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Changes in fatty acids contents and growth characteristics in transformed oilseed rape (*Brassica napus*)

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Abstract

Spring oilseed rape *Brassica napus* L. ssp. *oleifera* cv. HM-81 was transformed with T_L-DNA of the Ri plasmid of the agropine strain *Agrobacterium rhizogenes* 15834. Selfed progenies (R_2 and R_3 generations) were studied for changes in values of growth characteristics and fatty acids contents. Transformants are 'homozygous' for T_L-DNA. Both generations of transformants differed significantly from the nontransformed control plants in reduced length, lower number of pods per plant, lower total mass of seeds and the higher number of branches. The contents of palmitic, linoleic and linolenic acids were significantly higher in transformants when compared with the control. On the contrary, the contents of both stearic and oleic acids were in most of transformants significantly lower. Only traces of erucic acid (less than 0.05 %) were found, both in transformed and nontransformed plants.

Introduction

Plants with integrated T-DNA of the root-inducing plasmid pRi perform some specific changes in morphology, *e.g.* wrinkled leaves, reduced apical dominancy and plagiotropic roots (Tepfer 1984, Ooms *et al.* 1985, Hrouda *et al.* 1988, Dusbábková *et al.* 1989). The *rol* genes, located in the left part (T_L) of T-DNA of agropine Ri plasmids have specific functions for these changes (White *et al.* 1985, Schmülling *et al.* 1988, Schmülling *et al.* 1989). The transformants with T-DNA of pRi are often known either to be sterile or to contain less seeds per pod than nontransformed control plants (Spano and Costantino 1982, Guerche *et al.* 1987). As for agronomic traits, Fladung (1990) described changes in internodal length, in leaf size and number, and in tuber shape and eye number in potato with the integrated *rolC* gene. Arnoldo *et al.* (1992) evaluated transgenic canola plants and their progeny transformed with *Agrobacterium* binary vector, for their intrinsic agronomic

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properties in the field. In *Brassica napus* transformed with unchanged T-DNA we do not know any detailed report involving the alterations in values of quantitative characters and fatty acids contents in transformed plants except of data dealing with 'sterility' (Ooms *et al.* 1985) and 'fertility' (Guerche *et al.* 1987).

Here we describe alterations in plant length, number of pods per plant, total seed mass and number of branches in *Brassica napus* transformants containing entire T_L-DNA of the plasmid pRi 15834. Simultaneously we present the changes in fatty acids contents in oil of transformants when compared with their contents in control plants.

Materials and methods

Plant material: We studied the R_2 and R_3 generations of the progeny of *B. napus* 'HM-81-JZ' transformant ('JZ') and the original spring cultivar 'HM-81'. Self-pollinations were performed in the greenhouse of the Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, České Budějovice. Plants for growth characteristics determinations and for oil analyses were grown in an experimental field of the Plant Breeding Station, Sempra, Slapy near Tábor. Although no selective advantage for transformants or their hybrid progeny was observed, they were grown about 500 m far from other fields with normal oilseed rape plants.

The method of construction of 'JZ' transformant was described previously (Hrouda *et al.* 1988, Dusbábková *et al.* 1989). The 'JZ' transformant is 'homozygous' for T_L-DNA of the Ri plasmid; it contains T_L-DNA in both members of one pair of homologic chromosomes. There is a strict correlation between the presence of T_L-DNA and typical changes in morphology of a plant. The phenotype of our transformant and its progenies was unambiguously visually distinguishable from the normal phenotype of nontransformed individuals. We could expect the segregation of a recombinant genotype without T_L-DNA in frequency about 1:1000 within the autogamic progeny (Dusbábková *et al.* 1992). No significant differences in the resistance of both transformed and non-transformed plants to the infection with cauliflower mosaic, turnip yellow mosaic and turnip mosaic viruses were revealed (Špak *et al.* 1991).

Growth characteristics: Growth characteristics were determined by current methods usually using groups of twenty plants. The progeny of three R_1 plants (JZ-21, JZ-28 and JZ-36) was studied in R_2 and R_3 generations.

Determination of fatty acids: The contents of fatty acids in seeds of individual plants (15 seeds of each plant) were analyzed by the gas chromatography (Hougen and Bodo 1973). The method of sample adjustment was modified after Bezecná and Tenkl (1987).

Statistic evaluation: Differences were statistically evaluated by *t*-test. The *t*-values were calculated with a respect to differences in values of variance and differences in

number of members within the individual groups (Roth *et al.* 1962, Weber 1964). The correlation coefficients were determined by the Spearman's method.

Results

The groups of transformed progeny (R_2 and R_3 generation differ significantly ($P \leq 0.05$) from nontransformed plants by lower plant length, lower number of pods per plant and lower total mass of seeds per plant (Table 1). The average number of branches in transformed plants is in some individual cases significantly lower, in other cases higher in comparison with the control. In some other groups of transformants the differences in values of this property are not statistically significant. The lower length of plants can obviously determine lower number of pods and lower total mass of seeds.

Only the correlation coefficient between the number of pods and the total mass of seeds per plant has the statistically significant value ($r = 0.833$). The correlation coefficients calculated for plant length and number of pods, for plant length and mass of seeds, for plant length and number of branches, for number of pods and mass of seeds, for number of pods and number of branches and for mass of seeds and number of branches were not statistically significant (data not shown).

The preliminary determination, done in 1989, indicated that the contents of palmitic, linoleic and linolenic acids were higher and the contents of stearic, oleic and eicosenoic acids were lower in transformants. These tests were carried out in random samples of seeds and made thus any statistical evaluation impossible (Table 2).

In 1990 (Table 3) the significantly higher contents of palmitic, linoleic and linolenic acids were determined in most of transformant progenies. On the contrary, the contents of stearic and oleic acids were mostly significantly lower. The results obtained in both seasons 1989 and 1990 coincide remarkably. Only several groups of progenies had significantly lower contents of eicosenoic acid as compared to the control. In model experiments the determination of eicosenoic acid has, however, ten-fold higher error (coefficient of variability $v = 53.19$) than that of other fatty acids contents (v were between 3.8 and 5.4).

Only traces of erucic acid were found both in transformants and control plants in both seasons (1989 and 1990). The expression 'traces' corresponds to a content of erucic acid lower than 0.05%.

Discussion

The incorporation of T-DNA of the Ri plasmid causes the changes in a phenotype of plants and in yielding attributes: the reduced length of plants due to the shortened internodia, and in spite of higher number of branches the lower number of pods per plant and the lower total mass of seeds per plant. The morphological changes may correlate with effects of T_L-DNA of the Ri plasmid to the apical dominancy of plants

Table 1. Growth characteristics in R_2 transformants (row 2 - 4), R_3 transformants (row 5 - 13) and nontranformed control (row 1). Mean ± error of the mean, *n* - number of plants in a progeny, *differences significant at $P \leq 0.05$.

Row	Progeny	n	Lenght [cm]	Number of pods	Mass of seeds [g]	Number of branches
1	HM-81	20	81.40 ± 2.27	173.25 ± 21.61	2.00 ± 0.27	4.20 ± 0.22
2	JZ-21	20	39.35 ± 1.93*	76.30 ± 12.53*	0.49 ± 0.08*	3.50 ± 0.31*
3	JZ-28	17	32.29 ± 1.61*	37.82 ± 7.30*	0.41 ± 0.05*	5.64 ± 0.36*
4	JZ-36	20	30.15 ± 0.70*	54.15 ± 7.85*	0.15 ± 0.05*	5.45 ± 0.19*
5	JZ-21-7	20	38.70 ± 1.39*	48.55 ± 8.25*	0.23 ± 0.11*	5.60 ± 0.27*
6	JZ-21-8	19	45.94 ± 2.14*	102.00 ± 22.22*	1.45 ± 0.38	4.42 ± 0.22
7	JZ-21-10	20	38.85 ± 1.10*	76.55 ± 7.42*	0.46 ± 0.10*	4.25 ± 0.15
8	JZ-28-5	20	43.90 ± 1.14*	43.10 ± 4.29*	0.32 ± 0.07*	5.85 ± 0.29*
9	JZ-28-6	20	36.65 ± 0.85*	67.00 ± 8.47*	0.43 ± 0.11*	4.60 ± 0.25
10	JZ-28-9	20	43.35 ± 2.00*	87.15 ± 15.31*	0.51 ± 0.13*	3.65 ± 0.21*
11	JZ-36-5	9	33.00 ± 2.52*	49.77 ± 10.92*	0.24 ± 0.09*	4.44 ± 0.17
12	JZ-36-8	20	36.95 ± 1.61*	125.30 ± 17.22	0.98 ± 0.08*	5.92 ± 0.25*
13	JZ-36-11	20	39.00 ± 1.74*	124.40 ± 13.14	1.00 ± 0.21*	4.95 ± 0.23*

(Tepfer 1984, Schmülling *et al.* 1988). Histological analyses of dwarf plants with *rolC* gene, reported by Oono *et al.* 1990, indicated that shortening in the internodal length was associated with a smaller length in epidermal cells.

Table 2. Content of fatty acids [%] in R_2 transformants (row 2 - 4), R_3 transformants (row 5-13) and nontransformed control (row 1). Preliminary estimation performed in 1989. 16:0 - palmitic; 18:0 - stearic; 18:1 - oleic; 18:2 - linoleic, 18:3 - linolenic, 20:1 - eicosenoic acid.

Row	Progeny	16:0	18:0	18:1	18:2	18:3	20:1
1	HM-81	4.3	1.9	73.8	11.6	6.0	1.5
2	JZ-21	7.2	2.3	58.5	24.9	6.5	0.7
3	JZ-28	6.5	1.7	55.0	25.2	9.7	1.1
4	JZ-36	5.3	0.9	59.1	23.5	10.0	1.2
5	JZ-21-7	4.8	1.4	66.8	17.7	8.9	0.8
6	JZ-21-8	5.2	0.8	62.6	19.8	10.3	0.9
7	JZ-21-10	5.7	1.1	59.0	22.1	11.6	0.7
8	JZ-28-5	5.3	1.9	61.1	21.6	7.9	1.1
9	JZ-28-6	6.5	1.4	54.9	26.5	10.0	0.7
10	JZ-28-9	6.0	1.5	62.4	22.0	7.7	1.1
11	JZ-36-5	5.9	1.7	57.6	24.2	9.1	0.9
12	JZ-36-8	5.5	1.8	59.4	22.7	8.7	1.0
13	JZ-36-11	5.3	1.9	63.9	19.4	7.8	1.4
means of transformants		5.7	1.5	63.0	22.4	9.0	0.9
% of HM-81		133	80	85	193	150	64

We did not observe such a dramatic increase of lateral branching of stems in the progeny of oilseed rape transformed with Ri T-DNA as Fladung (1990) in transgenic potatoes with the incorporated *rolC* gene, where the tuber number was higher in comparison with normal plants but their yield was lower. The interaction of gene product of the *rolC* gene with the hormonal status of the plant was considered to be responsible for these morphological and physiological changes (Fladung 1990). Effects of T_L-DNA to the apical dominancy, especially the extent of the *rolC* gene expression may differ in individual cases (Schmülling *et al.* 1988, Fladung 1990). Pollen fertility in our transformants was, however, reduced to 62% in R_0 generation only (Dusbábková *et al.* 1992). Nevertheless, the changes caused by integration of T_L-DNA into the plant genome are similar to those evoked by the *rolC* gene transfer. These conclusions seem to be supported by results of Arnoldo *et al.* (1992) who did not observe either the reduction of the yield and maturity or changes in oil and protein contents in canola (*Brassica napus* ssp. *oleifera* L.) transgenic plants and their progeny containing the NPTII gene for kanamycin resistance introduced via *Agrobacterium*-mediated transformation. Although the expression of NPTII gene did not have any adverse effects on studied important agronomic traits, it may not concern other genes generally, especially those directly or indirectly influencing the yield. The location of insertion of any gene into the plant genome could be also considered.

Table 3. Content of fatty acids [%] in R_2 transformants (row 2 - 4), R_3 transformants (row 5 - 13) and nontranformed control (row 1). 16:0 - palmitic; 18:0 - stearic; 18:1 - oleic; 18:2 - linoleic; 18:3 - linolenic; 20:1 - eicosenoic acid. Mean ± error of the mean, *n* - number of plants in a progeny, *differences significant at $P \leq 0.05$.

Row	Progeny	n	16:2	18:0	18:1	18:2	18:3	20:1
1	HM-81	20	3.99 ± 0.04	1.48 ± 0.05	66.74 ± 0.43	17.40 ± 0.32	8.93 ± 0.22	1.05 ± 0.05
2	JZ-21	19	5.01 ± 0.16*	1.29 ± 0.05*	57.36 ± 0.74*	24.18 ± 0.49*	10.65 ± 0.27*	1.01 ± 0.05
3	JZ-28	13	5.46 ± 0.19*	1.11 ± 0.06*	54.74 ± 0.84*	26.49 ± 0.50*	11.21 ± 0.33*	0.83 ± 0.03*
4	JZ-36	16	6.00 ± 0.17*	1.18 ± 0.04*	53.80 ± 0.81*	27.20 ± 0.58*	10.56 ± 0.55*	1.01 ± 0.07
5	JZ-21-7	20	6.13 ± 0.16*	1.40 ± 0.06	53.64 ± 0.25*	25.41 ± 0.48*	9.73 ± 0.24*	0.90 ± 0.06
6	JZ-21-8	20	5.07 ± 0.21*	0.98 ± 0.05*	60.15 ± 1.17*	23.39 ± 0.79*	9.28 ± 0.31	0.83 ± 0.04*
7	JZ-21-10	20	4.98 ± 0.17*	1.08 ± 0.05*	55.49 ± 0.61*	26.56 ± 0.41*	10.59 ± 0.33*	0.95 ± 0.04
8	JZ-28-5	19	5.05 ± 0.10*	1.37 ± 0.11	56.71 ± 0.55*	25.02 ± 0.42*	10.15 ± 0.36*	1.04 ± 0.07
9	JZ-28-6	18	4.88 ± 0.12*	1.07 ± 0.07*	56.80 ± 0.73*	25.65 ± 0.52*	10.41 ± 0.25*	0.81 ± 0.06*
10	JZ-28-9	20	5.03 ± 0.15*	1.16 ± 0.06*	56.08 ± 0.51*	26.43 ± 0.38*	10.42 ± 0.25*	0.89 ± 0.05*
11	JZ-36-5	9	5.46 ± 0.25*	1.16 ± 0.10*	54:24 ± 0.79*	28.36 ± 0.58*	9.63 ± 0.31*	0.87 ± 0.10
12	JZ-36-8	20	4.82 ± 0.12*	1.21 ± 0.12*	56.48 ± 0.72*	25.69 ± 0.48*	10.59 ± 0.37*	0.88 ± 0.04*
13	JZ-36-11	20	4.49 ± 0.20*	1.11 ± 0.04*	56.58 ± 0.61*	25.42 ± 0.46*	11.05 ± 0.29*	0.90 ± 0.05*

Changes in the fatty acids contents in the rapeseed oil described in this paper may be meaningful in both theoretical and practical considerations for gene engineering of plants. Transformations could provide valuable genotypes for further recombination breeding. Here the statistically significant changes in contents of palmitic, oleic, linoleic and linolenic acids are remarkable, because coefficients of heritability (h^2) of their contents in rapeseed oil are roughly 0.9 (Pleines and Friedt 1988). In transformants the contents of both desirable linoleic and undesirable linolenic acids have increased. Nevertheless, the values of their contents differed substantially from the demanded state, which is 40 % and 3 %, respectively (Roy and Tarr 1987).

The low yield of transformed rapeseed might be prohibiting for the use of transformants in breeding programs. In some cases, however, the plant breeders use low yielding even wild species for changes in high yielding cultivars (Mayo 1987). The lowering of height exhibits an alteration valuable for plant breeders (Thompson 1983).

The changes in oil composition could be useful in near future. Besides the food industry, the rapeseed oil is used in technical branches, where the demands for its quality are quite different. Moreover, now it appears that the rapeseed oil could be a component of a fuel for diesel motors (Zaher 1990).

References

Arnoldo, M., Baszczynski, C.L., Bellemare, G., Brown, G., Carlson, J., Gillespie, B., Huang, B., MacLean, N., MacRae, W.D., Rayner, G., Rozakis, S., Westecott, M., Kemble, R.J.: Evaluation of transgenic canola plants under field conditions. - Genome **35:** 58-63, 1992.

Bezecná, L., Tenkl, L.: Some remarks to the rapid methods for an estimation of erucic acid and glukosinolates for winter rapeseeds. - In: 7 International Rapeseed Congress. Pp. 1520-1526. Poznań 1987.

Dusbábková, J., Nečásek, J., Hrouda, M.: [Transformation of oilseed rape with T-DNA of *Agrobacterium* plasmids.] - Genet. Šlecht. (Praha) **25:** 1-9, 1989. [In Czech.]

Dusbábková, J., Nečásek, J., Hrouda, M., Doležel, J., Hart, M.: Seggregation in the progeny of transformed rapeseed (*Brassica napus*). - Biol. Plant. **34:** 53-61, 1992.

Fladung, M.: Transformation of diploid and tetraploid potato clones with the *rolC* gene of *Agrobacterium rhizogenes* characterization and of transgenic plants. - Plant Breed. **104:** 295-304, 1990.

Guerche, P., Jouanin, L., Tepfer, D., Pelletier, G.: Genetic transformation of oilseed rape (*Brassica napus*) by the Ri T-DNA of *Agrobacterium rhizogenes* and analysis of inheritance of the transformed phenotype. - Mol. gen. Genet. **206:** 382-386, 1987.

Hougen, F.W., Bodo, V.: Extraction and methanolysis of oil from whole or crushed rapeseed for fatty acid analysis. - J. amer. Oil Chem. Soc. **50:** 230-234, 1973.

Hrouda, M., Dusbábková, J., Nečásek, J.: Detection of Ri T-DNA in transformed oilseed rape regenerated from hairy roots. - Biol. Plant. **30:** 234-236, 1988.

Mayo, O.: The Theory of Plant Breeding. - Clarendon Press, Oxford 1987.

Ooms, G., Bains, A., Karp, A., Twell, D., Wilcox, E.: Genetic manipulation in cultivars of oilseed rape (*Brassica napus*) using *Agrobacterium*. - Theor. appl. Genet. **71:** 325-329, 1985.

Oono, Y., Kanaya, K., Uchimiya, H.: Early flowering in transgenic plants possessing the *rolC* gene of *Agrobacterium rhizogenes* Ri plasmid. - Jap. J .Genet. **65:** 7-16, 1990.

Pleines, S., Friedt,W.: Breeding for improved C 18-fatty acids composition in rapeseed (*Brassica napus* L.). - Fat Sci. Technol. **90:** 167-171, 1988.

Roth, Z., Jozífko, M., Malý, V., Trčka, V.: Statistické Metody v Experimentální Medicíně. [Statistic Methods in Experimental Medicine.] - SZN, Praha 1962. [In Czech.]

Roy, N.N., Tarr, A.W.: Prospects for the development of rapeseed (*Brassica napus* L.) with improved linoleic and linolenic acid content. - Plant Breed. **98:** 89-96, 1987.

Schmülling, T., Schell, J., Spena, A.: Single genes from *Agrobacterium rhizogenes* influence plant development. - EMBO J. **7:** 2621-2629, 1988.

Schmülling, T., Schell, J., Spena, A.: Promoters of the *rolA, B* and *C* genes of *Agrobacterium rhizogenes* are differentially regulated in transgenic plants. - Plant Cell **1:** 665-670, 1989.

Špak, J., Dusbábková, J., Kubelková, D., Nečásek, J.: Resistance of transformed and non-transformed oilseed rape cv. HM-81 to the infection with cauliflower mosaic, turnip yellow mosaic and turnip mosaic viruses. - Biol. Plant. **33:** 234-239, 1991.

Spano L., Costantino, P.: Regeneration of plants from callus cultures of roots induced by *Agrobacterium rhizogenes* on tobacco. - Z. Pflanzenphysiol. **106:** 87-92, 1982.

Tepfer, D.: Transformation of several species of higher plants by *Agrobacterium rhizogenes*: sexual transmission of the transformed genotype and phenotype. - Cell **37:** 959-967, 1984.

Thompson, R.F.: Breeding winter oilseed rape (*Brassica napus*). - Adv. appl. Bot. **7:** 1-104, 1983.

Weber, E.: Gründriss der biologischen Statistik. 5.Ed. - Fischer Verlag, Jena 1964.

White, F.F., Taylor, B.H., Huffman, G.A., Gordon, M.P., Nester, E.W.: Molecular and genetic analysis of the transformed DNA regions of the root inducing plasmid of *Agrobacterium rhizogenes*. - J. Bacteriol. **164:** 33-44, 1985.

Zaher, F.A.: Vegetable oil as alternative fuel for diesel engines. - Grasas Aceites **90:** 82-91, 1990.

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