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Role of permanent dicentric systems in carrot somatic embryogenesis

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Summary. A permanent dicentric chromosome system was studied on carrot cultures and regenerated somatic embryos at different stages of development. The large chromosomal variability of the cultures and the presence of the breakage-fusion bridge cycle did not interfere with the initial developmental process up to the seedling stage but subsequent growth proceeded only if healing of the broken ends or dicentric loss had occurred. The behaviour of the dicentric chromosome in culture and during somatic embryogenesis is discussed in relation to chromosomal variability, abnormal development and the somaclonal variation that such mechanisms may generate in regenerated plants.

Key words: Carrot – Somatic embryogenesis – Permanent dicentric system – Chromosome variability

Introduction

The study and characterization of specific genetic mechanisms generating variability in culture and their transmission to regenerated plants are of particular interest in view of the role that somaclonal variation presents for plant improvement. Chromosomal aberrations and/or somatic gene rearrangements occurring early in culture may be responsible for such variability with the possibility, through plant regeneration, of transmitting such rearrangements into the germ line cells (Larkin and Scowcroft 1981).

Having ascertained the frequent presence of dicentric chromosomes, permanently transmitted through the breakage-fusion-bridge cycles in carrot cell cultures of different origins, we have utilized the in vitro somatic embryogenesis process to study the fate and the possible role of such aberrations on the development of the different embryonic phases (globular, heart, torpedo stages) and subsequent plant growth.

Material and methods

Three cell lines were studied: C15 – the carrot double mutant (α-amanitin resistant, 8-azaguanin resistant) unable to regenerate (Lo Schiavo et al. 1983), and F5 and F11 – two derivatives that have re-acquired regenerating potential after hybridization with wild-type protoplasts (Lo Schiavo et al. 1983).

Media and experimental procedures for the isolation of mutants and fusion experiments have been described by Lo Schiavo et al. (1983).

Regeneration was carried out as in Vergara et al. (1982) and consisted of the transfer of the cells, at a known density, after proper filtration through nylon sieves, to a hormone-free medium (as cellular units or C.U./ml). To enhance differentiation, some cultures were treated, prior to embryogenesis, for 15 days with 1% proline (Nuti Ronchi et al. 1984). The number of somatic embryos was counted under 40X magnification 20 days after transfer of cultures to the hormone-free medium.

Cytological analyses were performed on cells and embryonic stages both pretreated for 3 h with 0.1% colchicine, or lacking this pretreatment, fixed in Carnoy (Alcohol/acetic acid 3:1 v/v) and submitted to Feulgen reaction. Single developmental stages were squashed and analyzed separately. Only complete well-spread metaphase and medium-final anaphases were studied.

Results

Figure 1a shows the chromosome distribution of C15, F5 and F11 cultures: depending on ploidy, one or more dicentric were always present. The range was always 25–100 but C15 and F5 has a mode around 30 whereas for F11 the mode was 60. In all lines, from 50% to 70% of the anaphases showed a criss-cross or interlocked

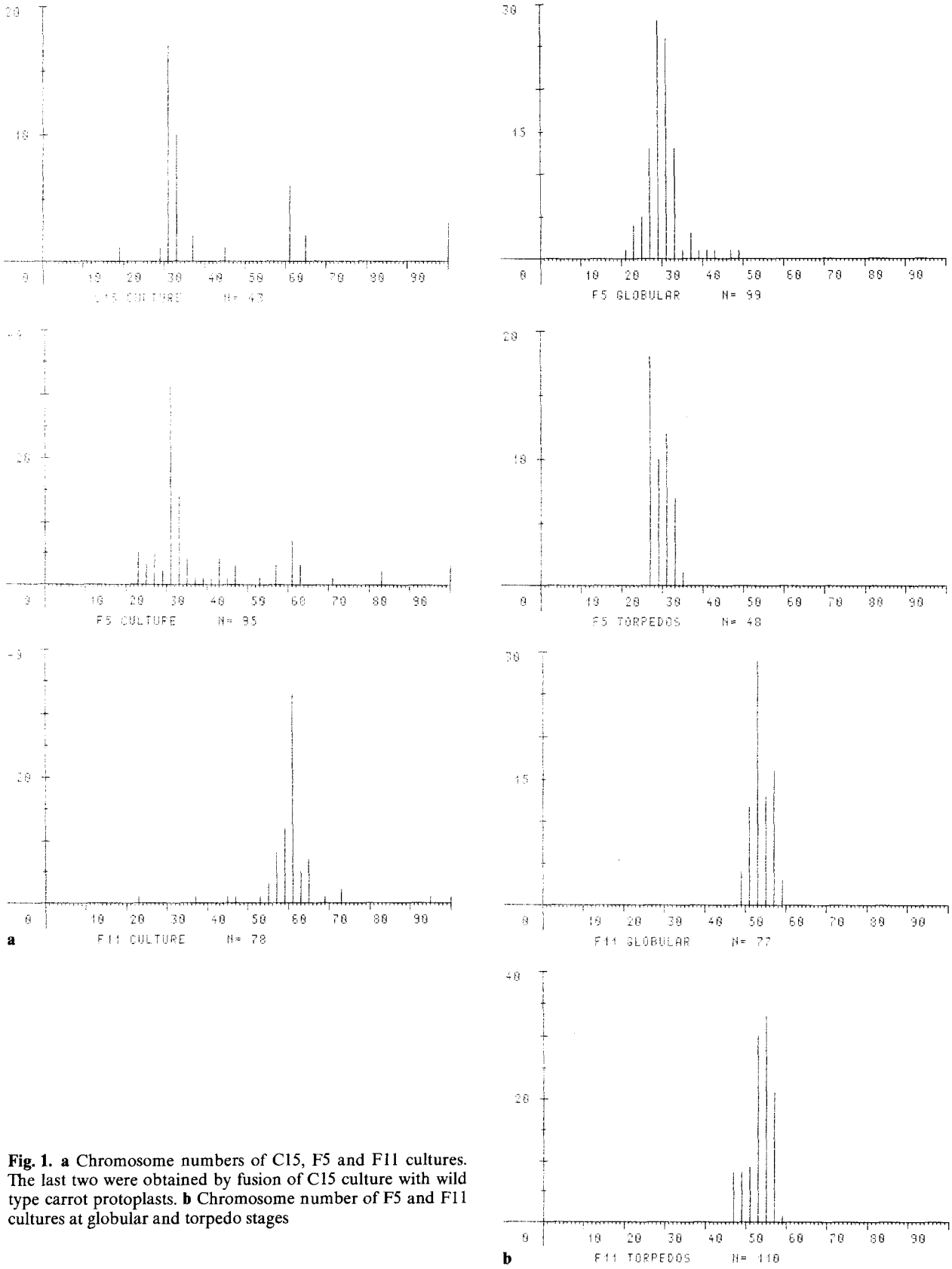

Fig. 1. **a** Chromosome numbers of C15, F5 and F11 cultures. The last two were obtained by fusion of C15 culture with wild type carrot protoplasts. **b** Chromosome number of F5 and F11 cultures at globular and torpedo stages

chromosome separation, confirming that a breakage-fusion-bridge cycle was responsible for the perpetuation of the dicentric condition (Darlington and Wylie 1953). Such dicentric chromosomes persisted throughout regeneration since it was always detected in single globular and torpedo stages developed from the F5 and F11 cultures.

Figure 1 b shows that the F5 globular stage had a reduced variability which was significantly more restricted when compared with F5 cultures: the former having a mode around 30 at the torpedo stages. No relevant differences were found between the F11 globular and torpedo stages; in both stages the range of chromosomes being between 50 and 60.

In all cases, single globular and torpedo stages showed different chromosomal numbers in the same structure. In the analyzed forms, cell numbers were clustered around 29–33 for F5 and 50–54 for F11 but, as shown by Fig. 1 b, only F5 torpedos showed such range of variability in the majority of metaphases. Such variability derived from the alternative transmission of the dicentric which can produce cells with different numbers of centromeres (± 2) even from cells with one dicentric (depending on the casual separation of centromeres, misdivision, lagging, non-disjunction etc. at anaphases) (Sears and Camara 1952).

Most of the regenerated forms of the F5 cultures were aberrant, showing, after 20 days of development, structures ranging from hypertrophic globular forms (with or without chloroplasts) with internal central xylematic differentiation to monstrous torpedos lacking cotyledons and tap-roots, rootless plantlets or single roots without cotyledons.

Table 1 shows the percentage of fairly normal plantlets obtained from these aberrant structures after transfer to fresh medium for 20 additional days of culture. Rootless plants were able to resume growth, forming lateral adventitious roots.

Table 1. Percentage of normal plantlets obtained, after 20 additional days of culture in fresh medium, from F5 regenerated aberrant stages

Stages	Total no.	No. of plantlets	%
Hypertrophic green globular stages	40	3	7.5
Hypertrophic globular stages	40	17	42.5
Hypertrophic heart stages	40	31	77.5
Roots	10	6	60
Cotyledonless and rootless structures	40	32	80
Torpedo stages	20	18	90
Rootless plantlets	40	39	97.5

One hundred and seventy plantlets obtained from the aberrant forms were again submitted to a third passage to fresh medium and allowed to grow, but the majority died or stopped growing, only 24 (14%) surviving to the stage of permitting transfer to pots for further observations. According to the analysis of the root-tips the chromosome numbers of 17 of such plants showed no dicentric bridge configurations, three plants having 28, nine plants 29, and five, 30 chromosomes. The analysis on F11 was not carried out as only aberrant regenerant were obtained that died before reaching adult stage.

Discussion

Our data confirm previous observations in *Nicotiana* (Nuti Ronchi et al. 1981) that even extreme chromosomal variability does not interfere with the initial developmental process, though such variability is certainly reduced in comparison to the original cultures. There is, however, a greater restriction on the chromosomal constitution when the meristem has to exercise control over the differentiation of its cellular products during plant growth.

It is well documented that gross chromosomal changes, while possible in culture, are selected against during plant regeneration (Shepard et al. 1980; Burk and Chaplin 1980; Grout and Crisp 1980; Novak 1980) but our data suggest that it is only at the growth stage that selection works since extremely unbalanced shoots and embryos can be regenerated but only balanced genomes, at least in *Nicotiana* and carrot, are able to survive beyond seedling stage. As already suggested (Nuti Ronchi et al. 1981; Nuti Ronchi 1981), different controls may operate in the blocking out of tissue at different stages of plant growth (Steeves and Sussex 1972). The analysis of regenerated globular and torpedo stages revealed that inside every single structure different chromosomal numbers beyond the variability expected by the random segregation of dicentric were present. Since the single cell origin of somatic embryos is well documented (McWilliam et al. 1976), such range of variability may be explained by a self-perpetuating instability at the cellular level (Nuti Ronchi et al. 1981). If this is the case, the reduction in variability seen in late embryos as compared with early stages should mean either that this endogenous source of variability stops operating in the course of development, or that selection is more stringent on later rather than on earlier stages.

Since the occurrence of the breakage-fusion-bridge cycle is fairly frequent (Bayliss 1975), together with other gross karyotypic changes, plants arising from cells which had been undergoing such a cycle would presumably offer a great

variability since although the healing of the broken ends apparently may restore normal karyotype, numerous deficiences, duplications, transpositions or other events have occurred. The role of these aberrations in creating somaclonal variation is probably much more relevant than previously considered. The hypothesis that transposition-like events could be related to chromosomal instability (McClintoch 1941, 1942) is an attractive one, especially because it would be the most efficient mechanism to generate and propagate heritable changes for quick adaptability to new environments. Only the development of an appropriate marker and molecular genetic techniques would, in the future, allow such a hypothesis to be tested.

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