Theor Appl Genet (1984) 67:267–271



# Karyotypic variability in plants of *Solanum melongena* regenerated from callus grown in presence of culture filtrate of *Verticillium dahliae* *

R. Alicchio, C. Antonioli and D. Palenzona
Institute of Genetics, University of Bologna, Via F. Selmi 1, I-40126 Bologna, Italy

Received July 1, 1983; Accepted August 10, 1983
Communicated by G. Wenzel

**Summary.** Plantlets were regenerated from calli derived from leaf explants of three genotypes of *Solanum melongena* (two parental genotypes and their hybrid). The cytological analysis showed that a) plants regenerated were all mixoploid, b) toxic medium (basal medium added with filtrate culture of *Verticillium dahliae*) was able to evidence karyotypic differences between genotypes not displayed by plants regenerated from callus grown on control medium, c) chromosomal mosaicism persists up to plant maturity and also in the selfed progeny. The results are discussed in terms of a selective process involving genes controlling chromosome number and/or a direct effect of toxic medium on the activity of the same genes.

**Key words:** Callus culture – Chromosome number – Regeneration – *Solanum melongena* – *Verticillium dahliae*

## Introduction

There is general agreement on the cytological instability of callus cultures although contrasting reports are available concerning the chromosomal number in regenerated plantlets. Many authors have found that in some species the changes of ploidy have such deleterious effects as to prevent plant regeneration (Torrey 1967; Murashige and Nakano 1965, 1967) and actually only diploid plants have been regenerated in many species independently from the degree of stability at the diploid level in the callus culture. On the other hand, findings by Sacristan and Melchers (1969), who have obtained polyploid and aneuploid plants regenerated from tobacco callus, have not confirmed the relationship between the increase of chromosome variation and loss of regenerative potential.

Mixoploidy in regenerated plants has been recently reported by Liu and Chen (1976), Sheridan (1975), Ogura (1976), Bennici (1978), Bennici and D'Amato (1978) and Mix et al. (1978). The presence of haploid, polyploid and aneuploid cells in regenerated plants suggests various considerations which need to be thoroughly examined:

a) the heterogeneous cell population in regenerated plants may reflect a similar chromosome number mosaicism in the callus;

b) the final chromosome distribution in regenerated plants may be determined either by selective equilibria (genetic, epigenetic and environmental) or by random drift;

c) a diplontic selection may take place throughout plant development for the euploid chromosome number.

Research was undertaken to evaluate some of the above considerations and in particular the relation between a cell selection for resistance to a fungal toxin and the successive chromosome dynamics in regenerated plants and in their selfed progeny.

## Materials and methods

Two lines of *Solanum melongena* (Lunga violetta di Romagna, LVR, and a plant introduction, P.I. 23327) and their $F_1$ hybrid were analyzed. Seeds were surface-sterilized with Na hypochlorite (5% w/v) and germinated on a filter paper bridge soaked in LS liquid medium (Linsmayer and Skoog 1965). Young leaves were excised from seedlings, cut into small pieces and put on solid medium LS plus 2,4 D 0.4 mg/l. After about one month callus was separated from the explant and subcultured every three weeks.

Callus at the fourth transfer was placed on a toxic medium obtained adding the filtrate culture of the fungus *Verticillium dahliae* to LS+2,4 D (control medium). In a previous work (Alicchio et al., submitted) we have demonstrated that the filtrate is able to inhibit callus growth of *Solanum melongena* and to induce, in young plants (by inoculating roots), some

* This research is supported by a grant from ERSO (Ente per la Ricerca e Sperimentazione in Ortoflorifrutticoltura e Sementi) – Regione Emilia Romagna

symptoms of wilt disease caused "in vivo" by the fungus. The isolation and purification of toxins from the filtrate culture of *Verticillium* has been reported by Pegg (1965), Stoddard and Carr (1966), Keen et al. (1970) and Nachmias et al. (1982).

The pieces of resistant callus which grew on toxic medium after one month were twice subcultured on control medium and then placed on a differentiating medium (without hormones) under 4000 lux with a 16/8 light/dark cycle at 25 °C. Buds appeared on the surface of the callus which, once separated from the callus and transferred onto fresh medium, gave origin to plantlets: these were placed in an unsterile bottle on a filter paper bridge soaked in tap water in order to develop roots (Alicchio et al. 1982). Plants were also regenerated from callus grown on control medium following the same culture procedure (subculturing, environmental conditions and media).

Chromosome counts were performed on the root tips of regenerated plants (R) pretreated with 8-hydroxyquinoline 0.02% for 5 h at 18 °C, fixed in ethanol-acetic acid 3 : 1 (v/v) and stained with aceto-orcein. Counts were obtained from a minimum of five cells and from at least two root apices for plant.

From a sample of LVR plants regenerated from toxic medium, only three plants survived when planted in soil; they were allowed to self pollinate and produced berries which gave a total yield from one to three hundred seeds for each plant. To control the karyotypic constitution of the selfed progeny (S), chromosome counts were made on root apices of germinated seeds belonging to different berries.

## Results

The cytological analysis, performed on root apices of plants regenerated from callus grown on control and toxic media reveals that no plant from each genotype consists exclusively of diploid cells, whereas the condition of mixoploidy and aneusomaty within each plant and each root is the rule (Fig. 1 a, b). These findings agree with the observations of various authors who found that "in vitro" regeneration frequently produces a mixoploid condition in root apical meristems.

In Fig. 2 the distribution of grouped chromosome number is drawn for regenerated plants; the modal karyotype of plants regenerated from callus grown on control medium is diploid ($2n=24$) in the three genotypes. Mean chromosome numbers reported in Table 1 are always lower than 24, the distance from diploid number being higher in hybrid genotype than in the two parental ones.

When plantlets are regenerated from callus grown in the toxic medium an increase in chromosome number variability and mean value in LVR and hybrids is evident (Table 1). In the first genotype the modal karyotype becomes hyperdiploid with a fairly large dispersion of low values, in the second one the modal karyotype is diploid but coexists with relatively high frequencies of chromosome numbers ranging from values lower than haploid and higher than tetraploid. The comparison among mean chromosome number of plants regenerated from control and toxic media are

**Table 1.** Comparison of mean and distribution of chromosome number among plants regenerated from callus grown on control (Co) and toxic (T) medium

| Genotypes | Medium | No. of mitoses | Mean chromosome no. | SD | "t" |
|---|---|---|---|---|---|
| LVR | Co | 214 | 22.54 | 5.06 | 11.17* |
| | T | 210 | 31.51 | 10.58 | |
| P.I. 23327 | Co | 69 | 22.52 | 7.18 | 0.108 |
| | T | 147 | 22.41 | 7.54 | |
| $F_1$ | Co | 59 | 21.66 | 5.57 | 4.60* |
| | T | 45 | 31.20 | 14.61 | |

* $P<0.01$

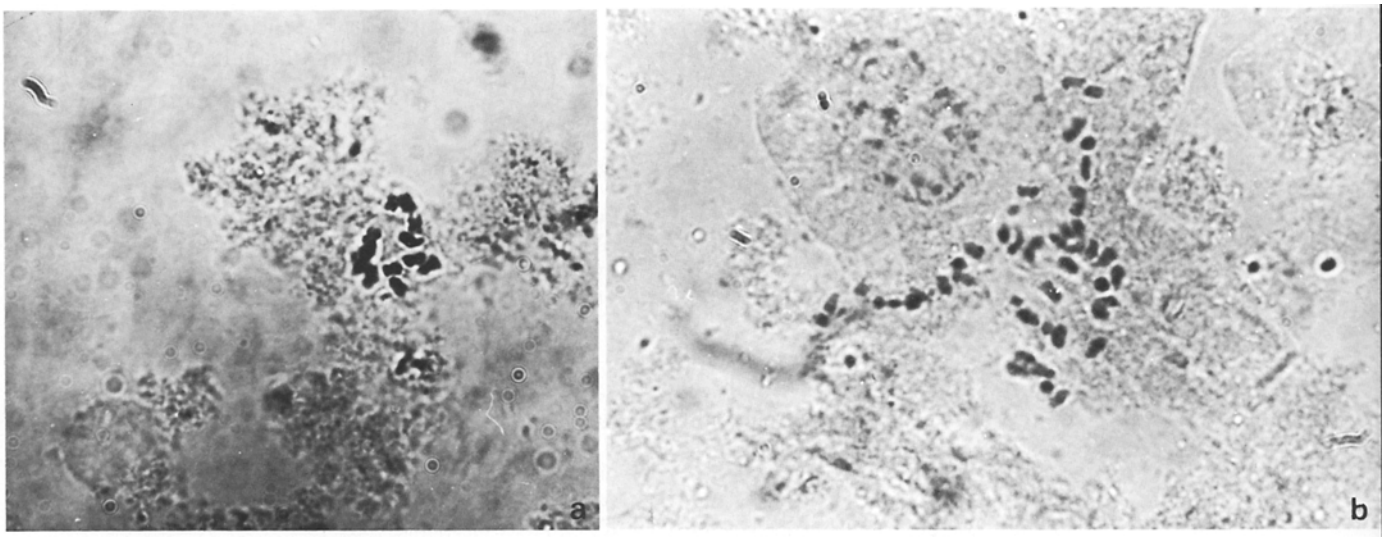


**Fig. 1.** Karyotypic variability in plants regenerated from leaf callus of *Solanum melongena* (LVR genotype) **a** hypodiploid cell, **b** hyperdiploid cell

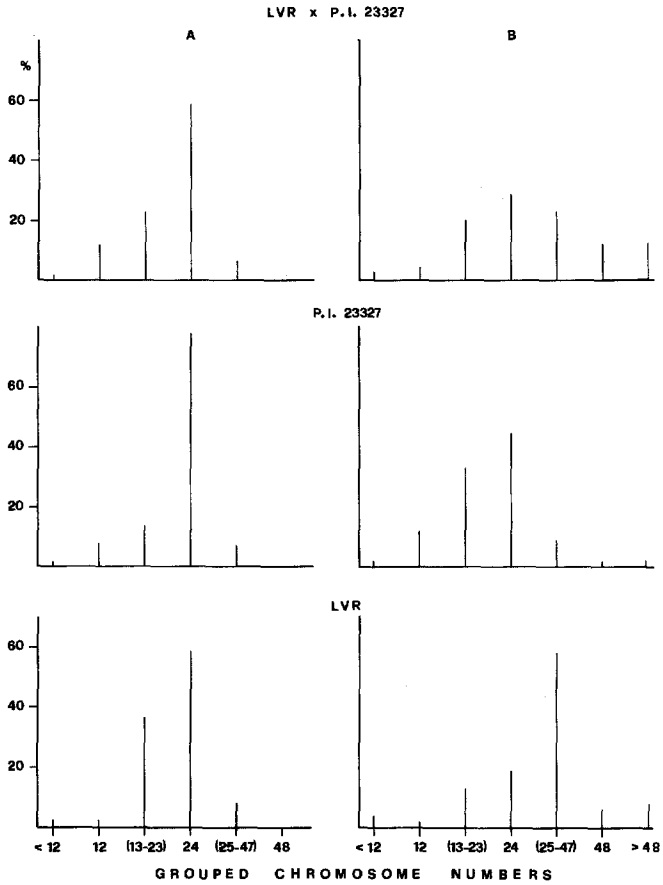


**Fig. 2.** Distribution of grouped chromosome numbers in root tip cells of plants regenerated from callus grown on control medium (**A**) and toxic medium (**B**)

highly significant in these two genotypes. The situation is quite different in the other genotype (P.I. 23327): in fact mean chromosome numbers and standard deviation values are very similar in plantlets regenerated from the two types of callus.

A cytological analysis was made to test if the toxic medium has a direct effect on cell division: root apices from germinated seeds soaked in a filtrate culture of *Verticillium dahliae* at 24, 36, 48, 72 and 96 h intervals have shown that in no instance were there changes in mitotic phase proportions which generally accompany a disturbance of spindle assembly.

The extensive variation and mixoploidy observed in chromosome numbers of regenerants from toxic medium is still present in their selfed progeny (S) (Fig. 3), where it is to be noticed that the modal karyotype is shifted from hyperdiploid to diploid values. No difference is detectable among seeds belonging to different berries.

## Discussion

The results reported in the present paper can be summarized as follows:

a) plants regenerated from callus grown on non-toxic medium show a similar degree of chromosomal mosaicism and a modal diploid karyotype in the three genotypes considered;

b) in a parental line (LVR) and in the hybrid, when callus was grown on toxic medium we observed an

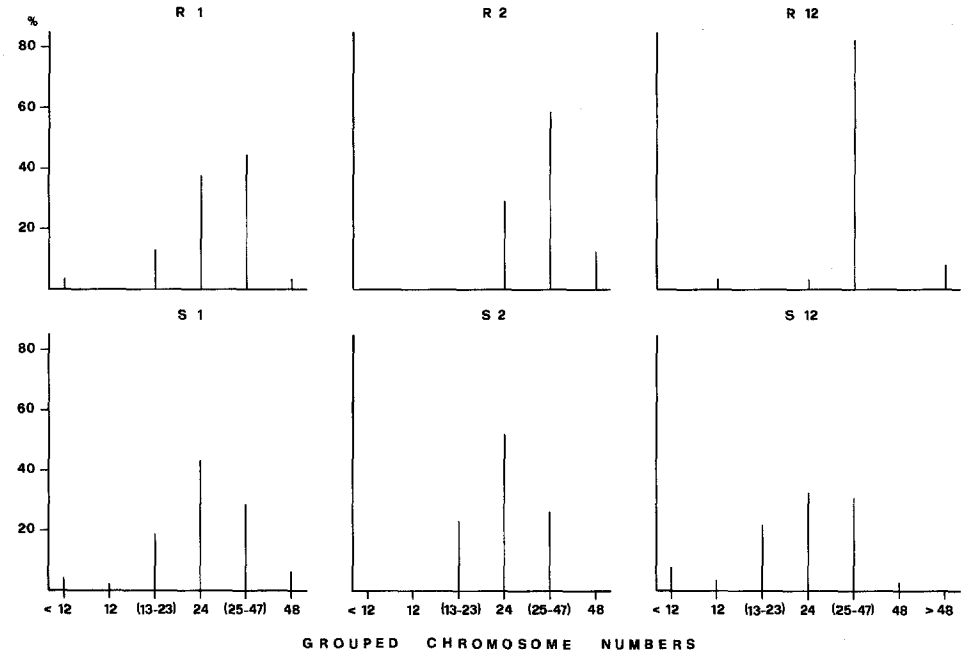


**Fig. 3.** Distribution of grouped chromosome numbers in root tip cells of three regenerated plants (*R*) and of their first selfed progeny (*S*) – LVR genotype

increase in the mean value and variability of chromosomal number during regeneration. No significant changes were observed in the other parental genotype;

c) chromosomal mosaicism persists up to plant maturity and it is also found in the selfed progeny.

The observations reported at this point suggest that mixoploidy in regenerated plants must reflect a genetic heterogeneity proper of callus culture; furthermore the similar chromosomal distribution and the presence of a diploid modal karyotype can be explained assuming a selective advantage of diploid cells rather than a random drift which might occur when inocula were transferred.

Points (a) and (b), on the whole, show that the toxic medium is able to evidence differences between genotypes which are not displayed by plants regenerated from callus grown on control medium. The possibility exists that toxic medium acts as a selective factor at the cellular level allowing hyperdiploid cells to divide more rapidly than diploid ones; the difference in competitivity originates a cell population able to regenerate plants with a more extensive chromosomal heterogeneity than populations not grown in the toxic medium. Lazar et al. (1981) have also found a correlation between the increase in variability of chromosome numbers of calli and their ability to grow in the presence of a chemical growth inhibitor.

The rôle of selection during regeneration on the expression of chromosomal variability has been extensively demonstrated in the work of many authors (Shimada and Tabata 1967; Sacristan and Melchers 1969; Yamane 1974; Novak and Vystot 1975; Bennici 1978; Orton 1980; Lupi et al. 1981).

The results reported in the present paper suggest that the toxic medium may affect the chromosomal number by interacting with cell genotypes. This may be explained both by a selective process involving genes controlling chromosome number and/or a direct effect on the activity of the same genes. The existence of a genetic control on spindle abnormalities which may lead to chromosomal mosaicism in intact higher plants is reported by various studies (Vaarama 1949; Watanabe 1962; Mok and Peloquin 1975). Unfortunately we do not have enough data to discriminate between the two hypotheses. Cytological observations on root apices of seeds soaked in fungal filtrate culture allow us only to exclude a direct effect of the toxic substances on cell division of meristematic tissues. Of course the test does not exclude that the toxic medium has a direct influence on callus divisions. The response to toxic substances, we observed, conveys the hypothesis that these compounds interact with different genes controlling mitosis.

The question is often raised as to whether mosaicism of regenerated plants, which is a clear-cut proof of multicellular origin of "in vitro" regenerated shoots and roots, is maintained in successive generations. Many authors have found a persistence of mixoploid condition during plant development, but plants were highly or completely sterile (Sacristan and Melchers 1969; Sheridan 1975; Mix et al. 1978); in other cases a diplontic selection has taken place (Orton 1980; Lupi et al. 1981). Little information on the chromosome behaviour of selfed progeny is available so far. In our experiments the persistence of mosaicism observed in the first selfed progeny of LVR parental line, even if associated with a partial diplontic selection, could be explained by supposing that the altered genic control of division induced by fungal toxic substances has resulted in a stabilization of cytological instability. Similar results were reported by Ogura (1976, 1978) who found that the variation of chromosome number persisted at least for three generations being under genic control involving cytoplasmatic factors.

More extensive studies are in progress regarding the persistence in successive selfing generations of chromosomal mosaicism of plants regenerated from callus of other genotypes exposed or not to the fungal toxic substances.

*Acknowledgements.* The authors are grateful to Mrs. C. Brunaleschi and Mrs. M. Bioni for their technical assistance.

## References

Alicchio R, Del Grosso E, Boschieri E (1982) Tissue culture and plant regeneration from different explants in six cultivars of *Solanum melongena.* Experientia 38:449–450

Bennici A (1978) Cytological chimeras in plants regenerated from *Lilium longiflorum* tissue grown *in vitro.* Z Pflanzenzücht 82:349–353

Bennici A, D'Amato F (1978) In vitro regeneration of *Durum* wheat plants. 1. Chromosome numbers of regenerated plantlets. Z Pflanzenzücht 81:305–311

Keen NT, Wang MC, Long M (1970) Induction of *Verticillium* wilt symptoms in cotton cuttings by lipopolysaccharide but not by endopolygalacturonase from *Verticillium albo-atrum.* Abs. Phytopathol 60:1298

Lazar GB, Dudits D, Sung ZR (1981) Expression of cycloheximide resistance in carrot somatic hybrids and their segregants. Genetics 98:347–356

Linsmayer EN, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 18:100–127

Liu MC, Chen WH (1976) Tissue and cell culture as aids to sugarcane breeding. 1. Creation of genetic variation through callus culture. Euphytica 27:273–282

Lupi MC, Bennici A, Baroncelli S, Gennai D, D'Amato F (1981) In vitro regeneration of *Durum* wheat plants. 2. Diplontic selection in aneusomatic plants. Z Pflanzenzücht 87:167–171

Mix G, Wilson HM, Foroughi-Wehr B (1978) The cytological status of plants of *Hordeum vulgare* L. regenerated from microspore callus. Z Pflanzenzücht 80:89–99

Mok, DWS, Peloquin SJ (1975) The inheritance of three mechanisms of diplandroid (2n pollen) formation in diploid potatoes. Heredity 35:295–302

Murashige T, Nakano R (1965) Morphogenetic behaviour of tobacco tissue cultures and implication of plant senescence. Am J Bot 52:819–827

Murashige T, Nakano R (1967) Chromosome complement as a determinant of the morphogenetic potential of tobacco cells. Am J Bot 54:963–970

Nachmias A, Buchner V, Krikun J (in press) Comparison of protein-lipopolysaccharide complexes produced by pathogenic and non-pathogenic strains of *Verticillium dahliae* Kleb from potato. Physiol Plant Pathol 20

Novak FJ, Vystot B (1975) Karyology of callus cultures derived from *Nicotiana tabacum* L. haploids and ploidy of regenerants. Z Pflanzenzücht 75:62–70

Ogura H (1976) The cytological chimeras in original regenerates from tobacco tissue cultures and in their offsprings. Jpn J Genet 51:161–174

Ogura H (1978) Genetic control of chromosomal chimerism found in a regenerate from tobacco callus. Jpn J Genet 53:77–90

Orton, TJ (1980) Chromosomal variability in tissue cultures and regenerated plants of *Hordeum*. Theor Appl Genet 56:101–112

Pegg, GF (1965) Phytotoxin production by *Verticillium albo-atrum*. Reinke et Berthold. Nature 208:1228–1229

Sacristan MD, Melchers G (1969) The karyological analysis of plants regenerated from tumorous and other callus cultures of tobacco. Mol Gen Genet 105:317–333

Sheridan WMF (1975) Plant regeneration and chromosome stability in tissue cultures. In: Ledoux L (ed) Genetic manipulation with plant material. Plenum Publ Corp, New York, pp 266–295

Shimada T, Tabata M (1967) Chromosome members in cultured pith tissue of tobacco. Jpn J Genet 42:195–201

Stoddart JL, Carr JM (1966) Properties of wilt-toxins produced by *Verticillium albo-atrum*. Reinke et Berth. Ann Appl Biol 58:81–92

Torrey JG (1967) Morphogenesis in relation to chromosomal constitution in long-term plant tissue cultures. Physiol Plant 20:265–275

Vaarama A (1949) Spindle abnormalities and variation in chromosome number in *Ribes nigrum*. Hereditas 35: 136–162

Watanabe Y (1962) Chromosome-mosaics observed in a variety of wheat, 'Shirahada'. Jpn J Genet 37:194–206

Yamane Y (1974) Induced differentiation and buckwheat plants from subcultured calluses in vitro. Jpn J Genet 49:139–146