Molecular evaluation of four pepper androgenic regenerants

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INTRODUCTION

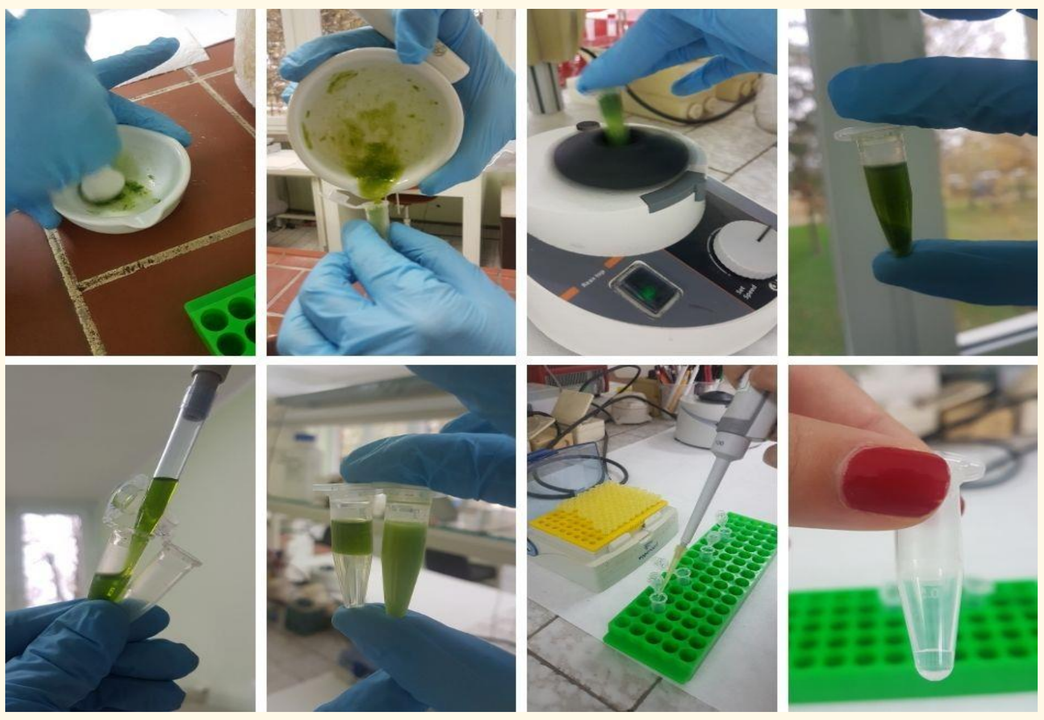
The method of androgenesis is used to induce development of haploid and spontaneous dihaploid plants. Haploids and dihaploids have the same genotype as the gametes from which they originated, and they are excelent material for conducting different genetic and plant breeding tests. The effectiveness of anther culture is highly dependent on growth, growing conditions and physiological state of donor plants, pollen or microspore stage at the time of anther dissection, genotype and composition of the nutrient media. The efficiency of anther culture is high for some plant species, while there are limitations in successful induction of androgenesis in other species. Unfortunately, pepper (*Capsicum annuum* L.) is categorized as plant species with low to moderate androgenic potential.

MATERIAL AND METHODS

The anthers from six pepper genotypes were used in two-year androgenesis experiment:
- Hybrids: Edita F1 and Homera F1
- Cultivars: Duga bela, Una, Amfora and Kurtovska kapija

Dumas de Vaulx et al. (1981) medium was used as induction medium for pepper androgenesis. The isolated anthers were cultivated on Cp medium and incubated on 35°C in dark for 8 days followed by their transfer to 25°C, 12 hours light/12 hours dark for 4 days. After 12 days of temperature treatment, the anthers were transferred to R_1 mediumand incubated at 25°C, 12 hours dark/12 hours light . Every 30 days the anthers were transferred to fresh R_1 medium. The fully developed androgenic embryos were placed on rooting medium V3.

Four androgenic regenerants (Edita_R1, Edita_R2, Edita_R3 and Edita_R4) from the donor hybrid Edita F1 were used for molecular evaluation and compared with the donor genotype.



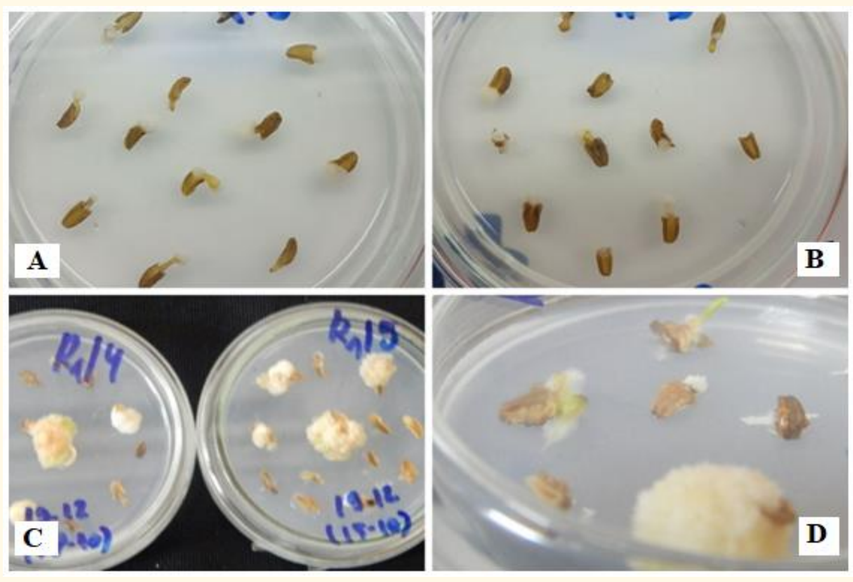
DNA extraction from regenerants leaves. according to the CTAB protocol, modified by Somma (2004).

CONCLUSION

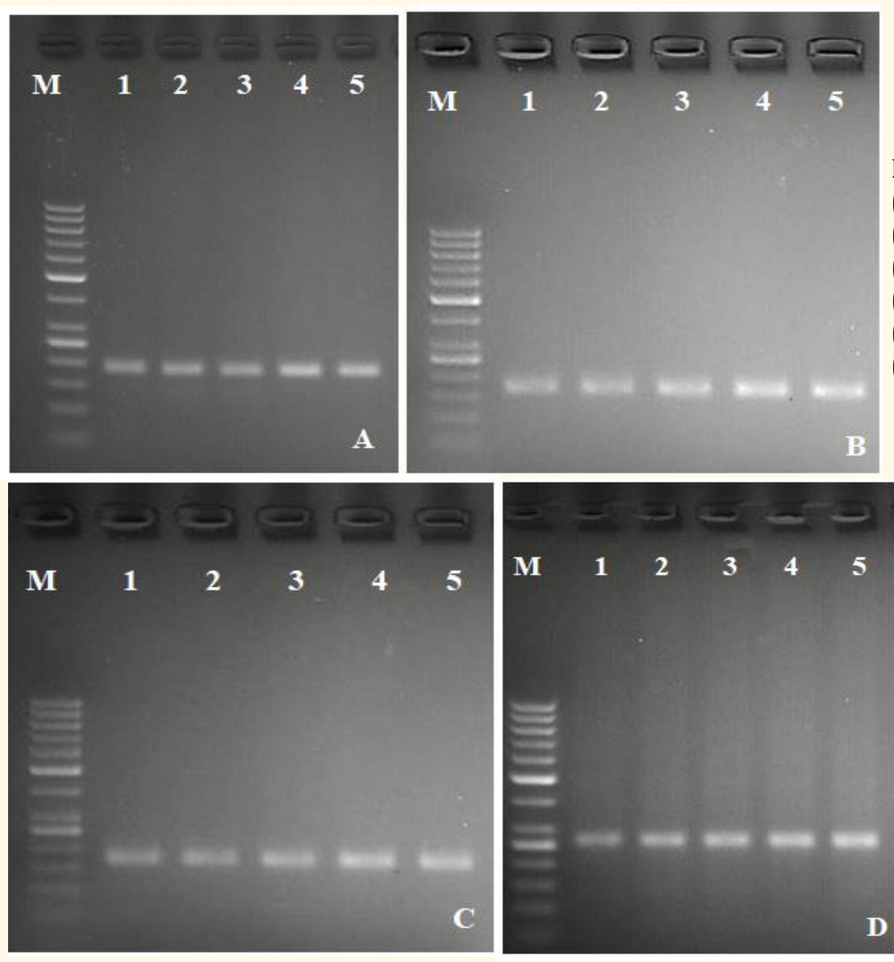
The molecular evaluation of the regenerants showed that all androgenic regenerants had the same allele for all SSR loci as donor genotype Edita F1.
All androgenic regenerants were homozygotes for the five tested loci.

RESULTS

All tested pepper genotypes responded differently in terms of callus and androgenic embryos formation.



Response of isolated anthers to Cp and R_1 medium and temperature treatments.
A, B) Anthers cultivated on R1 medium
C, D) Callus formation and emergence of embryos.



Legend:
(1) donor genotype Edita F1
(2) regenerant Edita_R1
(3) regenerant Edita_R2
(4) regenerant Edita_R3
(5) regenerant Edita_R4
(M) marker (50 bp DNA ladder)

Visualisation of amplified SSR loci by 2 % agarose gels
A) Hpms1-117 marker, size of the product 190 bp,
B) Hpms1-168 marker, size of the product170 bp,
C) Hpms1-274 marker, size of the product 170 bp,
D) EPMS-650 marker, size of the product 260 bp,
E) CAMS 117 marker, size of the product 220 bp.