FIELD RESISTANCE OF CANOLA TRANSFORMANTS (BRASSICA NAPUS L.) TO IGNITE® (PHOSPHINOTRICIN)

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INTRODUCTION

IGNITE® is a new herbicide with non-selective effects on all green parts of plants. L-phosphinotricin (L-ppt or glufosinate-ammonium), the active ingredient, shows no soil effect and extraordinarily high biodegradability in the soil. Under Saskatchewan summer conditions, a half-life time of only one week can be expected. Therefore, it is highly recommended as a replacement for the older herbicides which persist for longer periods of time in the soil. As a result, there will be very little selection pressure on weeds to develop resistant mutants as has been observed with other classes of herbicides (ie. sulfonylureas, imidazolinones, triazines; modified after Donn et al. 1990). To use this herbicide however, the crop must be resistant and this can be accomplished by inserting the resistance gene into the chromosomes by transformation.

Brassica napus has been subjected to several methods to achieve stable heritable genetic transformants in the past. There is an impressive list of successful transformation attempts which need to be reviewed later showing that the choice of tissue culture methods is not a critical factor for transformation of B. napus. However, only a few of these papers report a reliable, repeatable routine transformation in B. napus. Methods which produce haploid transformants have the advantage to be fast in tissue culture and in the following breeding as well.

MATERIALS AND METHODS

Our vector has been described previously (Donn et al. 1990). The following cultivars or breeding lines have been used: Petranova, Westar, ACS-N3 (Agriculture Canada, Dr. Rakow), Topas clone 4079 (Plant Biotechnology Institute, Dr. Keller), Excel, Profit, Ceres.

As a first approach, we compared several shoot transformation methods, in particular, the use of stem sections (Pua et al. 1987) and thin layer floral stem explants (Charest et al. 1988) and stem disks of cold-treated plants (Fry et al. 1987).

As a second approach, mesophyll protoplasts (Kartha et al. 1974) of selected shoot cultures (Oelck and Schieder 1983) were

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cocultivated with Agrobacteria (Marten et al. 1979) or treated with electroporation (Hofmann et al. 1991).

As the third approach, microspores were isolated (Keller and Armstrong 1978), cold-treated at 1°C for up to 6 months and cultured as described (Phan et al. 1988). The embryo derived shoots were cut into pieces with a blender and cocultivated with A. tumefaciens for two days as described (Phan et al. 1988; Swanson and Erickson 1990). After antibiotic treatment and recovery for two weeks, kanamycin selection was carried out with 50-60 mg/ℓ for two periods of 14 days. A second selection step on L-ppt (50 mg/ℓ) under high light intensity was carried out after the embryogenic development was completed. Rooting in the presence of L-ppt was taken as an indicator of transformation.

Plantlets from the systems described were propagated in incubators (10 h photoperiod, 20°C) prior to transfer to soil. Microspore-derived plantlets were treated with a solution containing 0.1% colchicine + 0.1% SDS for 8 h overnight hanging upside down to minimize colchicine damage to the root system. This procedure was carried out in a moist chamber between transfer from sterile medium to soil.

All plants were allowed a selfing generation in the growth chamber (16/8 h photoperiod, 15/10°C). Plants from those seeds were grown in the greenhouse at 20°C with high light intensity. They were sprayed with the equivalent of 1 or 2 kg/ha Ignite® at the 4-6 leaf stage.

After a second selfing generation and spray treatment, one clone 19/2 from cv. Topas was crossed onto an Agriculture Canada breeding line (H8-89-5691-5699). The hybrid F_1 seeds as well as the parents, clone 19/2 and cv. Westar were sown under isolation conditions (Kalous and Duke 1989) in 2 x 8 m plots in the field at Saskatoon in 1990. Seed quality analysis was investigated by Agriculture Canada in Saskatoon in their standard set-up for Co-op trials.

RESULTS

Protoplasts from suspension cultures, hypocotyl explants and mesophyll tissue (Oelck and König, unpublished results) were all suitable for plant transformation and regeneration. The stem explant cultures were also suitable for plant transformation and regeneration but they were all very labour intensive (Oelck and König, unpublished results). Microspore derived embryogenic tissue was preferred because of haploidy. The up to six month storage of embryos saved much work and even improved the quality of the embryo response on plant formation.

Selection for L-ppt resistance had to be carried out at a stage where the cultures were not undergoing shoot differentiation or somatic embryogenesis (Oelck and Heil 1991), otherwise selection was misleading because meristematic and embryogenic tissue show transient tolerance to the herbicide (De Block et al. 1989; Oelck and Heil 1991). Three to five days of selection with 60 mg/ℓ L-ppt with established shoot cultures were sufficient to confirm transformation. Transformation was further confirmed by PAT assays (De Block et al. 1987) and Southern hybridizations (Southern 1975). Inheritance of the resistance was stable and segregated in the Mendelian way. During the greenhouse generations, consisting of two or three selfings and several crosses (1988-1991), it

was remarkable to observe that the herbicide resistance trait was expressed stronger. This was not further investigated, but there was an obvious correlation between plant vigour and herbicide resistance.

Our most advanced transformant line, Topas 19/2 was produced from microspores (Phan et al. 1988) in 1988 hand-crossed with the Westar-related male in 1989/90 and field-grown near Saskatoon in 1990. Westar, Topas 19/2 and the F_1 (Topas 19/2 x Westar) were sprayed at the 6-8 leaf stage with Ignite® SL 18 (0.75 kg/ha a.i.). All Westar plants either died or were badly damaged, while all the 19/2 plants and the Westar/Topas hybrids survived equally well compared to the unsprayed controls.

Plants were harvested and seeds were analyzed for oil content (Table 1a), fatty acid composition (Table 1b) and glucosinolate content (Table 1c). The results are very encouraging, but somewhat preliminary because they were taken from only one field location (4 replications) and the 1990 growth conditions were unusually wet for the prairies. The mean of the oil content of the 19/2 plants (39.4%) was 0.9% lower than Westar, but the hybrid showed a strong heterosis and yielded 41.3% oil, 0.8% higher than Westar.

Table 1 Quality Analysis from 1990 Transgenic Brassica napus field test in Saskatoon
Mean and standard deviation of seed oil analysis (% dry matter) from replications

1a) Oil content

19/2 transformant	39.4 ± 0.86
F_1 hybrid	41.32± 0.57
Westar	40.72± 1.12

1b) Acyl composition of the oil (% of total fatty acids)

	16:0	16:1	18:0	18:1	18:2
19/2t.	3.62±0.17	0.30±0.00	0.05±0.06	61.87±1.30	21.47±0.78
hybrid	3.25±0.10	0.20±0.00	0.07±0.05	66.10±0.29	18.55±0.21
Westar	3.72±0.25	0.12±0.15	0.05±0.06	64.90±1.82	19.07±1.03

	18:3	20:0	20:1	22:0	22:1
19/2t.	8.37±0.10	0.5±0.0	1.27±0.05	0.30±0.00	0.02±0.05
hybrid	7.4 ±0.00	0.6±0.0	1.40±0.00	0.30±0.00	tr
Westar	7.3 ±0.22	0.6±0.0	1.60±0.47	0.35±0.06	0.17±0.35

1c) Glucosinolate content in μmoles per g oilfree meal

	Butenyl	Pentenyl	Hydroxy butenyl	Hydroxy pentenyl
Topas 4079*	1.4 ±0.00	0.45±0.10	1.95±0.10	0.05±0.05
Hoe 19/2t.	1.82±0.57	0.17±0.05	0.65±0.21	tr
hybrid	2.80±0.29	0.45±0.13	2.65±0.81	0.02±0.05
Westar	3.55±0.44	0.32±0.10	6.72±1.04	0.05±0.06

* Analysis under separate conditions from experimental material

in cases where the figure is 0.0 no ± SDV is possible.

The fatty acid composition (Table 1b) showed canola quality, even in the 19/2 line. The F_1 material showed again a better performance than cv. Westar. The slight increase in the 18:3 linolenic acid content of 19/2 was significant. Erucic acid patterns were undetectably low (22:1) and oleic acid was also on the low side (18:1). The hybrid was high due to heterosis.

The glucosinolate analysis (Table 1c) was investigated in all three lines. The original quality of Topas 4079 before transformation was maintained in spite of the repeated tissue culture process. A surprising drop in the alkenyl-glucosinolates (Hydroxybutenyl) derived from the Topas 4079 nature which is given as comparison. It resulted in a decrease of 10 to 50% less of the Westar content (Table 1c). The hybrid scored intermediate between the parents. The alkenyl glucosinolate content of the 19/2 transformant was very low and thus, should be used for future breeding work in lines where the alkenyl is too high. Also the Pentenylglucosinolates are low which is also the case in the original Topas 4079. It is a very important result that the transformation did not change the biochemical nature of the plants.

Observations on earliness and vigour showed that the hybrid plants flowered two days earlier than the early maturing Westar and seed set appeared to be at least equal.

DISCUSSION

There is a wide range of cell biological approaches possible to achieve transformation in B. napus. We compared several of them and the success was generally related to the regeneration frequency. Consequently, an excellent regeneration system is necessary before transformation becomes feasible. On the other hand, the suitability of the vector constructs for use in Brassica seems to be a limiting factor in obtaining reliable and relatively high transformation events and compatibility with the Brassica genome is not always the case (Oelck et al. in prep.). Several of the constructs used were efficient. This was independent from the selectable markers used as long as they were strong markers. Also the nature of the insertion and the unaffected quality of the resulting plant is more important than the ease and high frequency of transformation. Therefore, field results which demonstrate that the traits of the cultivar were unaltered by the transformation procedure are more important than test results produced in the laboratory, growth chamber and greenhouse. We have achieved the production of transformants using various methods and reported on preliminary data of the field performance of our most advanced line Topas clone 19/2 showing that its combining ability with Westar is good enough for future Canola hybrid breeding work.

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