Artikel: Plant cancer at the cell and organismic level, in the absence of pathogens, and the resulting loss of organogenic totipotency

Autor: Gaspar, Thomas / Penel, Claude / Greppin, Hubert

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Plant cancer at the cell and organismic level, in the absence of pathogens, and the resulting loss of organogenic totipotency

THOMAS GASPAR
CLAUDE PENEL
HUBERT GREPPIN
& JEAN-MICHEL FOIDART

ABSTRACT

GASPAR, Th., C. PENEL, H. GREPPIN & J.-M. FOIDART (1994). Plant cancer at the cell and organismic level, in the absence of pathogens, and the resulting loss of totipotency. *Saussurea* 25: 63-68. In English, English and French abstracts.

A comparative investigation of habituation (hormone independence) in plant calli and vitrification (malformations associated to hyperhydricity) of shoots under vegetative multiplication *in vitro*, has led to draw neoplasic progressions in the absence of pathogens at the plant cellular and organismic levels. The cancer terminal phase of habituation in calli is characterized by the irreversible loss of capacity for undifferentiated cells to organize themselves into regenerative primary meristems. Vitreous shoots subcultured in vitrifying conditions become fully habituated with many characters similar to habituated calli and animal cancerous cells. Progressive reduction of cell-cell adhesion inducing shoot breakability (friability is the term used for calli) leads to apex necrosis. This is considered as whole plant death through abandon of the meristems, just as arrest (death) of the whole animal (human) being is caused by heart arrest. The still surviving cells in culture are further uncapable to organize themselves in primary meristems. The irreversible loss of organogenic totipotency represents the typical plant cancer trait.

RÉSUMÉ

GASPAR, Th., C. PENEL, H. GREPPIN & J.-M. FOIDART (1994). Cancer végétal à l'état cellulaire et à l'état organismique, en l'absence de pathogènes, et perte de totipotence organogénétique. *Saussurea* 25: 63-68. En anglais, résumés anglais et français.

Une étude comparative des processus d'habituation (autonomie ou indépendance hormonale) dans des cals *in vitro* et de vitrification (malformations associées à l'hyperhydricité) des pousses feuillées en cours de micropropagation a conduit à esquisser des schémas de progressions néoplasiques (en l'absence de pathogènes) aux niveaux cellulaire et organismique. Le cancer à l'état cellulaire, dans des cals habitués en phase terminale, est caractérisé par la perte irréversible des cellules indifférenciées de leur capacité à s'organiser en méristèmes primaires organogènes. Des pousses feuillées vitrifiées, subcultivées en conditions vitrifiantes, deviennent progressivement habituées aux hormones et acquièrent des caractéristiques de cals habitués et de cellules cancéreuses animales. En particulier, la réduction progressive des mécanismes d'adhésion cellulaire rend les pousses cassables (on parle de friabilité et de cellules dissociables chez les cals). Les nécroses d'apex généralisées (nécroses cellulaires dans les cals) qui en résultent provoquent la mort

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de la plante entière, en tant que telle, comme la mort de l'animal entier résulte de l'arrêt du cœur. Les cellules survivantes sont incapables de s'organiser en méristèmes primaires organogènes. La perte irréversible de la totipotence organogénétique au niveau organismique caractérise leur état cancéreux, comme au niveau cellulaire.

The concept of plant tumors and cancers has been mostly associated with pathogens (PENGELLY, 1989; BEDNAR & LINSMAIER-BEDNAR, 1989). Plant genetic tumors and habituated calli however are recognized as neoplastic diseases in the absence of pathogens (BRAUN, 1978; BAYER, 1982) but their progression to cancers and a clear-cut distinction between tumors and cancers had never been discussed. Investigating habituation and vitrification, a physiological disorder affecting shoots under micropropagation (GASPAR, 1991), we collected sufficient informations to draw neoplasic progressions leading to cancer at the cell and organismic levels (GASPAR & al., 1991). We now have data to precise this concept of cancer at the termination of plant cell and organismic neoplasic progressions, with typical traits common to animal cancerous cells, but also with at least a specific plant characteristic.

We keep in culture in parallel from 1980 three calli generated in 1979 (DE GREEF & JACOBS, 1979; KEVERS & al., 1981) from the same sugar beet strain: a normal (N) hormone-dependent callus which is green and compact, and two fully habituated, auxin- and cytokinin- independent lines; one of the latters is green and self shoot-regenerating (HO for habituated organogenic), the second is white and nonorganogenic (HNO) whatever the different physical and chemical conditions imposed in thirteen years of assays. The hormone autonomous HNO callus is very juvenile since composed of small cells with a high nucleus/cytoplasm ratio, actively dividing (frequently through a very primitive manner by simple strangulation which may remain incomplete). These traits together with the absence of cellular differentiation (no xylem cells are differentiated as in N and HO cells) are characters of tumor cells. These HNO cells exhibit additional peculiarities such as a high number of irregularly shaped and segmented nuclei per cell (HAGÈGE & al., 1992a; CRÈVECOEUR & al., 1992), polyploidy and aneuploidy (HAGÈGE & al., 1992a) and a reduced cell — cell adhesion (LINERS & al., 1994) which are typical traits of animal cancer cells, and as animal cancer cells they most probably are of a monoclonal origin, since they originated in rare white cell clumbs at the surface of the green HO callus (DE GREEF & JACOBS, 1979; KEVERS & al., 1981). The HNO callus grows through the individual growth of its cells and not through primary meristematic points as do N and HO calli. We therefore propose that the *irreversible loss of totipotency*, that is the loss of cell capacity to organize primary meristems, as a typical plant trait of plant cancerous cells, *at the terminal phase of their neoplasic progression*. The latter precision is of importance since habituation and loss of organogenic potential have been seen as reversible in most cases (MEINS, 1987, 1989).

HNO cells exhibit several biochemical traits, resulting of disturbed metabolism of nitrogen (LE DILY & al., 1993), sugar (BISBIS & al., 1993), tetrapyrrole compounds (HAGÈGE & al., 1992c), or related to stress reactions involving reducing agents and protective enzymes against activated forms of oxygen (HAGÈGE & al., 1992b; GASPAR & al., 1992) which are also characteristics of animal cancerous cells (ALBERTS & al., 1989). The HNO callus, due to its watery-translucent aspect and hyperhydric nature, also to its hypolignification (CRÈVECOEUR & al., 1987) and its altered nitrogen metabolism (LE DILY & al., 1993) has been qualified as vitrified tissue by comparison with hyperhydric and hypolignified so-called vitreous shoots under micropropagation (GASPAR & al., 1991). Vitrification of shoots appears as an adaptative response to stress conditions, involving a reorientation of several metabolisms (KEVERS & al., 1984), and resulting in

Plant material	*Independence to auxins and cytokinins*	*Presence or capacity to form primary meristems*	*Organogenic totipotency*
Organs and tissues of a plant	—	+	+
Normal callus	—	+	+
Habituated organogenic callus	+	+	partial
Habituated nonorganogenic callus cells as cancerous cells	+	—	— (irreversible)

Table 1. — A few steps of an *in vitro* cellular neoplasic progression issuing in fully habituated nonorganogenic callus cells.

Plant material	*Hormonal autonomy (auxins + cytokinins)*	*Presence or capacity to form primary meristems (M)*	*Organs formed*	*State of the meristems (M)*
Explants from a whole normal plant or from an *in vitro* prolierating cluster	—	Well functioning M	Normal stems and leaves	Normal
Vitrified cluster	—	Well functioning M	Abnormal stems and leaves	All of the M still capable of normal functioning
Vitrified cluster subcultured in vitrifying conditions	+	Bad functioning of the M	Fasciated stem. Abnormal leaves	Only some of the M capable of normal functioning
Continued subculture in vitrifying conditions	+	Abnormal functioning	Aberrant organs	Irreversible loss of M through necrosis
Callus formed at stem basis	+	—	—	—

Table 2. — A few steps of an *in vitro* organismic neoplasic progression issuing in cancerized shoots and fully habituated nonorganogenic callus.

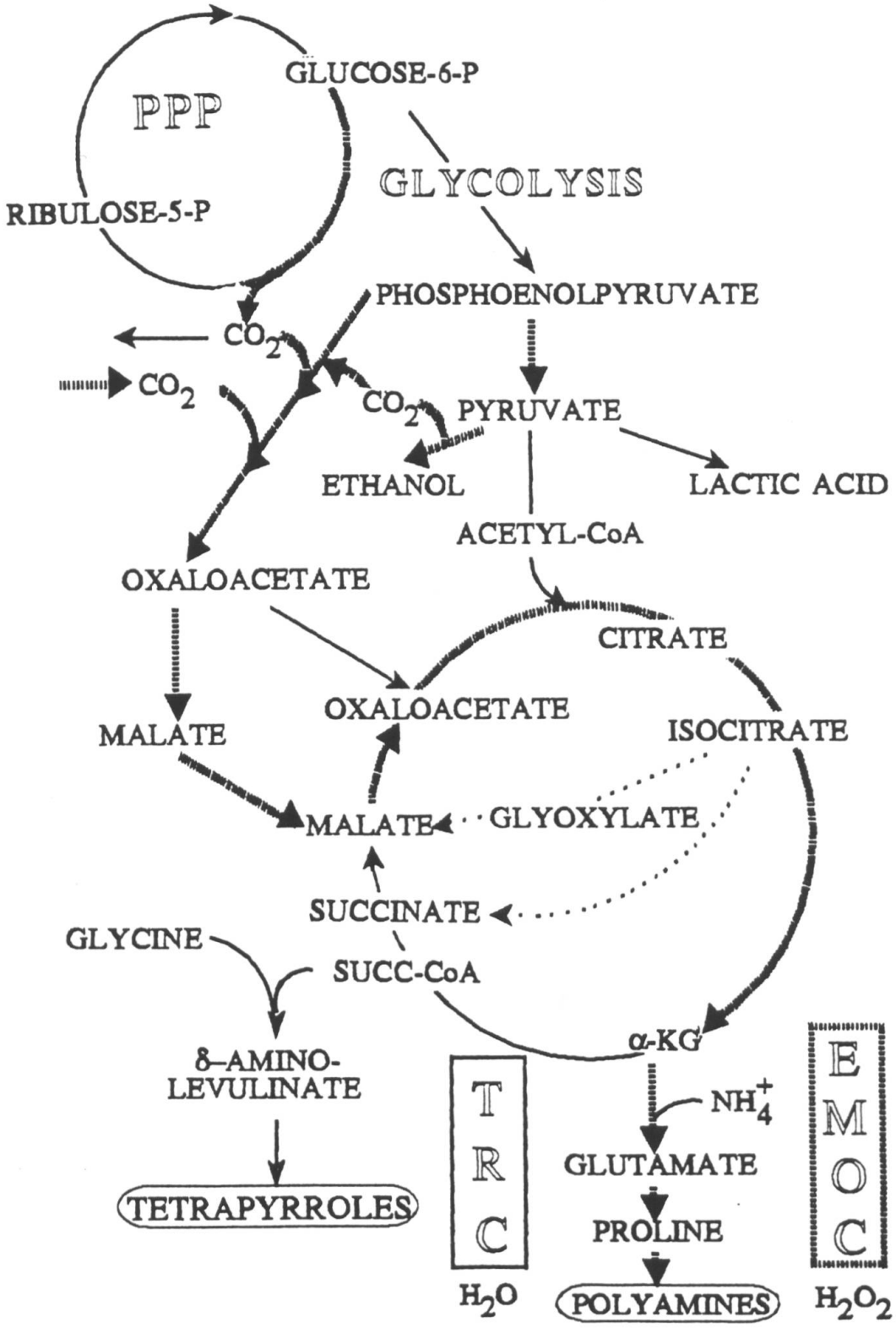

Fig. 1. — Tentative scheme showing the privileged glucose respiratory pathway of the HNO cells (heavy lines) and the relationship with overproduction of polyamines and deficiency in tetrapyrole — containing compounds. **EMOC:** extra-mitochondrial oxidative pathway. **TRC:** terminal respiratory chain. **PPP:** pentose phosphate pathway. The glyoxylate cycle (interrupted lines) still has not been evaluated.

malformed organs (ZIV, 1991). Vitrification is considered as a reversible phenomenon which means that meristems of vitrified shoots still are able to function normally when returned under non-vitrifying conditions. We have noticed that the continued assignment of vitrifying conditions to vitrified shoots at successive subcultures leads to their fully habituation (GASPAR & al., 1991). Leaves and stems become more and more malformed. Stem fasciation is an early sign of disturbance of primary meristem functioning. Necroses of (meristematic) leaf borders and apices become more frequent. The chances of survival of "shoot" explants diminish at each subculture and one can assist to the death of the whole population through necroses of all apices. May we declare that sudden death of whole shoots occurred through arrest-abandon of their primary meristems, due to generalized cancers, as animal or human being death occurs through heart arrest? In the absence of metastases, may we substitute the breakability character of vitrified shoots (friability was used to characterize easy cell dissociation in calli), that is a quite comparable loss of cell-to-cell adhesion? Shoots died as whole correlated organisms and became unable to regenerate any side shoots through axillary or adventitious shoots. The only thing they could do, whether some cells still remain living at the bases, was generating white nonorganogenic calli. Cancer at the plant organismic level therefore results in apex-meristem necroses and also is characterized by the irreversible loss by the still living cells of the capacity to organize other primary meristems. These precisions for plant cancer cells make them distinguishable from tumor cells which may reverse partially or completely and keep their totipotency (SACRISTAN & MELCHERS, 1977; BRAUN, 1982). The Table 1 and Table 2, redrawn from GASPAR & al. (1994), illustrate neoplasic progressions at the cell and organismic levels. The Figure 1, redrawn from CARRIÉ & al. (1994a), schematizes the priviledged routes of sugar metabolism in the NHO cancerous cells, justifying the previously shown

- favoured pentose phosphate pathway (BISBIS & al., 1993);
- accumulation of nitrogenous compounds (LE DILY & al., 1993);
- nonphotosynthetic CO_2 fixation through phosphoenolpyruvate (BISBIS & al., 1994);
- high malate dehydrogenase and alcohol dehydrogenase activities (CARRIÉ & al., 1994b);
- deficiency in tetrapyrrole-containing compounds (KEVERS & al., 1981; HAGÈGE & al. (1992c);
- expected formation of oxidizing agents (H_2O_2) through an extramitochondrial oxidative chain (CARRIÉ & al. 1994a).

REFERENCES

ALBERTS, B., D. BRAY, J. LEWIS, M. RAFF, K. ROBERTS & J. D. WATSON (1989). *Molecular Biology of the Cell.* Ch. 21: Cancer. Garland, New York, pp. 1187-1218.

BAYER, M. H. (1982). Genetic tumors : physiological aspect of tumor formation in interspecies hybrids. *In:* KHAL, G. & J. S. SCHELL (eds.), *Molecular Biology of Plant Tumors.* Acad. Press, New York, pp. 33-67.

BEDNAR, T. W. & E. M. LINSMAIER-BEDNAR (1989). Chemical carcinogenesis in plants and interaction with viruses and cancer causation. *In:* KAISER, H. E. (ed.) *Comparative Aspects of Tumor Development.* Kluwer Acad. Publ., Dordrecht, pp. 240-248.

BISBIS, B., J. DE RIEK, C. KEVERS & Th. GASPAR (1994). Sucrose metabolism and CO_2 uptake by chlorophyllous and non-chlorophyllous sugarbeet calli. *Bull. Rech. Fac. Sci. Agron. Gembloux.*

BISBIS, B., F. LE DILY, C. KEVERS, J.-P. BILLARD, C. HUAULT & Th. GASPAR (1993). Disturbed sugar metabolism in a fully habituated nonorganogenic callus of Beta vulgaris (L.). *Pl. Growth Regul.* 13: 257-261.

BRAUN, A. C. (1978). Plant tumors. *Biochim. Biophys. Acta* 516: 167-191.

BRAUN, A. C. (1982). A history of the crown gall problem. *In:* KAHL, G. & J. S. SCHELL (eds.) *Molecular Biology of Plant Tumors*. Acad. Press, New York, pp. 191-210.

CARRIÉ, B., B. BISBIS, C. PENEL, Th. GASPAR & H. GREPPIN (1994a). Disturbed sugar metabolism in a fully habituated nonorganogenic callus of Beta vulgaris. Additional data. (In press.)

CARRIÉ, B., Th. GASPAR, H. GREPPIN & C. PENEL (1994b). Redox characteristics of normal and habituated cell lines of sugarbeet. *Pl. Cell Environ.* 17: 457-461.

CRÈVECŒUR, M., D. HAGÈGE, A. M. CATESSON, H. GREPPIN & Th. GASPAR (1992). Ultrastructural characteristics of cells from normal and habituated sugar beet calli. *Pl. Physiol. Biochem.* 30: 87-95.

CRÈVECŒUR, M., C. KEVERS, H. GREPPIN & Th. GASPAR (1987). A comparative biochemical and cytological characterization of normal and habituated sugarbeet calli. *Biol. Pl.* 29: 1-6.

DE GREEF, W. & M. JACOBS (1979). In vitro culture of the sugarbeet: description of a cell line with high regeneration capacity. *Pl. Sci. Lett.* 17: 55-61.

GASPAR, Th. (1991). Vitrification in micropropagation. *In:* BAJAJ, Y. P. S. (ed.), *Biotechnology in Agriculture and Forestry.* Vol. 17 *"High-Tech and Micropropagation"*. Springer-Verlag, Berlin, pp. 116-126.

GASPAR, Th., D. HAGÈGE, C. KEVERS, C. PENEL, M. CRÈVECŒUR, I. ENGELMANN, H. GREPPIN & J.-M. FOIDART (1991). When plant teratomas turn into cancers in the absence of pathogens. *Physiol. Pl.* 83: 696-701.

GASPAR, Th., D. HAGÈGE, C. PENEL, J.-M. FOIDART & H. GREPPIN (1992). Peroxidases and plant cancer in the absence of pathogens. *In:* PENEL, C., Th. GASPAR & H. GREPPIN (eds.), *Plant Peroxidases 1980-1990. Topics and Detailed Literature on Molecular, Biochemical, and Physiological Aspects.* Univ. of Geneva, Switzerland, pp. 125-137.

GASPAR, Th., C. KEVERS, B. BISBIS, M. CRÈVECŒUR, C. PENEL, H. GREPPIN, F. LE DILY, J.-P. BILLARD, C. HUAULT & J.-M. FOIDART (1994). Cancer végétal in vitro: aspects morphogénétiques et biochimiques. *Compt. Rend. 4e Journ. Sci. Réseau "Biotechnologies végétales" de l'UREF* (in press).

HAGÈGE, D., R. CATANIA, H. MICALEF & Th. GASPAR (1992a). Nuclear shape and DNA content of fully habituated non-organogenic sugarbeet cells. *Protoplasma* 166: 49-54.

HAGÈGE, D., C. KEVERS, P. SALABERT & Th. GASPAR (1992b). Protective systems against activated oxygen species compared in normal and fully habituated nonorganogenic sugarbeet calluses. *In Vitro Cell. Dev. Biol.* 28P: 143-147.

HAGÈGE, D., D. WERCK-REICHHART, P. SCHMITT & Th. GASPAR (1992c). Deficiency in tetrapyrrole-containing compounds in a non-organogenic habituated sugar beet cell line. *Pl. Physiol. Biochem.* 30: 649-654.

KEVERS, C., M. COUMANS, M. F. COUMANS-GILLÈS & Th. GASPAR (1984). Physiological and biochemical events leading to vitrification of plants cultured in vitro. *Physiol. Pl.* 61: 69-74.

KEVERS, C., M. COUMANS, W. DE GREEF, M. HOFINGER & Th. GASPAR (1981). Habituation in sugarbeet callus: auxin content, auxin protectors, peroxidase pattern and inhibitors. *Physiol. Pl.* 51: 281-286.

LE DILY, F., J.-P. BILLARD, Th. GASPAR & C. HUAULT (1993). Disturbed nitrogen metabolism associated with the hyperhydric status of fully habituated callus of sugarbeet. *Physiol. Pl.* 88: 129-134.

LINERS, F., Th. GASPAR & P. VAN CUTSEM (1994). Acetyl- and methyl-esterification of pectins of friable and compact sugarbeet calli: consequences for intercellular adhesion. *Planta* 192: 545-556.

MEINS, F. Jr. (1987). Hormones and the molecular basis of determination in plants. *In:* JACKSON, M. B., S. H. MANTELL & J. BLAKE (eds.), *Advances in the Chemical Manipulation of Plant Tissue Cultures.* British Plant Growth Reg. Group, Monograph 16, pp. 19-28.

MEINS, F. Jr. (1989). Habituation: heritable variation in the requirement of cultured plant cells for hormones. *Annual Rev. Genet.* 23: 395-408.

PENGELLY, W. L. (1989). Neoplastic progression in plants. *In:* KAISER, H. E. (ed.), *Comparative Aspects of Tumor Development*. Kluwer Acad. Publ., Dordrecht, pp. 15-23.

SACRISTAN, M. D. & G. MELCHERS (1977). Regeneration of plants from "habituated" and "Agrobacterium-transformed" single-cell clones of tobacco. *Molec. Gen. Genet.* 152: 111-117.

ZIV, M. (1991). Vitrification: morphological and physiological disorders of in vitro plants. *In:* DEBERGH, P. C. & R. H. ZIMMERMAN (eds.), *Micropropagation. Technology and Application.* Kluwer Acad. Publ., Dordrecht, pp. 45-69.

Address of the authors: Th. Gaspar, Hormonologie végétale, Institut de botanique B22, Université, Sart Tilman, B-4000 Liège, Belgium.

C. Penel & H. Greppin, Laboratoire de physiologie végétale, Université de Genève, 3, Place de l'Université, 1211 Genève 4, Switzerland.

J.-M. Foidart, CHU, Université-Sart Tilman, B 23, B-4000 Liège, Belgium.