SELECTION OF PATHOGEN RESISTANT MUTANTS IN RAPESEED, BRASSICA NAPUS

Adelheid Spanier

Institut für Pflanzenbau und Pflanzenzüchtung, Georg August Universität, D-3400 Göttingen, Bundesrepublik Deutschland

SUMMARY

Four reliable cell-to-plant regeneration systems of *Brassica napus* were developed as a prerequisite for successful in vitro selection of mutants: haploid mesophyll protoplasts, embryogenic cell suspensions, haploid shoot embryogenic material and microspores. These plant materials were used for selection of resistance against the pathogens *Phoma lingam*, *Alternaria brassicae* and *Verticillium dahliae*. The selection was mediated by the toxic culture filtrates of the fungi. Those filtrates from *Phoma* and *Alternaria* caused necrosis on leaves and growth inhibition in vitro. Whether the culture filtrate of *Verticillium* is a reliable tool for mutant selection is still to be established. From our first experiments material could be obtained with reduced sensitivity to the culture filtrate of *Phoma lingam* and *Alternaria brassicae*.

INTRODUCTION

The blackleg disease caused by *Leptosphaeria maculans* (imperfect stage of *Phoma lingam*) is one of the most important diseases of oilseed rape, although there partial resistance is present in some European varieties, e. g. Jet Neuf (CARGEEG AND THURLING 1980, SACRISTAN 1982). Furthermore, in recent years two pathogens, *Verticillium dahliae* and *Alternaria brassicae* have led to significant yield reductions of rapeseed in Western Germany (KRÜGER 1986, AHLERS 1987). Genetic sources of resistance genes are not known in *Brassica*. Hence, the aim of the present work was to select for pathogen resistance in in vitro culture systems of rapeseed using toxin-containing culture filtrates of the fungi. Cell culture systems, such as protoplasts, microspores and cell suspensions permit work with large populations of haploid individuals which reduce the time required for obtaining novel resistant plants. In fact, the application of selective in vitro techniques using culture filtrates of fungi has been successful in producing resistant plants in several other host-pathogen systems (GENGENBACH ET AL. 1977, 1981; SACRISTAN ET AL. 1982, HARTMAN ET AL. 1984).

MATERIAL AND METHODS

All pathogen cultures used were isolated from rapeseed plants in 1986, Phoma lingam by Dr. D. Ahlers, Hannover, Alternaria brassicae by Dr. A. von Tiedemann, Göttingen and Verticillium dahliae by Dr. Nierenberg, Berlin. The fungi, the supply of which is gratefully acknowledged to the mentioned colleagues, were maintained in sterile "soil culture" at 4 °C. Culture filtrates were obtained and tested for toxicity as described by SACRISTAN (1982). Purified filtrates were used in MS medium (MURASHIGE AND SKOOG 1962) or S medium (SCHENCK AND RÖBBELEN 1982) in a 1:1 ratio (v/v), called T1 and T2 medium respectively.

Four kinds of plant materials were used for selection:

1. Microspores of rapeseed line JL344 (derived from Jet Neuf and Loras) were isolated and cultivated as described by LICHTER (1982) with slight modifications (see MATHIAS, this meeting). Embryoids were UV-irradiated (254 nm, 10 min) and cultivated on hormone-free T1 medium.

2. Protoplasts were isolated in an enzyme solution, containing 1 % cellulase, 0.1 % PATE, 0.6 M mannitol, pH 7.0 by incubation for 16 h on a roller as described by SCHIEDER (1984). After one week of culture in V47 + 0.5 mg/l NAA, BAP and 2,4-D the protoplasts were embedded in agarose, transferred to liquid medium (SHILLITO ET AL. 1983) and further cultivated with some modifications according to THOMAS ET AL. (1976). Two months after protoplast isolation the liquid medium was replaced by the T1 medium + 1 mg/l Zea, 1 mg/l Kin, 0.1 mg/l IES. After the induction of embryoids on K3-Medium (NAGY AND MALIGA 1976) + 2 mg/l 2,4-D, 0.1 mg/l NAA, 0.1 mg/l BAP, 10 % coconutmilk, shoots were regenerated on S-medium.

3. Cell suspension was initiated from haploid shoot embryogenic material of 'Loras 6' by cultivating on MS + 0.5 mg/l 2,4-D, 0.8 % agar for 3 months with a final transfer to MS + 10 mg/l Kin, 0.2 mg/l 2,4-D liquid medium on a rotary shaker at 20 °C. The cell suspension was plated on T1 medium + 10 % coconutmilk. Shoots were regenerated on MS medium + 1 mg/l BAP.

4. Shoot embryogenic material of different genotypes were initiated from microspores and cultivated on S-medium. For selection, material was EMS-mutagenized (1 % EMS, 2-3 h on a rotary shaker) and plated on T2 medium.

RESULTS AND DISCUSSION

Shoot redifferentiation of the used four haploid in vitro culture systems (microspores, protoplasts, cell suspension and shoot embryogenic material) was achieved via somatic embryogenesis in a percentage and time suitable for mutant selection (Table 1).

Table 1: Shoot regeneration of haploid in vitro systems of *B. napus*

Stages of development	Material			
	Microspores	Protoplasts (10^4 pp/ml)	Cell suspension	Shoot embryos
Callus		80-90% of isol. pp		
Proembryoids	yes	70-80% of colonies	80-100% of colonies	
Embryoids	200-400 per 25 buds	0.5-1% of proembry.	yes	
Callus + secondary embryogenesis	yes	yes	yes	yes
Shoots	70-80% of embryoids	80-90% of embryoids	yes*	yes
Time until shoots (months)	2	5	3-4	1-2

* = not yet finished

In order to obtain disease resistant plants by plating cells on culture filtrate-containing medium, a causal relationship of the toxin to pathogenecity must be ascertained. Culture filtrates of *Phoma lingam* and *Alternaria brassicae* induced necrotic and chlorotic spots when applied to detached leaves, similar to the host's response against the pathogen (Table 2; see also SACRISTAN 1982, MACDONALD AND INGRAM 1984). However, a strict host specificity of the toxin containing culture filtrate was not observed (see also SACRISTAN 1982, MACDONALD AND INGRAM 1984). We have evidence, that in addition to host-specific toxins, unspecific toxins are also present in the culture filtrates. Similarly a series of toxins has been characterized in other *Alternaria* species (YODER 1981, NISHIMURA AND KOHMOTO 1983).

The inhibitory effect of the filtrates is apparent a few days after cultivation on T1 or T2 medium, respectively. In early selection experiments material was obtained surviving one to

three transfers onto Phoma- or Alternaria-toxins (Table 2). Because of a high variation of filtrate toxicity, depending on preculture of the fungus (soil, oat meal plate, age of the fungus), efforts are made to concentrate the toxins.

In the host-parasite system Verticillium dahliae-Solanum melongena resistant lines were selected by ALICCHIO ET AL. (1984) via culture filtrate of the fungus. NACHMIAS ET AL. (1982) reported on the separation of a host specific protein lipopolysaccharide complex in cultures of a potato isolate of Verticillium dahliae. We obtained necrotic spots and slight growth inhibition when rapeseed leaves were treated in vitro with culture filtrate of the fungus (Table 2). Wilting of the plants, e.g. the classical symptom of fungal infection, could not be observed. The influence of temperature (LATUNDE-DADA AND LUCAS 1986) and other conditions of preculture of the fungus is yet to be determined.

Table 2: The influence of culture filtrates of pathogens against B. napus and early selection for pathogen resistance

Effect	Phoma lingam	Alternaria brassicae	Verticillium dahliae
Germination inhibition	+	-	-
Necrotic spots on leaves	+	+	+
Growth inhibition of			
- embryoids from microspores	$+/+_1$	+	?
- pp-colonies from mesophyll	+	+	?
- shoot embryogenic material	$+/+_3$	$+/+_1$	(+)
- cell suspension	$+/+_2$	+	(+)

+ = yes; - = no; ? = not yet clarified;
(+) = slight inhibition;
$/+_x$ = material survived x transfers to toxin-containing medium

ACKNOWLEDGEMENTS

Thanks are due to Dr. R. Mathias for supply of microspore derived materials, to Prof. Dr. G. Röbbelen for continuous support of this research and to Mrs. F. Buggert and Mrs. B. Mesecke for excellent technical assistance. This work was sponsored by Niedersächsisches Zahlenlotto GmbH, Hannover.

REFERENCES

AHLERS, D., 1987: Untersuchungen über das Auftreten der krankhaften Abreife an Winterraps. Raps -Förderungsfond.

ALICCHIO, R., C. ANTONIOLI, D. PALENZONA, 1984: Karyotypic variability in plants of Solanum melongena regenerated from callus grown in presence of culture filtrate of Verticillium dahliae. Theor. Appl. Genet. 67, 267-271.

CARGEEG, L.A., N. THURLING, 1980: Contribution of host-pathogen interactions to the expression of the blackleg disease of spring rape (Brassica napus) caused by Leptosphaeria maculans (Desm.). Ces. et De Not. Euphytica 29, 465-476.

GENGENBACH, B.G., C.E. GREEN, C.M. DONOVAN, 1977: Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci. USA 74, 5113-5117.

GENGENBACH, B.G., J.A. CONNELLY, D.R. PRING, M.F. CONDE, 1981: Mitochondrial DNA variation in maize plants regenerated during tissue culture selection. Theor. Appl. Genet. 59, 161-167.

HARTMAN, C.L., T.J. McCOY, T.R. KNOUS, 1984: Selection of alfalfa (Medicago sativa) cell lines and regeneration of plants resistant to the toxin(s) produced by Fusarium oxysporum f. sp. medicaginis. Plant Sci. Lett. 34, 183-194.

KRÜGER, W., 1986: Verticillium dahliae Kleb.. Auftreten und Beurteilung des Befalls bei Raps in Schleswig-Holstein. Raps 1, 14-19, 1986.

LATUNDE-DADA, A.O., J.A. LUCAS, 1986: Influence of temperature on host resistance and fungal sensitivity to medicarpin in lucerne callus lines infected with Verticillium albo-atrum. Physiol. Molec. Plant. Pathol. 28, 89-97.

LICHTER,R., 1982: Induction of haploid plants from isolated pollen of Brassica napus. Z. Pflanzenphysiol. 105, 427-434.

MACDONALD, M.V., D.S. INGRAM, 1984: Secondary embryogenesis and selection for resistance to disease in oilseed rape. In: British crop protection conference - pests and diseases. 223-230.

MURASHIGE, T., F. SKOOG, 1962: A revised medium for rapid growth and bio- assays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

NACHMIAS, A., V. BUCHNER, J. KRIKUN: Comparison of protein-lipopolysaccharide complexes produced by pathogenic and non-pathogenic strains of Verticillium dahliae Kleb. from potato. Physiol. Plant Pathol. 20, 213-221.

NAGY, J.J., P. MALIGA, 1976: Callus induction and plant regeneration from mesophyll protoplasts of Nicotiana sylvestris. Z. Pflanzenphysiol. 78, 453-455.

NISHIMURA, S., K. KOHMOTO, 1983: Host-specific toxins and chemical structures from Alternaria species. Ann. Rev. Phytopathol. 21, 87-116.

SACRISTAN, M.D., 1982: Resistance responses to Phoma lingam of plants regenerated from selected cell and embryogenic cultures of haploid Brassica napus. Theor. Appl. Genet. 61, 193-200.

SCHENCK, H.R., G. RÖBBELEN, 1982: Somatic hybrids by fusion of protoplasts from Brassica oleracea and B. campestris. Z. Pflanzenzüchtg. 89, 278-288.

SCHIEDER, O., 1984: Isolation and culture of protoplasts: Datura. In: Vasil I. (ed) Cell culture and somatic cell genetics of plants 1. Academic Press, New York, 350-355.

SHILLITO, R.D., J. PASZKOWSKI, I. POTRYKUS, 1983: Agarose plating and a bead type culture technique enable and stimulate development of protoplast-derived colonies in a number of plant species. Plant Cell Rep. 2, 244-247.

THOMAS, E., F. HOFFMANN, I. POTRYKUS, G. WENZEL, 1976: Protoplast regeneration and stem embryogenesis of haploid androgenetic rape. Mol. Gen. Genet. 145, 245-247.

YODER, O.C., 1980: Toxins in pathogenesis. Ann. Rev. Phytopathol. 18, 103-129.