



The effect of medium composition on somatic embryogenesis from mature embryos of triticales cultivars with various resistance to *Parastagonospora nodorum*

L. Kowalska, E. Arseniuk

Plant Breeding and Acclimatization Institute, National Research Institute
Department of Plant Pathology

Radzikow, 05-870 Blonie <http://www.ihar.edu.pl>

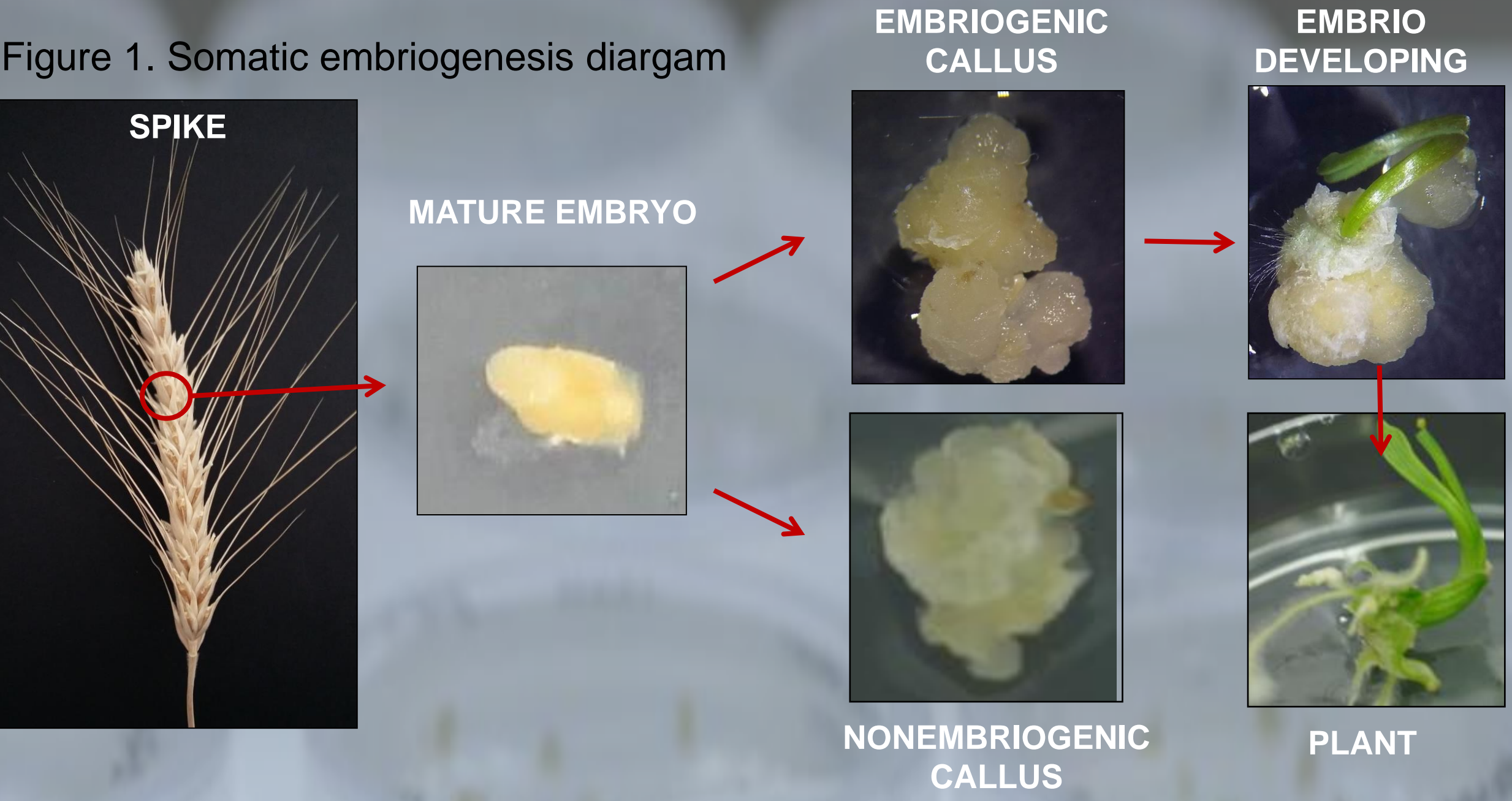
INTRODUCTION

Triticale belongs to the three most important cereal crops of the world and is grown under a wide variety of climatic and agricultural conditions. Fungal pathogens, especially *Parastagonospora nodorum*, represent the most relevant biotic stresses for cereals. High level of resistance among triticales cultivars to the pathogen is a rare feature.

OBJECTIVES

The main goal of the study was to support conventional methods of resistance breeding using the biotechnology tools, somatic embryogenesis (Figure 1.). This study was undertaken to improve callus induction and plant regeneration from mature embryos of five winter triticales cultivars with various resistance levels to *P. nodorum*.

Figure 1. Somatic embryogenesis diagram



METHODOLOGY

PLANT MATERIAL

Five winter triticales cultivars with various resistance to *P. nodorum* were tested. Table 1 presents the level of fungal infection assessed on 9-digit scale (– susceptible; ± moderately susceptible/resistant; + resistant).

Table 1. Characterization of resistance to *P. nodorum* triticales cultivars

TRITICALE CULTIVARS	LEAVES	GLUMES
Algoso	±	±
Borowik	±	±
Borwo	+	+
Cyrkon	±	-
Tomko	+	±

MEDIUM COMPOSITION

Triticale callus induction and plant regeneration are influenced by culture medium, initial plant organ and genotype. Mature embryos were cultured on standard Murashige-Skoog medium modified with three types of auxins and various sources of carbohydrates (Table 2.).

Table 2. Murashige – Skoog medium modification

	CARBOHYDRATES SOURCES		AUXINS		
	sucrose	maltose	2,4D	NAA	DICAMBA
S	+	-	-	-	-
M	-	+	-	-	-
S 2,4-D	+	-	+	-	-
M 2,4-D	-	+	+	-	-
S NAA	+	-	-	+	-
M NAA	-	+	-	+	-
S D	+	-	-	-	+
M D	-	+	-	-	+

PLANT REGENERATION PROCEDURES

1. Seeds surface-sterilization using 70% ethanol and 10% sodium hypochlorite.
2. Seeds imbibition in sterile water in 4°C for 24h.
3. Sterilization of seeds in 70% ethanol and HgCl₂.
4. Isolation of mature embryos from seeds and transfer on to the surface of callus induction media.
5. Incubation of plates with embryos in the dark (25°C ± 2°C) for 4 weeks.
6. Transfer embryogenic callus on regeneration medium.
7. Analysis of the somatic embryogenesis efficiency.

RESULTS

Callus growth and embryogenesis were observed from embryos cultured on all media. The highest percentage of embryos producing embryogenic callus was obtained for Algoso and Cyrkon while the smallest one for Borowik. The rate of embryogenic callus formation on media supplemented with dicamba was generally better than on media with NAA and 2,4-D. A significant difference was observed for Borowik. The percentage of embryogenic callus on dicamba containing medium was 53% when for 2,4-D 23% and for NAA barely 34%. In all triticales cultivars, carbohydrate sources used in the media had a significant effect on the production of callus from mature embryos. Three triticales cultivars showed regeneration capacity: Algoso, Cyrkon and Tomko (Figure 2.). Modified MS medium containing maltose, without auxins used in the study had a significant effect on the number of plantlets regenerated.

Figure 2. Comparison of reaction *in vitro* culture of triticales varieties

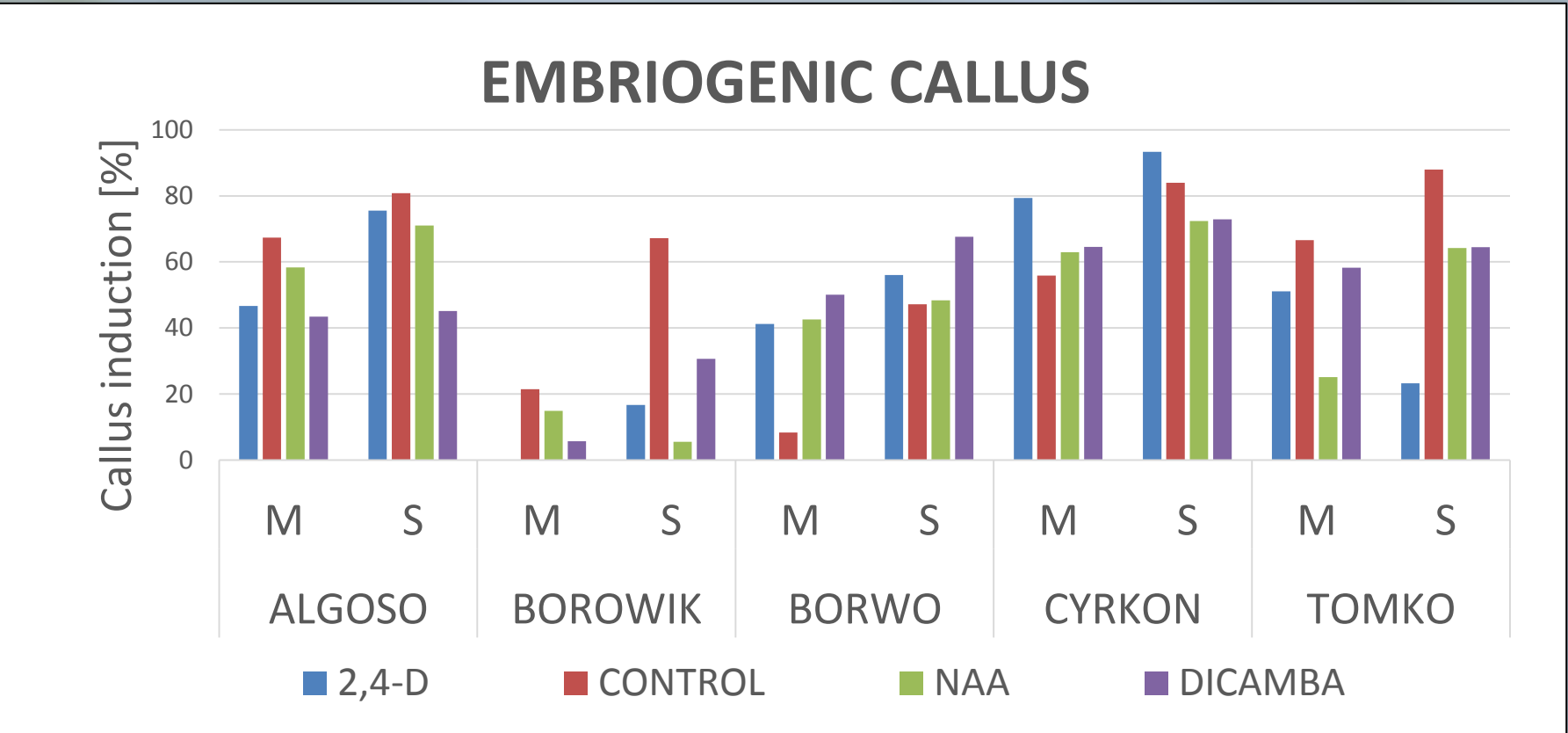


Table 3. Plant regeneration efficiency of triticales

CULTIVARS		CONTROL	2,4-D	DICAMBA	NAA
ALGOSO	AVERAGE	0	0	4,8	8,5
	M	0	0	0	4,8
	S	0	0	9,7	12,3
CYRKON	AVERAGE	0	0	3,3	4,4
	M	0	0	6,7	8,8
	S	0	0	0	0
TOMKO	AVERAGE	37,2	0	0	0
	M	3,8	0	0	0
	S	70,6	0	0	0
TOTALITY		12,4	0	2,7	4,3

SUMMARY

In conclusion, we have demonstrated significant effects of growth hormone type and carbohydrate sources interaction on embryogenic callus growth and plant regeneration from cultures initiated from mature embryos of triticales. Inducing media with dicamba and sucrose played a role in embryogenic callus growth, however, the highest efficiency of plant regeneration was obtained on medium without auxins. The results of this study can be applied to future practical application of triticales mature embryo culture for breeding, transformation and other biotechnological objectives.