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BRIEF NOTE

ABNORMAL THYLAKOID FORMATION IN AN ETHYL METHANESULFONATE-INDUCED MUTANT OF *RAPHANUS*[1]

KEVIN C. VAUGHN, JOHN J. FINER[2] and KENNETH G. WILSON, Dept. of Botany, Miami Univ., Oxford, OH 45056

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Although chlorophyll chimeras are frequently used as a measure of the effectiveness of a particular mutagen treatment (Gaul 1964, Blixt 1967), little work has gone on to describe the genetics, ultrastructure and biochemistry of these chimeras (Balkema 1972, Travis *et al* 1975). Work in our lab (Travis *et al* 1975, Fields *et al* 1978, Miller *et al* 1978) and by others(Hagemann 1976) indicated that a large number of plastome mutant chlorophyll chimeras could be isolated following seed treatment with various alkylating agents. As further proof of their genetics, mixed cells with wild type and mutant chloroplasts are found in several of the mutants (Hagemann 1976, Miller *et al* 1978). In the present report we describe the induction and ultrastructure of one of the more interesting mutants of *Raphanus sativus* L.

Groups of 200 seeds of *Raphanus sativus* L. (var. Cherry Belle) were immersed in 50 ml of a 1% (v/v) solution of ethyl methanesulfonate (EMS) in distilled H_2O for 6 hours. Seeds were then washed twice with distilled H_2O, planted in vermiculite, and the plants were grown under greenhouse conditions. Putative mutants were selected at the four-leaf stage.

Mutant leaf sectors from a green and cream sectorial chimera, isolated from the above treatment, were fixed in 3% glutaraldehyde in cacodylate buffer, post-fixed in 1% osmium tetroxide, dehydrated in acetone and infiltrated with a gradient of Ladd's Super Low Viscosity resin (Ladd Inc., Burlington, VT). Thin sections (gold to silver-grey reflectance), mounted on fine copper mesh grids, were post-stained with aqueous uranyl acetate and lead citrate. Specimens were examined with a Hitachi HS-9 electron microscope.

Previous work on a variety of genera (Fields 1977, Miller *et al* 1978) has shown

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[2]Present address: Plant Physiology Section, Texas A and M Univ., College Station, Texas 77843.

that a 1% EMS treatment for 6 hours is close to optimum for mutation (from 5 to 20%), while allowing a high percentage of germination (70%). At a mutagen dose of 1.0%, a variety of *albina*, *xantha* and *viridis* mutants (Gaul 1964) can be recognized in the leaves of the M_1 progeny of radish. One of these mutants, further characterized in our report, had a large cream-white and green mosaic marginal sector. The teeth of the leaves in mutant sectors were green, indicating a green epidermis or histogenic layer I (Burk *et al* 1964). Similar green marginal teeth are found on the leaves of *Hydrangea hortensis* variegata and *Pelargonium* 'Golden Brilliantissima' (Neilson-Jones 1969) and are epidermis-derived. A pattern of sorting-out rather than cell lineage variegation indicated that our mutant is probably a plastome mutant.

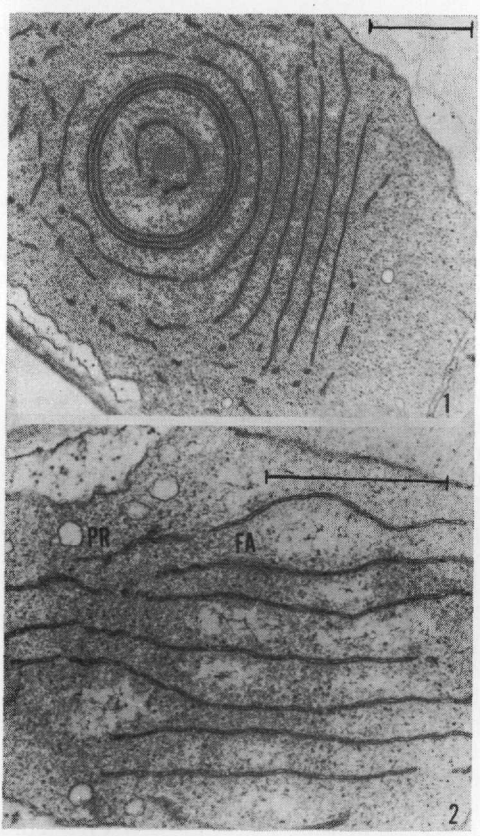

FIGURE 1. Electron micrograph of the plastids of the cream *Raphanus* mutant, showing concentrically arranged lamellae. Bar equals 1μ.

FIGURE 2. Electron micrograph of the cream *Raphanus* mutant, showing fibrillar areas (FA) of plastid DNA and peripheral reticula (PR) in the plastid. Bar equals 1μ.

When thin sections were examined, several ultrastructurally-distinct classes of plastids were observed in this mutant. Most notable in these plastids was the striking concentric lamellar system (fig. 1). Apparent linearly-arranged lamellae were those cut lengthwise rather than through this concentric lamellar system (fig. 2); these lamellae occurred singly or rarely as small doublets (fig. 2). Osmiophilic globules of varying electron densities were also noted (fig. 3). Less densely stained areas contained masses of plastid DNA between the rings of concentric lamellae (figs. 1, 2). Some invaginations of the inner membrane, which were ultrastructurally similar to peripheral reticula, appear to be a characteristic of these plastids (Sprey and Laetsch 1978). Normal green plastids of radish appeared to have a typical

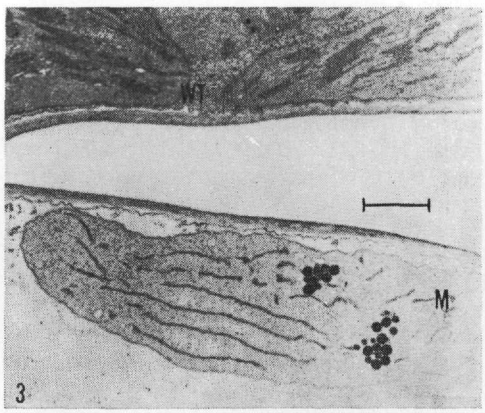

FIGURE 3. Electron micrograph showing a cell containing mutant (M) and wild type (WT) plastids. Bar equals 1μ.

chloroplast morphology (fig. 3) with a number of starch grains and normal thylakoid stacks (grana). These normal plastids occurred in cells adjacent to the mutant sectors, indicating that the mutant phenotype was not the result of a burning or wounding of the tissue due to

the mutagen treatment but to a true mutation.

The relative abundance of M_1 chlorophyll chimeras from EMS treatment offers a rich source of material for studies of the chloroplast mutants in a variety of organisms. The diplontic selection against the mutant sectors often encountered (Balkema 1972) seems to be reduced by the growing of the M_1 generation in the greenhouse rather than under constant lighting. Although these chlorophyll chimeras were often lost because they are lethal, with advances in all phases of tissue culture, such mutants may now be saved (Skirvin and Janick 1976, Skirvin 1978, Miller *et al* 1978). Several of the M_1 mutant sectors in radish are now in culture, although no full plant regeneration has been observed as yet.

Diers and Schotz (1968) described the ultrastructure of chloroplasts resulting from the disharmony between the nucleus and plastome in an *Oenothera* hybrid. In that hybrid the plastid ultrastructure observed was strikingly similar to that shown in fig. 2. Nuclear mutants of barley (Nielsen 1974) are of similar ultrastructure, indicating that a cooperative role of both nuclear and chloroplast genomes may be operative in at least this stage of plastid development.

Wettstein (1961) reported that, in the plastome mutants he had investigated, a normal granal system was formed, which was secondarily photobleached. Plastome mutants in *Oenothera* (Kutzellnigg *et al* 1975), *Mimulus* (Travis *et al* 1975) and *Hosta* (Vaughn *et al* 1978, Vaughn and Wilson 1979), appeared to have blocks in many of the developmental steps of normal chloroplast biogenesis. The ultrastructure of our chloroplast mutant of *Raphanus* appeared to be the result of one of these developmental blocks rather than a secondary destruction type; because no stages of degeneration were noted and none of the thylakoid arrangements of photobleached types were found (Fields *et al* 1978).

Chloroplasts mutants give some indication of the ways in which normal granal stacks are formed. Paolillo's (1970) interpretation of the stroma lamellae occurring in a right-handed helix is consistent with the ultrastructure observed in this mutant. If granal stacks were formed randomly at points along the helix, a normal granal arrangement could be formed and the thylakoids could then be connected *en masse* (Menke 1960). The *viridis* mutant of *Mimulus* (Travis *et al* 1975) may represent an intermediate between wild type granal stacking and this mutant of *Raphanus*.

Peripheral reticula, a character generally associated with carbon (C_4) metabolism (Laetsch 1974; Sprey and Laetsch 1978), were found in a mutant of *Raphanus*. Similar invaginations of the inner membrane are found in white and yellow mutants of *Betula* (Valanne and Valanne 1972), *Epilobium* (Anton-Lamprecht 1966) and *Hosta* (Vaughn, Wilson and Reibach 1979). Since the role of these structures in C_4 plants seems to be to facilitate transport from one plastid to another (Laetsch 1974) the peripheral reticulum in these mutants may serve a similar role. Thus, a plastid due to lack of chlorophyll may allow development of peripheral reticulum as a transport vehicle from the green to the mutant tissue.

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LITERATURE CITED

Anton-Lamprecht, I. 1966 Beitrage zum Problem der Plastidenabanderung. II. Uber das vorkmen von "Ruckmutationen" in einer spontanen standenen Plastiden schecke von Epilobium hirsutum. Z. Pflanzenphysiol. 54: 417–445.

Balkema, G. H. 1972 Diplontic drift in chimeric plants. Rad. Bot. 12: 51–55.

Blixt, S. 1967 Studies of induced mutation in peas. XXI. Effect of hydrogen ion concentration on seed-treatments with EMS. Agr. Hort. Genet. 25: 112–120.

Burk, L. G., R. N. Stewart and H. Dermen 1964 Histogenesis and genetics of a plastid-controlled variegation in tobacco. Amer. J. Bot. 51: 713–724.

Diers, L. and F. Schotz 1968 Ramuliche Bezeihungen zwischen osmiophilen Granuler und Thylakoiden. Z. Pflanzenphysiol. 58: 252–265.

Fields, M. B. 1977 Investigations of the biochemistry and genetics of mutant *Mimulus*. Unpub. Ph.D. thesis, Miami Univ., Oxford, OH.

———, K. C. Vaughn and K. G. Wilson 1978 Ultrastructural analysis of plastid degeneration in an ethyl methanesulfonate-induced mutant of *Mimulus*. J. Hered. 69: 263–265.

Gaul, H. 1964 Mutations in plant breeding. Rad. Bot. 4: 155–232.

Hagemann, R. 1976 Plastid distribution and plastid competition in higher plants and the induction of plastom mutations by nitrosourea compounds. pp. 331–38. *In:* Th. Bücher, W. Neupert, W. Sebald and S. Werner (eds.) Genetics and Biogenesis of Chloroplasts and Mitochondria. North Holland Pub. Co., Amsterdam.

Kutzellnigg, H., B. Meyer and W. Stubbe 1975 Untersuchangen on Plastommutanten von *Oenothera*. II. Überlick über die Ultrastruktur der Mutierten Plastiden. Biol. Zbl. 94: 513–526.

Laetsch, W. M. 1974 The C_4 syndrome: a structural analysis. Ann. Rev. Plant Physiol. 25: 27–52.

Menke, W. 1960 Das allgemaine Bauprimzip des Lamellar systems der Chloroplasten. Experientia 16: 537–538.

Miller, P. D., K. C. Vaughn and K. G. Wilson 1978 Induction, ultrastructure, and tissue culture of plastid mutants in carrot. *In:* Propogation of Higher Plants Through Tissue Culture: A Bridge Between Research and application. (Eds. K. W. Hughes, R. Henke and M. Constantine) U. Tenn. Press, Knoxville.

Neilson-Jones, W. 1969 Plant Chimeras. Methuen and Co. Ltd., London. 123 pp.

Neilson, O. F. 1974 Macromolecular physiology of plastids. VII. *Tigrina* mutants in barley: genetic, spectroscopic and structural characterization. Hereditas 76: 269–304.

Paolillo, D. J. 1970 The three dimensional arrangement of intergranal lamellae in chloroplasts. J. Cell Sci. 6: 243–255.

Skirvin, R. M. 1978 Natural and induced variation in tissue culture. Euphytica 27: 241–266.

——— and J. Janick 1976 Tissue culture-induced variation in scented *Pelargonium* spp. J. Amer. Soc. Hort. Sci. 101: 281–290.

Sprey, B. and W. M. Laetsch 1978 Structural studies of peripheral reticulum in C_4 plant chloroplasts of *Portulaca oleraceae* L. Z. Pflanzenphysiol. 87: 37–53.

Travis, D. M., K. D. Stewart and K. G. Wilson 1975 Nuclear and cytoplasmic chloroplast mutants induced by chemical mutagens in *Mimulus cardinalis:* genetics and ultrastructure. Theoret. Appl. Genet. 46: 67–77.

Valanne, H. and T. Valanne 1972 Structure of plastids of a variegated *Betula pubescens*. Canadian J. Bot. 50: 1835–1839.

Vaughn, K. C., K. G. Wilson and K. D. Stewart 1978 Light-harvesting pigment-protein complex deficiency in *Hosta* (Liliaceae). Planta 143: 275–278.

——— and ——— 1979 Plastome participation in chloroplast development in *Hosta* (Liliaceae). Plant Physiol. 63: 160.

———, ——— and P. H. Reibach 1979 Analysis of plastome mutants in *Hosta* (Liliaceae) that have apparent C–4 type ultrastructure. Cytobios. (In press) 1979.

Wettstein, D. 1961 Nuclear and cytoplasmic factors in the development of chloroplasts structure and function. Canadian J. Bot. 39: 1537–1545.