CHANGES IN PEROXIDASE AND POLYPEPTIDE PROFILES IN *Nicotiana tabacum* L. TRANSFORMED WITH *Agrobacterium rhizogenes*

MUDANÇAS NOS PERFIS DE PEROXIDASE E POLIPEPTÍDEOS EM *Nicotiana tabacum* L. TRANSFORMADA COM *Agrobacterium rhizogenes*

Sergio Echeverrigaray¹

SUMMARY

Morphological and proteic modifications in *Nicotiana tabacum* L. transformed by *Agrobacterium rhizogenes* were evaluated by the comparison of normal and transformant plants regenerated from hairy-roots formed by the strains A4 or IB-642 of *A. rhizogenes*. Changes in apical dominance were observed in IB-642 transformants, which exhibited an abnormal development of axillary buds. The electrophoretic analysis indicated an increase in peroxidase activity and the induction of several isozymes of this complex in the transformants. The SDS-PAGE patterns comparison allows to identify several changes, specially, the increase in 31-33 and 54 kD polypeptides in the transformants. Biochemical analysis suggests the induction of a pathogen or stress like response of the transformants due to the high auxin concentration codified by *A. rhizogenes* T-DNA incorporated to the plant genome.

Key words: genetic transformation, *Agrobacterium rhizogenes*, *Nicotiana tabacum*, electrophoretic analysis.

RESUMO

Modificações morfológicas e protéicas em transformantes de *Nicotiana tabacum* L. com *Agrobacterium rhizogenes* foram avaliadas através da comparação de plantas normais e transformantes regeneradas a partir de raízes pilosas formadas pelas linhagens A4 ou IB-642. Mudanças na dominância apical foram observadas em transformantes com IB-642, os quais exibiram um desenvolvimento anormal dos brotos axilares. As análises eletroforéticas mostraram um aumento na atividade de peroxidase e indução de várias isoenzimas deste complexo nos transformantes. A comparação dos perfis de SDS-PAGE permitiu identificar várias modificações, especialmente, o aumento dos polipeptídios de 31-33 e de 54 kD nos transformantes. As análises bioquímicas sugerem a indução de uma resposta do tipo patógeno ou estresse devida a alta concentração de auxina codificada pelo T-DNA de *A. rhizogenes* incorporado ao genoma.

Palavras-chave: transformação genética, *Agrobacterium rhizogenes*, *Nicotiana tabacum*, análise eletroforética.

¹Instituto de Biotecnologia, Universidade de Caxias do Sul, Caixa Postal 1352, 95001-970 - Caxias do Sul, RS.

INTRODUCTION

Agrobacterium rhizogenes is known to cause hairy-roots disease in many dicotyledoneous plants. This specie is closely related to the oncogenic *Agrobacterium tumefaciens* by both physiological and DNA homology criteria (HUFFMAN et al., 1984). Both species harbour high molecular weight DNA plasmids, known as Ri and Ti plasmids respectively, which are responsible for the virulence behavior and the morphological and chemical changes on the infected tissues. During the infective process part of the plasmid is replicated and transferred to the host cell. In *A. rhizogenes* the T-DNA, that in many cases can be found in two non-contiguous sequences (TL and TR), harbours genes which are responsible for the production of indolacetic acid and for the synthesis of opines, mainly agropine and/or mannopine. When transferred to the host cell, the T-region is stably integrated in the plant genome, stimulating the synthesis of IAA which promotes the differentiation to hairy-roots (BINNS & THOMASHOW, 1988).

Different phenotypes have been described in plants regenerated from a single root culture (TEPFER & CASSE-DELBART, 1987). The typical transformed (T) phenotype have moderately wrinkled leaves and are somewhat shorter than normal plants. The response of the cells to the T-DNA expression can be complex, the ultimate phenotype being determined not only by the transfer of the T-DNA but mainly by the plant cells response to the hormonal changes (PENGELLY et al., 1986). In the present study we report the changes in isozymes and polypeptides in *Nicotiana tabacum* transformed plants obtained by the regeneration of *A. rhizogenes* induced hairy-roots.

MATERIAL AND METHODS

Two strains of *Agrobacterium rhizogenes* were used to transform *Nicotiana tabacum* leaf fragments: A4 (kindly supplied by Dr. M. Tepfer, INRA-Versailles, France) and IB-642 (obtained from the Biological Institute of São Paulo, Brazil). The leaves of tobacco plants grown *in vitro* on 1/2 MS medium (MURASHIGE & SKOOG, 1962), were cut into 2cm fragments and transferred upside down to Petri dishes containing MS medium. A bacterial inoculum obtained by overnight culture of the strains on MYA medium (TEPFER & CASSE-DELBART, 1987), was used to inoculate the leaf fragments. After three days incubation at 25°C, the fragments were transferred to a new MS medium supplemented with 500mg/l of cefotaxime, and maintained at 25°C with a day length of 16 hours for 20 to

30 days. The hairy-roots formed from the incisions on the leaf fragments were subsequently transferred at 15 day intervals to fresh MS agar plates with cefotaxime, being incubated at 25°C in the dark. After three replicates the roots were transferred to MS medium without antibiotic and submitted to a 18 hours photoperiod at 25°C to stimulate plant regeneration. The transformant condition of the regenerated plants was confirmed by the electrophoretic evaluation of agropine and mannopine by the method reported by TEPFER & TEMPE (1981).

The samples used for the electrophoretic analysis of proteins and isozymes were obtained by the homogenization of 1 gram of leaves in 50mM Tris-HCl, pH 8.0 buffer at 4°C and centrifugation at 10000xg for 5 minutes. For total proteins analysis the samples were concentrated 10 times after precipitation with 2 vol. acetone (-20°C) for 2h. The electrophoretic separations were made by the methods of DAVIS (1964) for isozymes and LAEMMLI (1970) for SDS-PAGE polypeptides analysis. The SDS-PAGE (12%) gels were scanned with a Tecnow ARGOS-7 densitometer at 567nm to compare the relative intensity of the bands previously developed with Coomassie Brilliant Blue R-250.

RESULTS AND DISCUSSION

According to the procedures all the leaf fragments inoculated either with *A. rhizogenes* A4 or IB-642, developed hairy-roots after 20 days. The frequency of root induction was higher for the IB-642 strain. Roots grew rapidly when transferred to the MS medium with cefotaxime, giving in the sequence spontaneous regenerant plants. The higher regeneration was obtained from hairy-roots induced by IB-642. All the regenerated plants exhibited agropine production and were confirmed as real transformants. Those transformants which did not showed bacterial contamination were used for the subsequent studies in order to avoid possible confusion between effects due to the presence of the T-DNA region and those induced by the presence of the bacteria. When grown under normal conditions, the transformed plants exhibited the typical wrinkled leaves previously reported by TEPFER & CASSE-DELBART (1987) on *A. rhizogenes* transformed plants. In the case of transformants produced with IB-642 it was also observed the abnormal development of axillary buds, several of which grow until the production of seeds, giving to the plants a typical aspect (Figure 1). This phenotype is probably due to higher auxin induction by the IB-642 T-DNA region incorporated in the plant genome, in an analogous situation to that reported by AMASINO & MILLER (1982).

Figure 1. Initial stage in the development of axillary buds in IB-642 transformed plants (right) exhibiting the wrinkled leaf phenotype and normal plant (left) phenotype.

Table 1. Average relative concentration of SDS-PAGE bands of the leaf extracts profiles from transformants and original plants.

BAND No.	MW(D)	642	ORI	A4
1	98200	3.63	4.09	3.24
2	86900	1.27	1.51	1.25
3	85000	2.97	3.99	2.99
4	75200	1.51	2.91	1.25
5	71100	6.15	6.14	5.99
6	69600	0.00	3.77	0.00
7	62900	3.05	2.69	2.24
8	59500	3.14	3.02	3.24
9	56300	4.29	3.13	3.99
10	53900	8.52	6.57	8.73
11	52100	4.35	7.11	5.99
12	46100	4.65	5.82	4.99
13	43600	4.23	4.74	5.49
14	41700	6.10	6.25	5.49
15	40300	5.68	6.47	5.74
16	38100	6.16	6.36	6.23
17	32600	6.34	4.85	6.48
18	31600	8.05	3.88	8.23
19	26400	6.00	5.93	5.94
20	24200	3.81	4.31	4.49
21	23400	3.50	3.23	3.99
22	22100	3.87	3.23	4.04

Qualitative variations were observed between the peroxidase patterns of the normal and transformant plants (Figure 2). The transformants showed an increase in the number of bands which was accompanied by an increase in total peroxidase activity. The comparison between the polypeptide patterns (SDS-PAGE) of normal and transformant plants showed quantitative differences (Figure 2 and Table 1), specially at the bands with apparent molecular weights of 54, 33 and 31kD. Those bands have the same molecular weight of the quitinase and glucanase polipeptides reported by MAUCH & STARHLIN (1989). The variations in peroxidase and in polypeptide profiles indicate a stress like response of the plant to the presence of T-DNA or to one of its products.

At despite of the morphological variations observed between transformants induced by A4 and IB-642, we could not detect qualitative or quantitative differences in their esterase, peroxidase and polypeptide profiles (Figure 2 and 3).

Figure 2. Esterase (A) and peroxidase (B) profiles of Nicotiana leaf extracts from *A. rhizogenes* transformed and normal plants. a-IB-642 transformants, b - normal plant, c - A4 transformants.

Figure 3. SDS-PAGE electrophoretic profiles of *N. tabacum* leaves from IB-642 transformed plants (a,b,c), non transformed plants (d) and A4 transformed plants (e).

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