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Effect of auxin on thiophene synthesis and root morphology in *Tagetes patula* hairy-root cultures

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Tagetes species (marigolds) form thiophenes, heterocyclic sulphur-containing compounds with a strong nematicidal-activity. The highest thiophene concentrations are found in the roots. In in-vitro plant-cell cultures, thiophene accumulation increases 100-fold when root formation is initiated. This led us to hypothesize that the root-inducing phytohormone auxin is a factor regulating both thiophene biosynthesis and rhizogenesis.

Roots transformed by *Agrobacterium rhizogenes* displayed an increased sensitivity to indoleacetic acid (IAA). In these roots, thiophenes were labelled by adding [^{35}S]-sulphate to the culture medium. These experiments enabled us to quantify thiophene synthesis and degradation.

To evaluate the effects of auxin on root development and thiophene metabolism, roots were grown in absence or presence of 0·1 μM IAA. The formation of lateral roots was strongly enhanced by IAA. In contrast, IAA had an inhibitory effect on the ability to take up sulphate, on thiophene synthetic capacity, and on overall thiophene content.

Androgenic microspore cultures of the 2C1 line of *Zea mays* L.

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The maize line 4C1, developed by Morocz and colleagues at the Biological Research Centre in Szeged, Hungary, was used. Plants were grown in the greenhouse at 25°C with a 16/8 h light/dark regime. For microspore culture, N6 and YP media were used, both supplemented with 500 mg l^{-1} casein hydrolysate.

Only microspores, taken from tassels which had not yet emerged from the whorl, underwent androgenic pathways. To stimulate microspore division, several growth-regulators were tried. A combination of 0·1 mg l^{-1} 2,4-D and 2 mg l^{-1} dicamba, although reported to be effective for maize line 139/39–02 (Pescitelli *et al.* 1989, *Plant Cell Rep.* 7: 673–676), was ineffective for the 4C1 line. However, 2 mg l^{-1} PAA stimulated divisions, while highest rates were observed with 0·1 mg l^{-1} TIBA. Flotation of maize staminate-flowers at 8° for 10–14 days and refreshing the culture media each week (Genovesi, A.D. and Yingling, R.A., 1990, Abstr. 7th Int. Congr. Plant Tissue and Cell Culture, Amsterdam, p. 196) also stimulated divisions.

We were able to follow all androgenic pathways as have been described for other maize genotypes earlier (Pescitelli & Petolino 1988, *Plant Cell Rep.* 7: 741–744). The first division took place at 4–6 days after the onset of culture. Multinuclear stages were found after 9–10 days in culture. At 12 days the microcalli emerged from the burst pollen-grains. These microcalli further developed into loose or compact calli. The compact calli continued to develop into embryo-like structures.

Potentials and limitations of asymmetric hybridization in tomato

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An increase in genetic diversity is one of the main goals in plant breeding. Our approach was to establish plants after asymmetric somatic-hybridization, having the nuclear genome of one species but plastomes and chondriome components of the other species. In this research programme, the role of phylogenetic relatedness between the fusion partners in the maintenance of chloroplasts was investigated. Therefore, asymmetric hybridization experiments were carried out between protoplasts of *L. esculentum* and four donor species: *L. hirsutum*, *S. commersonii*, *S. etuberosum* and *S. nigrum*. These donor species were selected for their difference in phylogenetic relatedness to tomato. By using a cytoplasmic albino-mutant of *L. esculentum* (ALRC) it was possible to select the fusion products, based on chloroplast transfer. Because the donor protoplasts were gamma-irradiated, they were not able to divide.

In all combinations, except for the fusion between the ALRC and *S. nigrum*, regenerants were obtained from green-putative hybrid calli. These regenerants were analysed for their morphology, chloroplast

DNA and the nuclear DNA. The results of these experiments show that chloroplast transfer by asymmetric hybridization can surmount a considerable phylogenetic distance. In the fusion experiments with *L. hirsutum*, the hybrids had none of the donor nuclear DNA. However, in the combinations with less-related species, all asymmetric hybrids contained, besides acceptor nuclear-DNA, also various amounts of donor nuclear-DNA. These results suggest that donor nuclear-DNA is necessary for the maintenance of the donor chloroplasts in the hybrids, obtained between less phylogenetically related species. In the fusion with *S. nigrum* no hybrids at all were obtained, which can be due to incongruity between the parental genomes.

Effect of the phytotoxin of *Verticillium dahliae* on several developmental stages of tomato

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The interaction between a pathogen and a plant is studied using tomato as a model plant. Classical and molecular genetics of tomato are well developed and in-vitro techniques, including plant regeneration from protoplasts, are available. The fungal tomato pathogen *Verticillium dahliae* causes wilting and necrosis. A non-host specific toxin is involved in wilt development, i.e. resistant and susceptible plants are toxin sensitive. Resistance against *Verticillium*, conferred by the gene *Ve* is broken down by a new race of *Verticillium dahliae*.

Our work aims at understanding the existing *Ve*-resistance mechanism and at the development of new ways to induce disease-resistance. New resistances might be achieved by the application of in-vitro selection using the *Verticillium* toxin as a selective agent. To get more insight into the mechanism of *Ve* resistance and the mode of toxic action, the effects of the toxin on *Verticillium*-resistant sensitive tomato-cultivars as hosts and tobacco as non-host have been studied.

The toxin caused necrosis on leaves of both tomato cultivars in the light only. Protoplasts of the tomato cultivars were affected both in the light and the dark at low toxin concentrations. On tobacco or on other developmental levels of tomato (seed germination, shoot regeneration and callus growth), no significant effect was noticed. Selection for toxin insensitivity can be performed on tomato protoplasts, this insensitivity in principle is possible because tobacco protoplasts are far less affected.

After mutagenesis and selection experiments with protoplasts from a *Verticillium* susceptible tomato-cultivar, 966 putative toxin-insensitive calli were isolated. These calli now are being regenerated and the first shoots are emerging. Regenerated plants will be tested for toxin insensitivity and their level of disease resistance.

To compare induced forms of resistance to *Verticillium* with resistance conferred by the *Ve* gene, we mapped the *Ve* locus against morphological markers on position 147 of chromosome 12.

Effect of agar quality on in-vitro culture of plants

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Twenty agars were selected for physical and chemical analysis and for bioassays. Amongst these were the most commonly-used agars, including cheap and expensive ones and highly-purified agarose. It appeared that the agars had different effects on the pH of the media, indicating that agar is not an inert gelling-agent. The water content varied between 3 and 20%. The ash content of agar varied from 2·5 to 5·0%. Measurement of the EC of agar solutions showed that some agars are heavily contaminated with salts. With spectrophotometry and with neutron-activation analysis the contents of elements were determined. A direct relationship between the growth of plants on the agars and concentrations of elements has not been discovered till now, but the contribution of agar for some elements like copper may exceed that of the classical nutrient solution. An experiment with roses showed that the optimum agar concentration depended on the type of agar used. Organic contaminants were present as was demonstrated by determining the UV-absorption after washing the agars with ethanol. In this respect the auxin-like activity of some agars is interesting. On these agars, more roots and callus formed on explants.

In general, woody plants were more sensitive to the agar quality than herbaceous plants. *Syringa vulgaris* (lilac), being a salt-tolerant species *in vitro*, appeared to be an exception, only the agar concentration influenced the growth of the cultures. Roses were very sensitive: up to 100% of the explants died on one of the agars tested. The growth and development of bulbous plants *in vitro* was also affected by the type and brand of agar. *Hippeastrum*, tulip and hyacinth showed different growth characteristics on the agars that were used. Also *Gerbera* appeared to be very sensitive to agar quality (Pierik, R.L.M. 1991, *Acta Hortic* **289**: 45–55). In the bioassays there was no direct relationship between the price and degree of purification of the agars and the growth and development of the plants. Agarose sometimes inhibits the growth of the plants, indicating that agars, in contrast to agarose, may contain compounds which promote the growth of in-vitro cultures.

Karyological studies on different somatic hybrid calli of *Solanum tuberosum* L. + *Nicotiana Plumbaginifolia vivani*

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Leaf protoplasts of a monohaploid ($x=12$) potato H7322 were fused with tetraploid ($4x=40$) *N. plumbaginifolia* callus protoplasts, isolated from the nitrate reductase deficient (NR^-) mutants NA36, Cnx20, and Nia26, and from the histidin auxotrophic mutant RA132. The first combination was fused by using PEG treatment, the latter two by electrofusion, and the combination H7322(+)Cnx20 was fused in both ways. Heterologous fusion products were selected biochemically and in the case of H7322(+)Nia26 also mechanically, with a micropipet. The hybrid nature of the obtained calli was established by tests for nitrate-reductase activity or histidin prototrophy and by karyotyping. All calli showed variable chromosome-numbers, in general 6–20 potato chromosomes and 40–120 *N. plumbaginifolia* chromosomes, sometimes higher. Similar chromosome-numbers were found in NR^- calli obtained by biochemical selection from cell suspensions of H7322(+)Nia26 hybrid NR^+ calli. Intra- and interspecific chromosome-translocations frequently occurred in both NR^+ and NR^- calli. Air-dried spread-chromosome preparations showed less chromosome overlap than squash preparations, thereby revealing higher numbers of potato chromosomes. Irrespective of whether or not certain potato chromosomes had been eliminated, the somatic hybrids proved not suitable for mapping the complementing potato-genes: the chromosomal variation was too high. Regenerants from PEG-fused H7322 + Cnx20, by now cultured for over 5 years, have retained their almost complete potato genomes.

Somaclonal variation and screening for disease resistance in tomato

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Disease-resistant plants have been obtained by the screening of regenerants, or their progenies, at the whole-plant level. The perspectives of this approach were investigated in order to obtain tomato plants with resistance to bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis*. Somaclones were regenerated from leaf, cotyledon and hypocotyl explants of the susceptible tomato cv. Moneymaker. Various phenotypic alterations were observed among the regenerated plants (R1), but were not transmitted to the progenies. Aberrations of ploidy level, mainly tetraploidy, occurred in R1 plants and their R2 progenies. The frequency of polyploid plants depended on the explant source and showed a correlation with the percentage of polyploid cells present in the explant material. Several monogenic, recessive mutations were recovered in the R2 populations, four of which were shown to be allelic to known recessive single-gene mutants. Neither explant source nor duration of tissue-culture period influenced mutation frequency or mutation spectrum. In addition, somaclonal variation was compared with variation induced by treatment of seeds with ethyl methanesulphonate (EMS), with respect to a number of mutant types that could be scored unambiguously. The results showed that generally, the frequency of mutation was higher after EMS treatment. With respect to the mutant spectrum, no clear differences were observed between the spectra obtained after EMS treatment and tissue culture, except in the case of polyploid variants, which were not found after EMS treatment.

As genetic variation was observed, progenies of the somaclones were tested for resistance to bacterial canker. A fast screening method in the greenhouse, with a criterion for the selection of single, putatively-resistant plants based on the severity of wilting symptoms, was used. The evaluation of progenies of 279 somaclones for resistance showed that some variation for severity of wilting was present. However, somaclones with a major increase in resistance and thus valuable for plant breeding, were not found. The results suggest that the potential for somaclonal variation as a source of resistance to bacterial canker is limited.

Primary and secondary somatic embryogenesis in cassava

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A procedure has been established to obtain routinely somatic-embryos and plants from cassava. Four stages can be discerned: induction of embryos, germination of embryos, shoot development of germinated embryos (GE) and rooting of plants. Thirteen of the 15 tested clones originating from Africa, South America and Indonesia; GE and plants were obtained using these clones.

Both leaf-lobes of greenhouse grown and in-vitro grown plants were used as starting material. Lobes of greenhouse-grown plants gave the best results,

however the efficiency of regeneration depended on the growing conditions of the plants. For example M. Col 22 formed GE on 5–80% of the lobes. Lobes of in-vitro plants responded more stably but at a lower frequency (10–30% of the lobes formed GE).

Under optimal conditions, M. Col 22 lobes formed 24 GE/lobe and Tjurug 8 GE/lobe. As much as 71 GE could be isolated of one M. Col 22 lobe and 34 GE of one Tjurug lobe. Sixty per cent of the M. Col 22 GE developed into shoots and 12% of the Tjurug GE. The shoots were either normal-looking or deformed. However, after prolonged culture most of the deformed shoots reverted to normal-looking shoots.

Leaf-lobe derived somatic-embryos combined a high-embryogenic response and repeatability. Depending on the stage of the embryo and the used 2,4-D concentration in stage 1 between 50–90% of the primary somatic-embryos form new secondary-embryos. Even after eight cycles (stage 1→stage 2→stage 1, etc.) the GE retained their full embryogenic-capacity and were able to develop in morphological normal cassava shoots.

Experiments directed at improving the multiplication factor between the different cycles are performed with the final goal to obtain (single-cell originating) embryogenic cultures.