EFFECT OF DROUGHT AND SALT STRESSES ON THE GENE EXPRESSION LEVELS OF ANTIOXIDANT ENZYMES IN LENTIL (*Lens culinaris* M.) SEEDLINGS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

EMRE AKSOY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

SEPTEMBER 2008

Approval of the Thesis

EFFECT OF DROUGHT AND SALT STRESSES ON THE GENE EXPRESSION LEVELS OF ANTIOXIDANT ENZYMES IN LENTIL (*Lens culinaris* M.) SEEDLINGS

submitted by **EMRE AKSOY** in partial fulfillment of the requirements for the degree of **Master of Science in Biology Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Zeki Kaya
Head of Department, **Biology** _____

Prof. Dr. Meral Yücel
Supervisor, **Biology., METU** _____

Prof. Dr. Hüseyin Avni Öktem
Co-Supervisor, **Biology., METU** _____

Examining Committee Members:

Prof. Dr. Tülin Güray
Biology Dept., METU _____

Prof. Dr. Meral Yücel
Biology Dept., METU _____

Asst. Prof. Dr. Füsun İ. Eyidoğan
Education Faculty, Başkent University _____

Asst. Prof. Dr. A. Elif Erson
Biology Dept., METU _____

Dr. Ebru Özgür
Chemical Eng. Dept., METU _____

Date : _____

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Emre Aksoy

Signature:

ABSTRACT

EFFECT OF DROUGHT AND SALT STRESSES ON THE GENE EXPRESSION LEVELS OF ANTIOXIDANT ENZYMES IN LENTIL (*Lens culinaris* M.) SEEDLINGS

Aksoy, Emre
M.S., Department of Biology
Supervisor: Prof. Dr. Meral Yücel
Co-advisor: Prof. Dr. Hüseyin Avni Öktem

September 2008, 207 pages

This study was carried out for understanding of antioxidant mechanisms of lentil under abiotic stress conditions. For this aim, 14 days old lentil seedlings (*Lens culinaris* Medik cv. Sultan-1) were subjected to drought (20% PEG 6000), and salt (150 mM NaCl) stress for 6, 12 and 24 hours, for 3, 5 and 7 days. PCR conditions for Mn SOD, Cu/Zn SOD, chloroplastic/mitochondrial GR, CAT and chloroplast /stromal APX antioxidant enzymes were optimized. Then, total RNA was isolated from stressed and non-stressed plant roots and shoots. The gene expression levels of Mn SOD and Cu/Zn SOD were examined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique. *Arabidopsis* 18S rRNA was used as internal control in multiplex PCR technique.

Relative expression levels of Mn SOD were lower in shoots and roots under salt stress while no significant change was obtained under drought conditions in both tissues. Relative expression levels of Mn SOD were increased on 5th day of salt and drought applications in both shoots and roots. Relative expression levels of Cu/Zn SOD increased after 5th, and on 1st and 7th days of drough treatment in shoots and roots, respectively. On the other hand, expression levels of Cu/Zn SOD increased on 3rd and 5th days of salt treatment in shoot tissues.

Although it is nearly impossible to understand the whole antioxidant mechanism of plants under environmental stresses, this study was the first step to learn about molecular background of antioxidant defence mechanisms in lentil.

Key words: Lentil, *Lens culinaris*, drought stress, salt stress, antioxidant defence, RT-PCR, gene expression

ÖZ

KURAKLIK VE TUZ STRESLERİNİN MERCİMEK (*Lens culinaris* M.) FİDELERİNDE ANTİOKSİDANT ENZİMLERİN GEN İFADELERİ ÜZERİNE ETKİLERİ

Aksoy, Emre
Yüksek Lisans, Biyoloji Bölümü
Tez Yöneticisi: Prof. Dr. Meral Yücel
Tez Yardımcı Yöneticisi: Prof. Dr. Hüseyin Avni Öktem

Eylül 2008, 207 sayfa

Bu çalışma, abiyotik stres altında mercimek antioksidan mekanizmalarının 14 günlük mercimek (*Lens culinaris* Medik cv. Sultan-1) fideleri 6, 12, 24 saat ve 3, 5, 7 gün boyunca kuraklık (20% PEG) ve tuzluluk (150mM NaCl) streslerine maruz bırakılmıştır. Mn SOD, Cu/Zn SOD, kloroplastik/mitokondrial GR, CAT ve kloroplast/stromal APX antioksidan enzimleri için uygun PZR koşulları optimize edilmiştir. Daha sonra strese maruz bırakılmış ve bırakılmamış bitki kök ve gövdelerinden toplam RNA izole edilmiştir. Strese maruz bırakılan ve bırakılmayan fidelerde kuraklık ve tuzluluk stres uygulamaların Mn SOD ve Cu/Zn SOD'un gen ifade düzeyleri üzerine etkileri yarı-kantatif ters transkripsiyon polimeraz zincir reaksiyon tekniği (RT-PZR) kullanılarak karşılaştırılmalı bir biçimde incelenmiştir. Bu amaçla, çoklu PZR uygulamalarında internal kontrol olarak *Arabidopsis* 18S rRNA kullanılmıştır.

Kuraklık uygulamasında gövde ve kökte nispi Mn SOD ifade seviyelerinde herhangi anlamlı bir fark gözlenmezken tuz stresi altında her iki dokudaki nispi ifade seviyeleri azalmıştır. Nispi Mn SOD ifade seviyeleri tuz ve kuraklık uygulamalarının 5. gününde hem kök, hem de gövdede artmıştır. Nispi Cu/Zn SOD ifade seviyeleri, kuraklık uygulamasının 5. gününden sonra, köklerde ise uygulamanın 1. ve 7. günlerinde artmıştır. Öte yandan Cu/Zn SOD ifade seviyeleri tuz uygulamasının 3. ve 5. günlerinde artmıştır.

Her ne kadar bitkilerin çevresel streslere karşı geliştirdikleri tüm antioksidan mekanizmayı anlayabilmek olanaksız olsa da, bu çalışma mercimek antioksidan defans mekanizmasının moleküler altyapısının anlaşılması yolunda ilk adım olacaktır.

Anahtar Kelimeler: Mercimek, *Lens culinaris*, kuraklık stresi, tuz stresi, antioksidan defans, RT-PCR, gen eksresyonu

To fathers of revolutionary ideas to change the world for a better place to live in

ACKNOWLEDGEMENTS

I owe my supervisor Prof. Dr. Meral Yücel and my co-supervisor Prof. Dr. Hüseyin Avni Öktem a debt of gratitude for their precise, commanding and systematic scientific guidance both in theoretical background of and in experimental design of this research thesis. I will always be obliged to them for their endless support not only in this thesis, but also in changing my ethos.

I would like to express my reverence especially to Beray Gençsoy for her guidance throughout this study with her infinite patience. She was not an awesome tutor, but also an authentic friend with her polite and empathetic personality.

I would like to thank to my thesis juri members, Prof. Dr. Tülin Güray, Asst. Prof. Dr. Füsun İ. Eyidoğan, Asst. Prof. Dr. A. Elif Erson and Dr. Ebru Özgür, for their critics and suggestions.

I would like to thank to Dr. Ufuk Çelikkol Akçay, Hamdi Kamçı, A. Tahir Bayraç, Musa Kavas, Gülsüm Kalemtaş, Cengiz Baloğlu, Taner Tuncer, Nilüfer Afşar, Özge Şenyurt, Ferhunde Aysin, Sena Cansız, Fatma Kılbacak, Ceren Karakaş and Hamit Battal not only for being my Lab-mates, but also since I have drummed them deep into my brain as a big family of "Plant Biotechnology Research Laboratories". I believe that this family spirit lasts forever.

Being only three members of this big family, I especially thank to Oya Ercan, M. Tufan Öz and Ceyhun Kayıhan one by one for their critical ideas throughout my study with their knowledge obtained in previous scientific experiences.

This thesis work would not have been accomplished without the support of my parents, M. Ersin Aksoy and Sevim Aksoy, and my brother, Emrah Aksoy. They have always firmly believed in my success and supported me from their huge hearts. I believe they will support me wherever I go in the future. I want to dedicate this work to my deceased grandfather, Mehmet Aksoy, who has believed that I would have been a pioneer in the future and has exhorted me one time to be the best in any area I work.

Being aware of the power of friendship, I would like to set Tuğba İnanç Gök, Zeynep Bal and Murat Kavruk on a pedestal for their endless friendship, precious supports and their being my best friends. I know we will be in a closer contact wherever we go in the future.

My final acknowledgements go to Firdevs Yalçın from METU Central Laboratory both for her helps in analysis of RNA samples by UV-Vis Spectrophotometer and for her encouraging inspiration.

It is my duty to express my gratitude to TUBITAK for the support that I have received as scholarships both during my undergraduate and graduate education. This work was supported by BAP- 2006-08-11-12.

TABLE OF CONTENTS

APPENDICES

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ABA	Abscisic acid
AOX	Alternative oxidase
APX	Ascorbate peroxidase
CAT	Catalase
ETC	Electron transport chain
GR	Glutathione reductase
GSH	Reduced glutathione
GSSH	Oxidized glutathione
H_2O_2	Hydrogen peroxide
$HO_2^{\cdot}$	Hydroperoxyl radical
KCN	Potassium cyanide
MDA	Malondialdehyde
MAPK	Mitogen-activated protein kinase
O_2	Molecular oxygen
NO	Nitric oxide
PEG	Polyethylene glycol
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
RT-PCR	Reverse Transcription Polimerase Chain Reaction
SOS	Salt overly sensitive

$^1O_2$	Singlet oxygen
NaCl	Sodium chloride
$O_2^{\cdot -}$	Superoxide anion
SOD	Superoxide dismutase
Subsp.	Subspecies

CHAPTER 1

INTRODUCTION

1.1. Lentil

Lentil (*Lens culinaris* Medik.) is a self pollinating, diploid (2n=14), annual grain legume suitable for cultivation in warm temperate, subtropical and high altitude tropical regions of the world (Muehlbauer *et. al.*, 1995). In 2007, it was propagated in more than 40 countries on around 4 million hectares land (Andrews and McKenzie, 2007).

1.1.1. History and Origin

One of the oldest grain legumes, oldest remains of lentil dated to 11,000 BC were obtained from Greece's Franchthi cave (Hassen J., Renfrew, 1978).

Lens culinaris is originated in Near East and Central Asia (Ladizinsky, 1979) and spread to other parts of the world. Furgeson *et al.* (1998) showed that two centers exist for *L. culinaris* subsp. *orientalis*, (a) south-eastern Turkey and north-western Syria, and (b) southern Syria and northern Jordan.

1.1.2. Biodiversity and Ecology

The genus *Lens* is a member of the legume tribe *Vicieae* which includes the major legume crops such as faba bean, pea and lentil. *Lens* appears to be most closely related to *Vicia* (Kupicha, 1981). Scientific classification of *Lens culinaris* is given below:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Subfamily	Fadoideae
Tribe	Vicieae
Genus	*Lens*
Species	*L. culinaris*

After the publication of *Lens culinaris* by Medikus in 1787, the name of the species has been called as *Lens culinaris* Medik. Ferguson *et. al.* (2000) re-classified Lens into seven taxa:

L. culinaris Medikus

subsp. *culinaris*

subsp. *orientalis* (Boiss.) Ponert

subsp. *tomentosus* (Ladizinsky) Ferguson et al. (2000)

subsp. *odemensis* (Ladizinsky) Ferguson et al. (2000)

L. ervoides (Brign.) Grande

L. nigricans (M. Bieb.) Godr.

L. lamottei Czefr.

Lentil plants are slender, semi-erect annuals with compound leaves (4 to 7 pairs of leaflets) with a tendril at the tips. Height of plants normally ranges from 15 to 75 cm. Plants can have single stems or many branches depending upon the population in the field (Duke, 1981). *Lens culinaris* Medik. comes in two varieties: macrosperma (with large seeds and little pigmentation) and microsperma (with small seeds and some pigmentation). Depending on their variety and breed, however, lentil seeds can range in color from red-orange, to yellow, green, brown, or black (Sandhu and Singh, 2007).

Lentil is reported to require environments ranging from cool temperate steppe to wet through subtropical dry to moist forest life zones. It tolerates annual precipitation of 2.8-24.3 dm, annual mean temperature of 6.3-27.3°C and pH of 4.5-8.2 (Kay, 1973). In West Asia and North Africa, lentil is winter-sown at elevations below 850 meters and is usually spring-sown at higher elevations (Erskine *et.al.,* 1994).

1.1.3. Nutritional Value and Uses

Lentil is an important dietary source of energy, protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds as well as diverse non-nutritional components such as protease inhibitors, tannins, α- galactoside oligosaccharides and phytic acid (Urbano *et. al.*, 2007).

Protein concentration of lentils range from 22-34.6%, and 100 g of dried seeds contain 340-346 g calories, 12% moisture, 20.2 g protein, 0.6 g fat, 65.0 g total carbohydrate, about 4 g fiber, 2.1 g ash, 68 mg Ca, 325 mg P, 7.0 mg Fe, 29 mg Na, 780 mg K, 0.46 mg thiamine, 0.33 mg riboflavin, 1.3 mg niacin (Adsule *et al.,* 1989; Muehlbauer *et al.,* 1985). Lentil is an excellent supplement to cereal grain diets because of its good protein/carbohydrate content.

Among the cool season legume crops, lentil is the richest in the important amino acids such as lysine, arginine, leucine. However, nutritional value of lentil is low because lentil is deficient in methionine and cystine (Williams *et al.,* 1994).

1.1.4. Global Production

World lentil production has increased significantly in last decade from 2.65 million tons in 1991/1992 to 3.8 million tons in 2003/2004 (FAO, 2004). 4.1 million tons of lentils were produced in 2005 (IGEME, 2007). Top three lentil producing countries, India, Canada and Turkey, accounted for nearly 70 per cent of world production. Approximately 70 per cent of world lentil production is of the red variety with the remainder being primarily of the green variety (Lentil Market Report, 2004).

In Turkey, one of the major lentil producing countries, production was 570.000 and 662.000 tons in 2005 and 2006, respectively. Although an increase in total lentil production has been observed between 2000 and 2006, this rising trend was supported by the increase in production of red lentil. Green lentil production has declined from 200.000 tons (in 1986) to 42.000 tons (in 2006). The main reason for this decline is that poor yield of Turkish green lentil could not have compensated for the increased production of higher quality green lentil both in Canada and USA (IGEME, 2007).

1.1.5. Turkish Cultivars

Winter-sown red lentil cultivars are produced in South-Eastern Anatolia, whereas spring-sown green lentil cultivars are grown in Central Anatolia and in transition regions. Red lentil is produced mainly in Gaziantep, Şanlıurfa, Adiyaman, Diyarbakir and Mardin, whereas green lentil production is highest in Ankara,

Konya, Yozgat, Tokat, Nevsehir and Corum (IGEME, 2007). Molecular diversity of Turkish lentil cultivars was analyzed by two recent studies. Yuzbasioglu *et al.* analyzed different Turkish cultivars and breeding lines by RAPD markers (2006) and SDS-PAGE of seed proteins (2008).

1.2. Environmental Stresses

Biological stress is defined as any disadvantageous condition that reduces or inhibits the normal growth and/or survival of biological organisms such as plants (Jones *et. al.,* 1989). Being sessile, the plants are vulnerable to environmental stresses, which destroy the normal growth cycle and reduce the yield. Mahajan and Tuteja divides environmental stress into two main groups as biotic and abiotic (Mahajan and Tuteja, 2005). Biotic stresses are pathogens, insects, herbivores and rodents, whereas low and high temperatures, salinity, drought, excess water, radiation, heavy metals, pesticides, ozone, wind, nutrient deprivation in soil are some examples to abiotic stresses.

Drought stress has the highest percentage with 26%. It is followed by mineral stress with 20%, cold and freezing stress with 15%. All the other stresses get 29% whereas only 10% area is not exposed to any stress factors (Blum, 1986).

In the face of a global scarcity of water resources and the increased salinization of soil and water, abiotic stress is already a major limiting factor in plant growth and will soon become even more severe as desertification covers more and more of the world's terrestrial area. Drought and salinity are already widespread in many regions, and are expected to cause serious salinization of more than 50% of all arable lands by the year 2050 (Ashraf, 1994). In a world where population growth exceeds food supply, agricultural and plant biotechnologies aimed at overcoming severe environmental stresses need to be fully implemented (Vinocur and Altman, 2005).

Average lentil yields are low because of the limited yield potential of landraces, which are also vulnerable to an array of environmental stresses. Although Mediterranean climates are characterized by hot, dry summers and cold, wet winters, annual rainfall values have been notoriously variable in the last few years marking drought as a major problem. Major abiotic stress factors that limit lentil production are low moisture and high temperature stress in spring and, cold temperatures in winter at high elevations (Johansen *et. al.,* 1992). Moreover, extreme salinization by means of uncontrolled irrigation becomes a major trouble in some regions of Mediterranean countries. Mineral imbalances like boron, iron, and salinity and sodicity, though localized, do cause substantial yield losses. Among biotic stresses, the rust, vascular wilt and Ascochyta blight diseases caused by *Uromyces viciae-fabae* (Pers.) Schroet., *Fusarium oxysporum* f. sp. *Lentis* Schlecht and *Ascochyta fabae Speg.* f. sp. *lentis*, respectively, are globally important fungal pathogens of lentil (Bayaa & Erskine 1998).

Detrimental effects of all environmental stress types on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity (Parida and Das, 2005). They cause production of reactive oxygen species (ROS), which are cytotoxic in high concentrations.

1.2.1. Reactive Oxygen Species

A free radical is any species capable of independent existence that contains one or more unpaired electrons (Halliwell and Gutteridge, 2006). An unpaired electron is one that occupies an atomic or molecular orbital itself. The simplest free radical is the atomic hydrogen. There are many types of free radicals such as oxygen and nitrogen radicals in living systems.

Oxygen levels in atmosphere elevated over 2.2 billion years ago by activities of cyanobacteria. They used energy of sun to split water into hydrogen and oxygen atoms by means of photosynthesis. Since then, hydrogen atoms have been used as reducing agents while oxygen has released into the atmosphere as a by-product (Lane, 2002). Ever since the introduction of molecular oxygen (O_2) into the atmosphere, reactive oxygen species (ROS) have been the unwelcome companions of aerobic life (Mittler *et al.*, 2004).

Although molecular oxygen (O_2) is relatively unreactive due to its electron configuration (Elstner, 1987), it is thermodynamically a potent oxidizing agent. When O_2 tries to oxidize a nonradical by accepting a pair of electrons from it, both these electrons must have the same spin to fit into the vacant spaces in the uppermost free orbitals. A pair of electrons in an atomic or molecular orbital cannot meet this criterion since they have opposite spins. This spin restriction makes O_2 prefer to accept its electrons once at a time (Halliwell, 2006).
Ground state oxygen may be converted to the much more reactive ROS forms either by energy transfer or by electron transfer reactions. The former leads to the formation of singlet oxygen, whereas the latter results in the sequential reduction to superoxide, hydrogen peroxide, and hydroxyl radical (Apel, Hirt, 2004).

Singlet Oxygen

Singlet oxygen ($^1O_2$) is generated by an input of energy that rearranges the electrons in orbitals of O_2. Two forms of $^1O_2$ can be generated according to the access of one electron into the free orbitals. In both forms of $^1O_2$ the spin restriction is removed and the oxidizing ability greatly increased. (Foote *et. al.,* 1985).

The chlorophyll pigments associated with the electron transport system are the primary source of singlet oxygen. In PSII, $^3O_2$ is excited to $^1O_2$ by the reaction center chlorophyll of triplet excited state (Asada, 2006). Singlet oxygen may also arise as a by-product of lipoxygenase activity (Arora *et. al.*, 2002).

1.2.2. Superoxide

If a single electron is supplied to O_2, it enters one of the free antibonding orbitals, producing an anion called superoxide anion radical ($O_2^{\cdot -}$)(Fridovich, 1995).

Some enzyme systems reduce oxygen via tetravalent mechanisms (e.g. cytochrome c oxidase, alternative oxidases (AOX)], but some others reduce it in a stepwise manner, i.e. oxygen is accepting electrons one by one only. This leads to the formation of $O_2^{\cdot -}$. The major site of superoxide formation in mitochondria lies in the electron transport chain (ETC), especially at the level of Complex I and Complex III (Navrot *et. al.,* 2007). It was shown in animal mitochondria that the flavine mononucleotide (FMN) - containing subunit and an iron-sulfur cluster of the nicotinamide adenine dinucleotide (NADH) dehydrogenase of Complex I are the sites of $O_2^{\cdot -}$ generation (Chen et. al., 2005). In Complex III, the overreduction state of the ubiquinone pool can lead to a direct electron transfer to molecular oxygen, and to the formation of superoxide anions (Navrot *et. al.,* 2007).

One of the primary processes involved in ROS production in chloroplasts is the direct photoreduction of O_2 to the superoxide radical by reduced electron transport components associated with PSI (Apel, 2004). The majority of O_2 reduction *in vivo* proceeds via reduced ferrodoxin, which reduces molecular oxygen to the superoxide radical (Arora *et.al.,* 2002).

1.2.3. Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a broad-range chemical catalyst with both reducing and oxidizing properties. It belongs to the non-radical group of the ROS (Bienert *et.al.,* 2006). The half-life of H_2O_2 in lymphocytes is 1 ms (Reth, 2002).

Both $O_2^{\cdot -}$ and hydroperoxyl radical ($HO_2^{\cdot}$) undergo spontaneous dismutation to produce H_2O_2. H_2O_2 is generated in normal metabolism via the Mehler reaction in chloroplasts, electron transport chain in mitochondria and photorespiration in peroxisomes. Peroxisomes may also contain other systems that generate H_2O_2. Abiotic and biotic stresses enhance H_2O_2 generation via enzymatic sources such as plasma membrane localized NADPH oxidases, cell wall peroxidases or xanthine oxidase (Neill *et.al.,* 2002). Hydrogen peroxide can also be generated by specific enzymes. Most of the superoxide ions produced is efficiently converted to H_2O_2 by manganese-containing superoxide dismutase (MnSOD) in mitochondria (Rhoads *et.al.,* 2006), and copper/zinc- containing (Cu/ZnSOD) and iron-containing (FeSOD) in chloroplasts (Asada, 2006).

H_2O_2 can be converted into other more reactive ROS by various means including enzymes. In the presence of reduced transition metals such as Fe^{2+} in a chelated form, the formation of $OH^{\cdot}$ can occur in the Fenton reaction (Blokhina *et.al,* 2003).

Although hydrogen peroxide is a ROS, it also functions as a signaling molecule controlling different essential processes in plants. It functions in ABA-mediated stomatal closure (Zhang *et.al.,* 2001), gravitropism (Joo *et.al.,* 2001), blue light-induced photomorphogenesis (Chandrakuntal *et.al.,* 2004), and xylem differentiation and lignification (Barceló, 2005).

1.2.4. Hydroxyl Radical

Hydroxyl radicals are among the most highly reactive molecular species known. In the presence of suitable chelated transitional metals, particularly iron, hydroxyl radicals can also be formed from superoxide and hydrogen peroxide at ambient temperatures and neutral pH by the iron-catalyzed, superoxide-driven Fenton reaction (Babbs, 1989). The resulting Fe^{3+} can be reduced back to Fe^{2+} by the superoxide radical (O^{-2}), regenerating Fe^{2+} and allowing the reaction to continue. The sum of these two reactions is known as the Haber–Weiss reaction:

$$
\begin{aligned}
O_2^- + Fe^{3+} &\rightarrow O_2 + Fe^{2+} \\
Fe^{2+} + H_2O_2 &\rightarrow Fe^{3+} + OH^- + \cdot OH \\
O_2^- + H_2O_2 &\rightarrow O_2 + OH^- + \cdot OH
\end{aligned}
$$

1.2.5. Damage of ROS

The main components of membrane lipids, polyunsaturated fatty acids (PUFA), are susceptible to peroxidation. Hydroxyl radicals and singlet oxygen can react with the methylene groups of PUFA forming conjugated dienes, lipid peroxy radicals and hydroperoxides (Smirnoff, 1995).

Decomposition of lipid peroxides accelerated by iron and copper ions or by heating generates a complex mixture of toxic products including epoxides, aldehydes such as malondialdehyde, ketones and hydrocarbons. They bind avidly

to membrane proteins, inactivating enzymes and receptors. They can also attack DNA, forming mutagenic lesions (Esterbauer *et.al.,* 1991).

ROS damage on biological membranes can be modulated by regulation of membrane structures by adaptive mechanism such as alteration of composition and organization of lipids inside the bilayer in a way that prevents lipid peroxidation, modification of degree of PUFA unsaturation, mobility of lipids within the bilayer and preventive antioxidant systems (Blokhina, 2003).

ROS also attack to other macromolecules such as proteins and DNA, with the formation of nucleotide peroxides especially at the level of thymine (Cullis et.al., 1987).

Oxidative attack on proteins results in site-specific amino-acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, and altered electrical charge. After oxidative modification, proteins become sensitive to proteolysis and/or may be inactivated, or may show reduced activity. Other oxidative injuries include either random formation of intramolecular disulphide bridges, or uncontrolled oxidation of thiols to sulphonic acids (*Fança et.al.,* 2006).

ROS-induced DNA damage includes single- and double-strand breaks, abasic sites, and base damages. Furthermore, mitochondrial DNA is more sensitive to oxidative damage than nuclear DNA, in particular because of the absence of chromatin organization and lower mitochondrial DNA repair activities (Yakes and Van Houten, 1997). As major site of ROS production both in animal and plant cells is mitochondrial electron transport chain (ETC), the importance of ROS-dependent damage on mitochondrial proteins such as ETC proteins, and mitochondrial DNA becomes clearer.

1.3. Reactive Species Signaling Pathways

A moderate accumulation of ROS function as key inducers for secondary programmed metabolisms, defense signal, cell wall differentiation and activation of mitogen-activated protein kinases (MAPKs) leading to the environmental stress tolerance (Kotchoni, Gachomo, 2006).The rapid increase in ROS production, referred to as "the oxidative burst", was shown to be essential for many of these processes (Suzuki and Mittler, 2006).

Moderate accumulation of ROS significantly affects nuclear gene expression. Three principal modes of action indicate how ROS could affect gene expression. ROS sensors could be activated to induce signaling cascades that ultimately impinge on gene expression. Alternatively, components of signaling pathways could be directly oxidized by ROS. Finally, ROS might change gene expression by targeting and modifying the activity of transcription factors (Apel and Hirt, 2004).

Being small and containing no net charge, H_2O_2 is a susceptible candidate of ROS signaling in plant cells. H_2O_2 can oxidase or otherwise modulate signaling proteins, such as protein phosphatases, protein kinases including plasma membrane histidine kinase and MAPK cascades, transcription factors and calcium channels that are located in the plasma membrane or elsewhere. Elevated cytosolic calcium concentrations will initiate further downstream responses, via the action of calcium-binding proteins that include calmodulin (CaM), protein phosphatases and protein kinases. The oxidation of transcription factors may activate them and/or induce nuclear localization (Neill *et.al.,* 2002).

ROS modulate the expression of various genes, including those encoding antioxidant enzymes and modulators of ROS production, indicating the complex way in which intracellular ROS concentrations may be monitored and maintained at a constant level. The expression of different transcription factors is enhanced by ROS and includes members of WRKY, Zat, RAV, GRAS and Myb families (Vranova´ *et.al.,* 2002).

Although the receptors for ROS are unknown at present, it has been suggested that plant cells sense ROS via at least three different mechanisms: (I) unidentified receptor proteins; (II) redox-sensitive transcription factors; (III) direct inhibition of phosphatases by ROS (Mittler *et.al.,* 2004).

As ROS, reactive nitrogen species may generate signaling cascades under environmental stresses. Exogenous nitric oxide (NO) induces stomatal closure, ABA triggers NOS generation, removal of NO by scavengers inhibits stomatal closure in response to ABA, and ABA-induced stomatal closure is reduced in mutants that are impaired in NO generation (Bright *et.al.,* 2006). Similar to ROS NO also stimulates MAPK activity and cGMP production (Desikan *et.al.,* 2004). Both these NO-stimulated events are required for ABA-induced stomatal closure. ABA also stimulates the generation of H_2O_2 in guard cells, and pharmacological and genetical data demonstrate that NO accumulation in these cells is dependent on such production (Neill *et.al.,* 2008).

1.4. Protection Mechanisms against ROS

Because ROS are toxic but also participate in signaling events, plant cells require at least two different mechanisms to regulate their intracellular ROS concentrations by scavenging of ROS: one that will enable the fine modulation of low levels of ROS for signaling purposes, and one that will enable the detoxification of excess ROS, especially during stress (Mittler, 2002). Plants have evolved both non-enzymatic and enzymatic mechanisms to cope with deleterious effects of ROS in the cells. These systems are called as antioxidant protection mechanisms.

1.4.1. Non-Enzymatic Mechanisms

Glutathione, ascorbate, tocopherol, flavonoids, alkaloids and carotenoids are non-enzymatic antioxidant protection mechanisms.

The tripeptide glutathione (GSH) is the major low molecular weight thiol present in plant species. It is involved in the storage and the transport of reduced sulphur (Kopriva and Rennenberg 2004), in the detoxification of xenobiotics via glutathione-*S*-transferase (Dixon *et al.*, 2002), in the protection against heavy metals as a precursor in the synthesis of phytochelatins (Cobbett 2000), in the scavenging of active oxygen species by the ascorbate-glutathione cycle and in the regulation of the redox homeostasis of the cell together with its oxidized form (GSSG) (Noctor and Foyer 1998). Because of a central nucleophilic cysteine residue GSH scavenges cytotoxic H_2O_2, and reacts non-enzymatically with other ROS (Blokhina *et.al.,* 2003). It has been found in all cell compartments, namely cytosol, endoplasmic reticulum, vacuole and mitochondria (Jimenez *et.al.,* 1998). It was shown that GSH synthesis is controlled by NO (Innocenti *et.al.,* 2007).

Ascorbate is the most abundant small molecule antioxidant in plants and its concentration is 5-10 times much higher than that of GSH in the leaves (Luwe and Heber, 1995). Ascorbate occurs in almost all tissues, with the exception of dry seeds. It occurs in all subcellular compartments including the cell wall, but it has a low concentration in vacuoles (Franceschi and Tarlyn, 2002). Ascorbate anion readily loses an electron from its ene-diol group to produce the monodehydroascorbate (MDHA) radical. Further oxidation results in dehydroascorbate (DHA), which is uncharged. The ability of ascorbate to donate an electron and the relatively low reactivity of the resulting MDHA radical is the basis of its biologically useful antioxidant and free radical scavenging activity (Venkataraman *et.al.,* 2004). Ascorbate reacts with hydroxyl radical, superoxide, and singlet oxygen (Noctor and Foyer, 1998). In addition to its importance in photoprotection and the regulation of photosynthesis, ascorbate plays an important role in preserving the activities of enzymes that contain prosthetic transition metal ions (Padh, 1990). Ascorbate is also a powerful secondary antioxidant, reducing the oxidized form of α-tocopherol (Thomas *et.al.,* 1992).

The tocopherols and tocotrienols, in particular α-tocopherol, are required in the diet and are collectively known as vitamin E (or tocols). They are produced in higher plant plastids and by cyanobacteria. (Kamal-Eldin and Appelqvist, 1996). Vitamin E functions in protection against photo-oxidative damage, repairement of oxidizing radicals, stabilize membrane structure by changing the fluidity. Moreover, it reacts with alkoxy radicals, lipid peroxy radicals and with alkyl radicals derived from PUFA oxidation (Blokhina *et.al.,* 2003).

Carotenoids are lipophylic isoprenoid compounds synthesized by all photosynthetic organisms and also by some non-photosynthetic bacteria and fungi (Bartley and Scolnik, 1997). Most carotenoids are located, together with chlorophylls, in functional pigment-binding protein structures embedded in thylakoidal membranes. Besides acting as membrane stabilizers, carotenoids play

two main roles in photosynthesis. First, carotenoids act as accessory light-harvesting pigments that absorb in the range of 450–570 nm and transfer the energy to chlorophyll. And second, they channel excess energy away from chlorophyll for protection against photooxidative damage (Botella-Pavia and Rodriguez-Concepcion, 2006).

Plant phenolic compounds such as flavonoids and lignin precursors are potent antioxidants. Phenolics are chemical compounds characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups (Sakihama *et.al.,* 2002) It has been proposed that polyphenols function as antioxidants to support the primary ascorbate-dependent detoxification system as a backup defense mechanism of vascular plants (Yamasaki *et.al.,* 1997).

1.4.2. Enzymatic Mechanisms

To control the level of ROS and to protect cells under stress conditions, plant tissues contain several enzymes scavenging ROS (superoxide dismutase [SOD; E.C. 1.15.1.1], ascorbate peroxidase [APX; E.C. 1.1.1.11], catalase (CAT; E.C. 1.11.1.6], glutathione reductase [GR; E.C. 1.6.4.2], monodehydroascorbate reductase [MDHAR; E.C. 1.6.5.4], glutathione peroxidase, alternative oxidase and dehydroascorbate reductase [DHAR; E.C. 1.8.5.1]) and detoxifying lipid peroxidation products (glutathione S-transferase, and phospholipid-hydroperoxide glutathione peroxidase) (Blokhina *et.al.,* 2003).

Superoxide dismutases (SODs), the family of metalo-enzymes, catalyze the disproportion of superoxide ($O_2^{\cdot -}$) to molecular oxygen and H_2O_2 (Scandalios, 1993). It has been shown that phospholipid membranes are impermeable to charged $O_2^{\cdot -}$ molecules. Therefore, it is crucial that SODs are present for the removal of $O_2^{\cdot -}$ in the compartments where $O_2^{\cdot -}$ radicals are formed (Takahashi

and Asada, 1983). SODs decrease the risk of hydroxyl radical formation from superoxide via the metal-catalyzed Haber-Weiss type reaction.

Based on the metal co-factor used by the enzyme, SODs are classified into three groups as iron SOD (Fe-SOD), manganese SOD (Mn SOD), and copper-zinc SOD (Cu/Zn SOD). Although the enzyme is present in all aerobic organisms and in all sub-cellular compartments susceptible to oxidative stress (Bowler *et.al.,* 1992), SOD isozymes are located in different compartments (Alscher *et.al.,* 2002). Fe SODs are located in the chloroplast, Mn SODs in the mitochondrion and the peroxisome, and Cu/Zn SODs in the chloroplast, the cytosol, and possibly the extracellular space.

Comparison of deduced amino acid sequences from these three different types of SODs suggest that Mn and Fe SODs are more ancient types of SODs, and these enzymes most probably have originated from the same ancestral enzyme, whereas Cu/Zn SODs have no sequence similarity to Mn and Fe SODs and probably have evolved separately in eukaryotes (Kanematsu and Asada, 1990; Smith and Doolittle, 1992). The evolutionary reason for the separation of SODs with different metal requirements is probably related to the different availability of soluble transition metal compounds in the biosphere in relation to the O_2 content of the atmosphere in different geological eras (Bannister *et al.,* 1991).

The three SOD types can be identified by their different sensitivities to KCN and H_2O_2, and by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to their molecular weights. Cu/Zn SOD is characterized as being sensitive to both KCN and H_2O_2 while Fe SOD is sensitive only to H_2O_2 and Mn SOD is resistant to both inhibitors (Alscher *et.al.,* 2002).

The intracellular level of H_2O_2 is regulated by a wide range of enzymes including catalases and peroxidases. Catalase, which is a heme-containing enzyme, functions through an intermediate catalase-H_2O_2 complex (Compound I) and produces water and dioxygen (catalase action) or can decay to the inactive Compound II. In the presence of an appropriate substrate Compound I drive the peroxidatic reaction. Compound I is a much more effective oxidant than H_2O_2 itself, thus the reaction of Compound I with another H_2O_2 molecule (catalase action) represents a one-electron transfer, which splits peroxide and produces another strong oxidant, the hydroxyl radical(OH^-)(Elstner, 1987). Catalase is localized in mitochondria, glyoxisomes, and mostly in peroxisomes. There are three main isoforms in *Arabidopsis thaliana*: CAT1, CAT2, and CAT3. The expression of catalase genes is not only developmentally regulated but it is also sensitive to various environmental signals (Dat *et.al.,* 2000). Moreover, catalase is photoinactivated in moderate light under conditions to which plants are adapted (Streb and Feierabend, 1996). Because CAT does not require a supply of reducing equivalents for its function, it might be insensitive to the redox status of cells and its function might not be affected during stress, unlike other mechanisms (Mittler, 2002).

Another mechanism for reduction of H_2O_2 mainly in chloroplasts is ascorbate-glutathione (or Halliwell-Asada) cycle. Localization of ascorbate peroxidase (APX) and related enzymes indicates that chloroplasts reduce H_2O_2 with APX using the elements derived from water in PSII as seen in figure 1.3 (Arora *et.al.,* 2002).

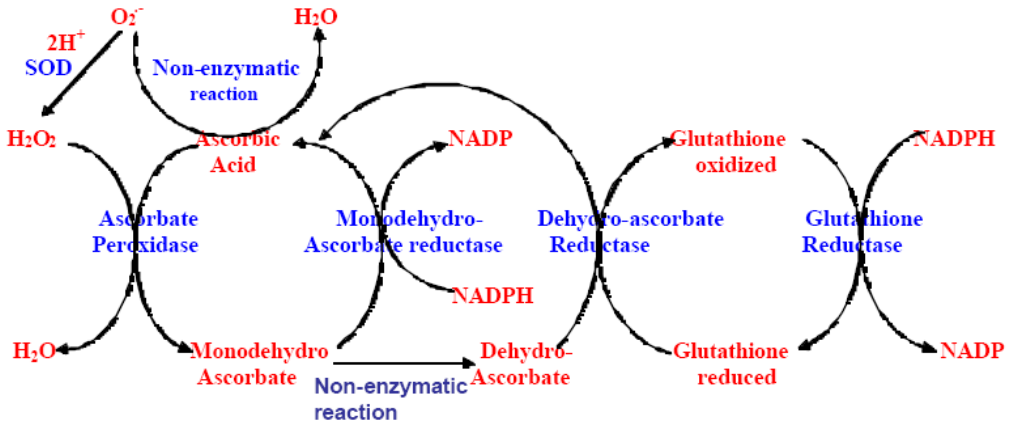

Figure 1.1. Halliwell-Asada cycle

APX is classified as class I peroxidase (Asada, 2006) and inhibited by thiol-modifying reagents (Raven, 2003). Chloroplastic APX is classified into thylakoid-bound (tAPX) and stroma-localized (sAPX) forms. tAPX binds in the vicinity of PSI so that it detoxifies even small quantities of H_2O_2 in its production site (Miyake *et.al.,* 1993). Hence, any H_2O_2 diffusion to other parts of the cell is inhibited by scavenging activity of tAPX enzyme. Except for *Arabidopsis thaliana*, a single gene codes tAPX and sAPX, and both isoforms are generated by alternative splicing (Yoshimura *et. al.,* 2002).

Halliwell-Asada cycle contains other enzymes for the regeneration of ascorbate. Glutathione participates in the generation of ascorbate from dehydroascorbate via the enzyme dehydroascorbate reductase (E.C. 7.8.5.1). In such reactions glutathione (GSH) is oxidized to glutathione disulphide (GSSG). GSH is regenerated by glutathione reductase (GR) in a NADPH-dependent reaction. GR is localized in chloroplast, mitochondrial and cytosolic fractions (Hernandez *et.al.,* 2000).

Different affinities of APX (μM range) and CAT (mM range) for H_2O_2 suggest that they belong to two different classes of H_2O_2-scavenging enzymes: APX might be responsible for the fine modulation of ROS for signaling, whereas CAT might be responsible for or the removal of excess ROS during stress (Mittler, 2002). In the thylakoidal system, local concentrations of SOD and tAPX are around 1mM in the vicinity of PSI (Asada, 1999).

In last decade, functional analysis of different antioxidant enzymes were done by different methods including reverse and forward genetics, functional genomics (Seki *et.al.,* 2001, 2002; Kreps *et.al.,* 2002; Cheong *et.al.,* 2002; Buitink *et.al.,* 2006), proteomics (Rossignol *et.al.,* 2006; Jorŕn *et.al.,* 2007; Sha Valli Khan *et.al.,* 2007; Vítámvás *et.al.,* 2007) and metabolomics (Cramer *et.al.,* 2007; Kim *et.al.,* 2007;.Shulaeva *et.al.,* 2008). Transgenic plants that overexpress genes of antioxidant enzymes have analyzed (McKersie, 1999, Kiyosue, 1993). Moreover, different knockout studies provided that more than one enzymatic activity per a particular ROS can be found in each of different compartments (Orvar and Ellis, 1997; Mittler *et.al.,* 1999; Pnueli *et.al.,* 2003). The advances in plant genomics and proteomics have revealed the reactive oxygen gene network (Mittler, 2004). Combination of extensive data obtained in different microarray analysis under different types of environmental stresses via different bioinformatics tools made the interaction of different environmental stresses easier to observe (Swindell, 2006). Moreover, a combination of different omic-methods will improve our understanding of stress tolerance pathways in plants (Thiellement *et.al.,* 2002). This will lead to a new era called as plant stress systems biology or plant stress interactomics (Weckwerth, 2008; Yuan *et.al.,* 2008; Morsy *et.al.,* 2008).

1.5. Drought and Salt Stresses

Plants experience drought stress when the water supply to roots becomes difficult or when the transpiration rate becomes very high. The two conditions often coincide under arid and semi-arid regions (Reddya *et.al.,* 2004). Effects of drought and salt stresses can be observed both in physiological and biochemical processes of plants.

1.5.1. Physiological and Biochemical Effects of Drought Stress on Plants

Under drought conditions the vegetative growth of the plants reduces. Reduced cyclin-dependent kinase activity results in slower cell division as well as inhibition of growth under drought conditions. Leaf growth is generally more sensitive than root growth. Reduced leaf expansion is beneficial to plants under water deficit condition as less leaf area is exposed resulting in reduced transpiration. In accordance, many plants such as cotton subjected to drought respond by accelerating senescence and abscission of the older leaves. This process is known as leaf area adjustment. Regarding root, the relative root growth may undergo enhancement, which facilitates the capacity of the root system to extract more water from deeper soil layers (Mahajan and Tuteja, 2005).

During drought, photosynthesis rate decreases due mainly to two reasons as stomatal closure occurring under moderate water deficit conditions and non-stomatal limitations occurring under extreme drought stress.

The first response of virtually all the plants to acute water deficit is the closure of their stomata to prevent the transpirational water loss (MansWeld and Atkinson, 1990). Closure of stomata may result from direct evaporation of water from the guard cells with no metabolic involvement. This process of stomatal closure is

referred to as hydropassive closure. Stomatal closure may also depend on metabolic activities and involve processes that result in reversal of the ion fluxes that cause stomatal opening. This process of stomatal closure, which requires ions and metabolites, is known as hydroactive closure. This process seems to be ABA regulated. Hormones, in particular ABA along with cytokinins and ethylene, have been implicated in the root–shoot signaling. This long distance signaling may be mediated particularly via ABA as well as ROS. Under the water deficit condition the pH of xylem sap increases therefore promoting the loading of ABA into the root xylem and its transport to the shoot (Hartung *et.al.,* 2002). Environmental conditions that increase the rate of transpiration also result in an increase in the pH of leaf sap, which can promote ABA accumulation and lead to reduction in stomatal conductance. ABA promotes the efflux of K^+ ions from the guard cells, which results in the loss of turgor pressure leading to stomata closure (Mahajan and Tuteja, 2005).

Stomatal closure in response to a water deficit stress primarily results in decline in the rate of photosynthesis. Very severe drought conditions also result in limited photosynthesis due to decline in Rubisco activity. It has been shown that the decline in the rate of photosynthesis in drought stress is primarily due to CO_2 deficiency. Decline in intracellular CO_2 levels results in the over-reduction of components within the electron transport chain and the electrons get transferred to oxygen at photosystem I (PS I). This generates ROS (Reddy *et.al.,* 2004).

1.5.2. Physiological and Biochemical Effects of Salt Stress on Plants

Salt in soil water inhibits plant growth for two reasons. First, it reduces the plant's ability to take up water, and this leads to slower growth. This is the osmotic or water-deficit effect of salinity. Second, it may enter the transpiration stream and eventually injure cells in the transpiring leaves, further reducing growth. This is

the salt-specific or ion-excess effect of salinity. The two effects give rise to a two-phase growth response to salinity (Munns, 2005).

The basic physiology of high salt stress and drought stress overlaps with each other during the first phase of salt stress since the mechanisms controlling this phase of the growth response are not specific to salinity; they are caused by factors associated with water stress. This is supported by evidence that Na^+ and Cl^- are below toxic concentrations in the growing cells themselves, in leaves (Fricke, 2004) and roots (Jeschke *et al*., 1986). Over the timescale of days, there is much evidence to suggest that hormonal signals, rather than water relations, are controlling growth in saline soils. The evidence for this is that leaf expansion in saline soil on the timescale of days does not respond to an increase in leaf water status (Munns, 2002). First phase of salt stress causes stomatal closure and a net decrease in photosynthesis as well as production of ROS as observed in drought stress.

Water potential and osmotic potential of plants become more negative with increase in salinity, whereas turgor pressure increases with increasing salinity (Parida and Das, 2005). Plasmolysis starts when the osmotic pressure of the solution is increased above that of the cells, causing protoplast to shrink, and the plasma membrane separates from the wall. Large gaps created between the plasma membrane and the wall may fill with solution and allow an artificial apoplastic pathway for salts to move across the root.

Moreover, salinity causes increases in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter in leaves of bean and cotton (Longstreth and Nobel, 1979). Salinity also reduces intercellular spaces in leaves and mitochondrial cristae and causes swelling of endoplasmic reticulum and mitochondria, and fragmentation of tonoplast and cytoplasm (Mitsuya *et.al.,* 2000).

The second phase of the growth response results from the toxic effect of salt inside the plant and is specific to salt stress. The salt taken up by the plant concentrates in old leaves. The cause of injury is probably the salt load exceeding the ability of cells to compartmentalize salts in the vacuole. Salts would then build up rapidly in the cytoplasm and inhibit enzyme activity. Alternatively, they might build up in the cell walls and dehydrate the cell (Munns, 2005). Increased treatments of NaCl induces increase in Na^+ and Cl^- and decrease in Ca^{2+}, K^+ and Mg^{2+} leaves in a number of plants (Khan *et. al.,* 1999; Khan *et. al.,* 2000). Physiological studies have indicated that during salt stress early effects (minutes to hours) on plant growth are due to water stress, whereas salt-specific effects only appear much later (days to weeks) (Denby and Gehring, 2005; Munns, 2002).

1.5.3. Drought and Salt Defense Mechanisms in Plants

Both drought and salt stresses cause osmotic and oxidative stress in plants. Plants have evolved different main defense mechanism activated under osmotic and oxidative stresses. These mechanisms are identical in both drought and salt stresses. One of the defense mechanisms is avoidance, which is the mechanism adopted by desert plants. Another defense mechanism is tolerance, which is crucial for survival under severe stress conditions (Kalefetoglu and Ekmekci, 2005). Plants have developed a plethora of biochemical and molecular mechanism to tolerate osmotic and oxidative stresses. Biochemical strategies include synthesis of compatible solutes; change in photosynthetic pathway; alteration in membrane structure; induction of antioxidant enzymes; and induction of plant hormones (Iyengar and Reddy, 1996; Ingram and Bartels, 1996; Parida and Das, 2005).

Plants adopted to live in saline environments have developed some additional biochemical and molecular strategies to cope with deleterious effects of ion accumulation in cytosol. Biochemical strategies include selective accumulation or exclusion of ions; control of ion uptake by roots and transport into leaves; and compartmentalization of ions (Munns, 2002).

1.5.4. Molecular Responses to Drought and Salt Stresses

Responses to drought stress may occur within a few seconds (such as a change in the phosphorylation status of a protein) or within minutes and hours (such as a change in gene expression) (Zhu, 2002). Stress responsive genes can be divided into two as 'early-response genes' and 'delayed-response genes'. Early-response genes are induced very quickly and often transiently. Their induction does not require new protein synthesis because all signaling components are already in place. Delayed-response genes are activated by stress more slowly, and their expression is often sustained. They constitute the vast majority of the stress-responsive genes. The early-response genes typically encode transcription factors that activate downstream delayed-response genes, which take a role in stress tolerance (Kalefetoglu and Ekmekci, 2005; Sreenivasulu *et.al.,* 2007).

Early-Response Genes: Transcription Factors

The stress responses of plants are regulated by multiple signaling pathways, and there is a significant overlap between the patterns of gene expression that are induced in plants in response to different stresses (Glazebrook, 2001). This overlap is coordinately regulated by transcription factors (Singh *et.al.,* 2002). Transcriptome analysis using microarray technology, together with conventional approaches, has revealed that dozens of transcription factors are involved in the plant response to various stresses (Vinocur and Altman, 2005). Most of these

transcription factors fall into several large transcription factor families such as AP2/ERF, bZIP, NAC, MYB, MYC, Csy2His2 zinc-finger and WRKY (Umezawa et.al., 2006).

Gene expression under stress conditions is governed by ABA-dependent and ABA-independent regulatory pathways. (Yamaguchi-Shinozaki and Shinozaki, 2005). The promoter of a drought-, high salinity-, and cold inducible gene, RD29A/COR78/LTI78, contains two major cis-acting elements, ABRE (ABA-responsive element) and DRE (dehydration-responsive element)/CRT (C-Repeat), both of which are involved in stress-inducible gene expression (Yamaguchi-Shinozaki and Shinozaki, 1994). ABRE and DRE/CRT are cis-acting elements that function in ABA-dependent and ABA-independent gene expression, respectively, in response to abiotic stresses. The AREB/ABF proteins require an ABA-mediated signal for their activation. Transcription factors belonging to the ERF/AP2 family that bind to these DRE/CRT elements were isolated and termed CBF/DREB1 and DREB2 (Yamaguchi-Shinozaki and Shinozaki, 2005). The CBF/DREB1 genes are rapidly and transiently induced by cold stress (Jaglo-Ottosen *et al.,* 1998; Liu *et al.,* 1998), whereas DREB2 genes are induced by dehydration stress.

Recently, a drought-inducible RD26 gene encoding a NAC transcription factor was identified (Fujita *et al.,* 2004). Expression of this RD26 NAC transcription factor gene is induced by drought, high salinity, ABA, and JA treatments (Shinozaki and Yamaguchi-Shinozaki, 2007).

Delayed-Response Genes

Delayed-response genes can be divided into four groups such as osmolyte biosynthesis, protection of cell integrity, ion homeostatis, and antioxidant protection (Sangam *et.al.,* 2005; Valliyodan and Nguyen, 2006).

1) Genes responsible for osmolyte biosynthesis

Molecules having a protective function include small organic compounds that are called osmolytes, osmoprotectants or compatible solutes. These molecules are small, electrically neutral and non-toxic even at molar concentrations (Reddy *et.al.,* 2004). They would be important to adapt plants to drought, as they could enhance osmotic adjustment and allow turgor maintenance of cells that would otherwise dehydrate. Tissue dehydration is less likely in salinity than drought, as the uptake of NaCl promotes osmotic adjustment, but compatible solutes would be essential to balance the osmotic pressure of the cytoplasm where salts accumulated preferentially in the vacuole (Munns, 2005). There are four main classes of solute that could have an osmotic or protective role: N-containing solutes such as proline and glycine betaine; sugars such as sucrose and trehalose; straight-chain polyhydric alcohols (polyols) such as mannitol and sorbitol; and cyclic polyhydric alcohols (cyclic polyols).

Many plants accumulate proline under stress conditions. Singh *et al.* (1972) were the first to assign a correlation between proline accumulation and drought resistance in barley cultivars. They showed that drought resistant cultivars accumulated many fold higher free proline than the susceptible cultivars. Proline also accumulates in leaves, stems and roots of *Pringlea antiscorbutica* (Aubert *et.al.,* 1999). Moreover, the gene for its synthesis, *P5CS* (Δ-1-pyrroline-5-carboxylate synthetase), is induced rapidly by stress (Hong *et.al.,* 2000). Proline

also operates as a chemical chaperon protecting native conformation of macromolecules under stress conditions (Mokhamed *et.al.,* 2006).

Glycine-betaine accumulation is observed in some higher plants (Khan *et.al.,* 2000; Wang and Nil, 2000) Most of the Glycine-betaine is synthesized in chloroplasts by two enzymes, namely choline monooxygenase and betaine aldehyde dehydrogenase. Its synthesis can be induced by both drought and salt stresses (Nakamura *et.al.,* 2001). Glycine-betaine has been shown to protect enzymes and membranes and also to stabilize PSII protein pigment complexes under stresses (Papageorgious and Morata, 1995).

Carbohydrates such as sucrose and trehalose accumulate under various stresses in higher plants (Guy, 1990; Balibrea *et.al.,* 1997; Ramos *et.al.,* 1999; Wang *et.al.,* 2000; Kerepesi and Galiba, 2000; El-Bashiti *et.al.,* 2005) They confer protective effects at two levels, by the stabilization of membranes and lipid assemblies at very low hydration, and by the stabilization of biological macromolecules (e.g., proteins) in the folded state under stress conditions that would normally promote their denaturation (Lou *et.al.,* 2008).

Polyols such as sorbitol, mannitol or pinitol function in two ways that are difficult to separate mechanistically: osmotic adjustment and osmoprotection. These compounds have hydrogen-bonding characteristics that allow them to protect macromolecules from the adverse effects of increasing ionic strength in the surrounding media (Crowe *et.al.,* 1992). Transgenic approaches for improvement of drought and salt tolerance in different types of plants by using genes of polyol synthesis pathways have proved that polyols mainly function in turgor maintenance (Bohnert and Jensen, 1996; Sheveleva *et.al.,* 1997; Gao *et.al.,* 2001; Abebe et.al., 2003).

Related to salt stres, compatible solutes would exert their effects in phase 1, but could also function in phase 2. The effect of osmoprotectants that accumulate to only low concentrations would be seen only in phase 2. These would protect metabolic function in cells in which salt concentrations are high. They are therefore most likely to function to affect growth in the long term (Munns, 2005).

2) Genes responsible for ion homeostatis

Because the stress, especially the salt stress, disrupts ion homeostatis in plant cells plants have adopted some strategies to attain ion homeostatis. There are two main mechanisms for maintenance of ion homeostatis: exclusion of ions, and compartmentalization of ions (Parida and Das, 2005). Ion concentrations in cytosol are maintained in a balance by help of various ion channels.

Removal of sodium from the cytoplasm or compartmentalization in the vacuoles is done by a salt-inducible enzyme, Na^+/H^+ antiporter (Apse et.al., 1999). Na^+ extrusion from plant cells is powered by the operation of the plasma membrane H^+-ATPase generating an electrochemical H^+ gradient that allows plasma membrane Na^+/H^+ antiporters to couple the passive movement of H^+ inside the cells, along its electrochemical potential, to the active extrusion of Na^+ (Yamaguchi and Blumwald, 2005). Molecular genetic analysis of *Arabidopsis sos* mutants have led to the identification of a plasma membrane Na^+/H^+ antiporter, SOS1, which play a role in salt stress sensing. The SOS1 transcript level is up-regulated under salt stress (Shi *et.al.,* 2002). Sodium efflux through SOS1 under salinity is regulated by SOS3-SOS2 kinase complex (Chinnusamy *et.al.,* 2005).

Na^+ sequestration into the vacuole depends on expression and activity of not only Na^+/H^+ antiporters, but also on V-type H^+-ATPase and H^+-PPase. These phosphatases generate the necessary proton gradient required for activity of Na^+/H^+ antiporters. The tonoplast Na^+/H^+ antiporter *NHX1* gene is induced by both salinity and ABA in *Arabidopsis* (Shi and Zhu, 2002) and rice (Fukuda et al., 1999). The *AtNHX1* promoter contains putative ABA responsive elements (ABRE), hence *AtNHX1* expression under salt stress is partially dependens ABA biosynthesis (Horie and Schroeder, 2004).

Another ion carrier channel, AtHKT1, has been shown to function as a selective Na^+ transporter in Arabidopsis (Yamaguchi and Blumwald, 2005). AtHKT1 was identified as a putative regulator of Na^+ influx in plant roots. Based on various research results, AtHKT1 was proposed to play a role in long-distance Na^+ transport and Na^+ circulation in the plant, with AtHKT1 mediating Na^+ loading into the leaf phloem and Na^+ unloading from the root phloem sap (Berthomieu *et.al.,* 2003).

3) Genes responsible for cellular integrity

Late Embryogenesis Abundant (LEA) proteins are formed during the late period of seed development accompanied by dehydration. They are proteins with small molecular weight ranging mainly from 10 to 30 kDa and above 30 kDa (Zhang and Zhao, 2003). Researchers detected their existence in different higher plants. They exist mainly in higher plant seeds, but have also been found in seedlings, roots and other organs. LEA proteins are mainly localized in cytoplasm and nuclear regions. They mainly function in whole-plant stress resistance to drought, salt, and cold (Hong-Boa *et.al.,* 2003).

Heat shock proteins (HSPs) are a family of proteins found in plants. They are located both in cytoplasm and organelles such as nucleus, mitochondria, chloroplast and endoplasmic reticulum. They take a role as molecular chaperones in protein folding and post-translational modification processes. They also prevent the aggregation of denatured proteins caused by drought, heat and other stresses and ensure the renaturation of aggregated proteins (Boston *et.al.,* 1996; Iba, 2002).

There are some recent physiological and biochemical studies related to effects of environmental stresses on antioxidant enzyme systems in lentil (Katerji *et.al.,* 2001; Cicerali, 2004; Saker *et.al.,* 2005; Ercan, 2007; Sidari *et.al.,* 2008) However, there are not any molecular studies in the literature about those systems in lentil

1.6 Aim of the Study

In this study, the effects of drought and salt stress applications on gene expression profiles of antioxidant enzymes in a Turkish lentil (*Lens culinaris* M.) cultivar, Sultan-1, was comparatively analyzed. Seedlings were grown for 7 days on hydroponic culture and then stresses applied. The following criteria were assayed in the study:

1) Short term (6 hours, 12 hours, and 24 hours of stress applications) and long term (3 days, 5 days, and 7 days of stress applications) effects of both drought (20% PEG) and salt (150mM NaCl) stresses,
2) Effects of both drought and salt stresses in two different tissues as shoot and root,
3) Analysis of gene expression levels of Mn-SOD, Cu/Zn-SOD antioxidant enzymes were analyzed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemical Materials and Kits

The chemicals and kits with their suppliers are listed in Appendix A.

2.1.2. Plant Materials

In this study, a Turkish lentil (Lens culinaris M.) cultivar, Sultan-1, was used. Sultan-1 is a spring-sown, green, macrosperma lentil cultivar and the weight of 1000 seeds is 59.1-62.3 grams. It is cultivated in Central Anatolia and in transition regions. The seeds were obtained from Eskisehir Anadolu Agricultural Research Institute (ATAEM).

2.2. Methods

2.2.1. Growth of Plants

Seeds were surface sterilized with 20% Ethanol for 30 seconds and then washed with distilled water for three times.

After sterilization, seeds were imbibed in distilled water for 14 hours at dark. Imbibed seeds were transferred to plastic pots covered with cheesecloth for support and were grown in hydroponic culture containing ½ strength Hoagland's Solution (Hoagland and Arnon, 1950). Each pot contains eight seeds. Planted seeds were grown for 7 days in a plant growth chamber at 25 ± 2ºC with 16 hours light and 8 hours dark photoperiod at a light intensity of 40 µmol $m^{-2}s^{-1}$.

2.2.2. Application of Drought and Salt Stress

Drought and salt stress treatments were initiated at 7th day of normal growth. ½ strength Hoagland's Solution was replaced with 20% polyethylene glycol 6000 (PEG-6000) or 150mM of sodium chloride (NaCl) for drought or salt stresses, respectively. Both treated (stress) and non-treated (control) plants were kept in the growth chamber at same growth conditions. Plants were removed from the growth chamber for sample collection at 6th, 12th and 24th hours, and on 3rd, 5th and 7th days of stress application.

2.2.3. Total RNA Isolation

A modified procedure of total RNA isolation was done from pre-frozen control and stress samples according to Chomczynski (1993). 0.2 g of pre-frozen tissue from control and stress samples were disrupted in liquid nitrogen and disrupted tissue samples were transferred into DEPC-treated and pre-cooled 1.5 mL-eppendorf tubes. Later, 1 mL of Trizol Reagent (the preparation of which is given in Appendix B) was added into the eppendorf tubes. The tubes were vortexed for 20 minutes in a block shaker at room temperature ($24^{O}C$). Then, the samples were centrifuged for 6 minutes and re-centrifuged for 3 minutes by converting the eppendorf tubes in reverse direction at room temperature at 21000 g (at max.speed). After 900µL of supernatant was transferred into new eppendorf tube 200µL chloroform was added and the tubes were shaken vigorously for 15

seconds, and later were incubated at room temperature for 6 minutes. Following the incubation, eppendorf tubes were centrifuged for 20 minutes at $4^{O}C$ at 21000 g. 450µL from upper phase was taken into new eppendorf tubes and 200µL chloroform was added onto them. Later the tubes were shaken vigorously for 15 seconds and were incubated at room temperature for 3 minutes, which is followed by centrifugation of eppendorf tubes for 5 minutes at room temperature at 21000 g. 350µL from upper phase was taken into new eppendorf tubes and 350µL of cold isopropanol was added onto them. The tubes were mixed by inverting several times and were incubated at room temperature for 10 minutes. After incubation, RNAs were collected as pellets at the bottom of the tubes by centrifugation for 10 minutes at room temperature at 21000 g. Later, the supernatant was taken off and 1.0mL 75% cold ethanol was added into tubes.

Following the ethanol addition the tubes were vortexed briefly to wash the pellet and then the tubes were left for 3 minutes at room temperature. Later, the tubes were centrifuged for 5 minutes at room temperature at 21000 g and the supernatant was taken off. The tubes were centrifuged for an additional 15 seconds at room temperature to collect the pellet at the bottom of the tubes. Finally, any visible liquid in the tubes were removed and the tubes were air dried in pre-sterilized hood for 10 minutes. 40µL of DEPC-treated water was added into the tubes and the tubes were incubated at $65^{O}C$ for 15 minutes by vortexing once in every 5 minutes to dissolve the pellet. The tubes were centrifuged for 5 seconds and kept at -80 ^{O}C for long term storage.

2.2.4. RNA Quality and Quantity Analysis

RNA quality and quantity was determined photometrically by using a double beam spectrophotometer (Carry 100 UV-Vis) at Molecular Biology and Biotechnology R&D Center Lab. Absorbance of sample in 10mM Tris/HCl (pH:8.0) at 260nm (A_{260}) and 280nm (A_{280}) was obtained [1 absorbance unit =

40µg/mL RNA]. The quality of RNAs was assayed by the ratio of A_{260} to A_{280}. The quantity of RNAs was calculated by the following equation:

Concentration = A_{260} X 0.04 X DF, where A_{260} is the absorbance of sample at 260 nm and DF is dilution factor (DF =200).

Later, the quality of RNA samples was verified also by gel electrophoresis. For that reason, RNase-free 1% agarose gels in 1X TAE solution was prepared. Pre-diluted (by DNase-treated water) RNA samples were loaded into the gels and run for 40 minutes at 100V. Observation of 25S rRNA and 18S rRNA bands in the gels clarified the integrity of RNA samples.

2.2.5. Removal of DNA

Any DNA contaminations were removed by using Fermentas DNase I according to the manufacturer's manual. 1µg of RNA sample, 1µL of 10X reaction buffer with $MgCl_2$, 1µL of Deoxyribonuclease I (1u/µL) was added into an RNase-free eppendorf tube and finally DEPC-treated water was added to make the final volume of the mixture 10µL. Then, the tube was incubated at 37ºC for 30 minutes. After incubation, 1µL of 25mM EDTA was added into the tube and finally, the tube was incubated at 65 ºC for 10 minutes.

2.2.6. Sodium Acetate Precipitation of RNA Samples

DNase treated RNA samples were precipitated by 3 M sodium acetate (NaOAc) solution (pH = 5.2). First, total volumes of DNase treated RNA samples in eppendorf tubes were calculated and required volume of 3 M sodium acetate solution was added into the tubes so as to make the final concentration of sodium acetate equal to 0.3 M. The tubes were vortexed. Required volume of previously cooled 100% ethanol (molecular biology grade) was added into the tubes so as to

make the final concentration of ethanol equal to 70%. The tubes were mixed by inverting several times and then incubated at -20ºC overnight. Later, the tubes were centrifuged for 30 minutes at 4ºC at 21000 g and ethanol was removed carefully. Then, 1 mL of 70% cold ethanol was added into the tubes and the tubes were incubated 3 minutes at room temperature. The tubes were centrifuged for 5 minutes at room temperature at 21000 g. After the removal of any visible liquid, the tubes were air dried in laminar flow for 10 minutes. Then, 15µL of DEPC-treated water was added to dissolve RNAs and the tubes were kept at 65 ºC for 5 minutes. Finally, samples were mixed and centrifuged for 5 seconds and kept at -80 ^{O}C for long term storage.

RNA quality and quantity was determined by spectrophotometry and electrophoresis as previously described. Later, any DNA contamination was also assayed by polymerase chain reaction (PCR) of a known sequence (Cu/Zn-SOD [amplicon length is 224 bp]). For this PCR, 1µL of RNA was put into a PCR tube as template. Then, the followings were added as indicated order: 7.75µL of PCR water, 5 µL of 10X complete reaction buffer, 5 µL of 2.0mM dNTP mix, 3 µL of both Cu/Zn-SOD right and left primers (concentration of each is 10 µM), 0.25 µL of Taq polymerase (5U/1 µL). 25 µL of PCR mixture of each samples were amplified by a PCR program, conditions of which was previously optimized (The PCR conditions were given in Appendix C). Then, PCR products were loaded into 2% agarose gels and run first for 20 minutes at 50V and then for 60 minutes at 100V. No band formation was expected at the end of gel electrophoresis since RNA samples were used as template in PCR amplification.

2.2.7. cDNA synthesis from RNA samples

cDNAs were synthesized from DNase-treated RNA samples according to the manual of Fermentas RevertAid™ 1st strand cDNA Synthesis Kit. 2.5µg of total RNA samples and 1 µL of oligo$(dT)_{18}$ primers (0.5µg/µL) were added into

DEPC-treated PCR tubes, and total volume was rolled up to 12 µL by addition of DEPC-treated water. Then, the compounds were mixed gently and spin down for 3-5 seconds in a microcentrifuge. PCR tubes were incubated at 70ºC for 5 minutes, chilled on ice and briefly centrifuged to collect drops. The tube was placed on ice and the following components were added in indicated order: 4 µL of 5X reaction buffer, 1 µL RiboLock™ Ribonuclease Inhibitor (20U/ µL) and 2 µL of 10mM dNTP mix. Later, they were mixed gently, centrifuged for 3-4 seconds and the tubes were incubated at 37 ºC for 5 minutes. After the incubation, 1 µL of RevertAid™ M-MuLV Reverse Transcriptase (200U/ µL) was added and the tubes were incubated at 42 ºC for 60 minutes. Finally, the tubes were incubated at 70 ºC for 10 minutes to stop the reaction and chilled on ice.

2.2.8. Degenerate PCRs

To date, there are no available data entry in GenBank database (Benson *et.al.,* 2008) related to genes that code for antioxidant enzymes in lentil (*Lens culinaris* M.). Degenerate PCR is a technique applied for identification of new genes or gene families (Telenius *et.al.,* 1992). It resembles standard PCR with an exception in primers, that is degenerate primers are used instead of specific primers. Degenerate primers are different from specific primers in that they contain wobbles so that there is more than one possibility for primer-template binding for PCR.

A protein search for antioxidant enzymes, namely SOD*, APX, CAT and GR, were done in NCBI (http://www.ncbi.nlm.nih.gov/). At one time, the word "Arabidopsis" and name of a different enzyme were searched together to obtain the protein sequence of that enzyme in *Arabidopsis*. Then, protein blast by "pblast" was done for each sequence obtained in previous search. At the end of pblast, protein sequences found in relatives of *Lens culinaris* were obtained in FASTA format. mRNA complete sequences (cds) for those protein sequences

were also obtained in FASTA format from NCBI. Later, both protein and mRNA sequences were aligned by CLUSTALW program (http://www.ebi.ac.uk/Tools/clustalw2/index.html). Protein and mRNA sequences are given in Appendix J. Then, degenerate primers were designed by an algorithm called as Consensus Degenerate Hybrid Oligonucleotide Primer (CODEHOP) (http://bioinformatics.weizmann.ac.il/blocks/codehop.html) (Rose *et.al.,* 1998). Both forward and reverse primers were tested whether they form homo- and/or hetero-dimers, and for their melting temperature (Tm), GC content and lengths by OligoAnalyzer 3.1, a publicly available program (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/Default.aspx). Unless the primers were verified to be appropriate for degenerate PCR degenerate primers were designed by comparison from the most conserved regions of aligned sequences. Wobbles were added at 3' end of the primers. Then, primers were tested out again by OligoAnalyzer 3.1 (The parameters and sequences of degenerate primers are given in Appendix D). Primers were obtained from Integrated DNA Technologies (Belgium).

Degenerate PCR conditions for all genes were optimized in a stepwise manner. First, initial denaturation time and temperature, denaturation time and temperature, primer annealing time and temperature, elongation time, and final extension time were optimized. Later, final concentrations of components of reaction mixture, namely PCR buffer, dNTP mix, Taq DNA polymerase, template, $MgCl_2$, forward and reverse primers, were also optimized. Finally, the best number of cycles was optimized. Critical steps of PCR optimization were given in the results whereas the resultant optimized PCR conditions were given in Appendix C.

* Specific primers of Barley Cu/Zn SOD were used to amplify the Lentil Cu/Zn SOD gene. Specific primers of Barley Cu/Zn SOD were designed after degenerate PCR amplification of related sequence in a previous study (Kayıhan, 2007).

2.2.9. Gel Elution of PCR Products & Sequencing

PCR products were extracted from agarose gels by GeneMark Gel Elution Kit (Cat. No.:DP03-300). Desired bands were cut out with a scalpel after electrophoresis of PCR products in % 0.8 agarose gels for 40 minutes at 75V. Then, the gel slices were transferred into a sterile 1.5mL microcentrifuge tube and equal volume of Binding solution was added into the tube, the tube was incubated at 60 ºC for 15 minutes. Then, according to the manual spin column was inserted into a collection tube and solution was transferred into the spin column. The tube was centrifuged at 13.000g for 1 minute and filtrate in the collection tube was discarded. Then, 500µL of binding solution was added into the spin column and the tube was centrifuged at 13.000g for 1 minute, filtrate was discarded. Later, 700 µL of washing solution was added into the spin column and the tube was centrifuged at 13.000g for 1 minute (This step was repeated one more time). The filtrate was discarded and the tube was centrifuged for 5 minutes at 13.000g. Finally, spin column was transferred into a new microcentrifuge tube and 15 µL of elution solution was added into the column, the tube was incubated at room temperature for 2 minutes. Later, the tube was centrifuged at 13.000g for 1 minute to elute DNA and the tube was stored at -20 ºC for further use.

Extracted PCR products were sent to RefGen Gen Araştırmaları ve Biyoteknoloji Ltd. Şti.for sequencing, which was done by ABI 310 Capillary DNA Sequencer in two directions as forward and reverse. Then, reverse sequence was inverted and the two sequences were aligned in CLUSTALW to check the sequencing. The sequences for all extracted PCR products were given in Appendix E.

A database analysis was done for each sequence in NCBI by tblastx and blastn search to test out the homology of the sequences. Results of each search were given in Appendix F and Appendix G, respectively.

2.2.10. PCR Optimizations

Specific primers for all genes were designed by PRIMER 3 program (http://fokker.wi.mit.edu/primer3/input.htm) and primers were checked by OligoAnalyzer 3.1. Primers were supplied by Integrated DNA Technologies (Belgium).

Initial denaturation time and temperature, denaturation time and temperature, primer annealing time and temperature, elongation time, final extension time, and number of cycles were optimized for the best PCR conditions. In addition, final concentrations of components of reaction mixture were also optimized. The optimized PCR conditions are given in Appendix C.

2.2.11. Multiplex PCR optimizations

Multiplex polymerase chain reaction (PCR) is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction (Henegariu *et.al.,* 1997). One of the usage areas of Multiplex PCR is reverse transcription PCR (RT-PCR) (Crisan, 1994), in which expression levels of genes can be quantified. *Arabidopsis* 18S rRNA gene was used as an internal control because it was found to be a house-keeping gene, the expression level of which is stable developmentally, under different environmental conditions and in different tissues (Brunner *et.al.,* 2004). This was also proven by this work and the results were presented in next chapter. *Arabidopsis* 18S rRNA and one of the antioxidant enzyme genes were simultaneously amplified in the same PCR tube and run in agarose gel.

Arabidopsis 18S rRNA was first amplified by optimized PCR conditions and run in 2% agarose gel. One band at 250bp was observed and then, this band was extracted from the gel and sequenced. Sequence results were given in Appendix E.

Parameters of multiplex PCR were first optimized according to Henegariu *et.al.* (1997). Optimized PCR conditions are given in Appendix H while the optimization results are presented in the next chapter.

2.2.12. Semi-Quantitative RT-PCR

Previously optimized PCR conditions for each gene, which are given in Appendix H, were used in semi-quantitative analysis of mRNA levels by RT-PCR (Weiss and Albermann, 2003). 25µL of PCR mix was prepared for each multiplex PCR. After PCR amplification, PCR products were run in 2% agarose gel electrophoresis in 1X TAE and photographs of ethidium-stained gels were taken by Vilber Gel Imager. A 50-1000bp DNA marker (Fermentas SMO371) was loaded into each gel to observe the precise band formation after electrophoresis. A negative control (which contains PCR product without template) was also loaded into the same gel to observe any type of contamination in PCR amplification. At least 3 separate multiplex PCR amplifications and agarose gel electrophoresis were done. The bands were quantified by Scion Image Analyzer (Scion Corporation, USA). Optical density of bands that represent *Arabidopsis* 18S rRNA and genes were obtained and ratio of gene band intensity to 18S rRNA band intensity was calculated for normalization of data.

2.2.13. Statistical Analysis

Data obtained in the study were analyzed with one-way analysis of variance (ANOVA) by using MINITAB 15 program (MINITAB Inc., USA). Differences were considered significant where P value was less than 0.05 ($p<0.05$) or 0.01 ($p<0.01$), where necessary, in Tukey's Test.

CHAPTER 3

RESULTS

A Turkish lentil (*Lens culinaris* M.) cultivar, Sultan-1, seedlings were exposed to 150mM of NaCl and 20% of polyethylene glycol (PEG) treatments after 7 days of normal growth in ½ strength Hogland's Solution (Hoagland and Arnon, 1950). The exposure time of plantlets varied within short term (6 hours, 12 hours, and 24 hours of stress applications) and long term (3 days, 5 days, and 7 days of stress applications) intervals. Both shoot and root tissues were used to isolate total RNA samples after indicated stress durations. Alteration in transcript expression levels of two superoxide dismutase isozymes, namely Mangane superoxide dismutase (Mn-SOD) and Copper/Zinc superoxide dismutase (Cu/Zn-SOD), were analyzed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique and the results were provided below.

3.1 Total RNA Isolation and Removal of DNA

Both the quality and the quantity of total RNA samples were determined photometrically by Varian Carry 100 UV Vis Spectrophotometer at Central Laboratory (METU). The quality of RNA samples was also verified by %1 agarose gel electrophoresis, the results of which are presented below.

Observation of 25S rRNA and 18S rRNA bands in the gels clarified the integrity of RNA samples. Any kind of DNA contamination might be observed as bands in the wells or any smear formation above the rRNA bands. The higher molecular weight species (hnRNA) might be visible above the 28S rRNA in some total RNA samples (Farrell, 2005).

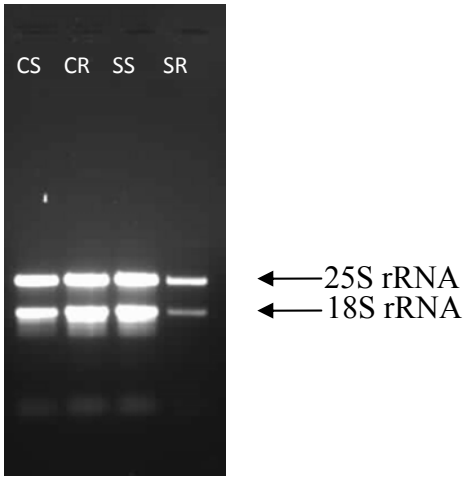

Figure 3.1. Gel electrophoresis results of total RNA samples taken from 150mM NaCl-treated plant tissues for 3 days after 7 days of normal growth. RNase-free 1% agarose gel in 1X TAE solution was run for 40 minutes at 100V. Each lane was loaded with 2µL of following total RNA samples, **CS:** Control shoot; **CR:** Control root; **SS:** Stressed shoot; **SR:** Stressed root.

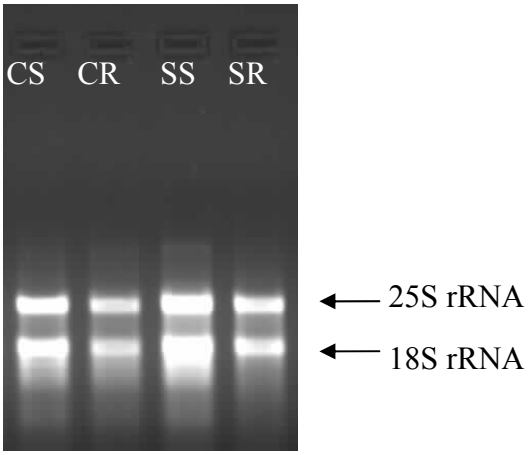

Figure 3.2. Gel electrophoresis results of DNase-treated total RNA samples taken from 150mM NaCl-treated plant tissues for 3 days after 7 days of normal growth. RNase-free 1% agarose gel in 1X TAE solution was run for 40 minutes at 100V. Each lane was loaded with 2µL of following total RNA samples, **CS:** Control shoot; **CR:** Control root; **SS:** Stressed shoot; **SR:** Stressed root.

As observed in Figure 3.2, visible DNA contamination was eliminated by DNase I treatment. The results of agarose gel electrophoresis were consistent with the spectrophotometric results as a decrease in the A_{260}/A_{280} ratios of samples was observed after DNase I treatment.

A previously known sequence of Barley Cu/Zn-SOD gene (amplicon length is 224 bp) was amplified by PCR conditions that were given in Appendix C to check any further DNA contaminations. For this reason, 1µL of RNA (final amount in PCR mixture was approximately 50ng) was used as template. 10µL of PCR products were run on 2% agarose gels. No band formation was observed at the end of gel electrophoresis since RNA samples were used as templates in PCR amplification. This check-point demonstrated a negative control for DNA contamination as given in Figure 3.3.

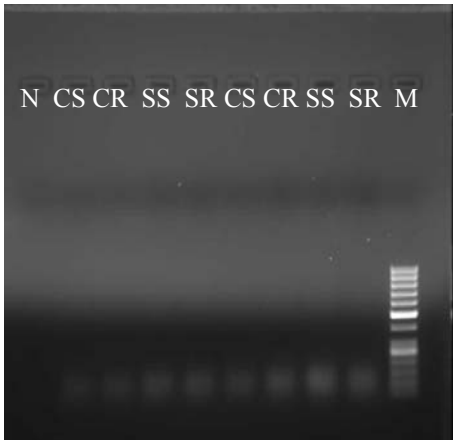

Figure 3.3. Gel electrophoresis results of PCR amplification of total RNA samples taken from 150mM NaCl-treated plant tissues for 3 days after 7 days of normal growth. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **CS:** Control shoot; **CR:** Control root; **SS:** Stressed shoot; **SR:** Stressed root; **N:** Negative control (no template); **M:** 50bp DNA Marker (Fermentas SM0371).

3.2 cDNA Synthesis from DNase Treated RNA Samples

To check if the cDNAs were properly synthesized, a previously known sequence of Barley Cu/Zn-SOD gene (amplicon length is 224 bp) was amplified by PCR conditions (Appendix C). These results were used as positive controls of cDNAs. Moreover, control primers and control template, which were supplied with Fermentas RevertAid™ 1st strand cDNA Synthesis Kit, were used as positive controls to check any further synthesis defects. The results of PCR amplification of 224 bp Cu/Zn-SOD amplicon are given in Figure 3.4.

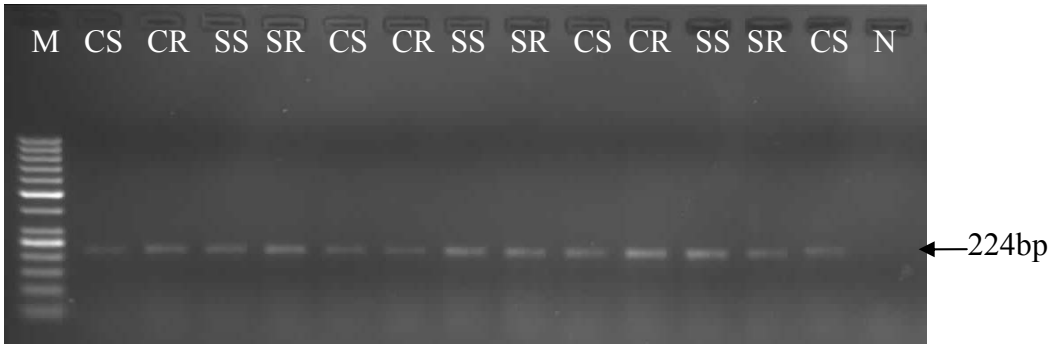

Figure 3.4. Gel electrophoresis results of PCR amplification of total RNA samples taken from 150mM NaCl-treated plant tissues for 3 days after 7 days of normal growth. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **CS:** Control shoot; **CR:** Control root; **SS:** Stressed shoot; **SR:** Stressed root; **N:** Negative control (no template); **M:** 50bp DNA Marker (Fermentas SM0371).

3.3 Degenerate PCR Optimizations

As there were no available data entry in GenBank database (Benson *et.al.,* 2008) related to genes that code for antioxidant enzymes in lentil (*Lens culinaris* M.), degenerate PCR was used to identify the genes encoding those enzymes. After "pblast" analysis done in NCBI database (the results of pblast analysis are provided in Appendix I) and multiple alignments of protein sequences found in those database searches, degenerate primers were obtained from Integrated DNA Technologies.

The main advantage of using degenerate primers is that they are expected to bind to many different regions including the desired target sequence and amplify those in normal PCR conditions. In common with normal PCR, degenerate PCR conditions have to be optimized to obtain the highest product yields. For this

reason, the final concentrations of some PCR ingredients were altered in a way that the yield of PCR products was observed to be gradually increased in each successive step.

3.3.1 Degenerate PCR Optimization of Mn SOD Transcript

Both forward and reverse degenerate primers for Mangane Superoxide Dismutase (Mn SOD) gene were tested if they form homo- and/or hetero-dimers, and for their melting temperature (Tm), GC content and lengths. All these verifications were done by OligoAnalyzer3.1 and the results were presented in Appendix D. According to those results, annealing temperature (T_A) of degenerate Mn SOD primers was expected to be within the range of 56.2-56.4ºC. Hence, the first conditional parameters to be checked were given in Table 3.1.a and Table 3.1.b. The results of PCR amplifications were demonstrated in Figure 3.5.

Table 3.1.a. Degenerate Mn SOD PCR Ingredients (Two different template concentrations were used)

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Mn SOD Right Degenerate Primer (10 µM)	1.2µM
Mn SOD Left Degenerate Primer (10 µM)	1.2µM
Taq DNA Polymerase (5U/1µL)	0.75U
Template	**50ng or 100ng**
25mM $MgCl_2$	1.5mM

Table 3.1.b. Degenerate MnSOD PCR Conditions (Two different PCR conditions were applied)

Condition A

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	2 min.
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	56ºC	45 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

Contidion B

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	2 min.
Cycle number = 5		
Denaturation	94ºC	30 sec.
Primer annealing	46ºC	45 sec.
Elongation	72ºC	1 min.
Cycle number = 35		
Denaturation	94ºC	30 sec.
Primer annealing	56ºC	45 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

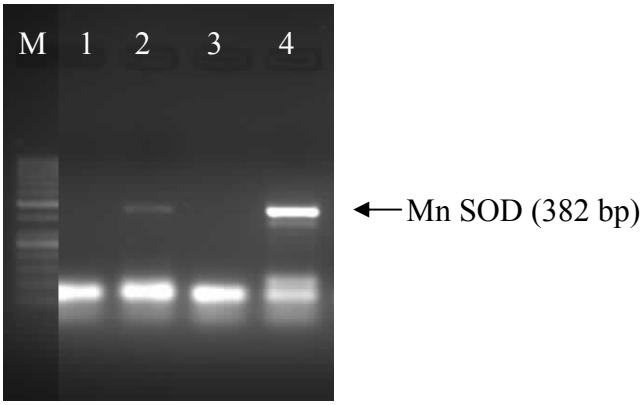

Figure 3.5. Gel electrophoresis results of PCR amplification of MnSOD transcript by degenerate primers. Amplicon length is 382 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products. **1:** Condition A with final template concentration of 50ng; **2:** Condition B with final template concentration of 50ng; **3:** Condition A with final template concentration of 100ng; **4:** Condition B with final template concentration of 100ng; **M:** 50bp DNA Marker (Fermentas SM0371).

Figure 3.5 clearly demonstrates that the doubled concentration of template is critical in addition to condition B, which includes a PCR step of 5 cycles during which primer annealing temperature is 10ºC lower than the expected T_A. By this way, degenerates primers were able to bind more easily both to specific and non-specific sequences. It is important to note that primer-dimers were observed in all lanes because of the degenerate nature of the primer pairs. As the degeneracy increases, the probability of primer-dimers and non-specific bindings increases.

When the condition of degenerate Mn SOD PCR were kept constant, but final primer concentrations were decreased to 0.8 µM while increasing final Taq

concentration to 0.1U, band intensity of PCR products was observed to be decreased (Figure 3.6).

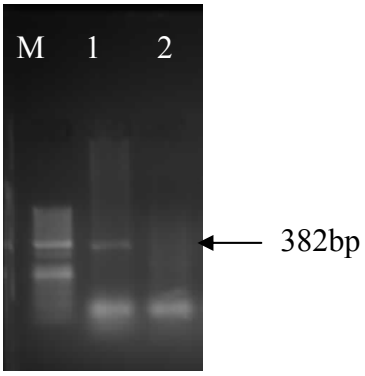

Figure 3.6. Gel electrophoresis results of PCR amplification of MnSOD transcript by degenerate primers. Amplicon length is 382 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** Mn SOD; **2:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

3.3.2. Degenerate PCR Optimization of CAT Transcript

The same verifications for catalase (CAT) gene were done by OligoAnalyzer 3.1 and the results were presented in Appendix D. According to those results, annealing temperature (T_A) of degenerate CAT primers was expected to be within the range of 53.4-57.4ºC. Firstly, best primer annealing temperature was tested by Graded PCR (T_A changed in the range of 45.0-57.0 ºC). Hence, the first conditional parameters to be checked were given in Table 3.2.a and Table 3.2.b. The results of PCR amplifications were demonstrated in Figure 3.7.

Table 3.2.a. Degenerate CAT PCR Ingredients

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
CAT Right Degenerate Primer (10 µM)	0.6µM
CAT Left Degenerate Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	0.05U
Template	50ng
25mM $MgCl_2$	1.5mM

Table 3.2.b. Degenerate CAT PCR Conditions

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	45-57ºC	30 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

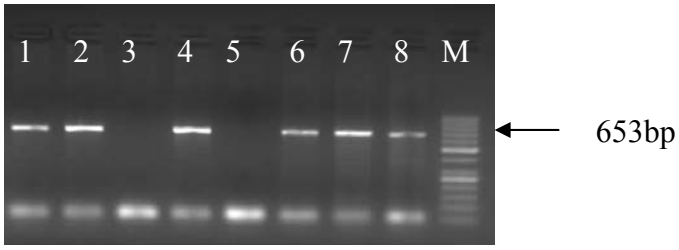

Figure 3.7. Gel electrophoresis results of PCR amplification of CAT transcript by degenerate primers. Amplicon length is 653 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** 45.0ºC; **2:** 45.8 ºC; **3:** 47.3 ºC; **4:** 49.4 ºC; **5:** 52.3 ºC; **6:** 54.5 ºC; **7:** 56.0 ºC; **8:** 57.0 ºC; **M:** 50bp DNA Marker (Fermentas SM0371).

Best working T_A was found to be 49.4 ºC as can be observed in Figure 3.7. Moreover, it was observed that both temperatures of 47.3 ºC and 52.3 ºC did not give any PCR product as they were out of T_A range, which was found by OligoAnalyzer3.1. Graded PCR for degenerate CAT primers has proved that OligoAnalyzer3.1 results were acceptable. Hence, it was decided to use T_A of CAT as 50 ºC as given by OligoAnalyzer3.1.

When final concentrations of both Taq and template were doubled while conditions were kept constant, the yield of PCR product has increased as shown in Figure 3.8.

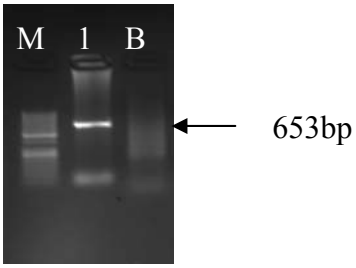

Figure 3.8. Gel electrophoresis results of PCR amplification of CAT transcript by degenerate primers. Amplicon length is 653 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** CAT; **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

3.3.3. Degenerate PCR Optimization of chloroplast/mitochondrial GR Transcript

After the same verifications for chloroplast/mitochondrial glutathione reductase (GR) gene were done the results were presented in Appendix D. According to those results, annealing temperature (T_A) of degenerate chloroplast/mitochondrial GR primers was expected to be within the range of 60.5-65.0ºC. First conditional parameters to be checked were given in Table 3.3.a and Table 3.3.b. The results of PCR amplifications were demonstrated in Figure 3.9.

Table 3.3.a. Degenerate chloroplast/mitochondrial GR PCR Ingredients

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
chloroplast/mitochondrial GR Right Degenerate Primer (10 µM)	0.6µM
chloroplast/mitochondrial GR Left Degenerate Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	0.1U
Template	50ng
25mM $MgCl_2$	1.5mM

Table 3.3.b. Degenerate chloroplast/mitochondrial GR PCR Conditions

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	57ºC	30 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

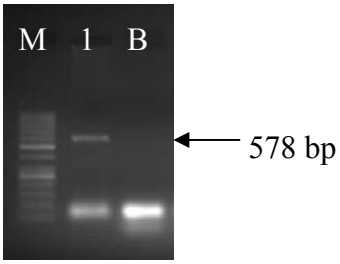

Figure 3.9. Gel electrophoresis results of PCR amplification of chloroplast/mitochondrial GR transcript by degenerate primers. Amplicon length is 578 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** chloroplast/mitochondrial GR; **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

To enhance the band intensity template concentration was tripled (150ng) and the resultant PCR product was run on 2% agarose gel in 1X TAE solution (Figure 3.10).

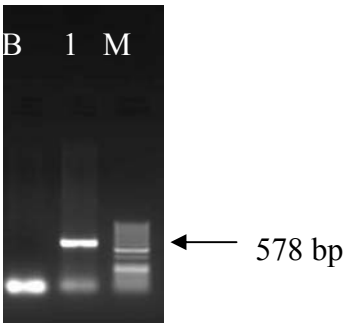

Figure 3.10. Gel electrophoresis results of PCR amplification of chloroplast/mitochondrial GR transcript by degenerate primers. Amplicon length is 578 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** chloroplast/mitochondrial GR; **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

3.3.4. Degenerate PCR Optimization of Chloroplast/Stromal APX Transcript

After the same verifications for Chloroplast/Stromal ascorbate peroxidase (APX) gene were done the results were presented in Appendix D. According to those results, annealing temperature (T_A) of degenerate Chloroplast/Stromal APX primers was expected to be within the range of 58.8–55.6ºC. First conditional parameters to be checked were given in Table 3.4.a and Table 3.4.b. The results of PCR amplifications were demonstrated in Figure 3.11.

Table 3.4.a. Degenerate Chloroplast/Stromal APX PCR Ingredients

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Chloroplast/Stromal APX Right Degenerate Primer (10 µM)	0.6µM
Chloroplast/Stromal APX Left Degenerate Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	0.1U
Template	50ng
25mM $MgCl_2$	1.5mM

Table 3.4.b. Degenerate Chloroplast/Stromal APX PCR Conditions (A_T range of 55-58ºC was analyzed in two different initial denaturation durations)

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	**2 min or 3 min.**
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	**55-58ºC**	30 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

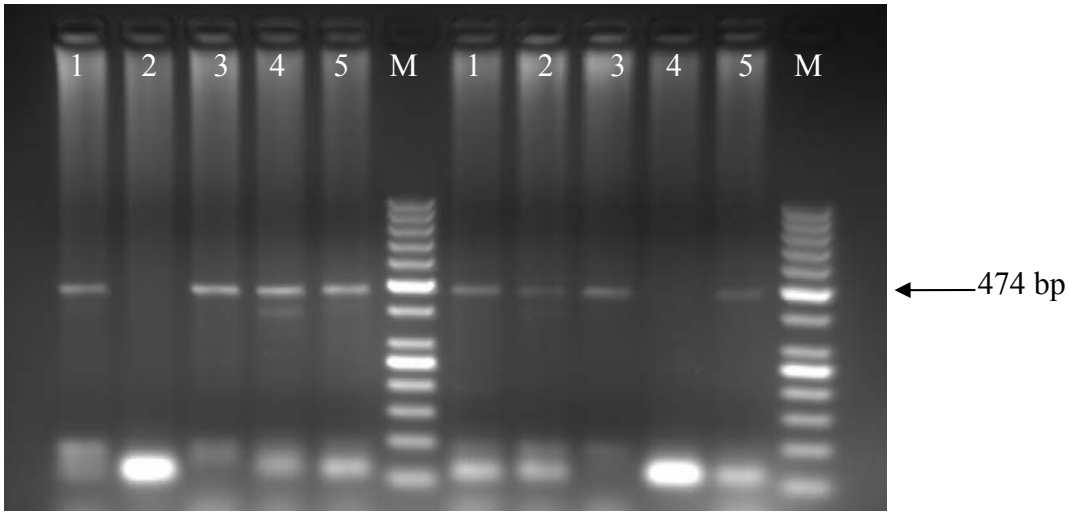

Figure 3.11. Gel electrophoresis results of PCR amplification of chloroplast/stromal APX transcript by degenerate primers. Amplicon length is 474 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** 55ºC; **2:** 56.1 ºC; **3:** 56.8 ºC; **4:** 57.4 ºC; **5:** 58.0 ºC; **M:** 50bp DNA Marker (Fermentas SM0371). First five lanes were PCR products with initial denaturation duration of 3 minutes while the last 5 lanes were PCR products with initial denaturation duration of 2 minutes.

As given in Figure 3.11., the best PCR condition was found to be the combination of initial denaturation duration of 3 minutes with T_A of 57.4 ºC. Later, duration of primer annealing step was doubled while final concentration of template was increased five times. Result of this PCR conditions was given in Figure 3.12.

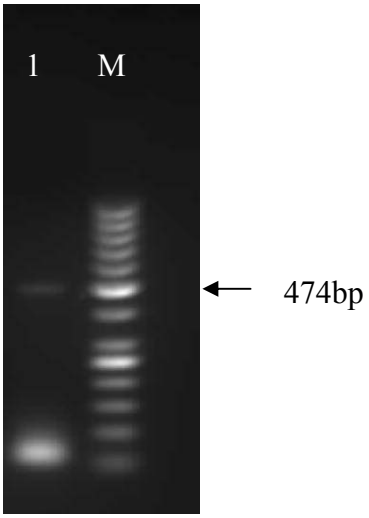

Figure 3.12. Gel electrophoresis results of PCR amplification of chloroplast/stromal APX transcript by degenerate primers. Amplicon length is 474 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of following PCR products, **1:** chl/str. APX; **M:** 50bp DNA Marker (Fermentas SM0371).

After testing different combinations of PCR parameters, the highest yield providing conditions were found and were given in Appendix C. 0.5µL of cDNA was used as template to amplify PCR products in a 50µL of PCR tube. Then, those PCR products were run on 0.8% agarose gel in 1X TAE solution for 40 minutes at 75V and the band which occurred at 382bp for Mn SOD, 653bp for CAT, 478 for chloroplast/mitochondrial GR, and 474 for chloroplast-stromal APX were eluted from the gel according to the GeneMark Gel Elution Kit. Finally, eluted PCR products were sequenced by ABI 310 Capillary DNA Sequencer in RefGen Gen Araştırmaları ve Biyoteknoloji Ltd. Şti. Forward and reverse sequences of all genes were given in Appendix E.

3.4. PCR Optimizations

According to Henegariu *et.al.* (1997), specific PCR conditions for individual genes have to be optimized before multiplex PCR optimizations were carried out. For that reason, PCR conditions of Mn SOD, Cu/Zn SOD, CAT, chloroplast/mitochondrial GR, chloroplast-stromal APX and 18S rRNA genes were optimized in a stepwise manner, some important steps of which were presented in this section.

3.4.1. PCR Optimization Results of MnSOD

Standard PCR ingredients were used during optimization of primer annealing step duration of Mn SOD PCR (Table 3.5) and the results of this optimization step were given in Figure 3.13.

Table 3.5.a. Mn SOD PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
MnSOD Right Specific Primer (10 µM)	0.6µM
MnSOD Left Specific Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.5mM

Table 3.5.b. Mn SOD PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 33		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	**30 sec. or 1 min. or 1.5 min**.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

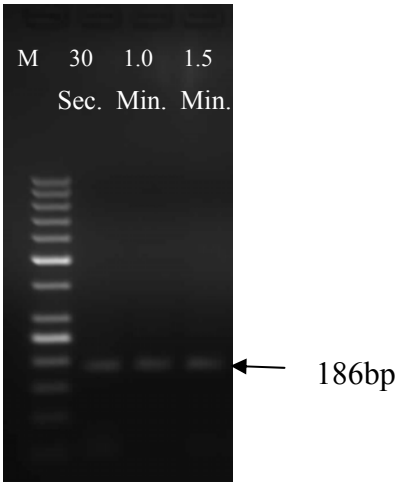

Figure 3.13. Gel electrophoresis results of PCR amplification of Mn SOD transcript by specific primers. Amplicon length is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

The most critical step in PCR optimization for any product is to find the optimum cycle number. This step is important to find the optimum cycle number, which gives a value in the exponential phase of PCR cycle number vs. observed density (O.D.) graph. Both the gel electrophoresis results and PCR cycle number vs. O.D. graph for cycle optimization of Mn SOD transcript are given in Figure 3.14. Each lane shows a different and gradually increasing number of cycle from left to right. According to the graph (Figure 3.15) 32 cycle will be the most optimum number of cycle for Mn SOD PCR conditions. Resultant PCR conditions are given in Appendix C.

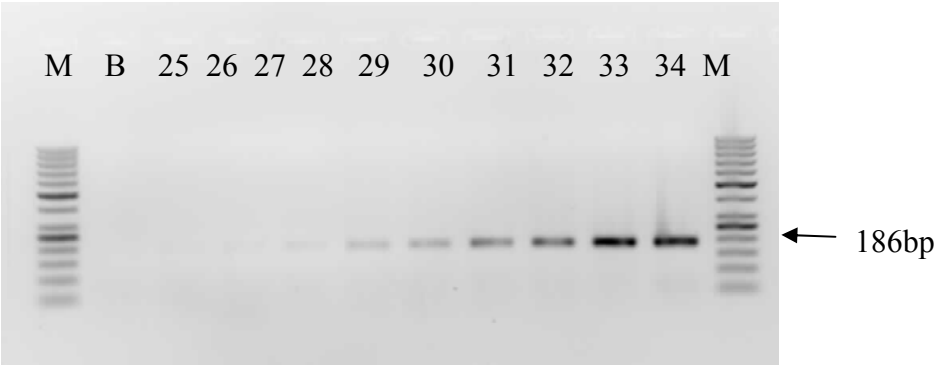

Figure 3.14. Gel electrophoresis results of PCR amplification of Mn SOD transcript by specific primers. Amplicon length is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

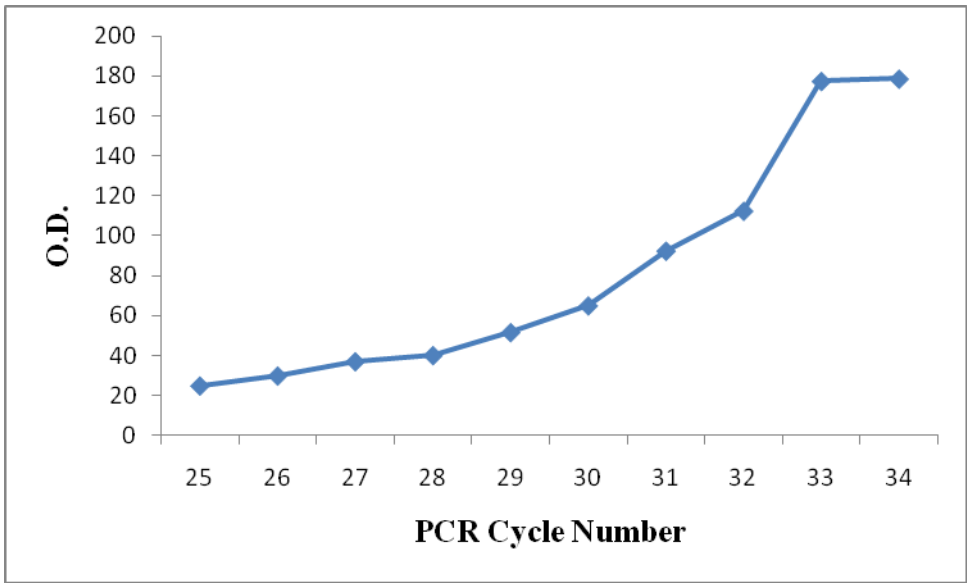

Figure 3.15. PCR cycle number vs. O.D. (mean intensity/defined area) graph for cycle optimization of Mn SOD transcript.

3.4.2. PCR Optimization Results of Cu/Zn SOD

Standard PCR ingredients were used during optimization of primer annealing step duration of Cu/Zn SOD PCR (Table 3.6) and the results of this optimization step were given in Figure 3.16.

Table 3.6.a. Cu/Zn SOD PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Cu/Zn SOD Right Specific Primer (10 µM)	0.6µM
Cu/Zn SOD Left Specific Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table 3.6.b. Cu/Zn SOD PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 30		
Denaturation	94ºC	30 sec.
Primer annealing	51ºC	**30 sec. or 1 min. or 1.5 min.**.
Elongation	72ºC	1 min. 30 sec.
Final Extension	72ºC	10 min.

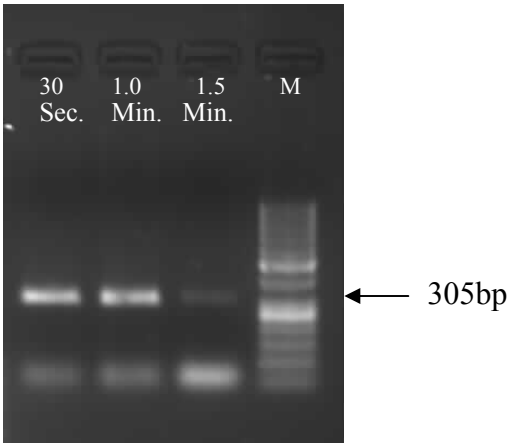

Figure 3.16. Gel electrophoresis results of PCR amplification of Cu/Zn SOD transcript by specific primers. Amplicon length is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

Both the gel electrophoresis results and PCR cycle number vs. OD graph for cycle optimization of Cu/Zn SOD transcript were given in Figure 3.17. Each lane shows a different and gradually increasing number of cycle from left to right. According to the graph (Figure 3.18.) 30 cycle was the most optimum number of cycle for Cu/Zn SOD PCR conditions. Resultant PCR conditions are given in Appendix C.

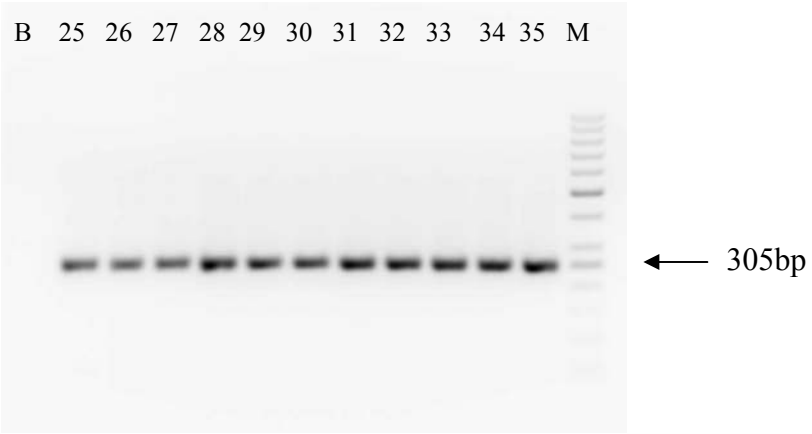

Figure 3.17. Gel electrophoresis results of PCR cycle optimization of Cu/Zn SOD transcript by specific primers. Amplicon length is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

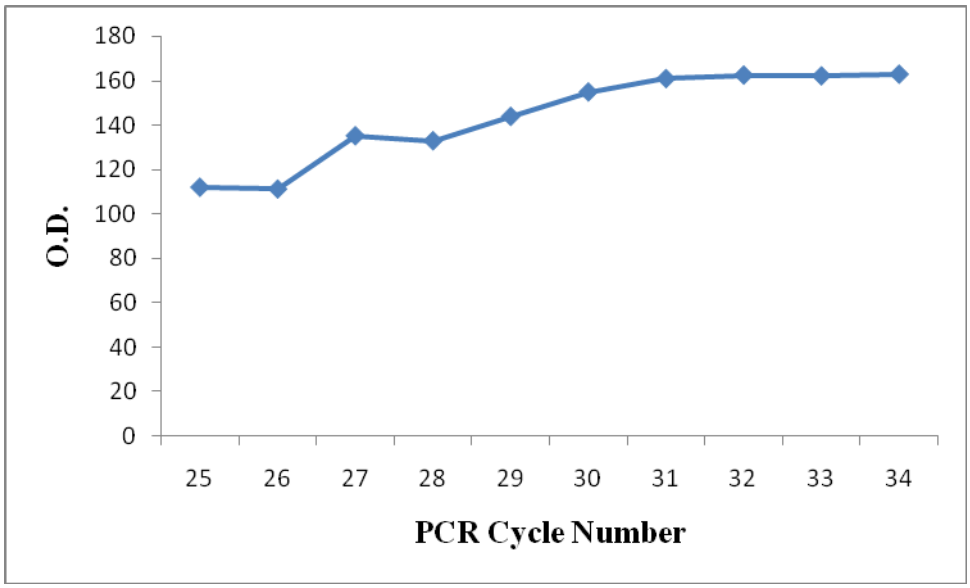

Figure 3.18. PCR cycle number vs. O.D. (mean intensity/defined area) graph for cycle optimization of Cu/Zn SOD transcript.

3.4.3. PCR Optimization Results of CAT

Standard PCR ingredients were used during optimization of primer annealing step duration of CAT PCR (Table 3.7) and the results of this optimization step were given in Figure 3.19.

Table 3.7.a. CAT PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
CAT Right Specific Primer (10 µM)	0.6µM
CAT Left Specific Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table 3.7.b. CAT PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 33		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	**30 sec. or 1 min. or 1.5 min.**.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

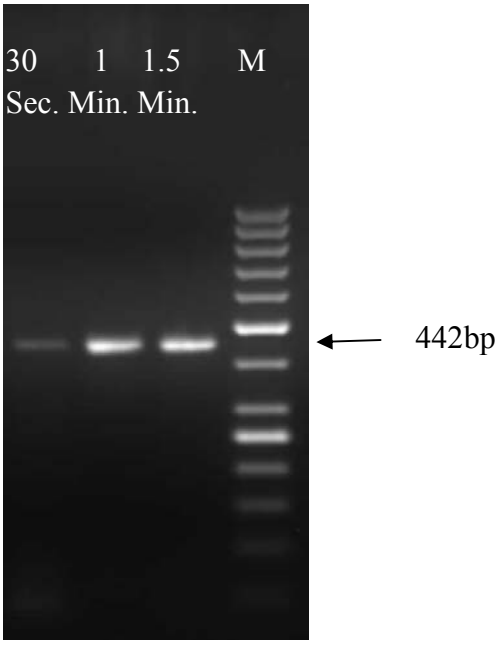

Figure 3.19. Gel electrophoresis results of PCR amplification of CAT transcript. Amplicon length is 442 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

Gel electrophoresis results and PCR cycle number vs. OD graph for cycle optimization of CAT transcript were given in Figure 3.20. Each lane shows a different and gradually increasing number of cycle from left to right. According to the graph (Figure 3.21) 34 cycle was the most optimum number of cycle for CAT PCR conditions. Resultant PCR conditions are given in Appendix C.

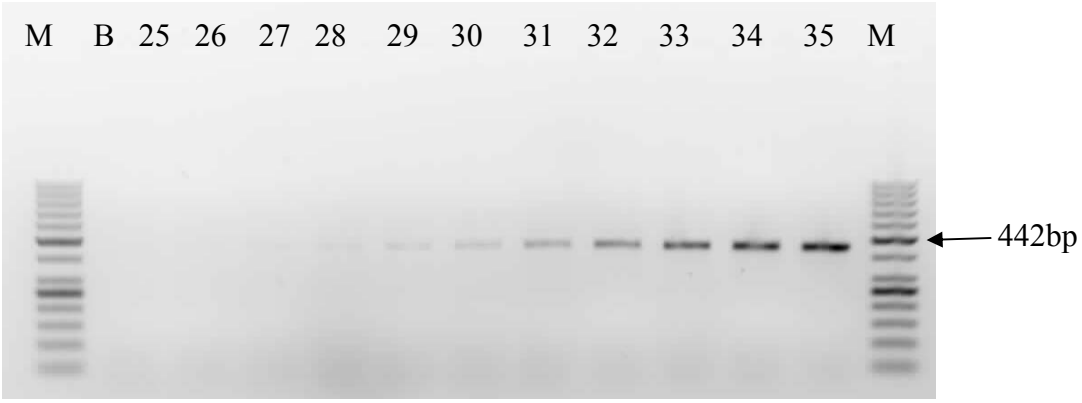

Figure 3.20. Gel electrophoresis results of PCR cycle optimization of CAT transcript by specific primers. Amplicon length is 442 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

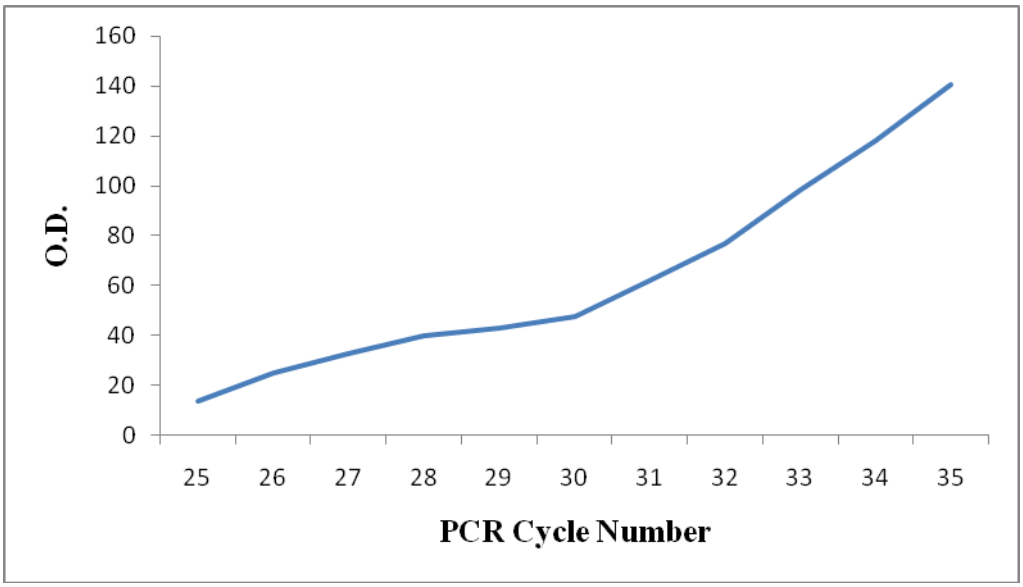

Figure 3.21. PCR cycle number vs. O.D. (mean intensity/defined area) graph for cycle optimization of CAT transcript.

3.4.4. PCR Optimization Results of Chloroplast/Mitochondrial GR

Standard PCR ingredients were used during optimization of primer annealing step duration of Chloroplast/Mitochondrial GR PCR (Table 3.8) and the results of this optimization step were given in Figure 3.22.

Table 3.8.a. Chloroplast/Mitochondrial GR PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Chloroplast/Mitochondrial GR Right Specific Primer (10 µM)	0.6µM
Chloroplast/Mitochondrial GR Left Specific Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table 3.8.b. Chloroplast/Mitochondrial GR PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 33		
Denaturation	94ºC	30 sec.
Primer annealing	49ºC	**30 sec. or 1 min. or 1.5 min.**.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

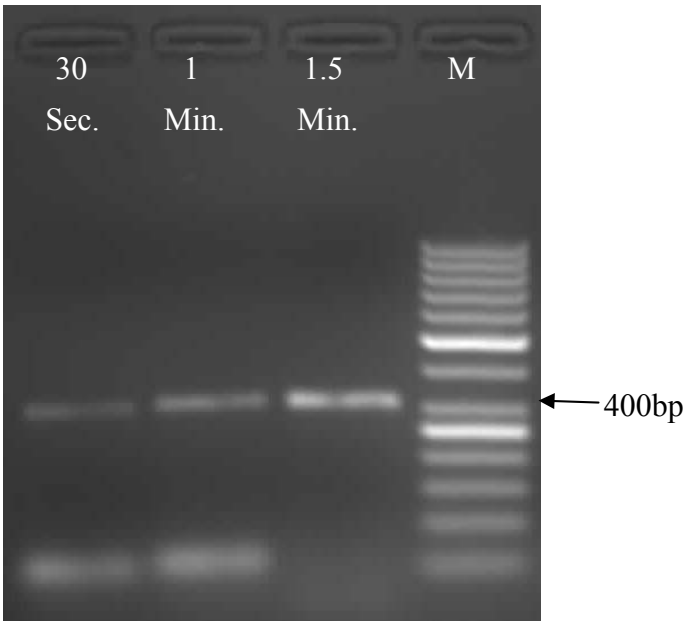

Figure 3.22. Gel electrophoresis results of PCR amplification of Chloroplast/Mitochondrial GR transcript by specific primers. Amplicon length is 400 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

Gel electrophoresis results and PCR cycle number vs. OD graph for cycle optimization of Chloroplast/Mitochondrial GR transcript were given in Figure 3.23. Each lane shows a different and gradually increasing number of cycle from left to right. According to the graph (Figure 3.24) 32 cycle was the most optimum number of cycle for Chloroplast/Mitochondrial GR PCR conditions. Resultant PCR conditions are given in Appendix C.

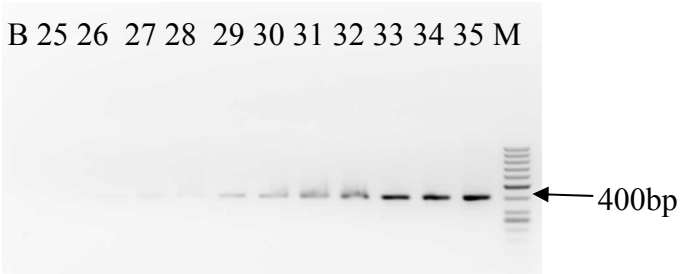

Figure 3.23. Gel electrophoresis results of PCR cycle optimization of Chloroplast/Mitochondrial GR transcript by specific primers. Amplicon length is 400 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

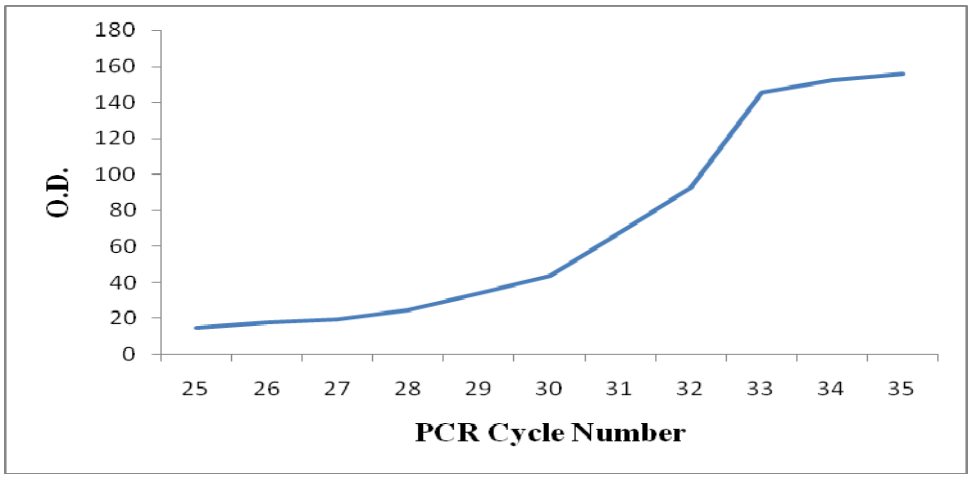

Figure 3.24. PCR cycle number vs. O.D. (mean intensity/defined area) graph for cycle optimization of Chloroplast/Mitochondrial GR transcript.

3.4.5. PCR Optimization Results of Chloroplast Stromal APX

Standard PCR ingredients were used during optimization of primer annealing step duration of Chloroplast Stromal APX PCR (Table 3.9) and the results of this optimization step were given in Figure 3.25.

Table 3.9.a. Chloroplast Stromal APX PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Chloroplast Stromal APX Right Specific Primer (10 µM)	0.6µM
Chloroplast Stromal APX Left Specific Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table 3.9.b. Chloroplast Stromal APX PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 34		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	**30 sec. or 1 min. or 1.5 min.**.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

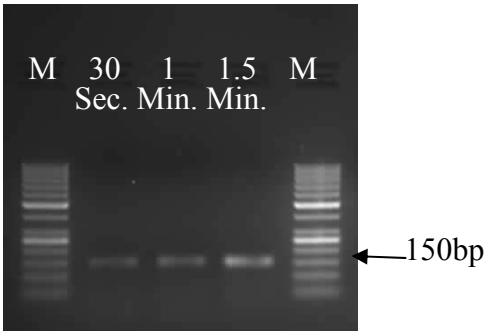

Figure 3.25. Gel electrophoresis results of PCR amplification of Chloroplast Stromal APX transcript by specific primers. Amplicon length is 150 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

Gel electrophoresis results and PCR cycle number vs. OD graph for cycle optimization of Chloroplast Stromal APX transcript were given in Figure 3.26. Each lane shows a different and gradually increasing number of cycle from left to right. According to the graph (Figure 3.27) 32 cycle was the most optimum number of cycle for Chloroplast Stromal APX PCR conditions. Resultant PCR conditions are given in Appendix C.

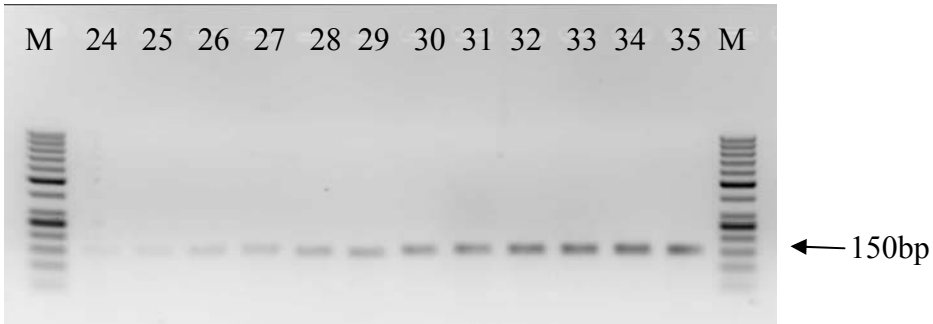

Figure 3.26. Gel electrophoresis results of PCR cycle optimization of Chloroplast Stromal APX transcript by specific primers. Amplicon length is 150 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

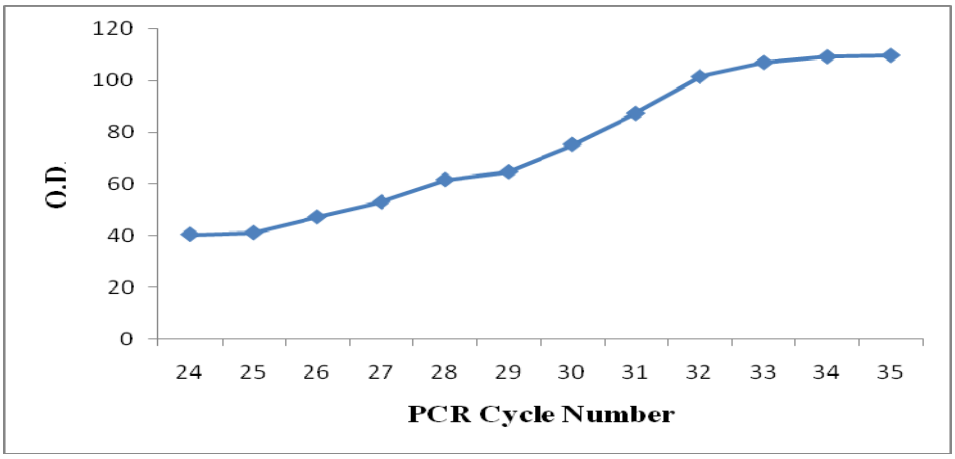

Figure 3.27. PCR cycle number vs. O.D. (mean intensity/defined area) graph for cycle optimization of Chloroplast Stromal APX transcript.

3.4.6. PCR Optimization Results of 18S rRNA

Standard PCR ingredients were used during optimization of primer annealing step duration of 18S rRNA PCR (Table 3.10) and the results of this optimization step were given in Figure 3.28

Table 3.10.a. 18S rRNA PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
18S rRNA Right Specific Primer (10 µM)	0.6µM
18S rRNA Left Specific Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1U
Template	50ng
25mM $MgCl_2$	1.25mM

Table 3.10.b. 18S rRNA PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 30		
Denaturation	94ºC	30 sec.
Primer annealing	51ºC	**30 sec. or 1 min. or 1.5 min.**
Elongation	72ºC	45 sec.
Final Extension	72ºC	10 min.

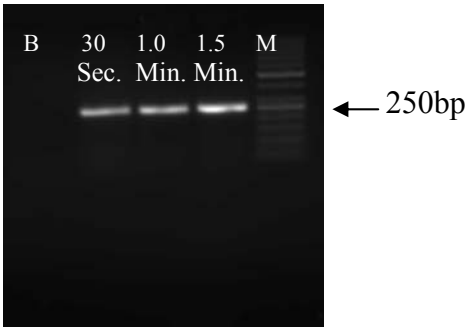

Figure 3.28. Gel electrophoresis results of PCR amplification of 18S rRNA transcript by specific primers. Amplicon length is 250 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

Gel electrophoresis results and PCR cycle number vs. OD graph for cycle optimization of 18S rRNA transcript were given in Figure 3.29. Each lane shows a different and gradually increasing number of cycle from left to right. According to the graph (Figure 3.30.) 30 cycle will be the most optimum number of cycle for 18S rRNA PCR conditions. Resultant PCR conditions are given in Appendix C.

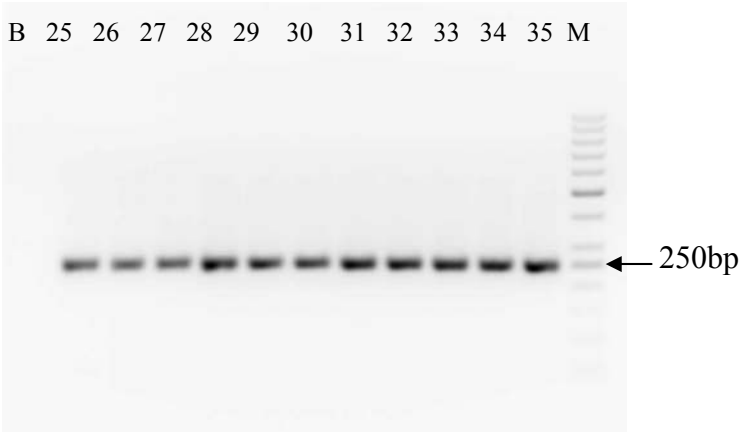

Figure 3.29. Gel electrophoresis results of PCR cycle optimization of 18S rRNA transcript by specific primers. Amplicon length is 250 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

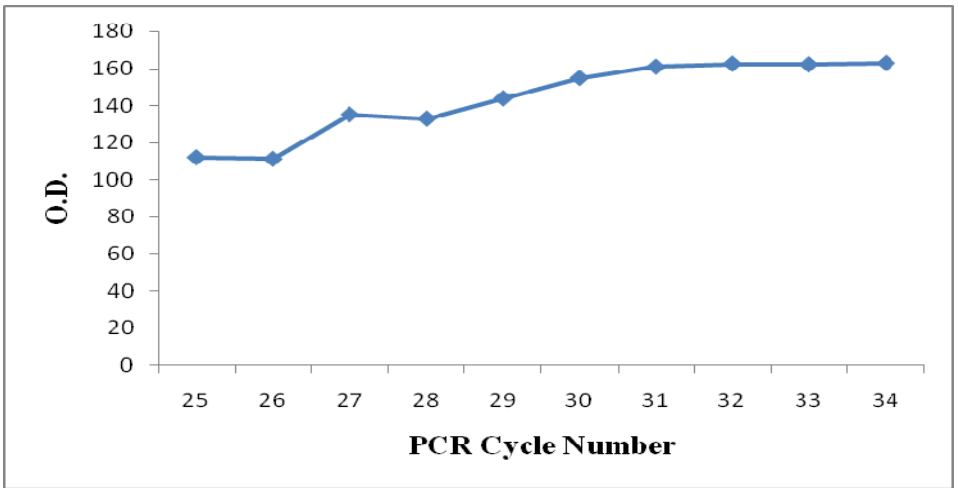

Figure 3.30. PCR cycle number vs. O.D. (mean intensity/defined area) graph for cycle optimization of 18S rRNA transcript.

3.5. Multiplex PCR Optimizations

Parameters of multiplex PCR were first optimized according to Henegariu *et.al.* (1997). Firstly, a standard PCR was carried out according to the conditions that were optimized for each individual gene. In this PCR, primers for both genes were added in equal amounts. Secondly, final concentration of reaction buffer was optimized. Then, different dilutions of primers were used to optimize primer conditions. Finally, optimum cycle number of multiplex PCR was found. Optimized PCR conditions were given in Appendix H.

3.5.1. Duplex PCR Optimization of Mn SOD and 18S rRNA

Equal amounts of right and left primers of Mn SOD and 18S rRNA transcripts were added into the same PCR mixture as given in Table 3.11. The results of PCR amplification were provided in Figure 3.31.

Table 3.11.a. Mn SOD Duplex PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Mn SOD Right Specific Primer (10 µM)	0.2 µM
Mn SOD Left Specific Primer (10 µM)	0.2 µM
18S rRNA Right Specific Primer (10 µM)	0.2µM
18S rRNA Left Specific Primer (10 µM)	0.2µM
Taq DNA Polymerase (5U/1µL)	1U
Template	50ng
25mM $MgCl_2$	1.25mM

Table 3.11.b. Mn SOD Duplex PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 34		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	1 min..
Elongation	72ºC	45 sec.
Final Extension	72ºC	10 min.

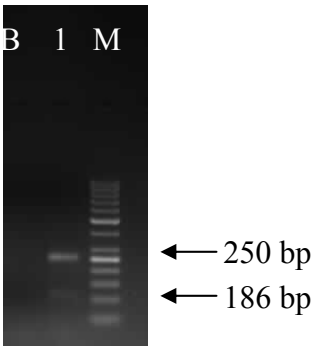

Figure 3.31. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Mn SOD is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. **B:** Blank; **1:** PCR Product; **M:** 50bp DNA Marker (Fermentas SM0371).

Because of low yield of Mn SOD, a serial dilution of both right and left primers of 18S rRNA was done. Hence, five different primer concentrations for 18S rRNA primers were used in next optimization step. For this reason, 0.2µM, 0.5 µM , 1.0 µM, 2.0 µM and 10 µM of primer stocks were prepared and 0.5µL of each new

primer stock was added into PCR tube. Hence, the effect of diluted primers was obviously observed in Figure 3.32.

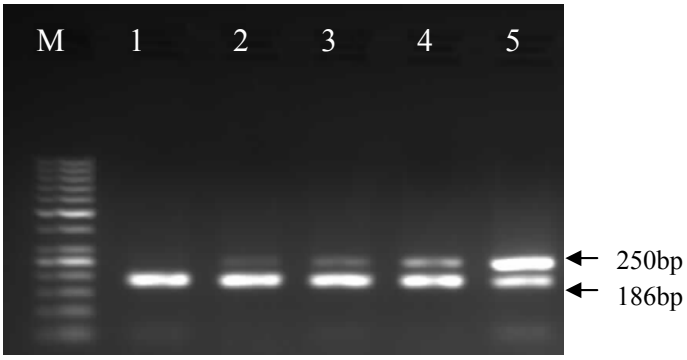

Figure 3.32. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript by different 18S rRNA primer dilutions. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Mn SOD is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. **1:** PCR Product with 0.2µM stock primers (50X dilution); **2:** Product with 0.5µM stock primers (20X dilution); **3:** Product with 1.0µM stock primers (10X dilution); **4:** Product with 2.0µM stock primers (5X dilution); **5:** Product with 10.0µM stock primers (without dilution); **M:** 50bp DNA Marker (Fermentas SM0371).

As can be observed, band intensity decreases with increasing dilution rate of 18S rRNA primers. It was expected to find the best dilution condition where the band intensities of Mn SOD and 18S rRNA were nearly similar. Hence, 2.0µM 18S rRNA stock primers with 5X dilution constant were decided to be used in further PCR amplifications.

Finally, cycle number for duplex PCR was optimized and the results were given in Figure 3.33. As can be observed from the figure, the best cycle conditions nearly coincide with those of PCR conditions of each single gene. Hence, 32 cycle was chosen for further PCR amplifications.

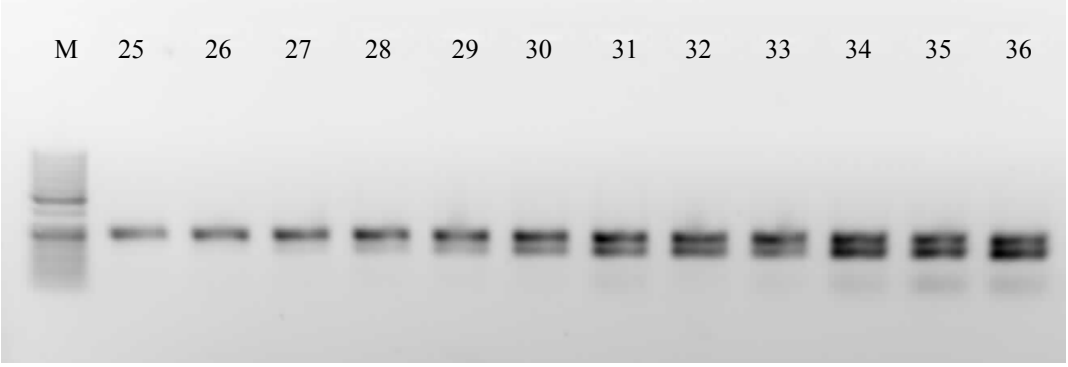

Figure 3.33. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript for cycle optimization. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

3.5.2. Duplex PCR Optimization of Cu/Zn SOD and 18S rRNA

Equal amounts of right and left primers of Cu/Zn SOD and 18S rRNA genes were added into the same PCR mixture as given in Table 3.12. The results of PCR amplification were provided in Figure 3.34.

Table 3.12.a. Cu/Zn SOD Duplex PCR ingredients.

Ingredient	Final Concentration
10X Taq Buffer	0.8X
2mM dNTP Mix	0.2mM of each
Cu/Zn SOD Right Specific Primer (10 µM)	0.2 µM
Cu/Zn SOD Left Specific Primer (10 µM)	0.2 µM
18S rRNA Right Specific Primer (10 µM)	0.2µM
18S rRNA Left Specific Primer (10 µM)	0.2µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.5mM

Table 3.12.b. Cu/Zn SOD Duplex PCR conditions.

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 33		
Denaturation	94ºC	30 sec.
Primer annealing	51ºC	1 min..
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

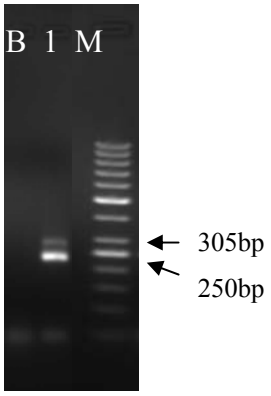

Figure 3.34. Gel electrophoresis results of Duplex PCR amplification of Cu/Zn SOD transcript. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Cu/Zn SOD is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. **B:** Blank; **1:** PCR Product; **M:** 50bp DNA Marker (Fermentas SM0371).

Because of low yield of Cu/Zn SOD, a serial dilution of both right and left primers of 18S rRNA was done. Hence, five different primer concentrations for 18S rRNA primers were used in next optimization step. For this reason, 0.2µM, 0.5 µM , 1.0 µM, 2.0 µM and 10 µM of primer stocks were prepared and 0.5µL of each new primer stock was added into PCR tube. Hence, the effect of diluted primers was obviously observed in Figure 3.35.

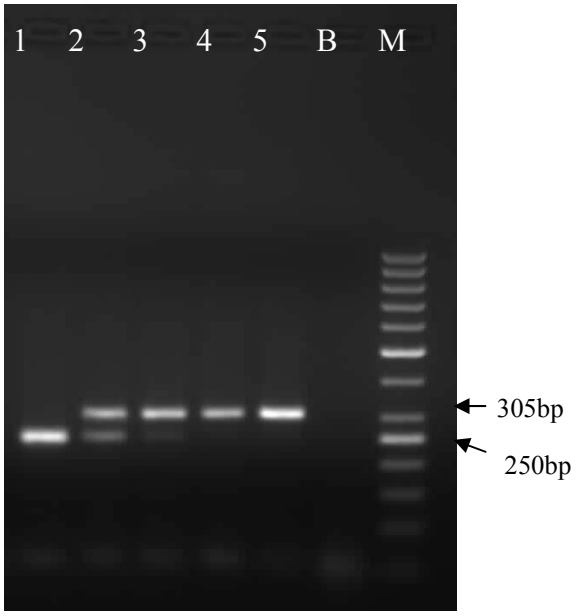

Figure 3.35. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript by different 18S rRNA primer dilutions. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Cu/Zn SOD is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. **1:** Product with 10.0µM stock primers (without dilution); **2:** Product with 2.0µM stock primers (5X dilution); **3:** Product with 1.0µM stock primers (10X dilution); **4:** Product with 0.5µM stock primers (20X dilution); **5:** PCR Product with 0.2µM stock primers (50X dilution); **B:** Blank; **M:** 50bp DNA Marker (Fermentas SM0371).

As can be observed, band intensity decreases with increasing dilution rate of 18S rRNA primers. It was expected to find the best dilution condition where the band intensities of Mn SOD and 18S rRNA were nearly similar. Hence, 2.0µM 18S rRNA stock primers with 5X dilution constant were decided to be used in further PCR amplifications.

Finally, cycle number for duplex PCR was optimized and the results were given in Figure 3.36. As can be observed from the figure, the best cycle conditions nearly coincide with those of PCR conditions of each single gene. Hence, 32 cycle was chosen for further PCR amplifications.

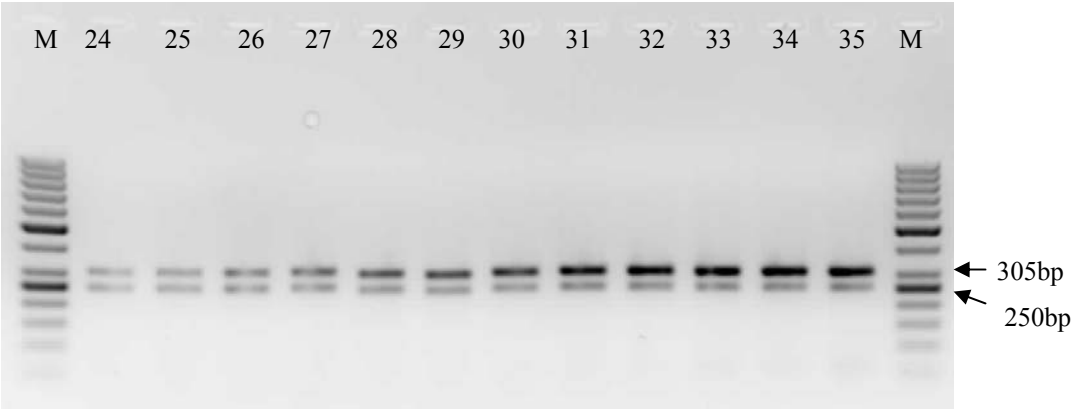

Figure 3.36. Gel electrophoresis results of Duplex PCR amplification of Cu/Zn SOD transcript for cycle optimization. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **M:** 50bp DNA Marker (Fermentas SM0371).

3.6. Results of Duplex RT-PCR

10 µL of duplex RT-PCR products for control shoot, control root, stress shoot and stress root samples which were taken at 6th, 12th hours and 1st, 3rd, 5th and 7th days of salt and drought stress applications were run in 2% agarose gel in 1X TAE solution for 60 minutes at 75V. 12 µL of 50-1000bp DNA marker (Fermentas SMO371) was loaded into each gel to observe the precise band formation after

electrophoresis. A negative control (which contains PCR product without template) was also loaded into the same gel to observe any type of contamination in PCR amplification. 10 µL of RT-PCR product of Barley Cu/Zn SOD cDNA was loaded into each gel as a positive control. Then, photographs of ethidium bromide-stained gels were taken by Vilber Gel Imager.

3.6.1. Results of Duplex RT-PCR for Salt Stress Treatments

The results of duplex RT-PCR for salt stress treatments were provided in this section.

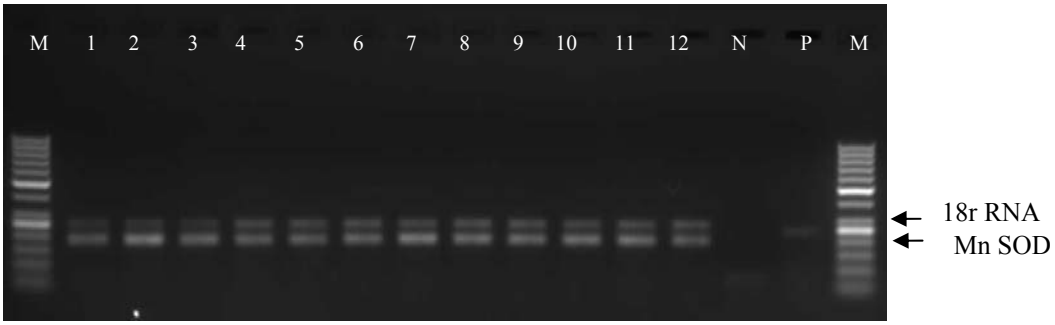

Figure 3.37. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript in shoot samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Mn SOD is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

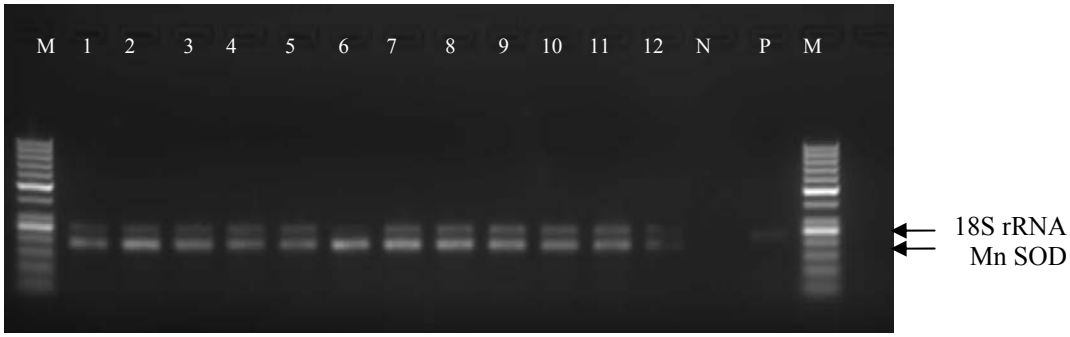

Figure 3.38. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript in root samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Mn SOD is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

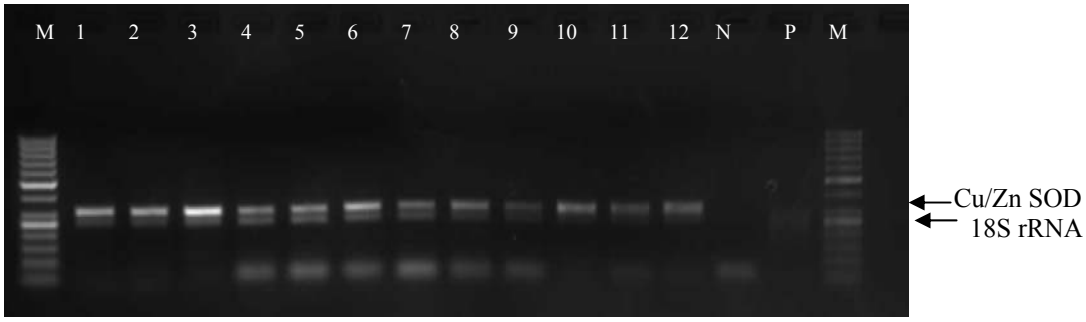

Figure 3.39. Gel electrophoresis results of Duplex PCR amplification of Cu/Zn SOD transcript in shoot samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Cu/Zn SOD is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

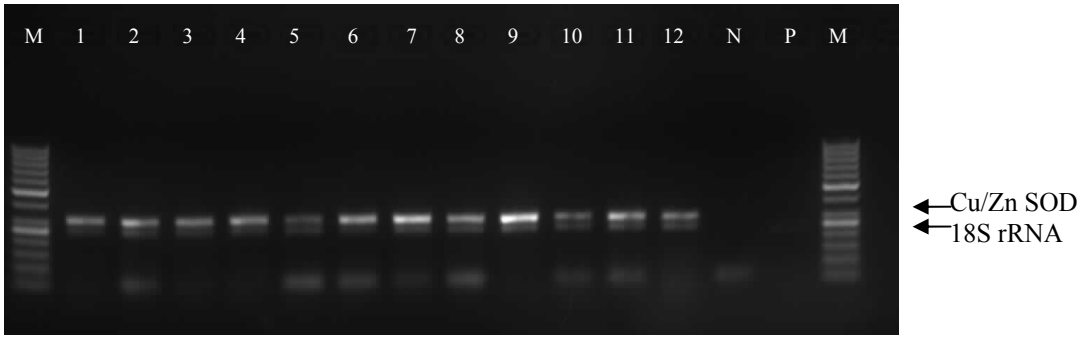

Figure 3.40. Gel electrophoresis results of Duplex PCR amplification of Cu/Zn SOD transcript in root samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Cu/Zn SOD is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

3.6.2. Results of Duplex RT-PCR for Drought Stress Treatments

The results of duplex RT-PCR for drought stress treatments were provided in this section.

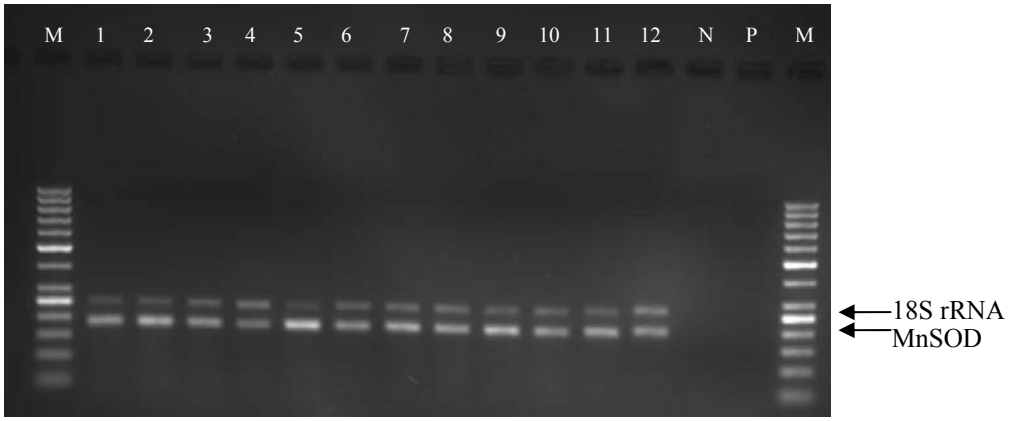

Figure 3.41. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript in shoot samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Mn SOD is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

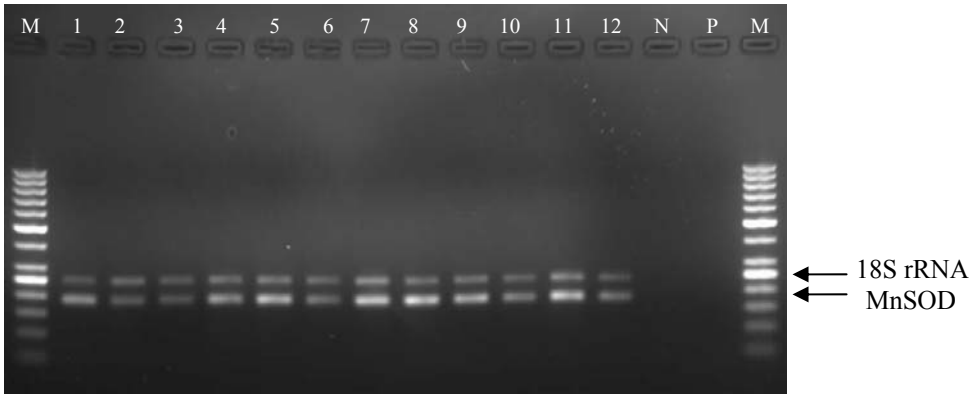

Figure 3.42. Gel electrophoresis results of Duplex PCR amplification of Mn SOD transcript in root samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Mn SOD is 186 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

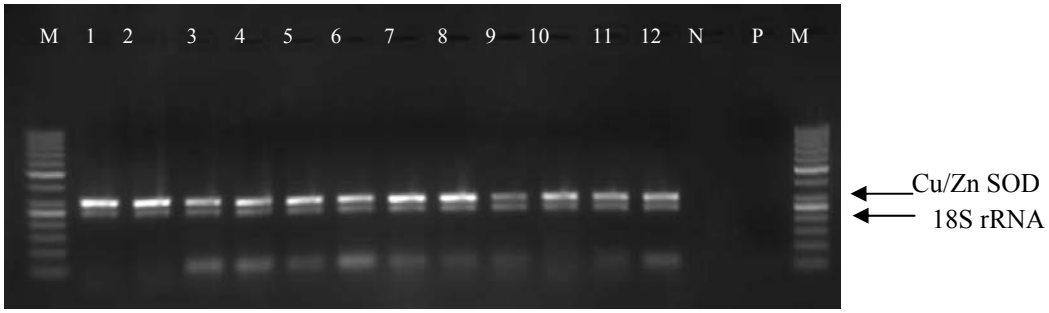

Figure 3.43. Gel electrophoresis results of Duplex PCR amplification of Cu/Zn SOD transcript in shoot samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Cu/Zn SOD is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

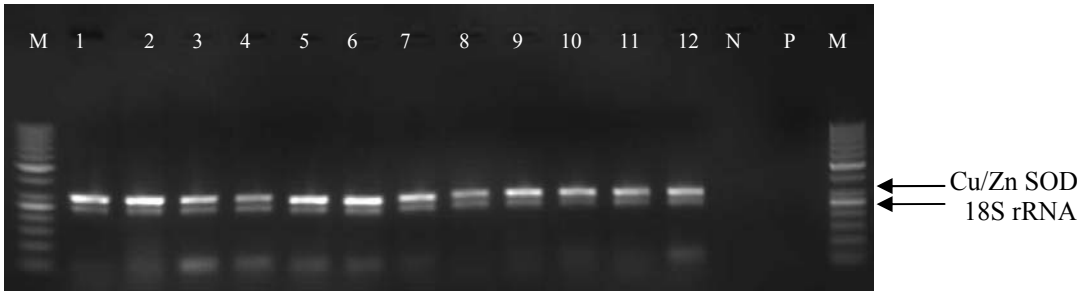

Figure 3.44. Gel electrophoresis results of Duplex PCR amplification of Cu/Zn SOD transcript in root samples. Amplicon length of 18S rRNA is 250 bp; Amplicon length of Cu/Zn SOD is 305 bp. 2% agarose gel in 1X TAE solution was run for 60 minutes at 100V. Each lane was loaded with 10µL of PCR products. **1:** 6 hours-control; **2:** 6 hours-stress; **3:** 12 hours-control; **4:** 12 hours-stress; **5:** 1 day-control; **6:** 1 day-stress; **7:** 3 days-control; **8:** 3 days-stress; **9:** 5 days-control; **10:** 5 days-stress; **11:** 7 days-control; **12:** 7 days-stress; **N:** Negative control; **P:** Positive control (Barley Cu/Zn SOD); **M:** 50bp DNA Marker (Fermentas SM0371).

3.7. Densitometric Analysis of Semi-quantitative RT-PCR Results

Densitometric analysis of RT-PCR results were carried out by Scion Image Analyzer (Scion Corporation, USA). Optical density of bands that represent Arabidopsis 18S rRNA and genes of SOD isozymes were obtained. Ratio of gene band intensity to 18S rRNA band intensity was calculated. Later, standard error of means (SEM) of each ratio were calculated by MINITAB 15 and were given by bars in the following graphs of "Relative Expression" vs. "Stress Duration". Data were analyzed by one-way ANOVA with Tukey's pairwise comparisons, where the family error rate was 5. Significant differences between samples were given in figures by an asterisk (*) when $p<0.05$, and by a double asterisk (**) when $p<0.01$.

3.7.1. Densitometric Analysis of Semi-quantitative RT-PCR Results for Mn SOD Transcript Expression

According to Figure 3.45 and Figure 3.46, for 6 hours of stress application, relative Mn SOD expression levels were significantly lower in drought applied shoot and root tissues with respect to control plants, while an increase in relative Mn SOD expression levels was observed both in shoot and root tissues after treatment with 150mM NaCl. This increase was significant in root tissues with respect to control samples. For 12 hours of stress application, the only significant change with respect to control plants was observed in root tissues after treatment with 20% PEG. The change was a 28%-decrease. Stress application did not affect the relative Mn SOD expression levels in shoot tissues significantly with respect to control plants. However, there was a significant (33%) decrease in drought-applied root tissues (with respect to control plants) while there was a significant increase (47%) in drought-applied root tissues with respect to salt-applied root tissues.

For 3 days of stress application, the decreases in relative Mn SOD expression levels both in shoot and root tissues were significant with respect to control plants. Relative Mn SOD expression levels decreased 16% and 33% in shoots and roots, respectively (compared to control plants). Differences in relative Mn SOD expression levels between salt-treated and non-treated plant shoot and roots were not statistically significant.

For 5 days of stress application, there were no significant changes in relative Mn SOD expression levels both in shoot and root tissues of drought-treated plants with respect to controls, while 150mM NaCl treatment increased relative Mn SOD expression levels both in shoot and root tisues. Those increases were obvious in shoots as 106% with respect to control and 113% with respect to drought, while in

roots 105% increase with respect to control and 100% increase with respect to drought were observed.

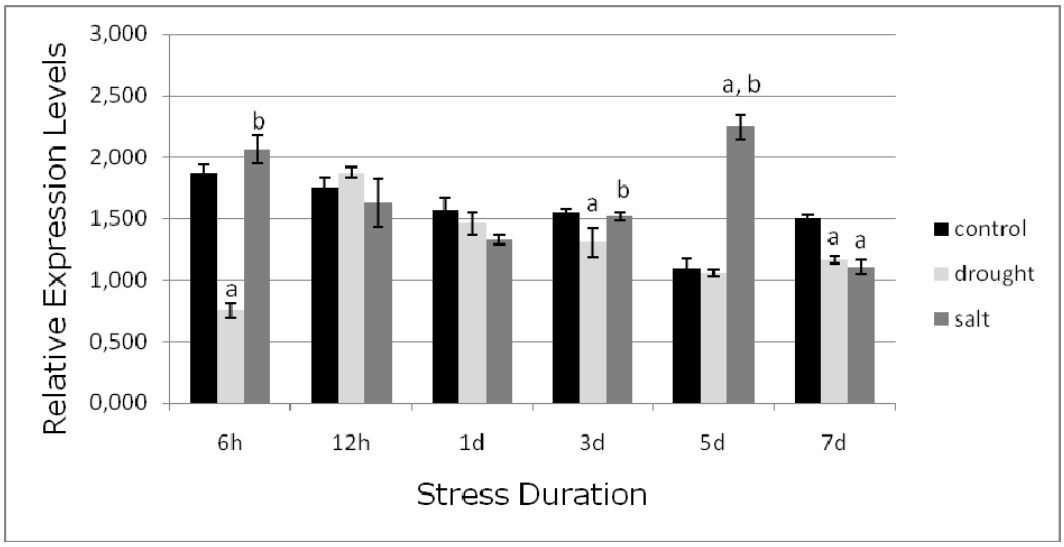

Figure 3.45. Relative gene expression levels of Mn SOD transcript of shoot tissues under salt (150mM NaCl) and drought (20% PEG) stresses. Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. [a] shows significant difference ($p<0.05$) with respect to control. [b] shows significant difference ($p<0.05$) with respect to drought. Bars indicate the mean values ± SEM (n=4).

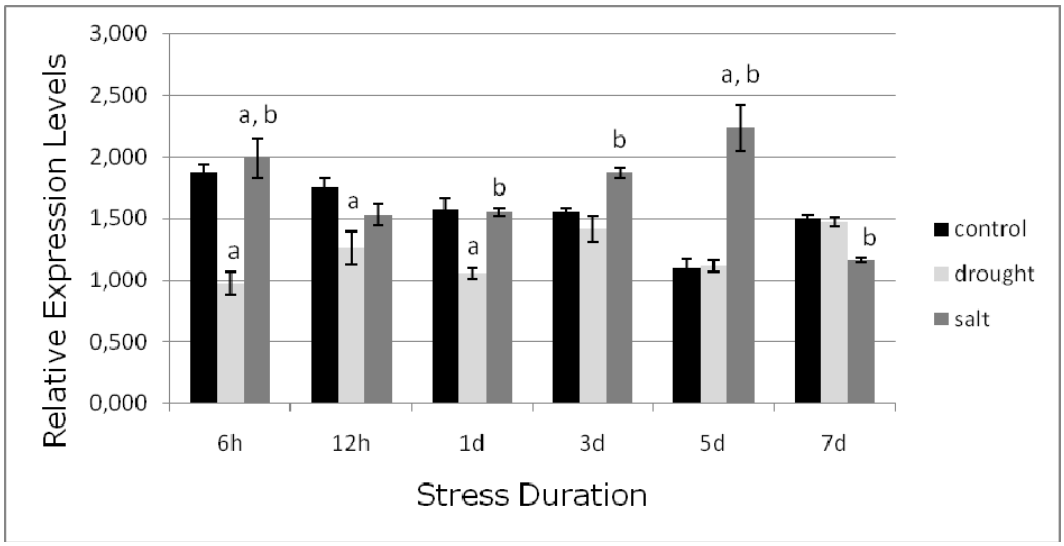

Figure 3.46. Relative gene expression levels of Mn SOD transcript of root tissues under salt (150mM NaCl) and drought (20% PEG) stresses. Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. [a] shows significant difference ($p<0.05$) with respect to control. [b] shows significant difference ($p<0.05$) with respect to drought. Bars indicate the mean values ± SEM (n=4).

For 7 days of stress application, decreased relative Mn SOD expression levels both in drought- and salt-treated shoots were significant with respect to controls.

Figure 3.47 and Figure 3.48 give change of Mn SOD expression levels in the course of drought application by comparing relative gene expression levels of stressed and non-stressed plants.Relative gene expression levels of stressed plants were lower in shoots than that of control plants except 5 days of drought treatment, in which a significant (13%) increase in relative Mn SOD expression level was observed in 20% PEG-applied plant shoots. Moreover, relative Mn SOD expression levels were decreased 63% and 25% in drought stress applied lentil

shoots for a period of 6 hours and 7 days, respectively (Figure 3.47). Although a significant decrease in relative gene expression levels were observed in root tissues, to which drought stress was applied for 6, 12 and 24 hours, a significant (26%) increase was observed in 7 days-stressed plant roots.

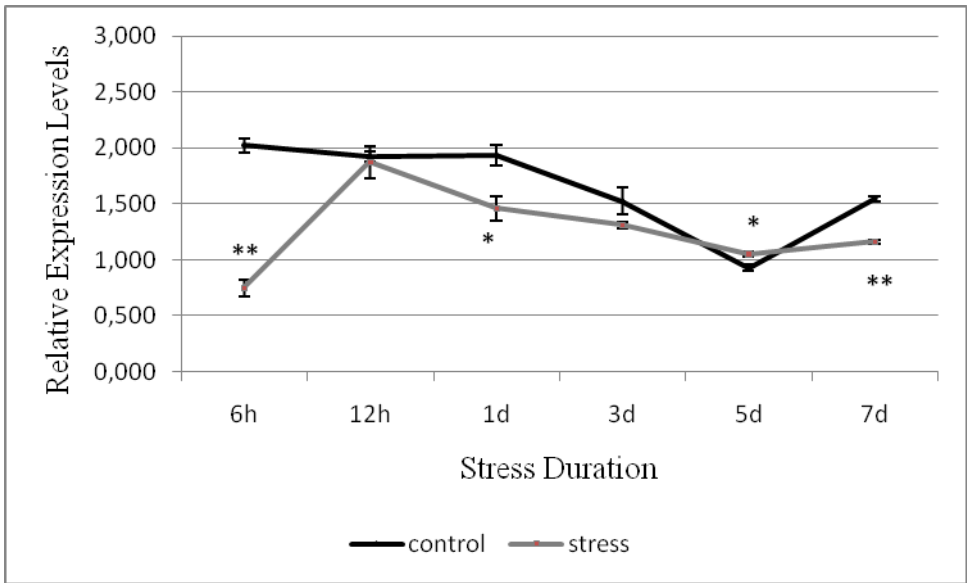

Figure 3.47. Relative gene expression levels of Mn SOD transcript of shoot tissues under drought stress (20% PEG). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

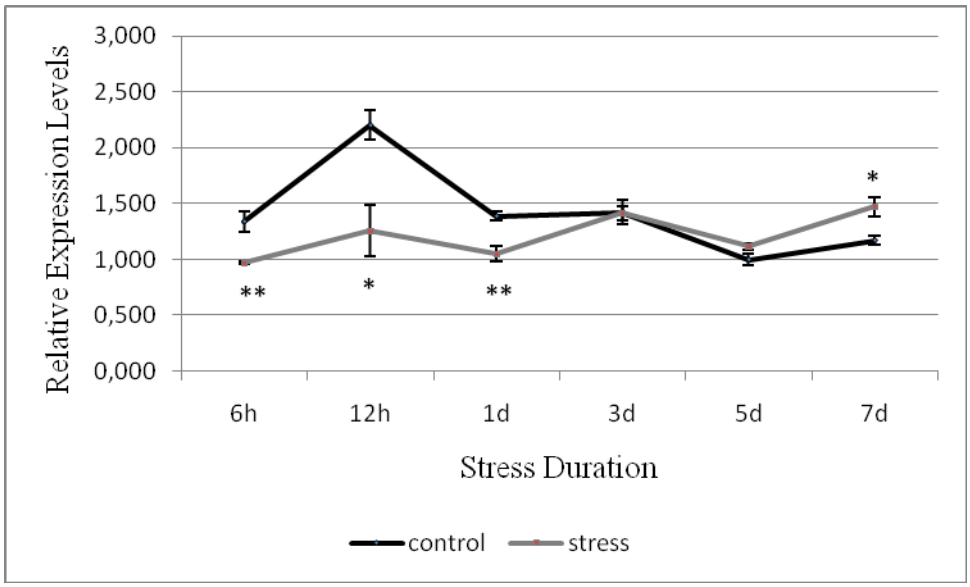

Figure 3.48. Relative gene expression levels of Mn SOD transcript of root tissues under drought stress (20% PEG). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

Figure 3.49 and Figure 3.50 show the change in Mn SOD expression levels in the course of salt application by comparing relative expression levels of stressed and non-stressed plants.Relative Mn SOD expression levels of stressed plants decreased in the course of 6 hours to 24 hours of salt application. Later, relative Mn SOD expression levels increased in stressed plants, but could barely reach to levels of control plants. Relative Mn SOD expression levels peaked on 5th day of salt stress application. Significant increases were observed both in shoot (58%) and root (42.8%) tissues on 5th day of salt (150mM NaCl) stress application. Then, relative Mn SOD gene expression levels of stressed plants decreased on 7th day of stress application both in shoot and root tissues.

The decrease was significant in shoot tissues on 7th day of salt stress application (Figure 3.50).

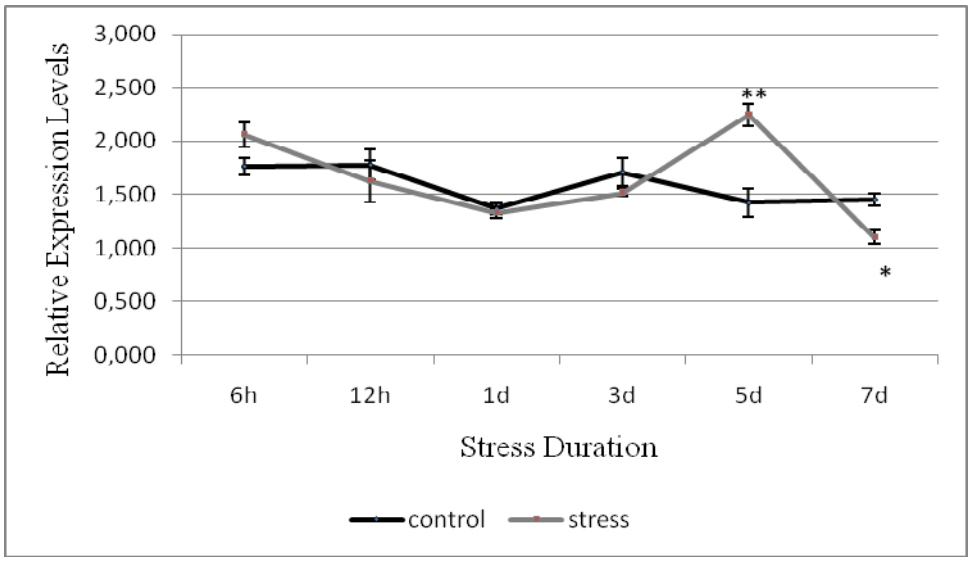

Figure 3.49. Relative gene expression levels of Mn SOD transcript of shoot tissues under salt stress (150mM NaCl). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

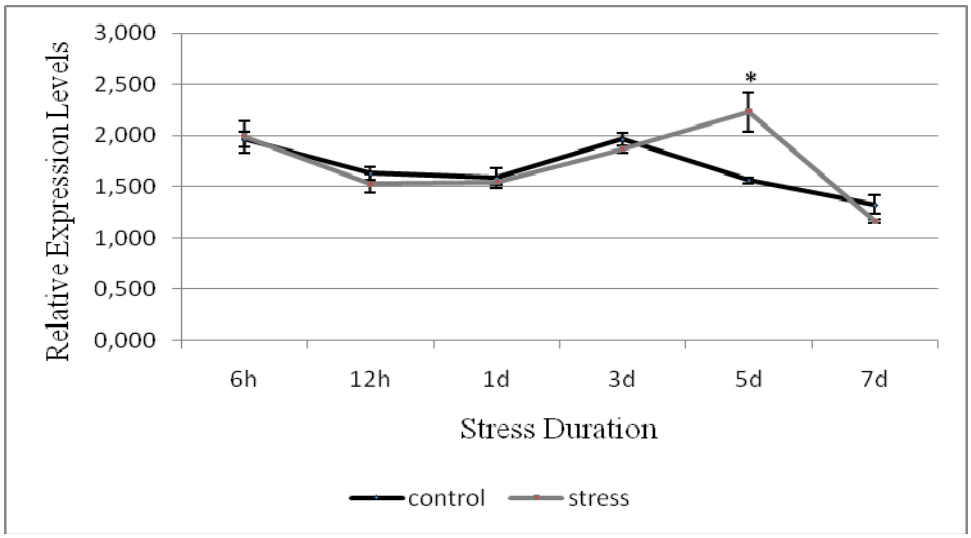

Figure 3.50. Relative gene expression levels of Mn SOD transcript of root tissues under salt stress (150mM NaCl). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

3.7.2. Densitometric Analysis of Semi-quantitative RT-PCR Results for Cu/Zn SOD Gene Expression

Relative Cu/Zn SOD gene expression levels for control (non-treated), salt- and drought-treated plant shoots and roots were given in Figure 3.51 and Figure 3.52, respectively. Relative Cu/Zn SOD gene expression levels increased in 6th, 12th and 24th hours of salt stress applications in root tissues where the increase was significant both in 6th and 24th hours of salt-treated roots with respect to controls and drought-treated roots. However, a significant decrease was observed in 7 days salt treated plant roots with respect to control and drought-treated root tissues.

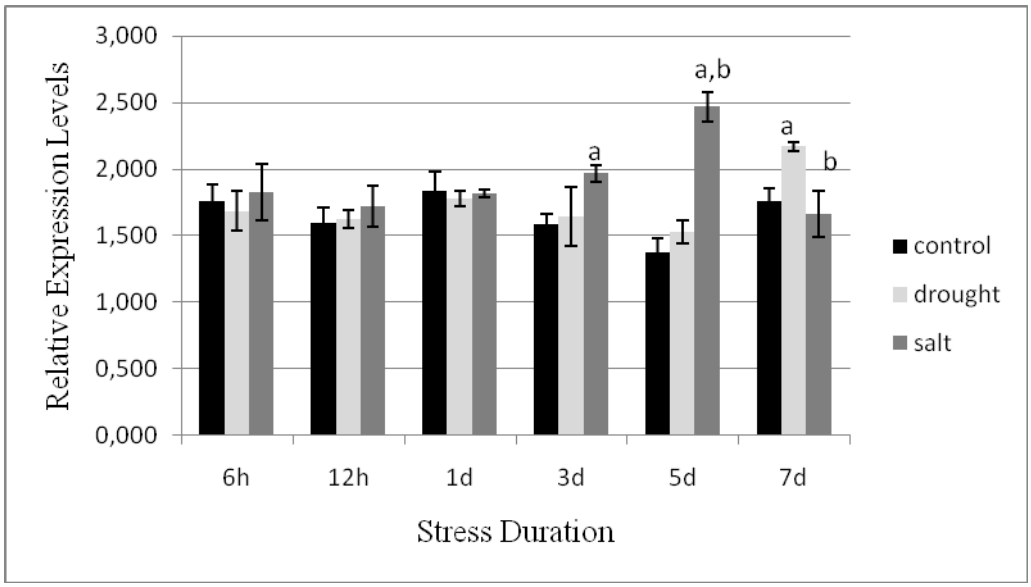

Figure 3.51. Relative gene expression levels of Cu/Zn SOD transcript of shoot tissues under salt (150mM NaCl) and drought (20% PEG) stresses. Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. [a] shows significant difference ($p<0.05$) with respect to control. [b] shows significant difference ($p<0.05$) with respect to drought. Bars indicate the mean values ± SEM (n=4).

Relative Cu/Zn SOD gene expression levels significantly increased in shoot tissues both in 3 and 5 days of salt stress application with respect to controls. Moreover, this increase was also significant with respect to drought-stressed plant shoots in 5 days of salt stress application. However, relative Cu/Zn SOD gene expression level was lower under salt stress than the level under drought stress for 7 days of stress-treated shoot tissues.

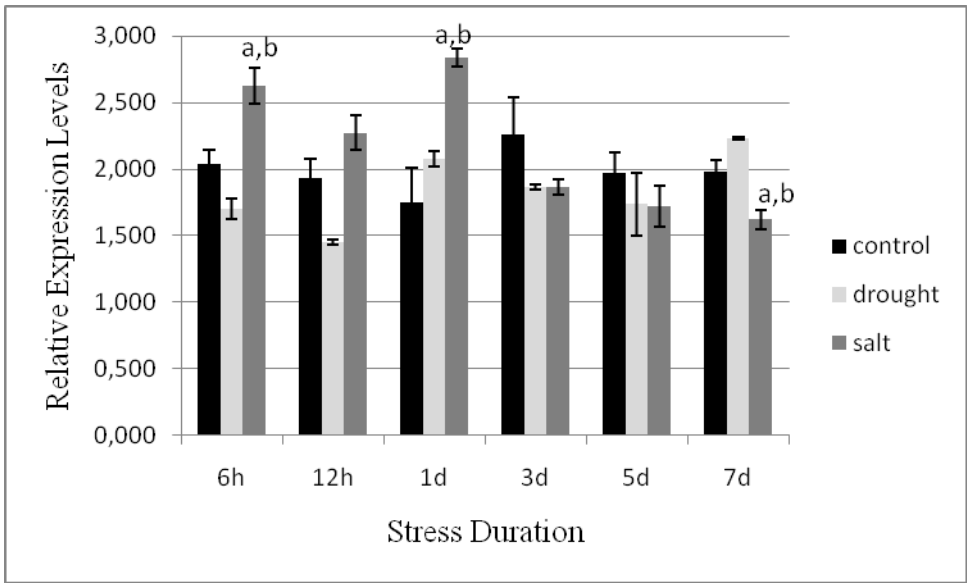

Figure 3.52. Relative gene expression levels of Cu/Zn SOD transcript of root tissues under salt (150mM NaCl) and drought (20% PEG) stresses. Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. [a] shows significant difference ($p<0.05$) with respect to control. [b] shows significant difference ($p<0.05$) with respect to drought. Bars indicate the mean values ± SEM (n=4).

Figure 3.53 and Figure 3.54 give change of Cu/Zn SOD gene expression levels in the course of drought application by comparing relative gene expression levels of stressed and non-stressed plants. There was not a pattern for Cu/Zn SOD gene expression under drought stress both in shoots and in roots. For instance, there is a significant increase (28%) in relative Cu/Zn SOD gene expression levels in 5 days-drought-applied shoots with respect to control plants, while the change is not significant in roots. However, there is a significant increase in relative Cu/Zn SOD gene expression levels both in 7 days-drought-applied shoots and roots with respect to controls.

Moreover, relative Cu/Zn SOD gene expression levels were increased (79%) significantly in 1 day-drought-applied root tissues.

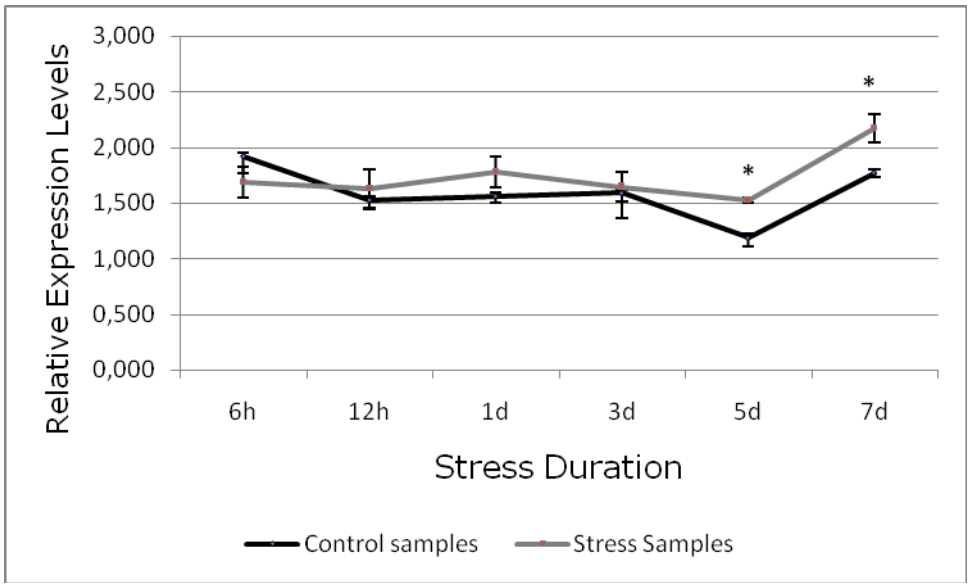

Figure 3.53. Relative gene expression levels of Cu/Zn SOD transcript of shoot tissues under drought stress (20% PEG). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

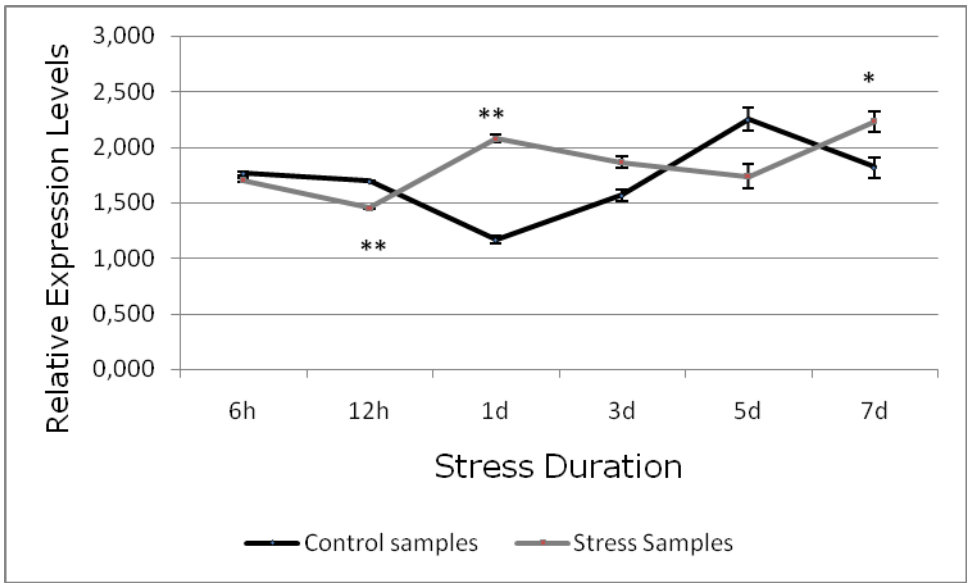

Figure 3.54. Relative gene expression levels of Cu/Zn SOD transcript of root tissues under drought stress (20% PEG). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

Figure 3.55 and Figure 3.56 give alteration of Cu/Zn SOD transcript expression levels in the course of salt application by comparing relative gene expression levels of stressed and non-stressed plants. There was a pattern of increase in relative Cu/Zn SOD gene expression levels in salt-treated shoots between 6 hours and 5 days of salt stress application. This pattern gives a peak at 5 days-salt-applied shoot samples. The increase of relative Cu/Zn SOD gene expression levels in salt-treated lentil shoots was 52%, which was significant with respect to controls. In contrast to ascending pattern observed in shoot tissues, there was a pattern of decrease in relative Cu/Zn SOD gene expression levels in salt-treated roots after 1 days of salt stress application. At 1st day of salt stress application, there was a significant increase (32%) in relative Cu/Zn SOD gene expression

levels in salt-treated plant roots with respect to controls. However, there occurred a significant decrease (33%) in relative Cu/Zn SOD gene expression levels in 3 days-salt-treated plant roots with respect to controls. A significant decreased was also observed in 7 days-salt-treated plant roots with respect to controls.

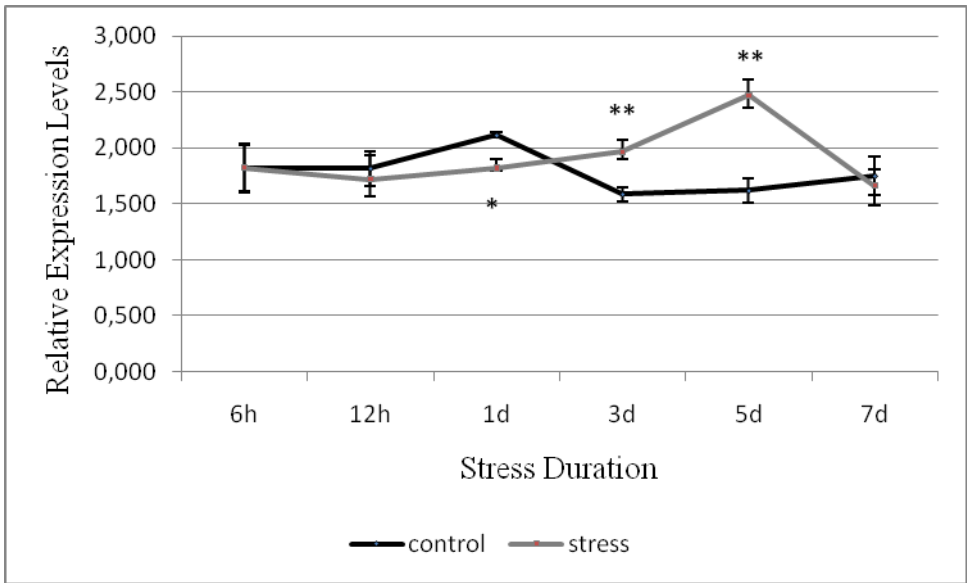

Figure 3.55. Relative gene expression levels of Cu/Zn SOD transcript of shoot tissues under salt stress (150mM NaCl). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

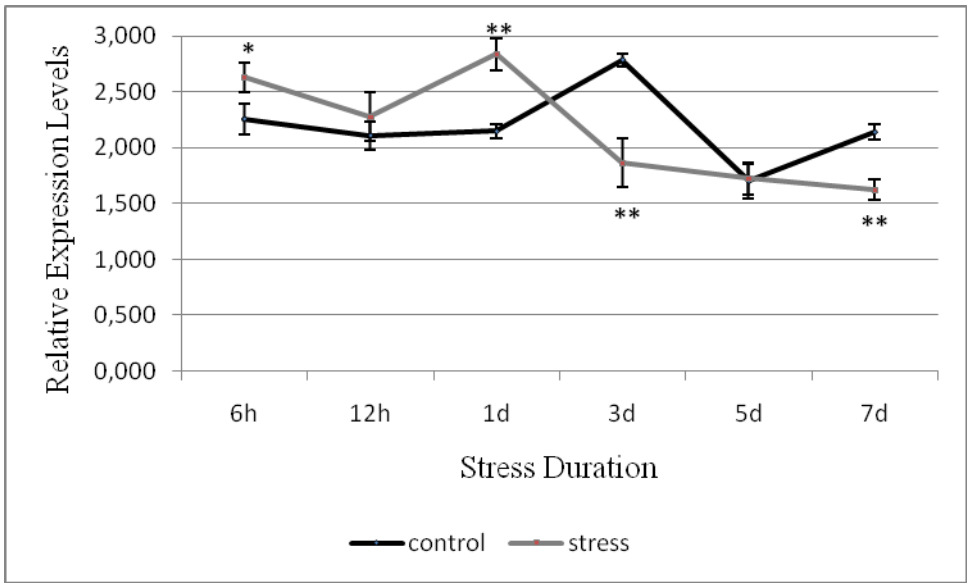

Figure 3.56. Relative gene expression levels of Cu/Zn SOD transcript of root tissues under salt stress (150mM NaCl). Samples were collected at 6th, 12th hours, and 1st, 3rd, 5th, and 7th days of stress applications. Asterisk (*) or double asterisk (**) shows significant difference with respect to control with p values <0.05 and <0.01, respectively. Bars indicate the mean values ± SEM (n=4).

CHAPTER 4

DISCUSSION

Drought and salt stresses are two widespread environmental stresses in many regions, and are expected to cause serious salinization of more than 50% of all arable lands by the year 2050 (Ashraf, 1994).

Physiological and biochemical effects of both stresses coincide because both of them cause oxidative stress. One of the best known results of oxidative stress is the production of ROS in plants.

Plants have evolved various enzymatic and non-enymatic protection mechanisms against toxic effects of ROS (Mittler, 2002). Enzymatic protection mechanisms consist of different antioxidative enzymes such as superoxide dismutase [SOD; E.C. 1.15.1.1], ascorbate peroxidase [APX; E.C. 1.1.1.11], catalase (CAT; E.C. 1.11.1.6], and glutathione reductase [GR; E.C. 1.6.4.2] (Blokhina *et.al.,* 2003).

Several environmental stresses induce the expression levels of antioxidative enzymes and their mRNAs (Edwards *et.al.*, 1994; Mittler and Zilinskas, 1994; Stevens, Creissen and Mullineaux 1997).

In this study, the effects of drought and salt stress applications on gene expression profiles of some antioxidant enzymes in a Turkish lentil (*Lens culinaris* M.) cultivar, Sultan-1, was comparatively analyzed. The experiment was designed to identify both short and long term effects of stress treatments on the gene expression levels of Mn SOD and Cu/Zn SOD genes in two different tissues as root and shoot.

4.1. Stress Application and Experimental Design

Both drought and salinity are serious environmental factors that affect crop yield in many areas of the world (Ashraf, 1994). The severity of yield loses will increase in following fifty years (Vinocur and Altman, 2005). Although there were some physiological and biochemical studies that distinguish the antioxidant behavior between drought and salt stresses (Reviewed by Bartel and Sunkar, 2005; Wang *et.al.,* 2003), there were no study reporting the relationship of antioxidant enzymes under the two stresses at molecular levels (Except a high body of experiment that was conducted in transcriptomics and proteomics fiels) (Salekdeh *et.al.,* 2002; Seki *et.al.,* 2002; Rossignol *et.al.,* 2006; Shulaeva *et.al.,* 2008).

Plants were grown in normal conditions (without any stress applications) for 7 days in growth chambers and 20% PEG or 150mM of NaCl were given to plants. Although there are different methods for drought application, polyethylene glycol 6000 was used in this experiment because a cytorrhytic rather than plasmolytic low water potential treatment can be imposed using solutions containing a high-molecular-weight solute such as PEG of molecular weight 6000 or above (Verslues *et.al.,* 2006). PEG 6000 or above cannot enter the pores of plant cells (Oertli, 1985) and thus causes cytorrhysis rather than plasmolysis. Polyethylene glycol is also a better choice for imposing low ww than the often used solute mannitol because mannitol has been shown to be taken up by plant cells and can cause specific toxic effects on growth (Hohl and Schopfer, 1991). In a previous study done on same lentil cultivar, the seedlings were treated with 10 %, 20% and 30 % PEG and 100 mM, 150 mM, 200 mM NaCl to induce drought and salinity stresses, respectively. Then, malondialdehyde (MDA) levels of stress-treated plant shoot and roots were analyzed. Hence, the highest MDA levels were found in 20% PEG and 150 mM NaCl treated plants (Ercan, 2008). Moreover, this data are in parallel to previous experiments since water potential of 20% PEG is nearly equal to water potential of 150mM of NaCl (van der Weele *et.al.,* 2000).

4.2. Effect of Drought Stress on Gene Expression Levels of Cu/Zn SOD and MnSOD Genes

Gene expression profile of Mn SOD enzyme (Figure 3.47) suggests that relative mRNA levels were very low in shoots at 6th hours of drought treatment. This is observed in shock treatments (Munns, 2002). Relative Mn SOD transcript expression levels increased at 12th hours of 20% PEG treatment by reaching to the levels obtained in control plants. Later, Mn SOD transcript expression decreased in drought-treated plants. As relative Mn SOD transcript expression levels in shoots of drought treated plants were always (except for 5 days) below the expression levels of same gene in control plants, it might be an indication of non-activated nature of Mn SOD gene under drought stress. These data are also proved by finding of Ercan (2008). No differences were previously indicated in H_2O_2 levels and Mn SOD activities in shoots of same cultivar under 20% PEG treatment for 7 days (Ercan, 2008). Similar results were obtained for root tissues (Figure 3.48) under same conditions with an exceptional increase in relative Mn SOD transcript expression levels reaching only to expression levels of control plants on 3rd days of drought application. This indicates that Mn SOD transcript expression is whether down-regulated or not affected by 20% PEG treatment for a course of 3 days. Finally, an increase in relative Mn SOD transcript expression levels over the levels of control plants were observed on 7th days of drought application. This result is in a good coherence with increased levels of H_2O_2 and Mn SOD activities in roots of 20% PEG treated Sultan cultivars for 7 days (Ercan, 2008).

Transcript expression profile of Cu/Zn SOD enzyme (Figure 3.53 and Figure 3.54) suggests that relative Cu/Zn SOD transcript expression levels increased at 7th days of 20% PEG application both in shoot and root tissues. Ercan (2008) found an increase in Cu/Zn SOD activities only in root tissues under drought stress. This may indicate a post-translational modification of Cu/Zn SOD protein

in Endoplasmic reticulum or in Golgi. The relationship between post-translational modifications of some enzymes and stress responsiveness of antioxidant enzymes were studied previously in *Arabidopsis* (Kang *et.al.,* 2008).

Moreover, there was a significant increase in relative Cu/Zn SOD transcript expression levels in root tissues of 1-day drought applied plants. Although transcript expression levels for stressed plants on 1st day was similar to the expression levels observed in stressed plants in other stress application days, there occurred a sharp decrease in Cu/Zn SOD transcript expression levels in control plants resulting in a significant difference between control and drought treated plants for 1 day. Hence, this indicates that relative gene expression levels of both enzymes change from time to time even in control plants. This shows that the expression levels are not stable developmentally, under different environmental conditions and in different tissues. Similar results were found in tobacco during development. Comparatively higher transcript expression levels of Cu/Zn SOD were found in young leaves and a gradual decrease was observed with leaf age (Kurepa *et.al.,* 1997). There is a parallel increase in transcript expression levels of Cu/Zn SOD both in control and stressed plants, and increase in stressed plants was much higher than the control ones. Hence, it is obvious that relative Cu/Zn SOD transcript expression levels increased in shoot tissues of 7 days drought treated plants because of application of 20% PEG.

In contrast to results presented here, Wu *et.al*. (1999) found that Mn SOD transcript expression had increased after 7 days of drought application while no change was observed in transcript expression of Cu/Zn SOD in wheat. These distinctive results indicate the importance of plant type used in experiments. Although there is a general antioxidative defence mechanism in all plants, there are some differences and conflicts between antioxidant defence mechanisms in different species.

4.3. Effect of Salt Stress on Gene Expression Levels of Cu/Zn SOD and MnSOD Genes

Transcript expression profile of Mn SOD enzyme (Figure 3.49 and Figure 3.50) suggests that relative gene expression levels decreased during first 24 hours of 150 mM of NaCl treatment both in shoot and root tissues. Then, relative transcript expression levels of Mn SOD increased between 1 day and 5 days of stress application and gave a peak value on 5th day of salt treatment. Later, it again decreased below control levels on 7th day of stress treatment.

Transcript expression profile of Cu/Zn SOD enzyme (Figure 3.55 and Figure 3.56) suggests that relative expression levels decreased in first 12 hours both in shoot and root tissues of 150mM NaCl-treated plants. Then, relative Cu/Zn SOD transcript expression levels increased starting at 12th hour of salt treatment, and gave a peak value on 1st and 5th days of stress application in roots and shoots, respectively. Later, expression levels decreased.

According to a previous biochemical study done on same cultivar, Mn SOD activity increased in shoots while significantly decreased in roots of 150mM NaCl-treated plants for 7 days. Cu/Zn SOD activities were higher both in shoots and roots of salt-treated plants. Again in that study, H_2O_2 concentrations increased significantly in shoots because total SOD activity increased in salt-treated plant shoots (Ercan, 2008). Being the scavengers of excess H_2O_2, activities of SOD enzymes are expected to be high in compartments susceptible to oxidative stress (Bowler *et.al.*, 1992). It is expected to observe high levels of Mn SOD and Cu/Zn SOD transcript expression and enzyme activity in shoots and roots of stressed plants. The main emphasis of the correlation between this study and Ercan's study would be better understood if the duration between levels of gene expression and

enzyme activity were compared. It was observed that transcript expression levels of Mn SOD gene peaked on 5th day of salt stress application while a high Mn SOD enzyme activity was observed in 7th day of salt application. There is a time gap between gene expression and protein functioning in all organisms. A decrease in Mn SOD transcript expression levels after peaked on 5th day of salt treatment may indicate that Mn SOD transcript expressions were produced during the first 5 days and then were degraded by cellular RNases. Hence, a decrease was observed after 5th day of stress application. The same time-gap in the induction of transcription and translation was also observed for Cu/Zn SOD. However, the correlation between the induction times of transcription and translation might be wrong since many gene products are regulated post-transcriptionally. Recently, it was found that Cu/Zn SOD genes were regulated post-transcriptionally by microRNAs in Arabidopsis. MicroRNA expression is downregulated transcriptionally by oxidative stresses, and this downregulation is important for post-transcriptional Cu/Zn SOD gene expression accumulation and oxidative stress tolerance (Sunkar *et.al.,* 2006).

Moreover, relative Cu/Zn SOD transcript expression levels were observed to increase in first 24 hours in roots of salt treated plants. This higher expression of Cu/Zn SOD may indicate that it was the major SOD enzyme which acted first in lentil to convert superoxide ($O_2^{\cdot -}$) to molecular oxygen and H_2O_2 Meanwhile, high expression of Cu/Zn SOD gene in first 24 hours of salt treatment may indicate that this gene was activated by transcription factors more earlier than Mn SOD gene or the two genes were activated by discrete signaling pathways. When plants are exposed to environmental stresses, some genes are activated by signal transduction. Hence, some genes are up-regulated and some are down-regulated while some others are not activated (Rabbani *et.al.,* 2003). Major stress responsive genes are the ones that function in detoxification, protein transport, protein turnover, protein folding, osmoprotection, photosynthesis, and water-ion movements (Sahi *et.al.,* 2006). Up-regulation of detoxification genes were observed under different environmental stresses including drought and salinity

(Seki *et.al.,* 2001, 2002; Kreps *et.al.,* 2002; Cheong *et.al.,* 2002; Rabbani *et.al.,* 2003; Buitink *et.al.,* 2006).

SOD isozymes are located in different sub-cellular compartments. Fe SODs are located in the chloroplast, Mn SODs in the mitochondrion and the peroxisome, and Cu/Zn SODs in the chloroplast, the cytosol, and possibly the extracellular space (Alscher *et.al.,* 2002). ROS production takes place in different organelles in a variety of activities. For that reason, expression levels of different SOD isozymes may vary under different environmental stresses. This is supported from a study of enzyme activity in yeast. The mutants showed the same SOD activity as the control strain, indicating that the deficiency in either cytosolic or mitochondrial enzyme had been overcome by an increase in activity of the remaining SOD. Hence, it was indicated that both Cu/Zn SOD and Mn SOD were important for improving survival, since the absence of only one isoform did not impair tolerance against dehydration (Pereira *et.al.,* 2003). Significance of both enzymes were given by over-expression studies in *Arabidopsis* (McKersie *et.al.,* 1999), tobacco (Gupta *et.al.,* 1993; Badawi *et.al.,* 2004), rice (Tanaka *et.al.,* 1999) and yeast (Pereira *et.al.,* 2003).

The basic physiology of high salt stress and drought stress overlaps with each other during the first phase of salt stress since the mechanisms controlling this phase of the growth response are not specific to salinity; they are caused by factors associated with water stress (Munns, 2002). Gene expression levels of Mn SOD and Cu/Zn SOD genes do also overlap with each other in 7 days of drought or salt treatments. One explanation for this overlap is that only the first phase of salt stress (associated with water stress) might be observed during the course of 7 days. Most probably 7 days is not sufficient to observe salt specific effects. Hence, plants need to be grown for a longer period of time to observe the effects of long term salt stress treatment on gene expression levels of antioxidant genes (Fidalgo *et.al.,* 2004; Gomez *et.al.,* 2004; Attia *et.al.,* 2008). Similar effects were obtained during the initial phase of salt stress in rice (Kawasaki *et.al.,* 2001).

CHAPTER 5

CONCLUSION

In this study, relative gene expression of Mn SOD and Cu/Zn SOD antioxidant enzymes in lentil (*Lens culinaris* M. cv. Sultan-1) were analyzed in a comparative manner both in shoot and root tissues under drought and salt stresses at different durations of stress applications.

Relative transcript expression levels of Mn SOD were lower in shoot and root tissues under salt stress while no significant change was obtained under drought conditions in both tissues. After 150mM NaCl application Mn SOD transcript expression levels increased between 1 and 5 days, by reaching to a peak on 5th day of stress application both in shoot and root tissues. Then expression levels decreased at 7th day of salt application. Mn SOD transcript expression levels were lower than control plants both in shoot and root tissues under drought conditions while expression of Mn SOD increased in drought-stressed plant shoots on 7 days.

Relative transcript expression levels of Cu/Zn SOD increased after 5th, and on 1st and 7th days of drought treatment in shoots and roots, respectively. On the other hand, transcript expression levels of Cu/Zn SOD increased on the 3rd and the 5th days of salt treatment in shoot tissues.

These results were in a good correlation with the results obtained in a previous study of the same antioxidant systems in the same cultivar (Ercan, 2008). This study supports Ercan's study in a way that antioxidant enzyme activities in lentil (*Lens culinaris* M. cv. Sultan-1) differed in salt and drought stresses and activities

were affected after gene expression of same antioxidant enzymes had been activated.

This study indicated that Mn SOD and Cu/Zn SOD are two important antioxidant enzymes in lentil and their genes were expressed under salt and drought stresses at different durations of stress applications.

Moreover, the results of both expression levels and activities of antioxidant enzyme systems in Sultan-1 cultivar of *Lens culinaris* signified that this cultivar might be more tolerant to drought than to salt stress.

Although it is nearly impossible to understand the whole antioxidant mechanism of plants under environmental stresses, this study was a step to learn about molecular background of some antioxidant enzymes. Gene expression profiles of CAT, chloroplast/mitochondrial GR and chloroplast/stromal APX will be the next step of this study. By this way, the comparison of gene expression profiles of different antioxidant enzymes with each other and also with enzyme activities will improve our knowledge of molecular protection mechanisms in lentil against salt and drought stresses.

CHAPTER 6

REFERENCES

Abe H., Urao T., Ito T., Seki M., Shinozaki K., Yamaguchi-Shinozaki K. (2003) "Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling" The Plant Cell: 15, pp. 63–78.

Abe H., Yamaguchi-Shinozaki K., Urao T., Iwasaki T., Hosokawa D., Shinozaki K. (1997) "Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression" The Plant Cell: 9, pp. 1859–1868.

Abebe T., Guenzi A. C., Martin B., Cushman J. C. (2003) "Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity" Plant Physiology: 131, pp. 1748–1755.

Alscher R. G., Erturk N., Heath L. S. (2002) "Role of Superoxide dismutases (SODs) in controling oxidative stres in plants" Journal of Experimental Botany: 53, pp. 1331-1341.

Apel K., Hirt H. (2004) "Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction" Annual Reviews of Plant Biology: 55, pp. 373-399.

Apse M. P., Aharon G. S., Snedden W. A., Blumwald E. (1999) "Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in Arabidopsis" Science: 285, pp. 1256–1258.

Arora A., Sairam R. K., Srivastava G. C. (2002) "Oxidative stres and antioxidative systems in plants" Current Science: 82, pp. 1227-1239.

Asada K (1999) "The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons" Annual Reviews in Plant Physiology and Plant Molecular Biology: 50, pp. 601–639.

Asada K. (2006) "Production and Scavening of Reactive Oxygen Species in Chloroplasts and Their Functions" Plant Physiology: 141, pp. 391-396.

Ashraf M (1994) “Breeding for salinity tolerance in plants” Critical Reviews in Plant Sciences: 13, pp. 17-42

Attia H., Arnaud N., Karray N, Lacha M. (2008) “Long-term effects of mild salt stress on growth, ion accumulation and superoxide dismutase expression of Arabidopsis rosette leaves” Physiologia Plantarum: 132 pp. 293–305.

Aubert, S., Hennion, F., Bouchereau, A., Gout, E., Blingy, R., Dome, A. J., (1999) “Subcellular compartmentation of proline in the leaves of the subantartic Kerguelen cabbage *Pringlea antiscorbutica* R-Br. In vivo C-13 NMR study” Plant, Cell & Environment: 22, pp. 255–259.

Babbs C. F., Pham J. A., Coolbaugh R. J. (1989) “Lethal Hydroxyl Radical Production in Paraquat-Treated Plants” Plant Physiology: 90, pp. 1267-1270.

Badawi G. H., Yamauchi Y., Shimada E., Sasaki R., Kawano N., Tanaka K., Tanaka K. (2004) “Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (Nicotiana tabacum) chloroplasts” Plant Science: 166, pp. 919–928.

Balibrea M. E., Rus-Alvarez A. M., Bolarín M. C., Pérez-Alfocea F. (1997) “ Fast changes in soluble carbohydrates and proline contents in tomato seedlings in response to ionic and non-ionic iso-osmotic stresses” Journal of Plant Physiology: 151, pp. 221–226.

Bannister WH, Bannister JV, Barra D, Bond J, Bossa F. (1991) “Evolutionary aspects of superoxide dismutase: the copperuzinc enzyme” Free Radical Research Communications: 12–13, pp. 349–361.

Barber J. (1998) “Photosystem two” Biochim. Biophys. Acta.:1365, pp. 269–77.

Barceló A. R. (2005) “Xylem-parenchyma cells deliver the H2O2 necessary for lignification in differentiating xylem vessels” Planta: 220, PP. 747–756.

Bartels D.; Sunkar R. (2005) “Drought and Salt Tolerance in Plants” Critical Reviews in Plant Sciences: 24, pp.23—58.

Bartley G.E., Scolnik P.A. (1995) “Plant carotenoids: pigments for photoprotection, visual attraction, and human health” Plant Cell: 7, pp. 1027–1038.

Baya B., Erskine W. (1998). “Diseases of lentil” In The Pathology of Food and Pasture Legumes (Eds D. J. Allen & J. M. Lenne), pp. 423–472. Wallingford, UK: CAB International.

Benson D. A., Karsch-Mizrachi I., Lipman D. J., Ostell J., Wheeler D. L. (2008) "GenBank" Nucleic Acids Research: 36, D25-D30.

Berthomieu P., Coné jé ro G., Nublat A., Brackenbury W. J., Lambert C., Savio C., Uozumi N., Oiki S., Yamada K., Cellier F., Gosti F., Simonneau T., Essah P. A., Tester M., Véry A-A., Sentenac H., Casse F. (2003) "Functional analysis of AtHKT1 in Arabidopsis shows that Na^+ recirculation by the phloem is crucial for salt tolerance" EMBO Journal: 22, pp. 2004–2014.

Bienert G. P., Schjoerring J. K., Jahn T. P. (2006) "Membrane transport of hydrogen peroxide" Biochimica et Biophysica Acta: 1758, pp. 994-1003.

Blokhina O., Virolainen E, Fagerstedt K. V. (2003) "Antioxidants, oxidative damage and oxygen deprivation stres: a review" Annals of Botany: 91, pp. 179-194.

Blum, A., (1986). "Breeding Crop Varieties for Stress Environments", Critical Reviews in Plant Sciences: 2, pp. 199-237

Bohnert H.J., Jensen, R.G. (1996) "Strategies for engineering waterstress tolerance in plants" Trends in Biotechnology: 14, pp. 89–97.

Boston R. S., Viitanen P. V., Vierling E. (1996) "Molecular chaperones and protein folding in plants" Plant Molecular Biology: 32, pp. 191-222

Botella-Pavia P., Rodriguez-Concepcion M. (2006) "Carotenoid biotechnology in plants for nutritionally improved foods" Physiologia Plantarum: 126, pp. 369–381.

Bowler C., Van Montagu M, Inze D. (1992) "Superoxide dismutase and stres tolerance" Annual Review of Plant Physiology and Plant Molecular Biology: 43, pp. 83-116.

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ. (2006) "ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H2O2 synthesis" The Plant Journal: 45, pp. 113–122.

Brunner A. M., Yakovlev I. A., Strauss S. H. (2004) "Validating internal controls for quantitative plant gene expression studies" BioMedCentral Plant Biology: 4,14.

Buettner G. R. (1993) "The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate" Archieves of Biochemistry and Biophysics: 300, pp. 535-543.

Buitink J., Leger J. J., Guisle I., Ly Vu B., Wuilleme S., Lamirault G., Le Bars A., Le Meur N., Becker A., Kuster H., Leprince O. (2006) "Transcriptome profiling uncovers metabolic and regulatory processes occurring during the transition from desiccationsensitive to desiccation-tolerant stages in *Medicago truncatula* seeds" The Plant Journal: 47, pp. 735–750.

Chandrakuntal K., Kumar P. G., Laloraya M., Laloraya M. M. (2004) "Direct involvement of hydrogen peroxide in curvature of wheat coleoptile in bluelight- treated and dark-grown coleoptiles" Biochemical and Biophysical Research Communications: 319, pp. 1190–1196.

Chen Y.R., Chen C.L., Zhang L., Green-Church K.B., Zweier J.L. (2005) "Superoxide generation from mitochondrial NADH dehydrogenase induces self-inactivation with specific protein radical formation" Journal of Biological Chemistry: 280, pp. 37339–37348.

Cheong Y., Chang H., Gupta R., Wang X., Zhu T., Sheng L. (2002) "Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal response in *Arabidopsis*" Plant Physiology: 129, pp. 661-677

Chinnusamy V., Jagendorf A., Zhu J-K. (2005) "Understanding and Improving Salt Tolerance in Plants" Crop Science: 45, pp.437–448.

Choi H., Hong J. H., Ha J., Kang J. Y., Kim S. Y. (2000) "ABFs, a family of ABA-responsive elements binding factors" Journal of Biological Chemistry: 275, pp. 1723–1730.

Chomczynski P. (1993) "A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples" BioTechniques: 15, pp. 532-537.

Cicerali I. N. (2004) "Effect of salt stress on antioxidant defense systems of Sensitive and resistant cultivars of Lentil (*Lens culinaris* M.)" M.Sc. Thesis, The Graduate School of Natural and Applied Sciences, Middle East Technical University.

Cobbett CS (2000) "Phytochelatins and their roles in heavy metal detoxification" Plant Physiology: 123, pp. 825–832.

Cramer G. R., Ergul A., Grimplet J., Tillett R. L., Tattersall E. A., Bohlman M. C., Vincent D., Sonderegger J., Evans J, Osborne C., Quilici D., Schlauch K. A., Schooley D. A., Cushman J. C. (2007) "Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles" Functional Integrative Genomics: 7, pp. 111–134.

Crisan D. (1994) "Molecular diagnostic testing for determination of myeloid lineage in acute leukemias" Annals of Clinical and Laboratory Science: 24, pp. 355-363.

Crowe J. H., Hoekstra F. A., Crowe C. M., (1992) "Anhydrobiosis" Annual Reviews of Plant Physiology: 54, pp. 579–599.

Cullis PM, Jones GD, Symons MC, Lea JS (1987) "Electron transfer from protein to DNA in irradiated chromatin" Nature:330, pp. 773–774. Current Opinion in Biotechnology: 16, pp. 123–132

Dat J., Vandenabeele S., Vranova E., Van Montagu M., Inze D., Van Breusegem F (2000) "Dual action of the active oxygen species during plant stres responses" Cellular and Molecular Life Sciences: 57, pp. 779–795.

DeGraaf J. (2004) "Turkey-Lentil Market Report" Last accessed date 05.06.2008 from World Wide Web http://www.sea.agr.gc.ca/europe/3864_e.htm

Denby K., Gehring C. (2005) "Engineering drought and salinity tolerance in plants: lessons from genome-wide expression profiling in Arabidopsis" Trends in Biotechnology: 23, pp. 547-552.

Desikan R., Cheung M-K., Bright J., Henson D., Hancock J. T., Neill S. J. (2004) "ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells" Journal of Experimental Botany: 55, PP. 205-212.

Dixon DP, Lapthorn A, Edwards R (2002) "Plant glutathione transferases" Genome Biology: 3, pp. 3004.1–3004.10.

Duke J.A. (1981). "Handbook of legumes of world economic importance" Plenum Press, New York. pp. 52-57.

Edwards E. A., Enard C., Creissen G. P., Mullineaux P.M. (1994) "Synthesis and properties of glutathione reductase in stressed peas" Planta: 192, pp. 137–143.

El-Bashiti T., Hamamcı H., Öktem H. A., Yücel M. (2005) "Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions" Plant Science: 169, pp. 47–54.

Elstner E.F. (1987) "Metabolism of activated oxygen species" In: Davies DD, ed.Biochemistry of plants, Vol. 11., London: Academic Press, pp. 253-315.

Elstner E.F., Osswald W. (1994) "Mechanisms of oxygen activation during plant stress" Proceedings of the Royal Society of Edinburgh: 102B, pp. 131-154.

Ercan O. (2008) "Effect of Drought and Salt Stresses on Antioxidant Defence Systems and Physiology of Lentil (*Lens culinaris* M.) Seedlings" M.Sc. Thesis, The Graduate School of Natural and Applied Sciences, Middle East Technical University.

Erskine W., Hussian A., Tahir M., Bahksh A., Ellis R. H., Summerfield R. J., Roberts E. H. (1994). "Field evaluation of a model of photothermal flowering responses in a world lentil collection" Theoretical and Applied Genetics: 88, pp. 423–428.

Esterbauer H, Schaur RJ, Zollner H (1991) "Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes" Free Radicals in Biological Medicine: 11, pp. 81–128.

FAO (2004), Last accessed date 05.06.2008 from World Wide Web http://faostat.fao.org/site/339/default.aspx

Farrell R. E. (2005) "RNA Isolation Strategies" in "RNA Methodologies: A Laboratory Guide for Isolation and Characterization" 3rd Edition, Elsevier Academic Press.

Ferguson M., Maxted N., Van Slageren M., Robertson L. D. (2000). "A re-assessment of the taxonomy of Lens Mill. (Leguminosae, Papilionoideae, Vicieae)" Botanical Journal of the Linnean Society: 133, pp. 41–59.

Fidalgo F., Santos A., Santos I., Salema R. (2004) "Effects of long-term salt stress on antioxidant defence systems, leaf water relations and chloroplast ultrastructure of potato plants" Annals of Applied Biology: 145, pp. 185-192.

Foote CS, Valentine JS, Greenberg A, Liebman JF, editors (1996) "Active Oxygen in Chemistry" Springer London, London, UK, pp. 6-11.

Foyer C.H. , Noctor G. (2000) "Transley Review No.112 Oxygen processing in photosynthesis: regulation and signaling" New Phytology: 146, pp. 359-388.

França M. B., Panek, A. D., Eleutherio E.C.A. (2007) "Eleutherio Oxidative stress and its effects during dehydration" Comparative Biochemistry and Physiology: 146, pp. 621–631.

Franceschi V. R., Tarlyn N. M. (2002) "L-ascorbic acid ıs accumulated in source leaf phloem and transported to sink tissues in plants" Plant Physiology: 130, pp. 649–656.

Fricke W. (2004) "Rapid and tissue-specific accumulation of solutes in the growth zone of barley leaves in response to salinity" Planta: 219, pp. 515–525.

Fridovich I. (1995) "Superoxide radical and SODs" Annual Reviews of Biochemistry: 64, pp. 97–112.

Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Ohme-Takagi M, Tran LSP, Yamaguchi-Shinozaki K, Shinozaki K. (2004) "A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway" The Plant Journal: 39, pp. 863–876.

Fukuda A., Nakamura A., Tanaka Y. (1999) "Molecular cloning and expression of the Na^+/H^+ exchanger gene in *Oryza sativa*" Biochimica et Biophysica Acta: 1446, pp. 149–155.

Furgeson M. (1998). "PhD Thesis: Studies of genetic variation within the genus *Lens*" School of Biological Sciences, University of Birmingham, Birmingham, UK

Gao M., Tao R., Miura K., Dandekar A. M., Sugiura A. (2001) "Transformation of Japanese persimmon (*Diospyros kaki* Thunb.) with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase" Plant Science: 160, pp. 837–845.

Glazebrook J (2001) "Genes controlling expression of defense responses in Arabidopsis— 2001 status". Current Opinion in Plant Biology: 4, pp. 301-308.

,
Gomez J. M., Jimenez A., Olmos E., Sevilla F. (2004) "Location and effects of long-term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (*Pisum sativum* cv. Puget) chloroplasts" Journal of Experimental Botany: 55, pp. 119-130.

Gupta A. S., Heinen J., Holaday S., Burke J. J., Allen R. D. (1993) "Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase" Proc. Nati. Acad. Sci. USA: 90, pp. 1629-1633.

Guy C. L. (1990) "Cold acclimation and freezing stress tolerance: role of protein metabolism" Annual Reviews in Plant Physiology.and Plant Molecular Biology: 41, pp. 187–223.

Halliwell B. (2006) "Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life" Plant Physiology: 141, pp. 312-322.

Halliwell B. , Gutteridge J.M.C. (2006) "Free Radicals in Biology and Medicine" Ed 4. Clarendon Press, Oxford, pp. 15-17.

Hartung W., Sauter A., Hose E. (2002) "Abscisic acid in the xylem: where does it come from, where does it go to?" Journal of Experimental Botany: 53, pp. 27–37.

Hassen J., Renfrew J. M. (1978). "Paleolithic- Neolithic seed remains at Franchthi cave, Greece" Nature: 71, pp. 349-352.

Henegariu O., Heerema N. A., Dlouhy S. R., Vance G. H., Vogt P. H. (1997) "Multiplex PCR: Critical Parameters and Step-by-Step Protocol" BioTechniques: 23, pp. 504-511.

Henzler T., Steudle E. (2000) "Transport and metabolic degradation of hydrogen peroxide in *Chara corallina*: model calculations and measurements with the pressure probe suggest transport of H_2O_2 across water channels" Journal of Experimental Botany:51, pp. 2053–2066.

Hernandez J. A., Jimenez A., Mullineaux P., Sevilla F. (2000) "Tolerance of pea (*Pisum sativum* L.) to long-term salt stres is associated with induction of antioxidant defenses" Plant Cell and Environment: 23, pp. 853–862.

Hohl M., Schopfer P. (1991) "Water relations of growing maize coleoptiles. Comparison between mannitol and polyethylene glycol 6000 as external osmotica for adjusting turgor pressure" Plant Physiology: 95, pp. 716–722.

Hong Z., Lakkineni K., Zhang Z., Verma D. P. S. (2000) "Removal of feedback inhibition of 1 pyrroline-5-carboxylase synthetase (P5CS) results in increased proline accumulation and protection of plants from osmotic stres" Plant Physiology: 122, pp. 1129–1136.

Hong-Boa S., Zong-Suo L., Ming-An S. (2005) "LEA proteins in higher plants: Structure, function, gene expression and regulation" Colloids and Surfaces B: Biointerfaces: 45, pp. 131–135.

Horie T., Schroeder J. I. (2004) "Sodium transporters in plants. Diverse genetic and physiological functions" Plant Physiology: 136, pp. 2457-2462.

Iba K. (2002) "Acclimative responses to temperature stress in higher plants: approaches of gene engineering for temperature tolerance" Annual Reviews in Plant Biology: 53, pp. 225-245.

IGEME, T.C Başbakanlık Dış Ticaret Müsteşarlığı İhracatı Geliştirme Etüd Merkezi. (2007) , BAKLİYAT RAPORU. Prepared by Akova, Y.

Ingram J., Bartels D. (1996) "The molecular basis of dehydration tolerance in plants" Annual Reviews of Plant Physiology and Plant Molecular Biology: 47, pp. 377–403.

Innocenti G., Pucciariello C., Gleuher M. L., Hopkins J.,de Stefano L., Delledonne M., Puppo A., Baudouin E., Frendo P. (2007) "Glutathione synthesis is regulated by nitric oxide in *Medicago truncatula* roots" Planta: 225, pp. 1597–1602.

Iyengar E. R. R., Reddy M. P. (1996) "Photosynthesis in highly salt-tolerant plants" In: Pesserkali, M. (Ed.) "Handbook of photosynthesis" Marshal Dekar, Baten Rose, USA, pp. 897–909.

Jaglo-Ottosen K. R., Gilmour S. J., Zarka D. G., Schabenberger O., Thomashow M. F. (1998) "Arabidopsis CBF1 overexpression induces coe genes and enhances freezing tolerance" Science: 280, pp. 104–106.

Jeschke WD, Aslam Z, Greenway H. (1986) "Effects of NaCl on ion relations and carbohydrate status of roots and on osmotic regulation of roots and shoots of *Atriplex amnicola*" Plant, Cell and Environment:9, pp. 559–569.

Jimenez A., Hernandez J.A., Pastori G., del Rio L. L., Sevilla F. (1998) "Role of the ascorbate-glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves" Plant Physiology: 118, pp. 1327-1335.

Jones H. G., Flowers T. J., Jones M. B. (1989). "Plants under Stress" Cambridge university Press, Cambridge, pp. 1–10.
Joo J.H., Bae Y.S., Lee J.S. (2001) "Role of auxin-induced reactive oxygen species in root gravitropism" Plant Physiology: 126, pp. 1055-1060.

Jorŕn J. V., Maldonado A. M., Castillejo Ma. A. (2007) "Plant proteome analysis: A 2006 update" Proteomics: 7, pp. 2947-2962.

Kalefetoglu T., Ekmekci Y., (2005) "The effects of drought on plants and tolerance mechanisms" G.U. Journal of Science: 18, pp. 723-740.

Kamal-Eldin A., Appelqvist L-A. (1996) "The chemistry and antioxidant properties of tocopherols and tocoprienols" Lipids: 31, pp. 671-701.

Kanematsu S, Asada K. (1990) "Characteristic amino acid sequences of chloroplast and cytosol isozymes of CuZn-superoxide dismutase in spinach, rice and horsetail" Plant Cell Physiology: 31, pp. 99–112.

Kang J. S., Frank J., Kang C. H., Kajiura H., Vikram M., Ueda A., S Kim, Bahk J. D., Triplett B., Fujiyama K., Lee S. Y., Schaewen A., Koiwa H. (2008) "Salt tolerance of Arabidopsis thaliana requires maturation of N-glycosylated proteins in the Golgi apparatus" *PNAS*:105, pp. 5933-5938.

Katerji N., van Hoorn J. W., Hamdy A., Mastrorillid M., Oweise T., Erskine W. (2001) "Response of two varieties of lentil to soil salanity" Agricultural Water Management: 47, pp. 179-190.

Kawasaki S., Borchert C., Deyholos M., Wang H., Brazille S., Kawai K., Galbraith D., Bohnert H. J. (2001) "Gene Expression Profiles during the Initial Phase of Salt Stress in Rice" The Plant Cell: 13, pp. 889–905.

Kay, D. (1979). "Food legumes" Tropical Development and Research Institute (TPI). TPI Crop and Product Digest No. 3. pp.48-71. UK

Kayıhan C. (2007) "Effect of Cold Stress on the Expression and Activity of Barley Cu/Zn SOD" M.Sc. Thesis, The Graduate School of Natural and Applied Sciences, Middle East Technical University.

Keates S.E., Tarlyn N.M., Loewus F.A., Franceschi V.R. (2000) "L-Ascorbic acid and L-galactose are sources for oxalic acid and calcium oxalate in *Pistia stratiotes*" Phytochemistry: 53, pp. 433-440.

Kerepesi I., Galiba G. (2000) "Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings" Crop Sciences: 40, pp. 482-487.

Khan M. A., Ungar I. A., Showalter A. M. (2000) "Effects of sodium chloride treatments on growth and ion accumulation of the halophyte *Haloxylon recurvum*" Communications in Soil Science and Plant Analysis: 31, pp. 2763–2774.

Khan, M.A., Ungar, I.A., Showalter, A.M. (1999) "Effects of salinity on growth, ion content, and osmotic relations in *Halopyrum mocoronatum* (L.) Stapf." Journal of Plant Nutrition: 22, pp. 191–204.

Khan, M.A., Ungar, I.A., Showalter, A.M. (2000) "Effects of sodium chloride treatments on growth and ion accumulation of the halophyte *Haloxylon recurvum*" Communications in Soil Science and Plant Analogy: 31, pp. 2763–2774.

Kim J. K., Bamba T., Harada K., Fukusaki E., Kobayashi A. (2007) "Time-course metabolic profiling in Arabidopsis thaliana cell cultures after salt stress treatment" Journal of Experimental Botany: 58, pp. 415–424.

Kiyosue T., Yamaguchi-Shinozaki K., Shinozaki K., (1993) "Characterization of two cDNAs (ERD11 and ERD13) for dehydration-inducible genes that encode putative glutathione S-transferases in Arabidopsis thaliana" FEBS Letters: 335, pp. 189–192.

Kopriva S, Rennenberg H (2004) "Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism" Journal of Experimental Botany: 55, pp. 1831–1842.

Kotchoni S. O, Gachomo E. W. (2006) "The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants" Journal of Bioscience: 31, pp. 389–404.

Kreps J. A., Wu Y., Hur-Song C., Zhu T., Wang X., Jeff F. Harper (2002) "Transcriptomic changes for *Arabidopsis* in response to salt, osmotic, and cold stres" Plant Physiology: 130, pp. 2129-2141.

Kupicha F. K. (1981). "Vicieae. *In* R. M. Polhill and P. H. Raven [eds.], Advances in legume systematics, part 1, 377–381. Royal Botanic Gardens, Kew, Richmond, UK

Kurban, H., Saneoka, H., Nehira, K., Adilla, R., Premachandra, G.S., Fujita, K. (1999) "Effect of salinity on growth, photosynthesis and mineral composition in leguminous plant *Alhagi pseudoalhagi* (Bieb.)" Soil Science and Plant Nutrition: 45, pp. 851–862.

Kurepa J., Herouart D., Montagu M. V., Inze D. (1997) "Differential Expression of CuZn- and Fe-Superoxide Dismutase Genes of Tobacco during Development, Oxidative Stress, and Hormonal Treatments" Plant Cell Phys:. 38, pp. 463-470.

Ladizinsky G. (1979). "The origin of lentil and its wild genpool" Euphytica: 28, pp. 179-187.

Laloi C., Apel K., Danon A. (2004) "Reactive oxygen signalling: the latest news" Current Opinion in Plant Biology: 7, pp. 323-328.Lam.) plantlets grown under light and dark conditions in vitro" Journal of Plant Physiology: 157, pp. 661–667.

Lane N (2002) "Oxygen, the Molecule That Made the World" Oxford University Press, Oxford, pp. 19-24

Liu Q., Sakuma Y., Abe H., Kasuga M., Miura S., Yamaguchi-Shinozaki K., Shinozaki K. (1998) "Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought- and low temperature-responsive gene expression, respectively, in Arabidopsis" The Plant Cell: 10, pp. 1491–1406.

Longstreth, D.J., Nobel, P.S., (1979) "Salinity effects on leaf anatomy" Plant Physiology: 63, pp. 700–703.

Luo Y., Li W-M., Wang W. (2008) "Trehalose: Protector of antioxidant enzymes or reactive oxygen species scavenger under heat stress?" Environmental and Experimental Botany: 63, pp. 378–384.

Luwe M., Heber U. (1995) "Ozone detoxification in the apoplasm and symplasm of spinach, broad bean and beech leaves at ambient and elevated concentrations of ozone in air" Planta: 197, pp. 448-455.

Mahajan S., Tuteja N. (2005). "Cold, salinity and drought stresses: An overview" Archives of Biochemistry and Biophysics: 444, pp. 139–158.

MansWeld T. J., Atkinson C. J. (1990) "Stomatal behaviour in water stressed plants" in: Alscher R.G., Cumming J.R. (Eds.) "Stress Responses in Plants: Adaptation and Acclimation Mechanisms" Wiley-Liss, New York, pp. 241–264.

Maurel C., Reizer J., Schroeder J. I., Chrispeels M. J. (1993) "The vacuolar membrane protein TIP creates water specific channels in *Xenopus* oocytes EMBO Journal: 12, pp. 2241–2247.

McKersie B. D., Bowley S. R., Jones K. S. (1999) "Winter survival of transgenic alfalfa overexpressing superoxide dismutase" Plant Physiology: 119, pp. 839–848.

Mitsuya, S., Takeoka, Y., Miyake, H., (2000) "Effects of sodium

,

Mittler R. (2002) "Oxidative stres, antioxidants and stres tolerance" Trends in Plant Science: 7, pp. 405-410.

Mittler R.,Vanderauwera S. ,Gollery M. ,Breusegem F. V.V. (2004) "Reactive oxygen gene network of plants" Trends in Plant Science: 9, pp. 490-498.

Mittler R., Hallak-Herr E., Orvar B. L., Van Camp W., Willekens H., Inze D., Ellis B. (1999) "Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyper-responsive to pathogen infection" Proceedings of National Academia of Sciences, USA: 96, pp. 14165-14170.

Mittler R., Zilinskas B. A. (1994) "Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought" Plant Journal: 5, pp. 397–405.

Miyake C, Cao WH, Asada K (1993) "Purification and molecular properties of thylakoid-bound ascorbate peroxidase from spinach chloroplasts" Plant Cell Physiology: 343, pp. 881–889.

Mizoguchi H., Hara S. (1996) "Effect of fatty acid saturation in membrane lipid bilayers on simple diffusion in the presence of ethanol at high concentrations" Journal of Fermentation Bioengineering: 81, pp. 406–411.,

Mokhamed A. M., Raldugina G. N., Kholodova V. P., and Kuznetsov Vl. V. (2006) "Osmolyte Accumulation in Different Rape Genotypes under Sodium Chloride Salinity" Russian Journal of Plant Physiology: 53, pp. 649–655.

Moon H., Lee B., Choi G., Shin S., Prasad D.T., Lee O., Kwak S.S., Kim D.H., Nam J., Bahk J. (2003) "NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants" PNAS: 100, pp.358-363.

Morsy M., Gouthu S., Orchard S., Thorneycroft D., Harper J. F., Mittler R., Cushman J. C. (2008) "Charting plant interactomes: possibilities and challenges" Trends in Plant Science:13, pp. 183-191.

Munns R. (2002) "Comparative physiology of salt and water stres" Plant, Cell and Environment: 25, pp. 239–250.

Munns R. (2005) "Genes and salt tolerance: bringing them together" New Phytologist: 167, pp. 645-663.

N. Sreenivasulu, B. Grimm, U. Wobus, W. Weschke (2000) "Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*)" Physiologia plantarum: 109, pp. 435–442.

Nakamura T., Nomura M., Mori H., Jagendroff A. T., Ueda A., Takabe T. (2001) "An isozyme of betaine aldehyde dehydrogenase in barley" Plant Cell Physiology:42, pp. 1088–92.

Navrot N., Rouhier N., Gelhaye E., Jacquot J. P. (2007) "Reactive oxygen species generation and antioxidant systems in plant mitochondria" Physiologica Plantarum: 129, pp. 185-195.

Neill S., Barros R., Bright J., Desikan R., Hancock J., Harrison J., Morris P., Ribeiro M., Wilson I. (2008) "Nitric oxide, stomatal closure, and abiotic stres" Journal of Experimental Botany: 59, pp. 165–176.

Neill S., Desikan R., Hancock J. (2002) "Hydrogen peroxide signalling" Current Opinion in Plant Biology: 5, pp. 388-395.

Noctor G., Foyer C. H. (1998) "Ascorbate and glutathıone: Keeping Active Oxygen Under Control" Annal. Reviews of. Plant Physiology and Plant Molecular Biolology: 49, pp. 249–79.

Nohmi T., Kim S.R., Yamada M., (2005) "Modulation of oxidative mutagenesis and carcinogenesis by polymorphic forms of human DNA repair enzymes" Mutation Research: 591, pp. 60–73.

Oertli J. J. (1985) "The response of plant cells to different forms of moisture stress" Journal of Plant Physiology: 121, pp. 295–300.

Orvar B. L., Ellis, B. E. (1997) "Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase show increased susceptibility to ozone injury" Plant Journal: 11, pp. 1297-1305.

Padh H. (1990) "Cellular functions of ascorbic acid" Biochemical Cell Biology: 68, pp. 1166–73.

Papageorgiou G. C., Morata N. (1995) "The usually strong stabilizing effects of glycine betaine on the structure and function in the oxygen evolving photosystem-II complex" Photosynthetic Research:44, pp. 243–52.

Parida A. K., Das A. B. (2005)." Salt tolerance and salinity effects on plants: a review" Ecotoxicology and Environmental Safety: 60, PP. 324–349

Pereira E. J., Panek A. D., Eleutherio E. C. A. (2003). "Protection against oxidation during dehydration of yeast" Cell Stress Chaperones: 8, pp. 120–124.

Pnueli, L. Liang H., Rosenberg M., Mittler R. (2003) "Growth suppression, altered stomatal responses, and augmented induction of heat shock proteins in cytosolic ascorbate peroxidase (Apx1)-deficient Arabidopsis plants" Plant Journal: 34, pp. 187–203.

Rabbani M. A., Maruyama K. , Abe H., Khan M. A., Katsura K., Ito Y., Yoshiwara K., Seki M., Shinozaki K., Yamaguchi-Shinozaki K. (2003) "Monitoring Expression Profiles of Rice Genes under Cold, Drought, and High-Salinity Stresses and Abscisic Acid Application Using cDNA Microarray and RNA Gel-Blot Analyses" Plant Physiology: 133, pp. 1755–1767.

Ramos M. L. G., Gordon A. J., Minchin F. R., Sprent J. I., Parsons R. (1999) "Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.)" Annals of Botany: 83, pp. 57–63

Raven E.L. (2003) "Understanding functional diversity and substrate specificity in haem peroxidases: what can we learn from ascorbate peroxidase?" Natural Product Reports: 20, pp. 367–381.

Reddy A. R., Chaitanya K. V., Vivekanandan M. (2004) "Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants" Journal of Plant Physiology: 161, pp. 1189–1202.

Rentel M. C., Lecourieux D., Ouaked F., Usher S. L., Petersen L., Okamoto H., Knight H., Peck S. C., Grierson S. C., Hirt H., Knight M. R. (2004) "OXI1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis" Nature: 427, pp. 858–861.

Reth M. (2002) "Hydrogen peroxide as second messenger in lymphocyte activation" Immunology: 3, pp. 1129–1134.

Rhoads D. M., Umbach A. L., Subbaiah C. C., Siedow J. N. (2006) "Mitochondrial oxygen species. Contribution to oxidative stres and interorganellar signalling" Plant Physiology: 141, pp. 357-366.

Rose T. M., Schultz E. R., Henikoff J. G., Pietrokovski S., McCallum C. M., Henikoff S. (1998) "Consensus-degenerate hybrid oligonucleotide primers for amplification of distantly-related sequences" Nucleic Acids Research: 26, pp.1628-1635.

Rossib R., Giustarinib D., Milzania A., Colombo R., Dalle-Donnea I. (2003) "Protein carbonyl groups as biomarkers of oxidative stres" Clinical Chimia. Acta: 329, pp. 23–38.

Rossignol M., Peltier J. B., Mock H. P., Matros A., Maldonado A. M., Jorrín J. V. (2006) "**Plant proteome analysis: A 2004-2006 update"** Proteomics: 6, pp. 5529-5548.

Sahi C., Singh A., Blumwald E., Grover A. (2006) "Beyond osmolytes and transporters: novel plant salt-stress tolerance-related genes from transcriptional profiling data" Physiologia Plantarum: 127, pp. 1-9.

Sakihama Y., Cohen M. F., Grace S.C.,Yamasaki H. (2002) "Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants" Toxicology: 177, pp. 67–80.

Sakuma Y., Maruyama K., Osakabe Y., Qin F., Seki M., Shinozaki K., Yamaguchi-Shinozaki K. (2006) "Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought responsive gene expression" The Plant Cell: 18, pp. 1292–309.

Salekdeh G. H., Siopongco J., Wade L. J., Ghareyazie B, Bennet J. (2002) "A proteomic approach to analyzing drought- and salt-responsiveness in rice" Field Crops Research: 76, pp. 199-219.

Sandhu J. S, Singh S. (2007) "History and Origin" in Edt.Yadav S. S., McNeil D., Stevenson P. C. "Lentil: An ancient crop for modern times" Springer.

Sangam S., Jayasree D., Reddy K. J., Chari P. V. B., Sreenivasulu N., Kavi Kishor P. B. (2005) "Salt tolerance in plants—transgenic approaches" Journal of Plant Biotechnology: 7, pp. 1–15.

Sarker A., Erskine W.,Singh M. (2005) "Variation in shoot and root characteristics and their association with drought tolerance in lentil landraces" Genetic Resources and Crop Evolution: 52, pp. 89-97.

Scandalios J. G. (1993) "Oxygen Stress and Superoxide Dismutases" Plant Physiology:1O1, pp. 7-12.

Seki M, **Narusaka M**, **Ishida J**, **Nanjo T**, **Fujita M**, **Oono Y**, **Kamiya A**, **Nakajima M**, **Enju A**, **Sakurai T**, **Satou M**, **Akiyama K**, **Taji T**, **Yamaguchi-Shinozaki K**, **Carninci P**, **Kawai J**, **Hayashizaki Y**, **Shinozaki K**. **(2002)** "Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant Journal: 31, pp. 279–292.

Seki M., Narusaka M., Abe H., Kasuga M., Yamaguchi-Shinozaki K., Carninci P., Hayashizaki Y., Shinozaki K.(2001) "Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray" Plant Cell: 13, pp. 61-72.

Sha Valli Khan P. S., Hoffmann L., Renaut J., Hausman J. F. (2007) "**Current initiatives in proteomics for the analysis of plant salt tolerance"** Current Science: 93, pp. 807-817.

Sheveleva E., Chmara W., Bohnert H. J., Jensen R. G. (1997) "Increased salt and drought tolerance by D-ononitol production in transgenic *Nicotiana tabacum* L." Plant Physiology: 115, pp. 1211–1219.

Shi H. Z., Quintero F. J., Pardo J. M., Zhu J. K. (2002) "The putative plasma membrane Na+/H+ antiporter SOS1 controls long-distance Na+ transport in plants" Plant Cell: 14, pp. 465–477.

Shi H., Zhu J.-K. (2002) "Regulation of expression of the vacuolar Na^+/H^+ antiporter gene AtNHX1 by salt stress and ABA" Plant Molecular Biology: 50, pp. 543–550.

Shinozaki K., Yamaguchi-Shinozak K. (2007) "Gene networks involved in drought stress response and tolerance" Journal of Experimental Botany: 58, pp. 221–227.

Shulaeva V., Cortesa D., Miller G., Mittler R. (2008) "Metabolomics for plant stress response" Physiologia Plantarum: 132, pp. 199–208.

Sidari M., Santonoceto C., Anastasi U., Preiti G., Muscolo A. (2008) "Variations in Four Genotypes of Lentil under NaCl-Salinity" Stress American Journal of Agricultural and Biological Science :3 pp. 410-416.

Singh K K. (2000) "The *Saccharomyces cerevisiae* SLN1P-SSK1P two component system mediates response to oxidative stress and in an oxidant-specific fashion" Free Radicals in Biological Medicine:29, pp.1043–50.

Singh K. B., Foley R. C., Oñate-Sánchez L. (2002) "Transcription factors in plant defense and stress responses" Current Opinion in Plant Biology: 5, pp. 430–436.

Singh T. N., Aspinall D., Paleg L. G. (1972) "Proline accumulation and varietal adaptation to drought in barley, a potential metabolic measure of drought resistance" – Nature: 236, pp. 188-190.

Smirnoff N. (1995) "Antioxidant systems and plant response to the environment" In: Smirnoff N., ed. Environment and plant metabolism: flexibility and acclimation Oxford, BIOS Scientific Publishers, pp. 217-243.

Smirnoff, N. (1993) "The role of active oxygen in the response of plants to water deficit and desiccation", New Phytology: 125, pp. 27-58.

Smith M.W., Doolittle R.F. (1992) "A comparison of evolutionary rates of the two major kinds of superoxide dismutases" Journal of Molecular Evolution: 34, pp. 175–184.

Sreenivasulu N., Sopory S. K., Kavi Kishor P. B. (2007) "Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches" Gene: 388, pp. 1–13.

Stevens R., Creissen G., Mullineaux P.M. (1997) "Cloning and characterization of a cytosolic glutathione reductase cDNA from pea (Pisum sativum L.) and its expression in response to stres" Plant Molecular Biology: 35, pp. 641–654.

Streb P., Feierabend J. (1996) "Oxidative stress responses accompanying photoinactivation of catalase in NaCl-treated rye leaves" Botanica Acta: 109, pp. 125–132.

Sunkar R., Kapoor A., Zhu J-K (2006) "Posttranscriptional Induction of Two Cu/Zn Superoxide Dismutase Genes in Arabidopsis Is Mediated by Downregulation of miR398 and Important for Oxidative Stress Tolerance" The Plant Cell: 18, pp. 2051-2065.

Suzuki N., Mittler R. (2006) "Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction" Physiologia Plantarum: 126, pp. 45-51.

Swindell W. R. (2006) "The Association Among Gene Expression Responses to Nine Abiotic Stress Treatments in *Arabidopsis Thaliana*" Genetics: 174, pp. 1811–1824.

Takahama U.(1992) "Hydrogen peroxide scavenging systems in vacuoles of mesophyll cells of *Vicia faba*" Phytochemistry: 31, pp. 1127–1133.

Takahashi M. A., Asada K. (1983) "Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids" Archives of Biochemistry and Biophysics: 226, pp. 558–566.

Tanaka Y., Hibino T., Hayashi Y., Tanaka A., Kishitani S., Takabe T., Yokota S. (1999) "Salt tolerance of transgenic rice over-expressing yeast mitochondrial Mn-SOD in chloroplasts" Plant Science: 148, pp. 131–138.

Telenius H., CarterN. P., BebbC. G., NordenskjöldM., PonderB. A. J., Tunnacliffe A. (1992) "Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer" Genomics: 13, pp. 718-725.

Thiellement H., Zivy M., Plomion C. (2002) "**Combining proteomic and genetic studies in plants"** Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences: 782, pp. 137-149.

Thomas C. E., McLean L. R., Parker R. A., Ohlweiler D. F. (1992) "Ascorbate and phenolic antioxidant interactions in prevention of liposomal oxidation" Lipids: 27, pp. 543-550.

Umezawa T., Fujita M., Fujita Y., Yamaguchi-Shinozaki K., Shinozaki K. (2006) "Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future" Current Opinion in Biotechnology: 17, pp. 113–122.

Uno Y., Furihata T., Abe H., Yoshida R., Shinozaki K., Yamaguchi-Shinozaki K. (2000) "Arabidopsis basic leucine zipper transcriptional transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions" Proceedings of the National Academy of Sciences, USA: 97, pp. 11632–11637.

Valliyodan B., Nguyen H. T. (2006) "Understanding regulatory networks and engineering for enhanced drought tolerance in plants" Current Opinion in Plant Biotechnology: 9, pp. 189–195.

van der Weele C. M., Spollen W. G., Sharp R. E., Baskin T. I. (2000) "Growth of Arabidopsis thaliana seedlings under water deficit studied by control of water potential in nutrient-agar media" Journal of Experimental Botany: 51, pp. 1555–1562.

Venkataraman S., Schafer F. Q., Buettner G. (2004) "Detection of Lipid Radicals Using EPR" Antioxidants & Redox Signaling: 6, pp. 631-638.

Verslues P. E., Agarwal M., Katiyar-Agarwal S., Zhu J., Zhu J. K. (2006) "Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status" The Plant Journal: 45, pp. 523–539.

Vinocur B., Altman A. (2005) "Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations" Current Opinion in Biotechnology: 16, pp. 123-132.

Vítámvás, P., Kosová, K., Prášil, I.T. (2007) "Proteome analysis in plant stress research" Czech Journal of Genetics and Plant Breeding: 43, pp. 1-6.

Vranova´ E., Atichartpongkul S., Villarroel R., Montagu M. V., Inze´ D., Camp W. V. (2002) "Comprehensive analysis of gene expression in *Nicotiana tabacum* leaves acclimated to oxidative stres" PNAS: 99, pp. 10870–10875.

Wang H. L., Lee P. D., Chen W. L., Huang D. J. Su J. C. (2000) "Osmotic stress-induced changes of sucrose metabolism in cultured sweet potato cells", Journal of Experimental Botany: 51, pp. 1991-1999.

Wang W., Vinocur B., Altman A. (2003) "Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance" Planta: 218, pp. 1–14.

Wang Y., Nil N., (2000) "Changes in chlorophyll, ribulose biphosphate carboxylase–oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stres" Journal of Horticultural Science and Biotechnology: 75, pp. 623–627.

Weckwerth W. (2008) "Integration of metabolomics and proteomics in molecular plant physiology - Coping with the complexity by data-dimensionality reduction" Physiologia Plantarum: 132, pp. 176-189.

Weiss J., Albermann N. (2003) "Quantification of mRNA levels with reverse transcription-polymerase chain reaction" Biochemical and Biophysical Research Communications: 311, pp. 561–562.

Wu G., Wilen R. W., Robertson A. J., Gusta L. V. (1999) "Isolation, Chromosomal Localization, and Differential Expression of Mitochondrial Manganese Superoxide Dismutase and Chloroplastic Copper/Zinc Superoxide Dismutase Genes in Wheat" Plant Physiology: 120, pp. 513–520.

Yakes FM, Van Houten B (1997) "Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stres" PNAS: 94, pp. 514–549.

Yamaguchi T., Blumwald E. (2005) "Developing salt-tolerant crop plants: challenges and opportunities" Trends in Plant Science: 10, pp. 615-620.

Yamaguchi-Shinozaki K., Shinozaki K. (2005) "Organization of cisacting regulatory elements in osmotic- and cold-stress-responsive promoters" Trends in Plant Science: 10, pp. 88–94.

Yamasaki, H., Sakihama, Y., Ikehara, N., (1997) "Flavonoidperoxidase reaction as a detoxification mechanism of plant cells against H_2O_2" Plant Physiology: 115, pp. 1405–1412.

Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S (2002) "Identification of a cis-element for tissue-specific alternative splicing of chloroplast ascorbate peroxidase pre-mRNA in higher plants" Journal of Biological Chemistry: 277, pp. 40623–40632.

Yuan J. S., Galbraith D. W., Dai S. Y., Griffin P., Stewart Jr. C. N. (2008) "Plant systems biology comes of age" Trends in Plant Science: 13, pp. 165-171.

Zhang L.S., Zhao W.M. (2003) "LEA protein functions to tolerance drought of the plant" Plant Physiology Communications: 39, pp. 61–66.

Zhang X., Zhang L., Dong F., Gao J., Galbraith D.W., Song C.P (2001) "Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*" *Plant Physiology:* 126, pp. 1438-1448.

Zhu J. K. (2002) "Salt and drought stress signal transduction in plants" Annual Reviews in Plant Biology: 53, pp. 247-273.

Zohad D. (1976). "The wild progenitor and plave of origin of the cultivated lentil *Lens culinaris*" Economic Botany: 26, pp. 326-332.

APPENDIX A

LIST of CHEMICAL MATERIALS and KITS

Chemicals / Kits	**Suppliers**
Molecular grade alcohol	Applichem
Chloroform	Applichem
Guanidine thiocyanate	Applichem
Isopropanol	Merck
β-Mercaptoethanol	Merck
Water-saturated Phenol	Applichem
Polyethylene glycol	Applichem
Potassium acetate	Applichem
Sarcosyl	Sigma
Sodium chloride	Applichem
Sodium citrate	Sigma
DEPC	Applichem
Deoxyribonuclease I	Fermentas
GeneRulerTM DNA Ladder	Fermentas
RevertAidTM First Strand cDNA Synthesis Kit	Fermentas
Taq DNA Polymerase	Applichem

APPENDIX B

The PREPARATION of TRIZOL REAGENT

1. Prepare 100mM Sodium citrate at pH 7.0.
2. Prepare 2M Potassium acetate at pH 4.8.
3. Prepare the following solution in a DEPC-treated flask:

- Weigh out 23.7 grams of guanidine thiocyanate,
- Add 12.5 mL of 100mM sodium citrate (pH: 7.0),
- Add 715 µL of 35% sarcosyl,
- Add 340 µL of β-mercaptoethanol,
- Bring the volume to 50 mL with DEPC-treated distilled water.

Autoclave the solution after mixing and dissolving all solid materials. After autoclaving, add 50 mL of water-saturated phenol and 10 mL of 2M potassium acetate (pH: 4.8) onto the solution.

APPENDIX C

PCR CONDITIONS

1) PCR CONDITIONS for DEGENERATE PRIMERS

Mn SOD (Amplicon Length is 382 bp)

Table C. 1. Final concentrations of PCR ingredients for Degenerate Mn SOD PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
MnSOD Right Degenerate Primer (10 µM)	1.2µM
MnSOD Left Degenerate Primer (10 µM)	1.2µM
Taq DNA Polymerase (5U/1µL)	0.05U
Template	100ng
25mM $MgCl_2$	1.5mM

Table C. 2. PCR Conditions of Mn SOD amplicon

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	2 min.
Cycle number = 5		
Denaturation	94ºC	30 sec.
Primer annealing	46ºC	45 sec.
Elongation	72ºC	1 min.
Cycle number = 35		
Denaturation	94ºC	30 sec.
Primer annealing	56ºC	45 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

CAT (Amplicon Length is 653 bp)

Table C. 3. Final concentrations of PCR ingredients for Degenerate CAT PCR

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
CAT Right Degenerate Primer (10 µM)	1.2µM
CAT Left Degenerate Primer (10 µM)	1.2µM
Taq DNA Polymerase (5U/1µL)	1U
Template	200ng
25mM $MgCl_2$	1.5mM

Table C. 4. PCR Conditions of CAT amplicon

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	45 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

Chloroplast/Mitochondrial GR (Amplicon Length is 578 bp)

Table C. 5. Final concentrations of PCR ingredients for Degenerate Chloroplast/Mitochondrial GR PCR

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Chloroplast/Mitochondrial GR Right Degenerate Primer (10 µM)	1.5µM
Chloroplast/Mitochondrial GR Left Degenerate Primer (10 µM)	1.5µM
Taq DNA Polymerase (5U/1µL)	1U
Template	200ng
25mM $MgCl_2$	1.5mM

Table C. 6. PCR Conditions of Chloroplast/Mitochondrial GR amplicon

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	57ºC	45 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

Chloroplast/stromal APX (Amplicon Length is 474 bp)

Table C. 7. Final concentrations of PCR ingredients for Degenerate chloroplast/stromal APX PCR

Ingredient	Final Concentration
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
chloroplast/stromal APX Right Degenerate Primer (10 µM)	1.2µM
chloroplast/stromal APX Left Degenerate Primer (10 µM)	1.2µM
Taq DNA Polymerase (5U/1µL)	0.5U
Template	100ng
25mM $MgCl_2$	1.5mM

Table C. 8. PCR Conditions of chloroplast/stromal APX amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 40		
Denaturation	94ºC	30 sec.
Primer annealing	57ºC	1 min.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

2) PCR CONDITIONS for SPECIFIC PRIMERS

Cu/Zn SOD Barley (To check any DNA contamination) (Amplicon length is 224 bp) *

Table C. 9. Final concentrations of PCR ingredients for Specific Cu/Zn SOD PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Cu/Zn SOD 1 Right Primer (10 µM)	0.6µM
Cu/Zn SOD 1 Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 10. PCR Conditions of Cu/Zn SOD 1 amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initian denaturation	94ºC	3 min.
Cycle number = 33		
Denaturation	94ºC	30 sec.
Primer annealing	54ºC	30 sec.
Elongation	72ºC	45 sec.
Final Extension	72ºC	10 min.

* Forward and reverse primers specific for barley (*Hordeum vulgare* L.) Cu/Zn SOD were used to amplify Cu/Zn SOD amplicon in lentil (*Lens culinaris* M.). Later, the amplicon was sequenced (result of which is given in Appendix E) and analyzed in databases for homology purposes (results are given in Appendices E and F).

Mn SOD (Amplicon Length is 186 bp)

Table C. 11. Final concentrations of PCR ingredients for Specific Mn SOD PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Mn SOD Right Primer (10 µM)	0.6µM
Mn SOD Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.5U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 12. PCR Conditions of Mn SOD amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initian denaturation	95ºC	1 min.
Cycle number = 32		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	1 min.
Elongation	72ºC	1 min. 30 sec.
Final Extension	72ºC	10 min.

18S rRNA (Amplicon Length is 250 bp)

Table C. 13. Final concentrations of PCR ingredients for Specific 18S rRNA PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
18S rRNA Right Primer (10 µM)	0.6µM
18S rRNA Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 14. PCR Conditions of 18S rRNA amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 30		
Denaturation	94ºC	45 sec.
Primer annealing	51ºC	30 sec.
Elongation	72ºC	45 sec.
Final Extension	72ºC	10 min.

Cu/Zn SOD Lentil (Amplicon Length is 305 bp)

Table C. 15. Final concentrations of PCR ingredients for Specific Cu/Zn SOD PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Cu/Zn SOD Right Primer (10 µM)	0.6µM
Cu/Zn SOD Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 16. PCR Conditions of Cu/Zn SOD amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 30		
Denaturation	94ºC	45 sec.
Primer annealing	51ºC	30 sec.
Elongation	72ºC	45 sec.
Final Extension	72ºC	10 min.

CAT Lentil (Amplicon Length is 442 bp)

Table C. 17. Final concentrations of PCR ingredients for Specific CAT PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
CAT Right Primer (10 µM)	0.6µM
CAT Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.30U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 18. PCR Conditions of CAT amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 34		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	1 min.
Elongation	72ºC	1 min. 30 sec.
Final Extension	72ºC	10 min.

Chloroplast/Mitochondrial GR Lentil (Amplicon Length is 400 bp)

Table C. 19. Final concentrations of PCR ingredients for Specific Chloroplast/Mitochondrial GR PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Chloroplast/Mitochondrial GR Right Primer (10 µM)	0.6µM
Chloroplast/Mitochondrial GR Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 20. PCR Conditions of Chloroplast/Mitochondrial GR amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 32		
Denaturation	94ºC	30 sec.
Primer annealing	49ºC	1 min.
Elongation	72ºC	1 min. 30 sec.
Final Extension	72ºC	10 min.

Chloroplast Stromal APX Lentil (Amplicon Length is 150 bp)

Table C. 21. Final concentrations of PCR ingredients for Specific Chloroplast Stromal APX PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Chloroplast Stromal APX Right Primer (10 µM)	0.6µM
Chloroplast Stromal APX Left Primer (10 µM)	0.6µM
Taq DNA Polymerase (5U/1µL)	1.30U
Template	50ng
25mM $MgCl_2$	1.25mM

Table C. 22. PCR Conditions of Chloroplast Stromal APX amplicon

PCR Step	**Step Temperature**	**Step Duration**
Initial denaturation	95ºC	1 min.
Cycle number = 31		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	1 min. 30 sec.
Elongation	72ºC	1 min.
Final Extension	72ºC	10 min.

APPENDIX D

PRIMER CONDITIONS

Amplicon Name	**Product Size (bp)**	**Tm (ºC)**	**T_A (ºC)**	**GC content (%)**
Mn SOD Degenerate	382	61.2 – 61.4	56	48.1 – 44.8
CAT Degenerate	653	53.4 – 57.4	48 – 52	34.8 – 48
Chl./Mitoch. GR Degenerate	578	60.5 – 65.0	55.5 – 60.0	44.4 – 51.8
Chl./Stromal APX Degenerate	474	63.8 – 59.7	58.8 – 54.7	51.8 – 46.4
Arabidopsis 18S rRNA	250	54.3 – 55.3	50 – 51	50 – 50
Mn SOD Specific	186	59.8 – 60.9	54 – 55	50 – 57.8
CAT Specific	442	54.2 – 56.5	49.2 – 51.5	50 – 55
Chl./Mitoc. GR Specific	400	52.8 – 55.3	48.8 – 50.3	40.9 – 45
Chl./Stromal APX Specific	150	60 - 61	55 – 56	50 – 55
Cu/Zn SOD Barley Specific	224	59.5 – 59.9	54	45 – 50
Cu/Zn SOD Lentil Specific	305	60	55	50 – 55

PRIMER SEQUENCES

Mn SOD Degenerate Forward Primer Sequence:

5’ – CTGCCTGATCTGGCTTATGATTAYGGWGC – 3’

Mn SOD Degenerate Reverse Primer Sequence:

5’ – GCACCCTTAGTAACCAGAGGATCYTGRTT – 3’

CAT Degenerate Forward Primer Sequences:

5’ – GGTTTCTTTGAAGTCACNCAYGAYG – 3’

CAT Degenerate Reverse Primer Sequences:

5’ - GTTACATCRAGAGGGTCAAARTC – 3’

Chloroplast/Mitochondrial GR Degenerate Forward Sequence:

5’ – GGTGCTTCTGCTGCTGTTTGYGARYTNCC – 3’

Chloroplast/Mitochondrial GR Degenerate Reverse Sequence:

5’ – TCTTCATCAAATCCCCKCARACCCTTC -3’

Chloroplast/stromal APX Degenerate Forward Primer Sequence:

5’ – AATGGCCACAAAGAGGTggwgcwaaygg – 3’

Chloroplast/stromal APX Degenerate Reverse Primer Sequence:

5’ – cwgtyagracctGACAAGTTACCGACTT – 5’

Arabidopsis 18S rRNA Forward Primer Sequence:

5’ – AAACGGCTACCACATCCAAG – 3’

Arabidopsis 18S rRNA Rewerse Primer Sequence:

5’ – CCCATCCCAAGGTTCAACTA – 3’

Mn SOD Specific Forward Primer Sequence:

5’ – GGCGGAGGTCATATTAACCA – 3’

Mn SOD Specific Reverse Primer Sequence:

5’ – AAGCCACACCCATCCAGAC – 3’

Cu/Zn SOD Barley Specific Forward Primer Sequence:

5’ – TTGCATTTCAACTGGACCAC – 3’

Cu/Zn SOD Barley Specific Reverse Primer Sequence:

5’ – GACCACCTTTCCCAAGATCA – 3’

Cu/Zn SOD Lentil Specific Forward Primer Sequence:

5’ – GCTTCCATATCCATGCCTTG – 3’

Cu/Zn SOD Lentil Specific Reverse Primer Sequence:

5’ – AGCTACTCTGCCACCAGCAT – 5’

CAT Specific Forward Primer Sequence:

5’ – CCTGTCATTGTGCGTTTCTC – 3’

CAT Specific Reverse Primer Sequence:

5’ – CTCCCACCTTAATGGCCTCT – 3’

Chloroplast/Mitochondrial GR Specific Forward Primer:

5’ – GAAATTGCTAGTCTATGCGTCA – 3’

Chloroplast/Mitochondrial GR Specific Reverse Primer:

5’ – AGCAAACTCCAAGGCAATGT – 3’

Chloroplast/Stromal APX Specific Forward Primer Sequence:

5’ – CTTCTCCAGCCGATCAAAGA – 3’

Chloroplast/Stromal APX Specific Reverse Primer Sequence:

5’ – AGGACATTGGTCAGGTCCAG – 3’

APPENDIX E

EXTRACTED PCR PRODUCT SEQUENCES

Cu/Zn SOD Forward Sequence *

TGNTTNCACTGGACCCATTTCATCCTATNNNTAGACAAGNAGNTCCTGAGGNTCCACTAGACATGCTGGTGA
TTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCTTCACCATTACTGACAACCAGATCCCTCTCACT
GGAACAAACTCCATCATAGGAAGGGCTGTTGTTGTCCATGCTGATCCTGATGATCTTGGGAAAGGTGGTCAT
NAGCTTANCAAANCTCTGGNACGCCNGCTGCCGAAACNTANNTNNANANNAANNNNNNNANATNNNTTTT
ATTTTCTTTANNNNGNNNATCGNNNNNNNNNNNNNANN

Cu/Zn SOD Reverse Sequence *

ATTTNTTAACNNATGNCCCCCTTTTCCCAAGGATCNTCNNTGNATCAGCATGGAACAACAACAGCCCTTCCTA
TGATGGAGTTTGTTCCAGTGAGAGGGATCTGGTTGTCAGTAATGGTGAAGCTTACAGTTCCATCATCACCAAC
ATTGATATTTCCTAAATCACCAGCATGTCTAGTCTCATCCTCAGGAGCACCATGTTCCTTCGCATTAGGATTG
AAATGTGGTCCAGTTGAAATGCAACCGTTCGTCGTGTCCCCCCAGGGCGANANA

* Sequencing of Cu/Zn SOD PCR product was done by ABI 310 DNA sequencer at METU Molecular Biology and Biotechnology R&D Center Laboratory.

CLUSTALW Analysis of Cu/Zn SOD Forward and inverse of Cu/Zn SOD Reverse sequences

```
CLUSTAL 2.0.8 multiple sequence alignment

CuZnSOD_Reverse_Inverse      ----------------------------ANANAG-CGGGACCCCCCTGTG 21
CuZnSOD_Forward              TGNTTNCACTGGACCCATTTCATCCTATNNNTAGACAAGNAGNTCCTGAG 50
                                                            *  ** *  *     **** *

CuZnSOD_Reverse_Inverse      CTGCTTGCCAACGTAAAGTTGACCTGGTG-----TAAAGTTAG-GATTAC 65
CuZnSOD_Forward                  GNTCCACTAGACATGCTGGTGATTTAGGAAATATCAATGTTGGTGATGAT
100
                                *      ** *   * ***  * *        ** *** * *** *

CuZnSOD_Reverse_Inverse          GCTTCCTTGTAC--CACGAGGACTCCTACTCTGATCTGTACGACC---AC
110
CuZnSOD_Forward                  GGAACTGTAAGCTTCACCATTACTGACAACCAGATCCCTCTCACTGGAAC
150
                             *   *  *   *  *** *  ***   *  * ****  *   **    **

CuZnSOD_Reverse_Inverse          TAAATCCTTTATAGTTACAACCACTACT----------ACCTTGACATTC
150
CuZnSOD_Forward                  AAACTCCATCATAGGAAGGGCTGTTGTTGTCCATGCTGATCCTGATGATC
200
                              ** *** * ****  *   *   *  *          * * ***   **

CuZnSOD_Reverse_Inverse          GAAGTGGTAATGACTGTTGGTCTAGGGAGAGT----GACCTTGTTTGAGG
196
CuZnSOD_Forward                  TTGGGAAAGGTGGTCATNAGCTTANCAAANCTCTGGNACGCCNGCTGCCG
250
                                *      **    *  *  **   *   *     **      **  *


CuZnSOD_Reverse_Inverse          TAGTATCCTTCCCGACAACAACAAGGTACGACTANGTNNCTNCTAGGAAC
246
CuZnSOD_Forward                  AAACNTANNTNNANANNAANNNNNNNNANATNNNTTTTATTTTCTTTANN
300
                              *   *   *    *  *         *        *   *      *

CuZnSOD_Reverse_Inverse      CCTTTTCCCCCNGTANNCAATTNTTTA 273
CuZnSOD_Forward              NNGNNNATCGNNNNNNNNNNNNNANN-- 325
                                     *  *   **
```

Mn SOD Forward Sequence

TTGCCCTGAGCTGCGCTTATGCGTCGAGAGATCGCACATCACTCATCAGAAACACCACCAGACTTATATTACC
AACTATAACAAAGCTCTCGAGCAGCTTCACGATGCCGTTGGTAAAGCTGATACATCTACCACTGTTAAGCTC
CAGAATGCCATCAAGTTCAACGGCGGAGGTCATATTAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTC
GGGAAGGAGGTGGTGAACCACCAAAGGAATCCCTAGGCTGGGCCATTGACACAAACTTTGGATCTTTGGAA
GCATTGATTCAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGGGTCTGGATGGGTGTGGCTTGGTCTTGAC
AAAGATTTGAAGAGGCTTGTGGTTGAAACCACTGCAAATCAAGATCCTCTGGTAACTAAGGGTGCTAAAAAC
TTATTTTATTTTTTTGTGTGCACCCCCCCCTACAAATTTAAACAAAAACACACGACGAACCCCC

Mn SOD Reverse Sequence

AAATAGGTGGTGAACACCACTGCCTCTTCGATCTTTGTCAGACCAAGCCACACCCCTCCAGACCCCTGAAGA
GCTGCACCTTCGGCATTAATCTTTTGAATCAATGCTTCCAAAGATCCAAAGTTTGTGTCAATGGCCCAGCCTA
GGGATTCCTTTGGTGGTTCACCACCTCCTTCCCGAACAGGAGCCAGATTTTTCCAGAAAATGGAATGGTTAAT
ATGACCTCCGCCGTTGAACTTGATGGCATTCTGGAGCTTAACAGTGGTAGATGTATCAGCTTTACCAACGGCA
TCGTGAAGCTGCTCGAGAGCTTTGTTATAGTTGGTAATATAAGTCTAGTGGTGTTTCTGATGATGGATTTGCA
TGATTTCGCCGCTAATGACAGGCTCCAAAGCTCCGTAATCATAAGCCAGATCAGGCAGACACATCCATCATC
AGAAACACCAGGGGAGTTATATTACCAAAAAAAAACCTGCGAGCCGTTGCCATGGTAAAGTGAAAAATACT
GTTTAACCCCAATAC

CLUSTALW Analysis of Mn SOD Forward and inverse of Mn SOD Reverse sequences

```
CLUSTAL 2.0.5 multiple sequence alignment

MnSOD1.ab1      ------------------------------------------------------------
MnSOD           GTATTGGGGTTAAACAGTATTTTTCACTTTACCATGGCAACGGCTCGCAGGTTTTTTTTT 60


MnSOD1.ab1      ------------------------------------------------------------
MnSOD           GGTAATATAACTCCCCTGGTGTTTCTGATGATGGATGTGTCTGCCTGATCTGGCTTATGA 120


MnSOD1.ab1      -----TTGCCCTG-AGCTGCGCTTATGCGTCGAGAGATCGCACATCACTCATCAGAAACA 54
MnSOD           TTACGGAGCTTTGGAGCCTGTCATTAGCGGCGAAATCATGCAAATCCATCATCAGAAACA 180
                       **  ** ***    * *  *** *** *    *** ***  ************

MnSOD1.ab1      CCACCAGACTTATATTACCAACTATAACAAAGCTCTCGAGCAGCTTCACGATGCCGTTGG 114
MnSOD           CCACTAGACTTATATTACCAACTATAACAAAGCTCTCGAGCAGCTTCACGATGCCGTTGG 240
                **** ******************************************************


MnSOD1.ab1      TAAAGCTGATACATCTACCACTGTTAAGCTCCAGAATGCCATCAAGTTCAACGGCGGAGG 174
MnSOD           TAAAGCTGATACATCTACCACTGTTAAGCTCCAGAATGCCATCAAGTTCAACGGCGGAGG 300
                ************************************************************

MnSOD1.ab1      TCATATTAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTCGGGAAGGAGGTGGTGA 234
MnSOD           TCATATTAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTCGGGAAGGAGGTGGTGA 360
                ************************************************************

MnSOD1.ab1      ACCACCAAAGGAATCCCTAGGCTGGGCCATTGACACAAACTTTGGATCTTTGGAAGCATT 294
MnSOD           ACCACCAAAGGAATCCCTAGGCTGGGCCATTGACACAAACTTTGGATCTTTGGAAGCATT 420
                ************************************************************

MnSOD1.ab1      GATTCAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGGGTCTGGATGGGTGTGGCTTGG 354
MnSOD           GATTCAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGGGTCTGGAGGGGTGTGGCTTGG 480
                ********************************************* *************

MnSOD1.ab1      TCTTGACAAAGATTTGAAGAGGCTTGTGGTTGAAACCACTGCAAATCAAGATCCTCTGGT 414
MnSOD           TCT-GACAAAGATC-GAAGAGGCA-GTGGTGTTCACCAC--CTATTT------------- 522
                *** *********  ********  *****    *****  * * *

MnSOD1.ab1      AACTAAGGGTGCTAAAAACTTATTTTATTTTTTTGTGTGCACCCCCCCCTACAAATTTAA 474
MnSOD           ------------------------------------------------------------
```

```
MnSOD1.ab1      ACAAAAACACACGACGAACCCCC 497
MnSOD           -----------------------
```

PLEASE NOTE: Showing colors on large alignments is slow.

Chl/Stromal APX Forward:

TTAAACAATTTTCAATGCTCGAGGTTGAAAATGGGAGGCACTGCTCGGACTTGTAAATGTATTGAAACTTCTC
CAGCCGATCAAAGACAAATATTCAGGCGTGACATATGCAGACTTATTTCAGTTAGCTGGTGCTACAGCTGTG
GAGGAAGCTGGAGGCCCAAAAATTCCCATGAAATATGGAAGAGTAGATACCTCTGGACCTGACCAATGTCCT
GAAGAAGGACGACTTCCTGATGCAGGGGCCCGTTCAGCTGCTGATCATTTGCGTGAAGTTTTTTACCGTATGG
GATTGGACGACAAGGAAATTGTTGCATTATCTGGAGCACACACAGTGGGGCGGTCTAGACCAGACCGTAGTG
GCTGGGGAAAACCTGAGACTAAATACACCAAAGACGGGCCAGGAGCACCTGGAGGACCATCCTGGACTGTC
CAATGGCTGAAAAAAACCTTTTCAGTTTTTGTGGGGTTTGATTTTTTTTTGTTTGTTCTGGATGGTTTTTTTTTC
TGCCTTTTCGGGGGGGGGGGTGGGTTGTGGGTTAGTTTAGTAAAAGAGTGGGTTCATTTATCTTTCCTTTCCG
GGGGGGGGCGCGGAGGCGGGGGGGGGGCCTTTAAAAAAAAAAAAAAAAAAAAAAAACACACCCGGGGG
GGGGGGGGGGGGGGGGG

Chl/Stromal APX Reverse:

CCCCCGCCTCCTTCTTCTAGTGGTGATTTTTAGAGCTCACGGTGTTTTCCCCCACGCCACTCACGCGGGCTGG
GGTCTAGACCGCCCCAGGTGTGTGTGCTCCAGATAATGGAACAATTTCCTTGTCGTCCCATCCCATACCGTAA
AAAACTTCACGCAAATGATCAGAGGGTGGAGGGGGGGCTGCATCAGGGAGTCGTCCTTCTTCAGGACATTGG
TCAGGTCCAGAGGTATCTACTCTTCCATAGTGCAGGGGAATTTTTGGGGCTCCAGCTTCCTCCACAGCTGTAG
CACCAGCTAACTGAAATAAGTCTGCATATGTCACGCCTGAATATTTGTCTTTGATCGGCTGGAGAAGTTTCAA
TGCATTTACAAGTCCAGCATTGGCTCCATGTTTCAACTCAGCCTCAAATCCTAAGCTACCGTTTGCTCCACCT
CTTTGTGGCCATTA

CLUSTALW Analysis of Chl/Stromal APX Forward and inverse of Chl/Stromal APX Reverse sequences

```
APX       ---------------------TTAAACAATT-TTCAATGCTCGAGG-----TTGAAAATG 33
Reversal  TAATGGCCACAAAGAGGTGGAGCAAACGGTAGCTTAGGATTTGAGGCTGAGTTGAAACAT 60
                                 ****  *   * *    * ****     ******

APX       GGAGGCACTGCTCGGACTTGTAAATGTATTGAAACTTCTCCAGCCGATCAAAGACAAATA 93
Reversal   GGAGCCAATGCT-GGACTTGTAAATGCATTGAAACTTCTCCAGCCGATCAAAGACAAATA 119
           **** ** **** ************* ********************************

APX         TTCAGGCGTGACATATGCAGACTTATTTCAGTTAGCTGGTGCTACAGCTGTGGAGGAAGC 153
Reversal    TTCAGGCGTGACATATGCAGACTTATTTCAGTTAGCTGGTGCTACAGCTGTGGAGGAAGC 179
            ************************************************************

APX        TGGAGGCCCAAAAATTCCCATGAAATATGGAAGAGTAGATACCTCTGGACCTGACCAATG 213
Reversal    TGGAGCCCCAAAAATTCCCCTGCACTATGGAAGAGTAGATACCTCTGGACCTGACCAATG 239
           ***** ************* ** * ***********************************

APX        TCCTGAAGAAGGACGACTTCCTGATGCAGGGGCCCGTTCAGCTGCTGATCATTTGCGTGA 273
Reversal    TCCTGAAGAAGGACGACTCCCTGATGCAGCCCCCCCTCCACCCTCTGATCATTTGCGTGA 299
           ****************** **********   *** * ** *  ****************
APX        AGTTTTTTACCGTATGGGATTGGACGACAAGGAAATTGTTGCATTATCTGGAGCACACAC 333
Reversal    AGTTTTTTACGGTATGGGATGGGACGACAAGGAAATTGTTCCATTATCTGGAGCACACAC 359
           ********** ********* ******************* *******************
APX        AG-TGGGGCGGTCTAGACCAGACCGTAGTGGCTGGGGAAAACCTGAGACTAAATACACCA 392
Reversal    ACCTGGGGCGGTCTAG-----ACCCCAGCCCGCGTGAGTGGCGTGGGGGAAAACACCGTG 414
           *  *************     ***  **     * *     * ** *   *** **
APX         AAGACGGGCCAGGAGCACCTGGAGGACCATCCTGGACTGTCCAATGGCTGAAAAAAACCT 452
Reversal     AGCTCTAAAAATCACCACTAGAAGAAGGAGGCGGGGG----------------------- 451
             *   *     *  * ***  * ** *  *  * **
APX         TTTCAGTTTTTGTGGGGTTTGATTTTTTTTTTGTTTGTTCTGGATGGTTTTTTTTTCTGCC 512
Reversal   ------------------------------------------------------------

APX          TTTTCGGGGGGGGGGGTGGGTTGTGGGTTAGTTTAGTAAAAGAGTGGGTTCATTTATCTT 572
Reversal    ------------------------------------------------------------

APX          TCCTTTCCGGGGGGGGGCGCGGAGGCGGGGGGGGGGCCTTTAAAAAAAAAAAAAAAAAA 632
Reversal    ------------------------------------------------------------

APX         AAAAAACACACCCGGGGGGGGGGGGGGGGGGGGG 667
Reversal    -----------------------------------
```

PLEASE NOTE: Showing colors on large alignments is slow.

CAT Forward:

TTTCGCCCCTCCATTGTGGCAGATTTCCTTCGAGCCCCTGGTGTTCAGACACCTGTCATTGTGCGTTTCTCAAC
TGTCATTCATGAACGTGGCAGCCCTGAAACCTTGAGGGACCCTCGAGGTTTTGCTGTAAAATTTTACACCAGA
GAGGGTAACTATGACCTTGTTGGAAACAACTTTCCTGTCTTCTTCGTTCATGACGGGATGAATTTTCCAGATA
TGGTCCACGCTCTTAAACCCAATCCCCAGACCCACATCCAGGAGAATTGGAGAATTCTTGACTTCTTCTCCCA
CTTTCCAGAAAGCCTTCACATGTTCTCCTTCCTATTTGATGATGTGGGTGTCCCTCAAGATTATAGGCATATG
GATGGTTTTGGAGTCAACACATATACCCTGATCAACAAGGCTGGGAAATCAGTGTTTGTGAAATTTCACTGG
AAGCCCACCTGTGGTGTGAAGTGTCTATTGGAAGAAGAGGCCATTAAGGTGGGAGGATCCAACCACAGTCAT
GCTACTAAAGACCTTTATGACTCAATTGCTGCTGGTAACTATCCTGAGTGGAAACTTTATATTCAAACAATAG
ATCCTGCTCATGAAGACAGATTTGACTTTGACCTCCTCCGATGTAACA

CAT Reverse:

AATGGGCTTCCTGAGCAGGATCTATTGTTTGAATATAAAGTTTCCACTCAGGATAGTTACTCGCAGCAATTGA
GTCATAAAGGTCTTTAGTAGCATGACTGTGGTTGGATCCTCCCACCTTAATGGCCTCTTCTTCCAATAGACAC
TTCACACCACAGGTGGGCTTCCAGTGAAATTTCACAAACACTGATTTCCCAGCCTTGTTGATCAGGGTATATG
TGTTGACTCCAAAACCATCCATATGCCTATAATCTTGAGGGACACCCACATCATCAAATAGGAAGGAGAACA
TGTGAAGGCTTTCTGGAAAGTGGGAGAAGAAGTCAAGAATTCTCCAATTCTCCTGGATGTGGGTCTGGGGAT
TGGGTTTAAGAGCGTGGACCATATCTGGAAAATTCATCCCGTCATGAACGAAGAAGACAGGAAAGTTGTTTC
CAACAAGGTCATAGTTACCCTCTCTGGTGTAAAATTTTACAGCAAAACCTCGAGGGTCCCTCAAGGTTTCAG
GGCTGCCACGTTCATGAATGACAGTTGAGAAACGCACAATGACAGGTGTCTGAACACCAGGGGCTCGAAGG
AAATCTGCACATGTGAGGTGCGAAACATCATGCGTGACTTTCAAGAAACCA

CLUSTALW Analysis of CAT Forward and inverse of CAT Reverse sequences

```
CAT1            --------------------------TTTCGCCCCTCCATTGTGGCAGATTTCCTTCGAG 34
CAT2            TGGTTTCTTGAAAGTCACGCATGATGTTTCGCACCTCACATGTG-CAGATTTCCTTCGAG 59
                                          ****** ****   **** ***************

CAT1            CCCCTGGTGTTCAGACACCTGTCATTGTGCGTTTCTCAACTGTCATTCATGAACGTGGCA 94
CAT2            CCCCTGGTGTTCAGACACCTGTCATTGTGCGTTTCTCAACTGTCATTCATGAACGTGGCA 119
                ************************************************************

CAT1            GCCCTGAAACCTTGAGGGACCCTCGAGGTTTTGCTGTAAAATTTTACACCAGAGAGGGTA 154
CAT2            GCCCTGAAACCTTGAGGGACCCTCGAGGTTTTGCTGTAAAATTTTACACCAGAGAGGGTA 179
                ************************************************************

CAT1            ACTATGACCTTGTTGGAAACAACTTTCCTGTCTTCTTCGTTCATGACGGGATGAATTTTC 214
CAT2            ACTATGACCTTGTTGGAAACAACTTTCCTGTCTTCTTCGTTCATGACGGGATGAATTTTC 239
                ************************************************************

CAT1            CAGATATGGTCCACGCTCTTAAACCCAATCCCCAGACCCACATCCAGGAGAATTGGAGAA 274
CAT2            CAGATATGGTCCACGCTCTTAAACCCAATCCCCAGACCCACATCCAGGAGAATTGGAGAA 299
                ************************************************************

CAT1            TTCTTGACTTCTTCTCCCACTTTCCAGAAAGCCTTCACATGTTCTCCTTCCTATTTGATG 334
CAT2            TTCTTGACTTCTTCTCCCACTTTCCAGAAAGCCTTCACATGTTCTCCTTCCTATTTGATG 359
                ************************************************************

CAT1            ATGTGGGTGTCCCTCAAGATTATAGGCATATGGATGGTTTTGGAGTCAACACATATACCC 394
CAT2            ATGTGGGTGTCCCTCAAGATTATAGGCATATGGATGGTTTTGGAGTCAACACATATACCC 419
                ************************************************************

CAT1            TGATCAACAAGGCTGGGAAATCAGTGTTTGTGAAATTTCACTGGAAGCCCACCTGTGGTG 454
CAT2            TGATCAACAAGGCTGGGAAATCAGTGTTTGTGAAATTTCACTGGAAGCCCACCTGTGGTG 479
                ************************************************************

CAT1            TGAAGTGTCTATTGGAAGAAGAGGCCATTAAGGTGGGAGGATCCAACCACAGTCATGCTA 514
CAT2            TGAAGTGTCTATTGGAAGAAGAGGCCATTAAGGTGGGAGGATCCAACCACAGTCATGCTA 539
                ************************************************************

CAT1            CTAAAGACCTTTATGACTCAATTGCTGCTGGTAACTATCCTGAGTGGAAACTTTATATTC 574
CAT2            CTAAAGACCTTTATGACTCAATTGCTGCGAGTAACTATCCTGAGTGGAAACTTTATATTC 599
                ****************************  ******************************

CAT1            AAACAATAGATCCTGCTCATGAAGACAGATTTGACTTTGACCTCCTCCGATGTAACA 631
CAT2            AAACAATAGATCCTGCTCAGGAAGCCCATT-------------------------- 629
                ******************* **** *   *
```

PLEASE NOTE: Showing colors on large alignments is slow.

Chloroplast/Mitochondrial GR Forward:

TTTTCTCACACTCTCCTCCCGATACAACCGCGAGGTGTCGCGCGGCACCTCGTGTAATACGTGGATGTGTCCC
TAAGAAATTGCTAGTCTATGCGTCAAAATTTTCTCATGAATTTGAAGAAAGCAATGGTTTTGGATGGAGATAT
GACAGTGAACCTAAGCATGACTGGAGTAGTTTGATTGCTAATAAAAATGCCGAGTTGCAGCGGCTTACTGGT
ATCTATAAGAATATTTTGAAAAATGCCGGTGTCAAGTTGATTGAAGGCCGTGGAAAGATTGTAGATCCTCAC
ACGGTTGATGTTGATGGGAAGTTATATTCAGCAAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCATTC
CTGATATTCCTGGAAAGGAATATGCAATAGATTCAGACGCTGCCCTTGATTTACCATCAAAGCCTCAGAAGA
TAGCCATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTATCTTTAATGGTTTGAAAAGTGAAGTTCA
TGTATTTATACGACAAAAGAAGGGTCTGCGGGGATTTTGATGAAGAA

Chloroplast/Mitochondrial GR Reverse:

TTTTGGTCGTATAATACATGAGCTTCACTTTTCAACCATTAAAGATACCAGCAAACTCCAAGGCAATGTAACC
CCCACCAACAATGGCTATCTTCTGAGGCTTTGATGGTAAATCAAGGGCAGCGTCTGAATCTATTGCATATTCC
TTTCCAGGAATATCAGGAATGAAGGGTCGACCTCCAACTGAAACTAAAATGTGTTTTGCTGAATATAACTTC
CCATCAACATCAACCGTGTGAGGATCTACAATCTTTCCACGGCCTTCAATCAACTTGACACCGGCATTTTTCA
AAATATTCTTATAGATACCAGTAAGCCGCTGCAACTCGGCATTTTTATTAGCAATCAAACTACTCCAGTCATG
CTTAGGTTCACTGTCATATCTCCATCCAAAACCATTGCTTTCTTCAAATTCATGAGAAAATTTTGACGCATAG
ACTAGCAATTTCTTAGGGACACATCCACGTATTACACAGGTGCCGCCGACACCTCCGGTGGTATCGGAGGAG
ATGGTGGAGAAAGGCAGCTCACAAACAGAACCCAGAAGCACACAACTCTCCTCCGATACCCCGGAGGGTGT
CGCGCGTGTATCGTGGAGTGTCTCACCAAATGGATTAGCAACATTCGAAATAGAAAAAACAGGGGTTTGGGA
TGGAAATATGACATGCACCTAACAGCTAGTGGGATATTTGATTGCTAATAAAAATGCAGGTCTGTAATCTAA
AATATTTGAAAAGCGACCTGATGGAAGCGAAAGATATAATCGATGGAATTTTTTGGACTTTCCTACGCCTCTC
AACAC

CLUSTALW Analysis of Chloroplast/Mitochondrial GR Forward and inverse of Chloroplast/Mitochondrial GR Reverse sequences

```
GR1.ab1          ------------------------------------------------------------
GR2_Tersini      GTGTTGAGAGGCGTAGGAAAGTCCAAAAAATTCCATCGATTATATCTTTCGCTTCCATCA 60

GR1.ab1          ------------------------------------------------------------
GR2_Tersini      GGTCGCTTTTCAAATATTTTAGATTACAGACCTGCATTTTTATTAGCAATCAAATATCCC 120

GR1.ab1          ------------------------------------------------------------
GR2_Tersini      ACTAGCTGTTAGGTGCATGTCATATTTCCATCCCAAACCCCTGTTTTTTCTATTTCGAAT 180

GR1.ab1          ------------------------------------------------------------
GR2_Tersini      GTTGCTAATCCATTTGGTGAGACACTCCACGATACACGCGCGACACCCTCCGGGGTATCG 240

GR1.ab1          ----------------------------------------TTTTCTCACACTCTCCTCC 19
GR2_Tersini      GAGGAGAGTTGTGTGCTTCTGGGTTCTGTTTGTGAGCTGCCTTTCTCCACCATCTCCTCC 300
                                                         ***   ***  ********
GR1.ab1          CGATACAACCGCGAGGTGTCGCGCGGCACCTCGTGTAATACGTGGATGTGTCCCTAAGAA 79
GR2_Tersini      -GATACCACCG-GAGGTGTCG-GCGGCACCT-GTGTAATACGTGGATGTGTCCCTAAGAA 356
                  ***** **** ********* ********* ****************************
GR1.ab1          ATTGCTAGTCTATGCGTCAAAATTTTCTCATGAATTTGAAGAAAGCAATGGTTTTGGATG 139
GR2_Tersini      ATTGCTAGTCTATGCGTCAAAATTTTCTCATGAATTTGAAGAAAGCAATGGTTTTGGATG 416
                 ************************************************************
GR1.ab1          GAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGATTGCTAATAAAAATGCCGA 199
GR2_Tersini      GAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGATTGCTAATAAAAATGCCGA 476
                 ************************************************************
GR1.ab1          GTTGCAGCGGCTTACTGGTATCTATAAGAATATTTTGAAAAATGCCGGTGTCAAGTTGAT 259
GR2_Tersini      GTTGCAGCGGCTTACTGGTATCTATAAGAATATTTTGAAAAATGCCGGTGTCAAGTTGAT 536
                 ************************************************************
GR1.ab1          TGAAGGCCGTGGAAAGATTGTAGATCCTCACACGGTTGATGTTGATGGGAAGTTATATTC 319
GR2_Tersini      TGAAGGCCGTGGAAAGATTGTAGATCCTCACACGGTTGATGTTGATGGGAAGTTATATTC 596
                 ************************************************************
GR1.ab1          AGCAAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCATTCCTGATATTCCTGGAAA 379
GR2_Tersini      AGCAAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCATTCCTGATATTCCTGGAAA 656
                 ************************************************************
GR1.ab1          GGAATATGCAATAGATTCAGACGCTGCCCTTGATTTACCATCAAAGCCTCAGAAGATAGC 439
GR2_Tersini      GGAATATGCAATAGATTCAGACGCTGCCCTTGATTTACCATCAAAGCCTCAGAAGATAGC 716
                 ************************************************************
GR1.ab1          CATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTATCTTTAATGGTTTGAAAAG 499
GR2_Tersini      CATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTATCTTTAATGGTT-GAAAAG 775
                 ***************************************************** ******
GR1.ab1          TGAAGTTCATGTATTTATACGACAAAAGAAGGGTCTGCGGGGATTTTGATGAAGAA 555
GR2_Tersini      TGAAGCTCATGTATT-ATACGACCAAAA---------------------------- 802
                 ***** ********* ******* ***
```

PLEASE NOTE: Showing colors on large alignments is slow.

18S rRNA Forward:

CCCAATTTTACTGGCACAACTTAAATATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTG
CCCTCCAATGGATCCTCGTTAAGGGATTTAGATTGTACTCATTCCAATTACCAGACTCAATGAGCCCGGTATT
GTTATTTATTGTCACTACCTCCCCGTGTTAGGATTGGGTAATTTGCGCGCCAGCTGCCTTCCTAGAGTTGGGT
AACCCGTTCAGAAAGCGGCAAAACGCACGTAAAGAGGATAGTTAATGATGACATTGGAGGTCTAATTGGGT
CTGGTAATTGGAATGAGTCCATAGAAATAATTAACGAGGGATAATTGAAGGGCAAGTCTGGTGAAAGCAGC
CGCGGAAATTCCAGCTCATTAGCGTATTTTTAAGTTAATGAGTAACAAGCCGTAGTTTAACCATGGGGATGG
GAAATTTTTGATTTTTAAAAAAAA

18S rRNA Reverse:

CCGACTTTACTGCACACTTAATATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCT
CCAATGGATCCTCGTTAAGGGATTTAGATTGTACTCATTCCAATTACCAGACTCAATGAGCCCGGTATTGTTA
TTTATTGTCACTACCTCCCCGTGTTAGGATTGGGTAATTTGCGCGCCTGCTGCCTTCCTTGGTGGTGGTAGCCG
TTTAACCACCTAACACGGGGAGGTAGTGACAATAAATAACAATACCGGGCATCATTGAGTCTGGTAATTGGA
ATGAGTACAATCTAAATCCCTTAACAGGATCCTTTTGGAGGGCGGTTCTAGTGCCAAATCCCATAATTTATAT
TTAAATTGTTGATTAAAAATTGTTTACGGGGGGGTGGAG

CLUSTALW Analysis of 18S rRNA Forward and inverse of 18S rRNA Reverse sequences

```
CLUSTAL 2.0.8 multiple sequence alignment


Forward         CCCAATTTTACTGGCACAACTTAAATATACGCTATTGGAGCTGGAATTACCGCGGCTGCT 60
Reverse         -CCGACTTTACTG-CACACTT--AATATACGCTATTGGAGCTGGAATTACCGCGGCTGCT 56
                 ** * ******* ****  *  ************************************

Forward         GGCACCAGACTTGCCCTCCAATGGATCCTCGTTAAGGGATTTAGATTGTACTCATTCCAA 120
Reverse         GGCACCAGACTTGCCCTCCAATGGATCCTCGTTAAGGGATTTAGATTGTACTCATTCCAA 116
                ************************************************************

Forward         TTACCAGACTCAATGAGCCCGGTATTGTTATTTATTGTCACTACCTCCCCGTGTTAGGAT 180
Reverse         TTACCAGACTCAATGAGCCCGGTATTGTTATTTATTGTCACTACCTCCCCGTGTTAGGAT 176
                ************************************************************

Forward         TGGGTAATTTGCGCGCCAGCTGCCTTCCTAGAGTTGGGTAACCCGTTCAGAAAGCGGCAA 240
Reverse         TGGGTAATTTGCGCGCCTGCTGCCTTCCTTGGTGGTGGTAGCCGTTT-----AACCACCT 231
                ***************** *********** *     **** **  **     * *  *

Forward         AACGCACGTAAAGAG-GATAGTTAATGATGACATTGGAGGTCTAATTGGGTCTGGTAATT 299
Reverse         AACACGGGGAGGTAGTGACAATAAATAACAATACCGGGCATC--ATTGAGTCTGGTAATT 289
                *** *  * *   ** ** * * *** *  * *  **   **  **** ***********

Forward         GGAATGAGTCCA--TAGAAATAATTAACGAGGGATAATTGAAGGGCAAGTCTGGTGAAAG 357
Reverse         GGAATGAGTACAATCTAAATCCCTTAACAGGATCCTTTTGGAGGGCGGTTCTAGTG---- 345
                ********* **     **    *****  *      *** *****   *** ***

Forward         CAGCCGCGGAAATTCCAGCTCATTAGCGTATTTTTAAGTTAATGAGTAACAAGCCGTAGT 417
Reverse         ---CC----AAATCCCA------TAATTTATATTTAAATTGTTGATTAAAAA----TTGT 388
                   **    **** ***      **   *** ***** **  *** *** **    * **

Forward         TTAACCATGGGGATGGGAAATTTTTGATTTTTAAAAAAAA 457
Reverse         TTA--CGGGGGGGTGGAG--------------------- 404
                ***  *  **** ***
PLEASE NOTE: Showing colors on large alignments is slow.
```

APPENDIX F

tblastx RESULTS

For Cu/Zn SOD:*

Sequences producing significant alignments:		Score (Bits)	E Value	N
dbj|AB189165.1|	Pisum sativum mRNA for copper zinc superoxide...	386	0.0	1
dbj|AB087845.1|	Pisum sativum SOD mRNA for superoxide dismuta...	384	0.0	1
gb|M63003.1|PEACUZNSD	Pea Cu-Zn superoxide dismutase mRNA, co...	451	1e-160	1
emb|AJ012691.1|CAR012691	Cicer arietinum mRNA for superoxide ...	361	2e-113	1
emb|AJ012739.1|CAR012739	Cicer arietinum mRNA for superoxide ...	361	8e-107	1
gb|EF530044.1|	Caragana jubata copper/zinc superoxide dismuta...	358	3e-98	1
gb|EF147959.1|	Populus trichocarpa clone WS0126_E03 unknown mRNA	347	9e-93	1
emb|AJ278669.1|PTR278669	Populus tremula x Populus tremuloide...	345	4e-92	1
dbj|AB190501.1|	Populus alba x Populus tremula var. glandulos...	343	2e-91	1
dbj|AB190500.1|	Populus alba x Populus tremula var. glandulos...	343	2e-91	1

For Mn SOD:*

```
                                                                    Score     E
Sequences producing significant alignments:                          (Bits)  Value
N

emb|X60170.1|PSSODR   Pisum sativum mRNA for manganese superoxi...   721     0.0
1
gb|U30841.1|PSU30841  Pisum sativum manganese superoxide dismu...   263     4e-161
1
gb|EF587264.1|  Glycine max MnSOD mRNA, complete cds                 444     7e-129
1
emb|X14482.1|NPMNSOD  Tobacco mRNA for manganese superoxide di...   431     6e-118
1
gb|BT013288.1|  Lycopersicon esculentum clone 134898R, mRNA se...   429     2e-117
1
emb|AJ238316.2|PPE238316  Prunus persica mitochondrial mRNA fo...   427     8e-117
1
gb|AF094832.1|AF094832  Zantedeschia aethiopica manganese supe...   427     1e-116
1
gb|AY137205.1|  Avicennia marina manganese superoxide dismutas...   425     4e-116
1
gb|DQ088820.1|  Gossypium hirsutum MnSOD mRNA, complete cds         424     7e-116
1
gb|EF470980.1|  Pistacia vera manganese superoxide dismutase-l...   423     2e-115
1
```

For CAT:*

```
emb|X60169.1|PSCATAL  Pisum sativum mRNA for catalase               495     2e-137
1
gb|AY424952.1|  Lotus japonicus catalase 1b (cat1) mRNA, compl...   478     2e-132
1
emb|AJ496418.1|PPE496418  Prunus persica mRNA for catalase (ca...   467     3e-129
1
dbj|D13557.1|VIRCAT  Vigna radiata var. radiata mRNA for catal...   467     4e-129
1
emb|Z36977.1|NPCAT3MR  N.plumbaginifolia mRNA for catalase (ca...   466     8e-129
1
emb|X52135.1|GHCAT1  Cotton mRNA for cottonseed catalase subun...   465     2e-128
1
dbj|AB333792.1|  Glycine max mRNA for peroxisomal catalase, co...   465     2e-128
1
gb|AF035255.1|AF035255  Glycine max catalase (cat4) mRNA, comp...   465     2e-128
1
gb|AY128695.1|  Capsicum annuum catalase 3 (CAT3) mRNA, comple...   463     7e-128
1
gb|AY173073.1|  Hypericum perforatum catalase (cat1) mRNA, com...   463     7e-128
1
```

For Chloroplast/Mitochondrial GR:*

emb|X60373.1|PSGLRED	P.sativum mRNA for glutathione reductase	385	2e-104
1			
emb|X90996.1|PSDNAGRGE	P.sativum GR gene	218	1e-102
1			
gb|L11632.1|SOYGLUTR	Soybean glutathione reductase (GR) mRNA,...	371	3e-100
1			
gb|DQ459505.1|	Phaseolus vulgaris dual-targeted glutathione r...	364	4e-98
1			
gb|DQ267474.1|	Vigna unguiculata glutathione reductase mRNA, ...	362	1e-97
1			
gb|AF105199.1|AF105199	Glycine max glutathione reductase (GR-...	202	4e-97
1			
emb|X76293.1|NCGMGRE	N.tabacum gor mRNA for glutathione reduc...	360	7e-97
1			
gb|AF109694.1|AF109694	Brassica juncea glutathione reductase ...	356	9e-96
1			
gb|AF019907.1|AF019907	Vitis vinifera glutathione reductase (...	354	3e-95
1			
gb|AF349449.1|AF349449	Brassica juncea glutathione reductase ...	353	8e-95
1			

For Chloroplast/stromal APX:*

gb|AY484493.1|	Vigna unguiculata chloroplast stromal ascorbat...	288	4e-75
1			
gb|AY484492.1|	Vigna unguiculata chloroplast thylakoid-bound ...	288	4e-75
1			
gb|AY148471.1|	Retama raetam stromal ascorbate peroxidase mRN...	284	5e-74
1			
dbj|D77997.1|SPICPAP	Spinacia oleracea mRNA for thylakoid-bou...	276	2e-71
1			
dbj|D83669.1|SPICPSAP	Spinacia oleracea mRNA for stromal asco...	276	2e-71
1			
dbj|AB090956.1|	Nicotiana tabacum sapx mRNA for stromal ascor...	275	2e-71
1			
gb|AF069316.1|AF069316	Mesembryanthemum crystallinum stromal ...	275	2e-71
1			
gb|AF069315.1|AF069315	Mesembryanthemum crystallinum thylakoi...	275	2e-71
1			
emb|CT828487.1|	Oryza sativa (indica cultivar-group) cDNA clo...	274	5e-71
1			
ref|NM_001053646.1|	Oryza sativa (japonica cultivar-group) Os...	274	5e-71
1			

* Only the first 10 results were given.

APPENDIX G

blastn RESULTS

For Cu/Zn SOD:*

```
>dbj|AB189165.1|    Pisum  sativum  mRNA  for  copper  zinc  superoxide  dismutase,
complete
cds
Length=857

 Score = 1546 bits (1714),  Expect = 0.0
 Identities = 857/857 (100%), Gaps = 0/857 (0%)
 Strand=Plus/Plus

Query  1    GACTATTCCATTTCATTTCTCTTATCTGTACTCGCTGTTGGGGTGTCCTGAGATCACATT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GACTATTCCATTTCATTTCTCTTATCTGTACTCGCTGTTGGGGTGTCCTGAGATCACATT  60

Query  61   GAACAATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGGGTACTATTA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GAACAATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGGGTACTATTA  120

Query  121  ACTTCAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTGGTCTTAAGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ACTTCAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTGGTCTTAAGC  180

Query  181  CTGGCCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTTGCATTTCAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CTGGCCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTTGCATTTCAA  240

Query  241  CTGGACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATGAGACTAGAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  CTGGACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATGAGACTAGAC  300


Query  301  ATGCTGGTGATTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCTTCACCATTA  360
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  ATGCTGGTGATTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCTTCACCATTA  360

Query  361  CTGACAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTGTTGTTGTCC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CTGACAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTGTTGTTGTCC  420

Query  421  ATGCCGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTACTGGAAATG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  ATGCCGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTACTGGAAATG  480

Query  481  CTGGTGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACTACTCTCCACT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CTGGTGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACTACTCTCCACT  540

Query  541  GTGCGTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTTGCTTGTTTAG  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  GTGCGTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTTGCTTGTTTAG  600

Query  601  GTCTGATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTCAATGGCTATAT  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  GTCTGATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTCAATGGCTATAT  660

Query  661  GACTGCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGTTGTCATGCTTG  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661  GACTGCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGTTGTCATGCTTG  720

Query  721  TTTTCAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAATAAAGACTTGTT  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721  TTTTCAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAATAAAGACTTGTT  780

Query  781  TTTAATAAAGACTTCATATTAGACTACAATATGGTGATAGAAAGTGTTATCTTGGaaaaa  840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  781  TTTAATAAAGACTTCATATTAGACTACAATATGGTGATAGAAAGTGTTATCTTGGAAAAA  840

Query  841  aaaaaaaaaaaaaaaaa  857
            |||||||||||||||||
Sbjct  841  AAAAAAAAAAAAAAAAA  857
```

>dbj|AB087845.1| Pisum sativum SOD mRNA for superoxide dismutase, complete cds

```
Length=850

 Score = 1525 bits (1690),  Expect = 0.0
 Identities = 849/850 (99%), Gaps = 1/850 (0%)
 Strand=Plus/Plus

Query  5    ATTCCATTTCATTTCTCTTATCTGTACTCGCTGTTGGGGTGTCCTGAGATCACATTGAAC  64
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATTCCATTTCATTTCTCTTATCTGTACTCGCTGTTGGGGTGTCCTGAGATCACATTGAAC  60

Query  65   AATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGGGTACTATTAACTT  124
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGGGTACTATTAACTT  120

Query  125  CAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTGGTCTTAAGCCTGG  184
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTGGTCTTAAGCCTGG  180

Query  185  CCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTTGCATTTCAACTGG  244
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTTGCATTTCAACTGG  240

Query  245  ACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATGAGACTAGACATGC  304
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATGAGACTAGACATGC  300

Query  305  TGGTGATTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCTTCACCATTACTGA  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TGGTGATTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCTTCACCATTACTGA  360

Query  365  CAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTGTTGTTGTCCATGC  424
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  CAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTGTTGTTGTCCATGC  420

Query  425  CGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTACTGGAAATGCTGG  484
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTACTGGAAATGCTGG  480

Query  485  TGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACTACTCTCCACTGTGC  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACTACTCTCCACTGTGC  540

Query  545  GTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTTGCTTGTTTAGGTCT  604
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  GTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTTGCTTGTTTAGGTCT  600

Query  605  GATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTCAATGGCTATATGACT  664
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  GATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTCAATGGCTATATGACT  660

Query  665  GCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGTTGTCATGCTTGTTTT  724
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661  GCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGTTGTCATGCTTGTTTT  720

Query  725  CAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAATAAAGACTTGTTTTTA  784
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721  CAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAATAAAGACTTGTTTTTA  780

Query  785  ATAAAGACTTCATATTAGACTACAATATGGTGATA-GAAAGTGTTATCTTGGAAAAAAAA  843
            |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||
Sbjct  781  ATAAAGACTTCATATTAGACTACAATATGGTGATATGAAAGTGTTATCTTGGAAAAAAAA  840

Query  844  AAAAAAAAAA  853
            ||||||||||
Sbjct  841  AAAAAAAAAA  850

>gb|M63003.1|PEACUZNSD  Pea Cu-Zn superoxide dismutase mRNA, complete cds
Length=738

 Score = 1285 bits (1424),  Expect = 0.0
 Identities = 719/722 (99%), Gaps = 1/722 (0%)
 Strand=Plus/Plus

Query  52   GATCACATTGAACAATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGG  111
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2    GATCACATTGAACAATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGG  61

Query  112  GTACTATTAACTTCAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTG  171
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  62   GTACTATTAACTTCAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTG  121

Query  172  GTCTTAAGCCTGGCCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTT  231
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  122  GTCTTAAGCCTGGCCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTT  181

Query  232  GCATTTCAACTGGACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATG  291
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  182  GCATTTCAACTGGACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATG  241

Query  292  AGACTAGACATGCTGGTGATTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCT  351
            |||||||||||||||||||| | |||||||||||||||||||||||||||||||||||||
Sbjct  242  AGACTAGACATGCTGGTGATCTTGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCT  301

Query  352  TCACCATTACTGACAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTG  411
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  302  TCACCATTACTGACAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTG  361

Query  412  TTGTTGTCCATGCCGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTA  471
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  362  TTGTTGTCCATGCCGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTA  421

Query  472  CTGGAAATGCTGGTGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACTA  531
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  422  CTGGAAATGCTGGTGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACTA  481

Query  532  CTCTCCACTGTGCGTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTTG  591
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  482  CTCTCCACTGTGCGTGCTGTTGAAGTTTTAGAAGAATAAATTGCACTCATCCCTCTCTTG  541

Query  592  CTTGTTTA-GGTCTGATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTCA  650
            |||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542  CTTGTTTAGGGTCTGATCTGTACTGCCGGATAATGTGTTTTTGTTTGTATTGAAATCTCA  601

Query  651  ATGGCTATATGACTGCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGTT  710
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  602  ATGGCTATATGACTGCACTTGGTGTTTAATCAGTTACTTCAGATGAAGTCTGTGGTTGTT  661

Query  711  GTCATGCTTGTTTTCAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAATA  770
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  662  GTCATGCTTGTTTTCAGTTTGCAGTATGATCTTAATTCTTAAGGAGTTGGGTTTTTAATA  721

Query  771  AA  772
            ||
Sbjct  722  AA  723

>emb|AJ012691.1|CAR012691  Cicer arietinum mRNA for superoxide dismutase
Length=809

 Score =  765 bits (848),  Expect = 0.0
 Identities = 599/724 (82%), Gaps = 45/724 (6%)
 Strand=Plus/Plus

Query  51   AGATCACATTGAACAATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCG  110
            |||||||||| ||||||||| |||||||||||||||||| ||| |||| ||   || ||
Sbjct  30   AGATCACATTCAACAATGGTTAAGGCTGTGGCAGTTCTTGGTAGCAGTGACACTGTGTCT  89

Query  111  GGTACTATTAACTTCAGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCT  170
            || |||||||||||||||||||||||| | |||||||||||||||||||||| |||||||
Sbjct  90   GGGACTATTAACTTCAGTCAGGAGGGAGACGGTCCAACCACTGTAACTGGAAATCTTGCT  149

Query  171  GGTCTTAAGCCTGGCCTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGT  230
```

```
            |||||||||||||| ||||| ||||||||||| |||||||| || ||||||||||| ||
Sbjct  150  GGTCTTAAGCCTGGTCTCCATGGCTTCCATATTCATGCCTTAGGGGACACCACAAATGGC  209

Query  231  TGCATTTCAACTGGACCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGAT  290
            ||||| ||||| |||||||||||||||||||||||||| ||||||||| ||||||||||
Sbjct  210  TGCATATCAACCGGACCACATTTCAATCCTAATGGGAAAGAACATGGTTCCCCTGAGGAC  269

Query  291  GAGACTAGACATGCTGGTGATTTAGGAAATATCAATGTTGGTGATGATGGAACTGTAAGC  350
              || | |||||||||| ||||||||||||||||||| |||||||||||||||||||||
Sbjct  270  CCGATTCGACATGCTGGCGATTTAGGAAATATCAATGTCGGTGATGATGGAACTGTAAGC  329

Query  351  TTCACCATTACTGACAACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCT  410
            ||| | ||||||||||| || ||||||||||||||| |||||||||||||||||||||||
Sbjct  330  TTCTCTATTACTGACAATCAGATCCCTCTCACTGGACCAAACTCCATCATAGGAAGGGCT  389

Query  411  GTTGTTGTCCATGCCGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACT  470
            |||||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390  GTTGTTGTTCATGCTGATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACT  449

Query  471  ACTGGAAATGCTGGTGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAGATCACT  530
            |||||||||||||| |||||||||||||||||||||||||||||||||||||| | |||
Sbjct  450  ACTGGAAATGCTGGCGGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAAACCACC  509

Query  531  ACTCTCCACT--------------------GTGCGTG-------CTGTTGAAGTTTTAG  562
            |||||| |||                     || |||        |||||||| |||||
Sbjct  510  ACTCTCAACTCCGGGATACTTGAAGTTGGAAATGTGTGATGATATATGTTGAAGCTTTAG  569

Query  563  AAGAATAAATTGCACTCATC-CCTCTCTTGCTTGTTTAGGTCTGATCTGTACTGCCGGAT  621
            ||||||||| ||||  |||| | || |||||||||||||||||||||||||||  | ||
Sbjct  570  AAGAATAAAATGCATGCATCTCATCACTTGCTTGTTTAGGTCTGATCTGTACTGTTGAAT  629

Query  622  AATGTGTTTTTGTTTGTATTGAAATCTCAATGGCTATATGACTGCACTTGGTGTTTAATC  681
              |||||||||  |||||  |||||                 |||| |||||  |||||
Sbjct  630  TGTGTGTTTTTCCTTGTAGCGAAAT----------------TTGCAATTGGTTCTTAATT  673

Query  682  AGTTACTTCAGATGAAGTCTGTGGTTGTTGTCATGCTTGTTTTCAGTTTGCAGTATGATC  741
               ||||| |  ||||||  |||||| ||||||||| |||||||| |||||| | |||||
Sbjct  674  TAGTACTTTACCTGAAGTTCGTGGTTATTGTCATGCCTGTTTTCACTTTGCATTGTGATC  733

Query  742  TTAA  745
            ||||
Sbjct  734  TTAA  737
```

For Mn SOD: *

```
>emb|X60170.1|PSSODR  Pisum sativum mRNA for manganese superoxide dismutase
Length=984

 Score =  592 bits (656),  Expect = 4e-166
 Identities = 361/383 (94%), Gaps = 0/383 (0%)
 Strand=Plus/Plus

Query  44   CATCAGAAACACCACCAGACTTATATTACCAACTATAACAAAGCTCTCGAGCAGCTTCAC  103
            |||||||||||||||||||||||||||||||||||| ||||||||||||||| |||||||||
Sbjct  247  CATCAGAAACACCACCAGACTTATATTACCAACTACAACAAAGCTCTCGAACAGCTTCAC  306

Query  104  GATGCCGTTGGTAAAGCTGATACATCTACCACTGTTAAGCTCCAGAATGCCATCAAGTTC  163
            |||||||||| ||||||||||||||||||||| |||||||||||||||||||||||| |||
Sbjct  307  GATGCCGTTGCTAAAGCTGATACATCTACCACCGTTAAGCTCCAGAATGCCATCAAATTC  366

Query  164  AACGGCGGAGGTCATATTAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTCGGGAA  223
            |||||||||||||||||| |||||||||||||||||||||||||||||||||||| | |||
Sbjct  367  AACGGCGGAGGTCATATCAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTAGTGAA  426

Query  224  GGAGGTGGTGAACCACCAAAGGAATCCCTAGGCTGGGCCATTGACACAAACTTTGGATCT  283
            |||||||||||||||||||||||||||||| ||||||||||||||||| || |||||||||
Sbjct  427  GGAGGTGGTGAACCACCAAAGGAATCCCTGGGCTGGGCCATTGACACCAATTTTGGATCT  486

Query  284  TTGGAAGCATTGATTCAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGGGTCTGGATGG  343
            |||||||||||||| |||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  487  TTGGAAGCATTGATACAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGCGTCTGGATGG  546

Query  344  GTGTGGCTTGGTCTTGACAAAGATTTGAAGAGGCTTGTGGTTGAAACCACTGCAAATCAA  403
            |||||||||||||| |||||||| |||||||||||||||||||||||||||||||| ||
Sbjct  547  GTGTGGCTTGGTCTCGACAAAGACTTGAAGAGGCTTGTGGTTGAAACCACTGCAAACCAG  606

Query  404  GATCCTCTGGTAACTAAGGGTGC  426
            || || ||||| ||||| || ||
Sbjct  607  GACCCACTGGTGACTAAAGGAGC  629
```

```
>gb|EF587264.1|  Glycine max MnSOD mRNA, complete cds
Length=1001

 GENE ID: 100101896 LOC100101896 | MnSOD [Glycine max]

 Score =  407 bits (450),  Expect = 3e-110
 Identities = 318/380 (83%), Gaps = 0/380 (0%)
 Strand=Plus/Plus

Query  44   CATCAGAAACACCACCAGACTTATATTACCAACTATAACAAAGCTCTCGAGCAGCTTCAC  103
            || ||||| ||||||||||||||| || |||||||| ||||| || ||||||||||| ||
Sbjct  196  CACCAGAAGCACCACCAGACTTACATCACCAACTACAACAAGGCCCTCGAGCAGCTCCAA  255

Query  104  GATGCCGTTGGTAAAGCTGATACATCTACCACTGTTAAGCTCCAGAATGCCATCAAGTTC  163
            || ||| | |  ||    ||| | ||  ||   ||||||||||||   ||||||||||||
Sbjct  256  GACGCCATCGCCAAGAAAGATTCCTCCGCCGTCGTTAAGCTCCAGGGCGCCATCAAGTTC  315

Query  164  AACGGCGGAGGTCATATTAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTCGGGAA  223
            ||||||||||||||| | |||||||| |||||||||||||||||| ||||||||||| |||
Sbjct  316  AACGGCGGAGGTCATGTCAACCATTCTATTTTCTGGAAAAATCTAGCTCCTGTTCGTGAA  375

Query  224  GGAGGTGGTGAACCACCAAAGGAATCCCTAGGCTGGGCCATTGACACAAACTTTGGATCT  283
            |||||||||||||||||| ||||  || || || ||||| ||||||||| | ||||| |||
Sbjct  376  GGAGGTGGTGAACCACCCAAGGGTTCACTGGGATGGGCTATTGACACACATTTTGGTTCT  435

Query  284  TTGGAAGCATTGATTCAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGGGTCTGGATGG  343
            || |||||||| || |||||  |||| || |||||||| || || |||||||||||||||
Sbjct  436  TTTGAAGCATTAATACAAAAAGTTAACGCAGAAGGTGCTGCACTACAGGGGTCTGGATGG  495

Query  344  GTGTGGCTTGGTCTTGACAAAGATTTGAAGAGGCTTGTGGTTGAAACCACTGCAAATCAA  403
            ||||||||||||||| |||||||| ||||||||||||||| ||||||||||||||| || ||
Sbjct  496  GTGTGGCTTGGTCTGGACAAAGAGTTGAAGAGGCTTGTAGTTGAAACCACTGCCAACCAG  555

Query  404  GATCCTCTGGTAACTAAGGG  423
            || || ||||| ||||||||
Sbjct  556  GACCCACTGGTTACTAAGGG  575
```

```
>emb|AJ440726.1|GMA440726    Glycine  max  partial  Mn-sod  gene  for  manganese-
superoxide dismutase
Length=431

 Score =  394 bits (436),  Expect = 2e-106
 Identities = 313/375 (83%), Gaps = 1/375 (0%)
 Strand=Plus/Plus

Query  49   GAAACACCACCAGACTTATATTACCAACTATAACAAAGCTCTCGAGCAGCTTCACGATGC  108
            ||| |||||||| ||||| || |||||||  ||||| || ||||||||||| || || ||
Sbjct  5    GAAGCACCACCA-ACTTACATCACCAACTTCAACAAGGCCCTCGAGCAGCTCCAAGACGC  63

Query  109  CGTTGGTAAAGCTGATACATCTACCACTGTTAAGCTCCAGAATGCCATCAAGTTCAACGG  168
            ||| |  ||    ||| | ||  ||   |||||||||||   |||||||||||||||||
Sbjct  64   CGTCGCCAAGAAAGATTCCTCCGCCGTCGTTAAGCTCCAGGGCGCCATCAAGTTCAACGG  123

Query  169  CGGAGGTCATATTAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTCGGGAAGGAGG  228
            |||||||||  | |||||||| ||||||||||||||||| ||||||||||| ||||||||
Sbjct  124  CGGAGGTCACGTCAACCATTCTATTTTCTGGAAAAATCTAGCTCCTGTTCGTGAAGGAGG  183

Query  229  TGGTGAACCACCAAAGGAATCCCTAGGCTGGGCCATTGACACAAACTTTGGATCTTTGGA  288
            ||||||||||||| ||||  || || || |||||||||||||||| | ||||| ||||| ||
Sbjct  184  TGGTGAACCACCCAAGGGTTCACTGGGATGGGCCATTGACACACATTTTGGCTCTTTTGA  243

Query  289  AGCATTGATTCAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGGGTCTGGATGGGTGTG  348
            ||||||  | |||||  ||||||| |||||||| || || ||||||||||||||||||||
Sbjct  244  AGCATTAGTACAAAAAGTTAATGCAGAAGGTGCTGCACTACAGGGGTCTGGATGGGTGTG  303

Query  349  GCTTGGTCTTGACAAAGATTTGAAGAGGCTTGTGGTTGAAACCACTGCAAATCAAGATCC  408
            ||||||||| |||||||| |||||||||||||| |||||||||||||| || || || ||
Sbjct  304  GCTTGGTCTGGACAAAGAGTTGAAGAGGCTTGTAGTTGAAACCACTGCCAACCAGGACCC  363

Query  409  TCTGGTAACTAAGGG  423
             ||||| ||||||||
Sbjct  364  ACTGGTTACTAAGGG  378
```

For Chloroplast/stromal APX:*

```
>gb|AY484493.1|  Vigna unguiculata chloroplast stromal ascorbate peroxidase mRNA,
complete cds; nuclear gene for chloroplast product
Length=1505

 Score =  497 bits (550),  Expect = 3e-137
 Identities = 357/410 (87%), Gaps = 1/410 (0%)
 Strand=Plus/Plus

Query  36   AGGCACTGCTCGGACTTGTAAATGTATTGAAACTTCTCCAGCCGATCAAAGACAAATATT  95
            || || |||| ||||| ||||||| |||||| ||||||||||| |||||||||||||| |
Sbjct  465  AGCCAATGCT-GGACTCGTAAATGCATTGAAGCTTCTCCAGCCAATCAAAGACAAATACT  523

Query  96   CAGGCGTGACATATGCAGACTTATTTCAGTTAGCTGGTGCTACAGCTGTGGAGGAAGCTG  155
            | || ||||||||||| |||||||||||||| |||||||| || ||||| ||||||||||
Sbjct  524  CTGGTGTGACATATGCGGACTTATTTCAGTTGGCTGGTGCCACTGCTGTTGAGGAAGCTG  583

Query  156  GAGGCCCAAAAATTCCCATGAAATATGGAAGAGTAGATACCTCTGGACCTGACCAATGTC  215
            ||||||||||| | || |||||||||||||||||||||||   ||||||||||| |||||||
Sbjct  584  GAGGCCCAAAACTCCCTATGAAATATGGAAGAGTAGATGTATCTGGACCTGAACAATGTC  643

Query  216  CTGAAGAAGGACGACTTCCTGATGCAGGGGCCCGTTCAGCTGCTGATCATTTGCGTGAAG  275
            | ||||||||  ||||||| ||||| ||  ||| |||| |||||||||||||||||| |||
Sbjct  644  CCGAAGAAGGGAGACTTCCCGATGCTGGTCCCCCTTCACCTGCTGATCATTTGCGTCAAG  703

Query  276  TTTTTTACCGTATGGGATTGGACGACAAGGAAATTGTTGCATTATCTGGAGCACACACAG  335
            |||| ||||| ||||| ||| | |||||||||||||||||||||||||||| |||||||||
Sbjct  704  TTTTCTACCGAATGGGTTTGAATGACAAGGAAATTGTTGCATTATCTGGTGCACACACAC  763

Query  336  TGGGGCGGTCTAGACCAGACCGTAGTGGCTGGGGAAAACCTGAGACTAAATACACCAAAG  395
            ||||| || |||||||||| |||||||| |||||||| ||||| |||||||| || ||||
Sbjct  764  TGGGGAGGGCTAGACCAGATCGTAGTGGTTGGGGAAAGCCTGAAACTAAATATACGAAAG  823

Query  396  ACGGGCCAGGAGCACCTGGAGGACCATCCTGGACTGTCCAATGGCTGAAA  445
            | |||||||||||||||||||||| ||| ||||| || |||||| |||||
Sbjct  824  ATGGGCCAGGAGCACCTGGAGGACAATCATGGACAGTGCAATGGTTGAAA  873


>gb|AY484492.1|      Vigna   unguiculata   chloroplast   thylakoid-bound   ascorbate
peroxidase
mRNA, complete cds; nuclear gene for chloroplast product
Length=1354

 Score =  497 bits (550),  Expect = 3e-137
 Identities = 357/410 (87%), Gaps = 1/410 (0%)
 Strand=Plus/Plus

Query  36   AGGCACTGCTCGGACTTGTAAATGTATTGAAACTTCTCCAGCCGATCAAAGACAAATATT  95
            || || |||| ||||| ||||||| |||||| ||||||||||| |||||||||||||| |
Sbjct  465  AGCCAATGCT-GGACTCGTAAATGCATTGAAGCTTCTCCAGCCAATCAAAGACAAATACT  523

Query  96   CAGGCGTGACATATGCAGACTTATTTCAGTTAGCTGGTGCTACAGCTGTGGAGGAAGCTG  155
            | || ||||||||||| |||||||||||||| |||||||| || ||||| ||||||||||
Sbjct  524  CTGGTGTGACATATGCGGACTTATTTCAGTTGGCTGGTGCCACTGCTGTTGAGGAAGCTG  583

Query  156  GAGGCCCAAAAATTCCCATGAAATATGGAAGAGTAGATACCTCTGGACCTGACCAATGTC  215
            ||||||||||| | || |||||||||||||||||||||||   ||||||||||| |||||||
Sbjct  584  GAGGCCCAAAACTCCCTATGAAATATGGAAGAGTAGATGTATCTGGACCTGAACAATGTC  643

Query  216  CTGAAGAAGGACGACTTCCTGATGCAGGGGCCCGTTCAGCTGCTGATCATTTGCGTGAAG  275
            | ||||||||  ||||||| ||||| ||  ||| |||| |||||||||||||||||| |||
Sbjct  644  CCGAAGAAGGGAGACTTCCCGATGCTGGTCCCCCTTCACCTGCTGATCATTTGCGTCAAG  703

Query  276  TTTTTTACCGTATGGGATTGGACGACAAGGAAATTGTTGCATTATCTGGAGCACACACAG  335
            |||| ||||| ||||| ||| | |||||||||||||||||||||||||||| |||||||||
Sbjct  704  TTTTCTACCGAATGGGTTTGAATGACAAGGAAATTGTTGCATTATCTGGTGCACACACAC  763
```

```
Query  336  TGGGGCGGTCTAGACCAGACCGTAGTGGCTGGGGAAAACCTGAGACTAAATACACCAAAG  395
            ||||| || |||||||||| |||||||| |||||||| ||||| |||||||| || ||||
Sbjct  764  TGGGGAGGGCTAGACCAGATCGTAGTGGTTGGGGAAAGCCTGAAACTAAATATACGAAAG  823

Query  396  ACGGGCCAGGAGCACCTGGAGGACCATCCTGGACTGTCCAATGGCTGAAA  445
            | ||||||||||||||||||||| ||| ||||| || |||||| |||||
Sbjct  824  ATGGGCCAGGAGCACCTGGAGGACAATCATGGACAGTGCAATGGTTGAAA  873


>gb|AY148471.1|  Retama raetam stromal ascorbate peroxidase mRNA, complete cds;
nuclear gene for chloroplast product
Length=1103

 Score =  466 bits (516),  Expect = 6e-128
 Identities = 350/410 (85%), Gaps = 1/410 (0%)
 Strand=Plus/Plus

Query  36   AGGCACTGCTCGGACTTGTAAATGTATTGAAACTTCTCCAGCCGATCAAAGACAAATATT  95
            || || |||| ||||||||||||| |||||| ||||| || || |||||||||||||| |
Sbjct  413  AGCCAATGCT-GGACTTGTAAATGCATTGAAGCTTCTTCAACCAATCAAAGACAAATACT  471

Query  96   CAGGCGTGACATATGCAGACTTATTTCAGTTAGCTGGTGCTACAGCTGTGGAGGAAGCTG  155
            | || || ||||||||||||||||||||||| || |||||||| |||||||||||||||||
Sbjct  472  CTGGTGTCACATATGCAGACTTATTTCAGTTGGCCGGTGCTACTGCTGTGGAGGAAGCTG  531

Query  156  GAGGCCCAAAAATTCCCATGAAATATGGAAGAGTAGATACCTCTGGACCTGACCAATGTC  215
            ||||||||||||| || ||||| ||||||||||| |||  | || | ||||| |||||||
Sbjct  532  GAGGCCCAAAAATCCCTATGAAGTATGGAAGAGTGGATGTCACTAGTCCTGAACAATGTC  591

Query  216  CTGAAGAAGGACGACTTCCTGATGCAGGGGCCCGTTCAGCTGCTGATCATTTGCGTGAAG  275
            ||||||||||  | ||||||||||| ||  |||  ||| |||||||||||||||| |||
Sbjct  592  CTGAAGAAGGGAGGCTTCCTGATGCTGGCCCCCCCTCACCTGCTGATCATTTGCGTCAAG  651

Query  276  TTTTTTACCGTATGGGATTGGACGACAAGGAAATTGTTGCATTATCTGGAGCACACACAG  335
            |||| ||||| ||||| ||| | ||||||||||| |||||| |||||||||||||||||
Sbjct  652  TTTTCTACCGGATGGGTTTGAATGACAAGGAAATCGTTGCACTATCTGGAGCACACACAC  711

Query  336  TGGGGCGGTCTAGACCAGACCGTAGTGGCTGGGGAAAACCTGAGACTAAATACACCAAAG  395
            ||||| |||||||||||||| || || |||||||| || |||||||| ||||| || ||||
Sbjct  712  TGGGGAGGTCTAGACCAGATCGCAGCGGCTGGGGCAAGCCTGAGACAAAATATACGAAAG  771

Query  396  ACGGGCCAGGAGCACCTGGAGGACCATCCTGGACTGTCCAATGGCTGAAA  445
            | ||||| |||||||||||||||| ||||||||| |  || ||| |||||
Sbjct  772  ATGGGCCGGGAGCACCTGGAGGACAATCCTGGACAGCACAGTGGTTGAAA  821
```

For CAT:*

```
>emb|X60169.1|PSCATAL  Pisum sativum mRNA for catalase
Length=1738

 Score =  989 bits (1096),  Expect = 0.0
 Identities = 599/630 (95%), Gaps = 2/630 (0%)
 Strand=Plus/Plus

Query  1    TTTCGCCCCTCCATTGTGGCAGATTTCCTTCGAGCCCCTGGTGTTCAGACACCTGTCATT  60
            |||||| |||    |||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  298  TTTCGCACCTGACATGTG-CAGATTTCCTTCGAGCCCCTGGTGTTCAGACACCTGTCATT  356

Query  61   GTGCGTTTCTCAACTGTCATTCATGAACGTGGCAGCCCTGAAACCTTGAGGGACCCTCGA  120
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| || |||
Sbjct  357  GTGCGTTTCTCAACTGTCATTCATGAACGTGGCAGCCCTGAAACCTTGAGGGATCCCCGA  416

Query  121  GGTTTTGCTGTAAAATTTTACACCAGAGAGGGTAACTATGACCTTGTTGGAAACAACTTT  180
            |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  417  GGTTTTGCTGTGAAATTTTACACCAGAGAGGGTAACTATGACCTTGTTGGAAACAACTTT  476

Query  181  CCTGTCTTCTTCGTTCATGACGGGATGAATTTTCCAGATATGGTCCACGCTCTTAAACCC  240
            || |||||||||||||||||||| |||||||||||||||||||||||| ||||||||||||
Sbjct  477  CCCGTCTTCTTCGTTCATGACGGTATGAATTTTCCAGATATGGTCCATGCTCTTAAACCC  536
```

```
Query  241  AATCCCCAGACCCACATCCAGGAGAATTGGAGAATTCTTGACTTCTTCTCCCACTTTCCA  300
            ||||||||||||||||||||||||||||||||||||||||||| ||||||| | ||||||||
Sbjct  537  AATCCCCAGACCCACATCCAGGAGAATTGGAGAATTCTTGATTTCTTCTACAACTTTCCA  596

Query  301  GAAAGCCTTCACATGTTCTCCTTCCTATTTGATGATGTGGGTGTCCCTCAAGATTATAGG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  597  GAAAGCCTTCACATGTTCTCCTTCCTATTTGATGATGTGGGTGTCCCACAAGATTATAGG  656

Query  361  CATATGGATGGTTTTGGAGTCAACACATATACCCTGATCAACAAGGCTGGGAAATCAGTG  420
            |||||||||||||||||||| |||||||| ||||||||||||||||||||| ||||| |||
Sbjct  657  CATATGGATGGTTTTGGAGTTAACACATACACCCTGATCAACAAGGCTGGAAAATCGGTG  716

Query  421  TTTGTGAAATTTCACTGGAAGCCCACCTGTGGTGTGAAGTGTCTATTGGAAGAAGAGGCC  480
            | ||| |||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  717  TATGTCAAATTTCACTGGAAGCCCACCTGTGGTGTGAAGTGTCTATTGGAGGAAGAGGCC  776

Query  481  ATTAAGGTGGGAGGATCCAACCACAGTCATGCTACTAAAGACCTTTATGACTCAATTGCT  540
            ||| |||||||||||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  777  ATTCAGGTGGGAGGATCCAACCACAGCCATGCTACTAAAGACCTTTATGACTCAATTGCT  836

Query  541  GCTGGTAACTATCCTGAGTGGAAACTTTATATTCAAACAATAGATCCTGCTCATGAAGAC  600
            |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||||
Sbjct  837  GCTGGTAACTATCCTGAGTGGAAACTTTACATTCAAACAATAGATCCTGCTCATGAAGAC  896

Query  601  AGATTTGACTTTGACCTCCTCCGATGTAAC  630
            |||||||| |||||||  ||  ||||||||
Sbjct  897  AGATTTGAGTTTGACCCACT-TGATGTAAC  925
```

>gb|AY424952.1| Lotus japonicus catalase 1b (cat1) mRNA, complete cds, alternatively spliced
Length=1756

```
 Score =  852 bits (944),  Expect = 0.0
 Identities = 557/612 (91%), Gaps = 1/612 (0%)
 Strand=Plus/Plus

Query  19   GCAGATTTCCTTCGAGCCCCTGGTGTTCAGACACCTGTCATTGTGCGTTTCTCAACTGTC  78
            |||||||||||||||||||||||| ||||||||||||| ||||| | |||||||| ||||||
Sbjct  375  GCAGATTTCCTTCGAGCCCCTGGAGTTCAGACACCTATCATTCTCCGTTTCTCGACTGTC  434

Query  79   ATTCATGAACGTGGCAGCCCTGAAACCTTGAGGGACCCTCGAGGTTTTGCTGTAAAATTT  138
            |||||||| ||||| |||||||||||||||||||| ||||| ||||||||||| || |||
Sbjct  435  ATTCATGAGCGTGGTAGCCCTGAAACCTTGAGGGATCCTCGTGGTTTTGCTGTGAAGTTT  494

Query  139  TACACCAGAGAGGGTAACTATGACCTTGTTGGAAACAACTTTCCTGTCTTCTTCGTTCAT  198
            |||||||||||||||||||| ||||||||| ||||||||||| |||||||||||||| | |
Sbjct  495  TACACCAGAGAGGGTAACTTTGACCTTGTGGGAAACAACTTCCCTGTCTTCTTCGTACGT  554

Query  199  GACGGGATGAATTTTCCAGATATGGTCCACGCTCTTAAACCCAATCCCCAGACCCACATC  258
            || || ||||| |||||||||||||||||| ||||| || ||||||||| || | ||||||
Sbjct  555  GATGGCATGAAATTTCCAGATATGGTCCATGCTCTGAAGCCCAATCCCAAGTCTCACATC  614

Query  259  CAGGAGAATTGGAGAATTCTTGACTTCTTCTCCCACTTTCCAGAAAGCCTTCACATGTTC  318
            |||||||||||||||||| |||||||||||||||||||||||||||||||||| ||||||
Sbjct  615  CAGGAGAATTGGAGAATCCTTGACTTCTTCTCCCACTTTCCAGAAAGCCTTCATATGTTC  674

Query  319  TCCTTCCTATTTGATGATGTGGGTGTCCCTCAAGATTATAGGCATATGGATGGTTTTGGA  378
             |||| |||||||||||||||||||| || |||||||||||||| |||||||||||||||
Sbjct  675  ACCTTTCTATTTGATGATGTGGGTGTTCCACAAGATTATAGGCACATGGATGGTTTTGGA  734

Query  379  GTCAACACATATACCCTGATCAACAAGGCTGGGAAATCAGTGTTTGTGAAATTTCACTGG  438
            || || |||||||||||||||||||||||||||||||  |||||  |||||||||||||||
Sbjct  735  GTTAATACATATACCCTGATCAACAAGGCTGGGAAAGTAGTGTACGTGAAATTTCACTGG  794

Query  439  AAGCCCACCTGTGGTGTGAAGTGTCTATTGGAAGAAGAGGCCATTAAGGTGGGAGGATCC  498
            |||||||| |||||||| |||||||||||||| |||||||||||||||||||||||||||||
Sbjct  795  AAGCCCACATGTGGTGTTAAGTGTCTATTGGAGGAAGAGGCCATTAAGGTGGGAGGATCC  854
```

```
Query  499  AACCACAGTCATGCTACTAAAGACCTTTATGACTCAATTGCTGCTGGTAACTATCCTGAG  558
            |||||||| ||||| ||| ||||||||||||| ||||||||||||||||||||||||||||
Sbjct  855  AACCACAGCCATGCCACTCAAGACCTTTATGAGTCAATTGCTGCTGGTAACTATCCTGAG  914

Query  559  TGGAAACTTTATATTCAAACAATAGATCCTGCTCATGAAGACAGATTTGACTTTGACCTC  618
            |||||||| |  |||||||| |||||||||| |||||||||||||||||| |||||||
Sbjct  915  TGGAAACTGTTCATTCAAACCATAGATCCTGATCATGAAGACAGATTTGATTTTGACCCA  974

Query  619  CTCCGATGTAAC  630
            ||  ||||||||
Sbjct  975  CT-TGATGTAAC  985


>dbj|AB333792.1|  Glycine max mRNA for peroxisomal catalase, complete cds
Length=1479

 Score =  821 bits (910),  Expect = 0.0
 Identities = 541/598 (90%), Gaps = 0/598 (0%)
 Strand=Plus/Plus

Query  19   GCAGATTTCCTTCGAGCCCCTGGTGTTCAGACACCTGTCATTGTGCGTTTCTCAACTGTC  78
            |||||||||||||||||||||||| |||||||| ||||| ||||| |||||||||||||||
Sbjct  259  GCAGATTTCCTTCGAGCCCCTGGAGTTCAGACGCCTGTAATTGTTCGTTTCTCAACTGTC  318

Query  79   ATTCATGAACGTGGCAGCCCTGAAACCTTGAGGGACCCTCGAGGTTTTGCTGTAAAATTT  138
            |||||||| ||||| |||||||||||||||||||||||||||||||||| || || |||
Sbjct  319  ATTCATGAGCGTGGTAGCCCTGAAACCTTGAGGGACCCTCGAGGTTTTGCCGTGAAGTTT  378

Query  139  TACACCAGAGAGGGTAACTATGACCTTGTTGGAAACAACTTTCCTGTCTTCTTCGTTCAT  198
            ||||||||||||||||||| ||||||||||||||||||| |||| |||||||| || | |
Sbjct  379  TACACCAGAGAGGGTAACTTTGACCTTGTTGGAAACAACCTTCCCGTCTTCTTTGTACGT  438

Query  199  GACGGGATGAATTTTCCAGATATGGTCCACGCTCTTAAACCCAATCCCCAGACCCACATC  258
            ||||| ||||| |||||||||||||| || ||||||||||||||||||| ||| |||||||
Sbjct  439  GACGGCATGAAATTTCCAGATATGGTTCATGCTCTTAAACCCAATCCCAAGAACCACATC  498

Query  259  CAGGAGAATTGGAGAATTCTTGACTTCTTCTCCCACTTTCCAGAAAGCCTTCACATGTTC  318
            || |||||||||||| || ||||||||||||||| |||||||||||||||||||||||||||
Sbjct  499  CAAGAGAATTGGAGGATCCTTGACTTCTTCTCTCACTTTCCAGAAAGCCTTCACATGTTC  558

Query  319  TCCTTCCTATTTGATGATGTGGGTGTCCCTCAAGATTATAGGCATATGGATGGTTTTGGA  378
             ||||  |||||||||||| ||||||| || |||||||| ||||||||||||||||||||
Sbjct  559  ACCTTTTTATTTGATGATTTGGGTGTTCCACAAGATTACAGGCATATGGATGGTTTTGGA  618

Query  379  GTCAACACATATACCCTGATCAACAAGGCTGGGAAATCAGTGTTTGTGAAATTTCACTGG  438
            || |||||||||||| ||||||||||||||||||||| |||||| |||||||||||||||||
Sbjct  619  GTTAACACATATACGCTGATCAACAAGGCTGGGAAAGCAGTGTATGTGAAATTTCACTGG  678

Query  439  AAGCCCACCTGTGGTGTGAAGTGTCTATTGGAAGAAGAGGCCATTAAGGTGGGAGGATCC  498
            ||| ||||  ||||| | ||||||||||||||| ||||||||||||||||||||||||| ||
Sbjct  679  AAGACCACTAGTGGTATAAAGTGTCTATTGGAGGAAGAGGCCATTAAGGTGGGAGGAGCC  738

Query  499  AACCACAGTCATGCTACTAAAGACCTTTATGACTCAATTGCTGCTGGTAACTATCCTGAG  558
            |||||||| ||||| ||| |||||||  |||| |||||||||||||||||||||||||||
Sbjct  739  AACCACAGCCATGCCACTCAAGACCTCCATGATTCAATTGCTGCTGGTAACTATCCTGAG  798

Query  559  TGGAAACTTTATATTCAAACAATAGATCCTGCTCATGAAGACAGATTTGACTTTGACC  616
            |||||||| | | |||| |||||||||||||  || ||||||| |||||||||||||
Sbjct  799  TGGAAACTGTTTGTTCAGACAATAGATCCTGAGCACGAAGACAAATTTGACTTTGACC  856
```

For Chloroplast/mitochondrial APX:*

```
>emb|X60373.1|PSGLRED  P.sativum mRNA for glutathione reductase
Length=2063

 Score =  870 bits (964),  Expect = 0.0
 Identities = 530/554 (95%), Gaps = 6/554 (1%)
 Strand=Plus/Plus

Query  2    TTTCTC-ACACTCTCCTCCCGATACAACCGCGAGGTGTCGCGCGGCACCTCGTGTAATAC  60
            |||||| ||  |||||||| ||||| |||| | ||||||| ||||||||| |||||||||
Sbjct  396  TTTCTCTACTATCTCCTCC-GATACCACCG-GTGGTGTCG-GCGGCACCT-GTGTAATAC  451

Query  61   GTGGATGTGTCCCTAAGAAATTGCTAGTCTATGCGTCAAAATTTTCTCATGAATTTGAAG  120
            | ||||||||||||||||||||||||||||||| |||||||| |||||||||||||||||
Sbjct  452  GGGGATGTGTCCCTAAGAAATTGCTAGTCTATGCCTCAAAATTCTCTCATGAATTTGAAG  511

Query  121  AAAGCAATGGTTTTGGATGGAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  512  AAAGCAATGGTTTTGGATGGAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGA  571

Query  181  TTGCTAATAAAAATGCCGAGTTGCAGCGGCTTACTGGTATCTATAAGAATATTTTGAAAA  240
            ||||||||||||| |||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  572  TTGCTAATAAAAACGCCGAGTTGCAGCGGCTTACTGGTATCTATAAGAATACTTTGAAAA  631

Query  241  ATGCCGGTGTCAAGTTGATTGAAGGCCGTGGAAAGATTGTAGATCCTCACACGGTTGATG  300
            |||||||||| |||||||||||||||||||||||||||||||||| ||||||| |||||||
Sbjct  632  ATGCCGGTGTTAAGTTGATTGAAGGCCGTGGAAAGATTGTAGATGCTCACACAGTTGATG  691

Query  301  TTGATGGGAAGTTATATTCAGCAAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCA  360
            |||||||||||||||||||||| |||||||||||||||||||||||||||||||||||||
Sbjct  692  TTGATGGGAAGTTATATTCAGCGAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCA  751

Query  361  TTCCTGATATTCCTGGAAAGGAATATGCAATAGATTCAGACGCTGCCCTTGATTTACCAT  420
            |||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct  752  TTCCTGATATTCCTGGAAAGGAATATGCAATAGATTCAGATGCTGCCCTTGATTTACCAT  811

Query  421  CAAAGCCTCAGAAGATAGCCATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTA  480
            |||||||||||||||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct  812  CAAAGCCTCAGAAGATAGCTATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTA  871

Query  481  TCTTTAATGGTTTGAAAAGTGAAGTTCATGTATTTATACGACAAAAGAAGGGTCTGCGGG  540
            |||||||||||||||||||||||||||||||||||||||| ||||||||||| | ||||||
Sbjct  872  TCTTTAATGGTTTGAAAAGTGAAGTTCATGTATTTATAAGACAAAAGAAGGTTTTGCGGG  931

Query  541  GATTTTGATGAAGA  554
            || |||||||||||
Sbjct  932  GA-TTTGATGAAGA  944


>gb|L11632.1|SOYGLUTR  Soybean glutathione reductase (GR) mRNA, complete cds
Length=2081

 GENE ID: 547793 GR | glutathione reductase [Glycine max]
(10 or fewer PubMed links)

 Score =  706 bits (782),  Expect = 0.0
 Identities = 493/554 (88%), Gaps = 9/554 (1%)
 Strand=Plus/Plus

Query  2    TTTCTC-ACACTCTCCTCCCGATACAACCGCGAGGTGTCGCGCGGCACCTCGTGTAATAC  60
            |||||| ||  |||||||| || || ||||    | |||| |||| |||| |||||||||
Sbjct  352  TTTCTCCACTATCTCCTCC-GAAACCACCG----GAGTCG-GCGGAACCT-GTGTAATAC  404

Query  61   GTGGATGTGTCCCTAAGAAATTGCTAGTCTATGCGTCAAAATTTTCTCATGAATTTGAAG  120
            | |||||||| || ||||| ||||| || ||||| || |||||||||||||||||||||||
Sbjct  405  GAGGATGTGTGCCGAAGAAGTTGCTGGTTTATGCATCTAAATTTTCTCATGAATTTGAAG  464
```

```
Query  121  AAAGCAATGGTTTTGGATGGAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGA  180
            |||| |||||||| |||||||||||||||||||| || |||||||| ||||||||||| |
Sbjct  465  AAAGTAATGGTTTCGGATGGAGATATGACAGTGAGCCCAAGCATGATTGGAGTAGTTTCA  524

Query  181  TTGCTAATAAAAATGCCGAGTTGCAGCGGCTTACTGGTATCTATAAGAATATTTTGAAAA  240
            | ||||||||||||| ||||||||||| |||||||| ||||| ||||||||||||| |
Sbjct  525  TAGCTAATAAAAATGCTGAGTTGCAGCGTCTTACTGGCATCTACAAGAATATTTTGAACA  584

Query  241  ATGCCGGTGTCAAGTTGATTGAAGGCCGTGGAAAGATTGTAGATCCTCACACGGTTGATG  300
            |||| || |||||| ||||||||||||| |||||||||  ||||||||||||||||||||
Sbjct  585  ATGCTGGGGTCAAGCTGATTGAAGGCCATGGAAAGATGATAGATCCTCACACGGTTGATG  644

Query  301  TTGATGGGAAGTTATATTCAGCAAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCA  360
            || |||||||| |||||||||| ||||||||||||||| ||||||||||||| |||||||
Sbjct  645  TTAATGGGAAGCTATATTCAGCCAAACACATTTTAGTTGCAGTTGGAGGTCGCCCCTTCA  704

Query  361  TTCCTGATATTCCTGGAAAGGAATATGCAATAGATTCAGACGCTGCCCTTGATTTACCAT  420
            |||||||||| ||||| |||||||  |||||||||||||| |||||||||||||||||||
Sbjct  705  TTCCTGATATCCCTGGGAAGGAATTAGCAATAGATTCAGATGCTGCCCTTGATTTACCAA  764

Query  421  CAAAGCCTCAGAAGATAGCCATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTA  480
            |||| |||  ||| |||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  765  CAAAACCTGTGAAAATAGCCATTGTTGGTGGTGGTTACATTGCCTTGGAGTTTGCTGGTA  824

Query  481  TCTTTAATGGTTTGAAAAGTGAAGTTCATGTATTTATACGACAAAAGAAGGGTCTGCGGG  540
            |||||||||||||||||||||| ||||||||||||||||| |||||||||| | ||||||
Sbjct  825  TCTTTAATGGTTTGAAAAGTGAGGTTCATGTATTTATACGGCAAAAGAAGGTTTTGCGGG  884

Query  541  GATTTTGATGAAGA  554
            || |||||||||||
Sbjct  885  GA-TTTGATGAAGA  897
```

```
>gb|DQ459505.1|  Phaseolus vulgaris dual-targeted glutathione reductase (dtGR)
mRNA, complete cds
Length=2055

 Score =  663 bits (734),  Expect = 0.0
 Identities = 484/554 (87%), Gaps = 6/554 (1%)
 Strand=Plus/Plus

Query  2    TTTCTCACAC-TCTCCTCCCGATACAACCGCGAGGTGTCGCGCGGCACCTCGTGTAATAC  60
            |||||||  | |||| ||| || || |||| |||| |||| |||| || | |||||||||
Sbjct  318  TTTCTCAACCGTCTCTTCC-GAAACCACCG-GAGGAGTCG-GCGGAACGT-GTGTAATAC  373

Query  61   GTGGATGTGTCCCTAAGAAATTGCTAGTCTATGCGTCAAAATTTTCTCATGAATTTGAAG  120
            | ||||| || || ||||| ||||| || ||||| ||||||||| |||||||||||||||
Sbjct  374  GAGGATGCGTGCCAAAGAAGTTGCTGGTTTATGCATCAAAATTTGCTCATGAATTTGAAG  433

Query  121  AAAGCAATGGTTTTGGATGGAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGA  180
            |||| ||||||||||| |||||||||| |||||| || |||||||| |||||||||||||
Sbjct  434  AAAGTAATGGTTTTGGCTGGAGATATGGCAGTGAGCCCAAGCATGATTGGAGTAGTTTGA  493

Query  181  TTGCTAATAAAAATGCCGAGTTGCAGCGGCTTACTGGTATCTATAAGAATATTTTGAAAA  240
            | ||||||||||||||| ||||||||||| |||||||| ||||| || ||||| ||||| |
Sbjct  494  TAGCTAATAAAAATGCTGAGTTGCAGCGTCTTACTGGCATCTACAAAAATATCTTGAACA  553

Query  241  ATGCCGGTGTCAAGTTGATTGAAGGCCGTGGAAAGATTGTAGATCCTCACACGGTTGATG  300
            |||| || |||||| |||| ||||||| |||||||||| ||||| |||||||||||||||
Sbjct  554  ATGCTGGAGTCAAGCTGATCGAAGGCCATGGAAAGATTATAGATTCTCACACGGTTGATG  613

Query  301  TTGATGGGAAGTTATATTCAGCAAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCA  360
            || ||||||||  ||||||||| ||||||||||||||| | || |||||||| |||||||
Sbjct  614  TTAATGGGAAGCAATATTCAGCCAAACACATTTTAGTTGCCGTCGGAGGTCGCCCCTTCA  673

Query  361  TTCCTGATATTCCTGGAAAGGAATATGCAATAGATTCAGACGCTGCCCTTGATTTACCAT  420
            ||||||||||||||||| |||||||||||||||||||||||   |||||||||||||||||
Sbjct  674  TTCCTGATATTCCTGGGAAGGAATATGCAATAGATTCAGATATTGCCCTTGATTTACCAT  733

Query  421  CAAAGCCTCAGAAGATAGCCATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTA  480
            |||| |||  ||| ||||| |||||||||||| ||||||||||||||||||||||||||||
Sbjct  734  CAAAACCTGGGAAAATAGCTATTGTTGGTGGTGGTTACATTGCCTTGGAGTTTGCTGGTA  793
```

```
Query  481  TCTTTAATGGTTTGAAAAGTGAAGTTCATGTATTTATACGACAAAAGAAGGGTCTGCGGG  540
            ||||||||||||||| |||| || |||||||| |||||||| |||||||||| |||| |||
Sbjct  794  TCTTTAATGGTTTGCAAAGCGAGGTTCATGTCTTTATACGGCAAAAGAAGGTTCTGAGGG  853

Query  541  GATTTTGATGAAGA  554
            || ||||| |||||
Sbjct  854  GA-TTTGACGAAGA  866
```

* Only the first three homologous sequences were taken.

APPENDIX H

OPTIMIZED MULTIPLEX PCR CONDITIONS

Mn SOD (Amplicon Length is 186 bp)

Table H. 5. Final concentrations of PCR ingredients for Multiplex Mn SOD PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Mn SOD Right Primer (10 µM)	0.6µM
Mn SOD Left Primer (10 µM)	0.6µM
18S rRNA Right Primer (2 µM)	0.12µM
18S rRNA Left Primer (2 µM)	0.12µM
Taq DNA Polymerase (5U/1µL)	1.5U
Template	50ng
25mM $MgCl_2$	1.25mM

Table H. 6. PCR Conditions of Mn SOD amplicon

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 32		
Denaturation	94ºC	30 sec.
Primer annealing	50ºC	1 min. 30 sec.
Elongation	72ºC	1 min. 30 sec.
Final Extension	72ºC	10 min.

Cu/Zn SOD Lentil (Amplicon Length is 305 bp)

Table H. 7. Final concentrations of PCR ingredients for Specific Cu/Zn SOD PCR

Ingredient	**Final Concentration**
10X Taq Buffer	1X
2mM dNTP Mix	0.2mM of each
Cu/Zn SOD Right Primer (10 µM)	0.6µM
Cu/Zn SOD Left Primer (10 µM)	0.6µM
18S rRNA Right Primer (2 µM)	0.12µM
18S rRNA Left Primer (2 µM)	0.12µM
Taq DNA Polymerase (5U/1µL)	1.25U
Template	50ng
25mM $MgCl_2$	1.5mM

Table H. 8. PCR Conditions of Cu/Zn SOD amplicon

PCR Step	Step Temperature	Step Duration
Initial denaturation	95ºC	1 min.
Cycle number = 30		
Denaturation	94ºC	45 sec.
Primer annealing	51ºC	30 sec.
Elongation	72ºC	45 sec.
Final Extension	72ºC	10 min.

APPENDIX I

PROTEIN and mRNA SEQUENCES with CLUSTALW ALIGNMENT of SEQUENCES

Mn SOD pBLAST Results (240 amino acids)

```
>gi|20902|emb|CAA42737.1| MnSOD [Pisum sativum]
MAARTLLCRKTLSSVLRNDAKPIGAAIAAASTQSRGLHVFTLPDLAYDYGALEPVISGEIMQIHHQKHHQTYITNYNKALEQ
LHDAVAKADTSTTVKLQNAIKFNGGGHINHSIFWKNLAPVSEGGGEPPKESLGWAIDTNFGSLEALIQKINAEGAALQASGW
VWLGLDKDLKRLVVETTANQDPLVTKGASLVPLLWIDVWEHAYYLQYKNVRPDYLKNIWKVINWKHASEVYEKESS
>gi|147945633|gb|ABQ52658.1| MnSOD [Glycine max]
MAARALLTRKTLATVLRNDAKPIIGVGITAAATHSRGLHVYTLPDLDYDYGALEPAISGDIMQLHHQKHHQTYITNYNKALE
QLQDAIAKKDSSAVVKLQGAIKFNGGGHVNHSIFWKNLAPVREGGGEPPKGSLGWAIDTHFGSFEALIQKVNAEGAALQGSG
WVWLGLDKELKRLVVETTANQDPLVTKGPNLVPLIGIDVWEHAYYLQYKNVRPDYLKNIWKVINWKYASEVYEKESS
>gi|3273751|gb|AAC24832.1| MnSOD [Arabidopsis thaliana]
MAIRCVASRKTLAGLKETSSRLLRIRGIQTFTLPDLPYDYGALEPAISGEIMQIHHQKHHQAYVTNYNNALEQLDQAVNKGD
ASTVVKLQSAIKFNGGGHVNHSIFWKNLAPSSEGGGEPPKGSLGSAIDAHFGSLEGLVKKMSAEGAAVQGSGWVWLGLDKEL
KKLVFDTTANQDPLVTKGGSLVPLVGIDVWEHAYYLQYKNVRPEYLKNVWKVINWKYASEVYEKENN
```

CLUSTALW Alignment of Protein Sequences

```
CLUSTAL 2.0.1 multiple sequence alignment


gi|20902|emb|CA      MAARTLLCRKTLSSVLRNDAKP-IGAAIAAASTQSRGLHVFTLPDLAYDY 49
gi|147945633|gb      MAARALLTRKTLATVLRNDAKPIIGVGITAAATHSRGLHVYTLPDLDYDY 50
gi|3273751|gb|A      MAIRCVASRKTLAGLKETSSRL----------LRIRGIQTFTLPDLPYDY 40
                     ** * :  ****: : ...::            : **::.:***** ***

gi|20902|emb|CA      GALEPVISGEIMQIHHQKHHQTYITNYNKALEQLHDAVAKADTSTTVKLQ 99
gi|147945633|gb      GALEPAISGDIMQLHHQKHHQTYITNYNKALEQLQDAIAKKDSSAVVKLQ 100
gi|3273751|gb|A      GALEPAISGEIMQIHHQKHHQAYVTNYNNALEQLDQAVNKGDASTVVKLQ 90
                     *****.***:***:*******:*:****:*****.:*: * *:*:.****

gi|20902|emb|CA      NAIKFNGGGHINHSIFWKNLAPVSEGGGEPPKESLGWAIDTNFGSLEALI 149
gi|147945633|gb      GAIKFNGGGHVNHSIFWKNLAPVREGGGEPPKGSLGWAIDTHFGSFEALI 150
gi|3273751|gb|A      SAIKFNGGGHVNHSIFWKNLAPSSEGGGEPPKGSLGSAIDAHFGSLEGLV 140
                     .*********:***********  ******** *** ***::***:*.*:

gi|20902|emb|CA      QKINAEGAALQASGWVWLGLDKDLKRLVVETTANQDPLVTKGASLVPLLW 199
gi|147945633|gb      QKVNAEGAALQGSGWVWLGLDKELKRLVVETTANQDPLVTKGPNLVPLIG 200
gi|3273751|gb|A      KKMSAEGAAVQGSGWVWLGLDKELKKLVFDTTANQDPLVTKGGSLVPLVG 190
                     :*:.*****:*.**********:**:**.:************ .****:

gi|20902|emb|CA      IDVWEHAYYLQYKNVRPDYLKNIWKVINWKHASEVYEKESS 240
gi|147945633|gb      IDVWEHAYYLQYKNVRPDYLKNIWKVINWKYASEVYEKESS 241
gi|3273751|gb|A      IDVWEHAYYLQYKNVRPEYLKNVWKVINWKYASEVYEKENN 231
                     *****************:****:*******:********..
```

Mn SOD mRNA Sequences

```
>gi|20901:55-777 Pisum sativum MnSOD
ATGGCCGCTCGAACCCTATTGTGCAGAAAAACCCTATCCTCCGTGCTCCGCAATGACGCAAAACCAATCGGAGCAGCCATAG
CAGCCGCATCAACTCAATCCCGCGGTTTGCATGTCTTCACGCTCCCGGATCTCGCTTACGACTACGGAGCTTTGGAGCCTGT
CATTAGCGGCGAAATCATGCAAATCCACCATCAGAAACACCACCAGACTTATATTACCAACTACAACAAAGCTCTCGAACAG
CTTCACGATGCCGTTGCTAAAGCTGATACATCTACCACCGTTAAGCTCCAGAATGCCATCAAATTCAACGGCGGAGGTCATA
TCAACCATTCCATTTTCTGGAAAAATCTGGCTCCTGTTAGTGAAGGAGGTGGTGAACCACCAAAGGAATCCCTGGGCTGGGC
CATTGACACCAATTTTGGATCTTTGGAAGCATTGATACAAAAGATTAATGCCGAAGGTGCAGCTCTTCAGGCGTCTGGATGG
GTGTGGCTTGGTCTCGACAAAGACTTGAAGAGGCTTGTGGTTGAAACCACTGCAAACCAGGACCCACTGGTGACTAAAGGAG
CAAGTTTGGTTCCATTGCTTTGGATAGATGTTTGGGAACATGCCTACTACTTACAGTACAAAAATGTTAGACCAGACTATTT
GAAGAACATTTGGAAAGTTATTAACTGGAAACATGCCAGTGAAGTATATGAGAAAGAGAGCTCTTAA
```

```
>gi|147945632:1-726 Glycine max MnSOD
ATGGCCGCGCGAGCTCTGTTGACCAGAAAAACCCTAGCCACCGTGCTCCGCAACGACGCGAAGCCCATAATCGGAGTTGGCA
TAACAGCAGCGGCTACTCATTCACGCGGGTTGCACGTGTACACGCTACCCGATCTGGATTACGACTATGGCGCACTGGAGCC
AGCCATCAGCGGCGACATCATGCAGCTGCACCACCAGAAGCACCACCAGACTTACATCACCAACTACAACAAGGCCCTCGAG
CAGCTCCAAGACGCCATCGCCAAGAAAGATTCCTCCGCCGTCGTTAAGCTCCAGGGCGCCATCAAGTTCAACGGCGGAGGTC
ATGTCAACCATTCTATTTTCTGGAAAAATCTAGCTCCTGTTCGTGAAGGAGGTGGTGAACCACCCAAGGGTTCACTGGGATG
GGCTATTGACACACATTTTGGTTCTTTTGAAGCATTAATACAAAAAGTTAACGCAGAAGGTGCTGCACTACAGGGGTCTGGA
TGGGTGTGGCTTGGTCTGGACAAAGAGTTGAAGAGGCTTGTAGTTGAAACCACTGCCAACCAGGACCCACTGGTTACTAAGG
GACCAAATTTGGTTCCATTGATTGGTATTGATGTTTGGGAGCATGCGTACTACTTACAGTACAAGAATGTTAGACCAGACTA
TCTGAAGAACATTTGGAAAGTTATTAATTGGAAATATGCCAGTGAAGTGTATGAGAAAGAGAGCTCTTAG
>gi|3273750:17-712              Arabidopsis              thaliana              MnSOD
ATGGCGATTCGTTGTGTAGCGAGTAGAAAAACCCTAGCCGGCTTGAAGGAGACATCATCGAGGCTATTGAGGATCAGAGGGA
TTCAGACTTTTACGCTTCCTGATCTTCCTTACGATTATGGCGCATTGGAACCGGCCATTAGTGGAGAGATCATGCAGATTCA
TCACCAGAAGCATCACCAGGCTTATGTTACTAATTACAATAATGCTCTTGAGCAGCTTGATCAAGCTGTGAACAAGGGAGAT
GCTTCCACTGTTGTTAAGTTGCAGAGCGCCATCAAATTCAACGGCGGAGGTCATGTCAACCATTCGATTTTCTGGAAGAACC
TTGCTCCTTCCAGTGAAGGTGGTGGAGAGCCACCAAAAGGRTCTCTTGGTAGTGCCATTGACGCTCACTTTGGCTCCCTTGA
AGGTCTGGTGAAAAARATGAGTGCTGAGGGTGCTGCAGTGCAAGGCTCAGGATGGGTGTGGCTCGGACTAGACAAAGAACTG
AAGAAGCTAGTTTTTGACACAACTGCCAATCAGGATCCATTAGTGACAAAAGGAGGAAGCTTGGTACCTCTGGTGGGTATAG
ATGTTTGGGAGCACGCCTACTACTTGCAGTACAAAAATGTGAGGCCTGAGTATCTGAAGAATGTATGGAAAGTGATCAACTG
GAAATATGCAAGCGAGGTTTATGAGAAGGAAAACAACTGA
```

CLUSTALW Alignment of mRNA Sequences

```
CLUSTAL 2.0.1 multiple sequence alignment


gi|20901_55-777        ATGGCCGCTCGAACCCTATTGTGCAGAAAAACCCTATCCTCCGTGCTCCGCAATGACGCA
60
gi|147945632_1-        ATGGCCGCGCGAGCTCTGTTGACCAGAAAAACCCTAGCCACCGTGCTCCGCAACGACGCG
60
gi|3273750_17-7       ------------------------------------------------------------


gi|20901_55-777        AAACC---AATCGGAGCAGCCATAGCAGCCGCATCAACTCAATCCCGCGGTTTGCATGTC
117
gi|147945632_1-        AAGCCCATAATCGGAGTTGGCATAACAGCAGCGGCTACTCATTCACGCGGGTTGCACGTG
120
gi|3273750_17-7        ---------------------------------------GGATCAGAGGGATT-CAGACT
20
                                                                **    ** ** **

gi|20901_55-777        TTCACGCTCCCGGATCTCGCTTACGACTACGGAGCTTTGGAGCCTGTCATTAGCGGCGAA
177
gi|147945632_1-        TACACGCTACCCGATCTGGATTACGACTATGGCGCACTGGAGCCAGCCATCAGCGGCGAC
180
```

```
gi|3273750_17-7        TTTACGCTTCCTGATCTTCCTTACGATTATGGCGCATTGGAACCGGCCATTAGTGGAGAG
80
                     *  ***** ** *****   ****** ** ** **  **** ** * *** ** ** **

gi|20901_55-777        ATCATGCAAATCCACCATCAGAAACACCACCAGACTTATATTACCAACTACAACAAAGCT
237
gi|147945632_1-        ATCATGCAGCTGCACCACCAGAAGCACCACCAGACTTACATCACCAACTACAACAAGGCC
240
gi|3273750_17-7        ATCATGCAGATTCATCACCAGAAGCATCACCAGGCTTATGTTACTAATTACAATAATGCT
140
                     ********  * ** ** ***** ** ****** ****  * ** ** ***** ** **

gi|20901_55-777        CTCGAACAGCTTCACGATGCCGTTGCTAAAGCTGATACATCTACCACCGTTAAGCTCCAG
297
gi|147945632_1-        CTCGAGCAGCTCCAAGACGCCATCGCCAAGAAAGATTCCTCCGCCGTCGTTAAGCTCCAG
300
gi|3273750_17-7        CTTGAGCAGCTTGATCAAGCTGTGAACAAGGGAGATGCTTCCACTGTTGTTAAGTTGCAG
200
                     ** ** *****  *  * **  *    **    *** * **  *    ****** * ***

gi|20901_55-777        AATGCCATCAAATTCAACGGCGGAGGTCATATCAACCATTCCATTTTCTGGAAAAATCTG
357
gi|147945632_1-        GGCGCCATCAAGTTCAACGGCGGAGGTCATGTCAACCATTCTATTTTCTGGAAAAATCTA
360
gi|3273750_17-7        AGCGCCATCAAATTCAACGGCGGAGGTCATGTCAACCATTCGATTTTCTGGAAGAACCTT
260
                         ******** ****************** ********** *********** ** **

gi|20901_55-777        GCTCCTGTTAGTGAAGGAGGTGGTGAACCACCAAAGGAATCCCTGGGCTGGGCCATTGAC
417
gi|147945632_1-        GCTCCTGTTCGTGAAGGAGGTGGTGAACCACCCAAGGGTTCACTGGGATGGGCTATTGAC
420
gi|3273750_17-7        GCTCCTTCCAGTGAAGGTGGTGGAGAGCCACCAAAAGGRTCTCTTGGTAGTGCCATTGAC
320
                     ******    ******* ***** ** ***** ** *  ** ** **  * ** ******

gi|20901_55-777        ACCAATTTTGGATCTTTGGAAGCATTGATACAAAAGATTAATGCCGAAGGTGCAGCTCTT
477
gi|147945632_1-        ACACATTTTGGTTCTTTTGAAGCATTAATACAAAAAGTTAACGCAGAAGGTGCTGCACTA
480
gi|3273750_17-7        GCTCACTTTGGCTCCCTTGAAGGTCTGGTGAAAAARATGAGTGCTGAGGGTGCTGCAGTG
380
                      *  * ***** **  * ****   *  *  ****  * *  ** ** ***** **  *

gi|20901_55-777        CAGGCGTCTGGATGGGTGTGGCTTGGTCTCGACAAAGACTTGAAGAGGCTTGTGGTTGAA
537
```

```
gi|147945632_1-        CAGGGGTCTGGATGGGTGTGGCTTGGTCTGGACAAAGAGTTGAAGAGGCTTGTAGTTGAA
540
gi|3273750_17-7        CAAGGCTCAGGATGGGTGTGGCTCGGACTAGACAAAGAACTGAAGAAGCTAGTTTTTGAC
440
                     ** *  ** ************** ** ** ********  ****** *** **  ****

gi|20901_55-777        ACCACTGCAAACCAGGACCCACTGGTGACTAAAGGAGCAAGTTTGGTTCCATTGCTTTGG
597
gi|147945632_1-        ACCACTGCCAACCAGGACCCACTGGTTACTAAGGGACCAAATTTGGTTCCATTGATTGGT
600
gi|3273750_17-7        ACAACTGCCAATCAGGATCCATTAGTGACAAAAGGAGGAAGCTTGGTACCTCTGGTGGGT
500
                     ** ***** ** ***** *** * ** ** ** ***  **  ***** **  ** *  *

gi|20901_55-777        ATAGATGTTTGGGAACATGCCTACTACTTACAGTACAAAAATGTTAGACCAGACTATTTG
657
gi|147945632_1-        ATTGATGTTTGGGAGCATGCGTACTACTTACAGTACAAGAATGTTAGACCAGACTATCTG
660
gi|3273750_17-7        ATAGATGTTTGGGAGCACGCCTACTACTTGCAGTACAAAAATGTGAGGCCTGAGTATCTG
560
                     ** *********** ** ** ******** ******** ***** ** ** ** *** **

gi|20901_55-777        AAGAACATTTGGAAAGTTATTAACTGGAAACATGCCAGTGAAGTATATGAGAAAGAGAGC
717
gi|147945632_1-        AAGAACATTTGGAAAGTTATTAATTGGAAATATGCCAGTGAAGTGTATGAGAAAGAGAGC
720
gi|3273750_17-7        AAGAATGTATGGAAAGTGATCAACTGGAAATATGCAAGCGAGGTTTATGAGAAGGAAAAC
620
                     *****  * ******** ** ** ****** **** ** ** ** ******** ** * *

gi|20901_55-777      TCTTAA 723
gi|147945632_1-      TCTTAG 726
gi|3273750_17-7      AACTGA 626
                        *
```

Chloroplast/Mitochondrial GR pBLAST Results (541 amino acids)

```
>gi|169965|gb|AAA33962.1| GR (Glycine max)
MATSLSVSPSLSLNTLFIAKALPLSRPSFLSLPLPKSLLSLSTRRRTFIVRAESQNGRDPVPAHYDFDLF
TIGAGSGGVRARRFAANYGASVAICELPFSTISSETTGVGGTCVIRGCVPKKLLVYASKFSHEFEESNGF
GWRYDSEPKHDWSSFIANKNAELQRLTGIYKNILNNAGVKLIEGHGKMIDPHTVDVNGKLYSAKHILVAV
GGRPFIPDIPGKELAIDSDAALDLPTKPVKIAIVGGGYIALEFAGIFNGLKSEVHVFIRQKKVLRGFDEE
IRDFVEEQMSVRGIEFHTEESPQAITKSADGSFSLKTNKGTVDGFSHIMFATGRRPNTQNLGLESVGVKL
AKDGAIEVDEYSQTSVYSIWAVGDVTNRINLTPVALMEGGALVKTLFQDNPTKPDYRAVPSAVFSQPPIG
QVGLTEEQAVQQYGDIDIFTANFRPLKATLSGLPDRVFMKLVVCAKTNEVLGLHMCGEDAPEIVQGFAVA
LKARLTKADFDATVGIHPSAAEEFVTMRTPTRKIRKSESSEGKSGSQAKAAAGV

>gi|1345568|emb|CAA42921.1| GR [Pisum sativum]
APPGTERETLMNQAMATPLSLSCCSPTLTRSTLFFTKTFPFSRSFSTPLPLSTKTLISLSPPHRTFAVRA
ESQNGADPARQYDFDLFTIGAGSGGVRASRFASNFGASSAVCELPFSTISSDTTGGVGGTCVIRGCVPKK
LLVYASKFSHEFEESNGFGWRYDSEPKHDWSSLIANKNAELQRLTGIYKNTLKNAGVKLIEGRGKIVDAH
TVDVDGKLYSAKHILVSVGGRPFIPDIPGKEYAIDSDAALDLPSKPQKIAIVGGGYIALEFAGIFNGLKS
EVHVFIRQKKVLRGFDEEIRDFVAENMALRGIEFHTEESPVAITKAADGSLSLKTNKGTEEGFSHIMFAT
GRSPNTKDLGLESVGVKVAKDGSIEVDEYSQTSVPSIWAIGDATNRVNLTPVALMEGVALAKTLFQNEPT
KPDYRAIPSAVFSQPPIGGVGLTEEQAAEQYGDIDVFTANFRPMKATLSGLPDRVFMKLIVSAETNVVLG
LHMCGEDAAEIAQGFAVGIKAGLTKADFDATVGIHPTAAEEFVTMRTPTRKVRKNQASQGKSDSKAKAVA
GS
>gi|451198|dbj|BAA03137.1| GR [Arabidopsis thaliana]
MASTPKLTSTISSSSPSLQFLCKKLPIAIHLPSSSSSSFLSLPKTLTSLYSLRPRIALLSNHRYYHSRRF
SVCASTDNGAESDRHYDFDLFTIGAGSGGVRASRFATSFGASAAVCELPFSTISSDTAGGVGGTCVLRGC
VPKKLLVYASKYSHEFEDSHGFGWKYETEPSHDWTTLIANKNAELQRLTGIYKNILSKANVKLIEGRGKV
IDPHTVDVDGKIYTTRNILIAVGGRPFIPDIPGKEFAIDSDAALDLPSKPKKIAIVGGGYIALEFAGIFN
GLNCEVHVFIRQKKVLRGFDEDVRDFVGEQMSLRGIEFHTEESPEAIIKAGDGSFSLKTSKGTVEGFSHV
MFATGRKPNTKNLGLENVGVKMAKNGAIEVDEYSQTSVPSIWAVGDVTDRINLTPVALMEGGALAKTLFQ
NEPTKPDYRAVPCAVFSQPPIGTVGLTEEQAIEQYGDVDVYTSNFRPLKATLSGLPDRVFMKLIVCANTN
KVLGVHMCGEDSPEIIQGFGVAVKAGLTKADFDATVGVHPTAAEEFVTMRAPTRKFRKDSSEGKASPEAK
TAAGV
```

CLUSTAL 2.0.1 multiple sequence alignment

```
gi|169965|      --------------MATSLSVS---PSLSLNTLFIAKALPLSRPS-FLSL 32
gi|1345568      APPGTERETLMNQAMATPLSLSCCSPTLTRSTLFFTKTFPFSRSF-STPL 49
gi|451198|      --------MASTPKLTSTISSSSPSLQFLCKKLPIAIHLPSSSSSSFLSL 42
                              :::.:* *     :  ..* ::  :* * .    .*

gi|169965|      PLP-KSLLSLST-----------RRRTFIVRAESQNGRDPVPAHYDFDLF 70
gi|1345568      PLSTKTLISLSP-----------PHRTFAVRAESQNGADPA-RQYDFDLF 87
gi|451198|      PKTLTSLYSLRPRIALLSNHRYYHSRRFSVCASTDNGAESD-RHYDFDLF 91
                * . .:* ** .             * * * *.::** :.   :******

gi|169965|      TIGAGSGGVRARRFAANYGASVAICELPFSTISSETTG-VGGTCVIRGCV 119
gi|1345568      TIGAGSGGVRASRFASNFGASSAVCELPFSTISSDTTGGVGGTCVIRGCV 137
gi|451198|      TIGAGSGGVRASRFATSFGASAAVCELPFSTISSDTAGGVGGTCVLRGCV 141
                *********** ***:.:*** *:**********:*:* ******:****

gi|169965|      PKKLLVYASKFSHEFEESNGFGWRYDSEPKHDWSSFIANKNAELQRLTGI 169
gi|1345568      PKKLLVYASKFSHEFEESNGFGWRYDSEPKHDWSSLIANKNAELQRLTGI 187
gi|451198|      PKKLLVYASKYSHEFEDSHGFGWKYETEPSHDWTTLIANKNAELQRLTGI 191
                **********:*****:*:****:*::**.***:::**************

gi|169965|      YKNILNNAGVKLIEGHGKMIDPHTVDVNGKLYSAKHILVAVGGRPFIPDI 219
gi|1345568      YKNTLKNAGVKLIEGRGKIVDAHTVDVDGKLYSAKHILVSVGGRPFIPDI 237
gi|451198|      YKNILSKANVKLIEGRGKVIDPHTVDVDGKIYTTRNILIAVGGRPFIPDI 241
                *** *.:*.******:**::*.*****:**:*::::**::**********

gi|169965|      PGKELAIDSDAALDLPTKPVKIAIVGGGYIALEFAGIFNGLKSEVHVFIR 269
gi|1345568      PGKEYAIDSDAALDLPSKPQKIAIVGGGYIALEFAGIFNGLKSEVHVFIR 287
gi|451198|      PGKEFAIDSDAALDLPSKPKKIAIVGGGYIALEFAGIFNGLNCEVHVFIR 291
                **** ***********:** *********************:.*******

gi|169965|      QKKVLRGFDEEIRDFVEEQMSVRGIEFHTEESPQAITKSADGSFSLKTNK 319
gi|1345568      QKKVLRGFDEEIRDFVAENMALRGIEFHTEESPVAITKAADGSLSLKTNK 337
gi|451198|      QKKVLRGFDEDVRDFVGEQMSLRGIEFHTEESPEAIIKAGDGSFSLKTSK 341
```

```
                 **********::**** *:*::*********** ** *:.***:****.*

gi|169965|       GTVDGFSHIMFATGRRPNTQNLGLESVGVKLAKDGAIEVDEYSQTSVYSI 369
gi|1345568       GTEEGFSHIMFATGRSPNTKDLGLESVGVKVAKDGSIEVDEYSQTSVPSI 387
gi|451198|       GTVEGFSHVMFATGRKPNTKNLGLENVGVKMAKNGAIEVDEYSQTSVPSI 391
                 ** :****:****** ***::****.****:**:*:*********** **

gi|169965|       WAVGDVTNRINLTPVALMEGGALVKTLFQDNPTKPDYRAVPSAVFSQPPI 419
gi|1345568       WAIGDATNRVNLTPVALMEGVALAKTLFQNEPTKPDYRAIPSAVFSQPPI 437
gi|451198|       WAVGDVTDRINLTPVALMEGGALAKTLFQNEPTKPDYRAVPCAVFSQPPI 441
                 **:**.*:*:********** **.*****::********:*.********

gi|169965|       GQVGLTEEQAVQQYGDIDIFTANFRPLKATLSGLPDRVFMKLVVCAKTNE 469
gi|1345568       GGVGLTEEQAAEQYGDIDVFTANFRPMKATLSGLPDRVFMKLIVSAETNV 487
gi|451198|       GTVGLTEEQAIEQYGDVDVYTSNFRPLKATLSGLPDRVFMKLIVCANTNK 491
                 * ******** :****:*::*:****:***************:*.*:**

gi|169965|       VLGLHMCGEDAPEIVQGFAVALKARLTKADFDATVGIHPSAAEEFVTMRT 519
gi|1345568       VLGLHMCGEDAAEIAQGFAVGIKAGLTKADFDATVGIHPTAAEEFVTMRT 537
gi|451198|       VLGVHMCGEDSPEIIQGFGVAVKAGLTKADFDATVGVHPTAAEEFVTMRA 541
                 ***:******:.** ***.*.:** ***********:**:*********:

gi|169965|       PTRKIRKSESSEGKSGSQAKAAAGV 544
gi|1345568       PTRKVRKNQASQGKSDSKAKAVAGS 562
gi|451198|       PTRKFRK-DSSEGKASPEAKTAAGV 565
                 ****.** ::*:**:..:**:.**
```

Chloroplast/Mitochondrial GR mRNA Sequences

```
>gi|169964:59-1693 Soybean GR
ATGGCGACGTCGCTCTCTGTTTCGCCATCTCTCTCTCTCAACACTCTCTTCATCGCCAAAGCGCTTCCAC
TATCTCGCCCCTCCTTTCTCTCTCTCCCTCTCCCCAAATCCCTACTCTCTCTCTCCACGCGCCGCCGCAC
CTTCATCGTCCGCGCCGAATCCCAAAACGGCCGCGACCCCGTTCCCGCCCACTACGACTTCGACCTCTTC
ACCATCGGCGCAGGCAGCGGCGGGGTCCGAGCCCGTCGCTTCGCCGCCAACTACGGCGCTTCTGTCGCCA
TCTGCGAGCTTCCTTTCTCCACTATCTCCTCCGAAACCACCGGAGTCGGCGGAACCTGTGTAATACGAGG
ATGTGTGCCGAAGAAGTTGCTGGTTTATGCATCTAAATTTTCTCATGAATTTGAAGAAAGTAATGGTTTC
GGATGGAGATATGACAGTGAGCCCAAGCATGATTGGAGTAGTTTCATAGCTAATAAAAATGCTGAGTTGC
AGCGTCTTACTGGCATCTACAAGAATATTTTGAACAATGCTGGGGTCAAGCTGATTGAAGGCCATGGAAA
GATGATAGATCCTCACACGGTTGATGTTAATGGGAAGCTATATTCAGCCAAACACATTTTAGTTGCAGTT
GGAGGTCGCCCCTTCATTCCTGATATCCCTGGGAAGGAATTAGCAATAGATTCAGATGCTGCCCTTGATT
TACCAACAAAACCTGTGAAAATAGCCATTGTTGGTGGTGGTTACATTGCCTTGGAGTTTGCTGGTATCTT
TAATGGTTTGAAAAGTGAGGTTCATGTATTTATACGGCAAAAGAAGGTTTTGCGGGGATTTGATGAAGAG
ATTAGAGATTTTGTTGAAGAACAAATGTCTGTAAGAGGTATTGAATTCCATACCGAGGAGTCTCCTCAAG
CTATCACGAAGTCAGCGGATGGCTCATTCTCTTTAAAGACCAACAAAGGTACAGTGGACGGTTTCTCACA
TATTATGTTTGCTACAGGACGCAGACCTAATACTCAGAACTTAGGCCTGGAGTCTGTTGGTGTGAAACTA
GCTAAAGATGGAGCTATAGAGGTTGATGAATACTCTCAAACATCAGTTTATTCAATTTGGGCAGTTGGAG
ATGTTACAAACAGGATAAATCTCACCCCAGTTGCTTTGATGGAGGGAGGAGCATTAGTAAAAACGCTGTT
TCAGGATAACCCAACAAAACCTGATTATAGAGCTGTTCCTTCTGCTGTATTTTCTCAACCACCAATTGGA
CAAGTTGGTCTTACTGAGGAACAGGCTGTACAACAATATGGAGATATTGACATCTTCACTGCAAATTTTA
GGCCGCTGAAAGCTACTCTCTCTGGGCTTCCAGACCGGGTTTTTATGAAATTAGTAGTCTGTGCAAAAAC
AAATGAAGTTCTAGGTTTGCATATGTGTGGAGAAGATGCTCCTGAAATTGTGCAGGGGTTTGCAGTTGCT
CTTAAAGCTCGCTTGACCAAGGCTGACTTTGATGCCACTGTAGGCATTCACCCAAGTGCAGCTGAGGAAT
TTGTTACCATGAGGACACCTACTAGGAAGATACGAAAGAGTGAATCGTCTGAGGGTAAGTCGGGCTCTCA
AGCAAAAGCTGCAGCAGGGGTTTAA
>gi|20746:52-1740 P.sativum GR
GCACCACCGGGAACAGAGAGGGAAACTCTAATGAACCAAGCAATGGCTACTCCACTTTCTCTCTCTTGTT
GTTCTCCAACTCTAACTCGCAGCACTCTCTTCTTCACCAAAACCTTTCCATTTTCTCGCTCCTTCTCTAC
ACCTCTCCCTCTCTCCACCAAAACCCTAATTTCCCTCTCTCCACCCCATCGCACCTTCGCCGTCCGCGCT
GAATCCCAAAACGGCGCCGACCCCGCCCGCCAGTATGACTTCGACCTTTTCACCATCGGCGCTGGAAGCG
GAGGCGTCCGTGCTTCCCGCTTTGCCTCCAATTTCGGCGCTTCTTCTGCTGTCTGCGAGCTCCCTTTCTC
TACTATCTCCTCCGATACCACCGGTGGTGTCGGCGGCACCTGTGTAATACGGGGATGTGTCCCTAAGAAA
TTGCTAGTCTATGCCTCAAAATTCTCTCATGAATTTGAAGAAAGCAATGGTTTTGGATGGAGATATGACA
GTGAACCTAAGCATGACTGGAGTAGTTTGATTGCTAATAAAAACGCCGAGTTGCAGCGGCTTACTGGTAT
CTATAAGAATACTTTGAAAAATGCCGGTGTTAAGTTGATTGAAGGCCGTGGAAAGATTGTAGATGCTCAC
ACAGTTGATGTTGATGGGAAGTTATATTCAGCGAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCA
TTCCTGATATTCCTGGAAAGGAATATGCAATAGATTCAGATGCTGCCCTTGATTTACCATCAAAGCCTCA
GAAGATAGCTATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTATCTTTAATGGTTTGAAAAGT
GAAGTTCATGTATTTATAAGACAAAAGAAGGTTTTGCGGGGATTTGATGAAGAGATTAGAGATTTTGTTG
CAGAAAATATGGCTCTGAGAGGTATTGAATTCCATACTGAGGAGTCTCCTGTAGCTATCACTAAGGCAGC
```

```
TGATGGTTCGCTCTCTTTAAAGACCAACAAAGGTACTGAGGAAGGTTTCTCTCATATTATGTTTGCCACT
GGACGCTCACCTAATACTAAGGATTTGGGCCTGGAGTCTGTTGGTGTGAAAGTGGCTAAAGATGGATCAA
TAGAGGTTGATGAATACTCTCAAACATCGGTTCCTTCTATTTGGGCAATTGGAGATGCTACAAATAGAGT
AAATCTCACTCCAGTTGCTTTGATGGAGGGAGTGGCATTAGCAAAAACTTTGTTTCAGAATGAGCCGACA
AAACCTGATTATAGGGCTATACCTTCTGCTGTGTTTTCCCAACCACCAATTGGAGGAGTTGGTCTTACAG
AGGAACAGGCTGCTGAACAATATGGTGATATTGACGTTTTCACAGCAAATTTTAGGCCGATGAAGGCCAC
CCTCTCTGGGCTTCCAGACCGGGTTTTTATGAAACTAATAGTCTCTGCAGAAACAAATGTTGTTCTTGGA
TTGCACATGTGTGGAGAAGATGCTGCTGAAATTGCACAGGGGTTTGCAGTTGGTATTAAAGCTGGATTAA
CAAAGGCGGACTTTGATGCCACAGTAGGCATTCATCCAACTGCAGCTGAGGAATTTGTTACCATGAGGAC
TCCCACTAGGAAGGTTCGAAAGAACCAAGCTTCACAGGGGAAGTCAGATTCTAAAGCAAAAGCTGTGGCT
GGATCTTAA
>gi|451197:19-1716 Arabidopsis thaliana GR
ATGGCTTCGACCCCGAAGCTTACCAGTACAATTTCATCATCTTCTCCATCTCTTCAATTCCTCTGCAAAA
AACTCCCAATCGCAATTCATCTACCATCATCTTCTTCCTCTAGCTTTCTCTCGCTTCCTAAAACCCTAAC
CTCTCTCTATTCTCTCCGTCCCCGTATCGCCCTACTCTCAAACCACCGCTATTACCACTCTCGCCGGTTT
TCTGTTTGTGCCAGTACCGATAATGGAGCTGAATCAGACCGCCACTACGATTTTGATCTCTTCACTATCG
GTGCCGGAAGCGGCGGCGTCCGCGCCTCTCGCTTCGCCACTAGCTTCGGTGCATCCGCCGCCGTTTGCGA
GCTTCCTTTTTCCACTATCTCTTCCGATACTGCTGGAGGCGTTGGAGGAACGTGTGTATTGAGAGGATGT
GTACCAAAGAAGTTACTTGTGTATGCATCCAAATACAGTCATGAGTTTGAAGACAGTCATGGATTTGGTT
GGAAGTATGAGACTGAGCCTTCTCATGATTGGACTACTTTGATTGCTAACAAGAATGCTGAGTTACAGCG
GTTGACTGGTATTTATAAGAATATACTGAGCAAAGCTAATGTCAAGTTGATTGAAGGTCGTGGAAAGGTT
ATAGACCCACACACTGTTGATGTAGATGGGAAAATCTATACTACGAGGAATATTCTGATTGCAGTTGGTG
GACGTCCTTTCATTCCTGACATTCCAGGAAAAGAGTTTGCTATTGATTCTGATGCCGCGCTTGATTTGCC
TTCCAAGCCTAAGAAAATTGCAATAGTTGGTGGTGGCTACATAGCCCTGGAGTTTGCGGGGATCTTCAAT
GGTCTTAACTGTGAAGTTCATGTATTTATAAGGCAAAAGAAGGTGCTGAGGGGATTTGATGAAGATGTCA
GGGATTTCGTTGGAGAGCAGATGTCTTTAAGAGGTATTGAGTTTCACACTGAAGAATCCCCTGAAGCCAT
CATCAAAGCTGGAGATGGCTCGTTCTCTCTGAAGACCAGCAAGGGAACTGTTGAGGGATTTTCGCATGTT
ATGTTTGCAACTGGTCGCAAGCCCAACACAAAGAACTTAGGGTTGGAGAATGTTGGCGTTAAAATGGCGA
AAAATGGAGCAATAGAGGTTGACGAATATTCACAGACATCTGTTCCATCCATCTGGGCTGTTGGGGATGT
TACTGACCGAATCAATTTGACTCCAGTTGCTTTGATGGAGGGAGGTGCATTGGCTAAAACTTTGTTTCAA
AATGAGCCAACAAAGCCTGATTATAGAGCTGTTCCCTGCGCCGTTTTCTCCCAGCCACCTATTGGAACAG
TTGGTCTAACTGAAGAGCAGGCCATAGAACAATATGGTGATGTGGATGTTTACACATCGAACTTTAGGCC
ATTAAAGGCTACCCTTTCAGGACTTCCAGACCGAGTATTTATGAAACTCATTGTCTGTGCAAACACCAAT
AAAGTTCTCGGTGTTCACATGTGTGGAGAAGATTCACCAGAAATCATCCAGGGATTTGGGGTTGCAGTTA
AAGCTGGTTTAACTAAGGCCGACTTTGATGCTACAGTGGGTGTTCACCCCACAGCAGCTGAGGAGTTTGT
CACTATGAGGGCTCCAACCAGGAAATTCCGCAAAGACTCCTCTGAGGGAAAGGCAAGTCCTGAAGCTAAA
ACAGCTGCTGGGGTGTAG
```

CLUSTAL 2.0.1 multiple sequence alignment

```
gi|169964_59-16        ------------------------------------------ATGGCGACGTCGCTCTCT
18
gi|20746_52-174        GCACCACCGGGAACAGAGAGGGAAACTCTAATGAACCAAGCAATGGCTACTCCACTTTCT
60
gi|451197_19-17        ---------------------------------------AACTCCCAATCGCAATTCAT
20
                                                                *  * *   *  *   *

gi|169964_59-16        GT---------TTCGCCATCTCTCTCTCTCAACACTCTCTTCATCGCCAAAGCGCTTCCA
69
gi|20746_52-174        CTCTCTTGTTGTTCTCCAACTCTAACTCGCAGCACTCTCTTCTTCACCAAAACCTTTCCA
120
gi|451197_19-17        CT---------ACCATCATCTTCTTCCTCTAGCTTTCTCTCGCTTCCTAAAACCCTAAC-
70
                      *           *  ** **    *    * *  *****   *  * *** *  *  *

gi|169964_59-16        CTATCTCGCCCCTCCTTTCTCTCTCTCCCTCTCCCCA---AATCCCTACTCTCTCTCTCC
126
gi|20746_52-174        TTTTCTCGCTCCTTCTCTACACCTCTCCCTCTCTCCACCAAAACCCTAATTTCCCTCTCT
180
gi|451197_19-17        CTCTCTC---TATTCTCTCCGTCCCCGTATCGCCCTA-----CTCTCAAACCACCGCTAT
122
                      * ****     * ** *    * *    ** * * *       *  *      * **

gi|169964_59-16        ACGCGCCGCCGCACCTTCATCGTCCGCGCCGAATCCCAAAACGGCCGCGACCCCGTTCCC
186
gi|20746_52-174        CCACCCCATCGCACCTTCGCCGTCCGCGCTGAATCCCAAAACGGCGCCGACCCCG--CCC
238
```

```
gi|451197_19-17        TACCACTCTCGCCGGTTTTCTGTTTGTGCCAGTACCGATAATGGAGCTGAATCAG--ACC
180
                         * *   ***   **    **  * **     ** * ** **    **  * *   **

gi|169964_59-16        GCCCACTACGACTTCGACCTCTTCACCATCGGCGCAGGCAGCGGCGGGGTCCGAGCCCGT
246
gi|20746_52-174        GCC-AGTATGACTTCGACCTTTTCACCATCGGCGCTGGAAGCGGAGGCGTCCGTGCTTCC
297
gi|451197_19-17        GCC-ACTACGATTTTGATCTCTTCACTATCGGTGCCGGAAGCGGCGGCGTCCGCGCCTCT
239
                     *** * ** ** ** ** ** ***** ***** ** ** ***** ** ***** **

gi|169964_59-16        CGCTTCGCCGCCAACTACGGCGCTTCTGTCGCCATCTGCGAGCTTCCTTTCTCCACTATC
306
gi|20746_52-174        CGCTTTGCCTCCAATTTCGGCGCTTCTTCTGCTGTCTGCGAGCTCCCTTTCTCTACTATC
357
gi|451197_19-17        CGCTTCGCCACTAGCTTCGGTGCATCCGCCGCCGTTTGCGAGCTTCCTTTTTCCACTATC
299
                     ***** *** * *  * *** ** **    **  * ******** ***** ** ******

gi|169964_59-16        TCCTCCGAAACCACCGGAG---TCGGCGGAACCTGTGTAATACGAGGATGTGTGCCGAAG
363
gi|20746_52-174        TCCTCCGATACCACCGGTGGTGTCGGCGGCACCTGTGTAATACGGGGATGTGTCCCTAAG
417
gi|451197_19-17        TCTTCCGATACTGCTGGAGGCGTTGGAGGAACGTGTGTATTGAGAGGATGTGTACCAAAG
359
                     ** ***** **  * ** *   * ** ** ** ****** *  * ******** ** ***

gi|169964_59-16        AAGTTGCTGGTTTATGCATCTAAATTTTCTCATGAATTTGAAGAAAGTAATGGTTTCGGA
423
gi|20746_52-174        AAATTGCTAGTCTATGCCTCAAAATTCTCTCATGAATTTGAAGAAAGCAATGGTTTTGGA
477
gi|451197_19-17        AAGTTACTTGTGTATGCATCCAAATACAGTCATGAGTTTGAAGACAGTCATGGATTTGGT
419
                     ** ** ** ** ***** ** ****    ****** ******** **  **** ** **

gi|169964_59-16        TGGAGATATGACAGTGAGCCCAAGCATGATTGGAGTAGTTTCATAGCTAATAAAAATGCT
483
gi|20746_52-174        TGGAGATATGACAGTGAACCTAAGCATGACTGGAGTAGTTTGATTGCTAATAAAAACGCC
537
gi|451197_19-17        TGGAAGTATGAGACTGAGCCTTCTCATGATTGGACTACTTTGATTGCTAACAAGAATGCT
479
                     ****  ***** * *** **    ***** **** ** *** ** ***** ** ** **

gi|169964_59-16        GAGTTGCAGCGTCTTACTGGCATCTACAAGAATATTTTGAACAATGCTGGGGTCAAGCTG
543
gi|20746_52-174        GAGTTGCAGCGGCTTACTGGTATCTATAAGAATACTTTGAAAAATGCCGGTGTTAAGTTG
597
gi|451197_19-17        GAGTTACAGCGGTTGACTGGTATTTATAAGAATATACTGAGCAAAGCTAATGTCAAGTTG
539
                     ***** *****  * ***** ** ** *******   ***  ** **    ** *** **

gi|169964_59-16        ATTGAAGGCCATGGAAAGATGATAGATCCTCACACGGTTGATGTTAATGGGAAGCTATAT
603
gi|20746_52-174        ATTGAAGGCCGTGGAAAGATTGTAGATGCTCACACAGTTGATGTTGATGGGAAGTTATAT
657
gi|451197_19-17        ATTGAAGGTCGTGGAAAGGTTATAGACCCACACACTGTTGATGTAGATGGGAAAATCTAT
599
                     ******** * ******* *  ****  * ***** ********  *******  * ***

gi|169964_59-16        TCAGCCAAACACATTTTAGTTGCAGTTGGAGGTCGCCCCTTCATTCCTGATATCCCTGGG
663
gi|20746_52-174        TCAGCGAAACACATTTTAGTTTCAGTTGGAGGTCGACCCTTCATTCCTGATATTCCTGGA
717
gi|451197_19-17        ACTACGAGGAATATTCTGATTGCAGTTGGTGGACGTCCTTTCATTCCTGACATTCCAGGA
659
                      *  * *   * *** *  ** ******* ** ** ** *********** ** ** **

gi|169964_59-16        AAGGAATTAGCAATAGATTCAGATGCTGCCCTTGATTTACCAACAAAACCTGTGAAAATA
723
```

```
gi|20746_52-174        AAGGAATATGCAATAGATTCAGATGCTGCCCTTGATTTACCATCAAAGCCTCAGAAGATA
777
gi|451197_19-17        AAAGAGTTTGCTATTGATTCTGATGCCGCGCTTGATTTGCCTTCCAAGCCTAAGAAAATT
719
                     ** ** *  ** ** ***** ***** ** ******** **  * ** ***  *** **

gi|169964_59-16        GCCATTGTTGGTGGTGGTTACATTGCCTTGGAGTTTGCTGGTATCTTTAATGGTTTGAAA
783
gi|20746_52-174        GCTATTGTTGGTGGGGGTTACATTGCCTTGGAGTTTGCTGGTATCTTTAATGGTTTGAAA
837
gi|451197_19-17        GCAATAGTTGGTGGTGGCTACATAGCCCTGGAGTTTGCGGGGATCTTCAATGGTCTTAAC
779
                     ** ** ******** ** ***** *** ********** ** ***** ****** * **

gi|169964_59-16        AGTGAGGTTCATGTATTTATACGGCAAAAGAAGGTTTTGCGGGGATTTGATGAAGAGATT
843
gi|20746_52-174        AGTGAAGTTCATGTATTTATAAGACAAAAGAAGGTTTTGCGGGGATTTGATGAAGAGATT
897
gi|451197_19-17        TGTGAAGTTCATGTATTTATAAGGCAAAAGAAGGTGCTGAGGGGATTTGATGAAGATGTC
839
                      **** *************** * ***********  ** ****************  *

gi|169964_59-16        AGAGATTTTGTTGAAGAACAAATGTCTGTAAGAGGTATTGAATTCCATACCGAGGAGTCT
903
gi|20746_52-174        AGAGATTTTGTTGCAGAAAATATGGCTCTGAGAGGTATTGAATTCCATACTGAGGAGTCT
957
gi|451197_19-17        AGGGATTTCGTTGGAGAGCAGATGTCTTTAAGAGGTATTGAGTTTCACACTGAAGAATCC
899
                     ** ***** **** ***  * *** ** * *********** ** ** ** ** ** **

gi|169964_59-16        CCTCAAGCTATCACGAAGTCAGCGGATGGCTCATTCTCTTTAAAGACCAACAAAGGTACA
963
gi|20746_52-174        CCTGTAGCTATCACTAAGGCAGCTGATGGTTCGCTCTCTTTAAAGACCAACAAAGGTACT
1017
gi|451197_19-17        CCTGAAGCCATCATCAAAGCTGGAGATGGCTCGTTCTCTCTGAAGACCAGCAAGGGAACT
959
                     ***  *** ****  **  * *  ***** **  ***** * ******* *** ** **

gi|169964_59-16        GTGGACGGTTTCTCACATATTATGTTTGCTACAGGACGCAGACCTAATACTCAGAACTTA
1023
gi|20746_52-174        GAGGAAGGTTTCTCTCATATTATGTTTGCCACTGGACGCTCACCTAATACTAAGGATTTG
1077
gi|451197_19-17        GTTGAGGGATTTTCGCATGTTATGTTTGCAACTGGTCGCAAGCCCAACACAAAGAACTTA
1019
                     *  ** ** ** ** *** ********** ** ** ***   ** ** **  ** * **

gi|169964_59-16        GGCCTGGAGTCTGTTGGTGTGAAACTAGCTAAAGATGGAGCTATAGAGGTTGATGAATAC
1083
gi|20746_52-174        GGCCTGGAGTCTGTTGGTGTGAAAGTGGCTAAAGATGGATCAATAGAGGTTGATGAATAC
1137
gi|451197_19-17        GGGTTGGAGAATGTTGGCGTTAAAATGGCGAAAAATGGAGCAATAGAGGTTGACGAATAT
1079
                     **  *****  ****** ** *** * ** *** ***** * *********** *****

gi|169964_59-16        TCTCAAACATCAGTTTATTCAATTTGGGCAGTTGGAGATGTTACAAACAGGATAAATCTC
1143
gi|20746_52-174        TCTCAAACATCGGTTCCTTCTATTTGGGCAATTGGAGATGCTACAAATAGAGTAAATCTC
1197
gi|451197_19-17        TCACAGACATCTGTTCCATCCATCTGGGCTGTTGGGGATGTTACTGACCGAATCAATTTG
1139
                     ** ** ***** ***   ** ** *****  **** **** ***  *  *  * *** *

gi|169964_59-16        ACCCCAGTTGCTTTGATGGAGGGAGGAGCATTAGTAAAAACGCTGTTTCAGGATAACCCA
1203
gi|20746_52-174        ACTCCAGTTGCTTTGATGGAGGGAGTGGCATTAGCAAAAACTTTGTTTCAGAATGAGCCG
1257
gi|451197_19-17        ACTCCAGTTGCTTTGATGGAGGGAGGTGCATTGGCTAAAACTTTGTTTCAAAATGAGCCA
1199
                     ** **********************  ***** *  *****  *******  ** * **
```

```
gi|169964_59-16        ACAAAACCTGATTATAGAGCTGTTCCTTCTGCTGTATTTTCTCAACCACCAATTGGACAA
1263
gi|20746_52-174        ACAAAACCTGATTATAGGGCTATACCTTCTGCTGTGTTTTCCCAACCACCAATTGGAGGA
1317
gi|451197_19-17        ACAAAGCCTGATTATAGAGCTGTTCCCTGCGCCGTTTTCTCCCAGCCACCTATTGGAACA
1259
                       ***** *********** *** * ** *  ** ** ** ** ** ***** ******  *

gi|169964_59-16        GTTGGTCTTACTGAGGAACAGGCTGTACAACAATATGGAGATATTGACATCTTCACTGCA
1323
gi|20746_52-174        GTTGGTCTTACAGAGGAACAGGCTGCTGAACAATATGGTGATATTGACGTTTTCACAGCA
1377
gi|451197_19-17        GTTGGTCTAACTGAAGAGCAGGCCATAGAACAATATGGTGATGTGGATGTTTACACATCG
1319
                       ******** ** ** ** *****     ********** *** * **  * * ***  *

gi|169964_59-16        AATTTTAGGCCGCTGAAAGCTACTCTCTCTGGGCTTCCAGACCGGGTTTTTATGAAATTA
1383
gi|20746_52-174        AATTTTAGGCCGATGAAGGCCACCCTCTCTGGGCTTCCAGACCGGGTTTTTATGAAACTA
1437
gi|451197_19-17        AACTTTAGGCCATTAAAGGCTACCCTTTCAGGACTTCCAGACCGAGTATTTATGAAACTC
1379
                       ** ********  * ** ** ** ** ** ** *********** ** ********* *

gi|169964_59-16        GTAGTCTGTGCAAAAACAAATGAAGTTCTAGGTTTGCATATGTGTGGAGAAGATGCTCCT
1443
gi|20746_52-174        ATAGTCTCTGCAGAAACAAATGTTGTTCTTGGATTGCACATGTGTGGAGAAGATGCTGCT
1497
gi|451197_19-17        ATTGTCTGTGCAAACACCAATAAAGTTCTCGGTGTTCACATGTGTGGAGAAGATTCACCA
1439
                        * **** **** * ** ***   ***** **  * ** *************** *  *

gi|169964_59-16        GAAATTGTGCAGGGGTTTGCAGTTGCTCTTAAAGCTCGCTTGACCAAGGCTGACTTTGAT
1503
gi|20746_52-174        GAAATTGCACAGGGGTTTGCAGTTGGTATTAAAGCTGGATTAACAAAGGCGGACTTTGAT
1557
gi|451197_19-17        GAAATCATCCAGGGATTTGGGGTTGCAGTTAAAGCTGGTTTAACTAAGGCCGACTTTGAT
1499
                       *****    ***** ****  ****   ******** * ** ** ***** *********

gi|169964_59-16        GCCACTGTAGGCATTCACCCAAGTGCAGCTGAGGAATTTGTTACCATGAGGACACCTACT
1563
gi|20746_52-174        GCCACAGTAGGCATTCATCCAACTGCAGCTGAGGAATTTGTTACCATGAGGACTCCCACT
1617
gi|451197_19-17        GCTACAGTGGGTGTTCACCCCACAGCAGCTGAGGAGTTTGTCACTATGAGGGCTCCAACC
1559
                       ** ** ** **  **** ** *  *********** ***** ** ****** * ** **

gi|169964_59-16        AGGAAGATACGAAAGAGTGAATCGTCTGAGGGTAAGTCGGGCTCTCAAGCAAAAGCTGCA
1623
gi|20746_52-174        AGGAAGGTTCGAAAGAACCAAGCTTCACAGGGGAAGTCAGATTCTAAAGCAAAAGCTGTG
1677
gi|451197_19-17        AGGAAATTCCGCAAAGAC---TCCTCTGAGGGAAAGGCAAGTCCTGAAGCTAAAACAGCT
1616
                       *****  * ** **        * **  **** *** *     ** **** *** * *

gi|169964_59-16        GCAGGGGTTTAA 1635
gi|20746_52-174        GCTGGATCTTAA 1689
gi|451197_19-17        GCTGGGGTGTAG 1628
                       ** **    **
```

CAT pBLAST Results (492 amino acids)

```
>gi|2661019|gb|AAB88171.1|[2661019] catalase (cat3) [Glycine max]
MDPYKNRPSSAFNSPFWTTNSGAPIWNNNSSLTVGSRGPILLEDYHLVEKLANFDRERIPERVVHARGASAKGFFEVTHDIS
HLTCADFLRAPGVQTPLIVRFSTVIHERGSPETLRDPRGFAVKFYTREGNFDLVGNNFPVFFVRDGLKFPDMVHALKPNPKS
HIQENWRILDFFSHHPESLHMFSFLFDDVGIPQDYRHMDGFGVNTYTLINKAGKAVYVKFHWKTTCGEKCLLDDEAIRVGGS
NHSHATQDLYDSIAAGNYPEWKLYIQTLDPENEDRLDFDPLDVTKTWPEDVLPLQPVGRMVLNKNIDNFFAENEQLAFCPAI
IVPGVYYSDDKLLQTRIFSYADTQRHRLGPNYLQLPANSPKCAHHNNHHDGFMNFMHRDEEVNYFPSRYDPVRHAERVPVPP
RTLGGKREKCMIEKENNFKQPGERYRSWPSDRQERFVRRWVDALSDPRVTHEIRSIWISYWSQADRSLGQKIASHLNLKPSI
>gi|218322|dbj|BAA02755.1|[218322] catalase [Vigna radiata]
MDPYKYRPSSAFNSPFWTTNSGAPVWNNNNSLTVGTRGPILLEDYHLVEKLANFDRERIPERVVHARGASAKGFFEVTHDVS
HLTCADFLRAPGVQTPVIVRFSTVIHERGSPETLRDPRGFAVKFYTREGNFDLVGNNLPVFFVRDGMKFPDMVHALKPNPKN
HIQENWRILDFFSHFPESLHMFSFLFDDLGVPQDYRHMDGFGVNTYTLINKAGKAVYVKFHWKTTSGVKCLLEEEAIKVGGA
NHSHATQDLHDSIAAGNYPEWKLFIQTIDPEHEDKFDFDPLDVTKTWPEDIIPLQPVGRLVLNKNIDNFFAENEQLAFCPAI
IVPGVYYSDDKMLQTRIFSYADSQRHRLGPNYLLLPANAPKSAHHNNHHEGFMNFIHRDEEVNYFPSRYDPVRHAEKFPIPP
AVFSGRREKIAIEKENNFKQAGERFRSWAPDRQDRFIRRWVDALSDPRVTHEIRSVWISYWSQADRSLGQKIASHLNMRPNI
>gi|20677|emb|CAA42736.1|[20677] catalase [Pisum sativum]
MDPYKHRPSSAFNSPFWTTNSGAPVWNNNSSLTVGSRGPILLEDYHLVEKLAQFDRERIPERVVHARGASAKGFFEVTHDIS
HLTCADFLRAPGVQTPVIVRFSTVIHERGSPETLRDPRGFAVKFYTREGNYDLVGNNFPVFFVHDGMNFPDMVHALKPNPQT
HIQENWRILDFFYNFPESLHMFSFLFDDVGVPQDYRHMDGFGVNTYTLINKAGKSVYVKFHWKPTCGVKCLLEEEAIQVGGS
NHSHATKDLYDSIAAGNYPEWKLYIQTIDPAHEDRFEFDPLDVTKTWPEDIIPLQPVGRMVLNKNIDNFFAENEQLAFCPAI
MLPGIYYSDDKMLQTRVFSYADSQRHRLGPNYLQLPVNAPKWSHHNNHHEGFMNAIHRDEEVNYFPSRHDTVRHAERVPIPT
THLSARREKCNIPKQNHFKQAGERYRTWAPDRQERFLRRWVEALSDTDPRITHEIRSIWVSYWSQADRSLGQKLASHLNMRP
SI
>gi|1246399|emb|CAA64220.1| catalase [Arabidopsis thaliana]

MDPYKYRPASSYNSPFFTTNSGAPVWNNNSSMTVGPRGPILLEDYHLVEKLANFDRERIPERVVHARGASAKGFFEVTHDIS
NLTCADFLRAPGVQTPVIVRFSTVIHERGSPETLRDPRGFAVKFYTREGNFDLVGNNFPVFFIRDGMKFPDMVHALKPNPKS
HIQENWRILDFFSHHPESLNMFTFLFDDIGIPQDYRHMDGSGVNTYMLINKAGKAHYVKFHWKPTCGVKSLLEEDAIRVGGT
NHSHATQDLYDSIAAGNYPEWKLFIQIIDPADEDKFDFDPLDVTKTWPEDILPLQPVGRMVLNKNIDNFFAENEQLAFCPAI
IVPGIHYSDDKLLQTRVFSYADTQRHRLGPNYLQLPVNAPKCAHHNNHHEGFMNFMHRDEEVNYFPSRYDQVRHAEKYPTPP
AVCSGKRERCVIEKENNFKEPGERYRTFTPERQERFIQRWIDALSDPRITHEIRSIWISYWSQADKSLGQKLASRLNVRPSI
```

CLUSTAL 2.0.1 multiple sequence alignment

```
gi|2661019|gb|A      MDPYKNRPSSAFNSPFWTTNSGAPIWNNNSSLTVGSRGPILLEDYHLVEK 50
gi|1246399|emb|      MDPYKYRPASSYNSPFFTTNSGAPVWNNNSSMTVGPRGPILLEDYHLVEK 50
gi|218322|dbj|B      MDPYKYRPSSAFNSPFWTTNSGAPVWNNNNSLTVGTRGPILLEDYHLVEK 50
gi|20677|emb|CA      MDPYKHRPSSAFNSPFWTTNSGAPVWNNNSSLTVGSRGPILLEDYHLVEK 50
                     ***** **:*::****:*******:****.*:***.**************

gi|2661019|gb|A      LANFDRERIPERVVHARGASAKGFFEVTHDISHLTCADFLRAPGVQTPLI 100
gi|1246399|emb|      LANFDRERIPERVVHARGASAKGFFEVTHDISNLTCADFLRAPGVQTPVI 100
gi|218322|dbj|B      LANFDRERIPERVVHARGASAKGFFEVTHDVSHLTCADFLRAPGVQTPVI 100
gi|20677|emb|CA      LAQFDRERIPERVVHARGASAKGFFEVTHDISHLTCADFLRAPGVQTPVI 100
                     **:****************************:*:***************:*

gi|2661019|gb|A      VRFSTVIHERGSPETLRDPRGFAVKFYTREGNFDLVGNNFPVFFVRDGLK 150
gi|1246399|emb|      VRFSTVIHERGSPETLRDPRGFAVKFYTREGNFDLVGNNFPVFFIRDGMK 150
gi|218322|dbj|B      VRFSTVIHERGSPETLRDPRGFAVKFYTREGNFDLVGNNLPVFFVRDGMK 150
gi|20677|emb|CA      VRFSTVIHERGSPETLRDPRGFAVKFYTREGNYDLVGNNFPVFFVHDGMN 150
                     ********************************:******:****::**::

gi|2661019|gb|A      FPDMVHALKPNPKSHIQENWRILDFFSHHPESLHMFSFLFDDVGIPQDYR 200
gi|1246399|emb|      FPDMVHALKPNPKSHIQENWRILDFFSHHPESLNMFTFLFDDIGIPQDYR 200
gi|218322|dbj|B      FPDMVHALKPNPKNHIQENWRILDFFSHFPESLHMFSFLFDDLGVPQDYR 200
gi|20677|emb|CA      FPDMVHALKPNPQTHIQENWRILDFFYNFPESLHMFSFLFDDVGVPQDYR 200
                     ************:.************ :.****:**:*****:*:*****

gi|2661019|gb|A      HMDGFGVNTYTLINKAGKAVYVKFHWKTTCGEKCLLDDEAIRVGGSNHSH 250
gi|1246399|emb|      HMDGSGVNTYMLINKAGKAHYVKFHWKPTCGVKSLLEEDAIRVGGTNHSH 250
gi|218322|dbj|B      HMDGFGVNTYTLINKAGKAVYVKFHWKTTSGVKCLLEEEAIKVGGANHSH 250
gi|20677|emb|CA      HMDGFGVNTYTLINKAGKSVYVKFHWKPTCGVKCLLEEEAIQVGGSNHSH 250
                     **** ***** *******: *******.*.* *.**:::**:***:****
```

```
gi|2661019|gb|A      ATQDLYDSIAAGNYPEWKLYIQTLDPENEDRLDFDPLDVTKTWPEDVLPL 300
gi|1246399|emb|      ATQDLYDSIAAGNYPEWKLFIQIIDPADEDKFDFDPLDVTKTWPEDILPL 300
gi|218322|dbj|B      ATQDLHDSIAAGNYPEWKLFIQTIDPEHEDKFDFDPLDVTKTWPEDIIPL 300
gi|20677|emb|CA      ATKDLYDSIAAGNYPEWKLYIQTIDPAHEDRFEFDPLDVTKTWPEDIIPL 300
                     **:**:**************:** :** .**::*************::**

gi|2661019|gb|A      QPVGRMVLNKNIDNFFAENEQLAFCPAIIVPGVYYSDDKLLQTRIFSYAD 350
gi|1246399|emb|      QPVGRMVLNKNIDNFFAENEQLAFCPAIIVPGIHYSDDKLLQTRVFSYAD 350
gi|218322|dbj|B      QPVGRLVLNKNIDNFFAENEQLAFCPAIIVPGVYYSDDKMLQTRIFSYAD 350
gi|20677|emb|CA      QPVGRMVLNKNIDNFFAENEQLAFCPAIMLPGIYYSDDKMLQTRVFSYAD 350
                     *****:**********************::**::*****:****:*****

gi|2661019|gb|A      TQRHRLGPNYLQLPANSPKCAHHNNHHDGFMNFMHRDEEVNYFPSRYDPV 400
gi|1246399|emb|      TQRHRLGPNYLQLPVNAPKCAHHNNHHEGFMNFMHRDEEVNYFPSRYDQV 400
gi|218322|dbj|B      SQRHRLGPNYLLLPANAPKSAHHNNHHEGFMNFIHRDEEVNYFPSRYDPV 400
gi|20677|emb|CA      SQRHRLGPNYLQLPVNAPKWSHHNNHHEGFMNAIHRDEEVNYFPSRHDTV 400
                     :********** **.*:** :******:**** :************:* *

gi|2661019|gb|A      RHAERVPVPPRTLGGKREKCMIEKENNFKQPGERYRSWPSDRQERFVRRW 450
gi|1246399|emb|      RHAEKYPTPPAVCSGKRERCVIEKENNFKEPGERYRTFTPERQERFIQRW 450
gi|218322|dbj|B      RHAEKFPIPPAVFSGRREKIAIEKENNFKQAGERFRSWAPDRQDRFIRRW 450
gi|20677|emb|CA      RHAERVPIPTTHLSARREKCNIPKQNHFKQAGERYRTWAPDRQERFLRRW 450
                     ****: * *.   ..:**:  * *:*:**:.***:*::..:**:**::**

gi|2661019|gb|A      VDALSD--PRVTHEIRSIWISYWSQADRSLGQKIASHLNLKPSI 492
gi|1246399|emb|      IDALSD--PRITHEIRSIWISYWSQADKSLGQKLASRLNVRPSI 492
gi|218322|dbj|B      VDALSD--PRVTHEIRSVWISYWSQADRSLGQKIASHLNMRPNI 492
gi|20677|emb|CA      VEALSDTDPRITHEIRSIWVSYWSQADRSLGQKLASHLNMRPSI 494
                     ::****  **:******:*:*******:*****:**:**::*.*
```

CAT mRNA Sequences:

```
>gi|2661018:6-1484 Glycine max CAT3
ATGGATCCCTACAAGAATCGGCCATCAAGCGCCTTCAATTCTCCCTTCTGGACTACTAATTCCGGTGCTC
CCATCTGGAACAACAACTCATCGCTGACTGTTGGATCTAGAGGTCCAATTCTGCTGGAAGATTACCATCT
GGTGGAGAAGCTTGCAAACTTTGACAGGGAACGGATCCCCGAACGTGTTGTCCATGCTCGTGGAGCTAGC
GCAAAGGGTTTCTTTGAAGTCACGCATGACATTTCTCACCTCACATGTGCCGATTTCCTCCGAGCCCCTG
GAGTTCAGACCCCTCTCATCGTCCGTTTCTCCACTGTTATCCACGAACGTGGCAGCCCTGAAACCCTCAG
GGATCCCAGAGGTTTCGCTGTGAAATTCTACACTAGAGAGGGTAACTTCGACCTTGTGGGAAACAACTTC
CCTGTCTTCTTCGTCCGCGATGGCCTCAAGTTTCCTGACATGGTCCATGCTCTCAAACCCAACCCCAAGT
CCCACATCCAGGAGAATTGGAGGATCCTCGATTTCTTCTCCCACCATCCCGAAAGCCTTCACATGTTCTC
CTTCTTATTCGATGACGTTGGTATCCCTCAAGATTACAGGCATATGGATGGTTTTGGTGTTAACACTTAC
ACCCTTATCAACAAGGCTGGCAAAGCCGTCTATGTCAAATTTCATTGGAAAACCACCTGTGGCGAAAAGT
GTCTCTTGGACGACGAGGCCATTAGGGTTGGAGGATCGAACCACAGTCATGCCACCCAAGACCTTTACGA
TTCAATTGCTGCTGGAAACTATCCTGAGTGGAAACTCTACATCCAAACATTGGATCCCGAAAACGAAGAC
AGGCTTGACTTTGACCCCCTTGATGTAACTAAGACCTGGCCTGAGGACGTTTTGCCCCTCCAGCCTGTTG
GCCGCATGGTCTTGAATAAGAACATTGATAACTTCTTTGCCGAGAACGAACAACTTGCATTTTGCCCTGC
AATTATTGTACCTGGTGTATACTACTCCGACGATAAATTGCTTCAGACTCGCATATTCTCCTATGCTGAT
ACCCAAAGACACAGACTTGGGCCAAATTATCTGCAGCTTCCTGCTAATTCCCCCAAGTGTGCCCATCACA
ACAATCACCATGATGGTTTCATGAATTTCATGCACAGGGATGAAGAGGTCAATTACTTCCCTTCAAGGTA
TGATCCTGTCCGTCACGCGGAAAGGGTCCCCGTTCCTCCACGTACCCTAGGTGGGAAGCGTGAAAAGTGC
ATGATTGAGAAGGAGAACAACTTCAAGCAACCTGGGGAGAGATACCGATCCTGGCCCTCTGACAGGCAAG
AAAGATTTGTCCGCCGATGGGTTGATGCTTTGTCTGACCCACGTGTCACCCATGAAATCCGCAGCATCTG
GATATCATACTGGTCTCAGGCTGATCGTTCTCTTGGACAAAAGATAGCATCTCACTTGAACTTGAAGCCA
AGTATCTAA

>gi|218321:54-1532 Vigna radiata CAT
ATGGATCCCTACAAGTACCGGCCATCCAGCGCTTTTAATTCTCCCTTCTGGACTACAAATTCTGGTGCTC
CTGTTTGGAACAACAACAACTCTTTAACTGTTGGAACTAGAGGTCCAATTCTCCTGGAGGATTATCATCT
TGTGGAAAAGCTTGCAAACTTTGACAGGGAACGGATCCCAGAACGAGTTGTCCATGCCAGGGGAGCTAGT
GCAAAGGGTTTCTTTGAGGTCACACATGACGTTTCTCACCTGACATGTGCAGATTTCCTTCGAGCACCTG
GAGTTCAGACACCAGTAATTGTCCGTTTTTCAACTGTCATCCATGAGCGTGGTAGCCCTGAAACTTTGCG
GGACCCTCGAGGTTTTGCTGTGAAATTTTACACCAGAGAGGGTAACTTTGATCTTGTTGGAAACAACCTT
CCCGTCTTCTTTGTACGTGACGGCATGAAGTTTCCAGATATGGTCCATGCTCTTAAACCCAATCCTAAGA
ACCACATCCAGGAGAATTGGAGGATCCTTGACTTCTTCTCTCACTTTCCGGAAAGCCTTCACATGTTCTC
CTTTTTATTTGATGATTTGGGTGTTCCACAAGATTACAGGCATATGGATGGTTTTGGAGTTAACACATAT
ACCTTGATCAACAAGGCTGGGAAAGCAGTGTATGTGAAATTTCACTGGAAGACCACGAGTGGTGTGAAGT
GTCTATTGGAGGAAGAAGCCATTAAGGTGGGAGGAGCCAACCATAGCCATGCTACACAAGACCTTCATGA
TTCGATTGCTGCTGGTAACTATCCTGAGTGGAAACTATTCATTCAGACAATAGATCCTGAGCACGAAGAC
AAATTTGACTTTGACCCTCTTGATGTAACCAAGACTTGGCCCGAGGACATTATACCCTTGCAGCCTGTTG
```

GTCGCTTGGTCCTGAATAAGAACATAGATAACTTCTTTGCTGAGAATGAACAACTTGCATTTTGCCCTGC
CATAATAGTTCCTGGTGTATATTACTCTGATGATAAGATGCTTCAAACCAGGATATTCTCTTATGCTGAT
TCACAGAGGCACAGGCTTGGACCAAACTATCTGCTACTTCCTGCCAATGCTCCCAAGTCTGCTCACCACA
ACAATCACCATGAAGGTTTCATGAATTTCATCCACAGGGATGAGGAGGTCAATTACTTCCCCTCAAGGTA
TGATCCTGTTCGTCATGCAGAAAAGTTCCCCATACCTCCTGCTGTCTTCTCTGGAAGGCGTGAAAAGATT
GCAATTGAGAAGGAGAACAACTTCAAGCAGGCTGGAGAGAGATTCCGATCTTGGGCACCTGACAGGCAAG
ATAGATTTATCCGCCGATGGGTTGATGCTTTATCTGACCCACGTGTCACCCATGAAATCCGCAGTGTCTG
GATATCATACTGGTCTCAGGCTGACCGTTCTCTTGGCCAAAAGATAGCATCTCATCTGAACATGAGGCCA
AATATTTAA

>gi|20676:57-1541 Pisum sativum CAT
ATGGATCCTTACAAGCATCGTCCTTCTAGCGCTTTCAATTCTCCTTTCTGGACTACGAACTCCGGTGCTC
CTGTTTGGAATAATAACTCTTCCCTAACCGTTGGATCTAGAGGTCCAATTCTATTGGAAGATTATCATCT
TGTGGAAAAGCTTGCCCAATTTGATAGGGAAAGGATCCCAGAACGTGTTGTCCATGCTAGGGGAGCAAGT
GCAAAGGGTTTCTTTGAAGTCACACATGATATTTCGCACCTGACATGTGCAGATTTCCTTCGAGCCCCTG
GTGTTCAGACACCTGTCATTGTGCGTTTCTCAACTGTCATTCATGAACGTGGCAGCCCTGAAACCTTGAG
GGATCCCCGAGGTTTTGCTGTGAAATTTTACACCAGAGAGGGTAACTATGACCTTGTTGGAAACAACTTT
CCCGTCTTCTTCGTTCATGACGGTATGAATTTTCCAGATATGGTCCATGCTCTTAAACCCAATCCCCAGA
CCCACATCCAGGAGAATTGGAGAATTCTTGATTTCTTCTACAACTTTCCAGAAAGCCTTCACATGTTCTC
CTTCCTATTTGATGATGTGGGTGTCCCACAAGATTATAGGCATATGGATGGTTTTGGAGTTAACACATAC
ACCCTGATCAACAAGGCTGGAAAATCGGTGTATGTCAAATTTCACTGGAAGCCCACCTGTGGTGTGAAGT
GTCTATTGGAGGAAGAGGCCATTCAGGTGGGAGGATCCAACCACAGCCATGCTACTAAAGACCTTTATGA
CTCAATTGCTGCTGGTAACTATCCTGAGTGGAAACTTTACATTCAAACAATAGATCCTGCTCATGAAGAC
AGATTTGAGTTTGACCCACTTGATGTAACTAAGACTTGGCCCGAGGACATCATACCCCTTCAGCCCGTAG
GTCGCATGGTCTTGAACAAGAACATAGATAATTTCTTTGCTGAGAATGAACAGCTTGCATTTTGTCCTGC
CATTATGCTGCCTGGTATATATTACTCAGATGACAAGATGCTTCAAACTAGGGTTTTCTCTTATGCTGAT
TCACAGAGGCACAGACTTGGACCGAACTACCTGCAACTTCCTGTTAATGCTCCCAAGTGGTCTCACCACA
ACAATCACCATGAGGGTTTCATGAATGCCATTCACAGGGATGAGGAGGTCAATTACTTCCCTTCAAGGCA
TGATACTGTTCGTCATGCAGAAAGGGTCCCCATTCCTACTACTCATTTATCTGCAAGGCGTGAAAAGTGC
AATATTCCGAAACAGAATCACTTCAAGCAGGCTGGAGAAAGATACCGAACTTGGGCACCTGACAGGCAGG
AAAGATTTCTCCGCAGGTGGGTAGAAGCTTTATCCGACACCGATCCACGCATCACCCATGAAATCCGCAG
CATTTGGGTATCATACTGGTCTCAGGCTGATCGTTCTCTTGGGCAGAAGTTAGCATCTCATCTGAACATG
AGGCCTAGCATTTAA

>(gi|1246398:1389-1403 A.thaliana CAT
ATGGATCCTTACAAGTATCGTCCAGCTAGTTCTTACAACTCTCCCTTCTTCACCACCAACTCTGGTGCTC
CTGTATGGAACAACAACTCCTCCATGACCGTTGGACCCAGAGGTCCTATCCTTCTTGAGGATTACCATCT
CGTTGAGAAGCTTGCCAATTTCGACAGGGAACGGATTCCAGAGCGTGTGGTTCATGCCAGAGGAGCCAGT
GCTAAAGGTTTCTTTGAGGTCACTCATGATATCTCTAACCTCACTTGTGCTGACTTTCTCCGAGCTCCCG
GTGTTCAGACTCCTGTCATTGTCCGGTTCTCCACCGTTATCCATGAGCGTGGAAGTCCCGAGACCTTGAG
AGACCCTCGTGGTTTTGCAGTCAAGTTCTACACCAGAGAGGGGAACTTTGATCTTGTTGGAAACAACTTT
CCTGTTTTCTTCATCCGCGATGGGATGAAGTTCCCTGACATGGTCCACGCTCTTAAGCCGAACCCAAAAT
CTCACATCCAAGAGAACTGGAGAATCCTTGACTTCTTCTCCCACCACCCTGAAAGTTTGAACATGTTCAC
TTTCCTCTTCGATGATATCGGTATCCCACAAGATTACAGGCACATGGATGGTTCAGGTGTCAATACATAC
ATGTTGATCAACAAAGCTGGCAAAGCTCACTACGTGAAGTTCCATTGGAAACCAACTTGTGGAGTCAAGT
CTCTTTTGGAAGAAGATGCAATTCGTGTTGGAGGAACCAACCACAGTCATGCGACTCAAGACTTGTATGA
CTCTATAGCTGCTGGAAACTACCCTGAATGGAAGCTCTTTATCCAAATCATTGATCCTGCTGATGAAGAC
AAGTTCGACTTTGACCCGCTCGATGTAACCAAGACCTGGCCTGAAGATATCTTGCCTCTCCAACCTGTTG
GACGTATGGTGTTGAACAAGAACATTGACAACTTCTTTGCAGAGAATGAGCAACTTGCCTTCTGTCCTGC
AATTATTGTTCCAGGGATACACTACTCAGACGACAAGCTGCTTCAAACCCGTGTCTTCTCCTATGCCGAT
ACTCAGAGACACCGTCTTGGACCAAACTACCTTCAGCTACCAGTCAATGCTCCAAAATGTGCTCACCACA
ACAACCACCATGAGGGATTCATGAATTTCATGCACAGGGACGAGGAGGTCAATTACTTCCCGTCGAGGTA
TGACCAGGTTCGTCATGCTGAGAAGTATCCAACTCCACCTGCTGTCTGCTCTGGAAAACGTGAGAGGTGT
GTTATTGAGAAGGAGAACAACTTCAAGGAGCCTGGAGAGAGATACCGTACCTTTACACCAGAGAGGCAAG
AACGATTCATCCAGAGATGGATTGATGCCCTATCCGACCCACGCATCACGCATGAAATCCGCAGTATCTG
GATCTCTTACTGGTCTCAGGCTGACAAGTCTTTGGGACAGAAGCTGGCAAGCCGTCTGAACGTGAGACCA
AGCATCTAA

CLUSTAL 2.0.1 multiple sequence alignment

```
gi|218321_54-15      --------------------------------------------------
gi|20676_57-154      ATGGATCCTTACAAGCATCGTCCTTCTAGCGCTTTCAATTCTCCTTTCTG 50
gi|2661018_6-14      ATGGATCCCTACAAGAATCGGCCATCAAGCGCCTTCAATTCTCCCTTCTG 50
_gi|1246398_138      ATGGATCCTTACAAGTATCGTCCAGCTAGTTCTTACAACTCTCCCTTCTT 50


gi|218321_54-15      --------------------CTGTTTGGAACAACAACAACTCTTTAACTG 30
gi|20676_57-154      GACTACGAACTCCGGTGCTCCTGTTTGGAATAATAACTCTTCCCTAACCG 100
gi|2661018_6-14      GACTACTAATTCCGGTGCTCCCATCTGGAACAACAACTCATCGCTGACTG 100
_gi|1246398_138      CACCACCAACTCTGGTGCTCCTGTATGGAACAACAACTCCTCCATGACCG 100
                                          *  * ***** ** ***   **  * ** *

gi|218321_54-15      TTGGAACTAGAGGTCCAATTCTCCTGGAGGATTATCATCTTGTGGAAAAG 80
gi|20676_57-154      TTGGATCTAGAGGTCCAATTCTATTGGAAGATTATCATCTTGTGGAAAAG 150
gi|2661018_6-14      TTGGATCTAGAGGTCCAATTCTGCTGGAAGATTACCATCTGGTGGAGAAG 150
_gi|1246398_138      TTGGACCCAGAGGTCCTATCCTTCTTGAGGATTACCATCTCGTTGAGAAG 150
                     ***** * ******** ** **  * ** ***** ***** ** ** ***

gi|218321_54-15      CTTGCAAACTTTGACAGGGAACGGATCCCAGAACGAGTTGTCCATGCCAG 130
gi|20676_57-154      CTTGCCCAATTTGATAGGGAAAGGATCCCAGAACGTGTTGTCCATGCTAG 200
gi|2661018_6-14      CTTGCAAACTTTGACAGGGAACGGATCCCCGAACGTGTTGTCCATGCTCG 200
_gi|1246398_138      CTTGCCAATTTCGACAGGGAACGGATTCCAGAGCGTGTGGTTCATGCCAG 200
                     *****  * ** ** ****** **** ** ** ** ** ** *****  *

gi|218321_54-15      GGGAGCTAGTGCAAAGGGTTTCTTTGAGGTCACACATGACGTTTCTCACC 180
gi|20676_57-154      GGGAGCAAGTGCAAAGGGTTTCTTTGAAGTCACACATGATATTTCGCACC 250
gi|2661018_6-14      TGGAGCTAGCGCAAAGGGTTTCTTTGAAGTCACGCATGACATTTCTCACC 250
_gi|1246398_138      AGGAGCCAGTGCTAAAGGTTTCTTTGAGGTCACTCATGATATCTCTAACC 250
                      ***** ** ** ** *********** ***** *****  * **  ***

gi|218321_54-15      TGACATGTGCAGATTTCCTTCGAGCACCTGGAGTTCAGACACCAGTAATT 230
gi|20676_57-154      TGACATGTGCAGATTTCCTTCGAGCCCCTGGTGTTCAGACACCTGTCATT 300
gi|2661018_6-14      TCACATGTGCCGATTTCCTCCGAGCCCCTGGAGTTCAGACCCCTCTCATC 300
_gi|1246398_138      TCACTTGTGCTGACTTTCTCCGAGCTCCCGGTGTTCAGACTCCTGTCATT 300
                     * ** ***** ** ** ** ***** ** ** ******** **  * **

gi|218321_54-15      GTCCGTTTTTCAACTGTCATCCATGAGCGTGGTAGCCCTGAAACTTTGCG 280
gi|20676_57-154      GTGCGTTTCTCAACTGTCATTCATGAACGTGGCAGCCCTGAAACCTTGAG 350
gi|2661018_6-14      GTCCGTTTCTCCACTGTTATCCACGAACGTGGCAGCCCTGAAACCCTCAG 350
_gi|1246398_138      GTCCGGTTCTCCACCGTTATCCATGAGCGTGGAAGTCCCGAGACCTTGAG 350
                     ** ** ** ** ** ** ** ** ** ***** ** ** ** **  *  *

gi|218321_54-15      GGACCCTCGAGGTTTTGCTGTGAAATTTTACACCAGAGAGGGTAACTTTG 330
gi|20676_57-154      GGATCCCCGAGGTTTTGCTGTGAAATTTTACACCAGAGAGGGTAACTATG 400
gi|2661018_6-14      GGATCCCAGAGGTTTCGCTGTGAAATTCTACACTAGAGAGGGTAACTTCG 400
_gi|1246398_138      AGACCCTCGTGGTTTTGCAGTCAAGTTCTACACCAGAGAGGGGAACTTTG 400
                      ** **  * ***** ** ** ** ** ***** ******** ****  *

gi|218321_54-15      ATCTTGTTGGAAACAACCTTCCCGTCTTCTTTGTACGTGACGGCATGAAG 380
gi|20676_57-154      ACCTTGTTGGAAACAACTTTCCCGTCTTCTTCGTTCATGACGGTATGAAT 450
gi|2661018_6-14      ACCTTGTGGGAAACAACTTCCCTGTCTTCTTCGTCCGCGATGGCCTCAAG 450
_gi|1246398_138      ATCTTGTTGGAAACAACTTTCCTGTTTTCTTCATCCGCGATGGGATGAAG 450
                     * ***** ********* * ** ** *****  * *  ** **  * **

gi|218321_54-15      TTTCCAGATATGGTCCATGCTCTTAAACCCAATCCTAAGAACCACATCCA 430
gi|20676_57-154      TTTCCAGATATGGTCCATGCTCTTAAACCCAATCCCCAGACCCACATCCA 500
gi|2661018_6-14      TTTCCTGACATGGTCCATGCTCTCAAACCCAACCCCAAGTCCCACATCCA 500
_gi|1246398_138      TTCCCTGACATGGTCCACGCTCTTAAGCCGAACCCAAAATCTCACATCCA 500
                     ** ** ** ******** ***** ** ** ** **  *    ********

gi|218321_54-15      GGAGAATTGGAGGATCCTTGACTTCTTCTCTCACTTTCCGGAAAGCCTTC 480
gi|20676_57-154      GGAGAATTGGAGAATTCTTGATTTCTTCTACAACTTTCCAGAAAGCCTTC 550
gi|2661018_6-14      GGAGAATTGGAGGATCCTCGATTTCTTCTCCCACCATCCCGAAAGCCTTC 550
_gi|1246398_138      AGAGAACTGGAGAATCCTTGACTTCTTCTCCCACCACCCTGAAAGTTTGA 550
                      ***** ***** ** ** ** *******   **   ** *****  *

gi|218321_54-15      ACATGTTCTCCTTTTTATTTGATGATTTGGGTGTTCCACAAGATTACAGG 530
gi|20676_57-154      ACATGTTCTCCTTCCTATTTGATGATGTGGGTGTCCCACAAGATTATAGG 600
```

```
gi|2661018_6-14      ACATGTTCTCCTTCTTATTCGATGACGTTGGTATCCCTCAAGATTACAGG 600
_gi|1246398_138      ACATGTTCACTTTCCTCTTCGATGATATCGGTATCCCACAAGATTACAGG 600
                     ******** * **  * ** *****  * *** * ** ******** ***

gi|218321_54-15      CATATGGATGGTTTTGGAGTTAACACATATACCTTGATCAACAAGGCTGG 580
gi|20676_57-154      CATATGGATGGTTTTGGAGTTAACACATACACCCTGATCAACAAGGCTGG 650
gi|2661018_6-14      CATATGGATGGTTTTGGTGTTAACACTTACACCCTTATCAACAAGGCTGG 650
_gi|1246398_138      CACATGGATGGTTCAGGTGTCAATACATACATGTTGATCAACAAAGCTGG 650
                     ** **********  ** ** ** ** ** *   * ******** *****

gi|218321_54-15      GAAAGCAGTGTATGTGAAATTTCACTGGAAGACCACGAGTGGTGTGAAGT 630
gi|20676_57-154      AAAATCGGTGTATGTCAAATTTCACTGGAAGCCCACCTGTGGTGTGAAGT 700
gi|2661018_6-14      CAAAGCCGTCTATGTCAAATTTCATTGGAAAACCACCTGTGGCGAAAAGT 700
_gi|1246398_138      CAAAGCTCACTACGTGAAGTTCCATTGGAAACCAACTTGTGGAGTCAAGT 700
                      *** *    ** ** ** ** ** *****  * **  **** *  ****

gi|218321_54-15      GTCTATTGGAGGAAGAAGCCATTAAGGTGGGAGGAGCCAACCATAGCCAT 680
gi|20676_57-154      GTCTATTGGAGGAAGAGGCCATTCAGGTGGGAGGATCCAACCACAGCCAT 750
gi|2661018_6-14      GTCTCTTGGACGACGAGGCCATTAGGGTTGGAGGATCGAACCACAGTCAT 750
_gi|1246398_138      CTCTTTTGGAAGAAGATGCAATTCGTGTTGGAGGAACCAACCACAGTCAT 750
                      *** ***** ** ** ** ***   ** ****** * ***** ** ***

gi|218321_54-15      GCTACACAAGACCTTCATGATTCGATTGCTGCTGGTAACTATCCTGAGTG 730
gi|20676_57-154      GCTACTAAAGACCTTTATGACTCAATTGCTGCTGGTAACTATCCTGAGTG 800
gi|2661018_6-14      GCCACCCAAGACCTTTACGATTCAATTGCTGCTGGAAACTATCCTGAGTG 800
_gi|1246398_138      GCGACTCAAGACTTGTATGACTCTATAGCTGCTGGAAACTACCCTGAATG 800
                     ** **  ***** *  * ** ** ** ******** ***** ***** **

gi|218321_54-15      GAAACTATTCATTCAGACAATAGATCCTGAGCACGAAGACAAATTTGACT 780
gi|20676_57-154      GAAACTTTACATTCAAACAATAGATCCTGCTCATGAAGACAGATTTGAGT 850
gi|2661018_6-14      GAAACTCTACATCCAAACATTGGATCCCGAAAACGAAGACAGGCTTGACT 850
_gi|1246398_138      GAAGCTCTTTATCCAAATCATTGATCCTGCTGATGAAGACAAGTTCGACT 850
                     *** ** *  ** ** *   * ***** *   * *******   * ** *
                                P2
gi|218321_54-15      TTGACCCTCTTGATGTAACCAAGACTTGGCCCGAGGACATTATACCCTTG 830
gi|20676_57-154      TTGACCCACTTGATGTAACTAAGACTTGGCCCGAGGACATCATACCCCTT 900
gi|2661018_6-14      TTGACCCCCTTGATGTAACTAAGACCTGGCCTGAGGACGTTTTGCCCCTC 900
_gi|1246398_138      TTGACCCGCTCGATGTAACCAAGACCTGGCCTGAAGATATCTTGCCTCTC 900
                     ******* ** ******** ***** ***** ** **  *  * **  *

gi|218321_54-15      CAGCCTGTTGGTCGCTTGGTCCTGAATAAGAACATAGATAACTTCTTTGC 880
gi|20676_57-154      CAGCCCGTAGGTCGCATGGTCTTGAACAAGAACATAGATAATTTCTTTGC 950
gi|2661018_6-14      CAGCCTGTTGGCCGCATGGTCTTGAATAAGAACATTGATAACTTCTTTGC 950
_gi|1246398_138      CAACCTGTTGGACGTATGGTGTTGAACAAGAACATTGACAACTTCTTTGC 950
                     ** ** ** ** **  ****  **** ******** ** ** ********

gi|218321_54-15      TGAGAATGAACAACTTGCATTTTGCCCTGCCATAATAGTTCCTGGTGTAT 930
gi|20676_57-154      TGAGAATGAACAGCTTGCATTTTGTCCTGCCATTATGCTGCCTGGTATAT 1000
gi|2661018_6-14      CGAGAACGAACAACTTGCATTTTGCCCTGCAATTATTGTACCTGGTGTAT 1000
_gi|1246398_138      AGAGAATGAGCAACTTGCCTTCTGTCCTGCAATTATTGTTCCAGGGATAC 1000
                      ***** ** ** ***** ** ** ***** ** **  * ** **  **

gi|218321_54-15      ATTACTCTGATGATAAGATGCTTCAAACCAGGATATTCTCTTATGCTGAT 980
gi|20676_57-154      ATTACTCAGATGACAAGATGCTTCAAACTAGGGTTTTCTCTTATGCTGAT 1050
gi|2661018_6-14      ACTACTCCGACGATAAATTGCTTCAGACTCGCATATTCTCCTATGCTGAT 1050
_gi|1246398_138      ACTACTCAGACGACAAGCTGCTTCAAACCCGTGTCTTCTCCTATGCCGAT 1050
                     * ***** ** ** **  ******* **  *  * ***** ***** ***

gi|218321_54-15      TCACAGAGGCACAGGCTTGGACCAAACTATCTGCTACTTCCTGCCAATGC 1030
gi|20676_57-154      TCACAGAGGCACAGACTTGGACCGAACTACCTGCAACTTCCTGTTAATGC 1100
gi|2661018_6-14      ACCCAAAGACACAGACTTGGGCCAAATTATCTGCAGCTTCCTGCTAATTC 1100
_gi|1246398_138      ACTCAGAGACACCGTCTTGGACCAAACTACCTTCAGCTACCAGTCAATGC 1100
                      * ** ** *** * ***** ** ** ** ** *  ** ** *  *** *


gi|218321_54-15      TCCCAAGTCTGCTCACCACAACAATCACCATGAAGGTTTCATGAATTTCA 1080
gi|20676_57-154      TCCCAAGTGGTCTCACCACAACAATCACCATGAGGGTTTCATGAATGCCA 1150
gi|2661018_6-14      CCCCAAGTGTGCCCATCACAACAATCACCATGATGGTTTCATGAATTTCA 1150
_gi|1246398_138      TCCAAAATGTGCTCACCACAACAACCACCATGAGGGATTCATGAATTTCA 1150
                      ** ** *   * ** ******** ******** ** *********  **
```

```
gi|218321_54-15      TCCACAGGGATGAGGAGGTCAATTACTTCCCCTCAAGGTATGATCCTGTT 1130
gi|20676_57-154      TTCACAGGGATGAGGAGGTCAATTACTTCCCTTCAAGGCATGATACTGTT 1200
gi|2661018_6-14      TGCACAGGGATGAAGAGGTCAATTACTTCCCTTCAAGGTATGATCCTGTC 1200
_gi|1246398_138      TGCACAGGGACGAGGAGGTCAATTACTTCCCGTCGAGGTATGACCAGGTT 1200
                     * ******** ** ***************** ** *** ****    **

gi|218321_54-15      CGTCATGCAGAAAAGTTCCCCATACCTCCTGCTGTCTTCTCTGGAAGGCG 1180
gi|20676_57-154      CGTCATGCAGAAAGGGTCCCCATTCCTACTACTCATTTATCTGCAAGGCG 1250
gi|2661018_6-14      CGTCACGCGGAAAGGGTCCCCGTTCCTCCACGTACCCTAGGTGGGAAGCG 1250
_gi|1246398_138      CGTCATGCTGAGAAGTATCCAACTCCACCTGCTGTCTGCTCTGGAAAACG 1250
                     ***** ** ** * *   **    **  *   *        **  *  **

gi|218321_54-15      TGAAAAGATTGCAATTGAGAAGGAGAACAACTTCAAGCAGGCTGGAGAGA 1230
gi|20676_57-154      TGAAAAGTGCAATATTCCGAAACAGAATCACTTCAAGCAGGCTGGAGAAA 1300
gi|2661018_6-14      TGAAAAGTGCATGATTGAGAAGGAGAACAACTTCAAGCAACCTGGGGAGA 1300
_gi|1246398_138      TGAGAGGTGTGTTATTGAGAAGGAGAACAACTTCAAGGAGCCTGGAGAGA 1300
                     *** * *      ***  ***  ****  ******** *  **** ** *

gi|218321_54-15      GATTCCGATCTTGGGCACCTGACAGGCAAGATAGATTTATCCGCCGATGG 1280
gi|20676_57-154      GATACCGAACTTGGGCACCTGACAGGCAGGAAAGATTTCTCCGCAGGTGG 1350
gi|2661018_6-14      GATACCGATCCTGGCCCTCTGACAGGCAAGAAAGATTTGTCCGCCGATGG 1350
_gi|1246398_138      GATACCGTACCTTTACACCAGAGAGGCAAGAACGATTCATCCAGAGATGG 1350
                     *** ***  * *   *  * ** ***** **  ****  ***   * ***

gi|218321_54-15      GTTGATGCTTTATCTGAC------CCACGTGTCACCCATGAAATCCGCAG 1324
gi|20676_57-154      GTAGAAGCTTTATCCGACACCGATCCACGCATCACCCATGAAATCCGCAG 1400
gi|2661018_6-14      GTTGATGCTTTGTCTGAC------CCACGTGTCACCCATGAAATCCGCAG 1394
_gi|1246398_138      ATTGATGCCCTATCCGAC------CCACGCATCACGCATGAAATCCGCAG 1394
                      * ** **  * ** ***      *****  **** **************

gi|218321_54-15      TGTCTGGATATCATACTGGTCTCAGGCTGACCGTTCTCTTGGCCAAAAGA 1374
gi|20676_57-154      CATTTGGGTATCATACTGGTCTCAGGCTGATCGTTCTCTTGGGCAGAAGT 1450
gi|2661018_6-14      CATCTGGATATCATACTGGTCTCAGGCTGATCGTTCTCTTGGACAAAAGA 1444
_gi|1246398_138      TATCTGGATCTCTTACTGGTCTCAGGCTGACAAGTCTTTGGGACAGAAGC 1444
                       * *** * ** *****************    *** * ** ** ***

gi|218321_54-15      TAGCATCTCATCTGAACATGAGGCCAAATATTTAA 1409
gi|20676_57-154      TAGCATCTCATCTGAACATGAGGCCTAGCATTTAA 1485
gi|2661018_6-14      TAGCATCTCACTTGAACTTGAAGCCAAGTATCTAA 1479
_gi|1246398_138      TGGCAAGCCGTCTGAACGTGAGACCAAGCATCTAA 1479
                     * ***   *   ***** ***  ** *  ** ***
```

Chl/Str. APX pBLAST Results (364 amino acids)

```
>gi|45268439|gb|AAS55853.1|[45268439] chloroplast stromal APX[Vigna unguiculata]
MALFSGAASARIIPSVSLSSSRSFFSLSSSSSSLQCLRSSPRISHLFLNQRRAEVRVSSGGYGTVSAPKSFASDPDQLKSAR
EDIKELLRSKFCHPILIRLGWHDAGTYNKNIEEWPQRGGANGSLRFEIELKHAANAGLVNALKLLQPIKDKYSGVTYADLFQ
LAGATAVEEAGGPKLPMKYGRVDVSGPEQCPEEGRLPDAGPPSPADHLRQVFYRMGLNDKEIVALSGAHTLGRARPDRSGWG
KPETKYTKDGPGAPGGQSWTVQWLKFDNSYFKDIKEKKDEDLLILPTDAALFEDPSFKVYAEKYAEDQETFFKDYAEAHAKL
SNLGAKFDPPEGIVIDESPNAGAEKFVAAKYSTGKE

>gi|4996604|dbj|BAA78553.1| stromal APX [Nicotiana tabacum]
MATNTLISASSPSPTMASSLTGAASRFLPSATIAATSSSSATTRLSLSSSSPSLKCLQSSPLLSHIFRYQ
KRSLVGTSSSGRFSTLASPKCAASDSDQLKSAREDIKELLKNTFCHPILVRLGWHDAGTYNKNIEEWPQR
GGANGSLRFEVELKHGANAGLVNALKLLQPIKDKYANVTYADLFQLASATAIEEAGGPKLPMKYGRVDVS
APEECPEEGRLPDAGPPSPASHLRDVFYRMGLNDKEIVALSGAHTLGRSRPERSGWGKPETKYTKDGPGN
PGGQSWTVQWLKFDNSYFKDIKERRDEDLLVLPTDAALFEDSSFKEYAEKYAVNQDVFFKDYAEAHAKLS
NLGAKFDPPEGFSIDNTPTQGQPEKFVAAKYSTGKD

>gi|1369920|dbj|BAA12039.1| stromal APX [Spinacia oleracea]
MASFTTTTAAAASRLLPSSSSSISRLSLSSSSSSSSSLKCLRSSPLVSHLFLRQRGGSAYVTKTRFSTKC
YASDPAQLKNAREDIKELLQSKFCHPIMVRLGWHDAGTYNKDIKEWPQRGGANGSLSFDVELKHGANAGL
VNALKLLQPIKDKYSGVTYADLFQLASATAIEEAGGPTIPMKYGRVDATGPEQCPEEGRLPDAGPPSPAQ
HLRDVFYRMGLDDKDIVALSGAHTLGRSRPERSGWGKPETKYTKDGPGAPGGQSWTAEWLKFDNSYFKDI
KEKRDADLLVLPTDAALFEDPSFKVYAEKYAADQEAFFKDYAEAHAKLSNQGAKFDPAEGITLNGTPAGA
APEKFVAAKYSSNKD
```

```
>gi|2392025|dbj|BAA22196.1| stromal APX [Cucurbita cv. Kurokawa Amakuri]
MAATALGSVAASSASSTTRFLSTATRATLPFSSRSSSLSSFKFLRSAPLISHLFLNQGRPSSCVSIRRFN
AASHPKCLASDPEQLKSAREDIKELLKTTFCHPILVRLGWHDAGTYNKNIEEWPQRGGANGSLRFDVELG
HGANAGLVNALKLIEPIKKKYSNVTYADLFQLASATAIEEAGGPKIPMKYGRVDVVGPEQCPEEGRLPDA
GPPSPAAHLREVFYRMGLNDREIVALSGAHTLGRSRPERSGWGKPETKYTKDGPGAPGGQSWTVQWLKFN
NSYFKDIKERRDEELLVLPTDAALFEDPSFKVYAEKYVEDQEAFFKDYAEAHAKLSNLGAKFDPPEGIVI
DDASSKPAGEKFDAAKYSYGKD

>gi|1419388|emb|CAA67425.1| stromal APX [Arabidopsis thaliana]
MAERVSLTLNVTLLSPPPTTTTTTMSSSLRSTTAASLLLRSSSSSSRSTLTLSASSSLSFVRSLVSSPRL
SSSSPLSQKKCRIASVNRSFNSTTAATKSSSSDPDQLKNAREDIKELLSTKFCHPILVRLGWHDAGTYNK
NIKEWPQRGGANGSLRFDIELKHAANAGLVNALNLIKDIKEKYSGISYADLFQLASATAIEEAGGPKIPM
KYGRVDASGPEDCPEEGRLPDAGPPSPATHLREVFYRMGLDDKDIVALSGAHTLGRSRPERSGWGKPETK
YTKEGPGAPGGQSWTPEWLKFDNSYFKEIKEKRDEDLLVLPTDAAIFEDSSFKVYAEKYAADQDAFFKDY
AVAHAKLSNLGAEFNPPEGIII
```

CLUSTAL W (1.83) multiple sequence alignment

```
gi|45268439|gb|AAS55853.1|----------------------MALFSGAAS----ARIIPSVSLSS-SRS 23
gi|4996604|dbj|BAA78553.1|------MATNTLISASSPSPTMASSLTGAASRFLPSATIAATSSSS-ATT 43
gi|2392025|dbj|BAA22196.1|-------------------MAATALGSVAASSASSTTRFLSTATRA-TLP 30
gi|1369920|dbj|BAA12039.1|----------------------MASFTTTTAAAASRLLPSSSSSIS-RLS 27
gi|1419388|emb|CAA67425.1|MAERVSLTLNVTLLSPPPTTTTTTMSSSLRSTTAASLLLRSSSSSSRSTL 50
                                                  :  :   :         : :  :

gi|45268439|gb|AAS55853.1|FFSLSSSSSSLQCLRSSPRIS-HLFLNQRRAEVRVSSGG-YGTVSAPKSF 71
gi|4996604|dbj|BAA78553.1|RLSLSSSSPSLKCLQSSPLLS-HIFRYQKRSLVGTSSSGRFSTLASPKCA 92
gi|2392025|dbj|BAA22196.1|FSSRSSSLSSFKFLRSAPLIS-HLFLNQGRPSSCVSIRR-FNAASHPKCL 78
gi|1369920|dbj|BAA12039.1|LSSSSSSSSSLKCLRSSPLVS-HLFLRQRGGSAYVTKTR-----FSTKCY 71
gi|1419388|emb|CAA67425.1|TLSASSSLSFVRSLVSSPRLSSSSPLSQKKCRIASVNRSFNSTTAATKSS 100
                            * *** . .: * *:* :*      *                  .*.

gi|45268439|gb|AAS55853.1|ASDPDQLKSAREDIKELLRSKFCHPILIRLGWHDAGTYNKNIEEWPQRGG 121
gi|4996604|dbj|BAA78553.1|ASDSDQLKSAREDIKELLKNTFCHPILVRLGWHDAGTYNKNIEEWPQRGG 142
gi|2392025|dbj|BAA22196.1|ASDPEQLKSAREDIKELLKTTFCHPILVRLGWHDAGTYNKNIEEWPQRGG 128
gi|1369920|dbj|BAA12039.1|ASDPAQLKNAREDIKELLQSKFCHPIMVRLGWHDAGTYNKDIKEWPQRGG 121
gi|1419388|emb|CAA67425.1|SSDPDQLKNAREDIKELLSTKFCHPILVRLGWHDAGTYNKNIKEWPQRGG 150
                           :**. ***.********* ..*****::************:*:*******

gi|45268439|gb|AAS55853.1|ANGSLRFEIELKHAANAGLVNALKLLQPIKDKYSGVTYADLFQLAGATAV 171
gi|4996604|dbj|BAA78553.1|ANGSLRFEVELKHGANAGLVNALKLLQPIKDKYANVTYADLFQLASATAI 192
gi|2392025|dbj|BAA22196.1|ANGSLRFDVELGHGANAGLVNALKLIEPIKKKYSNVTYADLFQLASATAI 178
gi|1369920|dbj|BAA12039.1|ANGSLSFDVELKHGANAGLVNALKLLQPIKDKYSGVTYADLFQLASATAI 171
gi|1419388|emb|CAA67425.1|ANGSLRFDIELKHAANAGLVNALNLIKDIKEKYSGISYADLFQLASATAI 200
                           ***** *::** *.*********:*:: **.**:.::********.***:

gi|45268439|gb|AAS55853.1|EEAGGPKLPMKYGRVDVSGPEQCPEEGRLPDAGPPSPADHLRQVFYRMGL 221
gi|4996604|dbj|BAA78553.1|EEAGGPKLPMKYGRVDVSAPEECPEEGRLPDAGPPSPASHLRDVFYRMGL 242
gi|2392025|dbj|BAA22196.1|EEAGGPKIPMKYGRVDVVGPEQCPEEGRLPDAGPPSPAAHLREVFYRMGL 228
gi|1369920|dbj|BAA12039.1|EEAGGPTIPMKYGRVDATGPEQCPEEGRLPDAGPPSPAQHLRDVFYRMGL 221
gi|1419388|emb|CAA67425.1|EEAGGPKIPMKYGRVDASGPEDCPEEGRLPDAGPPSPATHLREVFYRMGL 250
                           ******.:********. .**:*************** ***:*******

gi|45268439|gb|AAS55853.1|NDKEIVALSGAHTLGRARPDRSGWGKPETKYTKDGPGAPGGQSWTVQWLK 271
gi|4996604|dbj|BAA78553.1|NDKEIVALSGAHTLGRSRPERSGWGKPETKYTKDGPGNPGGQSWTVQWLK 292
gi|2392025|dbj|BAA22196.1|NDREIVALSGAHTLGRSRPERSGWGKPETKYTKDGPGAPGGQSWTVQWLK 278
gi|1369920|dbj|BAA12039.1|DDKDIVALSGAHTLGRSRPERSGWGKPETKYTKDGPGAPGGQSWTAEWLK 271
gi|1419388|emb|CAA67425.1|DDKDIVALSGAHTLGRSRPERSGWGKPETKYTKEGPGAPGGQSWTPEWLK 300
                           :*::************:**:*************:*** ******* :***

gi|45268439|gb|AAS55853.1|FDNSYFKDIKEKKDEDLLILPTDAALFEDPSFKVYAEKYAEDQETFFKDY 321
gi|4996604|dbj|BAA78553.1|FDNSYFKDIKERRDEDLLVLPTDAALFEDSSFKEYAEKYAVNQDVFFKDY 342
gi|2392025|dbj|BAA22196.1|FNNSYFKDIKERRDEELLVLPTDAALFEDPSFKVYAEKYVEDQEAFFKDY 328
gi|1369920|dbj|BAA12039.1|FDNSYFKDIKEKRDADLLVLPTDAALFEDPSFKVYAEKYAADQEAFFKDY 321
gi|1419388|emb|CAA67425.1|FDNSYFKEIKEKRDEDLLVLPTDAAIFEDSSFKVYAEKYAADQDAFFKDY 350
                           *:*****:***::* :**:******:***.*** *****. :*:.*****
```

```
gi|45268439|gb|AAS55853.1|AEAHAKLSNLGAKFDPPEGIVIDESPNAG-AEKFVAAKYSTGKE 364
gi|4996604|dbj|BAA78553.1|AEAHAKLSNLGAKFDPPEGFSIDNTPTQGQPEKFVAAKYSTGKD 386
gi|2392025|dbj|BAA22196.1|AEAHAKLSNLGAKFDPPEGIVIDDASSKPAGEKFDAAKYSYGKD 372
gi|1369920|dbj|BAA12039.1|AEAHAKLSNQGAKFDPAEGITLNGTPAGAAPEKFVAAKYSSNKD 365
gi|1419388|emb|CAA67425.1|AVAHAKLSNLGAEFNPPEGIII---------------------- 372
                            * ******* **:*:*.**: :
PLEASE NOTE: Showing colors on large alignments is slow.
```

Arabidopsis 18S rRNA Sequence:

```
>gi|16506|emb|X16077.1| Arabidopsis thaliana 18S rRNA gene
AAAAGATGACGGTCAAGACCTCGTCCTTTCTCTCTTTCCATTGCGTTTGAGAGGATGTGGCGGGGAATTG
CCGTGATCGATGAATGCTACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATG
CATGTGTAAGTATGAACGAATTCAGACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTGTTT
GATGGTAACTACTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCAACAAACCCCGACTTATGG
AAGGGACGCATTTATTAGATAAAAGGTCGACGCGGGCTCTGGCTTGCTCTGATGATTCATGATAACTCGA
CGGATCGCATGGCCTCTGTGCTGGCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGA
TAGTGGCCTACCATGGTGGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA
CGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAA
TAAATAACAATACCGGGCTCTTTCGAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGA
TCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGT
TGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGATGGGTCGGCCGGTCCGCCTTTGGTGTGCATTGGTCGG
CTTGTCCCTTCGGTCGGCGATACGCTCCTGGTCTTAATTGGCCGGGTCGTGCCTCCGGCGCTGTTACTTT
GAAGAAATTAGAGTGCTCAAAGCAAGCCTACGCTCTGGATACATTAGCATGGGATAACATCATAGGATTT
CGATCCTATTGTGTTGGCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTCGTATTTCA
TAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCATTTGCCAAGGATGTTTTC
ATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATACCGTCCTAGTCTCAACCATAAACGATG
CCGACCAGGGATCAGCGGATGTTGCTTATAGGACTCCGCTGGCACCTTATGAGAAATCAAAGTTTTTGGG
TTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAG
CCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGACTGA
GAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTA
ATTCCGTTAACGAACGAGACCTCAGCCTGCTAACTAGCTACGTGGAGGCATCCCTTCACGGCCGGCTTCT
TAGAGGGACTATGGCCGTTTAGGCCAAGGAAGTTTGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTT
CTGGGCCGCACGCGCGCTACACTGATGTATTCAACGAGTTCACACCTTGCCGACAGGCCCGGGTAATCTT
TGAAATTTCATCGTGATGGGGATAGATCATTGCAATTGTTGGTCTTCAACGAGGAATTCCTAGTAAGCGC
GAGTCATCAGCTCGCGTTGACTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAAT
GATCCGGTGAAGTGTTCGGATCGCGGCGACGTGGGTGGTTCGCCGCCCGCGACGTCGCGAGAAGTCCACT
AAACCTTATCATTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT
GTCGATACCTGT
```