Chromosome Constitution of Two Karyotypes in *Vicia macrocarpa*

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The chromosome complements of two cytotypes of *Vicia macrocarpa* were investigated and it was observed that the karyotypes changed in relation to the position of centromere, length of chromosome arms and number and nature of nucleolar chromosomes. At the same time changes in some morphological and physiological characters of the plants were also observed. In addition, the two chromosome complements were studied for their banding pattern at the mitotic prophase and metaphase. It was evident that the two cytotypes also showed differences in the amount and distribution of heterochromatin. One of the cytotypes (12 E) exhibited a larger number of C-bands than the other (12 C). It is concluded that the evolution of *V. macrocarpa* has been towards a decrease of the amount of heterochromatin, along with the changes of the karyotype. Various multivalents ranging from trivalents to hexavalents were observed in some cells at the meiosis in the F₁ and F₃ of 12 C×12 E. It was thus demonstrated that the two cytotypes differed by the presence of two reciprocal translocations. It was possible to identify the parent-derived individual chromosomes at both the mitotic stages and meiosis of the F₃ plants based on the differences in the heterochromatic bands and centromere position. The extra-chromosome of a trisomic plant in the F₃ was identified at the mitotic prophase as well as the chromosomes involved in the multivalents at the meiosis of the hybrids.

KEY WORDS: *Vicia macrocarpa*, Cytotype, Chromosome, Heterochromatin, C-band
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Introduction

Vicia macrocarpa (MORIS) BERTOL (2n=12), like *V. cordata*, *V. angustifolia*, *V. amphicarpa* and *V. pilosa*, is one of the related species in the *V. sativa* group. It is cultivated mainly for fodder in the central and western Mediterranean regions. Karyotypic polymorphism is not uncommon both within and between the species belonging to the *V. sativa* group (HANELT and METTIN 1966; METTIN and HANELT 1973; HOLLINGS and STACE 1974; LADIZINSKY 1978). In *V. macrocarpa*, however, karyotypic polymorphism had not been reported (COUTINHO 1940; HANELT and METTIN 1966; YAMAMOTO 1968) until a new karyotype was detected recently by YAMAMOTO (1984). The two complements have been designated as 12 C and 12 E, respectively (YAMAMOTO 1984). The present communication deals with the cytogenetic relationships of the two cytotypes within *V. macrocarpa*.

Materials and Methods

Seeds of the various strains used in the present experiment were obtained from different sources as listed in Table 1. For hybridizing 12 E with 12 C the strain 413 of 12 C was used as a parent.

Root tips were pretreated with cold water (0~2°C) for 24 hours, fixed in acetic ethanol (1:3). For karyotype analysis, the root tips were washed in ion exchanged water for 30 minutes, hydrolysed in 5 N HCl for 1 hour at about 20°C and stained by Feulgen re-

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Table 1. Seed source of *V. macrocarpa*

Strain No.	Karyotype	source
413	12 C	Portugal, 1979
397	12 C	HUJ, 1979
P-5	12 C	C. Barnard, Austl., 1963
P-6	12 C	C. Barnard, Austl., 1963
511	12 E	METTIN, D., GDR, 1980

gent for 1 hour. The stained root tips were washed in SO₂ solution before squashing in 45% acetic acid.

For C-banding the pretreated and fixed root tips were macerated in 45% acetic acid at 50°C for 15 minutes and subsequently squashed in 45% acetic acid. The slides were stored in a desiccator over silica gel for one day after the cover slips were removed by the dry ice method and air drying. The slides were treated with 5% aqueous barium hydroxide solution at 60°C for 5 minutes, rinsed in ion exchanged water for 30 minutes and then incubated in 2×SSC (0.3 M Sodium Chloride and 0.03 M Sodium Citrate, pH 7.0) at 60°C for 1 hour. After thorough washing, the slides were stained by Giemsa (GURR'S Improved R 66) diluted 50× with 1/15 M Sørensen phosphate buffer (pH 6.8) for 30 minutes.

To identify meiotic chromosomes with more accuracy, the pollen mother cells (PMCs) were first stained by acetocarmine and squashed. After observation under the microscope and photography, the preparations of which the cover slips had been removed were destained in 45% acetic acid at room temperature for about 30 minutes, and subsequently treated by the C-banding method described above.

Results

Plant morphology

The morphology of the 12 E strain was almost similar to that of the 12 C strains including the presence of coarsely dentate stipules, large leaflets, long flowers (3~3.5 cm), large pods (6~7 cm×0.8 cm) and seeds (5~6 mm in diam.). However, the plants differed in some other characters (Table 2). It was observed that 45% of the seeds in the 12 E strain were under a dormancy state as compared to the 100% germination of the 12 C strains, which is considered to be a characteristic of wild species.

Cytology

As shown in Fig. 1 a, the morphology of the chromosome pairs 1, 2 and 3 of 12 E was not similar to that of the corresponding chromosome pairs of 12 C, while the chromosome pairs 4, 5 and 6 were identical in both cytotypes. Besides, the 12 E had an additional pair of nucleolar chromosomes (chromosome 3) of which the satellites were very small.

Table 2. Morphology of 12 C and 12 E strains

Karyotype	Shape of leaflet	Color of pod	Shape of pod	Color of seed coat	Germination rate
12 C ¹⁾	emarginate	dark brown	flat	dark brown	100%
12 E	elliptic	yellowish	cylindrical	light brown with brown spots	55%

¹⁾ Includes all 12 C strains.

Fig. 1 Karyotypes of 12 C and 12 E. a : Metaphase. b : Prophase showing heterochromatic regions. c : C-banded metaphase. Horizontal lines indicate centromere position. Scale=10 μ .

At the Feulgen-stained prophase, the chromosomes of both complements could be distinguished based on the centromere position and the regions of constitutive heterochromatin distributed in various chromosomes (Fig. 1b). The heterochromatic regions in 12 E were more pronounced and the distinctive distribution of these regions facilitated the identification of each chromosome pair. In comparison, a smaller number of heterochromatic regions was observed in 12 C.

Fig. 2. Heterochromatin at interphase. 2 A : 12 C. 2 B : 12 E. Scale=10 μ .

The difference in the amount of heterochromatin between 12 C and 12 E was also reflected in the C-banded metaphase chromosomes as well as interphase nuclei (Fig. 1 c, Fig. 2). Moreover, the C-banding patterns in the metaphase chromosomes of the karyotypes of both cytotypes coincided well with the distribution of the Feulgen-stained heterochromatic bands observed in the prophase chromosomes (Fig. 1 b and c). Some chromosomes, in fact, were devoid of C-bands (Fig. 3). For example, four chromosomes of the haploid complement of 12 C did not show C-bands, implying thereby that the heterochromatin content was too small or that heterochromatin was lacking in these chromosomes. It was noticed that the C-bands in the majority of the chromosomes were present in the intercalary regions rather than in the centromeric or telomeric regions except for the chromosome 3 of 12 E in which small centromeric bands were observed.

Hybridization and meiotic chromosome pairing of the F_1 hybrid

Crosses between 12 C and 12 E strains were carried out. Interestingly, no seeds were obtained when 12 E was used as female parent though as many as 45 flowers were pollinated. In comparison, reciprocal crossing was successful and, of 20 flowers crossed, 12 seeds were collected from 9 pods.

Meiotic details at the first metaphase (MI) of the F_1 are shown in Table 3. Besides the 6 II configuration, 4 II+1 IV and 4 II+1 III+1 I configurations were observed in 25.7% and 9.9% of the cells, respectively. Formation of multivalents, i.e., one hexavalent or two trivalents, though in a low frequency, was also detected. The pollen fertility was 39%.

Identification of chromosomes in the F_3 plants

In the F_3 , a trisomic plant ($2n=13$) was obtained. Out of its 10 progenies, five were trisomic and five disomic ($2n=12$). From the Feulgen-stained metaphase chromosomes, it was difficult to determine whether the extra-chromosome in the trisomic plants was chromosome 3 or 4 as the chromosome pairs 3 and 4 of 12 C and 4 of 12 E have almost the same morphology (Figs. 1 a and 4 C). On the basis of the difference in the heterochromatic band at the *prophase*, however, it was demonstrated beyond doubt that the extra-chromosome was contributed by the chromosome 3 (Fig. 4 B). In the hybrids, the chromosomes 1, 2 and 3 of each parent could be easily distinguished at the metaphase unlike the chromosomes 4, 5 and 6 which were similar to each other in the two parents. At the prophase, however, the difference in the heterochromatic band between the chromosomes 6 of 12 C and 12 E (Fig. 1 b) made it possible to determine from which parent this pair of chromosomes was derived (Figs. 4 B and 5 B). As expected, interphase nuclei of the F_3 contained the same number of heterochromatic regions (chromocenters) as observed in the prophase chromosomes (Figs. 4 A and 5 A).

Fig 3 Idiograms of C-banded chromosomes.
Scale=1 μ .

Table 3. Chromosome pairing at MI of meiosis in F_1 hybrid of 12 C $\times$ 12 E

Configuration	No. of cells observed	Frequency %
6 II	51	50.50
4 II+1 IV	26	25.74
4 II+1 III+1 I	10	9.90
3 II+1 VI	5	4.95
3 II+2 III	5	4.95
5 II+2 I	4	3.96
Total	101	100

Note: I, II, III, IV and V indicate univalent, bivalent, trivalent, quadrivalent and hexavalent, respectively.

At the meiosis of the F_3 plants, in addition to the formation of bivalents, quadrivalents in the disomic plants and univalents, trivalents, quadrivalents and especially pentavalents in the trisomic plants were observed.

The individual chromosomes involved in the multivalents were identified on the basis of the difference in the C-bands and centromere position in reference to the known chromosome constitution and banding patterns in two F_3 plants (T 4 and D 7, see Figs. 4 B and 5 B). Figs. 7 B and 7 C show clearly that the chromosome 1 of 12 C (1 C, without band) was bound to the chromosome 3 of 12 E (3 E, with submedian centromere) within a pentavalent and a trivalent in the trisomic plant T 4. Furthermore, another pentavalent observed in the same plant showed that the chromosome 1 of 12 E (1 E, with one band) was associated with the chromosome 3 of 12 C (3 C) (Fig. 7 D). The same results were obtained from a quadrivalent at diakinesis in the disomic plant D 7 (Fig. 8 B 2). In fact, this quadrivalent was attached to the nucleolus (Fig. 8 B 1), implying that nucleolar chromosomes were involved in the rearrangement.

Figs. 4-5. Mitosis in F_3 plants of 12C $\times$ 12E. Figs. 4A-4C: Trisomic plant (T4). Figs. 5A-5C: Disomic plant (D 7). 4 A and 5 A: Heterochromatin at interphase. 4 B and 5 B: Prophase. 4 C and 5 C: Metaphase. Scale=10 μ .

Figs. 6-8. C-banded chromosomes of PMCs of parents and F_3 plants.

Figs. 6 A and 6 B. 12 C and 12 E with 6II, respectively.

Figs. 7 A-7 D. Trisomic plant T 4. 7 A : 6II + 1 I ; 7 B : 1V + 4II ; 7 C : 1III + 5II, note the connection between 1 C and 3 E by a thread (thickest arrow) ; 7 D : 1V + 4II. Thin arrows denote the C-band of chromosome 1E and thick ones indicate the centromere of chromosome 3 E.

Figs. 8 A and 8 B. Disomic plant D 7. 8 A : 6II ; 8 B 1 and 8 B 2 : 1VI + 4II, 8 B 1 was stained with acetocarmine. Arrow denotes the centromere of chromosome 1 C.

Discussion

On the basis of the results obtained it is evident that the chromosomes of the two cytotypes (12 C, 12 E) of *V. macrocarpa* differed not only in the position of the centromere, length of chromosome arms and number and nature of nucleolar chromosomes but also exhibited marked differences in the heterochromatic banding pattern. The chromosomes of the two complements, based on the cumulative data described earlier, can be divided into three categories A, B and C (Fig. 3). The chromosomes belonging to category A are quite distinct from each other in 12 C and 12 E in terms of C-banding as well as outer morphology of chromosomes. The category B, however, has chromosomes which are similar both from the two aspects mentioned above. Although the chromosomes of category

C are similar to each other in their morphology they differ completely in their C-banding pattern. Thus it is suggested that during the course of differentiation the two cytotypes have not only diverged in the chromosome morphology but also in the amount of heterochromatin. In the case of category C, the variation of heterochromatin may have occurred independently of that of the chromosome morphology in the present species.

C-bands have been usually found to occur at the centromeric and telomeric regions along with intercalary regions in some plant species. Interestingly, the C-bands in both karyotypes of *V. macrocarpa* were mainly intercalary and they were almost absent in other regions. The paucity of the centromeric bands, which if any were very thin, is in agreement with the C-banding data reported by RAMSAY (1984) in some other *Vicia* species.

Heterochromatin polymorphism within plant species is not uncommon. It has been reported in a few plant species (TANAKA 1969; BENTZER *et al.* 1975; WEIMARCK 1975; LELLEY *et al.* 1978; LINDE-LAURSEN 1978) and also found in *V. macrocarpa* in the current study. The phenomenon of deheterochromatinization which has been reported to influence the size of plant organs (TANAKA 1969) may possibly apply to *V. macrocarpa*. The percentage of seed germination of the 12 E strain was 45% lower than that of the 12 C strains. This character of partial dormancy is indicative of a primitive nature. The process of deheterochromatinization from 12 E to 12 C was probably associated with a variation in the morphological features though not to the extent observed in *Spiranthes sinensis* (TANAKA 1969).

The formation of multivalents in the F_1 and F_3 suggests that the two cytotypes differed from each other by two reciprocal translocations. In consideration of the fact that the frequencies of the multivalents were not significantly high, it is assumed that the regions of exchanged chromosomes were small in size.

Regarding the meiotic behaviour, the results of the karyotype analysis suggest that the translocational multivalents may have involved the chromosomes 1, 2 and 3 as these were structurally different from each other in the two karyotypes. The detection of pentavalents in the trisomic plant also implies that the extra-chromosome 3 was possibly included. On the other hand, it has been reported that the C-banding technique is effective in identifying meiotic chromosomes involved in translocations (SINGH *et al.* 1977; ROBERT 1981). In the present experiment, the C-banding patterns of the chromosome associations at the diakinesis and MI revealed that the chromosomes 1 and 3 derived from the two parents were involved in the formation of translocational multivalents.

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Vicia macrocarpa における二つの核型の染色体構成

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Vicia macrocarpa ($2n=12$) は, *V. sativa* (コモンベッチ) の近縁種であり, 牧草として地中海地方に栽培される. *V. sativa* とその近縁種には種内, 種間における核型多型が広く認められている. しかしながら, *V. macrocarpa* においては YAMAMOTO (1984) の最近の報告まで核型多型について報告されていなかった. 本研究は, 本種において検出された両核型 (12C と 12E) の細胞遺伝学的な関係を明らかにするため, 形質調査を加えて, 核型分析ならびにその雑種における成熟分裂の観察を行い, 両核型の分化について考察した.

両核型の間には動原体の位置, 染色体腕の長さ及び仁染色体の数と特徴について変異が観察され, 同時に, 形態形質及び生理的形質の差異も認められた. 更に, フォイルゲン染色による有糸分裂前期における異質染色質バンドパターンと分裂中期のギムザ C-バンドパターンを観察したが, 両核型間に異質染色質領域の差異が確実に認められた (Fig. 1). また, 祖先型と思われる 12E 型では C-バンドの数が 12C 型より多かった. しかし, C-バンドの分布は, 12E の第 3 染色体を除いて, 両核型共に主に長腕の介在部に現れた. 上述の染色体の形態的及び異質染色質領域の変異に基づいて, 両核型の染色体は三つのカテゴリーに分けられる. すなわち, 染色体の形態と異質染色質領域が異なる A カテゴリー. 両方とも差異が認められない B カテゴリー及び前者は似ているが, 後者では違った C カテゴリーである (Fig. 3). それらのことから, 本種の分化過程においては核型の変異だけではなく, 異質染色質領域の減少も起こったと推定された.

雑種 F_1 および F_3 の花粉母細胞の成熟分裂にみられる三価から六価までの多様な多価染色体の出現 (Table 3) は, 12C と 12E の間における二つの相互転座の存在を示している.

異質染色質バンド及び動原体の位置により, 本種の多型核型系統間の雑種における体細胞並びに花粉母細胞の各染色体の識別と同定は可能であった. それによって, F_3 のトリソミックスの過剰染色体は有糸分裂前期において識別され, 雑種の成熟分裂における多価接合に関与する染色体も同定された.