MOLECULAR GENETIC IDENTIFICATION OF INDUCED VARIANTS IN MILK THISTLE (*SILYBUM MARIANUM* L.)

By

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2003
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ABSTRACT

Abdoallah Aboelnasr Aboelnasr Sharaf: Molecular Genetic Identification of Induced Variants of Milk thistle (*Silybum marianum* L.). Unpublished Ph.D. Thesis. Department of Genetics, Faculty of Agriculture, Ain Shams University, 2012.

Five ecotypes from milk thistle (*Silybum marianum*) were used in this study to improve its morphological characters and\or active ingredients properties. Results of HPLC analysis of (*Silybum marianum*) Seeds from five different ecotypes and ten of its induced types by two doses of ethylmethanesulfonate (EMS) and Sodium Azide respectively, and irradiated by 4 doses of gamma rays showed that the percentages of silymarin ranged from 0.25 % to 3.3 %. The highest was Accession L.2 which can be considered as an important source for silymarin. And the lowest percentage was in accession 5 L.5.

Degenerate primers were designed for a given multiple alignment of DNA sequences of Leucocyanidin oxygenase (LDOX) gene using ClustalW algorithm. The results of designed degenerate primer showed that there was a homology found between the designed primers and the DNA templates for the accession numbers with at least 80% identity. The result of sequencing of eluted band of the amplified RT-PCR products of the four accessions were detected at the molecular weight (250 bp) were BLASTed to prove the role of Leucocyanidin oxygenase (LDOX) gene in the taxifolin synthesis and to detect the mutation effect in its DNA sequence.

Keywords: Milk thistle, ClustalW tool, degenerate PCR, Leucocyanidin oxygenase (LDOX) gene, *insilico* PCR.

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I.INTRODUCTION

Medicinal plants have been used to treat illness and disease for thousands of years; even now they are economically important, being used in the pharmaceutical, cosmetic, perfumery, and food industries. Recent years have witnessed an explosion in the popularity of natural products and cosmetic products containing natural extracts. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Medicinal plants provide very little amount of the active constituents, so large amounts of the plant material shall be harvested to maintain the desired amount, which leads to the risk on the plants biodiversity and on the ecosystem. The global market value of medicinal plants exceeds 60 billion USD annually, so protection of wild life ecosystem diversity is crucial for the continued collection of medicinal plants. Half of the 20 000 medicinal plants used today are threatened with extinction.

S. marianum, a plant that grows throughout the Mediterranean, southern Europe and parts of the US, has been used for some 2000 years as both food and medicine. Silybum species are members of family Compositae/Asteraceae which has many folklore names, *e.g*., holy thistle, marian thistle, Mary thistle, MT, Our Lady's thistle, St. Marythistle, wild artichoke, Mariendistel. The active chemical component of milk thistle (MT) is silymarin, which is a combination of three flavonoids: 50% silybinin, 25% silychristin and 25% silydianin **(Karen and Walterova 2005)**.

Silymarin is widely used as a hepatoprotectant and as a supportive therapy of liver disorders such as cirrhosis, hepatitis and fatty acid infiltration due to alcohol and toxic chemicals. Silymarin also showed the beneficial effects in the case of radiation injury to the membrane of liver cells. Studies suggested that silymarin and its polyphenolic fraction could have beneficial effects on some risk factors of atherosclerosis. The results have shown that silymarin has hypolipidemic effect **(Soboleva *et al*.,**

2006) Silymarin has anticarcinogenic and cancer chemopreventive effects. While almost the entire plant can be used for food, its seeds that are used to make MT seed extract. The seeds are the only part of this plant that has silymarin. Also, 25% of seed weight is a fixed oil with a great potential as edible oil **(Rizk *et al*,. 1970)**.

Mutation induction is a useful technique in plant breeding used to improve traits without disrupting the original genetic constitution of the crop. It has been used extensively in the improvement of several crops, especially vegetative propagated species without extensive hybridization and backcrossing. Mutation breeding has led to release of more than 2,250 plant varieties in the past 70 years **(Sleper and Poehlman 2006)**.

Phenylpropanoids are basic components that feeds into various biosynthetic pathways that generate a wide range of structurally related polyphenolic compounds **(Oliver and Jez 2008**), Anthocyanidin synthase (ANS) catalyzes the penultimate step in the biosynthesis of anthocyanin. This reaction is responsible for the formation of the colored anthocyanidins from the colorless leucoanthocyanidins **(Wei *et al*., 2010)**, The anthocyanin and proanthocyanidin (PA) biosynthetic pathways share common intermediates until leucocyanidin, which may be used by leucoanthocyanidin dioxygenase (LDOX) to produce anthocyanin **(Abrahams *et al*., 2003). A**n inhibition of gene expression was detected for anthocyanidin synthase (EC 1.14.11.19). These effects are interpreted as a feedback regulation by changed polyphenol levels **(Fischer *et al*., 2006).**

Our objectives in this study:

- Inducing mutant plants with higher active ingredients and\or useful morphological characters.
- Proving the role of Leucocyanidin oxygenase (LDOX) gene which a key enzyme in the taxifolin synthesis (the most important precursor of silymarin biosynthesis) and isolate it.

II- REVIEW OF LITERATURE

1. Description of silybum

Milk thistle is a tall, biennial herb, five to ten feet high, with hard, green, shiny leaves that have spiny edges and are streaked with white along the veins. The solitary flower heads are reddish-purple with bracts ending in sharp spines. The small hard fruits in the flowers, known technically as achenes, resemble seeds and are the part of the plant used medicinally.

1.1. Agronomic characterization

Omer *et al*. (1993) indicated that the seeds of *S. marianum* are used for the treatment of liver diseases. Seeds were collected from Al-Ayat province, and plants were cultivated using a combination of two row spacings (25 and 50 cm), 2 N levels (70 and 140 kg/ha) and 3 K levels (55, 85 and 115 kg K_2O/ha). At the wider row spacing, seed flavonolignan content was higher, but seed yield declined by 7.4-7.8% when compared with the narrow row spacing. The highest rates of N and K fertilization increased seed yield, oil yield and seed oil content when compared with lower rates, but N and K rates had no meaningful effect on seed flavonolignan content.

Hetz *et al*. (1995) mentioned that in crossing experiments between *S. marianium* and *S. eburneum,* the number of fruits produced was relatively high as compared to the two parental species. All the F1 plants showed the variegated leaf characteristic of *S. marianum*, whereas after selfing the F2 plants had completely green and variegated leaves in a ratio of about 3:1 indicating that the leaf colour is monofactorially inherited. This proves that the two species are only variants. Using leaf colour as the genetic marker, the outcrossing rate in field experiments was studied. Since the outcrossing rate was only about 2% on the average, Silybum is predominately a self-pollinator.

Ram *et al*. (2005) studied phenotypic and genotypic coefficients of variability, heritability in the broad sense and genetic advance which