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Resistance of Transformed and Non–Transformed Oilseed Rape cv. HM–81 to the Infection with Cauliflower Mosaic, Turnip Yellow Mosaic and Turnip Mosaic Viruses

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Abstract. Resistance of transformed and non–transformed spring oilseed rape cv. HM–81 to the infection with cauliflower mosaic virus (CaMV), turnip yellow mosaic virus (TYMV) and turnip mosaic virus (TuMV) was studied, to determine the influence of transformation on susceptibility of plants to viruses. For experiments the non-segregating R 1 generation of primary transformant HM–81–JZ and control plants of cv. HM–81 were used. The primary transformant was obtained by inoculation of stems with *Agrobacterium rhizogenes* 15834. All transformed plants of R 1 generation had typically „transformed" phenotype. No significant differences were revealed in the resistance of both transformed and non-transformed plants to each virus, as proved by qualitative and quantitative ELISA and visual evaluation of symptoms. Transformed plants infected with turnip yellow mosaic virus showed significantly lower reduction of green mass yield than non-transformed. In the case of CaMV and TuMV infection reduction of yield of transformed and non-transformed plants was almost the same.

Transformation of T–DNA of pRi or pTi plasmid (present in *Agrobacterium rhizogenes* or *A. tumefaciens*) causes changes in several plant characters. The changes in the sensitivity of pRi or pTi transformants against pathogenes were studied in a few cases only. Hairy roots induced with T–DNA of pRi in *Lycopersicon esculentum, Cucumis sativus, Linum grandiflorum* and some other plant species are resistant against *Fusarium oxysporum* (Mugnier 1988). Tumor tissue of tobacco transformed with pTi lost the resistance against *Thielaviopsis basicola* (Gasser *et al.* 1988). In another case the tumors of tobacco, obtained with the application of pTi, contained higher level of pathogenesis – related (PR) proteins, than the control plants (Antoniw *et al.* 1983). Linthorst *et al.* (1989) showed that the presence of individual PR proteins, caused through incorporation of genes controlling their production, does not increase the resistance against tobacco mosaic virus; higher level of resistance, however, may be obtained by simultaneous production of several different PR proteins. Ooms *et al.* (1988) studied tobacco transformants obtained

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with application of *A. rhizogenes* A4; in comparison with non–transformed control there were no differences in sensitivity for tobacco mosaic virus and potato virus Y.

The purpose of our work was to investigate the resistance against cauliflower mosaic virus, turnip vellow mosaic virus and turnip mosaic virus in oilseed rape, transformed with T–DNA of agropine plasmid pRi 15834.

MATERIAL AND METHODS

Seeds of *Brassica napus* var. *oleifera* cv. HM–81 were obtained from the Research Institute for Technical Crops and Oil Plants, Experimental Station Slapy at Tábor. Primary transformant HM–81–JZ regenerated from hairy roots induced with *A. rhizogenes* 15834 (Dusbábková *et al.* 1989). This transformant was fertile and had the typical „transformed" phenotype. The presence of T_L and T_R regions of pRi 15834 has been proved with Southern hybridization (Hrouda *et al.* 1988). R 1 progeny of primary transformat with „transformed" phenotype (wrinkled leaves, reduced apical dominance, shortening of internode) was used for virus infection.

The seeds of cv. HM–81 and transformant HM–81–JZ were sown on March 13 th 1989 and their germinative capacity was determined. A week later single seedlings were placed in pots filled with sterilized soil. On April 7 th all plants were mechanically inoculated.

The inoculum was prepared grinding leaves of Chinese cabbage (*Brassica pekinensis* [Lour.] Rupr.) cv. Nozaki three weeks after infection with each virus in a mortar with 0.01 M Mc Ilvanie buffer, pH 7.2, in the ratio 1:2 (w/v). The Czechoslovak isolate Trávčice of cauliflower mosaic virus (CaMV) (Špak 1989), strain ČSSR 2 of turnip yellow mosaic virus (TYMV) (Polák and Špak 1987) and isolate Ruzyně of turnip mosaic virus (TuMV) (Procházková and Polák 1985) were applied. Inoculations were carried out with a glass rod on the two lowest true leaves using carborundum 600 mesh as abrasive. With each virus 33 of transformed and non–transformed plants were infected. The same number of plants was left as healthy control. Control plants were also dusted with carborundum and inoculated with buffer. Plants were grown throughout the experiment in an insect–free glasshouse at 20–25°C.

Two weeks after infection symptoms on inoculated leaves were evaluated. On May 5th systemic symptoms were noted down, plants were cut off on ground level and weighed up. From each plant the fifth true leaf was taken for ELISA.

For qualitative and quantitative ELISA of CaMV and TYMV immunoglobulins G (IgG) labelled with horseradish-peroxidase were used according to modification of the method described by Polák and Křístek (1988).

The samples for CaMV ELISA were homogenized in the extraction buffer PBS pH 7.2 containing 0.05 % Tween 20, 0.5 % BSA, 1 % PVP 25, 2.5 % Triton X–100 and 1 M urea. Concentration of IgG for coating plates was 2.5 $\mu g\ ml^{-1}$, dilution of plant sap 1:2 and dilution of conjugated antibodies 1:1 000.

Extraction buffer PBS pH 7.2 with 0.05 % Tween 20 and 0.5 % BSA, concentration of coating IgG 3 μg ml^{-1}, sap dilution 1 :6 and dilution of the conjugate 1 :8 000 were used for TYMV ELISA. The absorbance values were measured at 492 nm in a Labsystems Multiscan MCC 340 ELISA reader.

For statistical evaluation of experimental data the *t* – test was used.

RESULTS AND DISCUSSION

The seeds of non–transformed rape showed 90.6 % germinative capacity, those of transformed was 88.6 %.

At the time of picking, non–transformed seedlings were higher, with distinct tap root and relatively poor root system. On the other hand transformed plants showed lower growth, shorter main root and rich branching lateral roots. During vegetation the transformed plants had lighter green leaves and slower growth as compared with non–transformed plants (see Fig.1.).

The change in a root morphology of transformed seedlings has an interesting character. Transformed plants have often a reduced apical dominance, their leaves are wrinkled, roots are plagiotropic and the pollen viability is reduced (Tepfer 1984). The structure and function of agropine plasmids pRi is relatively well known (Schmülling *et al.* 1988). In regard to the function of rol-genes situated in T_L-DNA of pRi it is possible to suppose that the formation of hairy roots is not caused only by the change of auxin/cytokinins ratio (Vilaine and Casse–Delbart 1987).

The CaMV isolate induced chlorotic local lesions on inoculated leaves, more numerous in transformed plants. The systemic infection evoked distinct vein mosaic on leaves in both cases (see Fig. 2).

Similarly, the TYMV isolate induced chlorotic local lesions on the inoculated leaves. The systemic symptoms of sharply limited yellow–green mosaic were induced in both transformed and non – transformed plants, more severe in those of transformed ones (see Fig. 3.). Generally, no conspicuous differences in severity of symptoms were observed between tested non–transformed or transformed plants.

The response of leaves which were inoculated with TuMV was formation of necrotic local lesions. Severe mosaic, curling, necrosis of leaves, stunting and in one case the dying back of the plant appeared as a systemic reaction to TuMV infection with transformed and non–transformed plants (see Fig. 4).

Mean mass of healthy and infected plants at the time of harvest are presented in Table 1. It shows that in all cases production of green mass from non–transformed rape is nearly twice that from transformed one. Nevertheless, lower yield should be compensated by new quality of the transformed plants.

Table 1 illustrates also that reduction of mass of plants (in % of healthy control) after inoculation of rape with each virus is almost the same in both transformed and non–transformed plants, excluding those infected with TYMV. Transformed plants

TABLE 1

Mean mass of cv. HM–81 non-transformed and transformed plants one month after inoculation with CaMV, TYMV and TuMV.

	Non-transformed [g]			Transformed [g]		
	x̄	s	[% of control]	x̄	s	[% of control]
Control	45.1	6.5	100.0	23.1	5.7	100.0
CaMV	30.6	5.4	67.8	16.1	5.2	69.7
TYMV	32.8	8.6	72.7	22.0	3.7	95.6
TuMV	26.8	7.3	59.3	14.1	5.5	61.0

infected with TYMV showed significantly lower reduction of yield ($P < 0.001$) in comparison with non–transformed ones. The highest loss of green mass was observed with TuMV infection, the lowest one with TYMV. The values of yield decrease in % of control are similar as those obtained by winter oilseed cultivar Jet Neuf (Polák *et al.* 1989) with the exception of infection with TuMV, where losess of HM–81 cv. were lower. This data is in the correlation with the higher resistance of spring cultivars of oilseed rape as was stated in our previous work (Špak *et al.* 1987).

The comparison of A 492 values with TYMV infection, as summarised in Fig. 5., indicates higher values in transformed plants. Nevertheless this difference was not statistically significant at the $P < 0.05$ level. Absorbance values of healthy non-

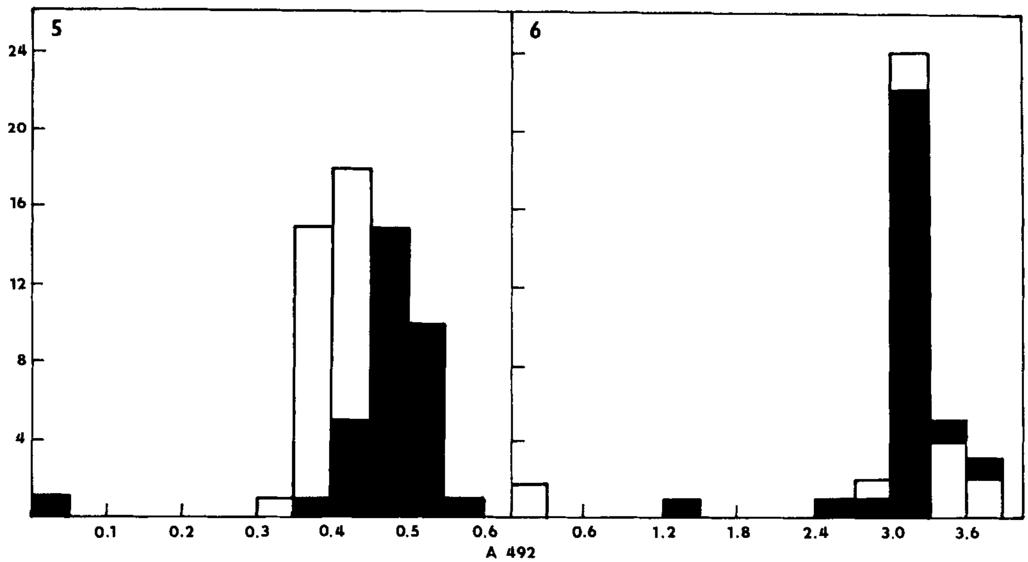

Fig. 5. The comparison of ELISA absorbance values of transformed (full columns) and non-transformed (empty columns) plants infected with turnip yellow mosaic virus.

Fig. 6. The comparison of ELISA absorbance values of transformed (full columns) and non-transformed (empty columns) plants infected with cauliflower mosaic virus.

–transformed plants varied between 0.001 to 0.011, those of transformed from 0.001 to 0.038.

The detection of CaMV by ELISA (Fig. 6) showed 100 % infection of transformed plants, while two non-transformed plants remained healthy. No significant differences were found between absorbance values of transformed and non-transformed plants. Absorbance values of healthy non-transformed plants varied between 0.086 and 0.158, those of transformed from 0.113 to 0.231.

The infection with TuMV induced symptoms on all transformed and non-transformed plants. Unfortunately TuMV ELISA could not be done to complete the investigation.

From the comparison of sensitivity with transformed and non-transformed plants of spring oilseed rape cv. HM-81 to the infection with CaMV and TuMV it is evident, that transformation with *A. rhizogenes* did not decrease resistance to the viruses. Our results confirm conclusions published by Ooms *et al.* (1984) and are auspicious for present intensive breeding programmes with "00" winter oilseed rape.

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Figs. 1–4 at the end of the issue.

BOOK REVIEW

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting, Volume 47. – International Agency for Research on Cancer, Lyon 1989. Pp. 534. Sw. Fr. 85.–.

The IARC Monographs are recognized as an authoritative source of information on the carcinogenicity and genotoxicity of chemicals and complex exposures. The first part of this forty-seventh IARC volume comprises 6 monographs on organic solvents; petroleum solvents, toluene and xylene (used primarily in paints), cyclohexane (used as an intermediate in the production of nylon), dimethylformamide (a polymer and resin solvent) and morpholine (an intermediate in the production of rubber). The further monographs cover the solvent stabilizer 1,2-epoxybutane, resin monomers and related compounds such as bis (2,3-epoxycyclopenthyl) ether and glycidil ethers, and paint pigments such as titanium dioxide, antimony trioxide and antimony trisulphide. The monographs on individual chemicals include sections on chemical and physical data, production, use, occurrence and analysis, and biological data relevant to the evaluation of carcinogenicity, including data on mutagenicity. The monograph on the occupational exposures in paint manufacture and painting includes data on exposures in the workplace, on toxic effects and on genetic and related effects. The publication is appendixed by a summary table of genetic effects in prokaryotes, lower eukaryotes, plants, insects, and in animal and human cells *in vitro*.

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