

Organelle DNA compositions and isoenzyme expression in an interspecific somatic hybrid of *Daucus*

Benjamin F. Matthews¹ and Jack M. Widholm²

¹ Tissue Culture and Molecular Genetics Laboratory, USDA, SE, ARS, Beltsville, MD, 20705

² Department of Agronomy, University of Illinois, Urbana, IL, 61801, USA

Summary. Cell suspension cultures of *Daucus carota*, *D. capillifolius* and a somatic hybrid of these lines were analyzed to determine their chloroplast and mitochondrial DNA compositions. The plastid DNAs (pDNA) from the somatic hybrid and *D. carota* were identical and were different from that of *D. capillifolius* when analyzed on agarose electrophoretic gels after digestion by the restriction endonuclease HpaII. The endonuclease restriction patterns of the mitochondrial DNAs (mtDNA) from each cell line were different. Although the restriction pattern of the mtDNA from the somatic hybrid contained fragments in common with one or both parents, unique fragments not found in the restriction pattern of either parent were also present.

The amounts and feedback regulation of aspartokinase, homoserine dehydrogenase and dihydrodipicolinic acid synthase were quantified to define the effects of somatic hybridization upon the pathway leading to the biosynthesis of lysine, threonine, methionine and isoleucine. Regulation of each enzyme by end product inhibitors was not altered in the somatic hybrid, but levels of each enzyme appeared to be increased. However, isoenzyme analysis indicated two major forms of homoserine dehydrogenase were present in the hybrid, including one unique form not present in either parent.

hybrids generated from fusion of protoplasts from other plant families.

The effects of somatic hybridization on the regulation and expression of enzymes in basic biochemical pathways have not been rigorously examined in any system. Examination of biochemical pathways in somatic hybrids may reveal nuclear-cytoplasmic interactions heretofore unknown and may increase our understanding of the biochemistry and regulation of basic biochemical pathways and how these pathways may be manipulated, using the tools of cell culture and molecular biology. For example, enzymes involved in the synthesis of lysine and threonine have been localized in the chloroplast (Bryan et al. 1977; Mills and Wilson 1978; Wallsgrave et al. 1983). This pathway provides an opportunity to examine the effects of fusion upon amino acid biosynthesis that is regulated by end product inhibition and repression (Matthews and Widholm 1979a). Key enzymes in the pathway include aspartokinase, homoserine dehydrogenase and dihydrodipicolinic acid synthase which have been examined in carrot (Matthews and Widholm 1978 and 1979a; Davies and Mifflin 1977; Sakano 1979; Sakano and Komamine 1978), soybean (Matthews and Widholm 1979b, c), corn (Matthews et al. 1975; Gengenbach et al. 1978; Walter et al. 1979), and other crop plants (Lea et al. 1979). Of these crops carrot is currently most amenable to cell culture manipulation and protoplast fusion studies (Matthews et al. 1984). Furthermore, the key enzymes in carrot which regulate lysine and threonine synthesis have been subjected to numerous studies.

The *Daucus carota* cell culture system has been used for more than two decades as a model system for embryogenesis and cell culture studies due to its rapid growth in cell culture and ability to regenerate into plants (Steward et al. 1958). Protoplasts of *Daucus carota* have been fused with protoplasts of other *Daucus* species (Kameya et al. 1981; Harms et al. 1981) or Umbellifera family members (Dudits et al. 1979 and 1980). Proof of somatic hybridization has rested upon the use of biochemically selectable markers, chromosome number and isoenzyme patterns. There are no reports in the literature on characterization of the chloroplast and mitochondrial DNAs of these fusion products or the parent cell lines, or on the effect of somatic hybridization on the regulation of a biosynthetic pathway. In this paper we report the first characterization of chloroplast and mitochondrial DNAs from *D. carota*, *D. capillifolius* and a somatic hybrid of these two species. Furthermore, we report the first analysis of the effects of somatic hybrid-

Introduction

Protoplast fusion and somatic hybrid analysis have been useful in studying the interaction and inheritance of the chloroplast, mitochondrial and nuclear genomes of plants. Most studies have focused upon the *Solanaceae* family. Organelle encoded traits, such as cytoplasmic male sterility (Zelcer et al. 1978; Belliard et al. 1978; Aviv and Galun 1980; Galun et al. 1982; Bonnett and Glimelius 1983) and streptomycin resistance (Maliga et al. 1982; Fluhr et al. 1983) have been transferred from one cell line to another. Novel mitochondrial DNA combinations in somatic hybrids of *Nicotiana* have been observed by Belliard et al. (1979), Nagy et al. (1981), and Galun et al. (1982). Recently, these observations have been extended to include *Petunia* (Boeshore et al. 1983; Izhar et al. 1983). This extensive rearrangement of the mitochondrial genome following protoplast fusion has not been well documented in somatic

ization upon the regulation and activity of enzymes in a biosynthetic pathway, including the formation of a unique isoenzyme form of homoserine dehydrogenase in the somatic hybrid.

Materials and methods

Plant material. Cell lines of *Daucus carota* L. (garden carrot) ($2n=18$), *D. capillifolius* ($2n=18$), and the somatic hybrid ($2n=36$) were described previously by Kameya et al. (1981). The garden carrot was established in culture in 1967 and later selected for resistance to azetidine-2-carboxylate (A2C) and 5-methyltryptophan (5MT) (Widholm 1977). The *D. capillifolius* and somatic hybrid have been maintained as cell suspension cultures for the past 3 years. Cells were grown by inoculating 0.25 g fresh weight cells into 50 ml liquid Murashige and Skoog (1962) medium containing 0.4 mg/l 2,4-dichlorophenoxyacetic acid and shaking (150 rpm) at 28° C.

Organelle DNA isolation. Suspension culture cells were converted into protoplasts by incubating 3 h at 28° C in 0.5% cellulase "Onozuka" RS (Yakult Pharmaceutical Industry Co., Ltd., Nishinomiya, Japan), 0.05% Pectolyase Y-23 (Seishin Pharmaceutical Co., Ltd., Nishinomiya, Japan), in Solution I, containing 0.3 M sorbitol, 0.3 M mannitol, 3 mM MES (2 N-morpholino ethane sulfonic acid), 0.6 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.7 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (pH 5.6). The protoplasts were washed in Solution I, collected by centrifugation at $150 \times g$, resuspended in ice-cold lysis buffer containing 300 mM sorbitol, 100 mM Tris-OH, (pH 7.8), 1 mM EDTA, 0.1% bovine serum albumin and 0.1% β -mercaptoethanol, and incubated on ice for 20 min. The protoplasts were ruptured by gentle passage through an 18 gauge needle and the nuclei and cellular debris collected by centrifugation at $160 \times g$ for 10 min at 4° C. The supernate was centrifuged at $3000 \times g$ for 7 min at 4° C to collect the plastids. The plastid pellet was kept on ice while the supernatant was centrifuged at $6,000 \times g$ for 15 min. The pellet, containing both plastids and mitochondria, was discarded. The supernatant was then centrifuged at $12,000 \times g$ for 15 min at 4° C to collect the mitochondria. The plastid and mitochondrial pellets were individually resuspended in 2.0 ml containing 50 mM Tris-HCl (pH 8.0), 20 mM EDTA and 2.5% Sarkosyl. The suspensions were incubated at 65° C for 30 min with occasional swirling to aid mixing and organelle lysis. CsCl was added to 4.0 M and incubated at 4° C for 1 h. The preparations were centrifuged at $12,000 \times g$, 15 min, 4° C. The clear liquid was retained and adjusted to a refractive index of 1.3955 with CsCl. Bisbenzimidazole (DeBonte et al. 1984) was added (1.5 mg/9 ml), and the preparations were centrifuged at $106,000 \times g$ for 24 h at 4° C. The plastid and mitochondrial DNA bands were removed, extracted 3 \times with isopropanol to remove the bisbenzimidazole, dialysed exhaustively against TE buffer, containing 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. The DNAs were concentrated by overnight precipitation with cold ethanol, collected by centrifugation and resuspended in TE buffer.

Organelle DNA restriction analysis. Organelle DNAs were digested with several restriction endonucleases by methods recommended by the supplier (Bethesda Research Laboratory, Gaithersburg, MD). Approximately 2 μ g of DNA

were digested in a total volume of 30 μ l for 4 h at 37° C. The digested DNA was analyzed on 0.7% and 1.0% agarose gels. Gels were stained with ethidium bromide, and photographic negatives of the gels were scanned on a Gilford spectrophotometer with a microprocessor which allowed migration distances and areas under peaks to be computed.

Enzyme extraction and analysis. Cell suspension cultures were grown for 6 days and then harvested as described by Matthews and Widholm (1978). All enzyme extraction procedures were conducted at 4° C unless stated otherwise. Enzyme activities were extracted as described previously by Matthews and Widholm (1978). Aspartokinase activity was assayed using the hydroxamate assay procedure (Matthews and Widholm 1978). The assay mixtures, containing 40 mM ATP, 20 mM MgSO_4 and 50 mM L-aspartate, were incubated at 30° C for 60 min. One unit of aspartokinase activity is equal to the amount of enzyme necessary to produce 1.0 μ mole of β -aspartyl phosphate/h. All assays included controls lacking aspartate. Homoserine dehydrogenase activity was assayed as described by Matthews and Widholm (1978). Assay mixtures contained 15 mM homoserine, 15 mM NAD, and 0.1 M KCl. One unit of enzyme activity is equal to the amount of enzyme required to produce a 0.001/min change in A_{340} . Assay mixtures lacking homoserine were used as controls. Dihydrodipicolinic acid synthase activity was measured using the o-aminobenzaldehyde assay method of Yugari and Gilvarg (1965) as modified by Matthews and Widholm (1978). Reaction mixtures contained 1.5 mM D,L-aspartic- β -semialdehyde, 37 mM pyruvate, 0.02 M Tris buffer (pH 8.2) and 0.5 mg o-aminobenzaldehyde. One unit of enzyme activity is equal to the amount which produces 0.001/min change in A_{520} at 37° C. Assay mixtures lacking pyruvate were used as controls.

Protein was determined by using a Bio-Rad (Richmond, CA) Protein Assay Kit. Homoserine dehydrogenase activity in enzyme extracts were subjected to electrophoresis in horizontal, 0.7% agarose gels submerged in a 43 mM Tris-OH, 46 mM glycine (pH 8.91) buffer. Enzyme activity was located by enzyme-specific staining techniques reported previously (Matthews and Widholm 1978).

Amino acid analysis. Three independent extractions and determinations for amino acid content were made for each cell line. Amino acids were extracted according to the method of Bielski and Turner (1966). The extracts were evaporated to dryness, resolubilized in 0.2 M lithium citrate (pH 2.2) and analyzed using a Beckman model 119 CL amino acid analyzer.

Results

Organelle DNA analysis

Restriction patterns of plastid DNA from *Daucus carota*, *D. capillifolius* and the interspecific somatic hybrid were identical when digested with either HindIII, Sall or PstI. However, differences in the plastid DNAs of *D. carota* and *D. capillifolius* were apparent when the plastid DNAs were digested with HpaII (Fig. 1). Furthermore, digestion of the plastid DNA from the somatic hybrid with HpaII indicated that the hybrid's plastid genome was the same as the *D. carota* parent. No new fragments were present in any of

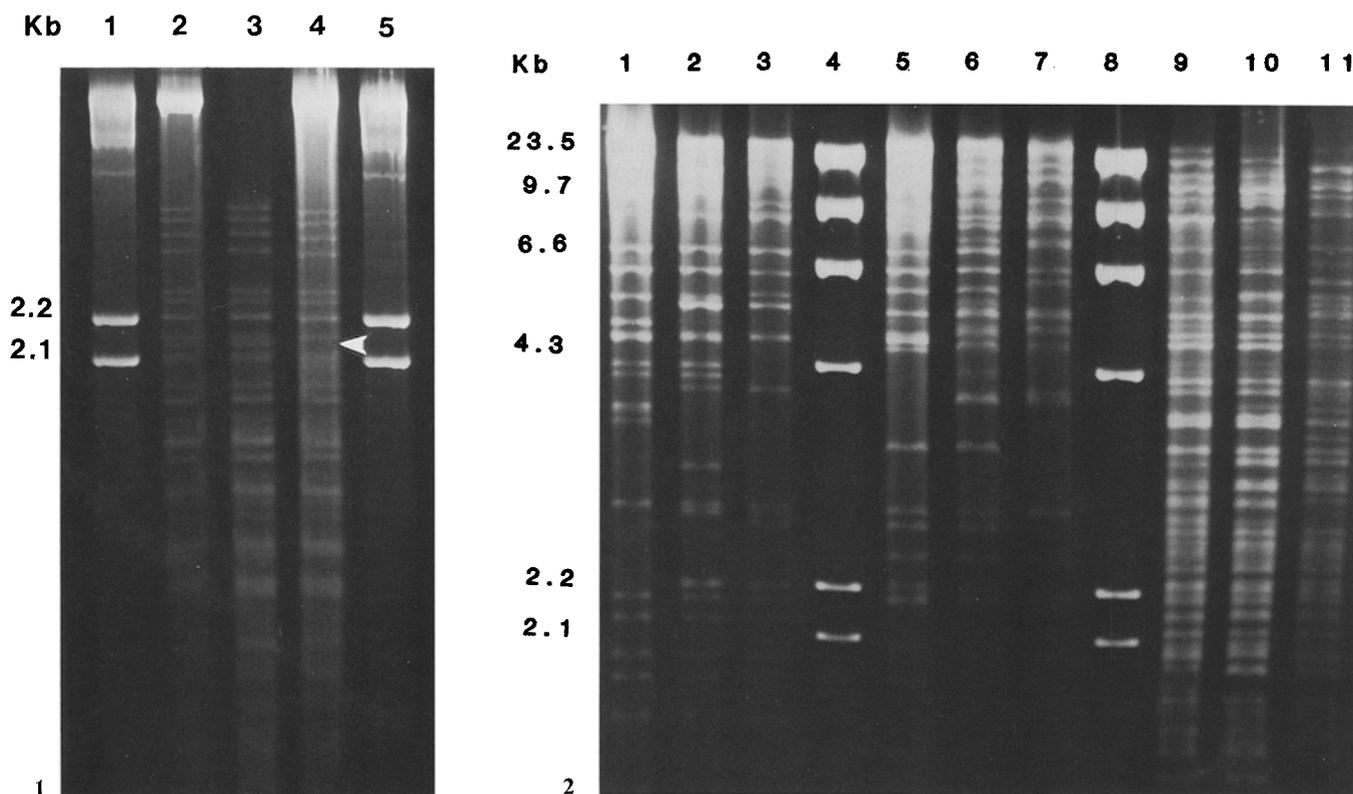


Fig. 1. Restriction fragment patterns of chloroplast DNA from *D. carota* (lane 2), somatic hybrid (lane 3) and *D. capillifolius* (lane 4) separated on a 0.7% agarose gel after digestion with HpaII. Lanes 1 and 5 are HindIII digests of λ DNA. The arrow indicates a missing band in lane 4

Fig. 2. Restriction fragment patterns of mitochondrial DNA from *D. carota* (lanes 1, 5, 9), somatic hybrid (2, 6, 10) and *D. capillifolius* (lanes 3, 7, 11). The carrot mtDNAs were digested with SalI (lanes 1–3), PstI (lanes 5–7) or HindIII (lanes 9–11). Lanes 4 and 8 are HindIII digests of λ DNA. DNA fragments were separated on a 0.7% agarose gel

the restriction patterns of the somatic hybrid plastid DNA, suggesting that no major rearrangements of the plastid genome occurred.

In contrast, the mitochondrial (mt) DNA restriction patterns of *D. carota* and *D. capillifolius* were different from each other and from the somatic hybrid (Fig. 2). Fragments unique to the DNA of the somatic hybrid indicate that rearrangements of the mtDNA occurred. Rearrangement of the mtDNA appears to be fusion-specific and not due to cell culture conditions. For example, prolonged culturing of selected sublines of *D. carota* has not noticeably affected its mtDNA. The parent cell line was initiated by Widholm in 1967. An S(2-aminoethyl)-L-cysteine (AEC) resistant subline was derived from it in 1973 and characterized as a lysine-uptake mutant in 1979 (Matthews et al. 1980). The A2C *D. carota* subline used in this paper for fusion was selected from the original cell line in 1977 (Widholm 1977). The mtDNAs from the parent and its two derivative lines have identical restriction patterns using SalI, HindIII and PstI. Other cell culture manipulations similarly indicate that the mtDNA is stable (Matthews and DeBonte 1984).

A composite of mtDNA restriction fragments from each cell line was assembled from analysis of 0.7% and 1.0% agarose gels. The composite of the SalI restriction patterns for the mtDNA of *D. carota* has 27 bands, while *D. capillifolius* has 28 bands. Of these bands, 16 appear to be in common with both *D. carota* and *D. capillifolius*, while 11 are specific to the *D. carota* and 12 are specific to the *D.*

capillifolius mitochondrial genome. The somatic hybrid mtDNA has 16 fragments in common with both parents, 6 fragments in common only with *D. carota*, 5 fragments in common only with *D. capillifolius* and 3 unique fragments. Thus, the somatic hybrid contained fragments in common with one or the other parent, or both parents. Three fragments were also present in the restriction pattern of the somatic hybrid mtDNA which were not present in either parent. Similar results were obtained when the mtDNAs were restricted with PstI and HindIII.

Although the complexity of the restriction pattern prevented accurate determination of the molecular weights of the mtDNAs, estimates of the sizes were made in which multiple fragments of a similar size were determined by the intensity of the DNA band. Using data from restriction patterns of SalI and PstI the size of the mtDNA of the somatic hybrid and parental cell lines were estimated to range between 400 and 450 kb. Digestion of the mtDNA from the somatic hybrid always generated more fragments than the mtDNA from either parent.

Enzyme and amino acid analysis

Electrophoretic analysis of enzyme extracts of the two parents and the somatic hybrid indicated that the hybrid contained two major forms of homoserine dehydrogenase (Fig. 3). The relative migration of the slower moving form coincided with that of the single form found in *D. capillifolius*

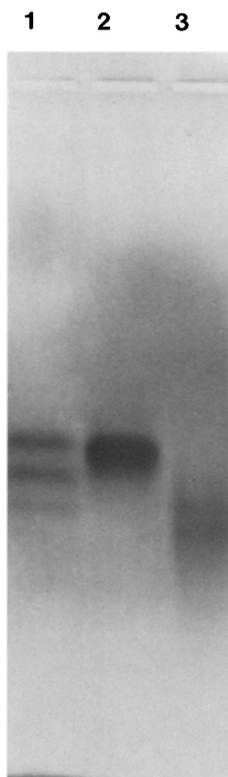


Fig. 3. Homoserine dehydrogenase activities of the somatic hybrid (lane 1), *D. capillifolius* (lane 2) and *D. carota* (lane 3) on a 0.7% agarose gel

Table 1. Enzyme activities in extracts from cell suspension cultures of line *D. carota*, *D. capillifolius*, and their somatic hybrid

	<i>D. carota</i>	Hybrid	<i>D. capillifolius</i>
Aspartokinase			
Specific activity	0.136	0.167	0.150
Relative activity	0.77	1.76	0.94
% activity (10 mM lysine)	19	16	17
% activity (10 mM threonine)	81	79	80
Homoserine dehydrogenase (NAD)			
Specific activity	129	124	93
Relative activity	688	944	682
% activity (10 mM threonine)	41	39	53
Dihydrodipicolinic acid synthase			
Specific activity	64	74	52
Relative activity	259	542	456
% activity (10 mM lysine)	95	95	94

lius. The faster moving form in the hybrid did not coincide with either the single *D. capillifolius* or two *D. carota* forms, but was unique to the somatic hybrid. The activity and feedback inhibition of aspartokinase, homoserine dehydrogenase and dihydrodipicolinic acid synthase were examined in enzyme extracts from both parents and the somatic hybrid (Table 1). The hybrid possessed slightly higher aspartokinase and dihydrodipicolinic acid synthase specific activities (units of enzyme activity/mg protein), however, the relative activities (units of enzyme activity/g fresh weight) of all three enzymes were greater in the somatic hybrid than in either parent cell line. No major differences

Table 2. Analysis of free amino acid pools^a

	<i>D. carota</i>	Hybrid	<i>D. capillifolius</i>
Amino acid (nmoles/g FrWt)			
Threonine	1300	220	300
Lysine	240	90	80
Methionine	95	40	70
Isoleucine	550	250	90
Tryptophan	325	75	4
Proline	9000	140	600

^a Data are averages of three determinations using 6-day-old cell suspension cultures

in sensitivity to end product amino acids were noted, although homoserine dehydrogenase activity from *D. capillifolius* was consistently slightly less sensitive to threonine inhibition than that from *D. carota* or the somatic hybrid.

The levels of the free amino acid pools present in the parental lines, the 5MT resistant *D. carota* and the *D. capillifolius* and the somatic hybrid were similar for most amino acids. The levels of the aspartate pathway amino acids, lysine and methionine were similar within experimental error also (Table 2). However, the level of the threonine free pool was higher in the 5MT resistant *D. carota* parental line than in the *D. capillifolius* parental line or in the hybrid, while isoleucine appeared to be only slightly higher in *D. carota*. The 5MT resistant *D. carota* cell line also contained higher levels of free tryptophan and proline than the somatic hybrid and *D. capillifolius*. The *D. carota* parental cell line was originally selected for 5-methyltryptophan resistance, and thus might be expected to contain higher levels of tryptophan. It was also selected for azetidine-2-carboxylate resistance, hence higher proline levels. The hybrid did not contain these higher levels of tryptophan and proline, although the tryptophan pool level was intermediate to the levels in the parent cell lines.

Discussion

The *D. carota* - *D. capillifolius* somatic hybrid described here is the first in the *Daucus* genus to be analyzed in depth for its organelle content and for changes in a biochemical pathway. In this hybrid the plastid DNA was the same as that of the parental *D. carota*. However, the mtDNA of the hybrid was a mixture of both parents. Rearrangements of the mitochondrial genome were indicated by the appearance of novel fragments not found in either parent. These observations are similar to those made with somatic hybrids of members of the *Solanaceae* family. In separate studies Belliard et al. (1979), Nagy et al. (1981), and Galun et al. (1982) analyzed somatic hybrids resulting from interspecific fusions and determined that no rearrangement of the chloroplast DNA occurred in the resulting hybrids, but that mtDNA rearrangements did occur. The hybrid mtDNA contained some, but not all, fragments of both parents, and in most hybrids, unique fragments were also found which were not present in the mtDNA restriction patterns of either parent.

Boeshore et al. (1983) have extended these observations to somatic hybrids derived from cytoplasmic male sterile and fertile *Petunia*. Although they did not observe a clear

correlation between the mtDNA restriction fragment pattern and cytoplasmic male sterility, they did observe extensive rearrangements of the mitochondrial genome. None of the mtDNA restriction patterns of the somatic hybrids resembled the restriction fragment pattern of either parent. This is in contrast to the observations of Belliard et al. (1979) and Galun et al. (1982) who observed restriction patterns of somatic hybrids to closely resemble those of one of the parents. The *Daucus* restriction pattern of the somatic hybrid we analyzed contained restriction fragments apparently from both parents, with few novel fragments, and did not appear to favor one parent greatly over the other.

Aspartokinase, homoserine dehydrogenase and dihydrodipicolinic acid synthase, enzymes in the pathway for lysine, threonine and methionine synthesis, were chosen for study because of their important roles in the synthesis of essential amino acids and because they appear to be nuclear encoded but compartmented for the most part in the chloroplast (Bryan et al. 1977; Lea et al. 1979; Mills and Wilson 1978; Wallsgrave et al. 1983; Sainis et al. 1981). This allows the study of nuclear-cytoplasmic interaction in somatic hybrids using an important metabolic pathway.

Analysis of several key regulatory enzymes in the pathway for lysine, threonine and methionine biosynthesis indicated that the enzyme activities in this pathway are somewhat higher in the somatic hybrid than in either parent cell line. The relative activities of all three enzymes were elevated, while the specific activities of both aspartokinase and dihydrodipicolinic acid synthase were also elevated. Elevation of the specific activities of these enzymes in relation to other cellular proteins was not necessarily expected because the levels of many proteins may be elevated in the hybrid due to the presence of both parental genomes. The presence of a unique isoenzymic form of homoserine dehydrogenase in the hybrid was also unexpected. One of the two major forms of homoserine dehydrogenase in the somatic hybrid comigrated with the *D. capillifolius* form, while only a faint band of homoserine dehydrogenase activity of the hybrid comigrated with the slower moving of the two *D. carota* homoserine dehydrogenase activities. The presence of a unique form of homoserine dehydrogenase in the somatic hybrid suggests that this enzyme is multimeric and the unique form may be a composite of subunits of the parental homoserine dehydrogenases. In fact, preliminary evidence (Matthews, unpublished) indicates that this enzyme is a dimer.

In this somatic hybrid the general regulation of the enzymes in this pathway does not appear to be grossly perturbed, in that the feedback inhibition of enzyme activities by end products was similar in both parents and the somatic hybrid.

The levels of the pools of free amino acids which are end products of the aspartate pathway indicated that only threonine levels were different between the two parental lines. More significant were the levels of tryptophan and proline which were overproduced by *D. carota*, the 5MT and A2C resistant parent cell line. It would be expected that the somatic hybrid would overproduce tryptophan, because the cybrid was selected from non-cybrids initially by its growth in 5MT levels inhibitory to *D. capillifolius* (Kameya et al. 1981). Resistance to 5MT was expressed as a semi-dominant character, and the intermediate level of the tryptophan free pool also indicates semi-dominance. However, callus from a regenerated somatic hybrid plant was

resistant to A2C as well as 5MT (Kameya et al. 1981). No indication of A2C resistance is apparent by the proline levels found in the somatic hybrid.

Somatic hybridization may be useful in revealing aspects of biochemistry of specific enzymes and may create new isoenzymic forms. These novel isoenzymes could have useful regulatory or catalytic properties which are different from those found in the parents.

Acknowledgements. This research was supported by the U.S. Department of Agriculture under Agreement No. 59-2246-1-1-737-0. The expert technical assistance of M. Woodworth and L. Nash is gratefully acknowledged.

Mention of trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

References

- Aviv D, Galun E (1980) Restoration of fertility in cytoplasmic male sterile (CMS) *Nicotiana sylvestris* by fusion with X-irradiated *N. tabacum* protoplasts. *Theor Appl Genet* 58:121-127
- Belliard G, Pelletier G, Vedel F, Quetier F (1978) Morphological characteristics and chloroplast DNA distribution in different cytoplasmic parasexual hybrids of *Nicotiana tabacum*. *Mol Gen Genet* 165:231-237
- Belliard G, Vedel F, Pelletier G (1979) Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. *Nature* 281:401-403
- Bielski RL, Turner NA (1966) Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Anal Biochem* 17:278-293
- Boeshore ML, Lifshitz I, Hanson MR, Izhar S (1983) Novel composition of mitochondrial genomes in *Petunia* somatic hybrids derived from cytoplasmic male sterile and fertile plants. *Mol Gen Genet* 190:459-467
- Bonnett HT, Glimelius K (1983) Somatic hybridization in *Nicotiana*: behavior of organelles after fusion of protoplasts from male-fertile and male sterile cultivars. *Theor Appl Genet* 65:213-217
- Bryan JK, Lissik EA, Matthews BF (1977) Changes in enzyme regulation during growth of maize. III. Intracellular localization of homoserine dehydrogenase in chloroplasts. *Plant Physiol* 59:673-679
- Davies HM, Mifflin BJ (1977) Aspartate kinase from carrot cell suspension culture. *Plant Sci Lett* 9:323-332
- DeBonte LR, Matthews BF, Wilson KG (1984) Variation of plastid and mitochondrial DNAs in the genus *Daucus*. *Am J Bot* 71:932-940
- Dudits D, Fejer O, Hadlaczky G, Koncz C, Lazar G, Horvath G (1980) Intergeneric gene transfer mediated by plant protoplast fusion. *Mol Gen Genet* 179:283-288
- Dudits D, Hadlaczky GY, Bajszar GY, Koncz CS, Lazar G, Horvath G (1979) Plant regeneration from intergeneric cell hybrids. *Plant Sci Lett* 15:101-112
- Fluhr R, Aviv D, Edelman M, Galun E (1983) Cybrids containing mixed and sorted-out chloroplasts following interspecific somatic fusions in *Nicotiana*. *Theor Appl Genet* 65:289-294
- Galun E, Arzee-Gonen P, Fluhr R, Edelman M, Aviv D (1982) Cytoplasmic hybridization in *Nicotiana*: Mitochondrial DNA analysis in progenies resulting from fusion between protoplasts having different organelle constitutions. *Mol Gen Genet* 186:50-56
- Gengenbach BG, Walter TJ, Green CE, Hibberd KA (1978) Feedback regulation of lysine, threonine, and methionine biosynthetic enzymes in corn. *Crop Sci* 18:472-476
- Harms CT, Potrykus I, Widholm JM (1981) Complementation and dominant expression of amino acid analogue resistance markers

- in somatic hybrid clones from *Daucus carota* after protoplast fusion. *Z Pflanzenphysiol* 101:377–390
- Izhar S, Schlichter M, Swartzberg D (1983) Sorting out in somatic hybrids of *Petunia* and prevalence of the heteroplasmon through several meiotic cycles. *Mol Gen Genet* 190:468–474
- Kameya T, Horn ME, Widholm JM (1981) Hybrid shoot formation from fused *Daucus carota* and *D. capillifolius* protoplasts. *Z Pflanzenphysiol* 104:459–466
- Lea PJ, Mills WR, Mifflin BJ (1979) The isolation of a lysine-sensitive aspartate kinase from pea leaves and its involvement in homoserine biosynthesis in isolated chloroplasts. *FEBS Lett* 98:165–168
- Maliga P, Lorz H, Lazar G, Nagy F (1982) Cytoplasm-protoplast fusion for interspecific chloroplast transfer in *Nicotiana*. *Mol Gen Genet* 185:211–215
- Matthews BF, DeBonte LR (1984) Chloroplast and mitochondrial DNAs of the carrot and its wild relatives. *Plant Mol Biol Reporter* (in press)
- Matthews BF, Widholm JM (1978) Regulation of lysine and threonine synthesis in carrot cell suspension cultures and whole carrot roots. *Planta* 141:315–321
- Matthews BF, Widholm JM (1979a) Expression of aspartokinase, dihydrodipicolinic acid synthase and homoserine dehydrogenase during growth of carrot cell suspension cultures on lysine- and threonine-supplemented media. *Z Naturforsch* 34C:1177–1185
- Matthews BF, Widholm JM (1979b) Enzyme expression in soybean cotyledon, callus, and cell suspension culture. *Can J Bot* 57:299–304
- Matthews BF, Widholm JM (1979c) Regulation of homoserine dehydrogenase in developing organs of soybean seedlings. *Phytochem* 18:395–400
- Matthews BF, Gurman AW, Bryan JK (1975) Changes in enzyme regulation during growth of maize. I. Progressive desensitization of homoserine dehydrogenase during seedling growth. *Plant Physiol* 55:991–998
- Matthews BF, Shye SCH, Widholm JM (1980) Mechanism of resistance of a selected carrot cell suspension culture to S(2-aminoethyl)-L-cysteine. *Z Pflanzenphysiol* 96:453–463
- Matthews BF, Wilson KG, DeBonte LR (1984) Variation in culture, isoenzyme patterns and plastid DNA in the genus *Daucus*. *In Vitro* 20:38–44
- Mills WR, Wilson KG (1978) Amino acid biosynthesis in isolated pea chloroplasts: metabolism of labeled aspartate and sulfate. *FEBS Lett* 92:129–132
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantarum* 15:473–497
- Nagy F, Torok I, Maliga P (1981) Extensive rearrangements in the mitochondrial DNA in somatic hybrids of *Nicotiana tabacum* and *Nicotiana knightiana*. *Mol Gen Genet* 183:437–439
- Sainis JK, Mayne RG, Walls Grove RM, Lea PJ, Mifflin BJ (1981) Localisation and characterisation of homoserine dehydrogenase isolated from barley and pea leaves. *Planta* 152:491–496
- Sakano K (1979) Derepression and repression of lysine-sensitive aspartokinase during *in vitro* culture of carrot root tissue. *Plant Physiol* 63:583–585
- Sakano K, Komamine A (1978) Change in the proportion of two aspartokinases in carrot root tissue in response to *in vitro* culture. *Plant Physiol* 51:115–118
- Steward FC, Mapes MO, Mears K (1958) Growth and organized development of cultured cells. II. Organization in cultures grown freely from suspended cells. *Am J Bot* 45:705–708
- Walls Grove RM, Lea PJ, Mifflin BJ (1983) Intracellular localization of aspartate kinase and the enzymes of threonine and methionine biosynthesis in green leaves. *Plant Physiol* 71:780–784
- Walter TJ, Connelly JA, Gengenbach BG, Wold F (1979) Isolation and characterization of two homoserine dehydrogenases from maize suspension cultures. *J Biol Chem* 254:1349–1355
- Widholm JM (1977) Relation between auxin autotrophy and tryptophan accumulation in cultured plant cells. *Planta* 134:103–108
- Yugari Y, Gilvarg C (1965) The condensation step in diaminopimelate synthesis. *J Biol Chem* 240:4710–4715
- Zelcer A, Aviv D, Galun E (1978) 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

Communicated by R.G. Hermann

Received May 25/Revised September 24, 1984