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In vitro techniques for selecting wheat (*Triticum aestivum* L.) for *Fusarium*-resistance. II. Culture filtrate technique and inheritance of *Fusarium*-resistance in the somaclones

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Summary

Calli of resistant, intermediary and susceptible wheat (*Triticum aestivum* L.) varieties were selected using culture filtrates of *Fusarium graminearum* and *F. culmorum* and the regenerants were evaluated for resistance up to R_3. Czapek-Dox broth medium was inoculated with mycelia of *Fusarium* isolates and incubated for 2–6 weeks. Filtrates were added to MS callus growing medium, then 5 weeks-old calli were transferred onto this medium (MST) for 4–5 weeks. MST containing 30% filtrate was found to be suitable for selection. Resistant calli were transferred again to fresh MST for further two selection cycles. The surviving calli produced less fertile regenerated lines (R_0) than the non-selected ones. Among 18 R_1 lines tested for *Fusarium*-resistance in the seedling stage by artificial inoculation in the greenhouse, two (11.1%) were significantly more resistant, one (5.6%) was more susceptible than the original cultivar and the rest (83.3%) behaved similarly to the donor plants. Among unselected R_3 lines of three varieties, practically the same number of resistant plants were found as among the related selected ones. When the R_3 selfed generations obtained through double-layer and culture filtrate selection techniques were tested for *Fusarium*-resistance, 35.7% of the lines were found to be more resistant than the original cultivars, none was more susceptible and 64.3% had a reaction similar to that of the source materials. Thus, inheritance of the disease reaction was not stable in all cases. Success of *in vitro* selection for *Fusarium*-resistance depended also on the genotype, and toxin analysis showed that although being effective, the selective media contained deoxynivalenol only exceptionally. In selecting wheat for *Fusarium*-resistance *in vitro*, the culture filtrate technique proved better than the double-layer procedure.

Introduction

Somaclonal variation and *in vitro* selection techniques have already been used for obtaining potentially disease-resistant plants of various crops (Wenzel, 1985; Daub, 1986; Gengenbach & Rines, 1986; Van den Bulk, 1991), but various problems still restrict the wider application of this approach (Daub, 1986; Chawla & Wenzel, 1987; Hammerschlag, 1988; Binarová et al., 1990; Ahmed et al., 1992).

In the first paper of this series, we have described the double-layer culture technique for *in vitro* selection of *Fusarium*-resistant wheats. Using this technique, resistant plants could be regenerated from resistant calli, however, only 3% of the R_2 populations was significantly more resistant than the original cultivars (Ahmed et al., 1992).

An alternative of the double-layer selection method, the culture filtrate technique is based upon toxic fungal metabolites incorporated into the culture media. Culture filtrates of *Fusarium* spp. have already been applied for obtaining somaclones resistant to toxic compounds produced by these pathogens in potato (Behnke, 1980), in alfalfa (Hartman et al., 1984; Arcioni et al., 1987; Binarová et al., 1990), in carna-

tion (Buiatti et al., 1985) and in wheat (Li & Huang, 1992).

In most cases the *in vitro* induced resistance was inherited in a stable manner (Wenzel, 1985; Daub, 1986; Van den Bulk, 1991). However, Wenzel & Foroughi-Wehr (1990) found that some progenies of several barley, wheat and potato regenerants selected for resistance to *Helminthosporium, Fusarium* or *Phytophthora* toxins did not express detectable resistance differences, as observed in the first *in vitro* generation (R_0). It was speculated that probably the R_0 somaclones were not uniform enough or the selection and the screening protocol were inadequate. Unfortunately, many *in vitro* selection studies do not include proof of stability and heritability of the induced resistance.

In the present paper we describe the *in vitro* selection of wheat calli for insensitivity to toxic metabolites of *F. graminearum* and *F. culmorum* via the culture filtrate technique, and inheritance of *Fusarium*-resistance in the third selfed generation (R_3) of R_0 regenerants obtained by the double-layer and the culture filtrate selection techniques. Preliminary results of toxin analysis of the selective media are also included.

Materials and methods

Plant materials and callus cultures

Callus cultures of Sakha 69 (resistant), Giza 157, Sakha 8 (resistant/intermediary), Giza 155, Giza 160 spring wheats and GK Ságvári winter wheat (susceptible) were initiated from immature embryos, mature embryos and immature inflorescences on solid MS medium (Murashige and Skoog, 1962) containing 1 or 2 mg l^{-1} 2,4-D, or from mesocotyls in 2,4-D solution (5–8 mg l^{-1}, Bartók and Sági, 1990). All cultures were grown at 26° C in darkness.

Culture filtrate technique

F. graminearum (isolate No. 12216) and *F. culmorum* (isolate No. 12375 or 12551) were used for toxin production by the aerated culture method (Mesterházy, 1977). The isolates were inoculated separately, each in 3 liter Czapek-Dox broth in 5 liter Erlenmeyer flasks and sterile air was blown through the medium at 26° C for one week. Then the cultures were kept in a refrigerator (5° C) for 2–6 weeks. The suspensions were homogenized and filtered to remove the conidia and mycelia. MS toxic medium (MST) was prepared by replacing part of bidistilled water with equivalent volume of culture filtrate before autoclaving and by addition of 1 mg l^{-1} 2,4-D plus 7% agar (40 ml medium pro 100 × 15 mm Petri dish). Five weeks-old calli were transferred to fresh MST medium for further two selection cycles (each for 4–5 weeks). Toxin sensitivity was assessed on the basis of callus weight or necrosis rating. The surviving calli were placed on MS regenerating medium supplemented with 0 or 0.1 mg l^{-1} 2,4-D and 10 mg l^{-1} $AgNO_3$. The pH of all culture media was adjusted to 5.8 before autoclaving at 120° C for 15 min (not degrading the *Fusarium* toxins, Patey & Gilbert, 1989; Ahmed et al., 1992). Primary (R_0) regenerants were transplanted in pots and grown to maturity in the greenhouse.

Analysis of Fusarium*-resistance*

In vivo Fusarium-resistance of 18 R_1 lines selected by the culture-filtrate technique and plants of further successive generations both from double-layer and culture-filtrate selections (including Lerma Rojo 64 and Tobari 66 spring wheats) as well as some unselected, regenerated plants were evaluated by means of a greenhouse seedling test (Mesterházy, 1978). Two replicates, each with ten uniform and healthy seeds sown per pot and inoculated with *F. graminearum* isolate No. 12216 or *F. culmorum* isolate No. 12375, and two replicates without *Fusarium* (controls) were used. Some inoculations were made with both fungal isolates.

Seed germination and number of killed seedlings were recorded every second day. The greenhouse regime was 25° C/20° C in 12/12 hour day/night cycles at 80% humidity. After three weeks, the experiment was evaluated based upon seed germination, number of killed plants, plant height and dry matter production, calculated for 60 seeds in six replications. Two resistant genotypes, 74-2, 84-4 and the original cultivars served as controls.

For statistical evaluation the LSD values from two-way ANOVA analyses were used. If the relative values of a line were significantly different from those of the original cultivar at least in two traits, the line was considered to be significantly more resistant or more susceptible.

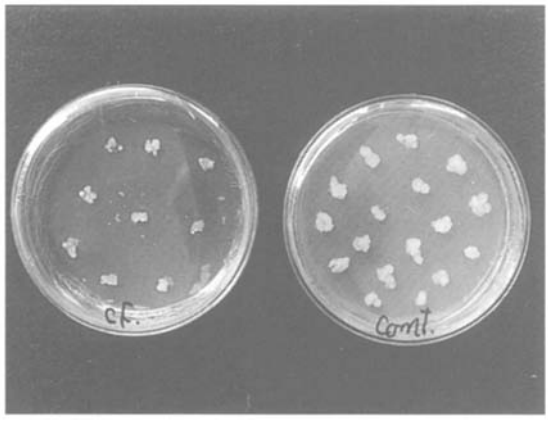

Figure 1. Wheat calli grown for 4 weeks on MST medium containing 30% *Fusarium* culture filtrate (left) and on MS medium (right).

Analysis of Fusarium *toxins in selective media*

For extraction and purification of the mycotoxins, 5 g of selective culture medium (upper layer of double-culture or MST containing 30% culture filtrate) was homogenized with 30 ml of acetonitrile/water (84/16, v/v) for 10 min and agitated overnight on a horizontal shaker. Fifteen ml of the homogenizate was filtered and passed through a 20 ml column filled with 3 g of neutral alumina packing. The filtrate was evaporated to dryness in a centrifugal evaporator, dissolved in 500 μl methanol and filtered using a cellulose acetate membrane filter into the autosampler vial. The mycotoxins were analyzed with a Hewlett-Packard HP 1090M liquid chromatograph using a diode array UV detector, a Hypersil ODS 250 × 2 mm microbore column and a gradient of water and acetonitrile/water (4/l, v/v) in 25 min at a flow rate of 0.45 ml/min.

Results

Growth of wheat calli on MST medium

Addition of *Fusarium* culture filtrates to MS callus growing medium at concentrations of 2%–15% affected callus growth only slightly, while concentrations above 25% were strongly inhibitory. The best selective effect was obtained at 30% culture filtrate concentration. The Czapek-Dox broth at 30% concentration was practically ineffective (Table 1 and Figure 1). Calli of different origin from the same variety responded similarly to the MS medium containing 30% culture filtrate.

Response of wheat genotypes to in vitro *selection and plant regeneration*

From the selected calli of the susceptible Giza 155 and Giza 160, only 9.6 and 11.5%, respectively, survived on MST medium, while percentages of the tolerant calli from resistant or resistant/intermediary genotypes were between 17.6% (Sakha 69) and 20.3% (Sakha 8). Regeneration frequency ranged from 17.8 to 38.5% and 2.0 to 7.8%, respectively, as per cent of the surviving or the total calli. Calli of all spring wheat genotypes not selected on MST medium regenerated better than the selected ones (data not shown). From selected calli of GK Ságvári fertile plants could not be obtained.

Fusarium-*reaction of the regenerated lines*

The greenhouse seedling test of the R_1 progeny from the MST-selected primary regenerants revealed that in the level of resistance relative to that of the original, both negative and positive differences occurred. For example, seed germination of Sakha 8 R_1 lines ranged from 72.5 to 102.7%, mortality of Sakha 69: 10.0 to 42.5%, shoot length of Giza 155: 65.4 to 112.1%, and dry matter of Sakha 69: 67.0 to 122.4% as related to the corresponding control values (Table 2). However, the majority of these variations was not significant. All regenerated lines of the susceptible variety Giza 155 were more resistant than the original, except germination percentage, but the difference was significant in the case of the CF/24-A R_1 line only.

When the R_3 generation of the R_1 or R_2 lines was subjected to the seedling infection test, the range of variation within the R_3 lines from selected calli was found to be similar to that of the R_1 lines. However, in case of Giza 157 the variation became lower. The majority of these variations was not significant again, but the number of lines with improved *Fusarium*-resistance in the R_3 progeny of Sakha 8 increased considerably. Except for the shoot length, quantitative characters of the unselected, inoculated R_3 lines of Sakha 8 varied similarly to those of the selected R_3 lines, and some unselected R_3 lines proved to be more resistant than the corresponding original cultivars (Table 3). Among the studied quantitative traits of both selected and non selected R_1 and R_3 plants, variation distances were greatest in mortality and smallest in germination percentage (Table 2).

Summing up, in the R_1 population from calli selected by culture filtrate, two lines (CF/5-3 and CF/24-A, 11.1%) showed significantly improved resistance over

Table 1. Fresh weight changes of mesocotyl-derived calli of GK Ságvári wheat after 4 weeks on MS medium (control I), MS + 30% Czapek-Dox broth medium (control II) and MST medium containing 30% *Fusarium graminearum* culture filtrate

	MS (control I)	MS + 30% Czapek-Dox broth medium (control II)	MST
Initial weight[a] (g)	1.18	1.16	1.07
Weight after 4 weeks (g)	1.73	1.60	1.14
Weight increase (g)	0.55	0.44	0.07
Weight increase (%)	46.61	37.93	6.54

[a] Total fresh weight of 30 calli each. Significant differences were found after 4 weeks between MST and both control calli (T-test, P = 0.1) only.

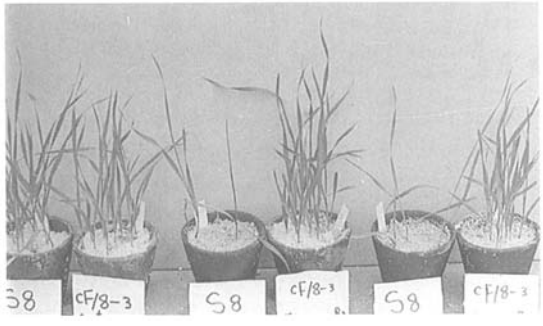

Figure 2. Fusarium resistance of Sakha 8 control and R_3 wheat seedlings derived from selected calli. From left to right: Sakha 8, CF/8-3 (controls), Sakha 8 + *F. graminearum*, CF/8-3 + *F. graminearum*, Sakha 8 + *F. culmorum*, CF/8-3 + *F. culmorum*.

the donor cultivars (Sakha 8 and Giza 155), and only one line (Sakha 69, CF/13-3, 5.6%), proved to be more susceptible. Rest of the lines (83.3%) behaved similarly to the original genotypes. Comparatively, in the culture filtrate-selected R_3 population (Giza 160 excluded) no one line was significantly less resistant than the corresponding donor, whereas 9 lines (Sakha 69: CF/13-5, CF/13-7; Sakha 8: CF/3-2, CF/3-5, CF/5-1, CF/5-5, CF/8-3, Figure 2; Giza 155: CF/24-A, 42.9%) were significantly more resistant, and 12 lines (57.1%) exhibited similar resistance as the original varieties. For all selected R_3 lines, the related data were: 10 more resistant (35.7%) and 18 with unchanged disease reaction (64.3%). The unselected R_3 lines of Sakha 69, Sakha 8 and Tobari 66 produced practically as many resistant plants (46.7%) as could be obtained from the related, selected R_3 population (44.44%). From the selected calli of the moderately resistant Giza 157, the susceptible Lerma Rojo 64 and Tobari 66, more resistant plants could not be regenerated, while two non-selected Tobari 66 regenerants clearly showed a good seedling resistance (Table 3).

Transmission of Fusarium *disease-reaction in selected wheat somaclones*

Stability of *Fusarium*-reaction was followed in six lines reacting differently from the donors. In the toxin-selected R_1 offspring, two lines (Sakha 8: CF/5-3, Giza 155: CF/24-A) became more resistant than their donor cultivars (Table 2). Then, the corresponding R_3 CF/5-3 line reverted to the original disease-reaction, while resistance of CF/24-A did not change in R_3 (Table 3). The improved resistance of the unselected DL2/18-B Tobari 66 R_3 somaclone (Table 3) was already present in R_2 and an *in vitro* unselected R_3 somaclone of the 84-4 reference cultivar also inherited the susceptible character from its R_2 predecessor (not shown). The control (parental type) disease-response turned into a more resistant type in two progeny lines (Sakha 8: CF/8-3 and Sakha 69: CF/13-7, Tables 2 and 3).

Deoxynivalenol content of the selective media

The main *Fusarium* toxin deoxynivalenol (DON) was found only incidentally, and in the positive cases, only in traces in both double-layer and MST media. Other UV-reacting *Fusarium*-mycotoxins could not be detected.

Table 2. Reaction of R_1 plants from calli selected by *F. culmorum* culture filtrate and of the original cultivars to *F. graminearum* and *F. culmorum* in the seedling infection test. Data are averages as % of the controls

Cultivar	Disease reaction[a]	Original cv. or R_1 line	Germination %	Mortality %	Shoot length %	Dry matter %
Sakha 69	R	original cv., control	100.0	0.0	12.0§	0.25•
		original cv., infected	97.5	17.5	94.2	92.5
		selected R_1				
		CF/13-3	70.0*−	42.5*−	72.7	67.0
		CF/13-4	92.5	10.0	111.0	115.5
		CF/13-6	100.0	41.7*−	90.6	82.9
		CF/13-7	95.0	10.0	113.5	108.3
		CF/15-3	95.0	20.0	87.5	122.4
Sakha 8	R/I	original cv., control	100.0	0.0	12.5§	0.32•
		original cv., infected	82.5	52.5	94.4	72.7
		selected R_1				
		CF/3-7	97.5	10.0*+	108.8	98.5
		CF/3-8	80.0	35.0	94.9	73.1
		CF/5-3	102.7	9.2*+	122.3	109.4*+
		CF/5-7	97.5	30.0	115.5	87.1
		CF/5-8	72.5	35.0	80.9	71.1
		CF/8-3	87.5	15.0*+	97.9	96.0
Giza 157	R/I	original cv., control	100.0	0.0	16.5§	0.43•
		original cv., infected	92.5	15.0	97.7	89.2
		selected R_1				
		CF/30-1	95.0	15.0	113.2	86.3
		CF/30-2	92.5	15.0	104.7	100.0
		CF/30-3	85.0	40.0*−	93.4	67.8
Giza 155	S	original cv., control	100.0	0.0	18.0§	0.45•
		original cv., infected	82.5	52.5	62.6	57.2
		selected R_1				
		CF/22- 2	76.3	35.0	87.9	72.1
		CF/23-1	74.2	41.8	74.8	64.8
		CF/23-5	67.0	47.2	65.4	58.7
		CF/24-A	92.5	22.5*+	112.1*+	92.8*+
84-4		reference cv., control	100.0	0.0	12.1§	0.36•
		reference cv., infected	92.5	15.0	120.2	90.2
74-2		reference cv., control	100.0	0.0	15.6§	0.42•
		reference cv., infected	92.5	12.5	77.2	91.7
		LSD 5%	23.3	24.1	33.2	31.5

[a] Disease reaction as in Table 2. § cm, • g/shoot, *− significantly inferior (less resistant), *+ significantly better (more resistant) than the donor cv.

Discussion

Growth of wheat calli on MST media

Inhibition of callus growth indicated that the MST medium containing 30% *Fusarium* culture filtrate was suitable for selecting wheat calli resistant to toxic metabolites of the fungi, resulting in 10% to 20% surviving calli in the first cycle, depending on the genotype (Table 1). In agreement with Arcioni et al. (1987), it was found that inhibition of callus growth was due to the *Fusarium* metabolites present in the selective media and not to the Czapek-Dox broth.

Table 3. Seedling infection test: reaction of R_3 plants from calli selected by double-layer (DL) or culture filtrate (CF) techniques, from unselected calli and of the original cvs. to *F. culmorum* (F.c.) and *F. graminearum* (F.g.) expressed in % of the uninfected controls. n.s. = not significantly different from the original (average data, if more than one n.s. line)

Cultivar	Original cv. or R_3 line	Isolate	Germination %	Mortality %	Shoot length %	Dry matter %
Sakha 69	cv.	F.c. + F.g.	82.5	32.5	55.9	55.9
	selected R_3					
	CF/13-5	F.c. 12375	90.0	15.0	88.8*	82.9*
	CF/13-7	F.c. 12375	100.0	4.2*	94.7*	90.1*
	4 n.s. lines	F.c. 12375	86.1	28.5	67.6	72.3
	unselected R_3					
	DL4/23-G	F.c. + F.g.	90.0	15.0	87.8*	89.0*
Sakha 8	cv.	F.c. + F.g.	63.2	52.6	38.3	44.5
	selected R_3					
	CF/3-2	F.c. 12375	90.0*	20.0*	69.7*	86.6*
	CF/3-5	F.c. 12375	87.5*	30.0	67.5*	71.4
	CF/5-1	F.c. 12375	86.7*	16.7*	75.7*	62.3
	CF/5-5	F.c. 12375	80.0	20.0*	61.2	79.4*
	CF/8-3	F.c. 12375	88.9*	15.6*	92.3*	77.1
	6 n.s. lines	F.c. 12375	78.5	36.2	58.5	57.8
	unselected R_3					
	CF/1-1	F.c. + F.g.	87.5*	27.5	70.4*	71.6*
	CF/1-2	F.c. + F.g.	82.5	22.5*	67.7*	66.9
	CF/1-16	F.c. + F.g.	85.0*	17.5*	66.7*	73.4*
	CF/1-18	F.c. + F.g.	91.7*	16.7*	90.4*	79.5*
	7 n.s. lines	F.c. + F.g.	77.1	38.9	52.7	51.9
Giza 157	cv.	F.c. + F.g.	90.0	32.5	54.3	53.1
	selected R_3					
	3 n.s. lines	F.c. 12375	90.5	25.8	70.5	72.8
Giza 155	cv.	F.c. + F.g.	77.5	52.5	26.7	28.7
	selected R_3					
	CF/24-A	F.c. 12375	92.5	24.5*	83.4*	87.8*
Giza 160	cv.	F.c. + F.g.	70.0	50.0	26.5	29.6
	selected R_3					
	CF/40-C	F.c. 12375	72.5	37.5	60.6*	56.3*
Tobari 66	cv.	F.c. + F.g.	79.0	42.1	45.5	53.9
	selected R_3					
	1 n.s. line	F.c. 12375	70.0	45.0	51.0	55.7
	unselected R_3					
	DL2/18-A	F.c. + F.g.	90.0	15.0*	85.4*	91.6*
	DL2/18-B	F.c. + F.g.	87.5	15.0*	88.1*	86.0*
	1 n.s. line	F.c. + F.g.	82.5	27.5	65.0	62.1
Lerma R.64	cv.	F.c. + F.g.	82.5	37.5	53.8	62.6
	selected R_3					
	5 n.s. lines	F.c. + F.g.	80.6	33.5	66.0	62.2
84-4	reference	F.c. + F.g.	108.3	13.9	85.0	103.1
74-2	reference	F.c. + F.g.	105.9	20.6	109.2	113.8
	LSD 5%		20.6	25.9	28.2	26.2

* Significantly better (more resistant) than the original cv.

Response of wheat genotypes to in vitro *selection and plant regeneration*

Seemingly, a positive relationship exists between *Fusarium*-resistance of the source material and insensitivity to the toxic fungal metabolites at cellular level. According to Wang & Miller (1988) and Fadel & Wenzel (1993), varietal susceptibility to *Fusarium* pathogens and toxin sensitivity correlate in wheat at both diploid and haploid level. Wolf and Earle (1990) also found that after HC toxin treatment the number of surviving maize calli varied depending on the genotype. Similar observations have been made by various authors also on different species (Pelcher et al., 1975; Arcioni et al., 1987; Chawla & Wenzel, 1987; Binarová et al., 1990; Liu et al., 1991). The 2% to 8% regeneration frequency of calli selected on MST medium was approximately ten times lower in average as compared to that under non-toxic conditions. This decline is probably due to the *Fusarium* metabolites present in the MST medium (Pauly et al., 1987; Latunde-Dada & Lucas, 1988; Ahmed et al., 1992; Posselt & Altpeter, 1994), causing loss of viability and partial or complete sterility. Fadel & Wenzel (1993) also mention that the regeneration ability of wheat anther calli can be lost when cultured on *Fusarium* toxin-containing medium for a long time. These reasons can explain, why resistant plants have infrequently been obtained in the majority of *in vitro* selection experiments (Pauly et al., 1987; Toyoda et al., 1989; Wenzel & Foroughi-Wehr, 1990; Wolf & Earle, 1990; Ahmed et al., 1992).

Fusarium-*reaction of the regenerated lines*

Lines from calli selected in vitro

Variation of the studied quantitative characters as related to *Fusarium*-susceptibility or -resistance in the culture filtrate-selected R_1 and R_3 lines of all but one genotype (Giza 157) did not change significantly. Variation in the parameters related to disease-reaction of selected R_1 progeny can be expected as the regenerants my be originated from cells of putative resistant calli unevenly exposed to the toxins, leading to a range of responses from susceptibility (regenerating cells escaped from the toxins or protected by the proximal, resistant cells) to resistance (Hammerschlag, 1988). It is also conceivable that the desired resistance selected for is present at cellular level only (Chawla & Wenzel, 1987; Mégnégneau & Blanchard, 1991) or that the *in vitro*-induced resistance will be lost during development (Ahmed et al., 1992). A resistant-susceptible segregation can also occur. However, the similar character variation in R_1 and R_3 may be apparent, since the population sizes and some of the lines were different. If the R_1 and R_3 lines were identical as in case of Giza 157, the R_3 variation became narrower, indicating the sorting out of more extreme gametal combinations.

Among the disease-related parameters, germination percentages varied least and mortality varied most, since in the seedling infection test the seminal roots are easier to invade and colonize by the fungi than the seeds. Then, early infection of the roots generally will result in a rapid seedling death, while the later fungal attacks can sometimes fail.

In the individual culture filtrate-selected R_3 families, a shift towards relative enrichment in more resistant lines could be observed (Table 2 vs. Table 3). This shift can be explained by reparation of some chromosomal disorders induced during the *in vitro* selection or by a favorable gamete selection. However, it can be a result of the altered host-parasite interaction due to generational change of the plant and pathogenic change of the fungus (Mesterházy, 1984). Irrespective of the shift mentioned above, disease-reaction of the regenerated wheats from *in vitro* selection exhibited a within-family variation, not disappearing even in the R_3 progeny. Between the different, regenerated families, a clear genotypic effect could be recognized in this respect (e.g. Sakha 8 vs. Giza 157, Table 3). Such variations of *in vitro*-selected, regenerated plants have been described in selecting oats, barley and wheat for *Helminthosporium*-resistance by Rhines & Luke (1985) & Chawla & Wenzel (1987), as well as for bacterial blight resistance by Pauly et al. (1987).

Lines from unselected calli

From unselected calli of some wheat varieties, plants with improved *Fusarium*-resistance could also be regenerated (Table 3). However, in the case of Sakha 8, 36.4% of the unselected R_3 lines had an improved resistance and this figure is close to the 45.4% more resistant lines from the selected counterpart. It is possible that cellular membranes of Sakha 8 are more accessible to the *Fusarium* toxins than those of the other varieties (Scowcroft et al., 1983). This mechanism was suggested to explain the relatively high incidence of resistant maize regenerants from callus cultures not subjected previously to T-toxin of *Drechslera maydis* (Brettell et al., 1980). It can also be hypothesized that the genome of Sakha 8 includes some minor or 'silent'

resistance genes readily activable under specific *in vitro* conditions. Presence and absence of such genes might explain, why sometimes the resistant, sometimes the intermediary or susceptible genotypes are found to be the better sources of resistant/tolerant regenerants from *in vitro* selected or non-selected cells (Rines & Luke, 1985; Daub, 1986; Wright & Lacy, 1988; Wenzel & Foroughi-Wehr, 1990; Ahmed et al., 1992).

Transmission of Fusarium *disease-reaction in wheat somaclones*

From six R_1 and R_2 lines studied for stability of their *Fusarium*-reaction, only three transmitted the induced, more resistant or more susceptible disease type into the next selfed generation, while the disease-reaction of two lines changed from less resistant to more resistant and that of one line from resistant to less resistant. This is in contrast to the often described stable inheritance of the *in vitro*-induced resistance (Wenzel, 1985; Daub, 1986; Van den Bulk, 1991). Reasons for instability of the *Fusarium*-reaction induced by selection of toxic media can be various: inhomogeneity of the primary regenerants (Wenzel & Foroughi-Wehr, 1990), chromosomal rearrangements and transpositional events (Larkin & Scowcroft, 1981) or changes in aggressiveness of the pathogen isolates (Mesterházy, 1984; Fadel & Wenzel, 1993). Obviously, disease-reaction of the progenies should be assessed over more generations by artificial inoculation with the related pathogen and not only with the selecting agent, since these two factors cannot be regarded always as to be associated (Binarová et al., 1990; Wenzel and Foroughi-Wehr, 1990; Posselt & Altpeter, 1994), and it is also possible that the toxic fungal compounds do not play a significant role in the pathogenesis (Daub, 1986).

Deoxynivalenol content of the selective media

DON, one of the major toxins of *F. graminearum, F. culmorum* and other *Fusarium* species, has been successfully used for improving *Fusarium*-resistance of wheat *in vitro* (Posselt & Altpeter, 1994). Although our double-layer and MST media were practically free of DON, this does not mean that other mycotoxins not absorbing in UV light were also absent. Fungi of the *Fusarium* genus can produce more than 100 toxic compounds of the trichothecene group and relatives of zearalenone (Mirocha & Christiansen, 1986), and many of them may have a selective value as good as that of DON. Fadel & Wenzel (1993) also preferred a purified mixture of toxic metabolites from different *Fusarium* isolates rather than DON. Moreover, Liu et al. (1991) could not induce tolerance by DON in wheat tissue cultures.

In conclusion, it is possible to select wheat calli with *Fusarium* culture filtrates and regenerate fertile plants with improved resistance from them and from the non selected calli as well. This is partly in contrast to the statement of Wenzel & Foroughi-Wehr (1990), working, however, with a quite different and rather limited wheat material. From the applied two techniques, the culture filtrate technique is simpler and more efficient, producing less susceptible somaclones, but a general drawback of both is the low output of resistant regenerants. Nevertheless, use of genotypes previously screened for presence of minor resistance genes would possibly improve the effectivity. The anther/microspore culture also offers new perspectives in selecting crop plants for *Fusarium*-resistance, via accessibility of recessive genes, recombination and homozigosity as stressed by Fadel & Wenzel (1993) and Simmonds et al. (1993).

Some of our toxin-selected R_3 regenerants exhibiting better *Fusarium*-resistance in the seedling inoculation test are under multiplication and will be further studied in the nursery.

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