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Differences in Gene Expression Through Callus Differentiation and Under Different Stresses in Alfalfa

By

Amal Abdelraheem Tageldin

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Supervised by:

Prof. H.I. Abdallah
Dr. E. M.M. El-Farash
Dr. H.M. El-Aref

Examined by:

Prof. Dr. E.A. Badr
Prof. Dr. M.R. El-Helw
Prof. Dr. H.I. Abdallah
Dr. E.M.M. El-Farash

APPROVAL SHEET

Title: Differences in gene expression through callus differentiation and under different stresses in alfalfa.

Name: Amal Abdelraheem Tageldin

Approved by:

Effat Bada

M. R. El-Hela

Hafez Abdallah

Effat M. Elfarash

(Committee in charge)

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important forage crop in Egypt and is relatively favourable for *in vitro* culture of various types of tissues and organs. Procedures have been developed for the *in vitro* culture of cotyledons, hypocotyles, shoots, leaf explants and protoplasts. It was demonstrated that the major regeneration pathway in alfalfa is via somatic embryogenesis (Bingham *et al*. 1988 and Parrott and Bailey, 1993). These methods have opened the possibilities for genetic studies (e.g. gene expression during differentiation and under stress conditions), screening and selecting plants which are tolerant to different stresses (Bingham *et al*, 1988 and Winicov and Shirzadegan, 1997).

The present investigation was carried out to focus on the utility of alfalfa tissue culture for studying the following objectives:

(1) Differential gene expression during plant differentiation from callus (white callus, green callus, somatic embryo, shoots and root developmental stages).

(2) The alterations in callus growth and gene expression under different environmental stress factors such as salinity, drought, cold and heat stresses.

REVIEW OF LITERATURE

When alfalfa callus tissue was initiated from seedling cotyledons and hypocotyls on MS-medium supplemented with 2,4-D (2 mg/l), Lupotto (1983) identified two distinct phenotypes, one fast- and the other slow-growing. He found that regeneration was possible only from the slow-growing hypocotyl-derived callus via somatic embryogenesis upon transfer of the callus to hormone-free medium followed by an additional transfer to media supplemented with yeast extract and inositol.

Through extensive experimentation *in vitro* and conventional breeding, a large number of cultivars and breeding lines have been regenerated. Bingham *et al* (1988) identified the following factors that apply to the *in vitro* culture of alfalfa :

a - Regeneration is genotype-specific and highly heritable.

b - Most cultivars contain genotypes capable of regeneration.

c - The frequency of regenerating genotypes in most germplasm stocks is about 10%.

d - Cultivars that are capable of forming adventitious shoots from roots may have excellent regeneration potential.

e - Any tissue explant from a regenerable genotype that will form callus will regenerate plants.

f - Regeneration efficiency can be increased by media manipulation.

g - The major regeneration pathway is via somatic embryogenesis.

Plant regeneration from alfalfa callus derived from a variety of explants has been reported. These explants included hypocotyls/cotyledons (Bingham *et al*. 1975; Stavarek *et al*. 1980; Novak and Konecna, 1982; Mitten *et al*. 1984; Brown and Atanassov, 1985; and Chen and Marowitch, 1987) and petioles (Walker *et al*. 1979; Novak and Konecna, 1982 and Kraic *et al*. 1994) as well as leaves (Novak and Konecna, 1982; Pezzotti *et al*. 1984; Chen and Marowitch, 1987 and Kraic *et al*. 1994). They also included internodes (Saunders and Bingham, 1972), stems (Novak and Konecna, 1982) and roots (Mariotti *et al*. 1984; and Chen and Marowitch, 1987).

Many different cultivars have been regenerated from these explants, but despite all these advances, it is still difficult to regenerate all alfalfa varieties. Genotypic variation in alfalfa culture was recognized by many investigators to be one of the most serious limitations of *in vitro*