MOLECULAR GENETIC STUDIES ON THE EGYPTIAN GRAY MANGROVE
(AVICENNIA MARINA)

BY

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B. Sc., Agric. Sc. (Genetics), Ain Shams University, 2003
M. Sc., Agric. Sc. (Genetics), Ain Shams University, 2008

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ABSTRACT

Alaa-Eldeen Abdel-Hady Mohamed Farag Elatawy, Molecular Genetic Studies on the Egyptian Gray Mangrove *(Avicennia marina)*. Unpublished Ph.D Thesis, Department of Genetics, Faculty of Agriculture, Ain-Shams University, 2018.

Mangrove is an ecological term for a diverse group of woody plant species that form the dominant vegetation in saline, tidal wetlands along tropical and subtropical coasts. DNA barcoding of Egyptian gray mangroves in Nabq protectorate was conducted sequencing of ITS1, ITS2. RT-PCR was conducted for four genes implicated for salt tolerance, oxidative and osmotic stresses in Egyptian gray mangroves within Nabq protectorate in South Sinai Governorate. The results showed over-expression of the mRNA of *ferritin* (*amFer1*) gene transcript, followed by slight increase in mRNA of *superoxide dismutase* (*amSOD1*) and even minute increase of ubiquitin *conjugation2* (*amUBC2*) compared with the actin gene. At the same time gene expression of *catalase* (*amCAT1*) was down regulated. 3'-UTR gene regulation and organizational area in the four genes above, were sequenced their homologies were compared to their counterparts in five different databases, the results confirmed that the variation in the 3'-UTR region is very limited because it is harboring important regulatory region that controls the efficiency and half-life time of mRNA and thus, it is highly conserved for effective gene expression.

Keywords: *Avicennia marina*, Gray mangrove, RT-PCR, *amSOD1*, *amCAT1*, *amFer1*, *amUBC2***,** DNA barcoding, 3'-UTR

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LIST OF ABBREVIATIONS

Abbreviation	**Full name**
ROS	Reactive oxygen species
EST	Expressed sequence tags
SOD	Superoxide dismutase
MDA	Malondialdehyde
CAT	Catalases
APX	Ascorbate peroxidases
AOC	Allene oxide cyclase
MT	Metallothioneins
ABA	Abscisic acid
RWC	Relative water content
RT-PCR	Reverse transcription polymerase chain reaction
amCat 1	*Avicennia marina* Catalase1
amSOD 1	*Avicennia marina* Superoxide dismutases 1
amFer 1	*Avicennia marina* Ferritin1
amUBC 2	*Avicennia marina* Ubiquitin conjugating E2
ITS	internal transcribed spacer
3'-UTR	3' untranslated region
EEAA	Egyptian Environmental Affairs Agency
ppm	Parts per million
ppt	Parts per thousand

INTRODUCTION

Mangrove plants are a very suitable system for the study of adaptation in marginal habitats. They are woody plants that inhabit the intertidal zones of tropical and subtropical coasts (**Palacios and Cantera, 2017)**. Mangroves are consisted of approximately 70 species from about 20 families (**Duke, 1992**), most mangroves tolerate fluctuations in environmental conditions, such as salinity, tidal currents, winds, temperature fluctuations, and muddy anaerobic soils. It was suggested that no other group of woody plants is so highly adapted to such extreme conditions (**Kathiresan and Bingham, 2001; Duke *et al.*, 2007; 2017**).

Mangrove is an ecological term for a diverse group of woody plant species that form the dominant vegetation in saline, tidal wetlands along tropical and subtropical coasts (**Tomlinson, 1986**). Salinity is one of the defining environmental features of mangrove habitats and ranges from seasonally freshwater to hypersaline conditions. Mangrove species are differentially distributed along these complex salinity gradients that vary in time and space. Like many other halophytic species (**Flowers and Colmer, 2008**), many studies have emphasized the effects of high salinity on water use. Mangroves typically have relatively low evaporation rates and high water-use efficiencies (**Ball and Farquhar, 1984; Clough and Sim, 1989**).

Gray mangrove (*Avicennia marina*) is a small tree 1-6 m high, bark is smooth and gray in color. Leaves are arranged in opposite direction and are hairy on the reverse side, having special glands for releasing excess salt. Yellow/orange bisexual flowers appear in late summer through early autumn in small dense clusters. Fruits are dispersed from the parent tree by tidal water movement. Pneumatophores (air breathing roots) supply oxygen to the root system. *Avicennia marina*, as a pioneer tree species of mangrove forest ecosystems, is widely distributed from east Africa and the Arabian Gulf, throughout Asia to China and Japan, to the southwestern Pacific, New Zealand and Australia

(**Duck, 2006**). The grey mangrove is one of the most widespread species of mangroves with a latitudinal range from 25^{o}N in Japan to 38^{o}S in Australia (**Tomlinson, 1986; Duke, 2006**). In nature, *A. marina* dominates highly saline habitats with salinities up to 1:5 times (**Nguyen *et al*., 2015**) or twice that of seawater (**Reef *et al*., 2012; Reef and Lovelock, 2015**). It is one of the most tolerant species of mangroves to salinity, aridity, water temperature and frost frequency (**Morrisey *et al*., 2010**). According to many growth studies, the optimum salinity for growth of *A. marina* varies from 10 to 90 % seawater (salinity 35 ppt) depending on the population source and growth conditions.

Salinity is one of the major environmental factors limiting plant growth and productivity. It is estimated that about one-third of world's cultivated land is affected by salinity (**Kaya *et al*., 2002**). Salt stress has toxic effects on plants and lead to metabolic changes, like loss of chloroplast activity, decreased photosynthetic rate and increase photorespiration rate which then leads to an increased reactive oxygen species (ROS) production (**Parida and Das, 2005**), such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical and singlet oxygen ($^1O_2$) (**Parida *et al*., 2004b**). In these cases, induction of antioxidant enzymes was shown to protect halophytes against ROS, thus preventing lipid peroxidation during salt stress. This suggests that these antioxidant enzymes are essential components of an adaptive defence mechanism against salt stress in halophytes like mangrove. Some of the major antioxidant enzymes involved in scavenging are SOD, CAT and POX **(Fang *et al*., 2005).**

Catalase (Cat) enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen, It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS), Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second **(Parida *et al*., 2004b; Negrão *et al*.,**