Theor. Appl. Genet. 57, 273-276 (1980)

Chromosome and Isoenzyme Studies on Cells Derived from Protoplast Fusion of *Nicotiana glauca* with *Glycine max-Nicotiana glauca* Cell Hybrids*

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Summary. The somatic hybrids of *Glycine max* (L)Merr.-*Nicotiana glauca* Grah. exhibited a preferential loss of *N. glauca* chromosomes. When protoplasts from such hybrid cells were 'back fused' twice to *N. glauca* protoplasts, a considerable increase in stability of the *N. glauca* chromosomes was observed. Gel electrophoresis studies of aspartate aminotransferase showed that the chromosome(s) responsible for this enzyme was stabilized in the 'back fused' hybrid cell lines. The data suggest that the 'back fusion' technique described in this study might aid in stabilizing somatic hybrids.

Key words: Somatic – Hybrids – Aspartate aminotransferase – 'Back fusion' – Chromosomes

Introduction

In previous papers (Kao 1977; Wetter 1977) we reported on the chromosome and isoenzyme behaviour of hybrid cells obtained from the fusion of soybean (*Glycine max* (L) Merr.) and *Nicotiana glauca* Grah. protoplasts. We found that most of the chromosomes of *N. glauca* were gradually eliminated and zymograms for alcohol dehydrogenase and aspartate aminotransferase of the hybrids changed to eventually resemble the soybean zymograms. The aspartate aminotransferase zymograms for the hybrids never possessed two slow moving bands which were present in *N. glauca*. This raised the question of whether this loss was the result of a rapid elimination of *N. glauca* chromosomes or whether the genes for these enzymes were suppressed in the hybrid.

This investigation demonstrates the effects of the 'back fusion' technique on chromosome stability in soybean-*N. glauca* hybrid cells. It also shows that the genes expressing aspartate aminotransferase in soybean and in *N. glauca* are expressed in the somatic hybrid.

* NRCC No. 18040

Materials and Methods

The procedure for isolation of protoplasts from hybrid cells of soybean-*N. glauca* was the same as previously described (Kao 1977). In the first 'back fusion', the *N. glauca* protoplasts were isolated from fresh leaves. In the second 'back fusion', the *N. glauca* protoplasts were isolated from leaf fragments which were preincubated in a cell culture medium (Kao 1977) for 24 hr to induce cell division. 'Back fusion' is defined as the fusion of *N. glauca* leaf protoplasts with protoplasts obtained from a soybean-*N. glauca* hybrid cell culture. The methods for fusion and isolation of hybrid cells have been described elsewhere (Kao 1977).

The first 'back fusion' was carried out about 27 months (March 6, 1978) after the initial fusion of *N. glauca* and soybean. Approximately 7 months later (October 11, 1978), the second 'back fusion' was performed with cells derived from the first 'back fusion'. When it had been ascertained that the chromosomes of one of the cell lines obtained from the second 'back fusion' had stabilized, individual cell clusters (designated as sublines) were selected at random and cultured for further studies.

The preparation and protein estimation of extracts, as well as the gel electrophoresis was carried out as described previously (Wetter 1977).

Results

1 Chromosomes

The chromosome composition of various somatic hybrid cell lines of soybean-*N. glauca* is presented in Figure 1. After 3 years of serial culture only a few *N. glauca* chromosomes with modified structures were observed in the soybean-*N. glauca* cell lines (Fig. 1a). Some of the larger chromosomes undoubtedly originated from *N. glauca* although there has been a considerable change in size and shape (see arrow in Fig. 1a). These no longer resembled the normal *N. glauca* chromosomes depicted in Figure 1e.

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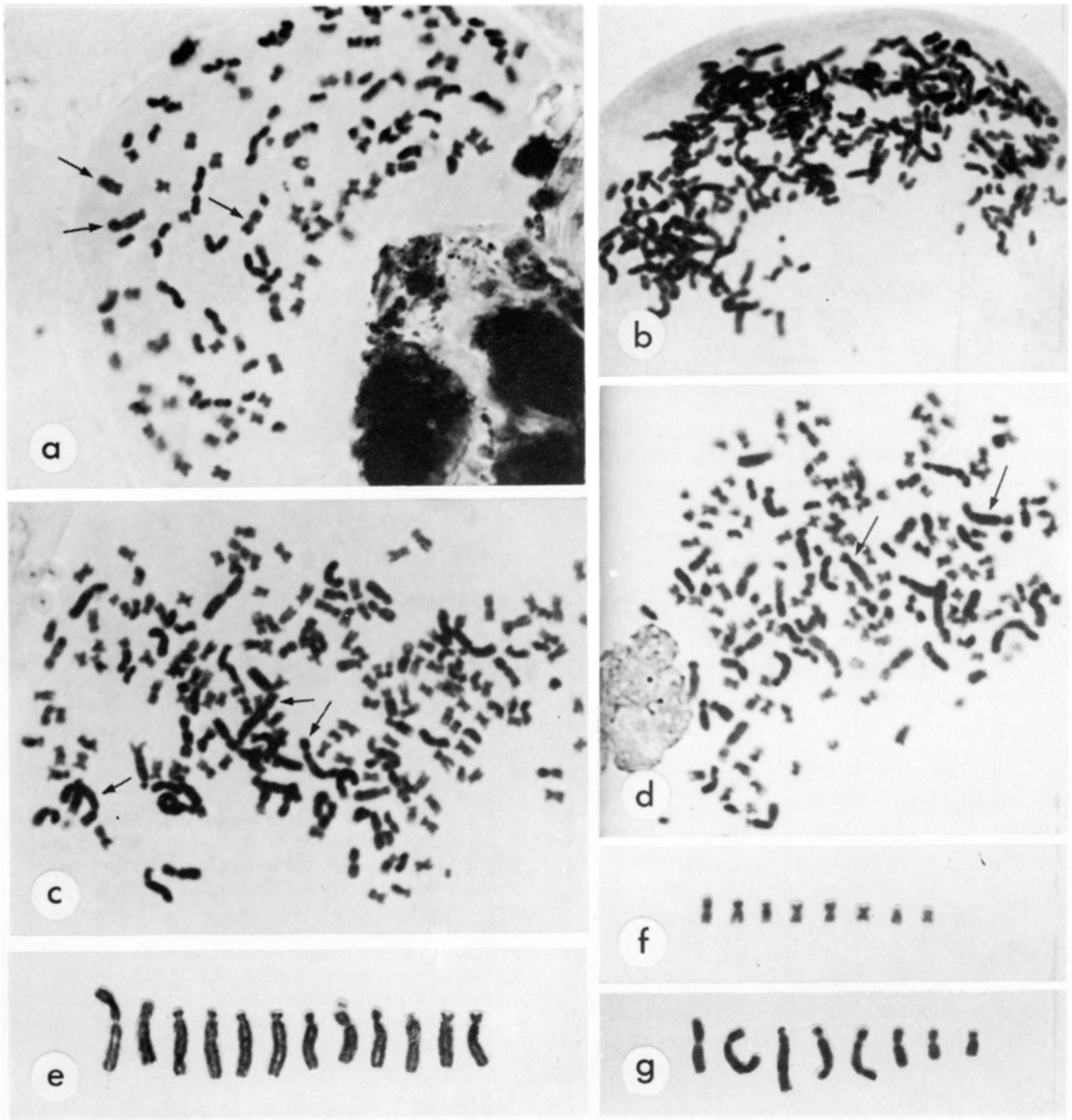

Fig. 1a-g. Chromosomes of various somatic hybrid cell lines of soybean-*Nicotiana glauca*. **a** After 3 years culturing in a liquid medium; **b** three months after the second 'back-fusion' of soybean-*N. glauca* to *N. glauca*; **c, d** six months after the second 'back-fusion'; **e** karyotype of *N. glauca* (n = 12) [Except chromosome 1 (medium constriction), 2 (submedium constriction) and 8 (subterminal constriction with satellite)]. All the others are subterminal and very similar in structure; **f** some typical soybean chromosomes (n = 20); **g** some typical *N. glauca* chromosomes in the somatic hybrid cell lines of soybean-*N. glauca* six months after second 'back-fusion'. The sizes and shapes were changed considerably in some of them.

Most of the chromosomes in these hybrid lines resembled those found in soybean (Fig. 1f). Considerable increase in *N. glauca* chromosomes was observed in some of the cell lines derived from the first 'back fusion'. The chromosome behaviour in the first 'back fusion' hybrids was much like that reported in earlier somatic cell hybrids (Kao 1977). The hybrid cell lines which had a higher proportion of *N. glauca* chromosomes grew much slower than those with fewer *N. glauca* chromosomes. A cell line derived from the first 'back-fusion' and, subsequently

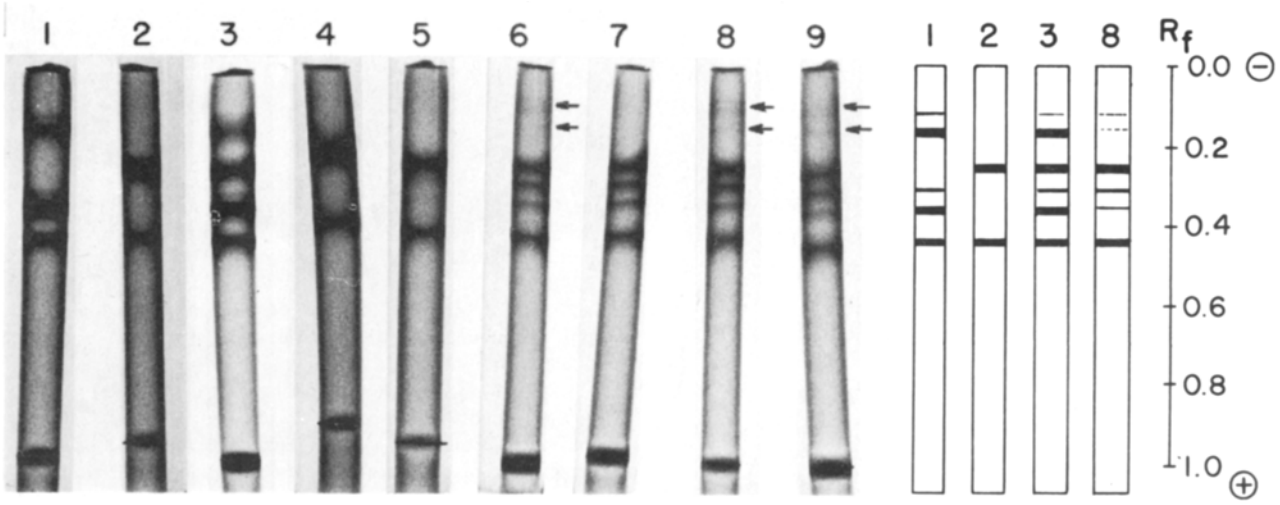

Fig. 2. Electrophoretic patterns of aspartate aminotransferase obtained for **1** *N. glauca*; **2** soybean; **3** equal mixture of *N. glauca* and soybean; **4** original fusion; **5** first 'back-fusion'; **6** second 'back-fusion'; **7-9** some sub-lines of the second 'back-fusion'. The schematic diagram on the right depicts patterns for Lanes **1-3** and **8**.

used for the second 'back-fusion' belonged to a fast growing culture which had few *N. glauca* chromosomes.

Generally speaking, the cell lines from the second 'back-fusion' had a higher number of *N. glauca* chromosomes and less chromosomal abnormalities than the hybrid parent. Three months after the 'back-fusion' numerous *N. glauca* chromosomes were observed in hybrid cells (Fig. 1b) and they were still present after 6 months of culturing (Fig. 1c, d). Most of the *N. glauca* chromosomes had a modified structure. However, a few of them, as shown by arrows in Figure 1c and d, resembled the standard *N. glauca* chromosomes (Fig. 1e). Some of the *N. glauca* chromosomes found in the 6 month old second 'back-fusion' hybrids are depicted in Figure 1g.

Once again, the cell lines which contained a higher number of *N. glauca* chromosomes grew more slowly. The morphology of the cells derived from the second 'back-fusion' was essentially the same as the hybrid parent. However, a few cell lines resembled *N. glauca* cells more than soybean, this was still evident after 6 months. The chromosome constituents of the cells in all of the 'back-fusion' cell lines were heterogeneous.

2 Isoenzymes

The results of the aspartate aminotransferase investigation is summarized in Figure 2. The normal zymograms obtained for *N. glauca* callus and soybean suspension cultures are shown in Lanes 1 and 2 respectively, an equal mixture of the two results in a zymogram represented in Lane 3. The original fusion which had been cultured for three years and the first 'back-fusion' for 2 years (Lanes 4 and 5, respectively) cannot be distinguished from soybean (Lane 2). This presumably is due to loss of *N. glauca* chromosome(s) which express aspartate aminotransferase. The second 'back-fusion' is depicted in Lane 6 and here, there is evidence of hybridization, i.e. four definite bands, band R_f 0.43 is derived from both soybean and *N. glauca*, band R_f 0.34 and 0.30, from *N. glauca* and band 0.26, from soybean. In addition, this selection shows very faintly the two slow moving bands, R_f 0.11 and 0.16, which are derived from *N. glauca*. These have not been observed in any previous initial fusions or in the first 'back-fusion'. The cell line presented in Lane 6 was used to select several sub-lines, some of which are shown in Lanes 7, 8 and 9. In these selections, all depicted the 4 fast bands but only 2, Lanes 8 and 9, showed the 2 slower moving bands.

Discussion

Fusion of mammalian cells at different cell cycles can greatly affect the stability of chromosomes (Johnson and Rao 1970 and Rao et al. 1975). Minna and Coon (1974) and Croce (1976) observed that in interspecific human × mouse somatic cell hybrids where embryonic mouse cells were employed, human chromosomes were retained while mouse chromosomes were lost. In previous reports, the opposite pattern had been observed. Hence, there appears to be reason to presume that plant cells fused at different stages could demonstrate similar effects. Therefore, advantages might exist in using protoplasts isolated from preincubated leaves of *N. glauca* for fusion with protoplasts from cultured soybean cells. One might assume that all the protoplasts from fresh *N. glauca* (Ng) leaves were in the G_1 stage. The protoplasts from cultured cells of soybean (SB) could be in the G_1, G_2 and perhaps S stage.

The possible combinations would be $Ng(G_1)SB(G_1)$, $Ng(G_1)SB(G_2)$ and perhaps $Ng(G_1)SB(S)$. Many cells isolated from *N. glauca* leaves, which have been incubated for 24 hr in the medium were entering mitosis. One would therefore expect that *N. glauca* protoplasts prepared from preincubated leaves would be in the G_1, G_2 and perhaps S stages. From this material one would expect new combinations, such as $Ng(G_2)SB(G_2)$, $Ng(G_2)SB(G_1)$ and perhaps $Ng(S)SB(G_1)$, $Ng(S)SB(G_2)$ and $Ng(S)SB(S)$. These new combinations might increase the chances of obtaining a stable soybean-*N. glauca* hybrid. Further experiments are required to test this hypothesis.

The slow moving band (R_f 0.15) is very distinct in the *N. glauca* zymogram, its intensity is equivalent to that of bands at R_f 0.33 and 0.43. In a previous study (Wetter 1977), this slow moving band was never detected in somatic hybrids of *N. glauca* and soybean even when the concentration of enzyme was doubled. Also in the present study there never was any evidence of these bands being present in either the original hybrid or in the hybrids from the first 'back-fusion'. However, the present results indicate that after two consecutive fusions of the hybrid cells of soybean-*N. glauca* with *N. glauca*, the slower bands observed in *N. glauca* are faintly visible. The faintness of the bands suggests that the gene dosage may be very low or that only a small portion of the cells in the population retained the genes for the two slow bands. The results clearly indicate that all the genes for aspartate aminotransferase from soybean and *N. glauca* can be expressed in the somatic hybrids.

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Received January 21, 1980
Communicated by G. Melchers and H.F. Linskens

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