BIOCHEMICAL STUDIES ON ISOLATION AND CHARACTERIZATION OF ANTIFUNGAL GENE AND TRANSFERRING TO PLANTS

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2003
M.Sc. Agric. Sci. (Agric. Biochem.), Fac. Agric., Cairo Univ., Egypt, 2008

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SUPERVISION SHEET

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Department: Agricultural Biochemistry **Approval**: 29 / 5 / 2016

ABSTRACT

This study describs cloning of plant defensin (PDF) gene isolated from seedlings of kidney bean *(Phaseolus vulgaris* L.) cultivar polesta, which designated as *pvPDF*. The resistant antifungal gene (*pvPDF*) was isolated directly from total DNA of kidney bean leaf tissue using Polymerase Chain Reaction technique (PCR). The corresponding full length gene, named *pvPDF* was cloned, sequenced and characterized further, confirmed by restriction endonucleases *Xba*1 and *Bam*H1 analysis. Its nucleotide sequence consists of 486 bp. The *pvPDF* sequence analysis showed a high significant homology to other known plant defensin gene sequences that presented in the database using the BLAST program. The *pvPDF* sequence has been deposited in the GenBank database with accession number kj939334. Furthermore, the characterized *pvPDF* DNA cloned in strata clone pSC-A vector for sequencing using *Escherichia coli* DH5 alpha competent cells. The presence of intron (non coding regions) in genomic *pvPDF* was deleted with SOEing PCR techniques. Then the *pvPDF* DNA sequence was fused to β- glucuronidase (GUS) using pBI121 binary vector under control of the CaMV 35S promoter and the NOS terminator region. This whole cassette was used for tobacco plant cells transformation *via Agrobacterium tumefaciens* (LB4404) for *pvPDF* function validation. Analysis of transgenic *pvPDF-GUS* tobacco plants indicated that GUS activity was observed with gene constructs with the strongest being in leaf. GUS activity was the highest with *pvPDF* gene. To verify the expression of *pvPDF*, it was constructed into pET29a plasmid under the control of T7 promoter then transformed into *E. Coli* BL21 bacteria. The analysis of resulted protein indicated that the *pvPDF* gene was expressed at 8 KDa.

Keywords: Gene Isolation, Cloning, *Phaseolus vulgaris*, Defensin Gene, *pvPDF*, gene construct.

DEDICATION

*First of all, I am grateful to **Allah** who blessed me with the opportunity to work at National Research Centre in the field of biotechnology.*

I dedicate this work to whom my heartfelt thanks; to my parents and my brothers and ALL MY FAMILY MEMBERS for their patience, help and support they lovely offered along the period of my post graduation.

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INTRODUCTION

Fungal diseases are the major constraints in agriculture crop production. A various range of fungal pathogens occur in the soils in which crops are grown and causes major losses in the crop yields (van der Weerden *et al*., 2013 and Sundaresha *et al*., 2016). The soil borne pathogens such fungi survive in the soil indefinitely as saprophytes and cause severe damage to the cultivated plants. Such kinds of fungi are confined to the vascular tissues and exhibit greater degree of parasitic specialization and decrease in the quality and safety of agricultural products (Montesinos, 2007). The effective and durable control for pathogens of crops has therefore become one of the major concerns in modern agriculture (Gao *et al.*, 2000 and Kaur *et al.*, 2011). The controlling measures such as use of synthetic chemicals, cultural practices, breeding for resistance and genetic engineering of crop plants are being used from time to time. Due to the dangerous effects and negative environmental impact of pesticides, antimicrobial peptides like plant defensins are considered attractive and promising candidates to be used in agricultural biotechnology due to their broad antifungal activity (Van der Biezen, 2001 and Sasaki *et al*., 2016). Plant defensins form part of a large family of cationic host defence peptides that are widely distributed throughout the plant kingdom. These peptides form part of the innate immune system and play a vital role in the protection of plants against invading fungal pathogens (Osborn *et al.*, 1995and Thevissen *et al.*, 1997).

Plant defensins have a broad spectrum antifungal activity and these peptides are not only active against the phytopathogenic fungi but also against baker's yeast, *Saccharomyces cerevisiae* and the human pathogenic fungi *Candida albicans* (Carvalho and Gomes, 2009, Stotz *et al.*, 2009 and Coninck *et al*., 2013). Defensin peptides are expressed during normal plant growth and development and are present in the peripheral cells of different plant organs which are the first barriers to pathogen invasion. Some plant defensins can be induced in response to fungal infection and mechanical wounding, whereas others are constitutively expressed.

Several defensin genes have been successfully transformed into various plant hosts (Gao *et al.*, 2000 and Lay and Anderson, 2005). Although the constitutive over expression of several plant defensins have shown to significantly enhance disease resistance of hosts under greenhouse-conditions, the effectiveness of defensins to maintain this resistance under field-conditions has only been demonstrated in a few cases (Koike *et al.*, 2002 and Kaur *et al.*, 2011).

The aim of the present study is to identify, isolate, cloning and characterize the precise sequence of the specific plant defensin gene *PvPDF* isolated from (*Phaseolus vulgaris* L.) plant and transfer it to tobacco plant.

REVIEW OF LITERATURE

Throughout a plant's lifespan, it is constantly threatened by various invading pathogens and pests. For protection against these pathogens, plants rely on their dynamic defence mechanisms. Plants have the ability to produce a wide variety of antimicrobial molecules, including several antimicrobial peptides (AMPs) (Broekaert *et al*., 1997 and Thevissen *et al*., 2007). These antimicrobial peptides are single gene products and plants produce these peptides effortlessly and rapidly, without excessive energy and biomass input (Boman and Hultmark, 1987).

In this regard, Broekaert *et al*. (1995) reported that numerous types of antimicrobial peptides produced by plants for protection against invading pathogens and pests is a class of peptides called plant defensins. Defensin peptides are present in vertebrates, invertebrates and plants and they all share structural and functional homology.

Thomma *et al*. (2003) mentioned that plant defensins are mostly located in the periphery of a range of organs. These locations are consistent with a role for these peptides as "first line of defence" against pathogens. These peptides can mostly be induced under pathogenic stress conditions or mechanical wounding and they are able to confer increased protection against pathogens in vegetative tissues.

El-Mounadi *et al*. (2016) reported that peptides play an important role in the protection of seeds where they are released upon mechanical wounding of seeds as well as during germination. During the latter critical phase of plant growth, peptides are released to form