IMPROVEMENT OF FUNGAL DISEASE TOLERANCE IN TOMATO PLANTS, USING GENE TRANSFER TECHNOLOGY

By

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B.Sc. Agric.Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2001

M.Sc. Agric.Sci. (Genetics), Fac. Agric., Cairo Univ., 2008

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APPROVAL SHEET

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ABSTRACT

Tomato yield is severely affected by *Fusarium* fungal disease. In order to investigate the influence of the genetic background on fungal resistance, 30-day old seedlings from four tomato cultivars (namely; Pomodora, Castle Rock, Super strain B, and Red star) were subjected to artificial infection with *Fusarium oxysporum lycopersici sp*. The data showed that the cultivars differ genetically for their response to fungal resistance. Based on fusarium specific ITS marker early diagnosis of fungal infection was detected. The disease severity data indicate that the cultivar Castle rock proved to be tolerant to Fusarium infection followed by the cv. Pomodora. Plant defense enzymes were increased under fungal infection. The regeneration capacity among the tested cultivars was investigated. The data indicate that, embryogenic calli were formed within 7 days in MS medium containing 1 mgl^{-1} 2,4-D. Adventitious shoots emerged from the embryonic calli in the presence of 2 mgl^{-1} BA. Shoot regeneration frequencies varied between tomato cultivars according to their genetic makeup. Regeneration frequency was higher in the cultivar Pomodora 48% compared with the other cultivars tested. The optimum condition of transformation was determined for the cultivars CastleRock and Super Strain B. The hypocotyl explants isolated from both cultivars were co-cultivated with *A. tumefaciens* strain LBA4404 harboring a binary vector pBI-121 containing the neomycin phosphotransferase-II gene (*npt-II*). The successful integration of the transgene was confirmed by PCR analyses. The *gus* gene expression can be detected only in the transgenic plants. To improve fungal resistance, cultivars Castle Rock and Super Strain B were transformed with *E.coli katE* genes. The transformation efficiency was 5.6 and 3.5% for cultivars Castle Rock and Super Strain B , respectively. PCR and DNA Dot blot techniques confirmed the integration of the *katE* genes into transgenic tomato genome. RT-PCR analysis confirmed that *kat-E* could be expressed normally in the transgenic plants. Green house experiment was conducted to investigate the effect of the introduced *kat-E* gene on tomato fungal tolerance. The data showed that the transgenic lines expressed different levels of fungal tolerance as expressed by the performance of plants disease severity and catalase concentration. These results show that the *kat-E* gene may enhance fungal tolerance by increasing catalase enzyme concentration.

Key words: Tomato, hypocotyl, *gus*, *kat-E*, *Fusarium*, *Agrobacterium*.

DEDICATION

I dedicate this work to whom my heartfelt thanks: to my father, mother, sisters, children and brother, for all the support and encouragement they continually offered along the period of my postgraduate studies.

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INTRODUCTION

Originating from the Andes, tomatoes (*Solanum lycopersicum L.)* were imported to Europe in the 16th century. At present, this plant is common around the world, and has become an economically important crop, tomato ranks 7th in worldwide production after maize, rice, wheat, potatoes, soybeans and cassava, reaching a worldwide production of around 160 million tons on a cultivated area of almost 4.8 million hectares in 2011 (FAOSTAT 2011). Tomato is considered a protective food because of its particular nutritive value, as it provides important nutrients such as lycopene, beta-carotene, flavonoids, vitamin C and hydroxycinnamic acid derivatives. Furthermore, this crop has achieved tremendous popularity especially in recent years with the discovery of lycopene's anti-oxidative activities and anti-cancer functions (Wu *et al.* 2011; Raiola *et al.* 2014). Thus, tomato production and consumption are constantly increasing. It is noteworthy that tomatoes are not only sold fresh, but also processed as soups, sauces, juices or powder concentrates.

It is a diploid plant with 2n = 24 chromosomes. The tomato belongs to the *Solanaceae* family, which contains more than 3,000 species, including plants of economic importance such as potatoes, eggplants, tobacco, petunias and peppers (Bai and Lindhout 2007).

Tomato is an excellent model for both basic and applied research programs. This is due to possessing a number of useful features, such as the possibility of growing under different cultivation conditions, its relatively short life cycle, seed production ability, relatively small genome (950 Mb),