Development of unreduced viable gametes in diploid potato clones using FDR in combination with antisense inhibition of Rad51

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The development of a system to generate clones capable of producing non-recombinant offspring has always challenged potato breeders. One strategy to achieve this goal is the combination of crossover suppression and unreduced gamete formation. Suppression of crossovers can be obtained through antisense silencing by the *Rec-A* like *Rad51* and other genes involved in meiotic pairing and recombination. However, the absence of crossovers gives rise to univalents at metaphase I and so to unbalanced games and hence led to sterility. To avoid this, we combined *Rad51* silencing with First Division Restitution (FDR), expecting that FDR could overcome sterility. FDR is a mechanism of meiotic chromosome restitution, which can take place if the following conditions are met: 1) univalents occur at metaphase I; 2) cell wall is not formed after telophase I and 3) spindles at anaphase II are fused or parallel allowing all chromatides to disjoin equally. As a consequence, gametes are balanced and have the same chromosome number as the mother. For this study we selected potato clones with different frequencies of FDR.

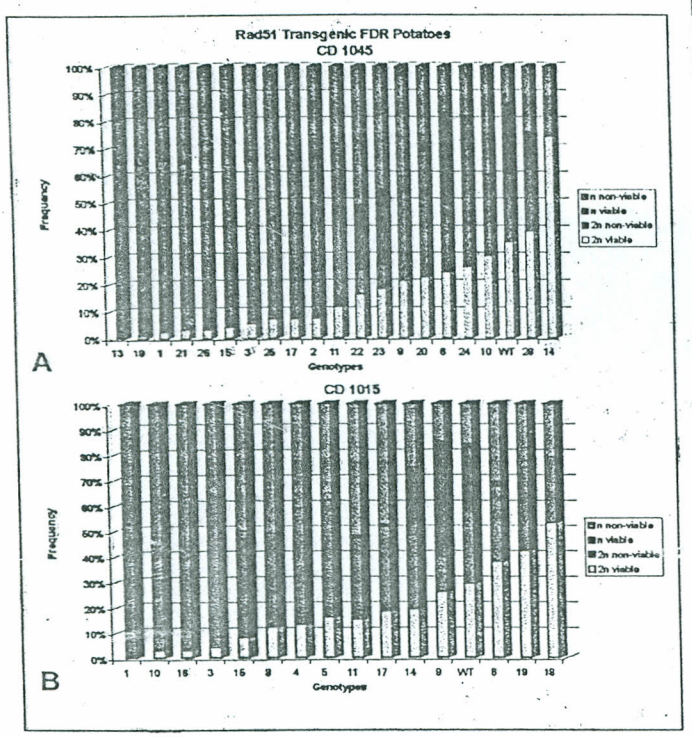

Fig. 2. Frequency of pollen grain classes in the transgenic and in the wild type clones, A. CD 1045 (WT) and B. CD 1015 (WT). Less yellow and red in the bar means higher level of sterility. In genotypes with yellow, but not red bars, only unreduced pollen grains were viable. Note that most of the clones show increased levels of sterility compared to the wild type.

Material and Methods

Four diploid potato clones (BE 1050, B92.7015.04, CD 1015, and CD 1045) with different degrees of FDR-gamete production were selected for transformation with *A. tumefasciens* containing tomato *Rad51*-antisense constructs. Both wild type and transgenic individuals were then planted in the greenhouse, where pollen grains and buds containing anthers at meiosis were collected. Pollen viability and ploidy analysis were performed by lactophenol-acid fuchsin staining (Fig. 1). For cytological analyses of meiotic stages we made cell spread preparations of pollen mother cells (PMCs), which were stained in 4',6-diamidino-2-phenylindole (DAPI) in Vectashield anti-fading (Vector Laboratories).

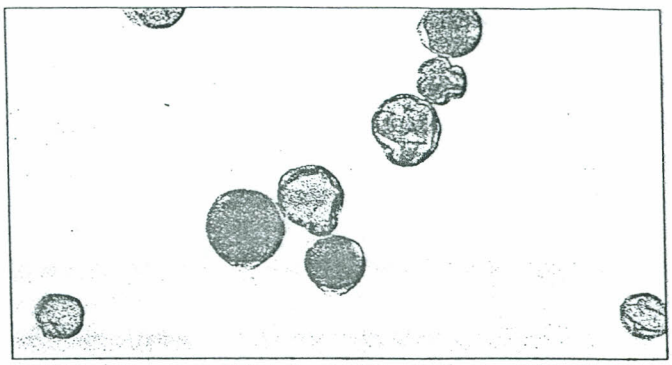

Fig 1. Pollen grains stained with lactophenol-acid fuchsin. Unreduced (2n) pollen are bigger than haploid (n) pollen. The shrunken, poorly stained grains are not viable.

Results and discussion

Pollen staining revealed that the wild type clone BE 1050 had a high rate of pollen sterility, while clone B92.7015-04 was actually a poor 2n pollen producer. In contrast, clones CD 1045 and CD 1015 produced more than 50% of 2n gametes (Figs. 2A and 2B). Therefore, evaluation efforts were concentrated on the 35 putative transgenic clones obtained from both CD wild types. Most of the CD transgenic clones showed strong viability reduction on both n and 2n pollen, as expected (Figs. 2A and 2B). In few of them, fertility was even negligible. In contrast, some clones displayed 10% to 73% of viable 2n pollen, along with no or hardly any n viable pollen. Some other clones, which showed a pattern quite similar to the wild types, can be considered either non-transformed or transformed, but non-expressing clones.

Microscopic analysis of DAPI stained spreads of PMCs showed that sterile genotypes had completely disturbed meiosis. Typically, chromosomes showed various degrees of degeneration throughout prophase I (Fig. 3) that were not observed in any other clone. In all genotypes with only 2n viable pollen, in contrast, meiosis was quite similar to the wild type controls, without indications of haploid gamete sterility.

The most promising clones will be submitted to a second round of pollen viability screening and PMC analysis, as well as to Southern and Northern blotting. If results confirm what was observed until now, these plants will be crossed to genotypes with known molecular patterns and the offspring will be studied for crossover recombination.

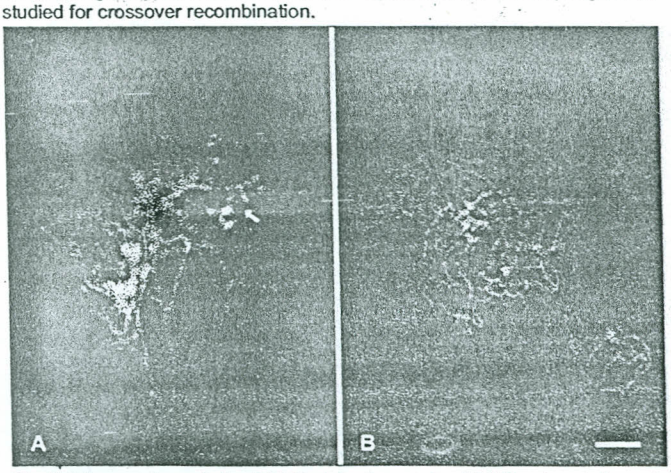

Fig. 3. Microscopic analysis of DAPI-stained spreads of PMC's at pachytene. A. Chromosomes show different degrees of degeneration and appear not only as threads, but also as dots of bright stained chromatin. B. Again different degrees of chromosome degeneration can be seen, as well as chromatides pulling apart. Bar equals 10 µm.