Chapter II

CHROMOSOME BANDING IN *Passiflora incarnata* L.

A. ORCEIN BANDING
B. TRYPSIN ORCEIN BANDING
C. GIEMSA BANDING

ORCEIN BANDING IN PASSIFLORA INCARNATA

INTRODUCTION

In plant systems, the banding techniques have been of limited use because of the difficulty of air drying of solid tissue, one of the essential steps for Giemsa and fluorochrome staining. To avoid this limitations some authors have tried Carmine, orcein or feulgen staining following controlled hydrolysis in HCl (Lavania, 1978). Sharma (1975, 1977) developed a new technique termed Orcein-banding (O-banding) for plant chromosomes providing a convenient method for securing bands with a precise understanding of the chemical reaction involved in the manifestation of the bands.

Among the abundant chromosome techniques now being routinely used in animal and human cytogenetics, not all have proved feasible with plant material. Moreover, plant tissues are comparatively less responsive to Giemsa reaction as compared to the animal system (Natarajan and Natarajan, 1972; Takehisa and Utsumi, 1973; Vosa, 1973 and Schweizer, 1974).

It is revealed from the previous literature that there are few reportes on chromosomal banding pattern in Passiflora. Therefore, it has been decided to undertake the study of banding pattern in Passiflora incarnata as a means to study the chromosomes in detail.

MATERIALS AND METHODS

Passiflora incarnata cuttings were grown in pots. Excised root tips of P. incarnata were treated with 0.2% aq. colchicine for 2 hours at 12 - 14°C. Root tips were washed thoroughly in distilled water, fixed in acetic-ethanol (1:2) for 12 hours and finally stored in 70% alchol. For the orcein banding the method developed by Sharma (1977) has been employed successfully.

Only metaphases showing maximum banding response were selected for detail analysis. The drawings were made with the aid of Camera-Lucida. The metaphases were photographed and enlarged.

In this study author used the term 'orcein banding' (O-banding) in a purely descriptive sense to design darkly staining chromosome segments obtained by a particular 'O'-banding method.

RESULTS

An estimate of the amount of banding was obtained from the sum of banded chromosome length expressed as percentage of total chromosome length (Table 2.1). In P. incarnata the position of the dark staining region is species specific and may be present either in centromeric, telomeric or intercalary position (Figs. 2.1,2.2,2.3). The main nucleolus (NOR-chromosome) usually have a very intensely staining terminal segment (Satellite or part of it) on their

Table 2.1

Proportion of orcein banded chromosome length expressed as percentage of total chromosome length.

Chromosome pair	Total chromatin length in μ	Total banded region in μ	Banding percentage
I	4.80	2.667	9.725
II	3.76	1.20	4.376
III	3.32	1.245	4.581
IV	3.27	0.9721	3.545
V	2.96	0.1184	0.4316
VI	a. 2.84	a. 0.4057	a. 1.8233
	b. 2.84	b. 0.0	b. 0.0
VII	2.50	0.3621	1.320
VIII	2.41	0.6025	2.199
IX	1.56	0.0	0.0

Fig. 2.1 Premetaphase : O-banded somatic chromosomes (X3500).

Fig.2.2 Metaphase : O-banded somatic chromosomes.

Fig. 2.3 Metaphase : O-banded somatic chromosomes.

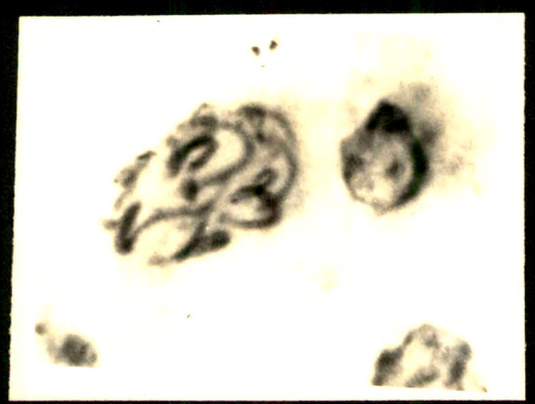

FIG. 2.1

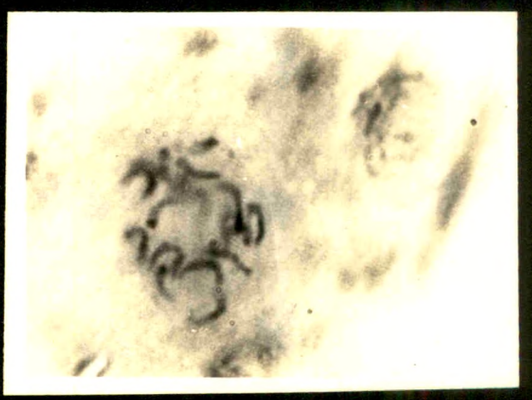

FIG. 2.2

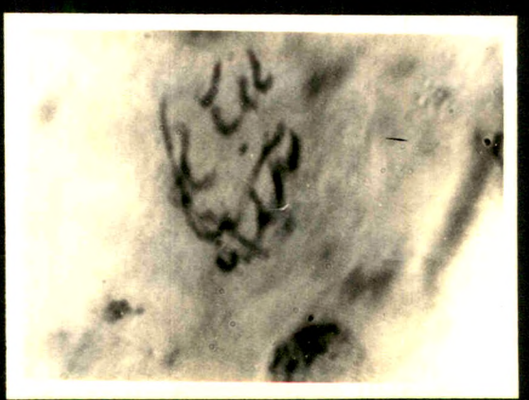

FIG. 2.3

long arms (Fig. 2.2). Orcein banding revealed a characteristic banding pattern which allowed the identification of 8 pairs of chromosomes of the diploid P. incarnata (2n = 18)complement in present investigation, while chromosome pair number 9 was without O-banded region. In P.incarnata percentage of total banded chromosome length is 27.2895 (Table 2.1).

DISCUSSION

As considered by Bobrow and Cross (1974) and Schweizer (1974) that in all chromosome banding techniques the quality of staining is somewhat erratic. As to overcome these effects author has selected only those metaphase cells showing maximum banding response. Uniformity in pretreatment, SSC treatment, and orcein staining was maintained.

In the O-banding procedure, a strong solution of sodium saline (preferably 6 x SSC) was used directly for root tips omitting the barium step in the Giemsa C-banding procedure of Vosa and Marchi (1972).

In the present investigation it was observed that in the chromosome pair number 6, the O-banding pattern of the homologues was certainly different (Table 2.1). Non-homology in chromosome pair indicates that the P. incarnata has had hybrid origin. NOR-chromosome homology of Passiflora incarnata to that of Passiflora edulis f. flavicarpa suggests that it might be derived either from Passiflora edulis or Passiflora

Passiflora edulis f. flavicarpa (Dixit, 1979). One more possibility is that they all have a common ancestor. The general banding features resemble other Passiflora species those described by earlier workers with minor deviations (Dixit, 1979). There may be minor variations in banding patterns at the inter-strain level. The differences in optimal conditions and treatments required for discerning the banding in different plant types may be mainly due to the differences in chromosomal condensation vis-a-vis differences in the amount, type and pattern of distribution of associated chromosomal proteins. Such differences may be responsible for the different classes of heterochromatin and heterochromatin stainability (Vosa, 1973; LaCour, 1978).

Narayan and Rees (1976) and Rees and Narayan (1977), were of the opinion that quantitative change in DNA mainly involves the repetitive, as distinct from unique, fraction of the chromosomal DNA which at the cytological level has been correlated with heterochromatin and euchromatin respectively. The bands however, may be due to preferential extraction of some protein components associated with DNA (Comings et al., 1973). Each banding treatment apparently produces specific qualitative and quantitative changes in the proteins of chromosomes. The diverse treatments producing the same type of chromosome banding have both common and unique effects on the chromosomal proteins. The manifestation of O-banding technique is dependent on DNA-protein linkage as well as treatment

procedures and comparative analysis of its correlation with Q, G, C, and R-bands will prove its usefulness in banding techniques.

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TRYPSIN-ORCEIN BANDING IN PASSIFLORA INCARNATA

INTRODUCTION

Cytogenetic studies have received tremendous impetus from chromosome banding techniques which allow understanding of chromosome structure and organization on the one hand, and chromosome identification and evolutionary relationships on the other. Introduction of banding techniques has facilitated the study of finger details of chromosomes and has provided a valuable tool for tracing species relationships (Vosa, 1975,1977).

Various techniques have been developed using Giemsa and fluorochrome dyes, and methods have been devised to give a variety of banding patterns by slightly modifying standard banding procedures (Layania, 1978). The use of proteolytic enzymes for demonstrating banding patterns in chromosomes was introduced by Dutrillaux et al., (1971) and this method was modified later by several authors (Seabright, 1972; Wang and Fedoroff, 1972). Recently, still other techniques have become available, one of which uses potassium permanganate (Utakoji, 1972). The present study deals with the trypsin-Orcein banding in P. incarnata.

MATERIALS AND METHODS

Root tips of P.incarnata have been pretreated and fixed according to method described in Orcein-banding technique. The method followed is that of Lavania and Sharma (1979).

The root tips are treated in 1% trypsin solution with 0.1 M phosphate buffer (pH 7.0) either for 5-10 minutes at 37°C or 30-60 minutes at 0°C, and rinsed in water; stained in 1.5% acetic-orcein : 1 N HCl mixture (19:1). Squashed as usual. If necessary, the duration of staining is varied to obtain proper contrast of differentiated regions. Dark and light stained regions on the chromosomes become clearly visible.

Only metaphases showing maximum banding response were selected for detail analysis. The drawings were made with the aid of Camera-Lucida. The metaphases were photographed and enlarged.

RESULTS

Table 2.2 indicates the proportion of Trypsin-orcein banded chromosome length expressed as percentage of total chromosome length. Trypsin-orcein banding technique allowed, the identification of atleast 6 pairs of chromosomes of the diploid P.incarnata (2n = 18) complement in the present study. (Figs. 2.4,2.5 & 2.6). In the present investigation the chromosome pair I (its homologues) and VI (its homologues) are different as banding region is concerned, although they are morphologically similar (Table 2.2) P.incarnata has a characteristic single trypsin-orcein band at the centromeres in chromosome pairs VII, VIII and IX (Fig. 2.4). In P.incarnata percentage of total banded chromosome length is 25.5754 (Table 2.2).

Table 2.2 Proportion of Trypsin-Orcein banded chromosome length expressed as percentage of total chromosome length.

Chromosome pair.	Total chromatin length in μ	Total banded regions in μ	Banding percentage
I	a 4.80	a 2.40	8.752
	b 4.80	b 0.0	0.0
II	3.76	0.0221	0.0805
III	3.32	0.0174	0.06345
IV	3.27	3.27	11.92
V	2.96	1.269	4.623
VI	a 2.84	a 0.0106	a 0.03647
	b 2.84	b 0.0	b 0.0
VII	2.50	0.0	0.0
VIII	2.41	0.0	0.0
IX	1.56	0.0	0.0

Fig. 2.4 Premetaphase : Trypsin - Orcein banded somatic chromosomes.

Fig. 2.5 Metaphase : Trypsin-Orcein banded somatic chromosomes.

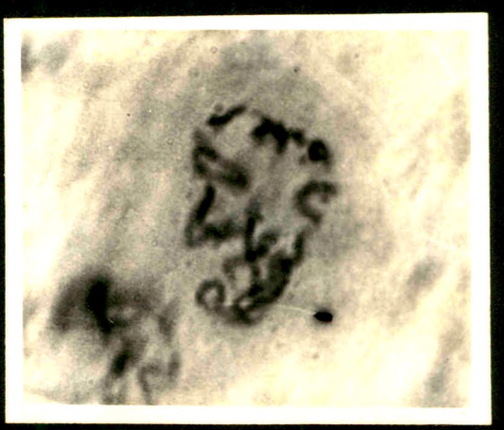

FIG. 2.4

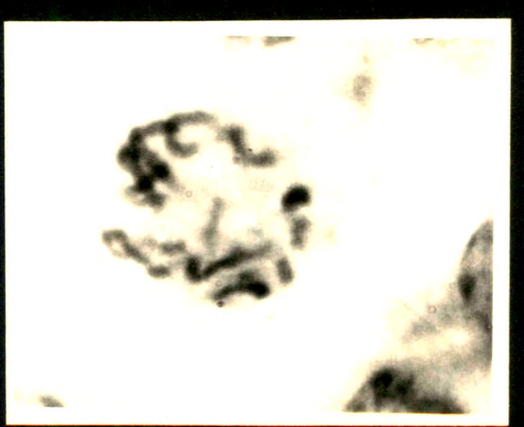

FIG. 2.5

DISCUSSION

Trypsin-orcein banding was a new technique which was successfully used for species belonging to Leguminosae, Commelinaceae and Liliaceae by Lavania and Sharma (1979). The position of these dark staining regions (O-bands) is species specific and may be present either in the centromeric, telomeric or intercalary position depending upon the species. The same is true in present investigation (Figs.2.4,2.5,2.6).

It has been proposed that a major factor in C-banding appears to be the presence of non-histone proteins that bind specifically to centromeric heterochromatin and protect it from extraction by sodium hydroxide and barium-hydroxide. This results in intense staining of C-bands and poor staining of the rest of the chromosome (Comings,1975). A similar mechanism is probably involved as well in O-banding, which may stain repeats of different categories (Sharma, 1975). Orcein is an amphoteric dye which stains both DNA and protein, and the primary reaction in chromosomes involves polypeptide tertiary amines (Sen, 1965).

In addition to the nucleic acid enzymes, trypsin and chymotrypsin are applied for the digestion of the basic protein while pepsin removes both histone and more acidic proteins (Kaufmann et al., 1950; Daly et al., 1951 and Kaufmann et al, 1951 In fact, the application of these 2 enzymes, combined with nucleas

has helped in the interpretation of chromosome structure as an interconnected system of 2 types of necleic acids and proteins. Controlled treatment with trypsin results in digestion of specific proteins from certain sites, where, possibly because of the unique DNA sequences, the binding is comparatively weak. However, the induction of C-bands with trypsin at freezing temperatures, may involve trypsin as a chelator as well (Dev. et al., 1972; Deavan and Petersen, 1973).

In the present investigation author has bome across with different banding patterns with various kinds of pretreatments, maceration treatments, enzyme treatments and staining procedures. However, the modification in Trypsin-orcein banding is useful to ascertain the structure and form of the chromosome than the other techniques, viz. C-banding and C-banding. It is essential to improve the technique which will be very practicable, gives better results than the techniques which are employed today.

GIEMSA BANDING IN PASSIFLORA INCARNATA

INTRODUCTION

The recent introduction of chromosome banding techniques has revolutionized cytogenetics by providing information on the complexity of DNA sequences discernible in the form of bands on the chromosomes, (Lavania, 1978). The differential Giemsa staining of heterochromatin was first observed in mouse chromosomes by pardue and Gall (1970). Subsequently extensive studies on Giemsa banding have been conducted and different techniques developed (Paris Conference, 1972, Nobel Symposium, 1972, Hsu, 1973, Vosa 1975). These banding techniques have proved to be valuable for identifying chromosomes as well as for providing information about their structural and molecular organization. Most early studies were based on mammalian chromosomes, but now several C-banding techniques applicable to Plant materials have been reported (Vosa and Marchi, 1972, Natarajan and Natarajan, 1972, Schweizer, 1973, Filion 1974, Kimber et al., 1976). Banding techniques have been applied to a relatively limited extents in plants because of the technical difficulties in making cell-wall free chromosome preparation (Sarma and Tandon 1974, Natarajan and Sarma, 1974, Noda and Kasha, 1978).

MATERIALS AND METHODS

For Giemsa C-banding, the technique of vosa and Marchi (1972) was followed. The roots were pretreated in 0.05% colchicine at room temperature for about 4 hours and

fixed overnight in Carnoy's fluid. Fixed root tips were hydrolysed in 1 N HCl for 40-60 seconds at 60°C. and squashed in 45% acetic acid. Cover glasses were removed in absolute ethanol and the preparations allowed to dry in air. Air dried samples were treated with a freshly prepared saturated aqueous solution of barium hydroxide at 10-15°C. for 10 seconds to 7.5 minutes depending upon the material; washed throughly with distilled water and incubated in 2 X SSC (0.3M NaCl and 0.03 M Na-Citrate, 1:1) at 60°C. for 30-60 minutes, rinsed in distilled water and stained in 3% diluted BDH Giemsa stain in M/15 phosphate buffer pH 6.8. The stained preparations were air-dried and mounted in DPX. Microphotographs were taken at x 1500.

In case of P. incarnata to obtain better differtiation, the technique had to be slightly modified by omission of the barium step according to Mok and Mok (1976) or author's own modification applying directly incubation in 0.1 M phosphate buffer pH 6.8, at 60°C for 1 hour followed by staining for C-banding patterns.

RESULTS

The length of each band and interband in the metaphase chromosomes was measured and expressed as the ratio of total chromosome length (Table 2.3). Giemsa banding technique allowed the identification of 8 pairs of chromosomes while chromosome pair VI was without banded.

Table 2.3 Proportionof Giemsa banded chromosome length expressed as percentage of total chromosome length.

Chromosome pair	Total Chromatin length in μ	Total banded region in μ	Banding percentage
I	4.80	3.20	11.69
II	3.76	0.9397	3.427
III	3.32	2.211	8.060
IV	3.27	1.31	4.777
V	2.96	2.96	10.79
VI	2.84	0.0	0.0
VII	2.50	1.25	3.605
VIII	2.41	1.20	4.375
IX	1.56	0.9354	3.475

Fig. 2.6 Premetaphase : Giemsa banded somatic chromosomes.

Fig. 2.7 Metaphase : Giemsa banded somatic chromosomes.

2.8 Metaphase : Giemsa banded somatic chromosomes.

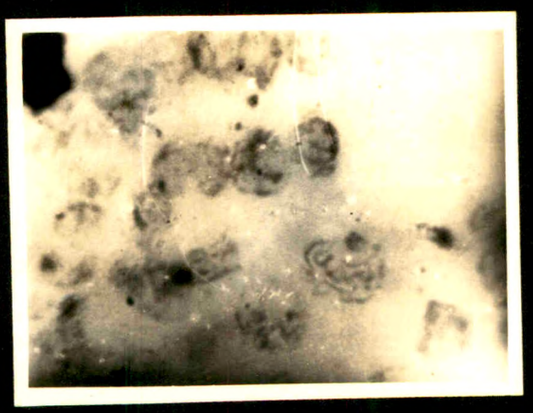

FIG. 2.6

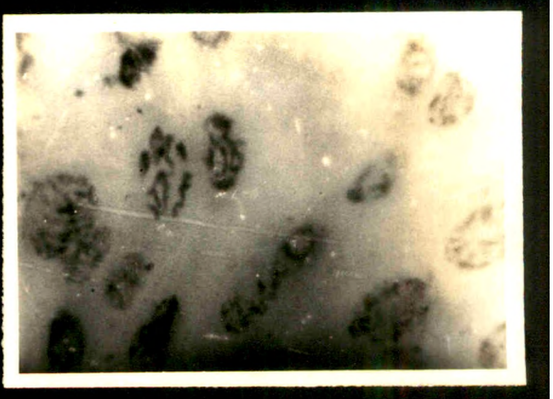

FIG. 2.7

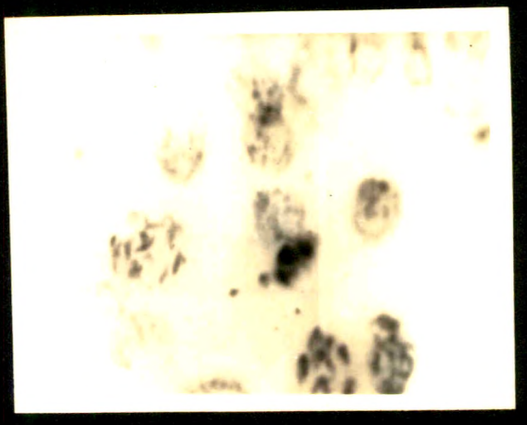

FIG. 2.8

In P.incarnata percentage of total banded chromosome length was 50.1864 (Table 2.3).

DISCUSSION

The Giemsa stain C-bands are thought to represent the constitutive heterochrmatin, to contain no structural genes, and to replicate late during the 'S' phase of cell cycle. Some researchers have considered constitutive heterochromatin to be composed primorily of repetitive DNA (Pardue and Gall 1970, Arrighi et al., 1970, Yunis and Yasmineh 1970, 1971, Hsu 1973, Vosa 1975).

The present study indicates that the banding pattern of chromosomes obtained after Giemsa staining technique is less, reproducible than Trypsin orcein banding pattern in P.incarnata. Various researchers have modified the original technique of Giemsa banding of plant chromosomes (Schweizer, 1974, Moda and Kasha 1978). In plant system these banding techniques have been of limited use because of the difficulty of air drying of solid tissue, one of the essential steps for Giemsa and flurochromes staining.

Present investigation reveals that the modifications which are suggested by Mok and Mok (1976) as well as by the author are not so satisfactory to produce distinct Giemsa banding pattern in chromosomes of P.incarnata. Further research is required to design a method which can be efficiently applied to the chromosomes like that of P.incarnata.