P L A N T B R E E D I N G A N D S E E D S C I E N C E
Volume 51 2005

Tomasz Góral, Edward Arseniuk

Department of Plant Pathology, Plant Breeding and Acclimatization Institute, Radzików, 05-870 Błonie, Poland

REACTION OF WINTER TRITICALE SOMACLONAL LINES TO *FUSARIUM* SPP. AT THE SEEDLING STAGE

ABSTRACT

Response of 136 somaclonal lines obtained from 15 winter triticale genotypes (Bolero, Dagro, Grado, Lasko, Moniko, Presto, Ugo, GRH 32-1, KS-24, MAH 15668-1, MAH 15833-1/1, T/8, T/9, T/28, T/47), to *Fusarium* seedling blight was evaluated. Mixture of five *Fusarium* species was used for inoculation. Somaclonal lines varied widely in their resistance to *Fusarium* spp. scored by coleoptile and root infection, and reduction of length of shoot and roots, and seedling weight. Most of the somaclonal lines differed significantly from parental genotypes in at least one of the above parameters. It was found that resistant parental genotypes (e.g. Grado, KS-24) produced mainly more susceptible somaclonal lines and susceptible ones (e.g. Ugo, MAH 15833-1/1) gave rise mainly to more resistant lines. However, some resistant lines originated from resistant genotypes (e.g Presto) were found.

Key words: *Fusarium*, resistance, seedling blight, somaclonal variation, triticale,

INTRODUCTION

Species of the genus *Fusarium,* under favourable conditions, can cause epidemics of fusarioses of cereals affecting different parts of cereal plant (Parry *et al.* 1995, Fernandez and Chen 2005). The most dangerous is *Fusarium* head blight; resulting in, together with yield reduction, contamination of grain with mycotoxins. Seedling resistance can be used as a early selection method for head blight resistance (Mesterhazy 1987). Resistance breeding is the most acceptable way to control *Fusarium* head blight. *In vitro* cultures of plant cells or organs, where somaclonal variation appears, may be a source of variation in disease resistance (Liang and McHughen 1987, Semal *et al.* 1988). This variation can be induced by process of *in vitro* culturing itself or induced *in vitro* by selecting factor (pathogen inoculum, mycotoxins or pathogen culture filtrate) (Ahmed *et al.* 1996, Van den Bulk 1991, Arseniuk *et al.* 1998, Góral *et al.* 1998, Yang *et al.* 1998). However, the existence of stable and applicable somaclonal variation is still in question. The aim of the study was assessment of somaclonal variation for resistance to *Fusarium* seedling blight in winter triticale. Resistance of somaclonal R_2 lines was compared to the resistance of parental genotypes to identify types of improved or decreased resis-

Communicated by Andrzej Anioł

tance. Differences in *Fusarium* seedling blight resistance were assessed among somaclonal lines generated from various parental genotypes, and among somaclonal lines from the same parental genotype.

MATERIALS AND METHODS

Resistance of winter triticale somaclonal lines to *Fusarium* seedling blight was tested under controlled environment conditions. The plant material used in the experiment included 136 somaclonal R_2 lines and 15 parental genotypes of winter triticale (Bolero, Dagro, Grado, Lasko, Moniko, Presto, Ugo, GRE3 32-1, KS 24, MAH 15668-1 = MAH 2, MAH 15833-1/1 = MAH 3, T/8, T/9, T/28, T/47). Somaclonal lines were obtained *in vitro* by the means of somatic embryogenesis without selecting factor (Arseniuk *et al.* 1998).

The inoculum comprised a mixture of 10 highly pathogenic isolates of *Fusarium* species (*F. avenaceum, F. culmorum, F. graminearum, F. sambucinum, F. sporotrichioides).* Two isolates per species were used. The isolates were grown at 20°C under near ultraviolet lamps with photoperiod 12/12 h to stimulate sporulation. After 2 weeks of incubation plates were washed out with distilled water. Obtained suspension was filtered through gauze to remove mycelial fragments. Spore density of all isolates was adjusted to 5×10^6 spores$\times$ml^{-1} with a haemocytometer. Equal volumes of spore suspensions of were mixed for inoculation. A sterile double layer filter paper was submerged into the spore suspension and placed in plastic Petri dishes (9 cm). For the control combination filter paper was soaked in distilled water.

Ten surface disinfected triticale seeds per replicate were placed into dishes. Three replications were applied. Seeds were incubated at 25°C with day length 16h. On the fifth day the following parameters were scored: coleoptile (CI) and root (RI) infection, length of shoot and roots, and seedling weight. Coleoptile infection was rated on a scale according to Grey and Mathre (1988): 0 - no discoloration, 1 - pinpoint lesions, 2 - extended linear lesions, 4 - discoloration of at least 50% and/or blighted seedling. CI parameter was calculated as a percentage of the highest infection (4). RI was a percentage of necrotic root area. Relative to control combination reductions of seedling weight (RW), shoot length (RSL) and roots length (RRL) were calculated. Resistance grade of parental genotypes was estimated on the basis of all five disease parameters.

Results were analysed with analysis of variance using the general linear model procedures, means were compared with the least significant difference test. Disease parameters were correlated with Pearson correlation procedure.

RESULTS AND DISCUSSION

An average disease reaction of triticale seedlings was as follows: RW = 40.9%, CI = 29.8%, RI = 55.9%, RSL = 55.6%, RRL = *73.3%* for parental genotypes and RW = 42.2%, CI = 29.0%, RI = 60.7%, RSL = 55.0%, RRL = 72.6% for somaclonal lines. Parental genotypes differed significantly in their disease reaction as mea-

sured with the above parameters. Wide range of variation for resistance in somaclonal lines was found for all parameters used.

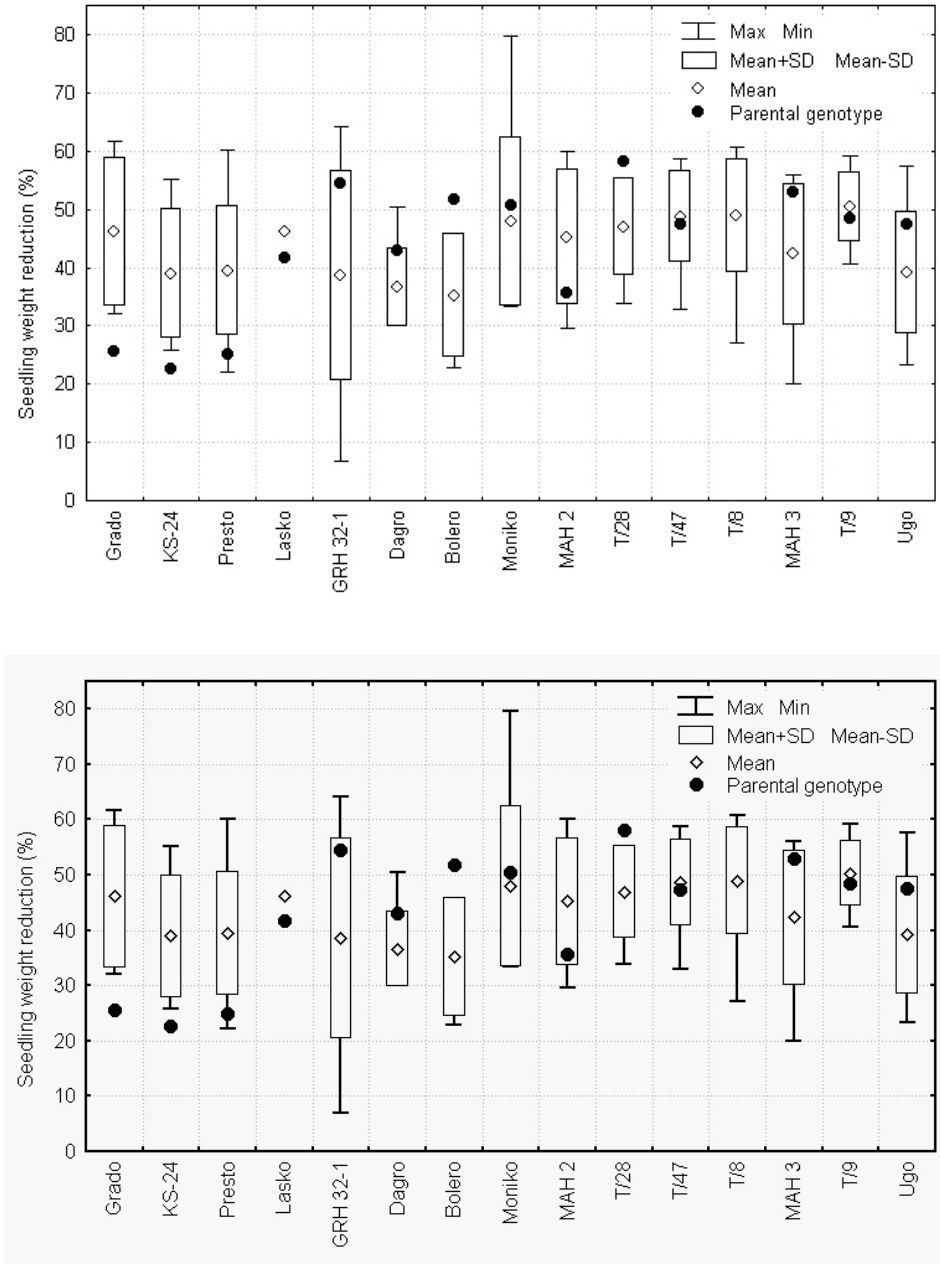

Fig. 1. Variation in seedling weight reduction in somaclonal R_2 lines of winter triticale inoculated with *Fusarium* spp. isolates. Parental genotypes are ranked according to their average resistance to seedling blight

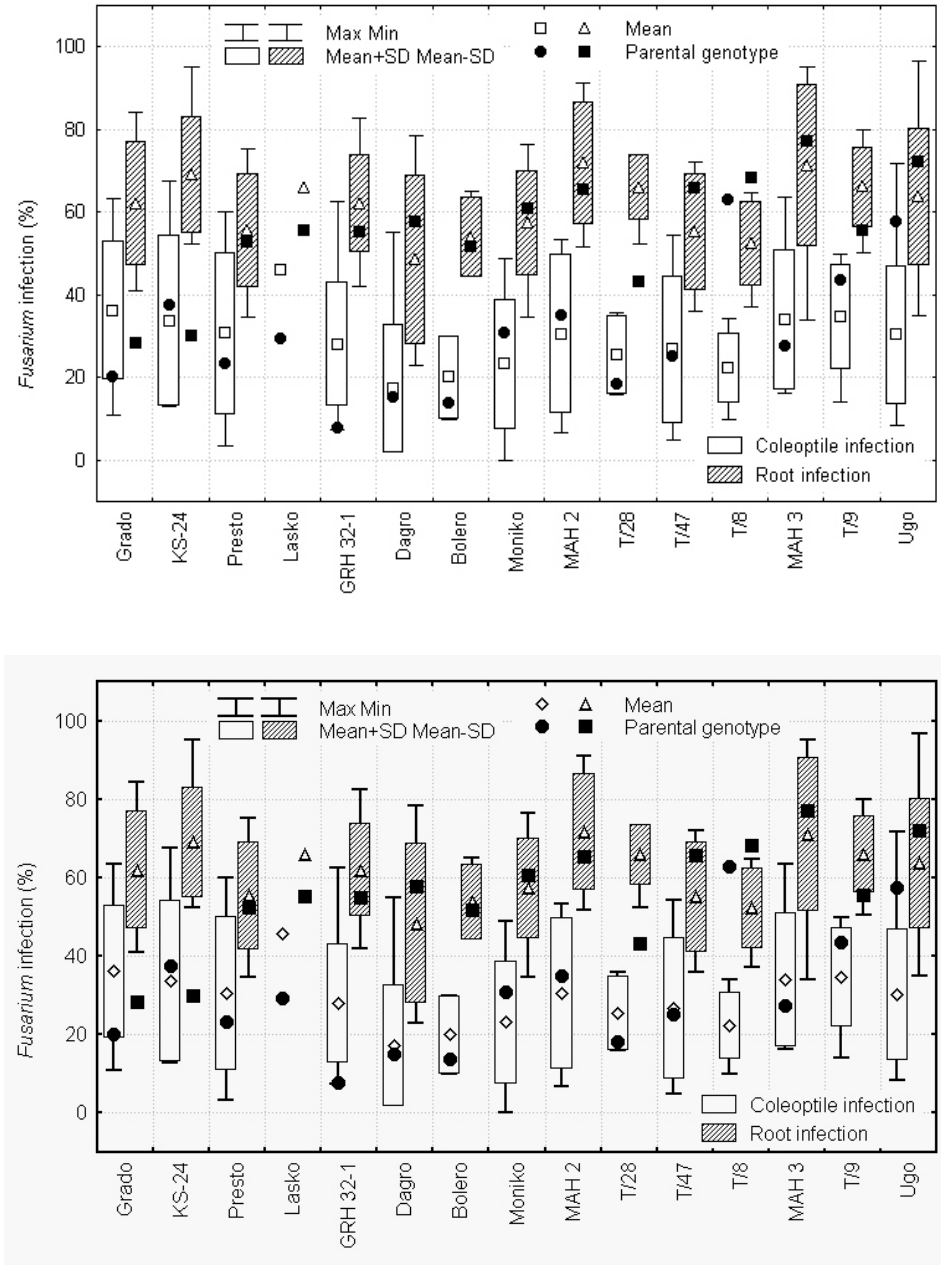

Fig. 2. Variation in coleoptile and root infection of somaclonal R_2 lines of winter triticale inoculated with *Fusarium* spp. isolates. Parental genotypes are ranked according to their average resistance to seedling blight

The examples of variation for seedling weight reduction and coleoptile and root infection are shown in Fig.1 and Fig.2. As shown in Table 1 the above parameters

were not correlated. Only root infection had small effect on the reduction of seedling weight. High coefficients were found for correlations between reduction of roots and shoot length and infections of coleoptile and roots. These data showed that for the most genotypes *Fusarium* infection caused decreasing of seedling growth. Also reduction of seedling weight correlated significantly with reductions of roots and shoot length. However, coefficients were lower showing that for some genotypes *Fusarium* infection caused shortening of shoot and roots without decreasing of seedling weight. It was observed, for example, for Moniko lines.

Table 1

Coefficients of correlations of parameters used to score resistance of winter triticale somaclonal lines to *Fusarium* seedling blight.

Parameters	Coleoptile infection [%]	Root infection [%]	Shoot length reduction [%]	Root length reduction [%]
Seedling weight reduction [%]	0.14	0.17*	0.51**	0.40**
Coleoptile infection [%]		0.70**	0.65**	0.63**
Root infection [%]			0.64**	0.66**
Shoot length reduction [%]				0.80**

*, ** - significant at P = 0.05 and P = 0.01, respectively

Table 2

Characteristics of winter triticale somaclonal R_2 lines generated from cultivars differing in resistance to *Fusarium* seedling blight.

Parental cultivar	Resistance grade	No. of lines	Percentage of lines and their disease reaction									
			Seedling weight reduction		Coleoptile infection		Root infection		Shoot length reduction		Root length reduction	
			Sa [%]	Ra [%]	S [%]	R [%]	S[%]	R [%]	S [%]	R[%]	S [%]	R [%]
Grado	R	9	55.6	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0
KS-24	R	8	25.0	0.0	0.0	12.5	100.0	0.0	50.0	0.0	37.5	0.0
Presto	R	15	46.7	0.0	20.0	6.7	0.0	6.7	20.0	0.0	20.0	13.3
Lasko	R	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GRH 32-1	I	17	0.0	29.4	29.4	0.0	17.6	0.0	11.8	11.8	0.0	23.5
Dagro	I	8	0.0	25.0	25.0	0.0	25.0	50.0	12.5	50.0	0.0	12.5
Bolero	I	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T/28	I	5	0.0	40.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Moniko	I	8	12.5	25.0	0.0	12.5	0.0	0.0	0.0	25.0	12.5	0.0
MAH 2	I	7	14.3	0.0	0.0	28.6	28.6	0.0	0.0	28.6	14.3	28.6
T/47	S	7	14.3	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T/8	S	11	-	-	0.0	100.0	0.0	36.4	0.0	18.2	0.0	72.7
T/9	S	13	0.0	0.0	0.0	7.7	0.0	0.0	0.0	7.7	0.0	15.4
MAH 3	S	7	0.0	28.6	0.0	14.3	14.3	28.6	0.0	28.6	42.9	14.3
Ugo	S	17	0.0	35.3	0.0	47.1	11.8	17.6	0.0	64.7	0.0	70.6
Total	-	136	12.5	14.7	6.6	19.1	19.1	10.3	7.4	19.1	8.1	23.5

R = resistant; I = intermediate; S =- susceptible; [a] - disease reaction significantly different from parental cv. reaction according to LSD test at P = 0.01

Somaclonal lines originated from different parental genotypes were analysed separately. Significant differences among lines were found for all parameters except: Bolero-lines for all parameters; Grado-lines for CI, RLL and RRL;

Moniko-lines for RI; T/9-lines for RW; T/28-lines for RI and RRL; and T/47-lines for RRL.

Most of the triticale genotypes produced somaclonal lines with significantly improved or decreased resistance (Table 2). However, resistant parental genotypes produced mainly more susceptible somaclonal lines and susceptible ones gave rise mainly to more resistant lines. Performance of intermediate parental genotypes was diverse, e.g., GRH 32-1, Moniko. Ahmed *et al.* (1996) found that the extent of the resistance of parental genotype of wheat did not correlate with the number of resistant regenerates. The authors stated that frequency of *in vitro*-derived resistant lines depended on the parental genotype. In this experiment we found some resistant lines originated from resistant genotypes (Presto 12/3) as well as susceptible lines originated from susceptible genotypes. Góral and Arseniuk (2003) studied variation of resistance of somaclonal lines of winter triticale to head infection with *Fusarium culmorum*. They used lines tested in this paper and found wide somaclonal variation of resistance to head blight. It was also possible to identify lines with significantly and stable improved resistance.

This experiment showed that only a few R_2 lines have improved resistance to three or more disease parameters (Table 3). However, disease reaction of lines obtained from susceptible parental genotypes was similar to reaction of the most resistant genotypes. It seems that *in vitro* generating somaclonal lines from resistant genotypes would be more efficient for obtaining lines with resistance improved over available in conventional genotypes. As regards *Fusarium*-resistance, mycotoxins produced by these fungi can be used for selection or induction of mutations in *in vitro* cultures. There are some reports showing that *in vitro* selection with deoxynivalenol can be efficient method for identifying or generating genotypes with improved *Fusarium*-resistance (Posslet and Altpeter 1994, Ahmed et. al. 1996, Yang *et al.* 1998, Góral *et al.* 2000). The authors obtained somaclonal lines

Table 3

Example of disease reaction of seedlings of winter triticale somaclonal R_2 lines showing *Fusarium* resistance improved over parental cultivars

Parental cultivar	Resistance grade	Line	Seedling weight reduction [%]	Coleoptile infection [%]	Root infection [%]	Shoot length reduction [%]	Root length reduction [%]
Presto	R		25.0	23.3	52.5	32.0	71.0
		12/3	27.5	7.5[a]	34.7[a]	17.0	52.4[a]
GRH 32-1	I		54.5	7.7	55.0	44.3	75.4
		4/5	9.9[a]	25.8	58.7	3.3[a]	51.3[a]
Dagro	I		43.0	15.0	57.7	57.8	72.6
		1/1	31.1[a]	10.8	34.0[a]	43.6[a]	64.7
		2/2	36.1	10.0	41.3[a]	28.4[a]	40.5[a]
MAH 3	S		53.0	27.5	77.0	71.2	75.2
		1/2	20.0[a]	20.8	34.0[a]	28.2[a]	40.9[a]
		4/1	42.5	16.3	59.5[a]	52.1[a]	68.9[a]

[a] - significantly more resistant than parental cultivar according to LSD test at p = 0.01

of wheat with improved resistance to *Fusarium* head blight and snow mold [caused by *Microdochium* (=*Fusarium*) *nivale*].

However, opposite results were also published. Hessemann and Maier (1998) did not find significant somaclonal variability in mature triticale plants. Such a variability was observed only in callus. Authors did not observed any effect of callus selection with deoxynivalenol on the resistance of regenerated plant under field conditions.

The results showed that increase or decrease of resistance in genotypes obtained through somatic embryogenesis depends to large extent on genotype used. Benslimane *et al.* (1988) and Chowdhury *et al.* (1994) stated that presence of somatic variability depends on explantate source, regeneration method (somatic embryogenesis or organogenesis) and source of regenerates (callus, protoplasts, cell suspension). Variability observed in regenerates can be also the effect of variability of parental genotypes (Brettell *et al.* 1986). In this experiment, however, relation between resistance of parental genotype and range of resistance variability in regenerates was not observed. Thus significant improving of resistance in regenerates requires large extent of somaclonal variability in particular parental genotype.

REFERENCES

Ahmed, K.Z., Mesterhazy, A., and Sagi, F. 1996. *In vitro* production of *Fusarium*-resistant wheat plants. In: Somaclonal Variation in Crop Improvement II, Ed.: Y.P.S. Bajaj, Springer-Verlag, Berlin Heidelberg, pp. 3-19.

Arseniuk E., H.J. Czembor, J. Zimny, A.L. Scharen & Z. Laudański 1998. Somaclonal variation as a tool for improvement of triticale resistance to Stagonospora nodorum blotch. Proceedings of the 4th International Triticale Symposium (July 26-31, 1998, Red Deer, Alberta, Canada), Vol. 1, pp. 148-163.

Benslimane A., Hartman C., de Buyse J., Henry Y., Picard E., Rode A. 1988. Ribosomal DNA as a convenient probe to follow segregation and possible divergency from expected homozygosity after haploidization of an androgenetic process. Theor. Appl. Genet. 75: 389-396.

Brettell R.I.S., Pallotta M.A., Gustafson J.P., Appels R. 1986. Variation at the *Nor* loci in triticale derived from tissue culture. Theor. Appl. Genet. 71: 637-643.

Bulk van den, R.W. 1991. Application of cell and tissue culture and *in vitro* selection for disease resistance breeding – a review. Euphytica 56: 269-281.

Chowdhury M.K.U., Vasil V., Vasil I.K. 1994. Molecular analysis of plants regenerated from embryogenic cultures of wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 87: 821-828.

Fernandez M.R., Chen Y. 2005. Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. Plant Dis. 89: 164-169.

Góral T., Arseniuk E. 2003. Reakcja linii somaklonalnych pszenżyta ozimego na porażenie grzybami z rodzaju *Fusarium*. Część I. Fuzarioza kłosów powodowana przez *F. culmorum* W.G. Smtih (Sacc.). Biul. IHAR (in press)

Góral T., Perkowski J., Stachowiak J., Arseniuk E. 1998. Resistance to *Fusarium* head blight in winter triticale somaclones generated from DON-selected and unselected calli. Proceedings of the 4th International Triticale Symposium (July 26-31, 1998, Red Deer, Alberta, Canada), Vol. 2, pp. 208-210.

Góral T., Perkowski J., Stachowiak J., Arseniuk E. 2000. Odporność linii somaklonalnych uzyskanych z odmian pszenżyta ozimego Moniko i Presto na fuzariozę kłosów oraz akumulacja deoksyniwalenolu w ziarnie. Fol. Univ. Agric. Stetin. 82: 73-78.

Grey W.E., Mathre D.E. 1988. Evaluation of spring barleys for reaction to *Fusarium culmorum* seedling and root rot. Can. J. Plant Sci. 68: 23-30.

Hessemann C.U., Maier F.J. 1998. Application of biotechnological methods as tissue and suspension culture techniques in triticale. Proc. 4th International Triticale Symp., July 26-31, 1998, Red Deer, Canada: 30-37.

Liang G.H., McHughen A. 1987. Novel approaches to wheat improvement. Wheat and Wheat Improvement - Agronomy Monograph no. 13: 472-488.

Mesterhazy A. 1987. Selection of head blight resistant wheats through improved seedling resistance. Plant Breeding 98; 25-36

Parry D.W., Jenkinson P., McLeod L. 1995. *Fusarium* ear scab in small grain cereals - a review. Plant Pathol. 44: 207-238.

Posslet U.K., Altpeter F. 1994. Improvement of snow mould resistance by conventional and *in vitro* techniques. Euphytica 77: 251-255.

Semal, J., Kummert, P., Lepoivre, P., Meulemans, Viseur, J., and Anceau, C. 1988. *In vitro* cultures producing pathogen-free plants and selecting disease resistant genotypes. Bull. Rech. Agron. Gembloux 23: 261-269.

Yang Z.P., Yang X.Y., Huang D.C. 1998. Studies on somaclonal variants for resistance to scab in bread wheat (*Triticum aestivum* L.) through *in vitro* selection for tolerance to deoxynivalenol. Euphytica 101: 213-219.