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Chromosomal and Isozyme Studies of *Nicotiana tabacum – Glycine max* Hybrid Cell Lines*

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Summary. The chromosomal stability of a number of somatic hybrids derived from soybean (*Glycine max* (L.) Merr.) and *Nicotiana tabacum* var. 'Xanthi' were investigated. Several of the hybrid cell lines retained more than half the complement of *N. tabacum* chromosomes after 7 months of culturing. A number of chromosomal abnormalities were observed. The hybrids were positively identified by employing isozyme analysis of several dehydrogenases and aspartate aminotransferase.

Key words: Isozymes – Somatic hybrids – Chromosomes

Introduction

Somatic hybridization has become an useful tool for studying the parasexual modification of plant cells and hopefully an experimental system for producing novel plants. Plants have been produced from somatic hybrid calli derived from different genera, e.g. *Solanum tuberosum – Lycopersicon esculentum* (Melchers et al. 1978), *Datura innoxia – Atropa belladonna* Krumbiegel and Schieder 1979), *Arabidopsis thalliana – Brassica napus* (Gleba and Hoffmann 1978, 1979) and *Daucus carota – Aegopodium podagraria* (Dudits et al. 1979).

Chromosome elimination is a common phenomenon in intergeneric fusion products (Binding and Nehls 1978; Gleba and Hoffmann 1978; Kao 1977; Power et al. 1975; Wetter and Kao 1980). Kao (1977) reported that in the somatic hybrids of soybean-*Nicotiana glauca* all of the soybean chromosomes and only a few *N. glauca* chromosomes were retained after 6 months of culturing. Still fewer *N. glauca* chromosomes were evident after 36 months (Wetter and Kao 1980). This paper reports on the hybrids resulting from the somatic fusion of soybean and *Nicotiana tabacum* protoplasts. The result indicates that greater chromosomal stability has been achieved as identified by chromosome and isozyme analyses of hybrid cell lines.

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Materials and Methods

Protoplasts of soybean (*Glycine max* (L.) Merr.) from a suspension culture were used as one of the fusion partners. The average number of chromosomes in the cells of these cultures was 60. Protoplasts from fragments of young leaves of *Nicotiana tabacum* var. 'Xanthi' were used as the other fusion partner, the chromosome number of which was 48.

The methods for isolation of protoplasts, fusion and culture of single heterokaryocytes in Cuprak dishes have been described elsewhere (Kao 1977). When the hybrids grew to a 100 to 200 cell cluster, they were transferred into the wells of Cooke Histo-plates (Dynatech Laboratories, Inc. 900 Slaters Lane, Alexandria, Virginia, USA 22314). The plates were sealed with Parafilm. The reason for the transfer is that the wells of Histo-plates are larger than the Cuprak dishes and allow for the utilization of more medium for vigorous growth. One month after fusion the hybrid calli were visible to the naked eye and they were then transferred to Falcon plastic dishes (60 × 15 mm) where they grew to colonies 2 to 3 mm in diameter. Cell samples taken at different stages were fixed with acetic acid-ethanol, stained with modified carbol fuchsin and examined under a Zeiss research microscope (Kao 1975).

The isozymes studied were performed with the hybrid calli described above. The preparation and protein estimation was carried out as described previously (Wetter 1977). The electrophoretic investigations were done, except for some modifications, as described in a previous report (Wetter 1977). The experiments were performed on a 5% gel rather than a 7% gel. The isozyme patterns for alcohol, 6-phosphogluconate and shikimate dehydrogenase were stained as described by Wetter and Kao (1976). The aspartate aminotransferase was visualized by employing the method of Wetter (1977).

Results

Chromosomes

Fixation and staining of cells, regenerated from protoplasts, 48 h after PEG treatment revealed that 14.8% of

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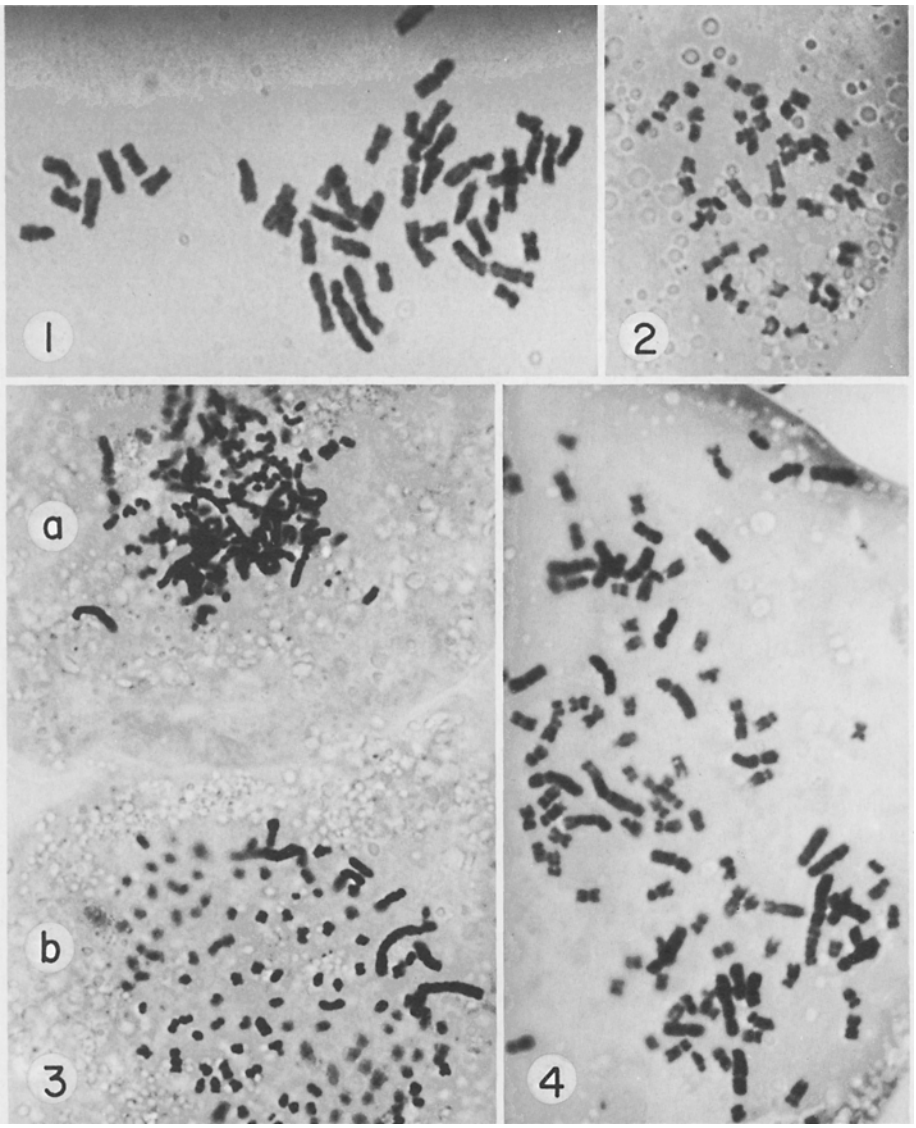
them were heterokaryocytes. The majority of them had one soybean and one *N. tabacum* nucleus, however multinuclear heterokaryocytes were also observed. *N. tabacum* chromosomes (Fig. 1) were easily distinguished from soybean chromosomes (Fig. 2), the former are thicker and longer than the latter. No premitotic nuclear fusion was observed in the heterokaryocytes.

Heterokaryocytes underwent their first division within 2 to 3 days as compared to soybean protoplasts which start to divide within 24 h and *N. tabacum* within 2 to 4 days. Within 4 days after fusion one could easily distinguish the hybrid clusters from the parental clusters by observing the arrangement of the green chloroplasts within the cells. Chloroplasts in dividing *N. tabacum* were evenly distributed while they tended to form clusters in the hybrid cells. Hybrid clusters could be recognized 20 days after fusion by the external morphology and trace of green coloration.

Considerable variation was noted in the chromosomal distribution in the heterokaryocytes. Complete sets of soybean and *N. tabacum* were retained in the



Figs. 1–4. **1** Chromosomes in a *Nicotiana tabacum* cell; **2** Chromosomes in a cultured soybean cell; **3a and b** Unequal distribution of *Nicotiana tabacum* chromosomes in two daughter cells of a *N. tabacum* – soybean hybrid. The hybrid was at the two cell stage; **4** Chromosomes in a cell of a nine month old *N. tabacum* – soybean hybrid cell line (Figs. 1, 2 and 4 are enlarged to the same magnification)

hybrid cells during the first division. Rearrangement in *N. tabacum* chromosomes was detected beginning with the second division. At this stage, loss of *N. tabacum* chromosomes were evident. The variation in number and morphology of *N. tabacum* chromosomes was dramatic as shown in Figure 3. The daughter cell "a" had a larger number of *N. tabacum* chromosomes, while the daughter cell "b" had only a few *N. tabacum* chromosomes. In both cells most of the *N. tabacum* chromosomes were abnormal in appearance.

A total of 21 hybrid cell lines were isolated. After 3 months of culturing, approximately two-thirds of the *N. tabacum* chromosomes were retained in some hybrid lines, many of these however contained abnormal chromosomes. After 6 to 7 months of culturing two very distinct classes could be distinguished in the hybrid cell lines. One class (hybrid lines No. 3, 4, 7, 9 and 12) had retained more than half the *N. tabacum* chromosomes in the majority of the cells (Fig. 4). In many of the cells there was still evidence of abnormal chromosomes, e.g. long chromosomes as well as chromosomal bridges. The second class (hybrid lines No. 5, 6, 10, 11, 13 and 14) had lost nearly all the *N. tabacum* chromosomes except for a very low number of cells which still retained a number of *N. tabacum* chromosomes. The remaining hybrid lines were intermediate in nature. None of the hybrid cell lines indicated any obvious changes in number and structure of the soybean chromosomes.

Isozymes

Several different isozyme systems, in addition to those found in Fig. 5, were investigated, e.g. lactate, formate and glucose-6-phosphate dehydrogenase, as well as esterase, acid phosphatase and superoxide dismutase. They were not utilized because pattern differences between parents were not great enough to be useful in identifying the hybrid lines. Twenty-one hybrid lines were assayed but only 6 representative lines are shown in Fig. 5. The study was carried out over a 7 month period.

The 4 enzyme systems chosen for the investigation, all show clear evidence of having achieved successful hybridization. The alcohol dehydrogenase zymogram (Fig. 5 a) shows that the hybrid lines have a 2-banded pattern (see hybrids 3, 4 and 12), the top band is derived from *N. tabacum,* the lower band from soybean. The majority of the hybrid cell lines expressed only the soybean band. The band derived from soybean is much more intense in the hybrids than in the parent while the opposite is true for the band derived from *N. tabacum.*

The shikimate dehydrogenase zymograms clearly illustrate that the same cell lines are hybrids (No. 3, 4 and 12 in Fig. 5 b). The doublet seen with an R_f of 0.56 and 0.60 is derived from *N. tabacum* while the broad zone over a R_f of 0.32 to 0.45 is an expression in part of soybean. This broad zone could also indicate the presence of genes from *N. tabacum* that are expressed by the 2 band seen at R_f 0.42 and 0.44. It is not possible to ascertain whether the 3 cell lines (No. 5, 10 and 11) are true hybrids or contain only soybean information.

The 6-phosphogluconate dehydrogenase is one of the more interesting systems studied in this investigation. Cell lines 3, 4 and 12 are undoubtedly hybrids (Fig. 5 c) as they have bands which are derived from both soybean and *N. tabacum.* However there is considerable variation, cell line 3 contains all the bands from

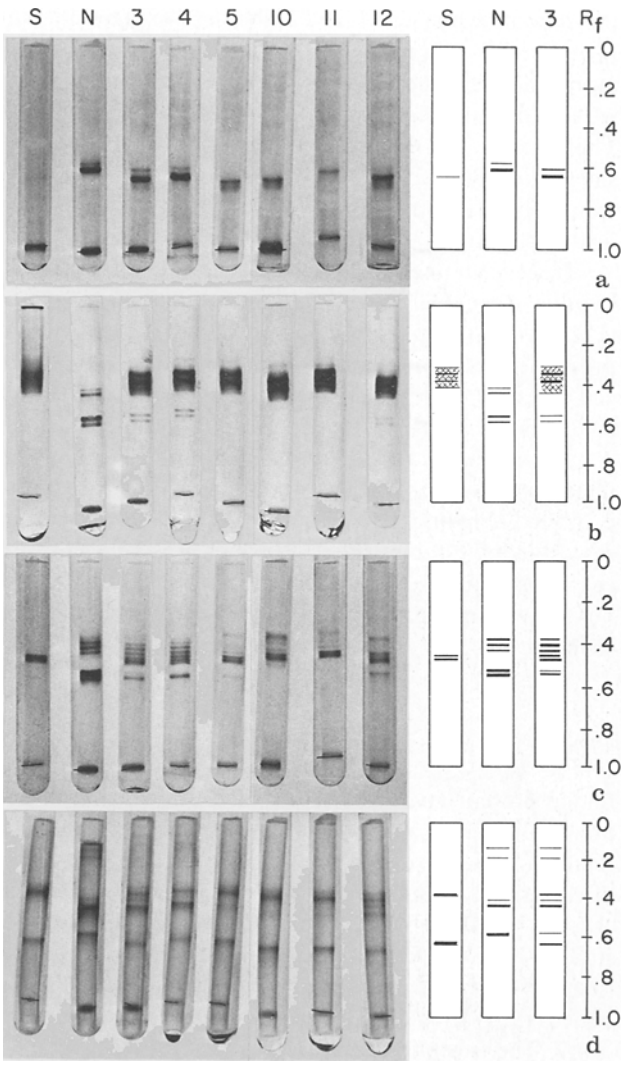


Fig. 5 a–d. **a** Electrophoretic patterns of alcohol dehydrogenase, **b** shikimate dehydrogenase, **c** 6-phosphogluconate dehydrogenase, **d** aspartate aminotransferase obtained for *N. tabacum* (N), soybean (S) and various hybrids. The schematic diagram on the right depicts patterns for the parents and one of the hybrids (3)

the parents while cell line 12 does not express all the *N. tabacum* bands in the R_f 0.38 to 0.42 region. Further more only cell line 3 expresses the doublet at R_f 0.56, while 4,5 and 12 exhibit a single band in this region. The remaining cell lines 10 and 11 as well as those not shown here indicate that they are also hybrids as they exhibit bands from soybean and *N. tabacum* in the R_f 0.40 to 0.48 area.

Three cell lines 3, 4 and 12 very definitely were hybrids when the enzyme, aspartate aminotransferase (Fig. 5 d), was employed. The 2 bands (R_f 0.40 and 0.65) present in soybean were always observed in the above cell lines, in addition the *N. tabacum* band with R_f of 0.46 was also present. It is interesting to note that the *N. tabacum* band at R_f 0.61 was only faintly evident in line 3 while absent in the other lines. The 3 hybrids exhibited a hybrid band at R_f 0.43. *N. tabacum* had a slow moving doublet (R_f 0.19) which was very faint in cell lines 3 and 4. The other cell lines including those not shown in Fig. 5 could not be distinguished from soybean.

Discussion

In the present investigation we have shown that more than half of the *N. tabacum* chromosomes have been retained in 5 hybrid cell lines for a period of 7 months of culturing, during which time there were no obvious changes in the soybean chromosomes. The isozyme studies have confirmed the above observations, in 3 isozyme systems studied the 5 hybrid lines have maintained their hybrid pattern throughout the 7 month study. The exception appeared to be alcohol dehydrogenase in which the hybrid pattern had disappeared at the end of the 7 month period. The results, excluding the alcohol dehydrogenase, suggested that genes on chromosomes expressing these enzymes were retained throughout the culture period. The continued presence of such abnormalities as ring chromosomes, long chromosomes and chromosomal bridges indicated that the hybrid cell lines have not yet stabilized.

N. tabacum is an amphidiploid derived from *N. sylvestris* and *N. tomentosiformis* and therefore has 4 sets of chromosomes. The double dose of chromosomes might account for the fact that the somatic hybrids of *N. tabacum*-soybean appear to be more stable than the *N. glauca*-soybean hybrids (Kao 1977). The protoplasts isolated from *N. tabacum* leaves divided more readily than those obtained from *N. glauca* leaves. Since soybean protoplasts also divided readily one might postulate that perhaps more nuclei of *N. tabacum* and soybean were in the same phase of the mitotic cycle and thus led to more stable hybrid lines. One might speculate that because of this the *N. tabacum* chromosomes were less likely to undergo premature chromosomal condensation, then fragmentation and finally elimination.

Acknowledgement

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