Preliminary investigation of molecular changes during ovule embryogenesis in sugar beet (*Beta vulgaris* L.)

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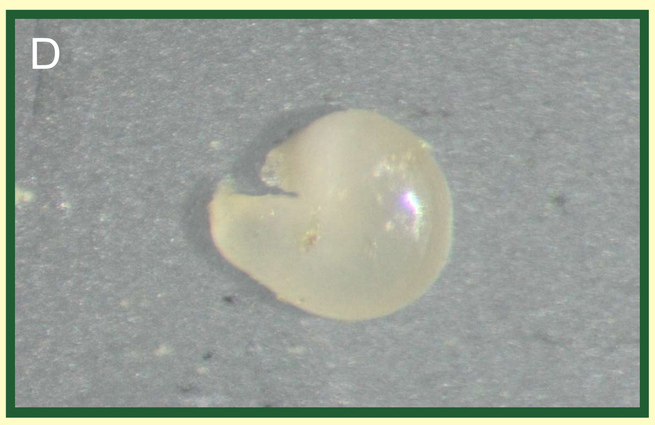

Fig. 1A-B. Maternal lines of sugar beet during cultivation on the field.
Fig. 1C-D. Unpollinated ovules of sugar beet: during *in vivo* conditions (**C**) and after isolation (**D**).

INTRODUCTION AND AIM OF THE STUDY

Currently, unpollinated ovules tissue culture has already had a huge impact on homozygous plants production in sugar beet (*Beta vulgaris* L.). Inbred lines developed from the heterozygous parents are traditionally achieved by performing time-consuming and labour-intensive backcrosses. Particularly haploid and doubled haploid technology can effectively help to enhance the efficiency to select superior plants. For sugar beet, gynogenesis is the most efficient and most promising of all the haploidization techniques tested so far. Unfortunately, the molecular mechanisms of embryogenesis induction are still unknown.

The aim of this study was to characterise received cDNAs identified by ISSR markers, that are expressed at each stage of ovary *in vitro* culture. In our investigation we were looking for candidate genes involved in embryo formation from single haploid egg cell.

MATERIALS AND METHODS

Breeding lines of sugar beet (Fig. 1A-B) were provided by the Sugar Beet Breeding Company (KHBC), Breeding Station in Straszków, Poland. Four genotypes, with different embryogenetic potential: higher and lower, were selected for molecular analyses. At the culture initiation stage, unfertilized ovules were isolated from unopened flower buds (Fig. 1C-D). The gynogenesis procedure was carried out as described by Gośka (1997) (Fig. 2A-B).

To analyze genes involved in ovule embryogenesis, mRNAs from unpollinated ovules at four different culture stages (after: 0, 7, 14, 28, days of ovule incubation on induction medium) were isolated with the use of commercial kit, reverse transcribed to cDNAs and used as templates for the following cDNA-PCR. A total of 15 ISSR previously selected primers were used for PCR amplifications. Amplification products were electrophoresed in 1.5 % agarose gel and TBE buffer, stained with ethidium bromide and visualized via Gel Doc 2000 UV transilluminator (BioRad, Poland). DNA fragments showing differential patterns were recovered from the agarose gel, cloned and sequenced. Using the BLASTn sequence alignment program, nucleotide sequences were compared with those in the NCBI databases.

Fig. 2A-B. Regenerating haploids of sugar beet from unpollinated ovules.

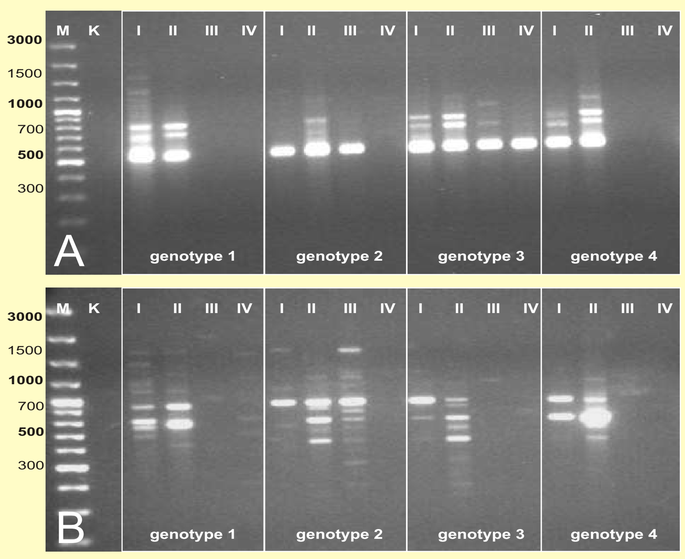

Fig. 3A-3B. Products of amplification obtained with selected ISSR primers (**A** - ISSR1; **B** - ISSR2) for four genotypes; **M** - DNA ladder; **K** - control; Unpollinated ovules after: **I** - isolation; **II** - 7 days of incubation on induction medium with growth regulators; **III** - 14 days of incubation on induction medium with growth regulators; **IV** - 28 days of incubation on induction medium with growth regulators.

PCR product No.	PCR product size (bp)	Homology
1	1094	PREDICTED: *Beta vulgaris* subsp. *vulgaris* dual specificity protein kinase *splA*
2	600	PREDICTED: *Beta vulgaris* subsp. *vulgaris BUD13* homolog
3	500	PREDICTED: *Beta vulgaris* subsp. *vulgaris* protein tweety-like
4	850	PREDICTED: *Beta vulgaris* subsp. *vulgaris* splicing factor 3A subunit 2
5	630	PREDICTED: *Beta vulgaris* subsp. *vulgaris* putative uncharacterized protein DDB_G0284695
6	881	PREDICTED: *Beta vulgaris* subsp. *vulgaris BUD13* homolog

Table 1. Characterization of differentially expressed cDNA products of amplification.

RESULTS

In the present study, of the 15 ISSR primers tested, five primers generated reliable products (Fig. 3A, 3B). A total of 52 bands were scored. The number of bands generated per primer varied from 1 to 17. The approximate size of the amplified products ranged from 230 to 1,958 bp. Six cDNA-ISSR products specifically expressed were detected (Table 1). Three cDNA fragments were detected in the ovules directly isolated from the donor plants: (band 2 and band 3) and one was typical of genotypes with higher embriogenetic potential (band 1). One differentially amplified cDNA fragment (band 6) was identified on the early stages of ovule *in vitro* culture. Whereas, two specific bounds (band 4 and 5) were obtained for ovules isolated from lines with low embryogenetic potential.

CONCLUSIONS

The results obtained in this study demonstrated that six differentially expressed cDNAs were identified during ovule embryogenesis. Sequence analysis and database searches revealed significant similarity with five candidate genes of sugar beet. However, the direct correlation between identified genes and their role in embryogenesis induction need to be confirmed in further studies.

ACKNOWLEDGEMENTS

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