EXPRESSION OF ENVIRONMENT STRESS RESISTANCE GENE (S) IN SOME POPLAR SPECIES

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003
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APPROVAL SHEET

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ABSTRACT

This study was carried out to establish reliable techniques which can be used for introducing GRX-2 gene into *Populus alba* by using *Agrobacterium tumefaciens* during the period from 2012 to 2016 at the Biotechnology Research Lab., and Plant Tissue Culture Lab., Horticulture Research Institute, Agricultural Research Center, Egypt. The aims of this work were to optimization of an efficient transformation system for *Populus alba* plants involve the GRX-2 gene by using *Agrobacterium tumefaciens* mediated technique. This gene donates recipient plant salinity tolerance and subjecting *P. alba* transgenic plants to salinity stress to evaluate their stress tolerance. Genetic transformation of an elite white poplar genotype (*Populus alba* L.) was performed with vector pRI 101-ON DNA in *Agrobacterium tumefaciens* strain LBA4404 containing the Glutaredoxin-2 gene in order to enhance the resistance to abiotic harmful stress. The time period of shoot tips infection with *Agrobacterium tumefaciens* suspension culture (0, 5, 10, 20 and 30 min) was tested for the transformation frequency. The highest frequency of transformation was obtained with shoot tip explants infected with *Agrobacterium* culture for 20 minutes at the concentration of $OD_{600}= 0.8$. Isoenzymes peroxidase and polyphenyl oxidase were used as indicators for GRX-2 expression transgenic poplars had highly total peroxidase and polyphenyl oxidase expression compared with that of the control. Molecular analysis using PCR proved the presence and integration of the transgenes in the genome of the transgenic plants. RT-PCR detected successfully the expression of GRX-2 gene. Field experiments were conducted to investigate the effect of the introduced GRX-2 gene on *Populus alba* at salt concentrations (0, 1000, 3000 and 6000 ppm). The data showed that the transgenic lines expressed different levels of salt tolerance as expressed by the performance of vegetative characters (plants plant height, number of leaves, root length and stem diameter), nutrient elements (N, P, K and Na^+) concentration, pigments contents (chlorophyll A, B and cartenoids), proline content and Isozymes electrophoresis (Peroxidase (POD) and Superoxide Dismutase (SOD)). These results show that the *GRX-2* gene enhance salt tolerance character.

Key words: *Agrobacterium*, *Populus alba*, Glutaredoxin-2, isozymes, RT-PCR, salinity, pigment content, proline, nutrient elements.

DEDICATION

*I dedicate this work to **my mother** and **my father** (God bless them).*

*As well as to my wife **Samah** for her encouragement during the course of this study.*

Also, I dedicate this work to all my friends.

WITH MY GRATITUDE AND LOVE

Amr

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CONTENTS

INTRODUCTION

Abiotic stresses are usually defined as nonliving environmental factors that have harmful effects on organisms (*e.g.*, drought, salinity, temperature extremes and pollutants) and limit the growth and development of an organism (Wang *et al.*, 2003a). They are generally classified in two categories according to their origin: edaphic stresses such as water deficit, salinity, lack of nutrients or soil pollution and atmospheric stresses, such as ozone, elevated carbon dioxide (CO_2), increased temperature, frost or high irradiance. Abiotic stress conditions cause extensive losses to agricultural production worldwide. Individually, stress conditions such as drought, salinity or heat have been the subject of intense research. (FAO, 2004)

Drought and soil salinity are rapidly emerging as a major impediment to plant growth and development in many parts of the world and projections indicate that by 2050 as much as 50% of total arable land will be under such pressure (Wang *et al*., 2003a). For example, approximately half of Chinas land mass is classified as arid, semi-arid or saline-alkali (Li *et al*., 2009).

Salinity stress causes adverse effects on different physiological parameters which affected the growth and survival of plants negatively. Different processes related to the photosynthetic pathway (Singh *et al*., 2010), the reduction of leaf area (Neuman *et al*., 1998), or to a general decrease in growth (Fung *et al*., 1998) have proved to be sensitive to salinity stress. Tolerance to environmental constraints is not yet taken into account as a selection criterion for new genotypes in breeding programmers.

In Egypt, the lack of summer rainfall, high evaporative demand and high salinity of irrigation water have caused salt to accumulate in soil. Such accumulation has been increased in some areas. The results of the inadequate irrigation practices and the lack of suitable leaching, which raised water levels are brining salts to the soil surface. Saline and water logged areas covered wide range of the country total area, making rehabilitation of those areas complex and expensive (FAO 2005). Using fast growing woody species such as *Populus* sp., which is known to have a higher water consumption, might be an opportunity to phytoremedate such areas, in the mean time, could decrease the water levels and to be used as an alternative to be considered in the recovery of marginal lands.

Large areas of irrigated land in semi arid areas have been converted into non profitable land due to the increase of salt content in the soils (Ghassemi *et al.*, 1995). Thus, selection of salt tolerant genotypes of forest tree species may be a solution from an economic and environmental point of view.

Populus spp. trees occupy a prominent position in many terrestrial forest ecosystems as well as our human social structure. In the autumn, aspens decorate the landscape with golden leaves providing relief against black-and-white checkered stems, scenes that have inspired generations of nature photographers. Cities and villages with names like “Aspen” and “Cottonwood” in the western U.S. are testament to the historic importance of poplars to rural communities and their economies. The columnar shape of the “Lombardi poplar” is a distinctive feature in many European gardens. The fragrant resin of balsam poplar signals springtime for many Canadians (Davis, 2008).

The genus’ taxonomy is complex because natural variation and hybridization are common among some sympatric species (Hamzeh and Dayanandan, 2004).

Populus is an important forest tree species worldwide. It is a fast growing deciduous hardwood, cultivated primarily for pulp production. A major factor limiting the establishment and management of short rotation *Populus* plantations is the lack of a broad spectrum herbicide which effectively controls weeds (Nelson and Haissig 1986).

Gordon (2001) said that poplar trees are known as “the trees of the people” because of their use to build homes, tools, to make medicines and protect river banks since 1000s of years.

Poplars are very desirable for biofuel production because of their fast growth, producing a significant amount of biomass in a short period of time. Their characteristics such as a small genome size, a rapid juvenile growth, a prolific sexual reproduction and the availability of extensive genetic maps have enabled *Populus* tree species to emerge as a model for woody plants (Confalonieri *et al.*, 2003). Poplars have been known as a “model” for forest tree in genetic and physiological research and as a potential bioenergy crop. Based in part on the long, collaborative history of research on poplars that have revealed its potential utility as a short-rotation woody crop (Bradshaw *et al.*, 2000). *Populus* is a member of a genus of forest trees which are amenable to manipulation *in vitro*. Shoot cultures of *Populus* can be maintained *in vitro* and used for clonal propagation thus providing a sterile source of explant material for bacterial co-cultivation experiments. In addition large numbers of propagules can be obtained rapidly for experimental or commercial purposes (McCown, 1985).

White poplar (*Populus alba L.*), of section *Populus* (syn. Leuce Duby), is native to the Mediterranean basin being widely distributed in floodplain forests throughout northern Africa, southern Europe and Central Asia (Brundu *et al.*, 2008). In Europe, *P. alba* hybridizes naturally with European aspen (*Populus tremula L.*) in regions where their ranges overlap (Lexer *et al.*, 2005).

The domestication and commercialization of *Populus* is extensive and well established as its position as the model tree for biological research owing to its fast growth, biomass producing, for CO_2 sequestration and bio remediation of degraded soils, extensive species diversity, typically wide natural distributions and associated within-species population variation, ease of sexual and asexual propagation, amenability to genetic transformation and small genome size, *i.e* (about 500 Mbp Bradshaw *et al*., 2000). Moreover, the recent sequencing of the *Populus trichocarpa* (black cottonwood) genome (Tuskan *et al*., 2006) has enhanced considerably the importance of the model designation.

In vitro cultivation of organs, tissues, cells, protoplasts was not seen as an alternative to stem cuttings for commercial propagation, already efficient and cheap. But it was considered as a gateway for the application of new techniques, such as *in vitro* selection of somaclonal variants, the rescue of embryos of difficult crosses and the development of homozygous lines. The most promising new technology is genetic trans-formation of poplars. This technology has two main advantages with respect to the conventional breeding:

1. Genes encoding specific proteins can be ‘cut’ from virtually any living being, from viruses to higher plants to animals and ‘pasted’ into poplars, thus broadening the range of genes available outside the current boundaries of the genus;

2. Individual genotypes can be modified for one or a small number of well defined traits while preserving the rest of the genome intact; targeted modification of commercial cultivars could add value to them without disrupting their genome.

Advanced methods for the genome analysis and identification of novel molecular markers have been recently and successfully applied to poplar.

Populus alba is differentiated from *P. tremula* in both biotic (species of insects and pathogens for which resistance is exhibited) and abiotic factors (tolerance of drought, wind, salinity and high temperatures (Imada *et al*., 2009).

Only one genetic map of *P. alba* originating from an inter-specific cross, *Populus adenopoda* × *P. alba* without anchored markers, has been reported among the 13 *Populus* mapping studies that have been published to date (Yin *et al*., 2009).

Transgenics involve the movement of specific and useful genes into the crop of choice and sometimes referred to as genetic engineering (Brummer *et al*., 2007).

Contribution of *in vitro* and molecular biological studies of *Populus* is apparent in virtually every area of forest biology and ecology. The future of *Populus* research appears bright and promise significant biotechnological advances toward our understanding of processes and interactions in forest eco system.

The main goals of this research work are:

1. Optimization of an efficient transformation system for *Populus alba* plants involving glutaredoxin (GRX-2) gene by using *Agrobacterium tumefaciens* mediation technique. This gene donates recipient plant salinity tolerance character.

2. Subjecting *Populus alba* transgenic plants to salinity stress to evaluate their higher stress tolerance characteristics.

REVIEW OF LITERATURE

1. Importance of the *Populus* species

Species from the genus *Populus* (Family *Salicaceae* Linnaeus) occur naturally over large areas in the northern hemisphere. The genus is divided into six sections: *Abaso eckenwalder* (Mexican poplar), *Aigeiros duby* (cottonwoods and black poplar), *Leucoides spach* (swamp poplars), *Populus* (white poplars and aspens, formerly called Leuce Duby), *Tacamahaca Spach* (balsam poplars) and *Turanga bunge* arid and tropical poplars (Dickmann and Kuzovkina, 2014).

Isebrands and Richardson (2013) stated that *Populus* spp. are light-demanding deciduous pioneer tree species. Some of the species are among the fastest growing tree species in the world and height growth can exceed 4 m/ year.

Soil properties that are known to affect the vitality of *Populus spp.* are pH, soil moisture, soil bulk density, soil nutrient composition and soil temperature (Mcivor *et al*., 2014).

Dickmann and Kuzovkina (2014) reported that the fast growth requires good water availability and well-drained soils to avoid oxygen deficit. Poplars and aspens have a natural vegetative regeneration which is the reason why dense stands of clone groups often are found in their natural habitats.

Populus species are grown worldwide for forest products, such as pulp fiber, dimension lumber, furniture components, flakes for oriented strand board (OSB), veneer and are now being proposed as a bioenergy crop (Davis, 2008). IPC (2012) reported that a twelve Many