Behaviour of MLO Evoking Potato Witches' Broom in Callus Tissue Culture of *Solanum laciniatum* AIT. and *Nicotiana tabacum* L. cv. Samsun

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Abstract. The growth of callus tissue cultures and the infectivity of twenty five *Solanum laciniatum* AIT. plants and of sixteen *Nicotiana tabacum* L. cv. Samsun plants were investigated. The plants were obtained from callus tissue cultures derived from stem pieces of the respective plants infected with a mycoplasma-like organism (MLO) evoking potato witches' broom. The tissues were cultivated on synthetic nutrient medium with kinetin and IAA. All *de novo* obtained *S. laciniatum* plants were healthy. On the contrary twelve of the sixteen reconstituted tobacco plants showed MLO presence.

Summarizing these and previous results, the authors suppose that the most important factor influencing MLO persistence in callus tissues cultivated on the applied nutrient medium may be the callus growth rate and the organogenesis set. Both these conditions are determined by the metabolism of the investigated plant species.

JACOLI and RONALD (1974) and JACOLI (1974) investigated the presence of a mycoplasma-like organism (MLO) in callus tissues derived from carrot root and aster stem. These authors demonstrated the presence of MLO in their electron microscopic studies but they failed to transmit aster yellows agent from callus tissue culture by leafhoppers. They did not investigate the fate of MLO in tissue cultures because they found them especially in primary explants and in subsequent transfers MLO gradually disappeared.

From the growth and infectivity point of view, callus tissue cultures derived from tomato and *Nicotiana glauca* GRAH. plants infected with potato witches' broom were investigated by Petrů *et al.* (1971, 1972). Cultivation conditions for MLO persistence in callus tissue cultures of *N. glauca* are given by Petrů and Ulrychová (1975). MLO persists in callus tissues cultivated *in vitro* on a synthetic medium with kinetin and IAA as growth regulators. This nutrient medium is suitable for organogenesis associated with the formation of sieve tubes where these microorganisms are mostly localized. However the nutrient medium alone does not determine MLO persistence in callus tissue cultures and the factor of primary importance may be the host plant. While the tissues derived from *N. glauca* stem grow on the above mentioned medium very well and the organogenesis appears

Received March 6, 1978; accepted April 11, 1978.

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relatively soon (Petrů and Ulrychová 1975) the growth of callus tissue cultures derived from Solanum nigrum L. proceeds very slowly and the formation of organs takes place on the same nutrient medium only after more than several months of cultivation (Petrů and Ulrychová 1977).

The trials to evoke organogenesis on the same nutrient medium in callus tissues derived from stems of tomatoes infected with potato witches' broom were negative in both healthy and infected tissues.

The endeavour to generalize these partial results of our studies led us to new experiments with additional Solanaceous plants. We chose Solanum lacinium Ait. and Nicotiana tabacum L. cv. Samsun. S. lacinium is economically a very important plant for its high content of the steroidal alcaloid solasodine serving as a precursor for the synthesis of steroid drugs and N. tabacum is very often used as experimental material in plant tissue cultures. The reaction on potato witches' broom infection of the first host plant has been described by Ulrychová and Jokes (1977) and of the second one by Valenta (1958).

Material and Methods

Callus tissue cultures were derived from stems of S. lacinium Ait. and N. tabacum L. cv. Samsun plants both healthy and infected with potato witches' broom. The infectivity of S. lacinium plants was verified by back transmission (by grafting) on tomatoes reacting very sensitively to this disease; S. lacinium plants are mostly a symptomless carrier of this disease.

The nutrient medium, the technique of callus tissue establishment and of infectivity assays are described in papers of Petrů and Ulrychová (1975) and Ulrychová and Petrů (1975).

Results and Discussion

Callus tissue developed on stem pieces of S. lacinium cultivated in vitro on the nutrient medium used is compact and there are no differences in growth rate and appearance between the tissues derived from healthy and diseased plants. Organogenesis appears during two to four months. The stems are formed relatively rarely (Fig. 1) and their growth is very slow. On the contrary, organogenesis is visible in callus tissue derived from N. tabacum cv. Samsun in some cases during a fortnight, it is very intense and proceeds very quickly (Fig. 2). Callus tissue originating from healthy and MLO infected plants behave identically.

Reconstituted plants of S. lacinium were obtained after six months on average, those of N. tabacum cv. Samsun even after two months of cultivation. Rooted plants were further cultivated after transmission into soil in a greenhouse and repeatedly assayed for the presence of MLO by grafting onto tomatoes. Twenty-five reconstituted plants of S. lacinium were assayed in two or three repetitions. These plants were derived from different stem parts and from cultures established in various seasons. We failed to demonstrate the presence of MLO in all 25 examined S. lacinium plants derived from diseased individuals. Sixteen reconstituted N. tabacum cv. Samsun plants also obtained from various explants were assayed similarly. Twelve plants demonstrated the presence of MLO in repeated assays, the
remaining four were healthy. Reconstituted plants derived from callus tissues of the controls of both studied plants were healthy according to our expectation.

Summarizing these results and comparing them with those obtained on previous experiments, we came to the conclusion that the most important factor influencing the MLO persistence in callus tissues cultivated in vitro on the used nutrient medium may be the callus growth rate and the organogenesis set. Both these conditions are determined by the metabolism of the respective plant species.

MLO persisted in callus tissue cultures of *N. glauca* and *N. tabacum* cv. Samsun, *i.e.* in fast growing tissues showing prompt organogenesis. The presence of MLO in *N. glauca* explants was determined after several subcultivations over a time period longer than two years. On the contrary, *Solanum nigrum* and *S. laciniatum* are plant species with relatively slow-growing calli and gently proceeding organogenesis on the applied medium and MLO were eliminated in both cases through the callus cultivation. MLO are localized *in vivo* and in callus tissue cultures *in vitro* mostly in sieve tubes and only rarely in their companion cells. The beginning of a callus culture derived from stem pieces is characterized by the tissue dedifferentiation which is followed by differentiation processes. Two essential types of differentiation are observed in tissue cultures. Some are histogenetic and lead to the formation of new tissues, while others are organogenetic, that is, they result in the formation of new organs such as roots, buds, flowers *etc.* (Gautheret 1966). According to our preliminary results (Petřů and Ulrychová 1976), scattered sieve tubes and tracheids appear in the parenchymatous tissue formed by the dedifferentiation process having no direct connection with vascular bundles of the original stem piece. Therefore we believe that MLO may penetrate through parenchymatous cells of callus tissue formed by dedifferentiation into sieve tubes of newly developed organs. In contradiction to Jacoli (1974) we do not suppose only a passive transport of MLO with nutrient flow but we presume that a very important factor in MLO spread in the host tissue is the affinity of these microorganisms towards more convenient nutrient conditions (Ulrychová and Limberk 1972). If the shift of MLO in callus tissue culture cannot be realized in a time limit the microorganisms perish and the host plant gets rid of the pathogen.

**References**


Figures at the end of the issue.

BOOK REVIEW


The volume is based on the concept that the biosynthesis of secondary natural substances is a widely found characteristic of cell specialization in almost all organisms. As in other specialization processes, the formation of the enzymes of secondary metabolic pathways is dependent on the stage of differentiation of the respective cell. For this reason the central theme of the book is the analysis of differentiation programmes.

The volume is divided into two parts. The first part deals with various aspects of cell specialization of microorganisms, higher plants and animals from the view of expression of secondary metabolism. The chapters include: coordinate and noncoordinate promotion of enzymes of secondary metabolism, regulatory effectors in this field and phase dependence of secondary metabolism and the organization of differentiation programmes.

The second part of the book concerns the particular aspects of the secondary natural substances in cell cultures of higher plants and problems of differentiation. The chapters present: the fate of secondary metabolism during initiation of plant cell cultures, realization of secondary metabolism (triggering factors, enzyme activities, comparison with the related intact plant), correlation between secondary metabolism and cellular structures and growth of plant cell cultures and formation of secondary substances.

This book will be very useful to plant physiologists and biochemists.
Fig. 1: Primary explant with callus and newly formed stems; explant derived from the stem of *Solanum laciniatum* Arr. plant infected with potato witches' broom; 11 weeks old culture.

Fig. 2: Primary explant with callus and newly formed stems; explant derived from the stem of *Nicotiana tabacum* L. cv. Samsun infected with potato witches' broom; 6 weeks old culture.