Preliminary detection of pectins and arabinogalactan proteins during ovule embryogenesis in sugar beet (*Beta vulgaris* L.)

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INTRODUCTION AND AIM OF THE STUDY

The use of haploid and doubled-haploid plants facilitate fundamental genetic studies, breeding approaches, and molecular genetics research in most crops, including sugar beet (*Beta vulgaris* L. ssp. *vulgaris*). The main advantage of using in vitro cultures to achieve genetic homozygosity is the greatly time reduction. Unfortunately, sugar beet is recalcitrant in response to androgenic in vitro culture, so that gynogenesis proved to be the most successful method to produce haploids in this species. Even though great progress has been made in the use of unpollinated ovules embryogenesis, a certain number of constraints still hinder breeding programs. Among these limitations, a genotype dependency of embryogenic potential seems to be the most notable. In many cases it determines the rate of success in generating doubled haploid lines. In relation to morphogenetic potential, changes in plant cell wall composition have been previously described. Cell wall pectins and arabinogalactan proteins (AGPs) are involved in different biological processes such as cell expansion and differentiation or tissue development. Moreover, these molecules have also been proven to play an important role in differentiation of floral organs and sexual reproduction.

Based on this, we compare the content of pectin and AGP epitopes in unfertilized ovules isolated from sugar beet genotypes of different embryogenic potential.

MATERIALS AND METHODS

Four sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) genotypes (Fig. 1A-D): two with low and two with high embryogenetic potential were grown in the field during normal season. The unfertilized ovaries were isolated from unopened floral buds of maternal lines at the culture initiation stage and cultured on induction medium described previously (Gośka 1997). Pectin and AGPs extracts were isolated from ovaries after 0 and 14 days of culture and characterized in terms of the binding capacity of several antibodies both: anti-pectic (JIM7, LM5) and anti-AGP (LM2, JIM13) by the dot-blot method (Wiśniewska and Majewska 2007).

RESULTS

Extracts from unfertilized ovaries of sugar beet genotypes with lower and higher embryogenetic potential showed similarities in terms of the presence and relative abundance of particular carbohydrate epitopes that typify pectins and arabinogalactan proteins. All extracts were characterized by the widespread occurrence of epitopes that bind LM2, JIM13, JIM7 antibodies (Fig. 2A, B, D), whereas epitopes that bind LM5 were not present (Fig. 2C). The differences were only seen among different ovaries developmental stages. Unfertilized ovaries *in vivo* proved to be richer in epitopes that binds to LM2 and JIM7 than ovaries after 14 days of *in vitro* culture. The difference was not so clearly seen between the presence of JIM13 epitopes, which showed to be quite stable.

CONCLUSIONS

In conclusion, the occurrence of specific pectin and AGPs epitopes in extracts of unfertilized ovaries demonstrate the importance of these molecules during sugar beet gynogenesis, but the exact biological function need to be determined in further studies.

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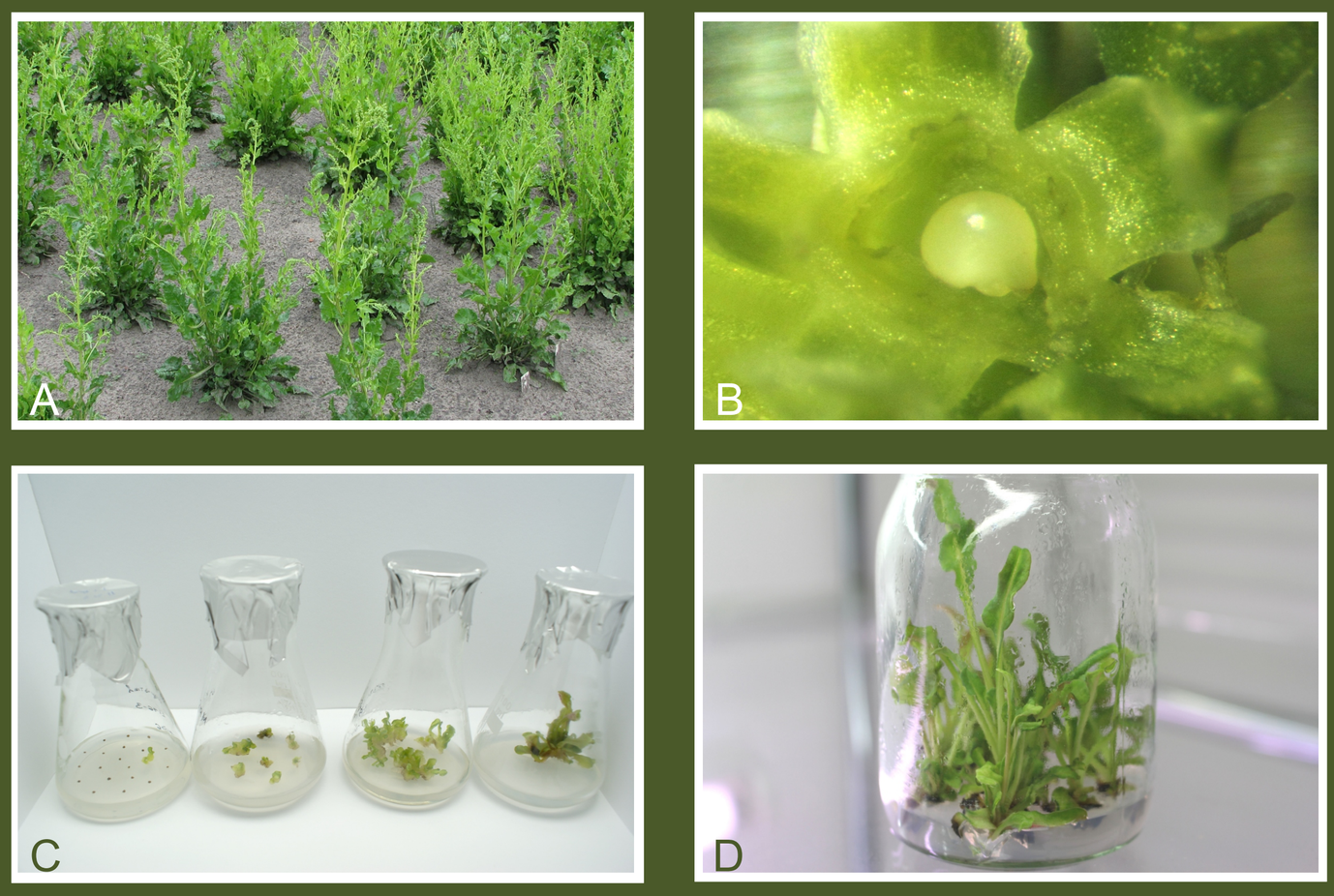
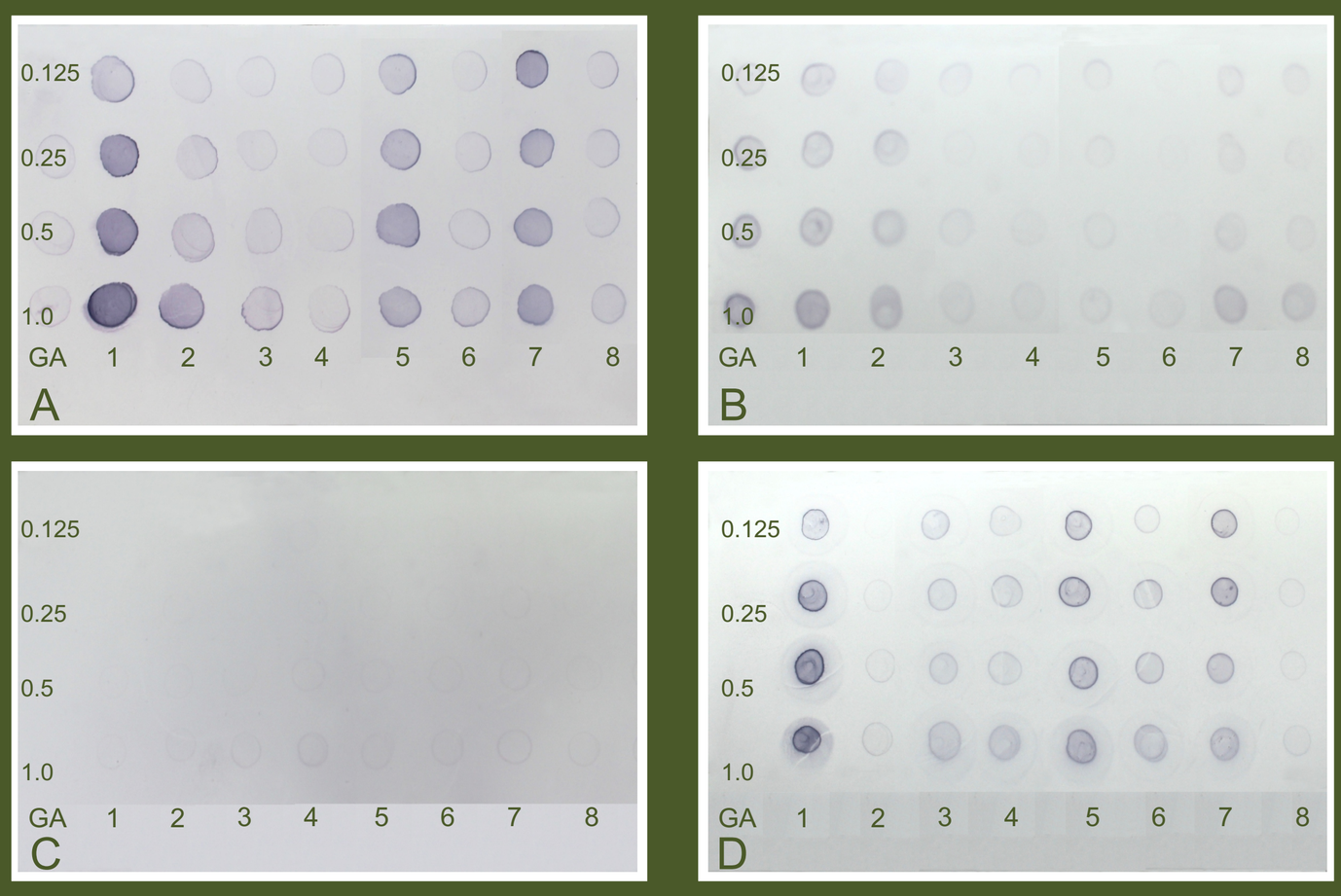


Figs. 1A-D. Successive stages of haploid plants regeneration: donor plants of *Beta vulgaris* L. during field cultivation (**A**), unfertilized ovary before isolation (**B**), unfertilized ovaries during regeneration (**C**), haploid plants (**D**).



Figs. 2A-D. Immunodetection of carbohydrate epitopes in pectin and AGP extracts by dot-blotting and antibody binding: LM2 (**A**), JIM13 (**B**), LM5 (**C**), JIM7 (**D**). Lines 1, 3, 5, 7 correspond to extracts form unfertilized ovaries *in vivo*, lines 2, 4, 6, 8 to extracts from unfertilized ovaries after 14 days of *in vitro* cultures. Genotypes with lower (lines 1, 2, 5, 6) and higher embryogenetic potential (lines 3, 4, 7, 8).

Genotype	Embryogenetic potential	Day of culture	LM2	JIM13	JIM7	LM5
1	low	0	++	++	++	-
		14	+	++	tr.	-
3	low	0	++	+	++	-
		14	+	+	+	-
2	high	0	+	+	+	-
		14	+	+	+	-
4	high	0	++	++	++	-
		14	+	++	tr.	-
GA			-	++	-	-

Table 1. The presence of carbohydrate epitopes in pectin and AGP extracts from unfertilized ovaries of genotypes with lower and higher embryogenetic potential.