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Chromosome Elimination in Trisomics of *Coix aquatica* Roxb.

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Summary. Somatic chromosome elimination was identified and its patterns studied in a trisomic (2n = 11) with marker genes in *Coix aquatica* Roxb. In a cross between a recessive trisomic with green base and white style (ccc ii ss) and a dominant disomic having purple base and purple style (CC II SS), all the F_1 seedling progeny were purple based because of the presence of C, I and S. For C to be expressed in seedling base, either I should be absent or S should be present with I. In style colour, however, irrespective of the presence of I and S, C produces purple phenotype.

In one trisomic (Ccc ii ss) plant (designated as 4 - 15) of the F_1 progeny, a part of the seedling base was green. All the tillers coming up from the green side of the main tiller also had green base, and those arising on the purple side were purple based. Similarly, the pistillate spikelets on the green side of the main culm and on the tillers with green base were white styled, and the male spikes showed 10 chromosomes. Female spikelets on the purple side of the main tiller and on the tillers with purple base were mostly purple styled and the male spikes had 11 chromosomes. In some of the purple based tillers, however, there were both 11 and 10 chromosomes in different regions or different inflorescence clusters on the tiller. In these tillers, where the chromosome number was 11, style colour was purple, and white style occurred when there were 10 chromosomes. In one tiller, the style colour was purple but the chromosome number was 10.

The recessive phenotype of the style in the trisomic conceivably resulted from an elimination of the extra chromosome carrying the dominant allele C. On the basis of the morphological features of the extra chromosome, such as length, centromere position and distribution pattern of the hetero and eupycnotic regions, it was identified as chromosome No. 2 in the complement. It was therefore possible to place with certainty the gene c on this chromosome. Sometimes, however, the extra chromosome carrying c also was eliminated giving 10 chromosomes and purple style.

In the other trisomic plants of the F_1 progeny, one plant showed 11 chromosomes but in a tiller there were only 10 chromosomes and white styles. In two other plants, although the chromosome number was 11 throughout, white style was present in a single cluster of inflorescences in one plant, and in one pistillate spikelet in the other. In the latter two cases, white style was believed to have arisen as a result of a mutation from C to c or somatic crossing over, giving the genotype ccc in the affected regions. In a single plant, chromosome elimination was observed in only one cell.

Apparently the 10-chromosome sectors arose from the 11-chromosome condition by selective elimination of the extra chromosome during mitosis in the primordium giving rise to these sectors. In the affected plants, elimination did not obviously occur at the same stage but at different times in their ontogeny. Instability is probably governed by one or a few major genes, associated with a number of modifiers, exhibiting incomplete penetrance and variable expression. Chromosome elimination did not apparently follow any particular pattern but was erratic. Probably some intracellular environment is necessary to trigger the mechanism governing the elimination into action. The unstable system, occurring in combination with other favourable features like the functional nature of the aneuploid gametes, sexual reproduction, monoecious condition favouring cross pollination and tolerance of extra chromosomes by the sporophyte, could be an important factor in the cytogenetic evolution of the species.

Introduction

The view that all tissues and cells of an organism have the same chromosome number had to be modified with the discovery of B-type chromosomes, whose number varies between different tissues or even different cells of the same tissue (Jones 1975). Also, there is evidence that the normal chromosomes of the complement in several species and hybrids show variation within the same individual (see Sachs 1952; Lewis 1962; Shahare and Shastry 1963; Khoshoo and Narain 1967; Davies 1974). While mosaics (or chimeras) involving genome reduplication or particular gene mutations causing phenotypic changes are well documented, the phenomenon of addition or deletion of single or fewer chromosomes resulting in mosaics of varying proportions in plants is believed to be rare. In trisomics of crop plants (e.g., maize, datura, tomato, spinach etc.) the presence of an extra chromosome is often associated with a recognizable phenotype. In spinach (Ellis and Janick 1959), loss of the extra chromosome from a portion of the trisomic plant brings about diploid features in a sector so that trisomic and disomic features can be recognized in the same individual. But in wild species (e.g., *Clarkia unguiculata*, Vasek 1956; *Collinsia heterophylla*, Dhillon and Garber 1960), the presence of

one or more additional chromosomes in the complement has no effect on the phenotype. In *Coix aquatica* (2n = 10), an asiatic member of the tribe Maydeae, in which chromosomal polymorphism exists in nature (Venkateswarlu and Chaganti 1973), the distinction between diploid and aneuploid plants in a population can be made only by ascertaining the chromosome numbers of the different plants. In such cases as this, the identification of loss of a chromosome in a trisomic would be greatly facilitated if genetic markers were available. Utilizing a combination of the trisomic condition and presence of marker genes, it has been possible to identify and study the patterns of somatic chromosome elimination in *C. aquatica*.

Materials and Methods

In *C. aquatica*, purple seedling base and purple style are dominant over green seedling base and pearly white style (hereafter referred as white style), respectively (Rao 1974). Purple colour of seedling base is controlled by three independent, dominant genes C, I and S. C, the basic colour factor, is necessary for anthocyanin formation. I is the inhibitor of C and S is the anti-inhibitor suppressing the action of I on C. Thus the genotypes C-I-S-, C-ii S- and C-ii ss show purple base and all others green base. C is pleiotropic and also affects the style colour. Its expression in style colour, however, is not inhibited by I irrespective of whether S is present or not. Thus, the genotype C- gives purple style and cc white style. The genotype of the parent with green base and white style is designated as cc ii ss and that of the other parent with purple base and purple style as CC II SS.

The genome of *C. aquatica* is unstable and the occurrence of intraplant variation in chromosome number is not uncommon, and aneuploids occur in selfed and open pollinated progenies of diploids (Venkateswarlu and Chaganti 1973; Venkateswarlu, Rao and Chaganti 1968; Rao, unpublished). Bearing these facts in mind, in the present study, whenever a plant appeared in the population that showed morphological features deviating from the normal (e.g., narrow leaves, larger capsular spathes etc.), a cytological study was made of it. In most instances, however, such phenotypic abnormalities were not associated with any cytological aberrations. During one such examination, one individual (appearing in the selfed progeny of the strain with green base and white style) was found to be a trisomic with 11 chromosomes, in which one of the longer chromosomes of the complement was in excess. From a comparison of the centromere position and distribution pattern of the hetero and eupycnotic regions of the extra chromosome at pachytene, diplotene and diakinesis, with the pachytene chromosome morphology established for this species (Venkateswarlu, Chaganti and Rao, unpublished), this chromosome was identified as the No. 2 chromosome. The genotype of the trisomic plant, depending on whether any of the c, i and s genes were carried on the No. 2 chromosome, would be ccc ii ss, cc iii ss or cc ii sss; if not cc ii ss.

The transmission of the extra chromosome to the progeny was studied by crossing the recessive trisomic as female with a diploid plant having the homozygous dominant characters of purple base and purple style. 38 seedlings were raised of the F_1 generation. All the F_1 seedlings showed, as expected, purple base but one of them exhibited a large sector of green base comprising about one-third of the culm circumference. This exceptional seedling, designated as 4 - 15, which was removed to the greenhouse, together with the rest of the F_1 progenies constituted the material for this investigation.

Results

Seedling 4 - 15 was transferred to a pot in the greenhouse at 4-leaf stage and the remaining F_1s were planted in the field. In plant 4 - 15, new tillers were examined for base colour as they came up. Those arising from the green side of the main culm showed green base. Tillers that developed later from these also showed green base. Tillers that arose from the purple side of the main tiller and all others originating from them showed purple base (Fig. 1). The ori-

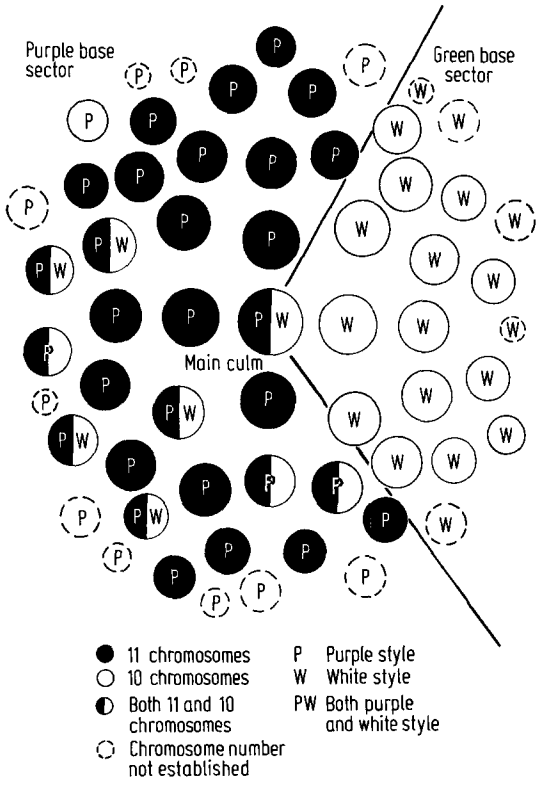

Fig. 1. Positions of tillers with reference to the main culm in an F_1 trisomic plant 4 - 15 (from the cross between green base white style trisomic × purple base purple style disomic) and patterns of chromosome elimination causing chromosome mosaics and phenotypic sectors for colour of seedling base and style in *C. aquatica* (2n = 10)

gin of green mosaic in an otherwise purple phenotype was presumed to be the result of a somatic mutation from C to c or S to s. If gene C was involved in the mutation, the basic colour factor would be absent, and if gene S was involved, the factor suppressing the action of I on C would be lacking. In either case the affected portions of the seedling base would be green.

When flowering commenced, the pistillate spikelets arising on the purple side of the main tiller showed purple style and those on the green side showed white style. The tillers that arose on the green side of the main culm showed only white style. It therefore appeared that gene C, rather than S, was involved in the mutation, as C would not be inhibited by I in its expression of style colour whether or not S was present or absent.

When randomly fixed male spikes of the main culm were examined cytologically, some showed 11 chromosomes which at meiosis formed one trivalent and 4 bivalents or 5 bivalents and one univalent, and others had 10 chromosomes forming 5 bivalents. This suggested that the plant might be a trisomic and the observed mosaicism could be the result of somatic elimination of the chromosome carrying the dominant allele C rather than gene mutation. In order to verify this alternative, 3 male spikes were fixed from the main culm from different inflorescence clusters having purple or white styles. Two of the spikes from a cluster with white styles had 10 chromosomes, confirming the assumption of chromosome elimination. The positions of all tillers with reference to the main culm were marked and similar fixations of cytological materials were made from each of them; at the same time the style colour in each was also recorded (Fig.1) to study the patterns of chromosome elimination. All the tillers showing white styles had 10 chromosomes. A large majority of culms with purple styles had 11 chromosomes, but one such tiller had 10 chromosomes; few tillers with purple style exhibited both the chromosome numbers of 11 and 10 and some tillers, like the main culm, had both white and purple styles (Fig.1). In all tillers where both white and purple styles were found, the two kinds were present distinctly on either side of the tiller, or the inflorescence clusters showing white styles were located terminally on the culm. Both kinds, however, were not present in the same cluster. The genotype of this trisomic should therefore be Ccc Ii Ss.

The presence of 10 chromosomes in one tiller and both 11 and 10 chromosomes in 3 tillers having purple style suggests that the chromosome eliminated here was the one carrying the recessive allele c, resulting in genotype Cc in the deficient portions. The occurrence of both 11 and 10 chromosomes in 6 tillers (including the main culm) having both purple and white style indicates elimination of the chromosome with the dominant allele C, resulting in recessive phenotype of the style and in genotype cc in the deficient regions. Only 45 tillers of this plant could be examined cytologically since staminate spikelets in the tillers arising in the later part of the growing season were either absent or rudimentary and, therefore, only base colour and style colour were recorded in these tillers (Fig.1). The extra chromosome, in addition to being eliminated in a portion of the main culm, was eliminated independently in 9 other tillers also. It is evident that the chromosome with the allele C was not the one involved in all cases. Since the genotype of the plant with reference to C is Ccc, if the elimination is equally frequent among the three chromosomes, the chances of the chromosome carrying c being eliminated are twice as great as that having C. But the observations (Fig.1) revealed that out of 10 cases, the chromosome with C was eliminated 6 times, and the one with c only 4 times, indicating that the former was preferentially eliminated. Since the main culm itself showed sectorial green base and that other tillers exhibiting mosaicism had clearly and spatially demarcated regions of purple and white style, it is conceivable that the elimination had occurred quite early in the ontogeny in the case of the main culm, and at various stages in the case of other tillers.

Of the remaining 37 F_1 plants, 33 grew to maturity. Table 1 shows their style colour and chromosome number. The occurrence of an entire tiller having white style and 10 chromosomes (plant 4-8, Table 1) might be due to somatic elimination of the chromosome carrying the dominant allele, as in plant 4-15. Where white style was observed in only one cluster of inflorescences that had 11 chromosomes forming one trivalent and 4 bivalents at meiosis (plant 4-1, Table 1), it might be due to a somatic mutation of C

Table 1. Chromosome number and style colour in the F_1 progeny (excluding plant 4 - 15) of the cross between green base white style trisomic × purple base purple style disomic in *C. aquatica*

Plant number	No. of plants	White Style		Purple style	
		No. of chromosomes	Portion of plant	No. of chromosomes	Portion of plant
4 - 8	1	10	One tiller	11	Rest of the plant
4 - 1	1	11	One cluster of pistillate inflorescences	11	Rest of the plant
4 - 21	1	11	One pistillate spikelet	11	Rest of the plant
4 - 5, 4 - 20, 4 - 22 and 4 - 30	4	--	--	11	Entire plant
4 - 2 to 4 - 4, 4 - 6 and 4 - 7, 4 - 9 to 4 - 14, 4 - 16 to 4 - 19, 4 - 23 to 4 - 29, 4 - 31 to 4 - 34	26	--	--	10	Entire plant

to c or somatic crossing-over, resulting in all recessive alleles (ccc) going into the same vegetative meristem giving rise to this particular cluster of inflorescences. The occurrence of a single female spikelet having white style (plant 4 - 21, Table 1) with the male spikes in this cluster showing 11 chromosomes might also be due to either of the two phenomena mentioned above, possibly occurring rather late in the primordium so that only a single spikelet was affected. The possibility, however, of localized somatic elimination affecting only one spikelet can not be ruled out.

Since tiller by tiller study of chromosome number was not made, in the F_1 plants having only purple style and 11 chromosomes (Table 1), it was not possible to determine whether elimination involving the chromosome carrying the recessive allele had occurred. Nevertheless, in one plant (4 - 20), in a single meiotic cells, among several observed within the same anther, 10 chromosomes forming 5 bivalents were observed. This indicates that such elimination, however infrequent it might be, did occur in these also but escaped attention as the style colour showed a dominant phenotype.

In a random cytological check of 2 to 3 tillers in each of the 26 F_1 plants having 10 chromosomes and purple style, no deviation in chromosome number was found.

It is conceivable that all plants in which chromosome elimination had occurred arose from zygotes with 11 chromosomes. The possibility that they might have started from zygotes with 10 chromosomes, with the sector having 11 chromosomes developing through mitotic non-disjunction, can be ruled out since the main tiller in all these plants (except in plant 4 - 15) showed purple base, purple style and 11 chromosomes. Even in plant 4 - 15, since a major portion of the main tiller showed purple sector and 11 chromosomes, it is believed that the 10-chromosome condition was derived from the 11-chromosome condition. The fact that, out of a total of 34 surviving F_1 plants from the trisomic × diploid crosses, 8 had 11 chromosomes and 26 had 10 chromosomes indicates that on the female side the extra chromosome is transmitted with a fairly high frequency (23.53%). The reciprocal cross was not attempted. However, other studies (Rao, unpublished) showed that the extra chromosome in trisomics is transmitted with high frequency by the male parent also.

Discussion

While it has frequently been reported that the chromosome number of an individual may vary, the factors and mechanisms underlying such instability are not

well understood. Polyploidy, intergeneric or interspecific hybridization, incompatibility of genes or cytoplasm, colchicine effect, and a combination of all these have been shown to be some of the factors affecting the stability of chromosomes in many species (see Thompson 1962; Shahare and Shastry 1963; Murray and Craig 1964; Yang 1965; Khoshoo and Narain 1967; Venkateswarlu and Krishnarao 1969; Davies 1974; Kasha 1974). The mechanisms capable of causing instability include fragmentation, breakage-fusion-bridge cycles, defects in chromosome reproduction, non-disjunction, selective chromatin elimination, spindle abnormalities, split metaphases, multipolar anaphases, some form of segregational mitosis and, in chemical terms, upsets in the ribonucleoprotein system and endonuclease attack, and incomplete DNA synthesis (see Sachs 1952; Kaufmann and Das 1955; Ehrendorfer 1959; Nielsen and Nath 1961; Lewis 1962; Yang 1965; Khoshoo and Narain 1967; Davies 1974; Kasha 1974).

C. aquatica, with 10 chromosomes, represents the base species in the polyploid series of chromosome numbers of 10, 20 and 40 in the genus *Coix*. The materials under study have not been subjected to colchicine or any other chemical treatment. They were originally derived from open pollination of 3 different populations from Andhra Pradesh, Madhya Pradesh and Orissa, grown sympatrically in the experimental garden. These 3 geographical populations are about 600 kilometres apart in nature, so that natural hybridization between them is not possible, although they cross readily when brought together. Interplant variation in chromosome number was observed in each of these populations (Venkateswarlu and Chaganti 1965, 1966, 1973) and at least a part of such variation could have arisen through intraplant variation, as observed by Koul (1970) in tapetal cells. It is likely that such instability is an inherent feature of the genome, and therefore the genic or cytoplasmic disharmony possibly occurring in interpopulation hybrids or their progenies is not exclusively responsible for the observed chromosomal instability.

The fact that instability affecting a portion of the plant was present in some plants of the progeny, while in others it was either absent or restricted to a few inflorescences of a tiller or a few meiocytes in an anther, suggests that it is probably governed by one or a few genetic factors; the genes concerned may lack full penetrance and are possibly associated with modifier complexes and hence the fluctuations in expressivity.

Cytological examination of the premeiotic cells in 11-chromosome sectors of plant 4 - 15 did not reveal any abnormalities usually known to be associated with instability, but earlier observations of instability in 10-chromosome plants (Rao, unpublished) showed a few cells at diakinesis with complementary chromosome numbers of 11 and 9 in the same anther. These obviously resulted from non-disjunction of a chromosome in premeiotic mitosis. In trisomic plants of the present study, it is conceivable that the instability occurred through selective elimination of the extra chromosome, by its failure to reach either pole during mitosis, from a portion of the primordium at different stages in different plants during development. The elimination, however, did not appear to have followed any particular pattern and seemed to be erratic. Probably a certain intracellular environment is necessary to trigger the modifier complexes governing the mechanism.

The occurrence of a trisomic plant originally in the selfed progeny of the recessive strain must have been due to union of gametes with 5 and 6 chromosomes, the latter apparently produced through meiotic non-disjunction or presence of somatic instability in the recessive parent. The genetic factors governing somatic instability and meiotic non-disjunction, whether or not the same, provide opportunities for increasing the chromosome number in the progeny. This unstable genetic system in *C. aquatica*, in combination with other favourable features such as the functional nature of gametes with aneuploid chromosome numbers, sexual reproduction, monoecious condition encouraging cross pollination and tolerance of extra chromosomes by the sporophyte, makes it an important force in the cytogenetic evolution of the species. The reasons why the chromosome carrying the dominant allele C (derived from the male parent) was preferentially eliminated, and why only the extra chromosome, and not any other chromosome of the complement, was selectively eliminated in mitosis are not known. It is also not clear whether the mutation from C to c or somatic crossing over inferred in plants 4 - 1 and 4 - 21 was only coincidental or had some-

thing to do with the instability factors. However, the frequent association between elimination of the No.2 chromosome and the appearance of the recessive phenotype of style colour permits the certain placement of gene c on this chromosome. Such incidental chromosome elimination in somatic cell hybrids between man and mouse has been used to assign specific biochemical genes to particular human chromosomes (see Ephrussi and Weiss 1969).

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