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ARE THERE HETEROMORPHIC SEX CHROMOSOMES IN SPINACH (*SPINACIA OLERACEA* L.)?

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INDEX WORDS

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SUMMARY

Four varieties and two introductions of *Spinacia oleracea* are cytologically studied in order to determine whether there is a heteromorphic sex chromosome pair in any of the sexes. It is observed that in one and the same preparation, and regardless of the sex of the plants, different cells had homomorphic and heteromorphic condition for chromosome 1 pair. It is concluded that as a result of differential contraction of chromosomes, the arm ratios and total lengths may vary, and give a distorted impression of certain pair of chromosomes. The absence of heteromorphic pair is confirmed in male and intersex plants by meiotic studies.

INTRODUCTION

Cultivated spinach, *Spinacia oleracea* L., as a dioecious species, has separate male and female plants. However, in some of the varieties there are also intersex individuals. With a view to the production of commercial hybrid varieties, so increasingly important for plant breeders and growers, SNEEP (1958) suggested creating an entirely female variety. Such a variety would help produce hybrid seed in a more economic way. A breeder should therefore understand the mechanism of sex determination in spinach. SNEEP (1958) presented an outline for developing an entirely female variety, based on the hypothesis of BEMIS & WILSON (1953) for sex determination in spinach. According to BEMIS & WILSON sex in spinach is determined by an XX and XY mechanism, which is further influenced by a pair of closely linked modifying autosomal genes. Subsequently, modified hypotheses were proposed (ELLIS & JANICK, 1960; SUGIYAMA & SUTO, 1964; DRESSLER, 1973).

In spite of divergent views on the mode of sex inheritance in spinach, there is general agreement to name the sex determining factors X and Y. These, X, Y factors have been genetically associated with the longest of the 6 pairs of small chromosomes of spinach using the trisomic method (ELLIS & JANICK, 1960; SUGIYAMA & SUTO, 1964). The longest chromosome pair, or 'sex chromosome' pair, has been the subject of several cytological investigations, and there are considerable differences of opinion regarding the homomorphic (= two similar in morphology) or the heteromorphic (= two dissimilar in morphology) condition in one of the sexes, the male (for review see SNEEP, 1962; IIZUKA & JANICK, 1971). BOSE & JANICK (1961) observed three 'distinct morphological types' of the longest chromosome, viz., 'standard' hetero-

brachial (long arm twice as long as the short arm), isobrachial (long and short arms of nearly equal length), and a 'variant type' distinguished by the presence of a satellite on the short arm. The presence of heteromorphic chromosomes has further been reported to occur in mitosis by DRESSLER (1958) and in meiosis by ZOSCHKE (1956).

During the course of our investigations on the sex determining mechanism in spinach, we have made cytological observations on four varieties and two introductions. The results are reported in this article.

MATERIAL AND METHODS

The four varieties used were Prévital, Nores, Wintra and Erasmo. The two introductions were P.I. 169671 and P.I. 169676, of which the former was said to carry an extra satellited chromosome and the latter a homobrachial variant of chro. 1 linked with X gene. All material save the varieties was kindly supplied by Unilevers Ltd.

All plants were grown in the greenhouse in small pots during the spring seasons of 1972, 73 and 74. Root tip fixations were made from 12–15 day old plants, which were carefully lifted from the pots. After the root tips had been excised the plants were transferred into slightly bigger pots and grown to maturity. In each case the karyotype was examined and the sex of the plant recorded.

Before fixation root tips were pre-treated, at room temperature, with colchicine 0.02% for 90 minutes; α-bromonapthalene (saturated solution) for 90 minutes; 8-hydroxyquinoline 0.002 m/l for 3 hours. Pre-treated root tips were fixed in a 3 : 1 solution of ethanol acetic acid for 24 hours, hydrolysed in 1 N HCl for 3 minutes at 60°C and stained with Feulgen stain for 2–3 hours. Dark stained root tips were squashed in a drop of 1% acetocarmine following the usual procedures.

The male and intersex plants, the karyotypes of which had already been studied at the seedling stage, were used for meiotic studies. Parts of young inflorescences were fixed in a mixture of 3 parts of ethanol and 1 part of propionic acid saturated with iron acetate for 48 hours or more. The anthers with suitable meiotic stages were squashed in a drop of acetocarmine.

RESULTS

The six pairs of spinach chromosomes can be classified as follows: Two pairs of sub-median chromosomes in which the longer arm is nearly twice as large as the short arm. (These chromosomes are also classed 'heterobrachial'). Four pairs of sub-terminal chromosomes in which the long arm is at least four times as large as the short arm. Of the four sub-terminal chromosomes, two pairs have satellites, all of which can be seen only in exceptionally good preparations. The above described karyotypes were observed in all the four varieties and two introductions investigated. For the sake of convenience the spinach chromosomes are numbered here according to SUGIYAMA & SUTO (1964). Accordingly, the two sub-median chromosome pairs are 1 and 2; the two sub-terminal chromosome pairs without satellites are 3 and 4, and the two sub-terminal chromosome pairs with satellite 5 and 6. Of the 6 pairs, 1 and 2 as well as 5 and 6 can be identified in many cases, whereas the pairs 3 and 4 sometimes may be confused with each other.

Effect of pre-treatment on chromosome contraction. The three pre-treatment chemicals, colchicine, α-bromonapthalene and 8-hydroxyquinoline, were included in this study in order to compare their effect on chromosome contraction and changes, if any, on the chromosome morphology. Of the three chemicals, colchicine and α-bromonapthalene generally produced optimum chromosome contraction in nearly 20–25% of the dividing cells. In the rest of the cells the chromosomes were either over-contracted or poorly spread. 8-hydroxyquinoline was not as favourable as the other two, although it produced a clearer differentiation of the centromeres. Since colchicine gave more repeatable results, all observations were confined to colchicine pre-treated preparations.

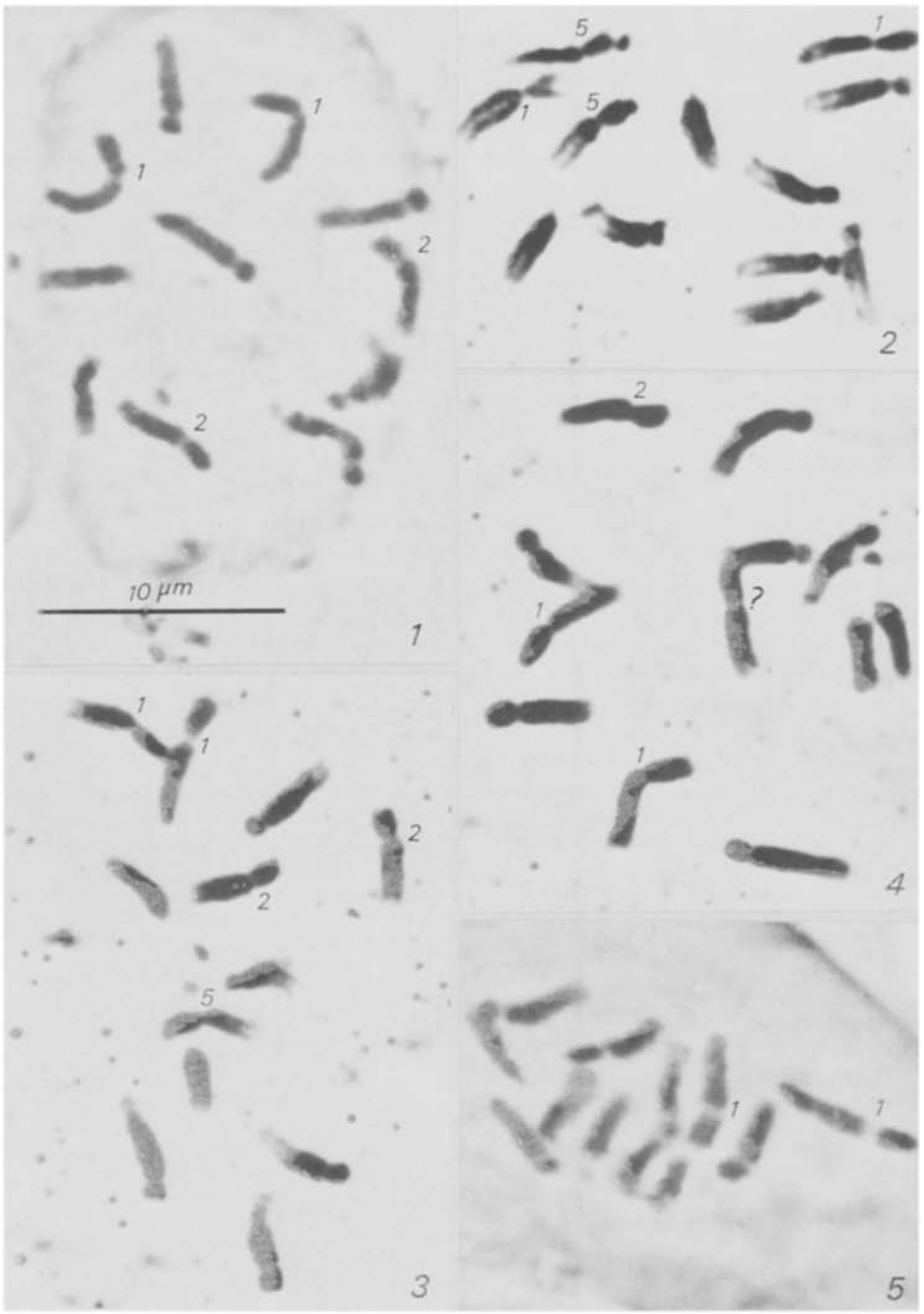
For a comparison of the effect of pre-treatment, chromosome 1 was examined critically in at least 20 cells, selected at random in each preparation for good spreading and contraction. Without exception, and regardless of the sex of the plant, the morphology of the chromosome varied from cell to cell in the same slide in all cases. In many of the cells a homomorphic condition was observed for the sub-median chromosome (Fig. 1), whereas in the same preparation a considerable number of cells also contained a sub-median and a median (both arms of nearly equal length) chromosome (Fig. 3), thus showing a heteromorphic condition. In a few cases, however, in spite of their properly contracted chromosomes, it was not possible to decide whether chromosome 1 was homo- or hetermorphic (Fig. 4). The number of cells showing homomorphic, heteromorphic and undecided cases in each plant (of different sexes) is given in Table 1. Similar results were also observed in the case of α-bromonapthalene and 8-hydroxyquinoline pre-treatments but the number of cells belonging to undecided categories was high.

Apart from the variation in arm ratios, clear differences were also observed in the total lengths of the two chromosomes of a pair (Fig. 5).

Table 1. Showing the number of cells having homomorphic, heteromorphic and undecided cases of chro. 1 in the same preparation in different types of plants.

Variety	Sex	Number of cells studied	Number of cells with pairs of		
			homomorphic*	heteromorphic	undecided
Prévital	female	21	19	2	–
	male	28	22	5	1
Nores	female	25	16	8	1
	male	31	27	2	2
Wintra	female	27	19	8	–
	male	25	18	6	1
Erasmo	female	28	21	6	1
	male	25	20	4	1
P.I. 169671	female	32	20	6	6
	male	28	16	8	4
	intersex	43	26	14	3
P.I. 169676	female	48	33	8	7
	male	37	19	11	7
	intersex	52	36	11	5

* Homomorphic for the sub-median chromosome.


10 µm
1
2
3
4
5

The longer chromosome of such a pair is, generally, light stained when compared with its homologue. The longer chromosome is comparable in length to the so-called 'giant' chromosome reported to occur in spinach by IIZUKA & JANICK (1963).

Observations on meiotic chromosomes. For the study of chromosome morphology, meiotic chromosomes in spinach are not favourable since only late stages of prophase and metaphse I can be obtained with relative ease. At these stages chromosomes are very much contracted and therefore no information on chromosome morphology can be obtained. Very rarely pachytene bivalents can be observed (Fig. 6), but is extremely difficult to get a large number of well spread-out cells for a comparison. Moreover, the centromeres are not properly differentiated, and therefore arm ratios of different chromosomes can not be determined. Nevertheless pachytene bivalents can be of great value to compare directly whether there is any differential segment between the so-called sub-median and median chromosomes.

At diakinesis and metaphase I no heteromorphic bivalents were observed in more than 120 plants examined in P.I. 169676. Normally, 6 bivalents were observed at diakinesis, of which two were invariably associated with the nucleolus (Fig. 7). At metaphase I, six bivalents, usually of the rod type (Fig. 8) were observed and in no case an association of more than two chromosomes was observed. Rarely, in some pollen mother cells one or two bivalents had a fragment of variable size (Fig. 9). This could be the exaggerated constriction of a sat-arm, but no definite explanation could be given for this structure.

DISCUSSION

The differences observed in the present study between karyotypes of different cells in the same preparation can only be explained as due to the differences in degree of contraction of the chromosome arms. As a result of differential contraction, the arm ratios and total lengths of chromosomes can vary to a considerable extent and give a distorted impression of a certain pair of chromosomes. Fig. 2 clearly illustrates that the second pair of sub-median chromosomes, normally present in other cells, is not at all distinguishable from the other sub-terminal chromosomes. Furthermore, the orientation of the very small chromosomes of spinach (2–5 μm) in a highly magnified, flat field of microscope enhances the difficulties for a proper identification of the respective pair and calls for extreme care. The presence of two pairs of satellite

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Fig. 1–5. Somatic chromosomes of *Spinacia oleracea*, P.I. 169676, ♀.
Fig. 1. Two sub-median chromosome pairs, 1 and 2. Both are homomorphic.
Fig. 2. Chromosome 1 pair is homomorphic. But chromosome 2 is not at all distinguishable from the other sub-terminal pairs. Chromosome 5 pair has satellites and therefore cannot be mistaken for chromosome 2.
Fig. 3. Chromosome 1 pair appears to be heteromorphic. Note the sub-median (below) and the median (above) chromosomes. Chromosome 2 pair is identifiable and only one of the chromosome 5 pair appears almost like a median chromosome.
Fig. 4. Chromosome 1 pair appears homomorphic but the median chromosome (?) also appears to be as large as the sub-median chromosome 1 of Fig. 3. Therefore it is an 'undecided' cell.
Fig. 5. Chromosome 1 pair consists of a very long ('giant') and a normal sub-median chromosome.

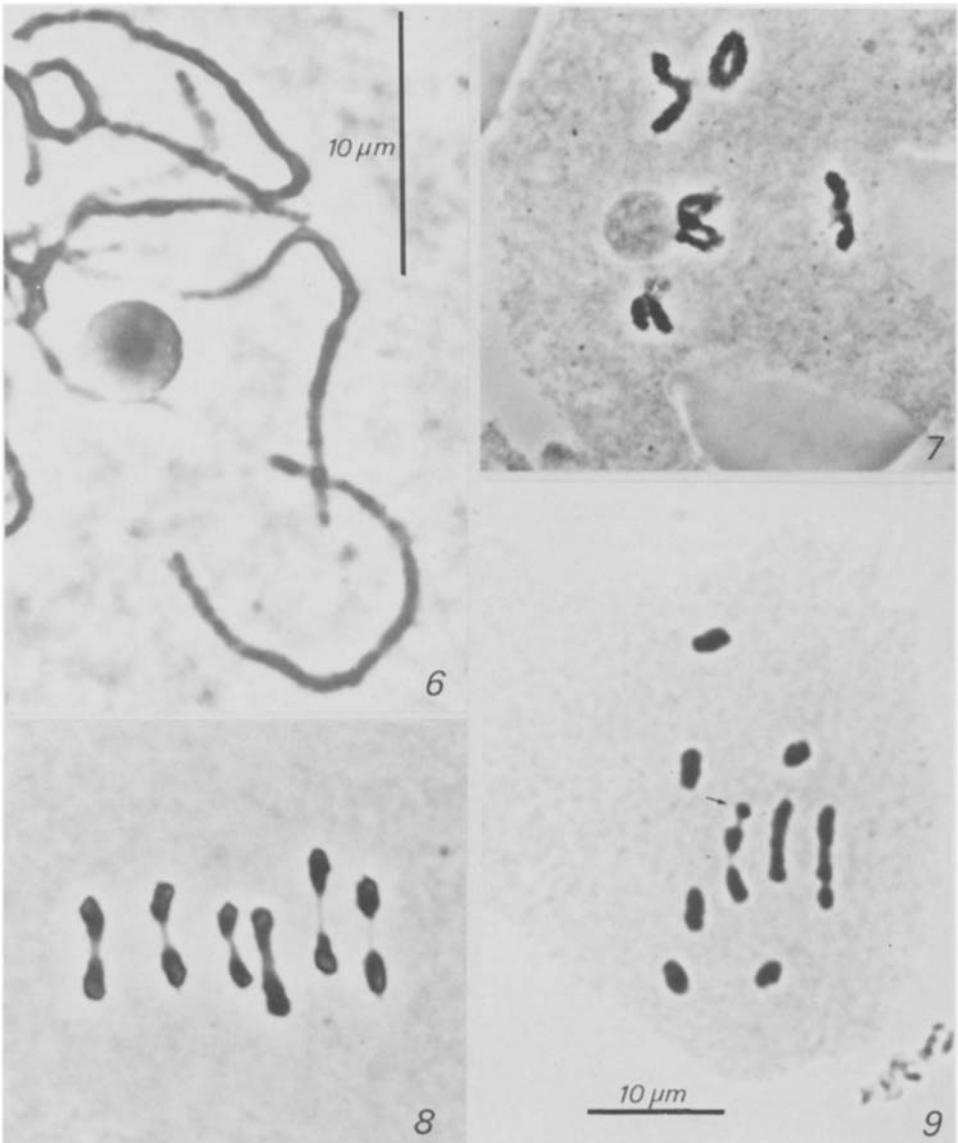

Fig. 6–9. Meiotic chromosomes of P.I. 169676 ♀.
Fig. 6. Pachytene chromosomes of spinach showing at least 3 complete bivalents.
Fig. 7. Diakinesis showing the attachment of two bivalents with the nucleolus.
Fig. 8. Metaphase I showing 6 normal road-bivalents.
Fig. 9. Late metaphase I showing a fragment attached to one of the bivalents (arrow); it does not indicate a heteromorphic condition in all Pmc's.

chromosomes in the somatic cells, as well as the invariable association of two bivalents with the nucleolus at diakinesis in male meiosis, has been recognized in the past (LORZ, 1937; SUGIYAMA & SUTO, 1964; TABUSHI, 1958).

The present study also confirms the above finding (Fig. 7). Yet some observers IIZUKA & JANICK, 1966; 1971; DRESSLER, 1958; and AKKOC, 1965) maintain that there is a single pair of satellite chromosomes, whereas the second pair (or a single chromosome) is only a variant, present in some of the varieties. This divergence of opinions appears to stem from the technical difficulty to reveal all the four sat-chromosomes in a single cell rather than their variation in number in different varieties. The size of a satellite may however vary, as in other plant species, thereby creating the wrong impression that a heteromorphic bivalent (Fig. 5) is present at meiosis. This may be the reason for ZOSCHKE'S (1956) observations of a 'heteromorphic' bivalent in male meiosis.

IIZUKA & JANICK (1971) reported the presence of a heteromorphic pair of chromosome 1 in 9.7% of the population in one of the introductions, P.I. 169676, which was also used in the present investigation. According to the above authors the median chromosome of the heteromorphic pair has arisen by a reciprocal translocation on the short arm of chromosome 1. Although the so-called heteromorphic pair is observed in some of the cells in the present investigation (see Table 1), there was no indication for the presence of any translocations in meiotic stages in more than 120 plants studied by us in the same introduction. It may be pointed out that slight morphological differences between the two chromosomes of a pair is a poor guide for detecting structural differences between chromosomes, especially in spinach. A more dependable approach would be to study meiotic pairing in a large number of male and intersex plants, and detect quadrivalent associations. Such studies however are not possible in female plants since meiosis cannot be studied in them.

As regards the localization of X and Y factors on a chromosome pair, there is agreement between the results of ELLIS & JANICK (1961), and SUGIYAMA & SUTO (1964). These authors observed a deviation from a 1 : 1 ratio of males and females in triplo-1, whereas in all other trisomics they observed, as expected, a ratio of 1 : 1 of males and females. The deviating male/female ratio in triplo 1 gives an impression that chromosome 1 is the sex chromosome pair. However, TABUSHI (1958) reported a deviation from the 1 : 1 ratio of males/females in a trisomic other than triplo 1. This clearly illustrates that more studies are needed before any conclusion can be reached regarding the so-called 'sex-chromosome pair' in spinach.

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