



Optimization of callus induction from mature embryos of wheat and triticales cultivars with various resistance to *Parastagonospora nodorum*

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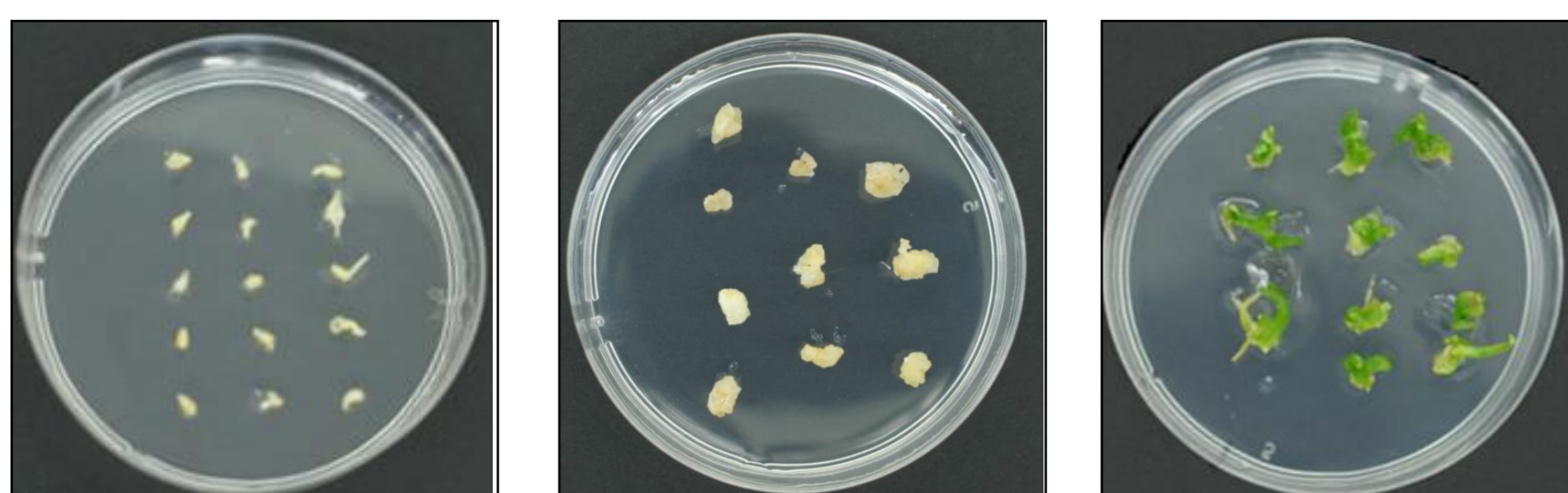
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BACKGROUND

Necrotrophic fungus *Parastagonospora nodorum* is a severe foliar and glume pathogen of triticales and wheat. *P. nodorum* causes worldwide losses in quantity and quality of grain yield. High level of resistance of wheat and triticales cultivars to the pathogen is a rare feature. Among the cereal crops complete resistance to *P. nodorum* and other species of *Parastagonospora* is not encountered. The effect of resistance breeding by conventional methods may be supported by the biotechnology tools e.g. by somatic embryogenesis and this is the ultimate goal of this study. However, wheat and triticales vary in capacity to callus induction. Therefore an effort was undertaken to optimize the callus induction procedures for both cereals.

Figure1. *In vitro* culture of mature embryos of wheat.



Somatic embryogenesis is the process in which a bipolar structure (containing both shoot and root apical meristems differentiated simultaneously at opposite poles) is formed from a single somatic cell or from a group of somatic cells. The ability of triticales and wheat callus induction are influenced by culture medium, initial plant organ and genotype. Standard Murashige-Skoog medium with modifications (Table 1.) was used to obtain the best callus formation.

Table 1. Media supplemented with different growth regulations and sources of carbohydrates

	S	M	S2-4D	M2-4D	SNA	MNA	SD	MD
sucrose	+	-	+	-	+	-	+	-
maltose	-	+	-	+	-	+	-	+
2,4-D	-	-	+	+	-	-	-	-
NAA	-	-	-	-	+	+	-	-
dicamba	-	-	-	-	-	-	+	+

RESULTS

All winter wheat genotypes gave rise to callus on different media. The rate of embryogenic callus formation on media supplemented with dicamba was generally better than on medium with NAA and 2,4-D. A significant difference was observed for Bamberka. The percentage of embryogenic callus on dicamba containing medium was 65.7% when for 2,4-D 21.5% and for NAA barely 18.9%. The type of sugar used in the media did not have any effect on the production of callus from mature embryos - the differences ranged from 1.4% for Wydma to 5.1% for Arkadia.

Callus induction was observed from triticales mature embryos cultured on all media. The highest percentage of embryos producing embryogenic callus also was obtained on dicamba containing media.

As opposed to wheat, type of sugar used in the media had a significant effect on the production of callus from mature embryos. In all triticales cultivars. Replacement of maltose by sucrose increased the mean callus percentage by 6.9% for Tomko to 20.7% for Borwo.

PLANT MATERIAL

Six winter wheat and five winter triticales cultivars, respectively, varying in resistance levels to *P. nodorum* for the purpose to optimize callus formation were tested. Assess the level of infection by pathogenic fungus in 9-digit scale (– susceptible; ± moderately susceptible/resistant; + resistant) was carried out (Table 2.)

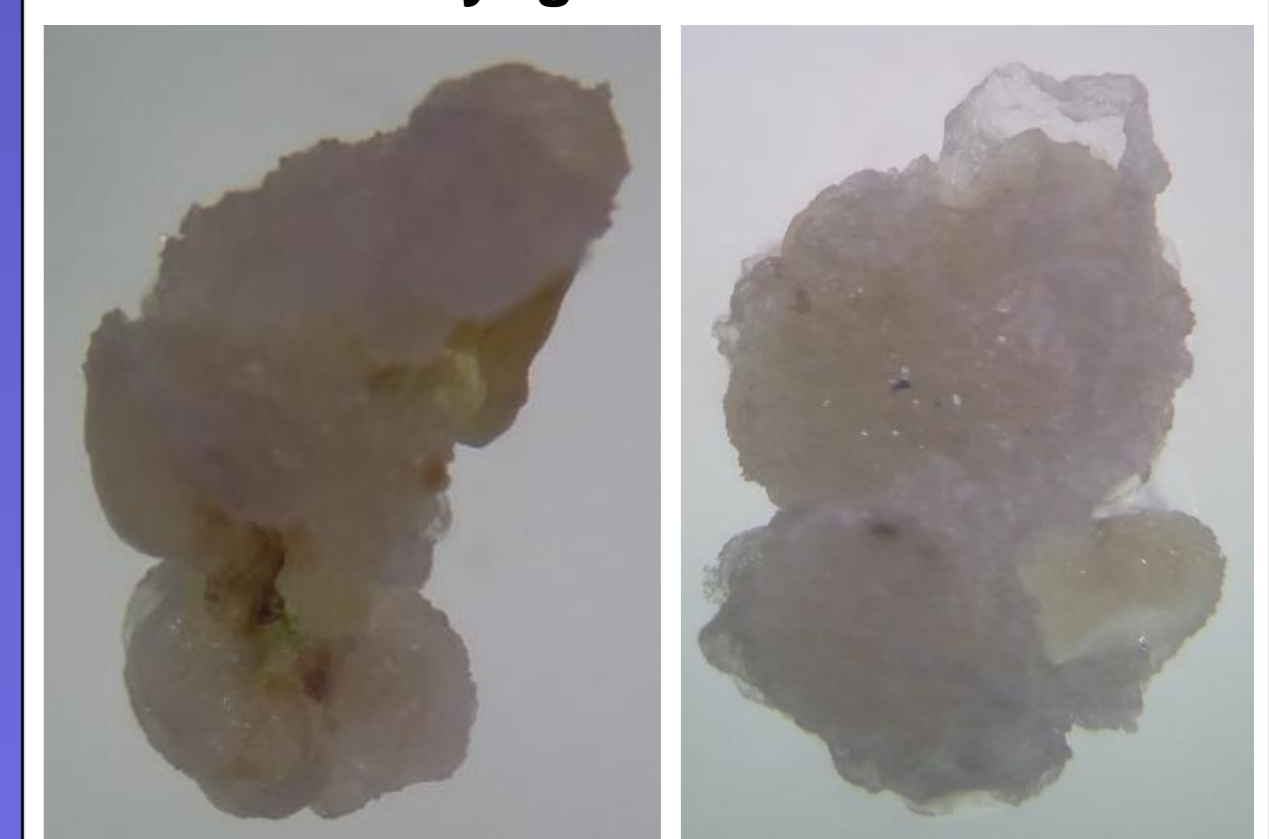
Table 2. Characterization of resistance levels to *P. nodorum* of wheat and triticales cultivars used in the study.

WHEAT CULTIVARS	LEAVES	GLUMES	TRITICALES CULTIVARS	LEAVES	GLUMES
Arkadia	-	-	Algoso	±	±
Astoria	-	-	Borowik	±	±
Bamberka	+	+	Borwo	+	+
Muza	±	±	Cyrkon	±	-
Ostroga	+	+	Tomko	+	±
Wydma	±	±			

DESCRIPTION OF PROCEDURES

1. Surface-sterilization of the seeds using 70% ethanol and 10% sodium hypochloride.
2. Pregermination (imbibition) of seeds in sterile water (4°C, 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. Assessment of the *in vitro* culture effect on the basis of the percentage of mature embryos producing embryogenic callus (Fig. 2.)

Figure 2.
A. Embryogenic callus;
B. Non-embryogenic callus



SUMMARY

The results demonstrate relatively high embryogenic potential of winter wheat and winter triticales cultivars. Callus production was strongly influenced by the type of growth hormone and sugar used in medium. The results showed that dicamba and sucrose in inducing media were most suitable for callus forming. The results obtained in the study increase knowledge about tissue culture response of wheat and triticales and bring closer the use of biotechnological methods for improving of the cereal species.

Acknowledgments

This work was financially supported by Ministry of Agriculture and Rural Development.

