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Karyological and cytophotometric study of explant derived clones of non-polysomatic and polysomatic species of *Kniphofia*

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Abstract

Cytological and cytophotometric analysis of root tips of regenerated plants, derived from rhizome explant of *Kniphofia nelsonii* and *Kniphofia uvaria*, revealed marked difference in behaviour of chromosomes and level of 4C nuclear DNA content. Karyotypic stability could be retained in all 52 regenerants of *K. nelsonii* whereas in *K. uvaria* out of 75 regenerants analysed, 12 plants were exclusively diploid and the rest 63 plants were predominantly diploid comprising variable amount of aneuploid and tetraploid cells. Cytological data was further confirmed by nuclear DNA content estimation. Alteration in the structure of chromosomes could also be noted in 57 regenerants of *K. uvaria* giving rise to two new karyotypes. The use of polysomatic tissue for securing variants *in vitro* in *K. uvaria* has been suggested.

Introduction

Plant regeneration *in vitro* is either associated with stability or instability of its genomic complements (Fish and Karp 1986, Armstrong and Phillips 1988, Evans *et al.* 1986). Most of the reports on cytogenetic analysis are made on plants regenerated from callus, whereas studies on the genetic constitution of direct explant derived regenerants are meagre (Cassels and Carney 1987, Detrez *et al.* 1989).

The present paper deals with behavioural patterns of chromosomes as well as 4C nuclear DNA content in regenerants derived from direct organogenesis of both polysomatic and non-polysomatic species of *Kniphofia*.

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Material and Methods

Plant regeneration: Plants could be regenerated directly from the rhizome explants of *K. nelsonii* and *K. uvaria* using Murashige and Skoog's (MS) 1962 basal medium following the method of Nayak and Sen (1990). A total of 52 regenerants of *K. nelsonii* and 75 regenerants of *K. uvaria* derived from one source plant, were subjected to cytological and cytophotometric analysis.

Cytological analysis: Root tips of source and regenerated plants of *K. nelsonii* and *K. uvaria* were pretreated with saturated solution of paradichlorobenzene : 0.002M hydroxyquinoline (1:2) at 10-12 °C for three hours. The fixative used was 1:3 acetic acid : ethanol. Fixation was followed by staining with aceto-orcein which involves heating the root-tip for 3-4 s in 2 % aceto-orcein and 1M HCl mixture (9:1), while keeping in the stain mixture for 1 h and squashing in 45% acetic acid. A minimum of 25 metaphase plates were scored from each regenerant of both the species by counting chromosomes in intact cells only. Morphology of chromosomes has been described following Battaglia's (1955) classification.

Cytophotometric analysis: For an estimation of nuclear DNA content, fixed root-tips of the source plant and regenerants were hydrolysed with 1M HCl at 58 °C for 10 min, stained with Schiff reagent for 1 h and squashed in 45 % acetic acid. Slides were made temporal. The DNA content was measured with *Leitz Wetzlar Aristophot MPV* microspectrophotometer by a single wavelength (550 nm) method (Macleish and Sunderland 1961) from metaphase plates using an objective of 40×. Measurements of the diaphragm were kept constant at a radius '40'. From each regenerant 50 metaphase plates were measured from 5 slides. For every slide 10 randomly selected metaphase plates were scored. DNA content was measured on the basis of absorbance expressed in terms of a relative arbitrary units, which were then converted to picograms by using Van't Hof's (1965) 4C nuclear DNA value of *Allium cepa* as a standard (67.1×10^{-12} g). Root-tips of *Allium cepa* were stained simultaneously with those of regenerants. To study the significant difference of 4C DNA content, if any, between the source plant and each regenerants *t*-test (Sokal and Rohlf 1973) was carried out.

Results

Stable regenerants from *K. nelsonii*: The source plant and 52 regenerated plants from a single clone of *K. nelsonii*, revealed total diploidy showing 2n = 12 chromosomes. Normal karyotype is represented as 8A+2B+2C (Fig. 1). Gross structural changes in any of the chromosomes of the regenerants could not be noted.

A cytophotometric estimation of nuclear DNA content revealed a unimodel type of DNA distribution with a single peak (Fig. 2). The 4C DNA content among regenerated plants varied from 31.3 - 31.5 pg, showing non-significant variation as compared to mean nuclear DNA content (31.4 pg) of the source plant (Table 1).

Variation in chromosome number, structure and DNA content in regenerated *K. uvaria* plant: In *K. uvaria*, out of 75 regenerants analysed, 12 plants were noted to be exclusively diploid (2n=12) and the remaining 63 plants were predominantly diploid.

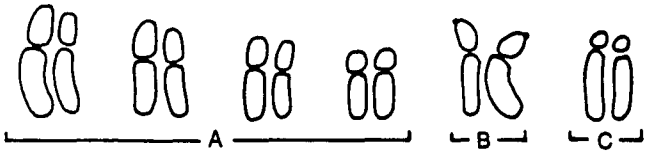

Fig. 1: Normal karyotype of the source plant of *K. nelsonii* showing 2n=12 chromosomes (x 1400).

Table 1. Karyotypic status and mean 4C DNA content of source and regenerated plants of *K. nelsonii* and *K. uvaria.*

Species		Number of plants analysed	Karyotypic status	Mode of DNA distribution	Mean 4C nuclear DNA content [pg] ± S.E.
K.. nelsonii	source plant	1	exclusively diploid	unimodal type with only 4C peak	31.4 ± 0.1
	regenerants	52	exclusively diploid	unimodal type with only 4C peak	31.1 - 31.5 ± (0.1 - 0.2)
K. uvaria	source plants	1	predominantly diploid	polymodal type with peaks 4C, <4C, >4C	31.8 ± 0.5
	regenerants	12	exclusively diploid	unimodal type with only 4C peak	31.4 - 31.7 ± (0.1 - 0.2)
		42	predominantly diploid	polymodal types with several peaks	29.5 - 31.2 ± (0.5 - 0.7)
		13	predominantly diploid	polymodal types with several peaks	28.3 - 28.9* ± (1.0 - 1.3)
		8	predominantly diploid	polymodal types with several peaks	27.3 - 27.8** ± (1.4 - 1.6)

* significant at 0.02 level
** significant at 0.001 level

Of the 3250 metaphase cells studied from those 63 plants, diploid cells predominated in 2600 cells. Ninety one cells were tetraploid showing 24 chromosomes and

aneuploids with different chromosome numbers as 4, 8, 9 could be recorded in 559 cells.

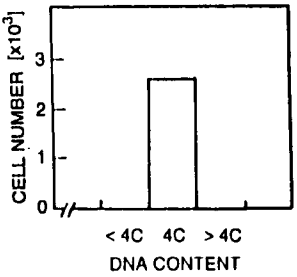

Fig. 2. Unimodal distribution of nuclear DNA content in regenerants of *K. nelsonii.*

The cells were intact and chromosome counts were carried out carefully. Such low aneusomatic cells do not possibly contribute to further development. The source plant, however, showed polysomaty in 5% of the cells showing both aneuploid and tetraploid chromosome numbers. Such polysomatic plants were reported earlier (Sen 1973).

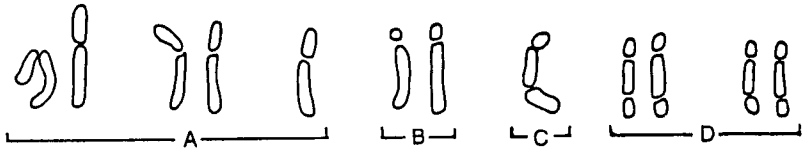

Fig.3. Normal karyotype of the source plant of *K. uvaria* showing 2n=12 chromosomes (× 1300).

Detailed structural analysis revealed a stable karyotype in 18 regenerants and alteration in karyotypes among 57 regenerants. Stable karyotypes were represented as 5A+2B+1C+4D (Fig. 3). Of remaining 57 plants, in 55 plants alteration could be located in the type 'A' chromosome where the constriction was shifted from submedian to almost median (Fig. 4). The new karyotype was represented as 4A+1A'+2B+1C+4D. Percentage of such variant cells was noted in about 75% of the cells among 55 regenerants. The type A' could be noted even in aneuploid cells. In the remaining two regenerants, in addition to an 'A' type, a 'D' type chromosome was also altered shifting one short arm to the opposite end (Fig. 5). Such altered karyotypes were represented as 4A+1A'+2B+1C+2D+2D'. These variants were noted in about 7% of the cells among two regenerants.

Cytophotometric analysis revealed that out of 75 regenerants analysed, 12 plants were exclusively diploid showing unimodal DNA distribution with a peak corresponding to 4C nuclear DNA content. The mean 4C nuclear DNA content of these regenerants varied from 31.4 pg to 31.7 (Table 1), whereas the source plant contained an average of 31.8 pg of DNA per nucleus. The remaining 63 plants which

were predominantly diploid showed a broad nuclear DNA content distribution of polymodal type (Fig. 6) with 4C levels occurring at a higher percentage (70%) along with cells of 4C and 4C values.

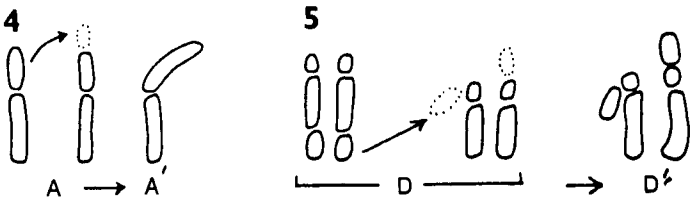

Fig. 4. Alteration of A type chromosome (× 1500).
Fig. 5. Alteration of D type chromosome (× 1500).

The mean 4C nuclear DNA content in 63 regenerants was always less than of the source plant indicating frequent occurrence of hypodiploid cells in the regenerated plants (Table 1). Statistical significance of values of mean DNA content of each regenerant was determined by *t*-test (Table 1).

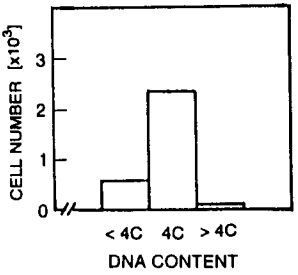

Fig. 6. Polymodal distribution of nuclear DNA content in regenerants of *K. uvaria*.

Discussion

Plants regenerated from explant by direct organogenesis mostly exhibit cytological stability (Krikorian *et al.* 1981, Sengupta and Sen 1987). A few cases are, however, reported showing variations among direct explant derived regenerated plants (Van Harten *et al.* 1981, Cassells and Carney 1987). The preexisting karyotypic status of the source plant influences the behavioural pattern of chromosomes in regenerants (Reisch 1983).

In *K. nelsonii*, all 52 regenerants analysed were exclusively diploid showing similar karyotype as that of the source plant. Cytophotometric analysis further confirms the cytological data revealing no significant variation in 4C nuclear DNA content among regenerated plants. Participation of only 4C cells in the *in vitro*

organization of shoot buds is evidently attributed to the absence of polysomaty in the source plant.

On the other hand, in *K. uvaria* out of 75 regenerants studied, 12 plants were exclusively diploid and 63 plants were predominantly diploid. The plants showing predominantly diploid chromosomes with aneuploid and tetraploid chromosome numbers can be attributed to the polysomatic state of the source plant. The polysomatic nature of the source plant has been confirmed both by chromosome counts and nuclear DNA estimations. Polysomaty also includes aneuploid cells. Therefore the species *K. uvaria* can be considered as a rare mixoploid. *In vitro* organisation of shoot buds resulted in the formation of exclusively diploid regenerants due to the fact that possibly polyploid cells did not participate in organogenesis. The significantly high and low participation rate of polyploid cells can lead to regenerants showing significant and non-significant amounts of variant cells respectively. Moreover, presence of more hypodiploid cells in predominantly diploid regenerants lowered the mean 4C nuclear DNA value ranging from 28.9 pg to 31.2 pg from the mean 4C nuclear DNA value (31.8 pg) of the source plant.

Supporting evidence of no or low participation of polyploid nuclei of source tissue during *in vitro* organisation of adventitious buds directly from explant tissue, has also been reported (Detrez *et al.* 1989) in *Beta vulgaris*.

Detailed cytological analysis revealed structural alteration of chromosomes among 57 regenerants; the origin of which could not be precisely traced to the source plant. Their origin from the source explant, however can not be ruled out. The genetic modifications, such as chromosomal rearrangements and variations in a direct explant derived population in a culture, are already on the record (Larkin *et al.* 1985).

Thus it can be concluded, that direct organogenesis from an explant of non-polysomatic species of *K. nelsonii* might be adopted for production of true to type diploid genotypes. The polysomatic species of *K. uvaria* may be explored for securing novel genotypes through propagation *in vitro*.

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