The influence of medium composition on embryogenic callus induction and plant regeneration from mature embryos of wheat cultivars with various resistance to *Parastagonospora nodorum*

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INTRODUCTION

PARASTAGONOSPORA NODORUM

Glume and leaf blotch (Figure 1.) is a fungal disease of wheat elicited by a necrotrophic fungus *Parastagonospora nodorum*. It is a serious pathogen of wheat worldwide, which by reducing assimilative area of plants affects adversely quantity and quality of grain yield. Among wheat cultivars complete resistance to species of *Parastagonospora* is not encountered.

Figure 1. Symptoms of glume and leaf blotch*

*Photos by S. Bartosiak

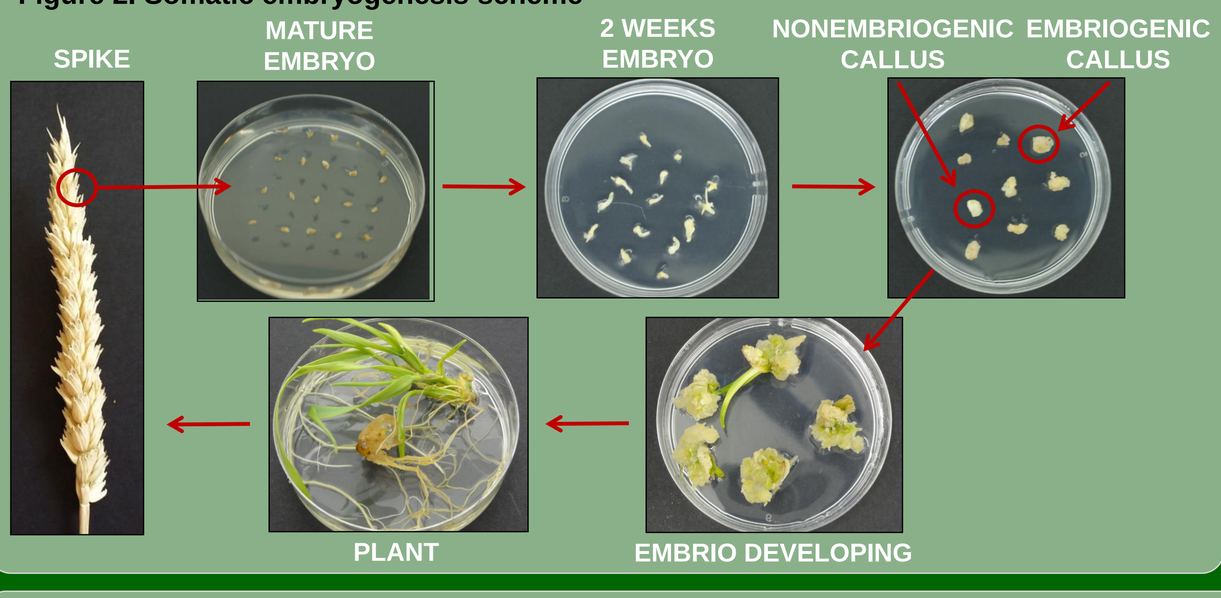
OBJECTIVES

This study was undertaken to improve embryogenic callus induction and plant regeneration from mature embryos winter wheat cultivars with various resistance to *P. nodorum*.

SOMATIC EMBRIOGENESIS

Somatic embryogenesis (Figure 2.) is commonly used method in biotechnology, influenced by culture medium, initial plant organ and genotype.

Figure 2. Somatic embryogenesis scheme



EXPERIMENTS

Six winter wheat cultivars with various resistance to *P. nodorum* were used as the source for mature embryo culture (Table 1). Mature embryos were cultured on standard Murashige-Skoog medium. The effects of three auxins and maltose vs. sucrose were evaluated (Table 2).

Table 1. Characterization of resistance to *P. nodorum* triticale cultivars. The level of fungal infection assessed on 9-digit scale (– susceptible; ± moderately susceptble/resistant; + resistant)

WHEAT CULTIVARS	Arkadia	Astoria	Bamberka	Muza	Ostroga	Wydma
LEAVES	-	-	+	±	+	±
GLUMES	-	-	+	±	+	±

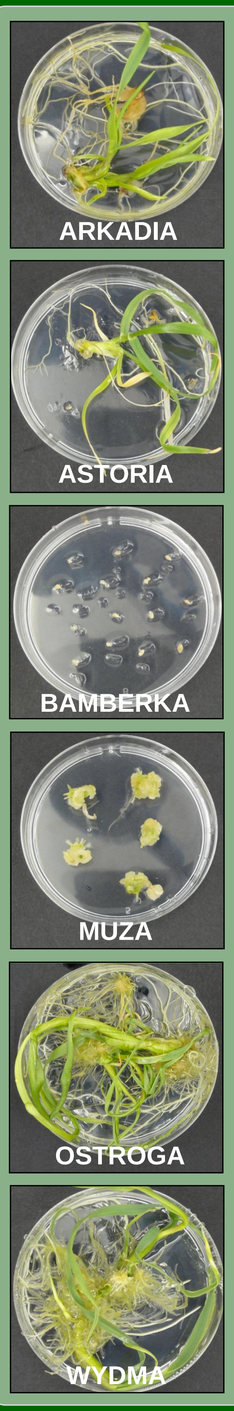
Table 2. Murashige – Skoog medium modification

	S	M	S 2,4-D	M 2,4-D	S NAA	M NAA	S D	M D
SUCROSE	+	-	+	-	+	-	+	-
MALTOSE	-	+	-	+	-	+	-	+
2,4D	-	-	+	+	-	-	-	-
NAA	-	-	-	-	+	+	-	-
DICAMBA	-	-	-	-	-	-	+	+

PLANT REGENERATION PROTOCOL

1. Seed surface-sterilization using ethanol and sodium hypochlorite.
2. Wheat seeds imbibition in sterile water in 4°C for 24h.
3. Re - sterilization of seeds in 70% ethanol and 10% sodium hypochlorite.
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 for 4 weeks.
6. Transfer embryogenic callus on regeneration medium.
7. Analysis of the somatic embryogenesis efficiency.

RESULTS



All winter wheat cultivars used in this study enhanced callus induction and plant regeneration on all tested media. The highest percentage of embryos producing embryogenic callus (EC) was obtained for cultivar Ostroga, while the smallest one for cv. Bamberka (Graph 1.). The rate of EC formation on media supplemented with dicamba was generally better than on media with NAA and 2,4-D. A significant difference was observed for cv. Bamberka. The percentage of EC on dicamba containing medium was 69.9% when for 2,4-D 52.4% and for NAA barely 47.1%. The type of sugar used in the media did not have any effect on the production of callus from mature embryos.

The largest rate of plant regeneration was observed for cv. Ostroga and the lowest one for cv. Bamberka (Table 3.). Media without growth hormones had a significant effect on the number of plantlets regenerated per EC. The largest difference was observed for cv. Ostroga. The percentage of plants regenerated on control medium was 16.3%, while for 2,4-D it barely reached 1.2%. Efficiency of plant regeneration was not strongly influenced by sugar type used in media.

Graph 1. Comparison of embryogenic callus induction *in vitro* culture of wheat varieties

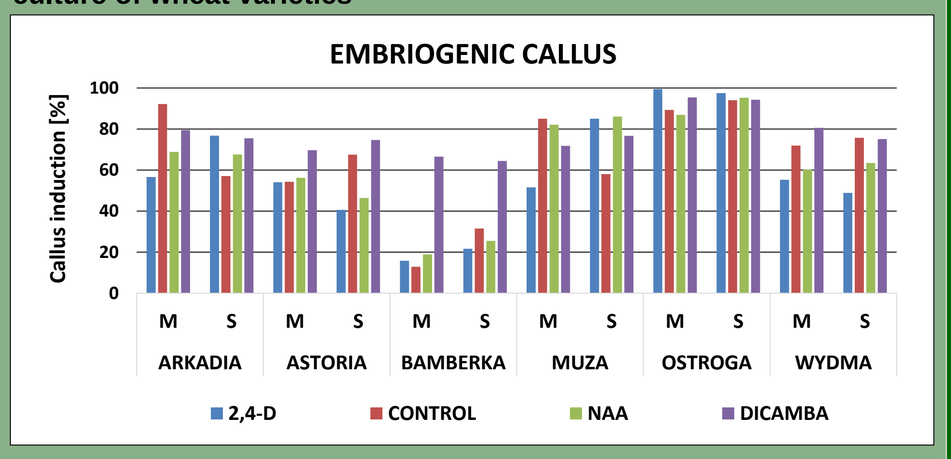


Table 3. Plant regeneration efficiency of wheat

CULTIVARS		CONTROL [%]	2,4-D [%]	DICAMBA [%]	NAA [%]
ARKADIA	M	8.2	0.0	0.0	1.4
	S	0.0	0.0	3.8	0.0
ASTORIA	M	0.0	0.0	0.0	2.3
	S	9.1	0.0	7.0	8.0
BAMBERKA	M	0.0	0.0	1.8	0.0
	S	0.0	0.0	0.0	0.0
MUZA	M	0.0	0.0	0.0	18.7
	S	13.0	0.0	0.0	1.2
OSTROGA	M	5.0	2.4	17.3	15.2
	S	27.7	0.0	12.1	9.8
WYDMA	M	7.1	0.0	0.0	2.9
	S	0.0	0.0	20.3	0.0

CONCLUSION

In summary, an efficient and reproducible protocol for wheat regeneration from mature embryos was developed.

The results demonstrate relatively high embryogenic potential of winter wheat cultivars. We found significant influence of type of auxins and carbohydrate sources on embryogenic callus growth and plant regeneration from wheat mature embryos cultures.

Inducing media supplemented with dicamba and sucrose played a role in embryonic callus growth, however, the highest efficiency of plant regeneration was obtained on medium without growth hormones.

The results obtained in the study increase knowledge about tissue culture response of wheat and bring closer the use of biotechnological methods for improving of the cereal species.

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