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Progeny Analysis of the Interspecific Somatic Hybrids: *Nicotiana tabacum* (CMS) + *Nicotiana sylvestris* with Respect to Nuclear and Chloroplast Markers

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Summary. The progeny of a fusion experiment involving *N. sylvestris* protoplasts and X-irradiated protoplasts of the cytoplasmic male sterile 'Line 92' (*N. tabacum* nucleus and alien, male-sterility inducing, cytoplasm) were analyzed. Three groups of somatic hybrid plants resulted: Type A, Type B-1 and Type B-2. These as well as their androgenic progenies and the progenies resulting from their pollination with *N. tabacum* or *N. sylvestris* were followed with respect to several nuclear and cytoplasmic traits. Those controlled by the nuclear genome were plant and flower morphologies; those controlled by genetic information in the cytoplasm were tentoxin sensitivity (affecting the coupling factor of chloroplast ATPase), the large subunit of ribulose bisphosphate carboxylase and the restriction endonuclease pattern of plastid DNA. A further cytoplasmic trait investigated (exact site of genetic control not known) was male sterility. The examinations of the somatic-hybrid groups and their respective progenies indicated that: Type A plants have *N. sylvestris* nuclei and 'Line 92' plastids; Type B-1 plants also have 'Line 92' plastids but their genome is composed of *N. sylvestris* and *N. tabacum* nuclei; Type B-2 plants with impaired male fertility had *N. sylvestris* plastids and *N. sylvestris* nuclei.

Key words: Tobacco – Protoplast fusion – Cybrids – DNA; RUBP carboxylase – Androgenic progeny – Male fertility – Tentoxin sensitivity – Chloroplast ATPase

Introduction

One of the obvious and repeatedly advocated (Melchers 1965) potential advantages of plant cells over mammalian cells for the production of somatic hybrids is their regeneration to sexually functional plants thus enabling a combination of parasexually and sexually based genetic studies. In recent years a considerable number of somatic hybrid angiosperms resulting from protoplast fusion were reported (Thomas et al. 1979). In a few of these reports studies were continued to the sexual hybrid progenies (e.g. Melchers and Labib 1974; Melchers and Sacristan 1977; Chen et al. 1977; Belliard et al. 1978; Schieder 1978; Gleba 1979; Izhar and Power 1979); however the analyses was usually incomplete and, with one exception (Belliard et al. 1978), not based on biochemical markers.

We recently reported the transfer of cytoplasmic male sterility (CMS) in *Nicotiana* from the CMS 'Line 92' (*N. tabacum* nuclei with alien cytoplasm) to *N. sylvestris* by protoplast fusion (Zelcer et al. 1978). Our fusion procedure included X-irradiation of the CMS 'Line 92' parental protoplasts and a nutritional selection against the *N. sylvestris* protoplasts. The somatic hybrid plants obtained were divided into three groups: Type A, Type B-1 and Type B-2. The plants of the first two types were male sterile, while Type B-2 plants were self-incompatible. In our previous publication we based our suggestion of the nuclear and cytoplasmic compositions of the three types on morphological features and male sterility. In the present publication we report on the androgenic and sexual progenies of these types and provide information on three chloroplast characteristics: tentoxin sensitivity, isoelectric focusing pattern of the large subunit of ribulose bisphosphate carboxylase (LS RUBPcase) and DNA-restriction-fragment patterns.

Part of these results were summarized at the Fifth International Protoplast Symposium, July 1979, Szeged, Hungary.

Material and Methods

Plant Material and Sexual Crosses

The *N. sylvestris* inbred line was originally obtained from Dr. P. Maliga (Szeged, Hungary). 'Line 92' is cytoplasmic male sterile

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(CMS) and has *N. tabacum* cv. Xanthi nuclei. The cytoplasm of this line, obtained from Prof. A. Marani (Faculty of Agriculture, Hebrew University) was presented as *N. suaveolens* but we found its plastid DNA and its LS RUBPcase to differ from those of 'authentic' *N. suaveolens*. We therefore tentatively concluded that the cytoplasm of 'Line 92' is of another tentoxin sensitive wild *Nicotiana* species, although the possibilities that there exists a cytoplasmic variability among *N. suaveolens* isolates cannot be ruled out. The somatic hybrid plants were those obtained in our previous study (Zelcer et al. 1978). Their morphological features are: Type A – plant and flower morphologies identical to *N. sylvestris* but stamens are stigmatoid and male sterile; Type B-1 – plant and flower morphologies similar to the F_1 (sexual): *N. sylvestris* × *N. tabacum*, cv. Xanthi with variable deformed leaves, but anthers are tapered and male sterile; Type B-2 – plant and flower morphologies identical to *N. sylvestris* but anthers with few or no functional pollen grains and self incompatible.

Sexual progeny was obtained by pollinating the somatic hybrids with either *N. tabacum* cv. Xanthi or *N. sylvestris*.

Anther Culture

Flower buds (1,5-2.0 cm long) were cultured on solidified Nitsch medium (Nitsch 1969) containing 0.25% charcoal; the resulting plantlets were first transferred to Jiffy pots and then raised to maturity in a temperature-controlled greenhouse.

Chromosome Analysis

Root tips were incubated with 0.1% colchicine for 3 h. Thereafter they were transferred into 2% acetocarmine in 45% acetic acid for cytological preparation.

Tentoxin Test

Seeds were placed on a filter paper in 0.3 ml water (control) or 0.3 ml water containing 20 μg/ml tentoxin (Durbin and Uchytil 1977a). On the fifth day they were transferred to light and on the tenth day their pigmentation was recorded. Seedlings which did not green in the presence of tentoxin were considered sensitive while those which greened were considered insensitive. No intermediate reactions were observed (Fig. 1).

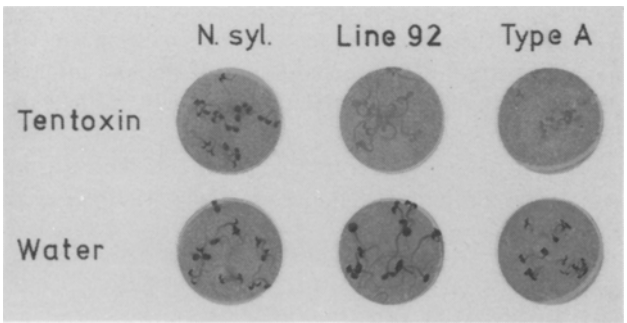

Fig. 1. Tentoxin seedling-test. Seeds were germinated as described under Material and Methods. Each well contains 0.3 ml tentoxin (upper row) or 0.3 ml water (lower row). Columns from left to right show: *N. sylvestris*. CMS 'Line 92' and f29s (Type A)

Isoelectric Focussing

Protoplasts were prepared from 5-6 leaves of each plant studied. The protoplasts were washed, resuspended in a small volume of buffer H (2.5 mM Tris-HCl, pH 7.4, 0.05 mM EDTA, 20 mM NaCl 1% pvp-40) and broken by passing several times through a syringe (no. 27 needle). The suspension was centrifuged (Sorval, SS 34 rotor) at 15 000 rpm for 15 min, the supernatant collected, layered on top of a 7-22% sucrose gradient in buffer H and centrifuged (Beckman L3-50, Sw 27 rotor) at 27 000 rpm for 25 h (Goldthwaite and Bogorad, 1971). Peak protein-containing fractions (A280/260> 1.5) in the lower part of the tube were pooled and the RUBPcase precipitated with $(NH_4)_2SO_4$. The precipitate was resuspended in buffer H and run through Sephadex G-25 to remove the $(NH_4)_2SO_4$. Carboxymethylation of the enzyme was performed according to Kung et al. (1974) and isoelectrofocussing carried out on disc urea-acrylamide gels (O'Farrell 1975).

Chloroplast DNA Isolation and Restriction Enzyme Fragmentation

Plastid DNA was isolated according to Frankel et al. (1979). Two units of restriction enzyme, BglI (New England Biolabs) in 66 mM KCl, 10 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM dithiotreitol, and 200 μg/ml bovine serum albumin were incubated with 1-2 μg of DNA at 37°C for 15 min.

Vertical slab gels (20 × 15 × 0.4 cm) of 0.7% agarose (Seakem) were run for 15 hrs in Tris-acetate 40 mM, sodium acetate 20 mM, 1 mM EDTA at 40 mA. The gel was stained for $\frac{1}{2}$ hr in 1 μg/ml ethidium bromide, washed briefly and photographed with short wave UV and a yellow filter.

Results

Morphology and Cytoplasmic Male Sterility

Shoot and flower morphologies of the original somatic hybrid plants of Type A and Type B-2 were identical with *N. sylvestris* and therefore it was assumed that they contain only the *N. sylvestris* genome (Zelcer et al. 1978). This assumption was confirmed when Type A and Type B-2 plants were pollinated with either *N. sylvestris* or *N. tabacum* and the morphology of the progeny checked (Table 1). The progenies of each of these crosses were homogenous. The original somatic hybrid plants of Type B-1 showed intermediate shoot and flower morphologies similar to the sexual hybrid *N. sylvestris* × *N. tabacum*, and their progeny displayed heterogeneity; some were more like *N. sylvestris* and some were more like *N. tabacum*. These results were consistent with the suggestion that Type B-1 contained both *N. sylvestris* and *N. tabacum* genomes, or parts of them.

Due to the relative late abortion of pollen in CMS *Nicotiana* (Frankel and Galun 1977) it was possible to obtain androgenic plants from male sterile plants (Table 2). The homogenous morphology of the androgenic plants obtained by anther culture of Type A plants is

Table 1. F_1 progeny of somatic hybrid plants pollinated with either *N. sylvestris* or *N. tabacum* cv. Xanthi. Several plants of each somatic hybrid type were used as female parents and 20 or more progeny plants of each sexual cross were characterized

Female Parent and chromosome number	Male Parent	
	N. sylvestris	*N. tabacum* (cv. Xanthi)
Type A (2n = 24)	Good seed setting; good germination. Shoot and floral morphologies identical to type A (2n = 24)	
(2n = 48)		Good seed setting; good germination. Shoot and floral morphologies like that of F_1 (*N. sylvestris* × *N. tabacum*). Anthers tapered and sterile. (2n = 48)
Type B-1 (2n = 48-80)		Very few seeds. Shoot and floral morphologies variable. Stamens stigmatoid/petaloid or with tapered anthers. Sterile. (2n = 48-72)
Type B-2 (2n = 48)		Good seed setting. Shoot and floral morphologies like F_1 (*N. sylvestris* × *N. tabacum*).[1] Pollen fertile but self sterile. (2n = 48)

consistent with the assumption that these plants contain only the *N. sylvestris* genome. On the other hand, all 23 plants contained stigmatoid stamens and were male sterile suggesting that their cytoplasm was contributed by the CMS parent.

We did not obtain an androgenic plant of Type B-1. Anther culture of Type B-2 plants yielded only one plant which was morphologically similar to *N. sylvestris*. It had normal, fertile anthers in contrast to the original Type B-2 plants which were self incompatible.

Chloroplast Markers

Tentoxin sensitivity: The cytoplasms of the original 'parental' protoplasts differed with respect to several characters. One of these was sensitivity to tentoxin, a cyclic tetrapeptide produced by *Alternaria tenuis*. Durbin and Uchytil (1977a) have shown that some *Nicotiana* species are sensitive to tentoxin while others are insensitive. This trait is cytoplasmic inherited (Durbin and Uchytil, 1977b) and is caused by specific binding of the toxin to the coupling factor (CF_1) of the chloroplast ATPase complex (Steele et al., 1976). *N. tabacum*, as well as *N. sylvestris*, which evolutionary is considered to be its maternal ancestor, are both insensitive to tentoxin. On the other hand, 'Line 92' (CMS), which contains a non-*tabacum* plasmon, is sensitive.

All 26 somatic hybrid plants were included in the tentoxin sensitivity tests (Table 3). The hybrid plants were the maternal parents and either *N. sylvestris* or *N. tabacum* were used as paternal parents. The test was performed using seeds derived from these crosses. As shown by Durbin and Uchytil (1977b) and by Burk and Durbin (1978), the reaction of *Nicotiana* seedlings to tentoxin is irrespective of the pollinating parent and depends solely

Table 2. Androgenic progeny of somatic hybrid plants. Anthers were removed from tested plants and the androgenic plantlets were raised to maturity as described in Material and Methods

Source of anthers and number of tested plants	Total number of anthers cultured	Number of plants obtained		Structure of stamens/male fertility
		with reduced chromosome number	with non-reduced chromosome number	
Type A (10 plants)	561	18	5	stigmatoid/sterile
Type B-1 (2 plants)	121	0	0	–
Type B-2 (2 plants)	82	1	0	normal/fertile

on the maternally contributed cytoplasm. In our experiments all Type A and Type B-1 seedlings were sensitive to tentoxin while Type B-2 seedlings were all insensitive (Table 3, Fig. 1). The results are consistent with the assumption that Type A and Type B-1 plants contain 'Line 92' plasmon while Type B-2 plants contain *N. sylvestris* plasmon.

Isoelectric Focussing Patterns of LS RUBPcase: Another plastid character in which *N. sylvestris* and 'Line 92' differed was the isoelectric focussing pattern (IEF) of the large subunit of RUBPcase. Kung et al. (1974) showed that when carboxymethylated RUBPcase was subjected to IEF under denaturing conditions purified large subunit migrated as 3 main bands of different isoelectric points. The IEF pattern was found to be species specific as well as maternally inherited in *Nicotiana*.

When isolated RUBPcase was subjected to carboxymethylation and IEF in the presence of urea we found that 'Line 92' and *N. tabacum* differed in the isoelectric point of one of the three main bands of the large subunit (Fig. 2a, b). This difference was moderate but consistent; when a mixture of the two enzymes was coelectrophoresed, four bands were detected (Fig. 2c). RUBPcase was then isolated from *N. sylvestris* and two representatives of Type A, Type B-1 and Type B-2 plants and electrophoresed. As expected, the IEF pattern of *N. sylvestris* large subunit was similar to that of *N. tabacum*. Due to the high polyphenol content of *N. sylvestris* we subsequently used *N. tabacum* enzyme preparation as our control. Each enzyme preparation studied was electrophoresed separately as well as mixed with enzymes from either *N. tabacum* or 'Line 92'. The results indicated that a mixture of Type B-2 large subunit (Fig. 2d) with *N. tabacum* large subunit gave 3 bands (Fig. 2e), while a mixture with 'Line 92' enzyme yielded four bands (Fig. 2f). This is consistent with the plasmons of Type B-2 plants being contributed by *N. sylvestris*. By analyzing Type A and Type B-1 enzymes in a similar way (not shown) we found that these contained the plasmon of 'Line 92'.

Restriction endonuclease patterns of plastid DNA: A third chloroplast character in which the two 'parental' plants differed was the restriction endonuclease digestion pattern of their plastid DNA. Atchison et al. (1976) were the first to show that the EcoRI endonuclease fragmentation pattern of plastid DNAs from several Australian *Nicotiana* species differed from that of several American species. Subsequently, Beillard et al. (1978) used an EcoRI digest of *Nicotiana* plastid DNA as a tool for analyzing somatic hybrids, while Frankel et al. (1979) compared EcoRI plastid DNA fragments from wild type *Nicotiana* species and lines obtained by recurrent crosses of such species with *N. tabacum*.

We analyzed plastid DNAs of 'Line 92' and *N. sylvestris* or *N. tabacum* by digestion with EcoRI, BglI and Bam HI restriction endonucleases. In each case differences in the restriction fragment pattern could be seen between the two parent species. Plastid DNAs were then isolated from representatives of Type A, B-1 and B-2 plants and digested with the same three enzymes. Type A and B-1 plants always gave a pattern identical to that of 'Line 92' whereas Type B-2 plants gave a pattern identical with *N. tabacum* or *N. sylvestris*. Fig. 3 shows typical results obtained with BglI – restricted plastid DNAs. These results add support to the conclusion that Type A and Type B-1 plants contain 'Line 92' plastons while Type B-2 plants contain the *N. sylvestris* plaston.

Table 3. Tentoxin sensitivity test. The tentoxin test as described in Material and Methods was performed with seeds obtained from the plant types listed below. All 26 somatic hybrid plants were used in this test

Plant type	Tentoxin sensitivity	
	sensitive	insensitive
N. tabacum		+
N. sylvestris		+
'Line 92'	+	
Type A	+	
Type B-1	+	
Type B-2		+

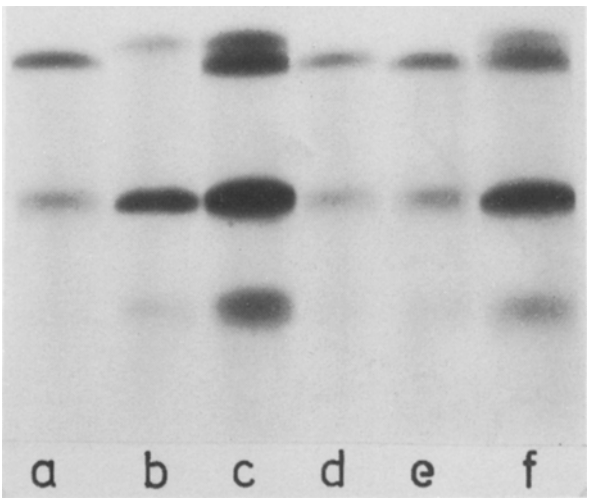

Fig. 2. Isoelectric focussing of LS RUBPcase. The enzyme was isolated from *N. tabacum*, CMS 'Line 92' and the somatic hybrid f22x (Type B-2), carboxymethylated and electrophoresed in urea polyacrylamide disc gels as described in Material and Methods. a = *N. tabacum*, b = CMS 'Line 92', c = mixture of a and b, d = f22x (Type B-2), e = mixture of a and d, f = mixture of b and d

Discussion

The fusion of X-irradiated protoplast of the CMS 'Line 92' (*N. tabacum* nucleus with alien cytoplasm) with *N. sylvestris* protoplasts yielded altogether 26 plants which were grouped into three different types (Zelcer et al. 1978). A detailed analysis of these plants and their progeny was possible as the two 'parental' protoplasts differed in their nuclear genomes as well as in several cytoplasmic characters. The results from the sexual crosses (Table 1) and analyses of the androgenic progeny plants (Table 2) indicate that Type A and Type B-2 plants contain the *N. sylvestris* nuclear genome while Type B-1 plants contain both *N. sylvestris* and *N. tabacum* nuclear genomes.

Three of the cytoplasmic characters analyzed resided in the chloroplasts, namely: tentoxin sensitivity, the large subunit RUBPcase IEF patterns and the plastid DNA restriction profiles. The forth cytoplasmic character studied was male sterility which results from a nuclear-cytoplasmic interaction. The nature of this interaction is still not known. Our results indicate that Type A hybrid plants consist of *N. sylvestris* genome plus 'Line 92' chloroplasts. Therefore they are actually cybrids. Type B-1 hybrid plants consist of both *N. tabacum* and *N. sylvestris* genomes plus 'Line 92' chloroplasts. Type B-2 plants consist of *N. sylvestris* genome and *N. sylvestris* chloroplasts. They differ from *N. sylvestris* only in as much as they are not fully fertile (they are self-incompatible). Our data does not exclude the possibility of Type B-2 plants being cybrids as well, conceivably they could have been derived from a fusion event in which *N. sylvestris* contributed the nucleus and the chloroplasts, while 'Line 92' contributed other cytoplasmic entities, e.g. mitochondria. Finally, our results confirm previous observations (Chen et al. 1977; Gleba 1979; Belliard et al. 1978) that when fusion involves two measureably different populations of chloroplasts, there is a rapid segregation to either one of the other organelle type. This is true for all three of the markers which we investigated.

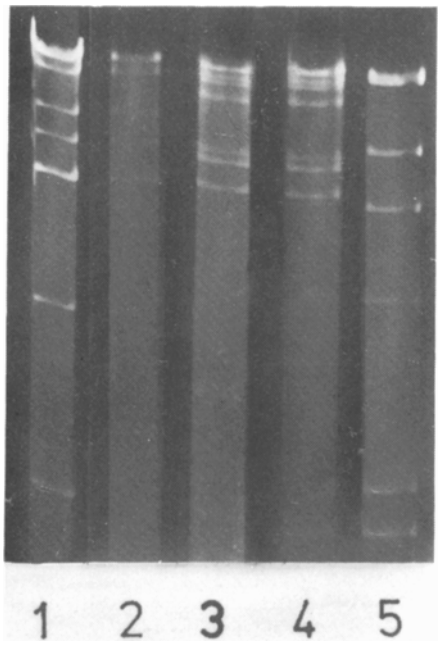

Fig. 3. BglI restriction pattern of plastid DNA. Chloroplast DNA was isolated then restricted and fractionated on 0.8% agarose gels as described in Material and Methods. **1** *Nicotiana tabacum*, **2** f22x (Type B-2), **3** f 26s (Type A), **4** CMS 'Line 92', **5** Hind III digest of λ phage DNA

The mechanism of male sterility is not yet understood. In maize, there is strong evidence that male sterility resides in the mitochondria (Levings and Pring 1976; Pring and Levings 1978). Frankel et al. (1979) favour the assumption that in tobacco the control of male sterility resides in the chloroplasts but strong evidence to support this assumption was not provided. Belliard et al. (1978), analyzing chloroplast DNA from the progeny of somatic hybrids between male sterile and male fertile tobacco plants, concluded that the control of male sterility probably does not reside in the chloroplasts and suggest the mitochondria as its possible site. Our Type A and B-1 plants contain 'Line 92' chloroplasts and are male sterile. However, it is not clear that the interaction of 'Line 92' chloroplasts with *N. sylvestris* (or *N. tabacum*) nuclear genome is responsible for the male sterility. Quite possibly, mitochondria, which may also be contributed by 'Line 92' cytoplasm, could be the cause. Indeed, if Type B-2 partial sterility is caused by a mixture of the two parental type mitochondria then the fertile androgenic plant that was obtained (Table 2) could have resulted from mitochondrial segregation.

The original fusion experiment consisted of X-irradiation of 'Line 92' protoplasts (Zelcer et al. 1978). We believe that this procedure explains the high percentage of 'cybrids' (19 out of 26) among the recovered plants. Such plants were also obtained in fusion experiments without any selection (Belliard et al. 1978; Gleba 1979), but there they composed only a small fraction of the recovered plants. Moreover, the presence of Type B-1 plants indicates that X-irradiation by a dose of 5Kr does not completely prevent a contribution from the irradiated genome. X-irradiation of protoplasts of one 'parental' plant should prove to be a useful technique for 'cybrid' production as well as for production of 'cybrids' containing one or few desired chromosomes of the irradiated genome along with the nuclear genome of the unirradiated 'parental' protoplasts.

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Literature

Atchison, B.A.; Whitefeld, P.R.; Bottomley, W.: Comparison of chloroplast DNAs by specific fragmentation with Eco RI endonuclease. Mol. gen. Genet. **148**, 263-269 (1976)

Belliard, G.; Pelletier, G.; Vedel, F.; Quatier, F.: Morphological characteristics and chloroplast DNA distribution in different cytoplasmic parasexual hybrids of *Nicotiana tabacum*. Mol. gen. Genet. **165**, 231-237 (1978)

Burk, L.G.; Durbin, R.D.: The reaction of *Nicotiana* species to tentoxin. J. Hered. **69**, 117-120 (1978)

Chen, K.; Wildman, S.G.; Smith, H.H.: Chloroplast DNA distribution in parasexual hybrids as shown by polypeptide composition of fraction I protein. Proc. Nat. Acad. Sci. (Wash.) **74**, 5109-5112 (1977)

Durbin, R.D.; Uchytil, T.F.: A survey of plant insensitivity to tentoxin. Phytopathology **67**, 602-603 (1977a)

Durbin, R.D.; Uchytil, E.F.: Cytoplasmic inheritance of chloroplast coupling factor 1 subunits. Biochem. Genet. **15**, 1143-1146 (1977b)

Frankel, R.; Galun, E.: Pollination mechanisms, Reproduction and Plant Breeding. Monographs on theoretical and applied genetics **2**. Berlin-Heidelberg-New York: Springer 1977

Frankel, R.; Scowcroft, W.R.; Whitfeld, P.R.: Chloroplast DNA variation in isonuclear male sterile lines of *Nicotiana*. Mol. gen. Genet. **169**, 129-135 (1979)

Gleba, Y.Y.: Non chromosomal inheritance in higher plants as studied by somatic cell hybridization. In: Plant Cell and Tissue Culture-Principles and Applications. The Fourth Ann. Coll. of Biol. Sci. Colloquium (in press). Ohio State Univers. Press, Columbus 1979

Goldthwaite, J.J.; Bogorad, L.: A one step method for the isolation and determination of leaf ribulose diphosphate carboxylase. Anal. Biochem. **41**, 57-66 (1971)

Izhar, S.; Power, J.B.: Somatic hybridization in *Petunia*: a male sterile cytoplasmic hybrid. Plant Sci. Letters **14**, 49-55 (1979)

Kung, S.D.: Sakano, K.; Wildman, S.G.: Multiple peptide composition of the large and small subunits of *Nicotiana tabacum* fraction I protein ascertained by fingerprinting and electrofocusing. Biochim. Biophys. Acta **365**, 138-147 (1974)

Levings, C.S.; Pring, D.R.: Restriction endonuclease analysis of mitochondrial DNA from normal and Texas cytoplasmic male-sterile maize. Science **193**, 158-160 (1976)

Melchers, G.: Einige genetische Gesichtspunkte zu sogenannten Gewebekulturen. Ber. Dt. Bot. Ges. **78**, 21-29 (1965)

Melchers, G.; Labib, G.: Somatic hybridization of plants by fusion of protoplasts. Mol. gen. Genet. **135**, 277-294 (1974)

Melchers, G.; Sacristan, M.D.: Somatic hybridization of plants by fusion of protoplasts. II. The Chromosome numbers of somatic hybrid plants of 4 different fusion experiments. In: La Culture des Tissus et des Cellules des Vegetaux. pp. 169-177. Paris: Mason 1977

Nitsch, J.P.: Experimental androgenesis in *Nicotiana*. Phytomorphology **19**, 389-404 (1969)

O'Farrel, P.H.: High resolution two dimensional electrophoresis of proteins. J. Biol. Chem. **250**, 4007-4021 (1975)

Pring, D.R.; Levings, C.S.: Heterogeneity of maize cytoplasmic genomes among male sterile cytoplasms. Genetics **89**, 121-136 (1978)

Schieder, O.: Genetic evidence for the hybrid nature of somatic hybrids from *Datura innoxia* Mill. Planta **141**, 333-334 (1978)

Steele, J.A.; Uchytil, T.F.; Durbin, R.D.; Bhatnagar, P.; Rich, D.E.: Chloroplast coupling factor 1: a species specific receptor for tentoxin. Proc. Nat. Acad. Sci. (Wash.) **73**, 2245-2248 (1976)

Thomas, E.; King, P.J.; Potrykus, I.: Improvement of crop plants via single cells in vitro – an assessment. Z. Pflanzenzücht. **82**, 1-30 (1979)

Zelcer, A.; Aviv, D.; Galun, E.: Interspecific transfer of cytoplasmic male sterility by fusion between protoplasts of normal *Nicotiana sylvestris* and X-ray irradiated protoplasts of male sterile *N. tabacum*. Z. Pflanzenphysiol. **90**, 397-407 (1978)

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