ASSESSMENT OF TRANSGENIC CANOLA PERFORMANCE UNDER FIELD CONDITIONS

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INTRODUCTION

Plants of transgenic canola (Brassica napus ssp. oleifera L.), cultivars Westar and Regent were evaluated in the field in two consecutive years of testing. The plants carried a neomycin-phosphotransferase (NPTII) gene for kanamycin resistance which was introduced via Agrobacterium-mediated transformation.

We decided to test transgenics in the field to ascertain whether they would suffer any repercussions due either to the introduction of a novel gene or to the in vitro transformation and culture process itself. In the field, we looked at the agronomic characters of maturity and yield. After harvest, we analyzed the seed to ensure that canola standards of the seed oil and meal were maintained.

The data clearly demonstrated that the transformed plants were statistically comparable to the non-transformed checks. These results indicate that canola can be genetically engineered with no measurable adverse effects on agronomic and qualitative traits.

MATERIALS AND METHODS

Vector Construction / Plant Transformation / Confirmation of Transformation

The vector construction strategy and transformation protocols are outlined and discussed in Arnoldo et al, 1991. NPTII enzyme assays and Southern analyses were conducted as per McDonnell et al, 1987 and Southern et al, 1974. Seed from the transgenics were germinated in the presence of 100 mg/L kanamycin to assess segregation and to confirm the stable, heritable transmission of the introduced antibiotic resistance gene.

Field Trials

The transgenic field testing was approved and monitored by the Plant Health Directorate, Seed Division, Agriculture Canada, Ottawa. Seed from 11 independent transgenics and 2 sets of non-transformed controls of both Westar and Regent were used. The first set was sown in the summer of 1989 and the next generation was sown in the summer of 1990. Approximately 100 seeds per sample per 2 metre rows was sown on heavy clay soil. Both isolation plots were located near Alloa, Ontario.

After approximately 1 month of growth, the plants were examined for maturity. Once the pods had filled the plants were harvested, and allowed to dry in drying rooms. Seed was collected.

Moisture, Oil and Protein Analysis

Seed was analyzed using a Dickey-John Instalab 600 Near Infrared Reflectance (NIR) apparatus. Samples were irradiated with specific wavelengths of light, and the reflected energy was measured. For water and protein, the 1.94 and 2.18uM bands were used respectively. Oil was measured with the 2.31 and 2.33uM bands. After NIR analysis, the seed from each sample was crushed, and used for both glucosinolate and fatty acid analysis.

Glucosinolate Analysis With Thymol

Glucosinolates were purified on DEAE-A25 Sephadex columns and reacted with thymol in sulfuric acid, following the method outlined by Brezinski and Mendelowski, 1984. The results represent total glucosinolates, including indolyl glucosinolates, of which 4-hydroxy-3-indolyl methyl glucosinolate is the major glucosinolate in rapeseed.

Fatty Acid Analysis

Using the method outlined by Hougen and Bodo, 1973, the fatty acid profile was determined. The oil was extracted in n-hexane. The fatty acids were esterified with a 0.5N methanolic base, and the hexane layer was removed and analyzed using a Perkin Elmer 8420 gas chromatograph. This was fitted with a 12 metre DB wax column, with an isothermal temperature of 230 C, injector temperature of 250 C and detector temperature of 300 C. The split ratio was 90:1, with a pressure of 7.0 psig.

RESULTS

Confirmation of Transformants

Transformants were identified by NPTII assays and Southern analysis on leaf samples from kanamycin resistant plantlets. Absolute confirmation of transformation was obtained by progeny segregation in the presence of kanamycin.

Analysis of Quality Characteristics From Field Trials

One month after sowing, plants in the field were examined. In both years of testing, there was no readily apparent difference in maturity between the transgenics and the non-transformed checks. The germination rates of the T1 generation transgenics were slightly lower than those of the checks (data not shown), probably due to the decreased fitness of the *in vitro* mother plant. The average yield of the transgenics was similar to that of the non-transformed checks; however, the range of yield values is large. (Table 1, column 4). We feel this is a consequence of two factors. First, the decreased germination rate of T1 generation transgenics resulted in high yields for surviving plants presumably due to decreased competition (1989 trial, T18, T19, T22). Second, those transgenics and checks that were placed near the edge of the plot visibly outperformed those located in the centre. This was particularly apparent in the 1990 plot. A comparison of yield between the two years demonstrates this point. T18

yielded 168% of check in 1989, but only 115% of check in 1990. T02 yielded 92% of check in 1989, but 177% of check in 1990. Hence, the cause does not seem to be genetic, but rather environmental.

The results of the NIR analyses for moisture, protein and oil are summarized in columns 5-7 of Table 1. All values are statistically similar, and most importantly, the oil and protein profiles are typical for canola.

In order to classify rapeseed as canola, glucosinolate levels must be less than 30uM/gm of oil-free meal. All field tested transgenics assessed for glucosinolates (Table 1, column 8) were below this level.

Analyses of the fatty acid compositions of the field test plants are presented in Table 2 (due to space limitations only the first year's results are shown). The second year's results confirm those of the first. The oil profiles of the checks are similar to those of the transgenics, and typical of canola oil. Of particular importance is the non-detectable erucic acid (C22:1) level; the analysis used ensures that detection is in the order of 0.01%.

DISCUSSION

In two years of field testing, we have examined the performance of transgenic canola plants. No deleterious effects were found in either the agronomic (maturity and yield) or quality (oil and meal) parameters. We utilized the 35S and nos promotors (both of which are believed to be expressed during most of the plant's development, and in most tissues in the plant) and the NPTII gene, and found no negative effects. This may not be true for other promotors or genes, and as such, future transgenics should be similarly assessed under field conditions. However, reports of field trials in other major crop plants indicate that genetic engineering can be used successfully. Recent examples include Hoekema et al.,(1989), Kaniewski et al.,(1990) and DeGreef et al.,(1989).

The average yield per plant of our transgenics was comparable to that of the non-transformed checks. The variability in absolute yield was large due to the factors presented in the "Results". It is important to note that the yield trials presented here were designed to demonstrate that genetic engineering can occur with no obvious negative effects on yield. Future analyses of transformants will utilize more conventional plot design to overcome some of the problems encountered in yield assessment.

With respect to the quality characteristics (% moisture, %oil, % protein, glucosinolates, and fatty acid analysis) the transgenics performed very well. The moisture content of both checks and transgenics were slightly high, but this was probably due to the early harvesting of seeds, necessary to eliminate the risk of seed pod shattering (as requested by the Plant Health Directorate, Seed Division, Agriculture Canada). The means of the controls and the checks were statistically comparable in all the parameters tested. The oil and protein

analyses indicate that all the transgenics were canola quality.

Examination of the 1989 transgenic field test plot in 1990 found no volunteer canola plants. The 1990 field test plot was similarly checked this spring. In 1991 we expect to field test transgenics carrying 'agronomically valuable' genes under the control of tissue- and stage-specific promotors. We hope that they too, will perform as well as the non-transformed checks.

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TABLE 1. Results from analysis of quality characteristics from field trials of B. napus transgenic plants and control cultivar checks.

Plant Code	Cultivar	Seed Source or Generation	Seed Weight per Plant (% of check)	NIR Analysis Moisture (%)	NIR Analysis Protein (%)	NIR Analysis Oil (%)	Glucosinolate Analysis (umol/g seed)
1989							
C1	Regent	Commercial	100	6.45	22.25	42.59	18.83
C3	Regent		100	6.50	21.92	43.62	15.34
C2	Westar	Certified seed	100	6.62	21.40	43.70	12.86
C4	Westar		100	6.63	22.14	43.12	15.76
Mean of Controls (±S.D.)				6.55±0.09	21.93±0.38	43.26±0.51	15.70±2.45
T02	Westar	T2	92	6.50	22.30	43.16	14.41
T03	Westar	T2	103	6.77	21.66	43.03	14.30
T04	Regent	T2	104	6.76	20.98	43.49	13.00
T07	Westar	T2	111	6.60	20.88	44.51	14.35
T08	Westar	T2	77	6.64	20.41	45.29	12.44
T09	Regent	T2	98	6.73	20.48	44.34	14.52
T10	Westar	T2	89	6.42	21.75	43.18	10.71
T18	Regent	T1	168	5.67	23.97	40.21	21.10
T19	Westar	T1	126	6.48	22.34	42.45	11.62
T20	Westar	T1	94	6.44	23.26	42.06	11.54
T22	Westar	T1	131	6.57	21.14	42.85	16.58
Mean of Transgenics (±S.D.)				6.51±0.30	21.74±1.14	43.14±1.36	14.05±2.89
1990							
C1	Regent		100	5.95	23.72	41.25	13.37
C3	Regent		100	5.82	23.57	40.92	14.11
C2	Westar		100	6.24	23.91	40.97	11.17
C4	Westar		100	6.36	23.78	41.21	11.03
Mean of Controls (±S.D.)				6.09±0.25	23.75±0.14	41.08±0.17	12.42±1.55
T02	Westar	T3	177	6.32	23.17	41.52	12.08
T03	Westar	T3	104	6.58	23.69	41.18	14.31
T04	Westar	T3	100	6.59	23.00	41.98	11.42
T07	Westar	T3	162	6.41	23.53	41.77	14.43
T08	Westar	T3	106	6.50	23.67	41.52	13.25
T09	Regent	T3	109	6.10	23.61	41.61	14.22
T10	Westar	T3	152	6.34	23.85	40.55	15.39
T18	Regent	T2	115	6.05	24.74	37.55	15.60
T19	Westar	T2	98	6.58	24.52	40.28	11.55
T20	Westar	T2	102	6.43	24.23	40.41	14.12
T22	Westar	T2	162	6.40	23.21	41.56	12.92
Mean of Transgenics (±S.D.)				6.39±0.18	23.75±0.56	40.90±1.25	13.57±1.44

TABLE 2. Analysis of Fatty Acid Composition of Transgenics and Non-Transformed Checks From Field Trials (% of Total)

Plant Code	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1	C24:0
C1		4.17	0.26	1.99	63.77	19.40	8.28	0.68	1.20	0.25		
C2		4.49		1.83	67.08	17.90	6.73	0.61	1.09	0.28		
C3		4.41		1.92	63.73	19.42	8.31	0.63	1.18	0.19		0.20
C4		4.42		1.82	65.99	18.30	7.40	0.65	1.16	0.26		
T02		4.25	0.33	2.10	63.76	19.82	7.79	0.66	1.11	0.20		
T03		4.56	0.35	1.96	64.98	19.28	6.87	0.61	1.12	0.26		
T04		4.39	0.34	1.86	65.28	19.22	6.70	0.63	1.10	0.26		0.22
T07		4.33		1.93	66.95	17.42	7.11	0.59	1.09	0.26		0.32
T08		4.28		1.96	67.77	17.02	7.05	0.62	1.07	0.24		
T09		4.63		1.98	64.89	19.02	7.54	0.63	1.09	0.23		
T10		4.30		1.82	66.71	18.03	7.20	0.58	1.16	0.20		
T18		4.59		1.71	62.24	20.26	9.25	0.57	1.15	0.24		
T19		4.31		1.84	66.36	17.65	7.79	0.62	1.17	0.26		
T20		4.45		1.61	65.07	18.95	7.80	0.60	1.14	0.19		
T22		4.68		1.78	63.91	20.30	7.39	0.61	1.11	0.22		