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Production of Cucumber Doubled Haploid Plants via Ovule Culture

Abouzar Asadi[1], Alireza Zebarjadi[2]* and Mohammad Reza Abdollahi[3]

1- Ph. D. in Plant Breeding, Department of Production Engineering and Plant Genetics, Faculty of Science and Agricultural Engineering, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran
2- ***Corresponding Author:** Associate Professor, Department of Production Engineering and Plant Genetics, Faculty of Science and Agricultural Engineering, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran (Zebarjadiali@yahoo.com)
3- Associate Professor, Department of Plant Breeding, Faculty of Agronomy and Plant Breeding, Bu-Ali Sina University, Hamadan, Iran

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Abstract

Background and Objectives
Production of pure lines is one of the most important tools due to production of high-yield seed's. Pure lines can have produced in a short time using in vitro techniques and therefore reduce several years in required time for conventional plant breeding programs. The purpose of this study was investigation of different concentration of TDZ on production of haploid plants in cucumber ovule culture.

Materials and Methods
The experimental layout was conducted in factorial arrangement in randomized completely design with three replications in greenhouse and laboratory of Razi university during 2015-2017. Factors were two cucumber genotypes (Esfahani and Beit Alfa) and different concentrations of TDZ (0, 0.01- .08 mg/l). Unfertilized ovaries were harvested 1 day before anthesis and they were sliced into 1 mm cross sections under sterile conditions and placed on solid MS medium. Immediately after placing the unfertilized ovary slices of each genotype on induction medium, they were exposed to a thermal shock pretreatment at 35±1°C for 3 days. The first visual structures formed after 3 days in culture. After two weeks of culture the frequency of embryo formation were recorded. After embryogenesis the embryos were sub-cultured in medium containing 1.5 mg/L-1 GA3 and finally cultured in medium containing 0.05 mg/L-1 NAA and 1.5 mg/L-1 BAP for organogenesis.

Results
Results indicated that there are statistical significant effect at 1% among TDZ concentrations. Genotypes and interaction between genotype and TDZ concentration did not have significant effect on embryo induction. According to the results of means comparison, M8 medium with average of 23.33 for Esfahani genotype and 20.66 for Beta Alfa genotype has highest embryo induction and M1 has the lowest embryo induction for both genotypes. Ploidy level of calli and embryos were identified by Flucytometry assay. Haploid embryos have been transferred to the regenerative medium. The chromosome content of haploid plants be doubled

spontaneously in all regenerated plants. Double haploid plants originated from megaspore and they are equivalent to haploid, which regards to homozygosity and can be used directly in breeding program.

Discussion

High number of embryos were obtained in high concentration of TDZ (0.08 mg/l). Flucytometry assay is prepare way to recognize of ploidy level in plant tissues, thus it could be useful to choose haploid tissue. Spontaneous chromosome doubling may occur via somatic cell fusion, endoreduplication, endomitosis and possibly many other mechanisms. In conclusion, the higher rates of embryo formation frequency achieved and doubled haploids obtained in the present study are promising for future work.

Keywords: Gynogenesis, Haploids plant, Ovary slice, Pure lines, TDZ