IMPROVEMENT OF STRAWBERRY PLANTS USING TISSUE CULTURE TECHNIQUES

By

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B.Sc. Agric. Sci. (Horticulture), Fac. Agric., Cairo Univ., 1998
M.Sc. Agric. Sci. (Vegetable Crops), Fac. Agric., Cairo Univ., 2004

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Improvement of strawberry plants using tissue culture techniques.
Supervisors: Dr. Mohamed Abdel-Mageed Badawi
Dr. Khaled El-Sayed Ali
Department: Vegetable Crops
Approval: 12 / 7 /2009

ABSTRACT

The experiments of the present study were conducted in the Tissue Culture Lab., Department of Vegetable Crops, Faculty of Agriculture, Cairo University, Giza, Egypt, during the period from 2005 to 2008. The objectives of this work were to improve strawberry plants using tissue culture techniques by developing a successful protocol for tetrahaploid production in strawberry cvs Camarosa and Sweet Charlie using anthers and unpollinated ovaries, and select new strains from these cvs tolerant to salt stress *in vitro*. Closed floral buds (2-4 days before anthesis) were pre-treated at 4˚C for different periods and cultured their anthers and ovaries on different media composition. Embryogenesis was performed from both anthers and ovaries and multiplication was induced to produce new shoots. Shoots were rooted and produced whole plantlets for adaptation. Results showed that cold pre-treatment was not efficient for producing the best ovule regeneration and production of tetrahaploid plants and both cultivars gave nearly similar results under the optimal conditions for ovule regeneration. On contrary, anthers needed 24 h cold pre-treatment for producing embryogenic calli with cv. Sweet Charlie. Determination of ploidy level was carried out and examinations showed that plantlets produced from the ovule culture were tetrahaploid but 8 plantlets from 11 produced from anther's embryogenic calli were tetrahaploid and the rest were octaploid. For achenes, achenes were isolated from strawberry mature fruits of the studied cvs, stored in dark at 4 ˚C for different periods and exposed to scarification by hydrochloric acid followed by sterilization and rinsing in sterile distilled water for different periods. The germination percentage was 95% with cv. Camarosa and 97% with cv. Sweet Charlie after 5 weeks of cultivation combined with one month cold storage pre-treatment in dark at 4˚C and 4h rinsing period at 10˚C, in both studied cvs without significant differences between them. Based on the results obtained on embryogenesis of both anthers and ovaries, and achenes germination, the best medium and the best conditions for each explant were used in salinity experiment by adding Rashedy salt to the chosen media. The highest concentration of Rashedy salt for treating ovaries that produced embryogenesis, shoot multiplication, recovery and adapted plantlets was 5000 ppm and cv. Sweet Charlie was, significantly, the best for producing salinity tolerant plantlets *in vitro* from ovaries culture. Only 2 plantlets from cv. Sweet Charlie succeeded to be stable under 5000 ppm after acclimatization in the greenhouse. All plants produced from achenes in all salt concentrations were not able to overcome these concentrations in the greenhouse. The effect of salt stress on strawberry genetic stability was allowed to be examined through RAPD test and both of the tolerant strains of cv. Sweet Charlie were have genetic variation when compared with the original plants by using RAPD-PCR.

Keywords: strawberry - *Fragaria* - anther culture - ovary culture - embryogenesis - androgenesis - gynogenesis - tetrahaploid – achene culture - salinity - RAPD-PCR.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my husband ***Mohamed Y. Hazman*** *and my sister* ***Maryam*** *for their patience and help, as well as to my parents and sons for all the support they lovely offered along the period of my post graduation.*

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INTRODUCTION

The cultivated strawberry (*Fragaria* ×*ananassa* Duch.) is grown in most arable regions of the world. It is one of the most valuable fruit crops in the world, there have been relatively little application of biotechnology in research or breeding efforts on this crop.

Strawberry (*F.* ×*ananassa* Duch.) is a natural hybrid of *F. chiloensis* (L.) P. Mill. and *F. virginiana* Duch. It is a perennial, stoloniferous herb which belongs to the Rosaceae family. Strawberries have traditionally been a popular delicious fruit for its flavor, taste, fresh use, freezing and processing. It contains relatively high quantities of ellagic acid, which has a wide range of biological activity (Sakila *et al*., 2007).

Cultivated strawberry is octaploid (2n = 8× = 56 chromosomes). Traditional breeding efforts to improve strawberry quality and yield are labor intensive, costly, and time-consuming, since many generations of crossing and selection are routinely required for cultivar development.

Tissue culture techniques in strawberries can contribute to reduction of both time and effort needed in a breeding programme aimed at achieving high homozygosity in octoploid strawberries (Hennerty and Sayegh, 1996).

Reducing the ploidy level of breeding material via gynogenesis may accelerate plant improvement efforts by more direct exposure of genetic traits at the haploid level, by phenotypic expression of gametes for assessment of ovule-donor potentials, and, together with

chromosome redoubling techniques, by the production of highly homozygous lines for further use as parental lines.

The technologies for haploid and dihaploid production in vitro are now widely used to produce new cultivars and for genetic analysis of molecular biological research on many important agricultural crops. Many problems, however, remain and these limit extensive application of the technologies in modern breeding programmes (Atanassov *et al*., 1995)

The *in vitro* germination of the strawberry plant achenes constitutes a necessary stage in the production of sterile seedlings essential to any process in biotechnology as genetic transformation. However, the germination of achenes is often poor. (El Hamduni *et al*., 2001)

In vitro selection of strawberry on salt containing media during germination and early seedling growth allowed obtaining clones with increased salinity tolerance. This confirms practical value of the presented approach. The method is relatively simple and inexpensive. It may become an important tool in strawberry stress tolerance breeding (Dziadczyk *et al*., 2003).

The objectives of the present study were to improve strawberry plants using tissue culture techniques by developing a successful protocol for tetrahaploid production in strawberry cvs Camarosa and Sweet Charlie using anthers and unpollinated ovaries, and select new lines from these cvs tolerant to salt stress *in vitro*.

REVIEW OF LITERATURE

Plant tissue culture refers to growing and multiplication of cells, tissues and organs on defined solid or liquid media under aseptic and controlled environment. Plant tissue culture technology is being widely used for large-scale plant multiplication. The commercial technology is primarily based on micropropagation, in which rapid proliferation is achieved from tiny parts. Plant tissue cultures are initiated from tiny pieces, called explants, taken from any part of a plant. Practically, all parts of a plant have been used successfully as a source of explants. In practice, the explant is removed surgically, surface sterilized and placed on a nutrient medium to initiate the mother culture, that is multiplied repeatedly by subculture. The cultured cells and tissue can take several pathways. The pathways that lead to the production of true-to-type plants in large numbers are the preferred ones for commercial multiplication. The process of micropropagation is usually divided into several stages i.e., prepropagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. These stages are universally applicable in large-scale multiplication of plants. The delivery of hardened small micropropagated plants to growers and mark*et al* so requires extra care (Ahloowalia *et al*., 2004).

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in