre is also less than that of 4 n. Though

case of recurrent triploids produced out of stocks 4 n and 2 n (e.g., 3 n water melon of Kihara). Vigorous and persistent selection should be made by raising a large population. Nowadays vegetative propagation in jute is resorted to. In order to multiply and to get more 3 n seeds as well as fibres, this method should be taken up and tried.

The idea among many persists that triploids are sterile due to numerical unbalance of chromosomes but careful examination of literature reveals that such is not the case. Progeny has been found in triploids as in jute. Chin (1942–43) recorded seed-setting in $4n \times 2n$ plant but not in $2n \times 4n$ owing to the failure of pollen tube growth. But success in jute is in reverse direction (i.e. $2n \times 4n$) and further details are to be worked out and cause of failure in seed-setting in $4n \times 2n$ cross is to be observed.

Length and breadth of leaves increased in autotriploid jute, when compared to its diploid. Johnsson (1940) observed extremely large leaves in triploid aspen. Stomatal size is also larger in breadth and length. MÜNTZING (1936), TOMETROP (1937) and BERGSTRÖM (1940) also recorded that triploid aspens had longer stomata.

Formation of full seeds is much less in 3 n than in 4 n and too much less than in 2 n. Pandey (1955) stated that in the reciprocal crosses of 2 n and 4 n, the rate of endosperm development is much slower than that of the embryo. Within six to eight days after pollination the whole endosperm is consumed by the fast growing embryo. There is, however, no conspicuous qualitative disturbance in endosperm development, which mostly shows normal nuclei. The disintegration of the embryo starts from the suspensor and proceeds to the main embryo which is affected at last.

Pollen fertility (potentially viable stained pollen — 63.5%) percentage is almost like that of 4 n but very much less than that of 2 n. This sterility may be due to so many cytological irregularities observed in these triploids. MUNTZING and PRAKKEN (1941)

recorded surprisingly good pollen in triploid rye plant. They stated that since most of the pollen grains will receive unbalanced chromosome complements, a considerable and visible degree of sterility should be expected, if the partial sterility in the ordinary population is haplontic and due to structural or numerical disturbances. They added that it is possible, however, that intra-chromosomal structural disturbances may have a more drastic effect than an inter-chromosomal lack of balance. Even if the sterility in rye population should turn out to be diplontic to a rather high extent, this does not exclude a casual connection between sterility and chromosomal disturbances. As a working hypothesis they suggested as follows:

The idea of a normal standard set of chromosomes in rye (and other allogamic organisms) may imply a rather rough schematizing. More probably there is no definite standard type of chromosome structure and definite deviation from this type. On the contrary, the chromosome complements received by the gametes after meiosis are probably rather frequently different in several structural details. Sometimes the differences carried out by two gametes forming individual are great enough to give rise to multiple associations or other conspicuous deviations. In most cases, however, the differences are too slight to influence the chromosome pairing observed to the M I. If really the chromosome complements differ in this way, containing chromosomes that are incompletely homologous, they should also influence the diplophase. Then it will often happen that the rye plants are quantitatively disturbed with respect to chromatin imbalance, i.e. they will represent more or less pronounced deficiencies and duplications. This will certainly influence vitality as well as fertility. Disturbances of this kind as well as the formation of special lethal categories of gametes must lead to sterility and partial sterility is, indeed, a phenomenon, which seems to characterize allogamous in contrast to autogamous diploid plant species (MUNTZING, 1938). MÜNTZING (1938) and MÜNTZING and PRAK-KEN's (1941) contention support the occurrence of minute structural alteration in triploid rye leading to sterility. It appears that this phenomenon does not happen in case of 3 n jute which is mainly autogamous (self-pollinated) species. The accumulation of the elements, so to say, to cause structural alterations with the change of ploidy level unexposed to selective force is quite evident from the observations of fragments, chromatin bridges, quadrivalents and pentavalents, which follow as a result. Thus the theory is supported in part by the actual occurrence of this sterility but also by the demonstration of chromosomal aberrations. Evidence of a similar kind was already discussed by MÜNTZING (1939). He (1933) observed percentage of good pollen in 3 n potato to vary from 13 to 37. In triploid tomato LESLEY (1926) counted 13% good pollen and Jorgensen (1928) recorded 14%. LAMM (1944) observed high percentage of pollen to be "good" but still "good" pollen was not functional, when the triploid was used as the male parent in crosses.

In autotriploid rice and its progeny, KARIBASAPPA (1961) recorded frequency of trivalents as 9.4 per cell. He, however, recorded 12 trivalents, as expected,

in 6 cells out of 50. Price and Ross (1957) recorded 10 trivalents as the maximum number in 3 n Sorghum vulgare. Müntzing (1936) showed in 3 n male aspen the presence of a varying number of trivalents and univalents, in addition to bivalents. Lamm (1944) found a twin rye plant — one 2 n (2 n = 14) and another 3 n (3 n = 21). Morphologically 3 n was slightly more vigorous and number of trivalents per cell varied from 0 to 6, the mean being 3.75 ± 0.36 . MÜNTZING and PRAKKEN (1941) recorded that the number of trivalents varied between 3 and 7 per cell, the mean number per cell is 4.9. Lesley (1926) found 12 trivalents in tomato and she recorded univalents to be dividing. UPCOTT (1935) found frequent occurrence of trivalents and a high percentage of univalents and observed that chiasma frequency is less in 3 n than in 4 n. This would lead to reduce the number of multivalents per cell in the 3 n. She could not find more than 7 III's, though LESLEY and Lesley (1929) found more. Most likely chiasma frequencies in those plants were higher due to genotypic differences or environmental conditions. Chand-LER, PORTERFIELD and STOUT (1937) observed in 3 n Lilium tigrinum trivalents ranging from 7-12. MÜNTZING (1933) observed 11 trivalents in 3 n potato and the range of univalents varied. He stated that the high proportions of chromosomes are united as trivalents indicating autotriploidy. DARLINGTON and MOFFETT (1930) found a maximum association of nine chromosomes in triploid Pyrus malus. In Festuca ovina group (Scottish Plant Breeding Station Report, 1959), the triploids form an average of about 4 III's per cell (maximum being 7 III's). RAMANUJAM (1938) discussed many references showing varying degrees of associations in autotriploids. It is now well known that the relative frequency of trivalents, bivalents and univalents is dependent on the frequency and nature of chiasmata. Plants in which chiasmata are frequent and interstitial will show more trivalents than plants with a low number of terminal chiasmata and within the same chromosome complement the small chromosomes will show a lower frequency of trivalents than the longer chromosomes. In jute plants chromosomes are very small and consequently the frequency is less but details are to be worked out to get a thorough idea of its nature.

Chromosome number has been found to be varied in the population of 2 n × 4 n jute. As regards this point Jones and Bamford (1942) had shown that the triploids contributed nearly every chromosome complement from 18 to 60. Rybin (1930) observed that chromosome number varied from 15 to 20 in triploids and that chromosomes may be eliminated. Sato (1937) observed in the progeny of triploid Lilium that somatic numbers varied markedly between 34 and 39 except 31 and karyotypes were not uniform and fragments were present. Varying number of fragments has been observed also in autotriploid jute species (olitorius).

Further work is in progress.

Summary

1. Height, base diameter, the number of branches, the number of pods, size of ultimate fibres, leaf measurements, pod measurements, measurements of guard cells, number of full seeds per pod in case of autotriploid jute were noted and compared with diploid and autotetraploid jute.

- 2. Mean numbers of chromosome associations per p.m.c. in 3 n jute show 2.48 univalents, 4.64 bivalents, 0.58 trivalents, 1.80 quadrivalents, 0.064 hexavalents and 0.064 fragments. Quadrivalents are noticed in the forms of ring and chain. Y-shaped trivalent is common. Chromosome numbers varied.
- 3. Laggards, occasional bridges due to stickiness, irregular segregation, late dividing bivalents etc. are noted. Elimination of chromatin bodies is observed.
- 4. Monads to octads with or without micronuclei are recorded.
- 5. Fertility percentage in 3 n (63.5%) is compared with 2 n and 4 n. Pollen diameters of 3 n is also compared with those of 2 n and 4 n.

Zusammenfassung

1. An Autotriploiden einer Juteart (Corchorus olitorius Linn., Linie Chinsurah Green) wurden die Merkmale Pflanzenhöhe, Basisdurchmesser, Verzweigung, Anzahl der Kapseln, Länge der letzten Faser, Blattumfang, Umfang der Kapseln, Ausmaß der Stomata und Zahl der Samen je Kapsel untersucht und mit denen der diploiden und autotetraploiden Typen verglichen.

2. Durchschnittlich wurden folgende Chromosomenkonfigurationen in den Pollenmutterzellen der Autotriploiden gefunden: 2,48 Univalente, 4,64 Bivalente, 0,58 Trivalente, 1,80 Quadrivalente, 0,064 Hexavalente und 0,064 Fragmente. Die Quadrivalente zeigten meist Ring- oder Kettenform. Y-förmige Trivalente traten häufig auf. Die Chromosomenzahl

variierte.

- 3. Außerdem wurden "laggards" als Folge gelegentlicher Bildung von Verklebungsbrücken, unregelmäßiger Verteilung der Chromosomen und später Trennung von Bivalenten usw. festgestellt, ebenso eine Elimination von Chromatinkörperchen.
- 4. Nach Abschluß der Meiose traten Monaden bis Oktaden mit und ohne Mikronuclei auf.
- 5. Schließlich wurde die Fertilität (63.5%) und der Pollendurchmesser der 3 n- mit den 2 n- und 4 n-Formen verglichen.

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KURZE MITTEILUNG

Tagungen der Arbeitsgemeinschaft Getreideforschung 1965

Die Arbeitsgemeinschaft Getreideforschung beabsichtigt, im Jahre 1965 folgende Tagungen durchzuführen:

1. Stärke-Tagung 28. – 30. 4. 1965

2. Getreidechemiker-Tagung 1.— 3. 6. 1965

Bäckerei-Tagung
 Müllerei-Tagung

7.— 9. 9. 1965 6.— 8. 10. 1965.

Sämtliche Tagungen finden in Detmold, im Roemer-Haus der Arbeitsgemeinschaft Getreideforschung e. V. statt.