Allele mining for drought related antioxidant genes in *Cucumis spp*.

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Lalita Mundalia

THESIS

Master of Science in Biotechnology

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Department of Biotechnology

COLLEGE OF AGRICULTURE, BIKANER

SWAMI KESHWANAND RAJASTHAN AGRICULTURAL UNIVERSITY

BIKANER-334006 (RAJ.)

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THESIS

Submitted to the
SWAMI KESHWANAND
Rajasthan Agricultural University, Bikaner
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE
In
Biotechnology

By

Lalita Mundalia
2012

Swami Keshwanand Rajasthan Agricultural University, Bikaner

College of Agriculture, Bikaner

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This is to certify that this thesis entitled **“Allele mining for drought related antioxidant genes in *Cucumis spp.”*** submitted for the degree of **Master of Science** in the subject of **Biotechnology** embodies bonafide research work carried out by **Ms. Lalita Mundalia** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation has been fully acknowledged. The draft of the thesis was also approved by the advisory committee on________________.

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Date: **(Lalita Mundalia)**
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Abbreviations and symbols

APOX - Ascorbate per oxidase
BLAST - Basic Local Alignment Search Tool
B-ME - β-mercaptoethanol
bp - Base Pair
C Control
CAT - Catalase
CAA Control APOX activity
CCA Control catalase activity
CGA Control GPOX activity
cDNA - Complementary DNA
CTAB - Cetyl Trimethyl Ammonium Bromide
°C - Degree centigrade
Conc. - Concentration
d day
ddH$_2$O - Double distilled water
DNA - Deoxyribonucleic acid
dNTPs - Deoxynucleotide triphosphates
ds - Double stranded
DW - Dry weight
EDTA - Ethylene Diamine Tetraacetic Acid
EST - Expressed Sequence Tag
EtBr - Ethidium Bromide
F - Forward
FW - Fresh weight
g - Gram
GPOX - Guaiacol per oxidase
kb - Kilo Base
mg - Milligram
mM - Milimolar
ml - Millilitre
M - Molar
MS - Murashige and Skoog (1962) medium

medium

min	-	Minute
mRNA	-	Messenger RNA
NCBI	-	National Centre for Biotechnology Information
nm	-	Nano meter
OD	-	Optical density
PCR	-	Polymerase Chain Reaction
Pmol	-	Pico mole
RDW	-	Root dry weight
RDWC		Root dry weight control
RDWT		Root dry weight treated
RFW	-	Root fresh weight
RFWC		Root fresh weight control
RFWT		Root fresh weight treated
RL	-	Root length
RLC		Root length control
RLT		Root length treated
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
rpm	-	Rotation per minute
SDW	-	Shoot dry weight
SDWC		Shoot dry weight control
SDWT		Shoot dry weight treated
sec	-	Second
SFW	-	Shoot fresh weight
SFWC		Shoot fresh weight control
SFWT		Shoot fresh weight treated
SL	-	Shoot length
SLC		Shoot length control
SLT		Shoot length treated
SNP	-	Single Nucleotide Polymorphism
SPP		Species
ss	-	Single stranded
T		Treatment
Taq	-	*Thermus aquaticus*

TAA Treated APOX activity
TGA Treated GPOX activity
TCA Treated catalase activity
TBE - Tris-borate EDTA
TE - Tris-EDTA
TF’s Transcription factors
Tm - Melting Temperature
Tris - Tris (hydroxymethyl) amino methane
µg - Microgram
µl - Microliter
V - Volts
Var - Varieties

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1. INTRODUCTION

Drought stress is usually accompanied by nutrient limitation, salinity, and oxidative stresses, thereby hampering plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al.,* 2008; Farooq *et al*., 2008; Farooq *et al*., 2009). The reactions of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth (Chaves *et al*., 2002; Jaleel *et al*., 2008).

Water deficit conditions, often results in imbalance between energy intake and consumption by photosynthetic organ causes the production of reactive oxygen species (ROS) and inability of the plant to control them, which eventually led to denaturation of functional and structural proteins (Smirnoff,1998). As a consequence, diverse environmental stresses often activate similar cell signaling pathways (Shinozaki and Yamaguchi-Shinozaki, 2000; Knight and Knight, 2001; Zhu, 2001b and 2002) and cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants and accumulation of compatible solutes (Vierling and Kimpel, 1992; Zhu *et al.,* 1997; Cushman and Bohnert, 2000). Usually, plants have mechanisms to reduce their oxidative damage by the activation of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase etc.) or expression of genes that are involved in osmolyte biosynthesis (glycine betaine, mannitol, proline, trehalose etc.).

At molecular level variation in allelic form of a gene can also play an important role in drought stress tolerance and hence prospecting the bio resources for novel genes and alleles for abiotic stress tolerance is urgently required to enhance and sustain productivity in the face of threats due to climate changes. These allelic variants may play a role in gene regulation and affect the expression level. Despite the presence of rich diversity, the available germplasm has not been effectively utilized for prospecting new genes and novel alleles for use in genetic improvement of agricultural species. The ongoing elucidation of the molecular control mechanisms of abiotic stress tolerance, which may result in the use of molecular tools for engineering more tolerant plants, is based on the expression of specific stress-related genes. However, quest for more efficient genes and their alleles from unexplored species needs to continue for continued improvement of crop species. The approach can be optimized by focusing on target sets of polymorphisms for example by using SNP detections. Identification and access to allelic variation that affects the plant phenotype is of the utmost importance for the utilization of genetic resources, such as in plant variety development.

In the simplest form, a single nucleotide polymorphism (SNP) is an individual nucleotide base difference between two DNA sequences. As a nucleotide base is the smallest unit of inheritance, SNPs provide the ultimate form of molecular genetic marker. They represent the most frequent source of polymorphisms (Rafalski, 2002). SNPs provide an important source of molecular markers that are useful in genetic mapping, map-based positional cloning, and detection of marker-trait-gene association relationships between individuals. The low mutation rate of SNPs makes them excellent markers for studying complex genetic traits and as a tool for the understanding of genome evolution (Syvanen, 2001).

The genus *Cucumis* comprises of a large amount of diverse of varieties with a major native diversity centre in tropical and southern Africa. It includes some commercially important and widely grown vegetables such as cucumber and melon. Cucurbitaceae plants have a high water requirement (Loomis and Crandall, 1977) and are considered to be sensitive to drought stress. Prolonged exposure to low soil moisture, due to a lack of rainfall or irrigation, has been shown to reduce significantly fruit yield and quality (Doss *et al*., 1977; Elkner, 1985). Transient water deficits are also observed in cucumber plants when transpiration rates exceed the rate of water uptake by the root system. Plant water deficits are evidenced by leaf wilting, closure of stomata, and, ultimately, a reduction in photosynthetic rate (Ackerson and Herbert, 1981; Genty *et al*., 1987). Nevertheless the wild species of *Cucumis* is a reservoir of potentially useful genes for genetic improvement of cucumber and melon. These Wild species represent a broad genetic base of valuable characters, such as resistance to diseases, pests, and abiotic stress.

The present experimentation was thus envisaged to study the within and between species variations present for drought tolerance and associated activity of antioxidant enzymes and allelic variations defining these differences in activity of enzymes:

- Screening of various genotypes of *Cucumis* species and sub species against drought stress at seedling stage.
- Determination of effect on antioxidant enzyme activity due to drought stress.
- Amplification and sequencing of antioxidant gene using specific primer pairs from tolerant and susceptible group.
- Identification of SNP's in the sequences.

2. REVIEW OF LITERATURE

In all societies, progress and development depend upon stable and rich food supplies that then provide the time that allows them to devote their energies to other activities. Abundant food production at specific geographical sites within climates that were suitable for a year-round supply of food allowed the emergence, construction and proliferation of permanent communities of ever greater civilization. Early communities were situated near reliable water sources with the consequence that crops became gradually selected/ adapted for growth under near optimal conditions of the available water supply (Hodge, 2004; Bressan *et al.,* 2009). On the other hand, soil is also important for agriculture and food supply, and it is the first protecting barrier for man so both agricultural and eco-environmentally sustainable development are closely linked with water and soil in relation to the successful civilization of man (Shao *et al.,* 2008; Zhou and Shao, 2008). With the increases in climate change, soil water deficit is one of the biggest challenges not only for crop productivity but also for vegetation diversity (Mittler, 2006; Rodríguez-Loinaz *et al.,* 2008).

Stress factors are divided into biotic and abiotic stresses. Biotic stresses include a variety of pathogenic microorganisms and higher animals including interferences from human whereas abiotic stresses includes water logging, drought , extreme of temperature, wind, storm, lightning, intense light, excessive soil salinity, inadequate or excess mineral nutrients and also treatment with plant growth regulators antibiotics. among the environment abiotic stresses, water deficit limit global food productivity more severely than any other environment factor (Boyer, 1982; Araus *et al*., 2002; Passioura, 2002).

The growth and productivity of crop plants depend largely on their vulnerability to environmental stresses. High salinity, Drought and temperature stresses are the major constraints that limit agricultural production. Plants respond to these conditions with an array of biochemical and physiological adaptations, which involve, the function of many stress related genes. Hence any attempt to improve the stress tolerance requires a better understanding of physiological, biochemical and molecular events (Cherian *et al*., 2006).

Abiotic stresses are the major constraint to agriculture production worldwide. The plants have an in built ability to respond to fluctuations circadian and seasonal environmental conditions. The reactions in plants due to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Chaves *et al*., 2003; Jung, 2004; Dacosta & Huang, 2007).

Drought is the most significant factor restricting plant production on majority of agricultural fields of the world (Tas and Tas, 2007) especially for arid and semi arid regions (Shao *et al.,* 2008). it is characterized by the reduction of water content, turgor, total water potential, wilting, closure stomata, and decrease in cell enlargement and growth (McKersie and Leshem, 1994; Kumar *et al.,* 2007). Severe water stress may result in the arrest of photosynthesis, the disturbance of metabolism, and finally death (Mckersie and Leshem, 1994). It influence the growth of weeds, agronomic management, nature and intensity of insect, pest and disease (Wheatley *et al.,* 1989; Whitman and Whitman, 1994). When water is limited, plants that use a finite water supply more efficiently would positively affect plant productivity and production (Wright *et al.,* 1993).

The adaptation strategies of plants to drought stress include drought escape, drought avoidance and drought tolerance. Escaping drought involves completion of the life cycle before onset of the drought period. Drought avoidance involves the maintenances of the plant water status in the presence of drought stress, while drought tolerance involves maintenance of the plant function in the presence of drought (Turner *et al.*, 1986).

Root growth under drought conditions might be a key factor to the understanding of the contribution of roots to drought adaptation (Kashiwagi *et al.,* 2005). An extensive root system that enhances the ability of the plant to capture water, is a fundamental adaptation mechanism against drought (Ludlow and Muchow, 1990; Passioura, 1982). In rice, root length, root length density and the number of thick roots, are important for the exploiting the available water (Ekanayake *et al.,* 1985).

Water deficit condition reduce the number of leaves per plant, leaf size, leaf area and leaf longevity (Nayar and Gupta, 2006; Cabuslay *et al.,* 2002). Leaf area plasticity is important in maintaining the control of water use in crops. Reduction in leaf area by water stress is an important cause of reduced crop yield through reduction in photosynthesis (Kramer, 1983).

Fresh and dry biomass have a profound effect on water limited conditions. A common adverse effect of water stress on crop plants is the reduction in fresh biomass production (Ashraf and O'Leary, 1996). Drought stress decreases the mean plant biomass and it increases both the relative variation in plant biomass and the concentration of mass within a small fraction of the population. (Duan and Zhao, 1996; Xin *et al.,* 1998; Wu and Wang, 1999).

Higher degree of drought resistance was associated with higher protein contents. However, the nature of plant species and the types of tissue modulate the concentration of soluble proteins under water stress (Ashraf and Mehmood,1990; Irigoyen *et al.,* 1992; Terri *et al.,* 1986).

Drought-inducible genes, based on their temporal responses and function, are classified in two major categories, functional genes and regulatory genes. (Ingram and Bartels,1996; Bray, 1997; Shinozaki and Yamaguchi-Shinozaki, 1997; Yamaguchi-Shinozaki *et al.,* 2002; Seki *et al.,* 2002a and 2002b). Genes that respond early after recognition of stress are more likely involved in the signal transduction pathway and have a regulatory role over down- stream responsive genes. Functional group proteins that are directly involved in stress tolerance are: (i) late embryogenesis abundant proteins (LEA), chaperones and mRNA binding proteins; (ii) compatible solutes like proline, glycine betain and sugars which functions as osmolytes (iii) water channel proteins, sugar transporters and proline transporters (iv) detoxifying enzymes, such as glutathione S-transferase, superoxide dismutase and a soluble epoxide hydrolase (v) proteinases and proteinase inhibitors. The regulatory genes includes protein kinases, transcription factors and enzymes in phospholipids metabolism (Yamaguchi-Shinozaki *et al.,* 2002).

All abiotic stresses lead to the production of reactive oxygen species (ROS) albeit different forms and in different sub-cellular compartments (Jaspers and Kangasjärvi, 2010; Asada, 2006). These abiotic stresses causes photo-oxidative damage creating an imbalance between the photosynthetic assimilation of CO_2 and photochemical activity by increasing the production of reactive oxygen species (ROS), such as superoxide (O2 -), hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH) and singlet oxygen ($1O_2$) (Forman *et al.,* 2010). However, under various abiotic stresses the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages (Almeselmani *et al*., 2006). Accumulation of reactive oxygen species (ROS) as a result of various environmental stresses is a major cause of loss of crop productivity (Mittler, 2002; Apel and Hirt, 2004; Bartels and Sunkar, 2005; Foyer and Noctor, 2005).

The H_2O_2 produced inside the peroxisomes can moved to the chloroplasts, mitochondria and cytosol via aquaporin mediated transport across membranes. (Mittler *et al*., 2002; Moller *et al*., 2007). H_2O_2 homeostasis is maintained by several redundant enzymatic pathways that involve peroxidases and other antioxidative enzymatic and non-enzymatic components (Moller *et al*., 2007).

The reduction of reactive oxygen species generation is essential for alleviation of plant damage under an abiotic stress (Hu *et al.*, 2007; Moradi and Ismail, 2007).The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signalling and/or damage will occur (Moller *et al*., 2007).

Detoxification of reactive oxygen species in plants, include enzymatic mechanisms such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), peroxidase (POX), glutathione reductase (GR) and mono dehydro ascorbate reductase (MDAR) as well as non-enzymatic mechanisms (such as, flavonoids, anthocyanins, carotenoids and ascorbic acid, AA) (Ramachandra *et al*., 2004). Damages caused by oxidative stress include: inactivation of enzymes, lipid peroxidation, protein degradation and destruction of DNA strands (Friso *et al*., 2004; Gechevet *et al*., 2006; Baruah *et al*., 2009).

The major elements of the enzymatic antioxidant system are SOD, APOX and CAT. these are the main enzymes present ubiquitously permitting the tightly control of ROS levels by scavenging directly ROS and converting them into less reactive and less harmful species. They can be considered as intracellular ROS sensors due to their direct interaction with ROS. Another group of enzymes, monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), is involved in the reduction of oxidized AsA or GSH, thus, balancing the redox status of the cell (Asada, 1999; Foyer & Noctor, 2011). Up-regulation of the enzymes involved in the antioxidant system both at the transcript and the protein levels in response to ROS accumulation has been shown for a variety of abiotic stresses (Gill & Tuteja, 2010).

The mode of action of these enzymes, is that superoxide dismutases react with superoxide radicals at almost diffusion- limited rates to produce hydrogen peroxide, which is disposed off by catalase and peroxidises. (Bowler *et al*.,1992). The peroxidises with broad specificities are often found in the cell wall, where they utilize H_2O_2 to generate phenoxy compounds, which then polymerize to produce cell wall components such as lignin (Greppin *et al.,* 1986). Glutathione reductase, the other key component, has a regulatory function because of the dependence of its activity on the availability of NADPH (Arora *et al*., 2002).

Catalase (EC1.11.1.6) a tetrameric heme protein, is an oxido-reductase, occurring in almost all aerobic organisms, and one of the few enzymes showing dual activity: it has hyperoxidase activity (catalytic activity) when it catalyzes the breakdown of hydrogen peroxide into water and oxygen. It also shows peroxidase activity (Luhova *et al.,* 2003). Catalase is also associated with scavenging of H_2O_2 and an increase in its activity is related with increase in stress tolerance (Upadhyay *et al.,*1990; Olmos *et al.,*1994; Kraus, 1995; Kumutha *et al.*, 2009; Khanna *et al.*, 2007)

Hydrogen peroxide scavenging in plants is essential for cellular protection and cellular signalling (Mittler, 2002). Plants contain at least five different enzymes capable of rapid and efficient H_2O_2 removal.

APOX (Ascorbate peroxidase) exists as iso-enzymes and plays an important role in the metabolism of H_2O_2 in higher plants (Shigeoka *et al.,* 2002). APOX iso enzymes are critical components that prevent oxidative stress in photosynthetic organisms. Additionally, recent studies on the response of APOX expression to some stress conditions and pathogen attacks indicate the importance of APOX activity in controlling the H_2O_2 concentration in intercellular signalling.

Exploitation of gene banks for efficient utilization depends on the knowledge of genetic diversity, in general, and allelic diversity at candidate gene(s) of interest, in particular. Hence, allele mining seems to be a promising in characterization of genetic diversity or allelic/genic diversity among the accessions of the collection in terms of its utility for improving a target trait (Kaur *et al*., 2008). The availability of sequence and sequence variation that affects the plant phenotype is of utmost importance for the utilization of genetic resources in crop improvement (Graner, 2006).

The existing allelic diversity in any crop species is caused by mutations, the evolutionary driving force (Kumar *et al*., 2010). Mutations create new alleles or cause variations in the existing allele and allelic combinations. They take place in coding and non-coding regions of the genome either as single nucleotide polymorphism (SNP) or as insertion and deletion (InDel).

The mutations in coding regions and/or regulatory regions may have tremendous effect on the phenotype by altering the encoded protein structure and/or function while those that occur in non-coding regions of a gene could often be silent without any effect on the phenotype. Even though most of the mutations are deleterious, in general 0.1% of the mutations are super vital leading to alterations in gene function which may be highly necessary for the survival of the plant.

The mutation frequency between any two nucleotides is not random but is dependent on the nucleotide base, the base sequence in its immediate proximity and the methylation status of the DNA. A major mechanism of spontaneous mutation is due to errors in DNA replication. Nucleotide bases in DNA can exist in two different structural forms (tautomers) called KETO and ENOL forms, but are predominantly found in the KETO form. Shifts to the ENOL form (tautomerisation) can alter pairing preferences, such that A may pair with C rather than T. Reversion of the tautomeric shift following DNA replication leads to fixation of a base mutation (Edwards *et al.,* 2007).

Sequence based allele mining involves amplification of alleles in diverse genotypes through PCR followed by identification of nucleotide variation by DNA sequencing. Sequencing-based allele mining would help to analyze individuals for haplotype structure and diversity to infer genetic association studies in plants. (Kumar *et al*., 2010)

With the decreasing cost of DNA sequencing and the accumulation of large libraries of genomic DNA and expressed sequence tag (EST) sequences, molecular marker technologies are shifting toward sequence variation. Single nucleotide polymorphisms (SNPs) are present at a greater frequency throughout the genome and usually associated with lower genotyping error rates than microsatellite markers (Kennedy *et al.,* 2003).

In the simplest form, a single nucleotide polymorphism (SNP) is an individual nucleotide base difference between two DNA sequences. As a nucleotide base is the smallest unit of inheritance, SNPs provide the ultimate form of molecular genetic marker. They represent the most frequent source of polymorphisms (Rafalski, 2002). SNPs provide an important source of molecular markers that are useful in genetic mapping, map-based positional cloning, and detection of marker-trait-gene association relationships between individuals. The low mutation rate of SNPs makes them excellent markers for studying complex genetic traits and as a tool for the understanding of genome evolution (Syvanen, 2001).

Single nucleotide polymorphisms (SNPs) are the source of DNA variation in most of the plant and animal genomes (Garcés-Claver *et al.*, 2007). SNPs can serve as a powerful tool for marker-assisted selection (MAS) and map-based cloning since they are considered as highly stable markers and often contribute directly to a phenotype (Andersen and Luebberstedt, 2003; Kim *et al.*, 2005).

SNPs can differentiate between related sequences both within individual and between individuals in a population. Within individual, the individual may be heterozygous at genetic locus, homologous gene copies may be differentiated by SNPs. The frequency of SNPs and the heterozygosity (haplotypic diversity) between two individuals are direct measures of genetic diversity. Under inbreeding conditions, lower frequency of SNPs and genetic diversity is observed. For domesticated crop plants, narrow genetic bases have contributed to corresponding reduced genetic diversity and low SNP frequency at nucleotide level. Out breeding species compared to inbreeding species generally have higher frequency of SNPs. It is generally well accepted that some species, for example maize, are highly polymorphic, whilst others, such as soybean and melon, are less polymorphic (Edwards *et al.,* 2007).

The distribution of SNPs is not random across genome. SNPs occur in coding and non-coding regions of the genome and at different frequencies. The uneven distribution may be due to differences in recombination rate, gene density, transmission pattern, selection strength and compositional pressure. Genomic regions with low recombination rates generally have reduced levels of polymorphisms (Rafalski and Morgante, 2004).

Several approaches have been appl for the SNP discovery including the mining of EST sequence data sets that offer a valuable resource for the SNP detection due to the relatively high redundancy of gene sequences, the diversity of genotypes represented in the databases, and the likelihood that each SNP is associated with an expressed gene (Picoult-Newberg *et al*., 1999; Batley *et al*., 2003a).

There are several methods that are used in genotyping SNPs. Matrix-assisted laser desorption/ionization time-off light mass spectrometry (MALDI-TOF) (Griffin and Smith, 2000) and the detection of hetero duplex through sequences by denaturing high performance liquid chromatography (DHPLC) and DNA microarrays (Hacia and Collins, 1999) are some of the effective methods for the detection of SNPs.

SNP poly-morphisms have been identified from many individuals through direct sequencing of DNA segments (amplified by PCR) (Gaut and Clegg, 1993). Compared to other SNP genotyping methods, sequencing is in particular, suited to identifying multiple SNPs in a small region, such as the highly polymorphic Major Histocompatibility Complex region of the genome (Rapley & Harbron 2004). SNPs are excellent markers for association mapping of genes controlling complex traits and provide the highest map resolution (Botstein and Risch, 2003; Brookes, 1999; Bhattramakki *et al.,* 2002).

Single nucleotide polymorphisms are becoming the marker of choice for genetic studies and due to their abundance they can provide a large supply of markers for crop improvement programs (Gupta *et al*., 2001, Nasu *et al*., 2002; Feltus *et al*., 2004). The advances in SNP-based marker technology have had a great impact on the generation of high-density genetic maps (Snelling *et al*., 2005), trait mapping (Hayashi *et al.,* 2004), association studies, and positional cloning of genes underlying complex traits (Monna *et al*., 2002; Shen *et al*., 2004).

3. MATERIALS AND METHODS

The present experimentation entitled "Allele mining for drought related antioxidant genes in *Cucumis spp*." was conducted at Plant Biotechnology Centre, Swami Keshwanand Rajasthan Agricultural University, Bikaner during 2011-12. In order to achieve the objectives of present investigation, the experiments were planned and executed as described below:

3.1 Materials

3.1.1. Plant Material

The experimental material for the present investigation consisted of 62 genotypes of seven *cucumis spp* viz. *C. prophetarum*, *C. melo*, *C.momordica, C. utilissimus*, *C.agretis*, *C.hardwickki*, *C.callosus* (Table 3.0). These were part of the core collection identified under NAIP project and available at Plant Biotechnology Centre, SKRAU, Bikaner.

Table 3.0: List of cucumis accessions used in the study

S.NO.	**GENOTYPE**	**S.NO.**	**GENOTYPE**	**S.NO.**	**GENOTYPE**
	C.prophetarum		***C.callosus***		***C.melo var melo***
1	IC-258181	1	IC-258113	1	IC-297507
2	IC-373402	2	AHK-200		***C.hardwickki***
3	IC-467722	3	SKY/DR/RS-96	1	IC-331459
4	IC-469509	4	SKY/DR/RS-27	2	IC-331619
5	IC-469551	5	SKY/DR/RS-100	3	IC-331620
	C. utilissimus	6	SKY/DR/RS-91	4	IC-331626
1	IC-258163	7	SKY/DR/RS-111		***C.momordica***
2	IC-276340	8	SKY/DR/RS-79	1	IC-265062
3	IC-276363	9	IC-91205	2	IC-371709
4	IC-276382	10	EC-428164	3	IC-415521
5	IC-276525		***C.melo***	4	IC-415531
6	IC-276541	1	BS-25	5	IC-415539
7	IC-276564	2	MM-1	6	IC-435555
8	IC-313031	3	BS-41	7	IC-433621
9	IC-315294	4	IIHR-3	8	IC-538103
10	IC-321448	5	IIHR-18	9	IC-588188
11	IC-347735	6	IIHR-81		***C.chate***
12	IC-398779		***C.trigonas***	1	ARYA
	C.sativus	1	IC-280785		
1	SPP-44	2	IC-349721		
2	SPP-93		***C.agretis***		
3	SPP-56	1	IC-258165		
4	SPP-58	2	IC-258167		
5	SPP-63	3	IC-276546		
6	SUPER VIGOUR		***C.cananon***		
7	SWARNA AGETI	1	IC-541846		
8	VR-101				

3.1.1.2. Chemicals

All the chemicals used were purchased from Himedia Laboratories Pvt. Ltd., Bombay and Sigma Chemicals Co., USA. PCR components were obtained from Bangalore Genei, Bangalore, India and chromous biotech limited. For all the experiments, Milli Q water was used. All the glasswares used in the present study were thoroughly cleaned, rinsed and dried by baking at 180^0C overnight before use. All reagents solutions, media, ependroff tubes, PCR tubes and tips were sterilized at 15 psi for 20 minutes in autoclave.

Throughout the course of investigation, Murashiage and Skoog (1962) medium were used for the nutrient supplementation. The chemical compositions of media have been presented in Table 3.1.

Table 3.1: Murashiage and Skoog Medium (1962)

Contents	**Concentration in medium (mg/l)**
Macronutrients	
NH_4NO_3	1650
KNO_3	1900
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MgSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CaCl_2.6H_2O$	0.025
Na_2EDTA	37.30
$FeSO_4.7H_2O$	27.80

Vitamins & other supplements	
Inositol	100
Glycine	2
Thiamine HCl	0.1
Pyridoxine HCl	0.5
Nicotinic Acid	0.5

Sucrose	3.0% (30 g/l)
Agar	0.8% (8 g/l)

Readymade MS media vials were used to prepare a given quantity of medium, final volume was made as per requirement. pH of MS medium was adjusted to 5.8-6.0 using 1N NaOH or 0.1N HCl.

3.1.1.3 Glassware

Borosil grade glasswares were used for all the experiments. Oven dried (250^0C) flasks, pipettes, petridishes, beakers, volumetric flasks and measuring cylinders (10, 25, 50, 100, 500 and 1000 ml) were used for media preparations.

3.1.1.4 Sterilization

For sterilization, the culture medium was poured in flasks and plugged with cotton and wrapped with aluminum foil. Autoclaving was done at 15-16 psi (1.06 kg/cm^2) for 18 minutes and stored at 27 ± 2^0C.

3.2 Methods

3.2.1 Screening of germplasm:-

For *in vitro* screening, seeds of cucumis spp. were grown in plastic pots filled with vermiculite soil saturated with ¼ MS (Murashige and Skoog, 1962) liquid media at 30º C in controlled condition of green house and targeted for drought stress at seedling stage. 30 ml water was given at the interval of one day. In order to develop water stress no water was supplemented from outside to 7 day old seedlings. Visual observations were recorded as desiccation symptoms developed in the plants. Data was recorded for root length, shoot length, fresh and dry weights of shoots and roots and activity of antioxidant enzymes CAT, APOX, GPOX was also measured simultaneously.

Germination and Growth Parameters

3.2.1.1 Seedling length

The shoot and root length (cm) were measured after 7 days of drought stress with the help of meter scale.

3.2.1.2 Fresh Weight of Root and shoot

Fresh weight of the root and shoot samples was taken with the help of weighing balance just after extracting them from soil and separating at juncture.

3.2.1.3 Dry weight of root and shoot

Both the root and shoot of each plant were dried to constant weight in an oven after putting them separately in a pre-weighed butter paper bag. Dry weight of the root and shoot samples was taken with the help of weighing balance along with the bag. Dry weight was then calculated.

3.2.2 Antioxidants

3.2.2.1 Enzyme Extraction

Leaf samples (0.2 g fresh wt) were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinyl polypyrrolidone (PVP). The homogenate was filtered through four layers of cheese cloth and then centrifuged at 4°C for 20 min at 15000 *g*. The supernatant was collected and an appropriate aliquot dilution of the crude extract was used for enzyme assays. All operations for enzyme extraction were performed at 0 - 4°C.

3.2.2.2 Enzyme Quantification

The total protein content of extract was determined by Bradford assay (Bradford, 1976).

I. 0, 10, 20, 30, 40 µl of standard protein (Bovine serum albumin 1mg/ml) were taken in separate test tube.

II. The volume was adjusted to 200 µl by adding double distilled water.

III. 1800 µl of Bradford reagent was added, mixed and kept at 37˚C for 10 min.

IV. Absorbance was measured at 595 nm.

V. Similarly, 20 µl of extracted enzyme was diluted in 180 µl of dd H_2O and 1800 µl of Bradford reagent was added to 200 µl of above diluted samples in a test tube.

VI. The mixture was allowed to cool and absorbance was measured at 595 nm.

VII. Graph was drawn between concentration of standard protein and absorbance. The quantities of unknown samples were measured on the basis of standard curve and dilution factor.

3.2.2.3 Catalase activity

CAT activity was measured by following the decomposition of H_2O_2 at 240 nm (coefficient of absorbance, $\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 as described by Chance and Maehly (1955) for 1 min. Enzyme activity was expressed as µmol of H_2O_2 decomposed mg^{-1} (protein) min^{-1}.

Preparation of Reagents

I. **Phosphate buffer (50mM):** Potassium hydrogen phosphate (0.2 M) was prepared by dissolving 34.83 g of it in 1000 ml dH_2O. Further, potassium dihydrogen phosphate (0.2 M) was prepared by dissolving 27.21 g of it in 1000 ml dH_2O. Potassium hydrogen phosphate and Potassium dihydrogen phosphate were mixed in the ratio of 72:28 and pH was adjusted to 7.2 with pH meter.

II. **H_2O_2 (6.66mM):** 13.6 µl of 30% H_2O_2 was diluted to 20 ml with dH_2O.

3.2.2.4 GPOX activity

For GPOX, the oxidation of guaiacol was measured by following the increase in absorbance at 470 nm ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) for 1 min. The assay mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 10 mM H_2O_2 as described by Chance and Maehly (1955). GPOX activity was expressed as µmol (tetraguaiacol formed) mg^{-1}(protein) min^{-1}.

Preparation of Reagents

i. **Phosphate buffer (50 mM):** Potassium hydrogen phosphate (0.2 M) was prepared by dissolving 34.83 g in 1000 ml dH_2O. Further, Potassium dihydrogen phosphate (0.2 M) was prepared by dissolving 27.21 g in 1000 ml dH_2O. Potassium hydrogen phosphate and Potassium dihydrogen phosphate were mixed in the ratio of 61: 39 and pH was adjusted to 7.0 with pH meter.

ii. **H_2O_2 (50 mM):** 102.04µl of 30% H_2O_2 was diluted to 20 ml with dH_2O.

iii. **EDTA (2mM):** 0.0358 gm dissolved in dH_2O and volume was made up to 100 ml.

iv. **Guaiacol (50 mM):** 55 µl of guaiacol was diluted to 10 ml with dH_2O.

3.2.2.5 APOX activity

APOX activity was measured by following the decrease in absorbance at 290 nm due to ascorbate oxidation (ε = 2.8 mM-1 cm-1) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 0.1 mM EDTA for 1 min according to the method of Nakano and Asada (1980). Enzyme activity will expressed as mmol(ascorbate oxidised) mg-1(protein) min-1.

Preparation of Reagents

I. **Phosphate buffer (50 mM):** as above

II. **H_2O_2 (6.66mM):** as above

III. **Ascorbic acid (0.5M): 4.4** mg dissolved in dH_2O and volume was made up to 100 ml.

IV. **EDTA (2mM):** 0.0358 gm dissolved in dH_2O and volume was made up to 100 ml.

3.2.3 Correlation Analysis

Correlation study was done among various morpho-physiological and biochemical parameter.

3.2.4 DNA isolation

0.5 gm of leaves was homogenized in liquid Nitrogen (liq. N_2) after through washing. The homogenized material was handled as per the method described by Doyle and Doyle (1990) in the following steps:

i. 0.5 g of tissue material was ground in liquid N_2 with the help of mortar-pestle.

ii. The homogenized material was transferred to 5 ml pre-warmed (60^0C) DNA Isolation Buffer (2 X CTAB DNA Extraction Buffer - 100 mM Tris, 20 mM EDTA, 1.4 M NaCl, 2 % CTAB and 2 µl/ml β-mercaptoethanol) in capped polypropylene tubes.

iii. Clumps were suspended by using spatula.

iv. Incubated for 1 h at 60^0C with occasional mixing by gentle swirling in water bath.

v. After removing from water bath one volume of chloroform: Isoamyl alcohol (24:1) was added and mixed by inversion for 15 minutes to ensure emulsification of the phases.

vi. Spun at 15,000 rpm for 15 minutes at 20ºC (Thermo Scientific* Heraeus Biofuge Stratos centrifuge)

vii. Aqueous phase was taken and transferred to another tube.

viii. 0.6 volume of ice-cold isopropanol was added to precipitate DNA.

ix. DNA-CTAB complex was precipitated as a fibrous network, lifted by Pasteur pipette and was transferred to washing solution. In some cases amorphous precipitation was collected by the centrifugation at 5,000 - 10,000 rpm for 5-10 minutes at 20°C.

x. 20 ml of 70 % alcohol was added to the pellet of DNA and was kept for 20 minutes with gentle agitation.

xi. The pellet was collected by centrifugation at 5,000 rpm for 5 minutes at 20°C.

xii. The tubes were inverted and drained on a paper towel. The pellet was dried over-night after covering with parafilm with tiny pores.

xiii. The pellet was re-dissolved in 500 µl of TE buffer by keeping over night at 4°C without agitation.

3.2.5 Purification of DNA

RNA was removed by treating the sample with DNase free RNase procured from Bangalore Genei, Bangalore. Protein including RNase was removed by treating with chloroform: Isoamyl alcohol (24:1). The purification was carried out through the following steps:

i. 2.5 µl of RNase was added to 0.5 ml of crude, DNA preparation (2.5 µl of RNase = 25 µg of RNase, so treatment was 50 µg / ml of DNA preparation).

ii. Gently it was mixed thoroughly and was incubated at 37 °C for 1 hr.

iii. After 1 hr, a mixture of 0.3 - 0.4 ml of chloroform: Isoamyl alcohol (24:1) was added and mixed thoroughly for 15 minutes till an emulsion was formed.

iv. Spun for 15 minutes at 15,000 rpm.

v. Supernatant was taken avoiding the whitish layer at interface.

vi. The DNA was re-precipitated by adding double the quantity of absolute alcohol.

vii. To pellet the DNA, the tube was centrifuged for 5 minutes at 5,000 - 10,000 rpm.

viii. The pellet was washed with 70% alcohol and dried over night.

ix. The DNA was re-dissolved in 50µl of TE buffer.

3.2.6 Gel Analysis

The integrity of DNA was judged through gel analysis in following steps:

i. Cast 150 ml Agarose gel (0.8%) in 0.5X TBE (Tris Borate EDTA) buffer containing 0.5 µg / ml of Ethidium Bromide (Appendix – 1).

ii. 2 µl of DNA per sample was loaded in each well.

iii. 2 µl of uncut Lambda phage DNA was also loaded as control.

iv. Electrophoresis was conducted at 50 V for 1 h.

v. Gel was visualized under UV light using Trans illuminator.

vi. Presence of single compact band at the corresponding position to λ phage DNA indicates intact and high molecular weight of isolated DNA.

3.2.7 Quantification of DNA

The quantification of DNA was done by recording its absorbance at 260 nm and 280 nm using a UV- VIS spectrophotometer (UNICAM) in the following steps:

i. 1000 µl T.E. buffer was taken in one cuvette and was set as a blank and spectrophotometer was calibrated at 260 nm as well as at 280 nm wavelengths.

ii. In second cuvette 990ul T.E. buffer and 10 µl of DNA was added mixed properly and the absorbance (A) at 260 nm and 280 nm was recorded.

iii. DNA concentration was calculated by employing the following formula:

$$\text{Amount of DNA } (\mu g / \mu l) = \frac{A_{260} \text{ x } 50 \text{ x dilution factor}}{1000}$$

iv. Quality of DNA was judged from the ratio of values recorded at 260 and 280 nm.

3.2.8 Dilution of DNA for PCR

The quantified DNA was diluted to a final concentration of 25 ng μl^{-1} in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) for further -PCR reactions.

3.2.9 PCR Reaction

3.2.9.1 Primer designing

Primers for PCR were designed using NCBI's Primer 3 tool (http://www.ncbi.nlm.nih.gov/tools/) with the parameters of optimum primer $T_m > 57\text{-}63^oC$, primer length 20-25 nucleotides, and an expected amplicon size of 300-790 bp. From NCBI home 1578bp linear DNA EST(EU798635.1) of "C.melo WMR 29 catalase 2" gene was taken. Genome blast of EST's was done with the available genome of C.sativus. for Catalase maximum homology was found with C. sativus (AC- ACHR01008134.1,contig number 8134).

Table 3.2: List of primers used for PCR reaction

Catalase	Forward primer(5'-3')	Reverse primer(5'-3')	Amplicon length

Cat a	AGCGTTATTGGGA CGTGTGGC	CCCCTGGGTCCAA CGGTCAAC	734
Cat b	ACGATGCAGCACC GACCTTCA	ACCAGGGGAGAGGC TTGCCA	307
Cat c	TCCCGGTCAAGGCT CGGAGA	GTTTCAGGGCTGCCC CGCTC	781
Cat d	GAGCGGGGCAGCCC TGAAAC	ACCTGAACCGTCCATG TGCCTG	676
Cat e	TCGTTGACCGTTGGA CCCAGG	ACCAACAAACAAATCTC CATGGCCT	387

Specific annealing temperature of these set of primers were obtained from temperature gradient between 45-68°C. Annealing temperature for each Catalase primer set was different i.e. for Cat a is 66°C, Cat c is 66°C and Cat d is 68°C, PCR for transcriptional factors(WRKY, ERF/AP family) was also done at their specific annealing temperature. PCR reactions were performed in a final volume of 50 µl containing 10 X Assay Buffer, 5.0 unit of Taq DNA polymerase, 200 µM each of dNTPs (chromous biotech), 10 pmols/reaction of specific primers and 25 ng of template (sample) DNA. The PCR was performed in ‘thermo cycler using the following cycling parameters:

Cycle 1

Denaturation (94 °C) 5 Minutes

Primer annealing (°C) 1 Minute (66°C- cat a, 64°C-cat c, and 68°C-cat d)

Primer extension (72 °C) 2 Minutes

Cycle 2 - 43

Denaturation (92 °C) 1 Minute

Primer annealing (°C) 1 Minute (As above)

Primer extension (72 °C) 2 Minutes

Cycle 44

Denaturation (92 °C) 1 Minute

Primer annealing (°C) 1 Minute (As above)

Primer extension (72 °C) 5 Minute

Table 3.3: Reaction component used for amplification

Component	**Concentration**	**Quantity/reaction(µl)**
Sterile Water	-	32.6
Buffer	10X	5
dNTPs	10mM	4
Primer (F+R)	10µM	4
DNA	-	4
Taq DNA polymerase	5 UNIT/µl	0.4
Total		**50**

Following the amplification, the PCR products were loaded on 1.5% Agarose gel (Himedia, molecular grade), which was prepared in 1XTBE buffer containing 0.5 µg/ml of the Ethidium Bromide. The amplified products were electrophoresed for 2.0 – 2.5 hrs at 100 V with cooling. After separation, visualized under UV transilluminator and gel photograph was taken by using gel documentation system (Alfa Innotech, USA).

3.2.10 Sequencing and sequence analysis

The amplified PCR product was sent to Chromous Biotech Pvt. Ltd. New Delhi, India; for double-pass Sanger sequencing. Sequences received were screened by BIOEDIT software http://www.mbio.ncsu.edu/BioEdit/bioedit.html for the presence of vector sequences. All sequences were further checked for quality and put to contig formation by Seqman^{TM}II 5.08 (DNASTAR.Inc. Lasergene Gene Corporation, Ann Arbor, MI).

sequencess were pro-assembled into contigs using pro-assembly parameters- match size (25), minimum match percentage (80%) and minimum sequence length (100).

3.2.10.1 SNP detection

SNPs were identified using Seqman^{TM}II 5.08 (DNASTAR.Inc. Lasergene Gene Corporation, Ann Arbor, MI) software. Different type of SNP's i.e transition, transversion and indels were detected.

4. RESULTS

The experiments implicated in the present investigation aiming at understanding plant responses to oxidative damage and to find out the allelic variation among the genotypes in *Cucumis spp*. were conducted at Plant Biotechnology Centre, SKRAU, Bikaner. The results obtained for various experiments have been presented below under following headings:

4.1 Visual observations

4.1.1 Screening of germplasm

As per previously standardized method 62 genotypes of *Cucumis spp.* were screened for drought stress tolerance at seedling stage under in vitro condition. For the purpose of screening initially all the genotypes were well watered with measured amount (30ml with interval of one day) under *in vitro* condition. Seedlings were exposed to stress after 7 d of growth by suspending water supply. Some of the seedlings remained healthy without showing any detrimental effect, while some of the seedling showed stressful characteristics like desiccation of leaf and stem, leaves either dried completely or curled at margins or tips, the stem lodged or wilted completely at the same level of water stress. Varietal differences were observed in relation to severity of the effect. According to visual observations all the genotypes were classified as tolerant, moderately tolerant, moderately susceptible and susceptible accessions. Among 62 genotypes 5 were considered as tolerant, 13 were considered moderately tolerant and 17 were considered as moderately susceptible and 9 were as susceptible. A number of genotypes (18) from different species of cucumis failed to germinate in multiple replicates, probably because of lost of seed viability. Genotype Distribution according to the level of tolerance was found to follow normal distribution. However, the curve was skewed towards susceptibility.

Table 4.0: Level of tolerance in cucumis accessions during water deficit condition

S.NO.	Genotype	Tolerance Level	S.NO.	*C. melo*	Tolerance Level
	C.pophetarum		1	IC-297507	MS
1	IC-258181	T		***C.callosus***	
2	IC-373402	T	1	IC-258113	T
3	IC-467722	T	2	AHK-200	S

S.NO.	Genotype	Tolerance Level	S.NO.	*C. melo*	Tolerance Level
4	IC-469509	NG	3	SKY/DR/RS-96	MT
5	IC-469551	NG	4	SKY/DR/RS-27	MT
	C.utilissimus		5	SKY/DR/RS-100	MS
1	IC-258163	T	6	SKY/DR/RS-91	MT
2	IC-276340	NG	7	SKY/DR/RS-111	MS
3	IC-276363	NG	8	SKY/DR/RS-79	MS
4	IC-276382	MT	9	IC-91205	MT
5	IC-276525	NG	10	EC-428164	MS
6	IC-276541	MT		***C.melo***	
7	IC-276564	MT	1	BS-25	MT
8	IC-313031	MS	2	MM-1	MS
9	IC-315294	NG	3	BS-41	NG
10	IC-321448	S	4	IIHR-3	S
11	IC-347735	NG	5	IIHR-18	MS
12	IC-398779	MT	6	IIHR-81	MS
	C.cinnamon			***C.chate***	
1	IC-541846	NG	1	ARYA	MS
	C.trigonous			***C.sativus***	
1	IC-280785	MT	1	SPP-44	NG
2	IC-349721	NG	2	SPP-93	MT
	C.agretis		3	SPP-56	S
1	IC-258165	NG	4	SPP-58	MS
2	IC-258167	MS	5	SPP-63	MS
3	IC-276546	S	6	SUPER VIGOUR	S
	C.momordica		7	SWARNA AGETI	S
1	IC-265062	NG	8	VR-101	MT
2	IC-371709	MT		***C.hardwikki***	
3	IC-415521	S	1	IC-331459	NG
4	IC-415531	MS	2	IC-331619	NG
5	IC-415539	MS	3	IC-331620	NG

S.NO.	Genotype	Tolerance Level	S.NO.	*C. melo*	Tolerance Level
6	IC-435555	S	4	IC-331626	MS
7	IC-433621	MS			
8	IC-538103	NG			
9	IC-588188	NG			

T- tolerant, MT- moderately tolerant, MS- moderately susceptible,

S- susceptible, NG- no germination

Figure 4.0: Differential phenotypic variations represented by *Cucumis* genotypes.

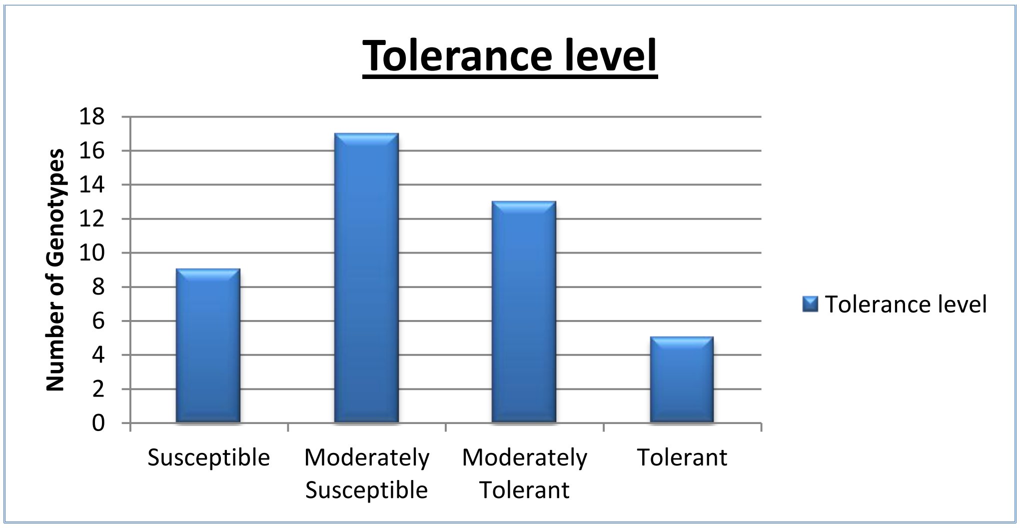


Figure 4.1: Frequency distribution of susceptible, moderately susceptible, moderately tolerant and tolerant genotypes among 44 *cucumis* genotypes*.*

4.1.1.1 Root Length

An average decrease in root length was observed under water deficit condition. Average root length was 12.5 cm in control plants with a range of 5 cm to 28.5 cm while in stressed condition it was 9.5 cm in the range of 2.50 cm to 21.2 cm (table 4.1). *C. melo* had longer roots (16.06 cm) while mean root length was less in *C. momordica* (9.42 cm). roots were of comparable length in other species, however, within spp. variation was considerable. maximum root length was observed for IC-276564 (21.2cm) followed by SKY/DR/RS-111 (16.0 cm), SKY/DR/RS-79 (15.5 cm) and IIHR-81 (14.5 cm), However, maximum reduction in root length was noted for BS-25 (15.5 cm) followed by IC-276382 (10.6 cm), SPP-58 (8.0 cm) while minimum reduction in root length was observed in IC-258113 and SPP-63 (0.05 cm) followed by SPP-93(1.5 cm) respectively.

4.1.1.2 Shoot Length

There was a decrease in average shoot length under water deficit condition with an exceptional increase in some genotypes. In control the average shoot length was 11.8 cm in the range of 4 cm to 25 cm and in stressed condition it was 8.45 cm in the range of 3.80 cm to 12.5 cm. The shoot length varied between and within species of *Cucumis* (table 4.1). Maximum shoot length was observed for EC-428164 and IC-415539 (12.5 cm), followed by IIHR-81 (12.0 cm), IIHR-3 (11.7 cm), However, maximum reduction in shoot length was observed for IC-276564 (15.0 cm) followed by EC-428164 (12.5 cm), IC-276382 (8.5 cm) while minimum reduction was noted for IC-258181 (0.2 cm) followed by IC-280785 and SPP-93 (0.5 cm) and VR-101 (0.8 cm) respectively.

4.1.1.3 Root Fresh Weight

There was a decrease in average root fresh weight under water deficit condition. Most species experienced drastic reduction in fresh weight of roots with variations at species and within species level. The reduction was comparatively much less in *C. prophetarum.* However, fresh weight in control was much less in this species. Under control condition the average root fresh weight was 0.52 mg in the range of 0.03 mg to

2.55 mg while in stressed condition the average root fresh weight was 0.06 mg in the range of 0.02 mg to 0.27 mg. maximum root fresh weight was noted for IIHR-3 (0.27 mg) followed by IC-276564 (0.15 mg) and IC-321448 (0.12 mg). However, maximum reduction in root fresh weight was observed for IIHR-18 (2.52 mg) followed by SUPER VIGOUR (1.94 mg), IIHR-81 (1.85 mg) and SPP-58 (1.7 mg) while minimum reduction in root fresh weight was noted for IC-258167 (0.01 mg), followed by IC-258113, IC-276541 and IC-467722 (0.02 mg).

4.1.1.4 Shoot Fresh Weight

The effect of water deficit condition on shoot fresh weight of different *Cucumis* genotypes under control and stressed condition was noted. Over all stress resulted in reduced shoot weight in all the species, however, within species variation seems to be high. Average shoot fresh weight under control condition was 0.65 mg in the range of 0.15 mg to 1.82 mg, while under water deficit condition the average SFW was 0.26 mg in the range of 0.05 mg to 0.62 mg. Maximum shoot fresh weight was observed for SPP-56 (0.62 mg) followed by ARYA (0.60 mg), IC-276564 and SPP-58 (0.56 mg) and IIHR-3 (0.50 mg). However, maximum reduction in shoot fresh weight was observed for IC-276546 (1.42 mg), followed by SPP-56 (1.20 mg), IC-415539 (1.16) while minimum reduction was observed for SKY/DR/RS-96 (0.03 mg) followed by IC-433621 (0.09 mg), IC-258163 (0.05 mg) respectively.

4.1.1.5 Root dry weight

The reducing effect of drought stress on root dry weight was observed. The *C. prophetarum* which had all tolerant genotypes retained much less water in its roots even in control as evident by less difference in fresh and dry root weight of this species. However, species and genotypes varied for DRW. Under control condition the average root dry weight was 0.02 mg in the range of 0.01 mg to 0.05 mg while in water deficit condition the average root dry weight was 0.01 in the range of 0.01 mg to 0.05 mg. maximum root dry weight was noted for IC-276382 and IC-276564 (0.05 mg) followed by IC-321448 (0.03 mg). Maximum root dry weight reduction was noted for IC-373402 (0.04 mg), VR-101(0.04 mg) followed by IC-415531 (0.03 mg), IC-91205 (0.03 mg) and MM-1

(0.03 mg), while minimum reduction in root dry weight was noted for IC-467722 (0.01 mg), IC-258163 (0.01 mg) respectively (Table 4.1).

4.1.1.6 Shoot dry weight

Shoot dry weight also reduced under water stress. Variation for SDW was evident both at species and genotypic level within species. In control condition the average shoot dry weight was 0.05 mg in the range of 0.01 mg to 0.14 mg and in water deficit condition the average root dry weight was 0.03 mg in the range of 0.01 mg to 0.05 mg. Maximum shoot dry weight was noted for IC-29507 and IC-331626 (0.05 mg), followed by IC-37302 (0.04 mg). However, maximum reduction was observed for IIHR-18 (0.11 mg) followed by SKY/DR/RS-111 (0.09 mg) and IIHR-81 (0.07 mg), while minimum reduction was observed in IC-313031, IC-258163 (0.01 mg) respectively.

Table 4.1: Effect of water deficit condition on various morphological parameters of *Cucumis* genotypes

S.No.	*GENOTYPE*	CONTROL PLANTS						TREATED PLANTS						*RLT/RLC*	RFWT/RFWC	RDWT/RDWC	SLT/SLC	SFWT/SFWC	SDWT/SDWC
		RL	SL	RFW	SFW	RDW	SDW	RL	SL	RFW	SFW	RDW	SDW						
	C.prophetarum																		
1	IC-258181	15.5	4	0.06	0.31	0.01	0.02	10.5	3.8	0.03	0.16	0.02	0.01	0.7	0.5	2.0	1.0	0.5	0.5
2	IC-373402	12.5	11	0.04	0.46	0.05	0.05	9.2	9.5	0.02	0.2	0.01	0.04	0.7	0.5	0.2	0.9	0.4	0.8
3	IC-467722	12	8	0.04	0.3	0.02	0.01	10	9.5	0.02	0.1	0.01	0.03	0.8	0.5	0.5	1.2	0.3	3.0
	SD	**1.89**	**3.51**	**0.01**	**0.09**	**0.02**	**0.02**	**0.66**	**3.29**	**0.01**	**0.05**	**0.01**	**0.02**	**0.08**	**0.00**	**0.96**	**0.17**	**0.09**	**1.37**
	Average	**13.33**	**7.67**	**0.05**	**0.36**	**0.03**	**0.03**	**9.90**	**7.60**	**0.02**	**0.15**	**0.01**	**0.03**	**0.75**	**0.50**	**0.90**	**1.00**	**0.43**	**1.43**
	C.utilissimus																		
1	IC-258163	7.5	5.5	0.03	0.15	0.02	0.04	3.4	5.5	0.03	0.1	0.01	0.01	0.5	1.0	0.5	1.0	0.7	0.3
2	IC-276382	22	13	0.16	0.88	0.02	0.06	11.4	4.5	0.07	0.23	0.05	0.03	0.5	0.4	2.5	0.3	0.3	0.5
3	IC-276541	11.3	8.5	0.04	0.49	0.01	0.03	8.5	7.3	0.02	0.2	0.01	0.02	0.8	0.5	1.0	0.9	0.4	0.7
4	IC-276564	28.5	24	0.39	0.87	0.03	0.06	21.2	9	0.15	0.56	0.05	0.02	0.7	0.4	1.7	0.4	0.6	0.3
5	IC-313031	6.7	8.5	0.6	0.4	0.05	0.02	2.5	8	0.03	0.21	0.01	0.01	0.4	0.1	0.2	0.9	0.5	0.5

S.No.	***GENOTYPE***	**CONTROL PLANTS**						**TREATED PLANTS**						***RLT/RLC***	**RFWT / RFWC**	**RDWT / RDWC**	**SLT / SLC**	**SFWT / SFWC**	**SDWT / SDWC**
		RL	**SL**	**RFW**	**SFW**	**RDW**	**SDW**	**RL**	**SL**	**RFW**	**SFW**	**RDW**	**SDW**						
6	IC-321448	7.5	9.5	0.36	0.5	0.03	0.02	5	4.8	0.12	0.2	0.03	0.02	0.7	0.3	1.0	0.5	0.4	1.0
7	IC-398779	9	13.5	0.09	0.53	0.02	0.02	4.8	10.7	0.02	0.27	0.01	0.02	0.5	0.2	0.5	0.8	0.5	1.0
	SD	**8.56**	**6.05**	**0.22**	**0.26**	**0.01**	**0.02**	**6.54**	**2.31**	**0.05**	**0.14**	**0.02**	**0.01**	**0.15**	**0.30**	**0.80**	**0.27**	**0.14**	**0.30**
	Average	**13.21**	**11.79**	**0.24**	**0.55**	**0.03**	**0.04**	**8.11**	**7.11**	**0.06**	**0.25**	**0.02**	**0.02**	**0.58**	**0.42**	**1.05**	**0.69**	**0.49**	**0.61**
	C.trigonous																		
1	IC-280785	11.5	10	0.06	0.31	0.01	0.02	9	9.5	0.02	0.1	0.01	0.025	0.8	0.3	1.0	1.0	0.3	1.3
	C.agretis																		
1	IC-258167	9.5	6	0.04	0.28	0.01	0.03	4.2	5	0.03	0.1	0.01	0.03	0.4	0.8	1.0	0.8	0.4	1.0
2	IC-276546	11.5	15.5	0.19	1.68	0.02	0.05	5.9	7	0.05	0.26	0.02	0.04	0.5	0.3	1.0	0.5	0.2	0.8
	C.momordica																		
1	IC-371709	11	5	0.06	0.21	0.01	0.01	4.2	3.8	0.03	0.05	0.01	0.01	0.4	0.5	1.0	0.8	0.2	1.0
2	IC-415521	8	10	0.15	0.8	0.01	0.03	4.5	6.2	0.02	0.06	0.01	0.02	0.6	0.1	1.0	0.6	0.1	0.7
3	IC-415531	14.5	11.7	0.2	1.03	0.04	0.05	9	7.5	0.02	0.16	0.01	0.03	0.6	0.1	0.3	0.6	0.2	0.6
4	IC-415539	10	14.5	0.2	1.3	0.02	0.04	6.5	12.5	0.02	0.14	0.01	0.02	0.7	0.1	0.5	0.9	0.1	0.5
5	IC-435555	7.5	10.5	0.08	0.38	0.01	0.03	8	8.5	0.03	0.2	0.01	0.02	1.1	0.4	1.0	0.8	0.5	0.7
6	IC-433621	5.5	5.8	0.06	0.17	0.02	0.02	3.5	4	0.02	0.08	0.01	0.01	0.6	0.3	0.5	0.7	0.5	0.5
	SD	**3.15**	**3.61**	**0.07**	**0.47**	**0.01**	**0.01**	**2.23**	**3.24**	**0.01**	**0.06**	**0.00**	**0.01**	**0.23**	**0.17**	**0.33**	**0.10**	**0.19**	**0.18**
	Average	**9.42**	**9.58**	**0.13**	**0.65**	**0.02**	**0.03**	**5.95**	**7.08**	**0.02**	**0.12**	**0.01**	**0.02**	**0.65**	**0.26**	**0.71**	**0.73**	**0.26**	**0.66**
	c melo var. melo																		
1	IC-297507	8.5	11.2	0.7	0.87	0.03	0.04	9.5	6.1	0.06	0.3	0.01	0.05	1.1	0.1	0.3	0.5	0.3	1.3
	C.callosus																		
1	IC-258113	14	9.5	0.06	0.16	0.02	0.01	13.5	9.5	0.04	0.06	0.02	0.01	1.0	0.7	1.0	1.0	0.4	1.0
2	AHK-200	19.5	9.8	0.15	0.56	0.01	0.03	14	10.2	0.03	0.18	0.01	0.03	0.7	0.20	1.00	1.04	0.32	1.00
3	SKY/DR/RS-96	7	8.2	0.03	0.25	0.01	0.01	8.5	10.2	0.06	0.22	0.02	0.02	1.2	2.00	2.00	1.24	0.88	2.00
4	SKY/DR/RS-27	9	11.5	0.07	0.4	0.01	0.02	7.5	9.5	0.02	0.06	0.01	0.01	0.8	0.29	1.00	0.83	0.15	0.50
5	SKY/DR/RS-100	9.5	11.5	0.09	0.4	0.01	0.03	7	9.5	0.05	0.21	0.01	0.02	0.7	0.56	1.00	0.83	0.53	0.67
6	SKY/DR/RS-91	11	9.5	0.11	0.35	0.03	0.02	11	8.5	0.04	0.2	0.02	0.02	1.0	0.36	0.67	0.89	0.57	1.00
7	SKY/DR/RS-111	17.7	12	0.22	1.13	0.02	0.11	16	9.5	0.03	0.12	0.01	0.02	0.9	0.14	0.50	0.79	0.11	0.18
8	SKY/DR/RS-79	19.5	11.5	0.28	0.87	0.03	0.04	15.5	9.8	0.06	0.45	0.02	0.03	0.8	0.21	0.67	0.85	0.52	0.75
9	IC-91205	8	14	0.23	0.6	0.04	0.04	14	7.2	0.03	0.18	0.01	0.01	1.8	0.13	0.25	0.51	0.30	0.25
10	EC-428164	20.5	25	0.4	1.44	0.02	0.06	12.5	12.5	0.04	0.4	0.01	0.04	0.6	0.10	0.50	0.50	0.28	0.67
	SD	**5.31**	**4.77**	**0.12**	**0.41**	**0.01**	**0.03**	**3.29**	**1.34**	**0.01**	**0.13**	**0.01**	**0.01**	**0.33**	**0.57**	**0.48**	**0.23**	**0.23**	**0.51**
	Average	**13.57**	**12.25**	**0.16**	**0.62**	**0.02**	**0.04**	**11.95**	**9.64**	**0.04**	**0.21**	**0.01**	**0.02**	**0.95**	**0.47**	**0.86**	**0.85**	**0.40**	**0.80**
	C.melo																		
1	BS-25	28	17.5	0.25	1.57	0.01	0.09	12.5	9.5	0.02	0.45	0.01	0.02	0.4	0.08	1.00	0.54	0.29	0.22
2	MM-1	9.8	9	0.1	0.67	0.04	0.02	6	5.5	0.03	0.12	0.01	0.02	0.6	0.30	0.25	0.61	0.18	1.00
3	IIHR-3	13	15.5	1.77	0.45	0.02	0.08	10.5	11.7	0.27	0.5	0.01	0.03	0.8	0.15	0.50	0.75	1.11	0.38
4	IIHR-18	17.5	14.8	2.55	0.54	0.02	0.14	11.2	9	0.03	0.5	0.01	0.03	0.6	0.01	0.50	0.61	0.93	0.21
5	IIHR-81	12	14	1.91	0.23	0.03	0.11	14.5	12	0.06	0.35	0.02	0.04	1.2	0.03	0.67	0.86	1.52	0.36
	SD	**7.24**	**3.16**	**1.08**	**0.52**	**0.01**	**0.04**	**3.15**	**2.61**	**0.11**	**0.16**	**0.00**	**0.01**	**0.29**	**0.12**	**0.28**	**0.13**	**0.57**	**0.32**
	Average	**16.06**	**14.16**	**1.32**	**0.69**	**0.02**	**0.09**	**10.94**	**9.54**	**0.08**	**0.38**	**0.01**	**0.03**	**0.74**	**0.12**	**0.58**	**0.67**	**0.80**	**0.44**
	C.chate																		
1	ARYA	11.5	10.5	0.64	0.38	0.01	0.09	13	11	0.12	0.6	0.01	0.04	1.1	0.19	1.00	1.05	1.58	0.44
	C.sativus																		
1	SPP-93	14	11	1.32	0.35	0.03	0.07	12.5	10	0.06	0.47	0.02	0.03	0.9	0.05	0.67	0.91	1.34	0.43
2	SPP-56	5	9.5	0.23	1.82	0.02	0.01	6.5	9.7	0.03	0.62	0.01	0.02	1.3	0.13	0.50	1.02	0.34	2.00
3	SPP-58	17.5	14.5	1.79	0.32	0.01	0.02	9.5	10	0.09	0.45	0.01	0.04	0.5	0.05	1.00	0.69	1.41	2.00
4	SPP-63	10.5	9.5	0.7	0.32	0.01	0.04	10	9	0.04	0.42	0.01	0.03	1.0	0.06	1.00	0.95	1.31	0.75

S.No.	***GENOTYPE***	**CONTROL PLANTS**						**TREATED PLANTS**						***RLT/RLC***	**RFWT / RFWC**	**RDWT / RDWC**	**SLT / SLC**	**SFWT / SFWC**	**SDWT / SDWC**
		RL	**SL**	**RFW**	**SFW**	**RDW**	**SDW**	**RL**	**SL**	**RFW**	**SFW**	**RDW**	**SDW**						
5	SUPER VIGOUR	14.5	15.5	1.99	0.32	0.02	0.1	10.5	9.8	0.05	0.43	0.01	0.04	0.7	0.03	0.50	0.63	1.34	0.40
6	SWARNA AGETI	10	14.5	1.65	0.31	0.03	0.05	8	9.8	0.09	0.1	0.01	0.02	0.8	0.05	0.33	0.68	0.32	0.40
7	VR-101	8.5	7	0.5	1.32	0.05	0.01	6.8	6.2	0.07	0.26	0.01	0.03	0.8	0.14	0.20	0.89	0.20	3.00
	SD	**4.20**	**3.22**	**0.69**	**0.63**	**0.01**	**0.03**	**2.15**	**1.37**	**0.02**	**0.17**	**0.00**	**0.01**	**0.23**	**0.04**	**0.31**	**0.15**	**0.57**	**1.04**
	Average	**11.43**	**11.64**	**1.17**	**0.68**	**0.02**	**0.04**	**9.11**	**9.21**	**0.06**	**0.39**	**0.01**	**0.03**	**0.86**	**0.07**	**0.60**	**0.82**	**0.90**	**1.28**
	C.hardwikki																		
1	IC-331626	13	11	1.07	0.2	0.03	0.05	7	8.5	0.08	0.1	0.01	0.05	0.5	0.07	0.33	0.77	0.50	1.00
	Overall SD	**1.77**	**4.95**	**0.71**	**0.08**	**0.01**	**0.02**	**2.47**	**3.32**	**0.04**	**0.04**	**0.01**	**0.03**	**0.10**	**0.30**	**1.18**	**0.13**	**0.01**	**0.35**
	Overall Average	**11.75**	**10.60**	**0.48**	**0.59**	**0.02**	**0.04**	**8.62**	**7.77**	**0.05**	**0.24**	**0.01**	**0.02**	**0.71**	**0.29**	**0.76**	**0.72**	**0.52**	**0.83**

4.1.2 Antioxidant enzyme activity

4.1.2.1 Catalase

Catalase activity increased with treatment in cucumis genotypes (Table 4.2). Average activity of catalase enzyme in control plants was 0.051 µmol mg^{-1}(protein) min^{-1} in the range of 0.002 to 0.009 µmol mg^{-1}(protein) min^{-1} while under stress condition average activity was 0.0057 µmol mg^{-1}(protein) min^{-1} in the range of 0.003 - 0.01 µmol mg^{-1}(protein) min^{-1}. Both within and between species variations were observed for catalase activity. The enzyme activity was higher in *C.melo, C.sativus*, and *C.hardwikki*. Maximum catalase activity was noted in IC-331626 (0.10 µmol mg^{-1}(protein) min^{-1}), followed by SPP-58 (0.09 µmol mg^{-1}(protein) min^{-1}), IIHR-81(0.008 µmol mg^{-1}(protein) min^{-1}). Maximum increase in catalase activity with treatment was recorded in genotypes IC-258163 (0.004 µmol mg^{-1}(protein) min^{-1}) followed by IC-415555 (0.005 µmol mg^{-1}(protein) min^{-1}), IC-258181 (0.005 µmol mg^{-1}(protein) min^{-1}). However, certain genotypes showed reduction in catalase activity in treatment that are SPP-58(0.003 µmol mg^{-1}(protein) min^{-1}), BS-25, C-258163 (0.002 µmol mg^{-1}(protein) min^{-1}) and IC-435555 (0.002 µmol mg^{-1}(protein) min^{-1}), AHK-200, SKY/DR/RS-111 (0.0001 µmol mg^{-1}(protein) min^{-1}), IC-276564, IIHR-3 and SUPER VIGOUR (0.0002 µmol mg^{-1}(protein) min^{-1}).

4.1.2.2 Ascorbate Peroxidase (APOX)

Average APOX activity in cucumis genotypes was decreased under water stress condition compared to control. Average activity of APOX enzyme in control plants was 0.21 µmol mg^{-1}(protein) min^{-1} in the range of 0.08 µmol mg^{-1}(protein) min^{-1} to 0.46 µmol mg^{-1}(protein) min^{-1} while under stress condition average activity was 0.19 µmol mg^{-1}(protein) min^{-1} in the range of 0.07 µmol mg^{-1}(protein) min^{-1} - 0.48 µmol mg^{-1}(protein) min^{-1}. Most genotypes (29) showed reduction in activity maximum reduction being in ARYA (0.17 µmol mg^{-1}(protein) min^{-1}), followed by IC-415539 (0.15 µmol mg^{-1}(protein) min^{-1}), SUPER VIGOUR (0.12 µmol mg^{-1}(protein) min^{-1}). Some genotypes (19) showed increased activity in treatment compared to control including SUPER VIGOUR and IIHR-81 (0.4 µmol mg^{-1}(protein) min^{-1}), SWARNA AGETI (0.46 µmol mg^{-1}(protein) min^{-1}), IC-258181 (0.45 µmol mg^{-1}(protein) min^{-1}) etc. Species level variations were also evident in the enzyme activity in addition to within species variations (Table 4.2)

4.1.2.3 Guaiacol Peroxidase (GPOX)

Average GPOX activity increased (0.05 µmol mg^{-1}(protein) min^{-1}) with treatment in cucumis genotypes (Table 4.2). Average activity of GPOX enzyme in control plants was 0.11 µmol mg^{-1}(protein) min^{-1} in the range of 0.01 µmol mg^{-1}(protein) min^{-1} to 0.30 µmol mg^{-1}(protein) min^{-1} while under stress condition average activity was 0.17 µmol mg^{-1}(protein) min^{-1} in the range of 0.72 µmol mg^{-1}(protein) min^{-1} - 0.34 µmol mg^{-1}(protein) min^{-1}. The increase in enzyme activity was reported in most of the genotypes (40 out of total 44). Moreover, inter and intra species variations were also recorded for GPOX activity (Table 4.2). The GPOX activity was higher in *C.sativus, C. melo, C. momordica*. Maximum GPOX activity was noted in SPP-93 (0.34 µmol mg^{-1}(protein) min^{-1}), followed by IIHR-18 (0.30 µmol mg^{-1}(protein) min^{-1}), IC-415531 (0.26 µmol mg^{-1}(protein) min^{-1}). However, certain genotypes showed reduction in GPOX activity in treatment that are IC-258113 (0.19 µmol mg^{-1}(protein) min^{-1}), IC-280785 (0.14 µmol mg^{-1}(protein) min^{-1}), ic-415531 (0.13 µmol mg^{-1}(protein) min^{-1}) etc.

Table: 4.2 effect of drought stress on activity of CAT, APOX and GPOX enzymes in *Cucumis* genotypes

S.NO.	GENOTYPE	CATALASE			APOX			GPOX		
		Control	**Treatment**	**T.CAT/C.CAT**	**Control**	**Treatment**	**T.APOX/C.APOX**	**Control**	**Treatment**	**T.GPOX/C.GPOX**
	C.prophetarum									
1	IC-258181	0.004	0.006	1.405	0.415	0.455	1.094	0.140	0.221	2.407
2	IC-373402	0.004	0.005	1.182	0.261	0.168	0.643	0.099	0.228	3.837
3	IC-467722	0.004	0.004	1.158	0.317	0.187	0.589	0.109	0.200	3.152
	SD	**0.000**	**0.001**	**0.136**	**0.078**	**0.160**	**0.277**	**0.022**	**0.015**	**0.715**
	Average	**0.004**	**0.005**	**1.248**	**0.331**	**0.270**	**0.776**	**0.116**	**0.216**	**3.132**
	C.utilissimus									
1	IC-258163	0.002	0.004	2.000	0.246	0.304	1.237	0.105	0.131	4.072
2	IC-276382	0.004	0.005	1.125	0.186	0.147	0.787	0.089	0.111	5.371
3	IC-276541	0.004	0.004	1.128	0.188	0.218	1.160	0.090	0.104	5.311
4	IC-276564	0.006	0.006	1.036	0.124	0.097	0.783	0.074	0.188	8.084
5	IC-313031	0.003	0.004	1.121	0.147	0.135	0.922	0.057	0.166	6.826
6	IC-321448	0.004	0.005	1.359	0.119	0.085	0.712	0.174	0.241	8.382
7	IC-398779	0.006	0.006	1.054	0.188	0.270	1.435	0.117	0.118	5.319
	SD	**0.001**	**0.001**	**0.343**	**0.045**	**0.086**	**0.274**	**0.037**	**0.050**	**1.607**
	Average	**0.004**	**0.005**	**1.260**	**0.171**	**0.179**	**1.005**	**0.101**	**0.151**	**6.195**
	C.cinnamon									
1	IC-280785	0.004	0.005	1.114	0.166	0.128	0.770	0.132	0.277	6.020
	C.trigonous									
1	IC-258167	0.004	0.004	1.079	0.159	0.125	0.785	0.019	0.096	6.301
2	IC-276546	0.005	0.006	1.077	0.168	0.220	1.305	0.075	0.110	5.945
	C.agretis									
1	IC-371709	0.004	0.004	1.081	0.157	0.205	1.301	0.066	0.122	6.353
2	IC-415521	0.005	0.007	1.308	0.222	0.150	0.677	0.178	0.237	4.515
3	IC-415531	0.008	0.008	1.065	0.166	0.130	0.786	0.129	0.268	6.031
4	IC-415539	0.005	0.004	0.917	0.093	0.245	2.630	0.074	0.073	10.730
5	IC-435555	0.004	0.006	1.541	0.207	0.080	0.386	0.083	0.129	4.836
6	IC-433621	0.006	0.007	1.048	0.185	0.112	0.602	0.081	0.131	5.402
	SD	**0.002**	**0.002**	**0.225**	**0.045**	**0.061**	**0.826**	**0.043**	**0.075**	**2.273**
	Average	**0.005**	**0.006**	**1.160**	**0.172**	**0.154**	**1.064**	**0.102**	**0.160**	**6.311**
	C.melo var. melo									
1	IC-297507	0.004	0.005	1.091	0.156	0.167	1.071	0.116	0.125	6.398
	C.momordica									
1	IC-258113	0.005	0.006	1.180	0.226	0.157	0.693	0.052	0.246	4.431
2	AHK-200	0.006	0.006	1.017	0.125	0.085	0.681	0.089	0.150	8.026
3	SKY/DR/RS-96	0.006	0.007	1.083	0.126	0.154	1.220	0.134	0.180	7.949
4	SKY/DR/RS-27	0.003	0.004	1.121	0.145	0.116	0.798	0.100	0.178	6.906
5	SKY/DR/RS-100	0.007	0.006	0.940	0.105	0.125	1.194	0.098	0.104	9.533
6	SKY/DR/RS-91	0.007	0.007	0.943	0.166	0.082	0.497	0.070	0.138	6.031
7	SKY/DR/RS-111	0.004	0.005	1.023	0.124	0.085	0.682	0.037	0.130	8.052
8	SKY/DR/RS-79	0.004	0.004	1.139	0.153	0.136	0.886	0.073	0.109	6.540
9	IC-91205	0.005	0.005	1.064	0.285	0.171	0.601	0.136	0.149	3.506
10	EC-428164	0.005	0.005	0.943	0.128	0.081	0.633	0.104	0.174	7.819
	SD	**0.001**	**0.001**	**0.087**	**0.056**	**0.035**	**0.244**	**0.032**	**0.041**	**1.825**
	Average	**0.005**	**0.005**	**1.045**	**0.158**	**0.119**	**0.789**	**0.089**	**0.156**	**6.879**
	C.melo									
1	BS-25	0.006	0.008	1.390	0.146	0.101	0.692	0.080	0.083	6.873
2	MM-1	0.003	0.004	1.194	0.119	0.115	0.969	0.137	0.073	8.410
3	IIHR-3	0.006	0.006	1.036	0.452	0.315	0.698	0.248	0.255	2.214
4	IIHR-18	0.005	0.006	1.058	0.388	0.411	1.060	0.307	0.310	2.578
5	IIHR-81	0.008	0.009	1.090	0.426	0.488	1.145	0.149	0.156	2.347
	SD	**0.002**	**0.002**	**0.145**	**0.161**	**0.174**	**0.208**	**0.091**	**0.105**	**2.936**
	Average	**0.006**	**0.006**	**1.153**	**0.306**	**0.286**	**0.913**	**0.184**	**0.175**	**4.484**
	C.sativus									
1	ARYA	0.008	0.008	1.053	0.105	0.275	2.624	0.060	0.119	9.542
1	SPP-58	0.006	0.009	1.586	0.163	0.220	1.351	0.126	0.179	6.143
2	SPP-56	0.004	0.005	1.136	0.239	0.184	0.770	0.304	0.266	4.188
3	VR-101	0.007	0.007	1.076	0.466	0.132	0.284	0.169	0.170	2.145
4	SPP-63	0.003	0.004	1.333	0.248	0.257	1.036	0.086	0.099	4.026
5	SUPER VIGOUR	0.008	0.008	1.025	0.359	0.488	1.361	0.208	0.218	2.789
6	SWARNA AGETI	0.004	0.005	1.216	0.379	0.461	1.217	0.141	0.213	2.642
7	SPP-93	0.006	0.005	0.762	0.083	0.115	1.387	0.215	0.343	12.034
	SD	**0.002**	**0.002**	**0.242**	**0.137**	**0.140**	**0.670**	**0.078**	**0.079**	**3.587**

S.NO.	GENOTYPE	**CATALASE**			**APOX**			**GPOX**		
		Control	**Treatment**	**T.CAT/C.CAT**	**Control**	**Treatment**	**T.APOX/C.APOX**	**Control**	**Treatment**	**T.GPOX/C.GPOX**
	Average	**0.006**	**0.006**	**1.149**	**0.255**	**0.267**	**1.254**	**0.164**	**0.201**	**5.438**
	C.hardwickki									
1	IC-331626	0.010	0.010	1.053	0.320	0.323	1.011	0.086	0.174	3.125
	Overall SD	**0.004**	**0.003**	**0.249**	**0.067**	**0.093**	**0.059**	**0.038**	**0.033**	**0.507**
	Overall Average	**0.005**	**0.005**	**1.045**	**0.201**	**0.190**	**0.919**	**0.112**	**0.159**	**5.331**

4.2 Correlation Analysis

Correlation study among all the morphological and biochemical parameters viz. root length, root fresh weight, root dry weight, shoot length, shoot fresh weight, shoot dry weight, Catalase, Guauicol Peroxidase (GPOX), Ascorbate peroxidase (APOX) under control and stress conditions has been presented below.

In *C.utilissimus* species significant correlation was found in various traits as RLC was associated with RLT, RDWT, SLC, SFWT, SDWC and RLT was with RFWT, RDWT and SFWT. CCA and CAA showed correlation with TCA and TAA.

In *C.momordica* highly significant correlation was observed for RLT with RFWC, RDWT and RFWT showed with RDWT. Correlation between TAA and RLT and RFWT was observed. TGA showed correlation with SLC and SDWC.

In *C.callosus* less correlation was observed between various traits. Correlation between RLT and RFWC, RFWT was observed along with correlation between RFW and RFWT, RFWT and RDWT, SLT and SDWT was also observed.

In *C.melo* species correlation study was done for a number of traits. Significant correlation was observed between various traits as RLC has shown with RFWT, RDWC, and RFWC has shown with RDWT, SDWT, CGA and TGA.SLC was associated with CCA, SLT, TCA. Antioxidants enzymes was also significantly correlated with each other.

Correlation was also observed in *C.sativus* species genotypes. significant correlation was observed in RLC associated with RFWC,RFWT, SDWT and RLT was associated with RFWC and CAA. Activity of antioxidant enzymes were found be associated with RFWC, RFWT, SDWT, SLC. correlation between CCA and TCA was also found.

Overall correlation taking all the species of *cucumis* together did not show correlation with tolerance level however; significant correlations among a number of traits were observed. Root length in control was associated with RLT, RFWT, RDWT and SLC, where as root length in treatment was associated with RFWC, SLT, SFWT and SDWT in addition to above parameters for RLC. Root fresh weight in control (RFWC) was associated with most growth parameters and enzymes but not the RDWC. On the contrary, Root fresh weight in treatment (RFWT) had positive correlations only with RLC and RLT where as RDWT had significant correlations with various growth parameters and enzyme activity (Table 4.3).

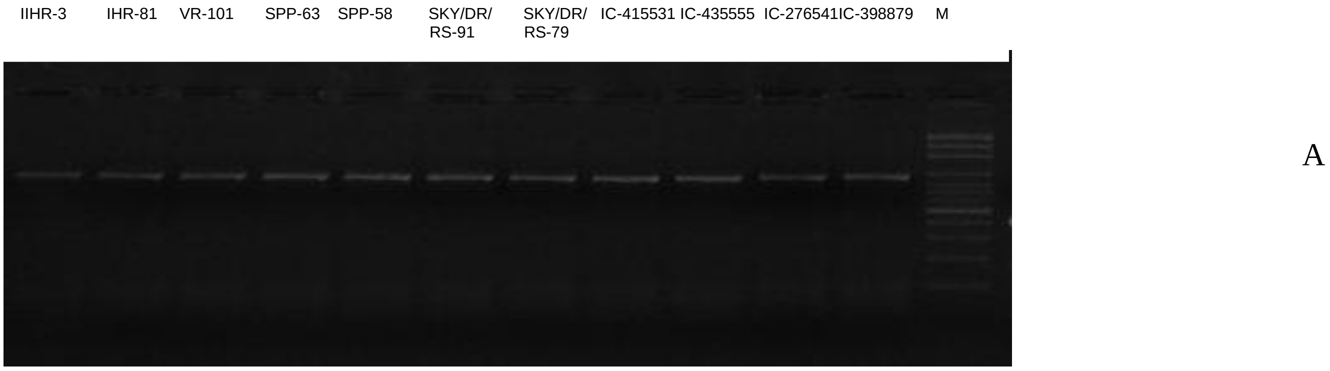
Table 4.3 correlation study among various morpho-physiological and biochemical parameters of various *Cucumis Species* under drought stress

	RLC	RLT	RFWC	RFWT	RDWC	RDWT	SLC	SLT	SFWC	SFWT	SDWC	SDWT	CCA	TCA	CAA	TAA	CGA	TGA	RLT/ RLC	RFWT/ RFWC	RDWT/ RDWC	SLT/ SLC	SFWT/ SFWC	SDWT/ SDWC	RANK
RLC	1																								
RLT	.747(**)	1																							
RFWC	0.167	0.203	1																						
RFWT	0.201	0.256	.409(*)	1																					
RDWC	-0.118	0.014	0.096	0.112	1																				
RDWT	.437(**)	.407(*)	-0.101	.353(*)	0.103	1																			
SLC	.672(**)	.606(**)	.343(*)	0.328	-0.008	0.227	1																		
SLT	0.242	.418(*)	.371(*)	0.173	-0.138	-0.239	.572(**)	1																	
SFWC	0.331	0.202	-0.247	-0.102	0.115	0.009	.371(*)	0.145	1																
SFWT	.405(*)	.442(**)	.578(**)	.395(*)	0.008	0.093	.519(**)	.507(**)	0.214	1															
SDWC	.520(**)	.515(**)	.630(**)	0.167	0.011	0.049	.522(**)	.340(*)	0.165	.417(*)	1														
SDWT	0.076	0.159	.360(*)	0.137	-0.12	-0.032	0.2	.334(*)	0.306	.648(**)	0.163	1													
CCA	0.166	0.247	.390(*)	0.118	-0.104	-0.112	0.23	.346(*)	-0.076	.409(*)	0.254	.455(**)	1												
TCA	0.175	0.277	0.295	0.057	-0.146	-0.041	0.158	0.279	-0.126	.409(*)	0.255	.373(*)	.806(**)	1											
CAA	-0.068	0.037	.747(**)	0.304	-0.132	-0.266	0.147	0.257	-.384(*)	.383(*)	.357(*)	0.25	0.28	0.217	1										
TAA	-0.063	-0.221	0.021	0.075	0.097	0.246	-0.006	-0.127	-0.229	-0.007	0.044	-0.25	-0.25	-0.259	0.172	1									
CGA	-0.129	-0.134	.544(**)	.422(*)	0.251	0.021	0.141	0.003	0.142	.515(**)	0.21	.420(*)	0.049	0.01	.383(*)	0.181	1								
TGA	0.097	-0.067	-0.101	0.196	.338(*)	.478(**)	0.092	-0.188	-0.125	0.032	-0.077	-0.297	-.352(*)	-0.283	-0.179	.729(**)	0.233	1							
RLT/RLC	-0.278	.369(*)	0.004	0.024	0.083	-0.046	-0.005	0.238	0.015	0.152	-0.017	0.253	0.136	0.182	0.148	-0.319	0.051	-0.327	1						
RFWT/RFWC	-0.216	-0.145	-.417(*)	-0.012	-0.276	0.224	-.344(*)	-0.179	-0.26	-0.313	-.363(*)	-0.084	-0.191	-0.09	-0.23	0.113	-0.182	0.074	0.124	1					
RDWT/RDWC	.346(*)	0.246	-0.227	0.149	-.503(**)	.708(**)	0.075	-0.139	-0.094	-0.021	-0.093	0.071	0.001	0.077	-0.22	0.086	-0.16	0.187	-0.028	.524(**)	1				
SLT/SLC	-.478(**)	-0.243	-0.145	-0.21	-0.111	-.384(*)	-.577(**)	0.276	-0.274	-0.081	-.345(*)	0.081	-0.01	0.056	-0.031	-0.048	-0.159	-0.12	0.227	.404(*)	-0.117	1			
SFWT/SFWC	0.06	-0.071	.359(*)	0.121	-0.061	-0.115	0.048	0.143	-.352(*)	0.329	-0.161	0.175	0.207	0.165	.397(*)	-0.002	0.11	0.122	-0.177	-0.166	0.015	0.064	1		
SDWT/SDWC	-.339(*)	-0.271	-0.108	-0.036	-0.103	-0.11	-0.298	-0.053	0.153	0.226	-.519(**)	.613(**)	0.078	0.048	0.015	-0.245	0.301	-0.167	0.247	0.271	0.085	.354(*)	0.317	1	
RANK	0.193	0.184	-0.138	0.227	-0.029	0.242	0.036	0.041	-0.158	-0.031	0.03	-.425(*)	-0.157	-0.052	0.057	0.276	-0.214	0.168	-0.019	.382(*)	0.219	0.103	-0.063	-0.264	1

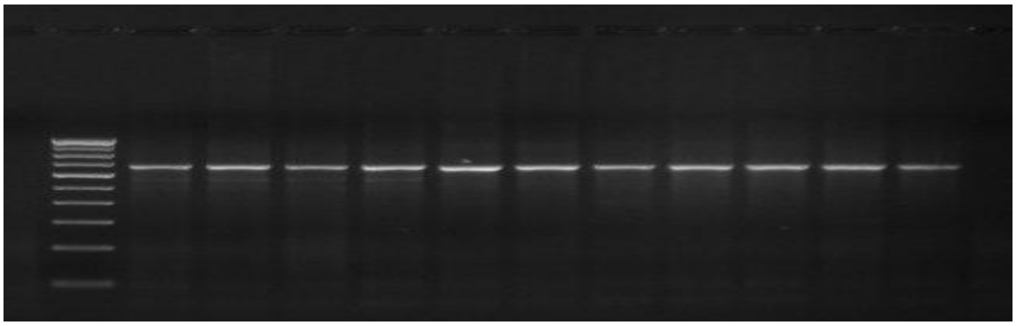
4.3 DNA isolation And PCR amplification

Total genomic DNA was isolated from the selected 11 genotypes (IIHR-3, IIHR-81, VR-101, SPP-63, SPP-58, SKY/DR/RS-91, SKY/DR/RS-79, IC-415531, IC-435555, IC-276541, IC-298779) representing various species and differing for catalase activity, with the CTAB method described by Doyle and Doyle (1990) and treated with RNase to eliminate RNA. DNA concentration was measured by UV absorbance method. the integrity of the isolated DNA was verified by visualization of DNA on Agarose gel (0.8%) with DNA standard lambda uncut. The quantity of DNA was determined as the ratio A_{260}/ $A_{280.}$ The quantified DNA was diluted to 25 ng/µl and using 2 µl of this per reaction for the PCR amplification.

Temperature gradient was set to obtained specific annealing temperature for each set of Catalase and transcriptional factors (ERF1 and WRKY) primers using SPP-63 as a template DNA. PCR for each set of Catalase and the TF (transcription factor) primers were done at their specific annealing temperature, amplified PCR product were verified on 1.5% Agarose gel. TF's showed genotype specificity in amplification. Amplified products of Catalase gene were sent for sequencing.



M IIHR-3 IIHR-81 VR-101 SPP-63 SPP-58 SKY/DR/ RS-91 SKY/DR/ RS-79 IC-415531 IC-435555 IC-276541 IC-398879



M IIHR-3 IIHR-81 VR-101 SPP-63 SPP-58 SKY/DR/ SKY/DR/ IC-415531 IC-435555 IC-276541 IC-398879
RS-91 RS-79

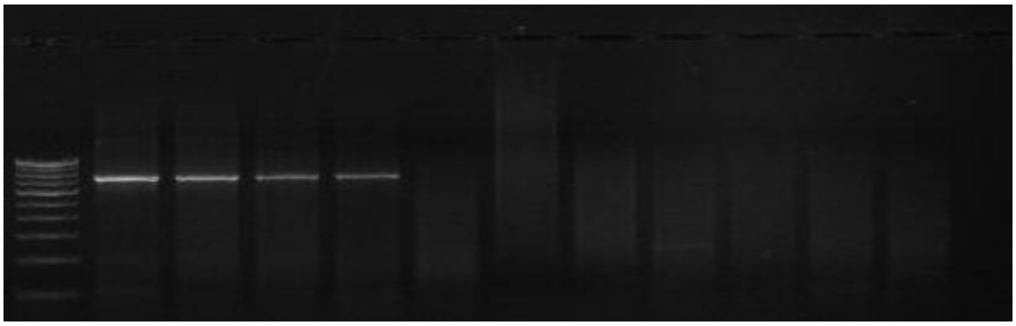
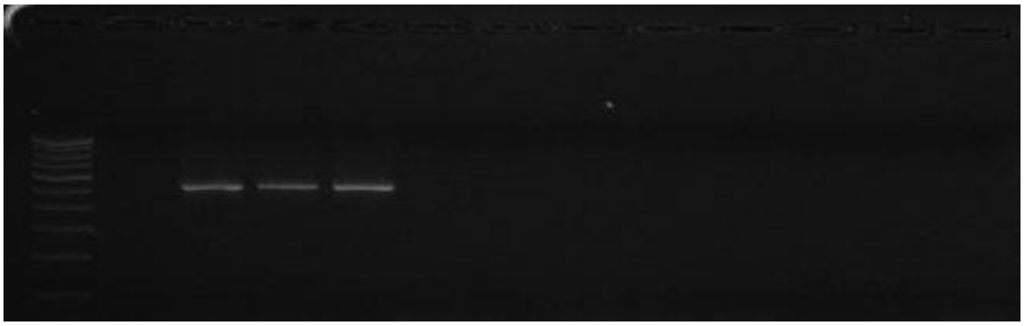
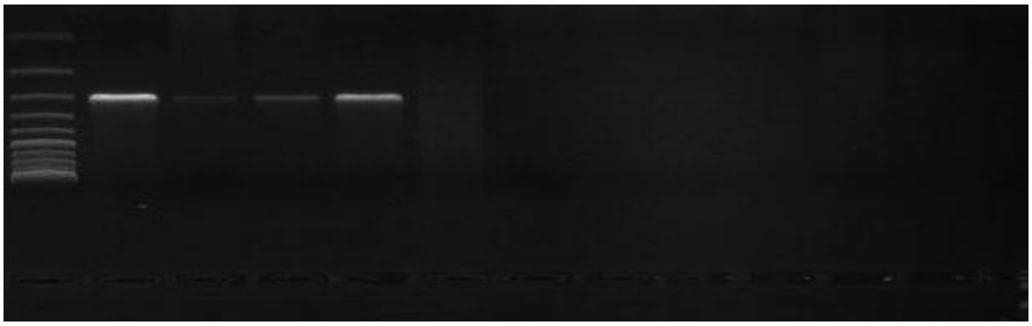


Figure 4.2: The PCR amplified products of Catalase primers for various genotypes of *Cucumis spp.*

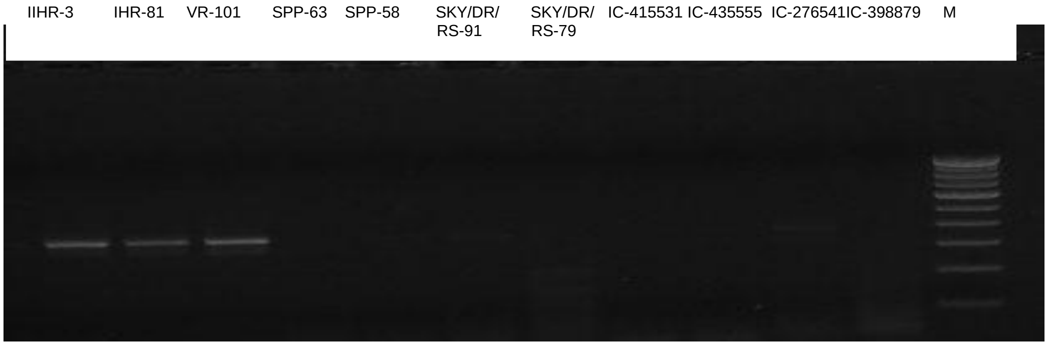
M IIHR-3 IIHR-81 VR-101 SPP-63 SPP-58 SKY/DR/ SKY/DR/ IC-415531 IC-435555 IC-276541 IC-398879
RS-91 RS-79



M IIHR-3 IIHR-81 VR-101 SPP-63 SPP-58 SKY/DR/ SKY/DR/ IC-415531 IC-435555 IC-276541 IC-398879
RS-91 RS-79



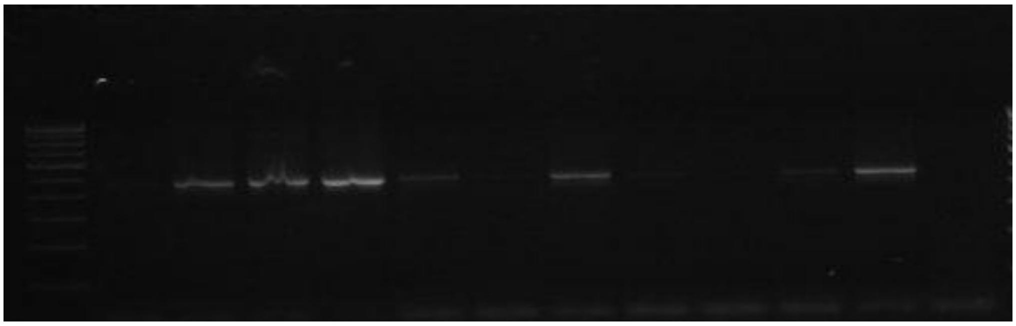
B



C

Figure 4.3: **The PCR amplified product of WRKY Transcription factor primer sets in various genotypes of *Cucumis spp.***

M IIHR-3 IIHR-81 VR-101 SPP-63 SPP-58 SKY/DR/ RS-91 SKY/DR/ RS-79 IC-415531 IC-435555 IC-276541 IC-398879



A

B

M IIHR-3 IIHR-81 VR-101 SPP-63 SPP-58 SKY/DR/ RS-91 SKY/DR/ RS-79 IC-415531 IC-435555 IC-276541 IC-398879

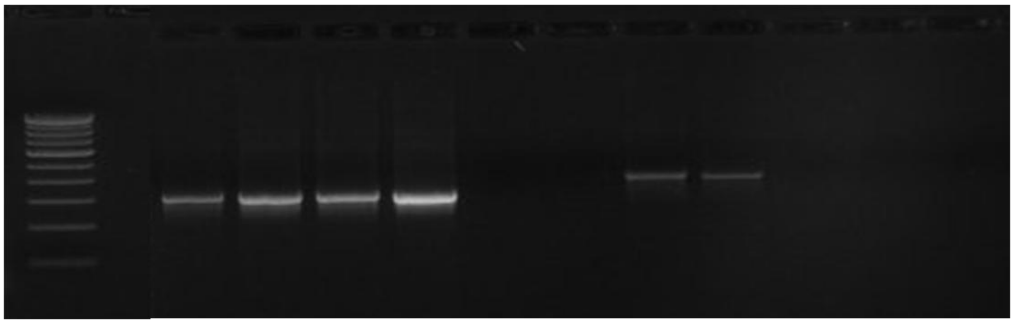


Figure 4.4: The PCR amplified product of ERF/APTELA primer sets in various genotypes of *Cucumis spp.*

4.4 Sequencing and sequence analysis

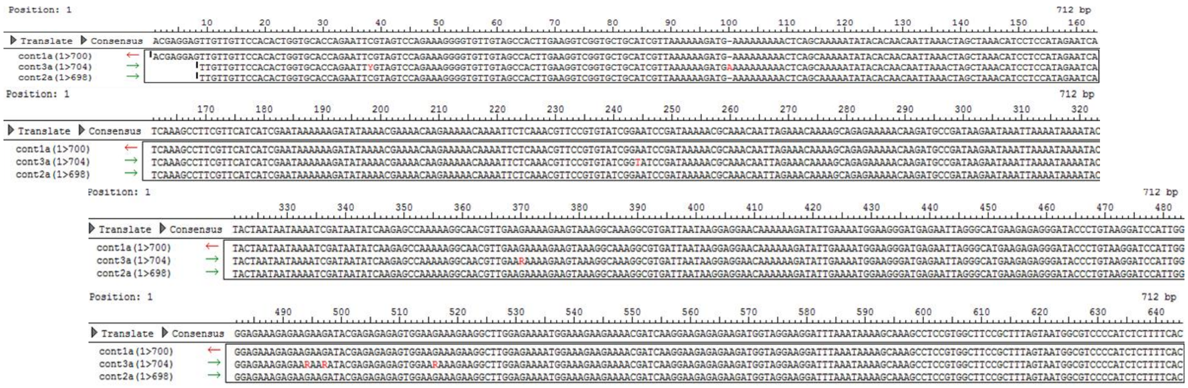
The genome database searched with catalase EST of *C.melo* identified full sequence of catalase of 2674 bp in the contig 8134 of *C.sativus* genome (http://www.ncbi.nlm.nih.gov genome/1639). In order to sequence full gene from different species and genotypes differing in catalase activity, 5 primer sets were developed from overlapping sequences. The amplicon size of these primer pairs ranged from 307-781 bp and these primer sets were named as a, b, c, d and e. The amplified products from selected genotypes of 5 species were sent for custom sequencing. Only 3 amplicon could be sequenced covering approximately 2200 bp of the gene. However, first amplicon could be sequenced only in two species *C.melo* (two genotypes) and one *C.sativus spp.* genotypes. Second amplicon was sequenced in four genotypes; two of *C.melo* and two *C.sativus*, while third amplicon was sequenced for genotypes from different species. The sequences amplified by same primer set were further clustered into contigs (Seqman^{TM}II 5.08, DNASTAR.Inc. Lasergene Gene Corporation, Ann Arbor, MI software), three contigs of different size were obtained viz. contig 1 (712 bp) containing three sequences, contig 2 (784 bp) containing four sequences and contig 3 (669 bp) containing 9 sequences. A sequence comparison revealed species specific variations as well as SNPs associated with catalase activity.

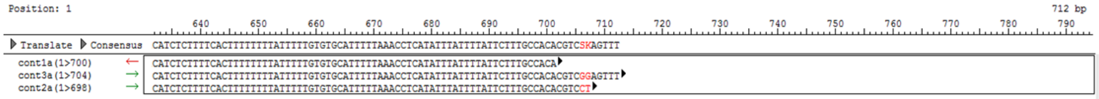
In *C.melo* genotypes (IIHR-81 and IIHR-3) no SNPs were detected in contig 1 and in contig 2 i.e. the coding region of the gene that were; transitions (A/G) and transversion (G/T), (A/T), (A/C) were found at 347,534, 556 sequence position and substitution of “A” nucleotide with ambiguous nucleotide was observed at sequence position 526 and 529 in IIHR-3. In contig three variation between these two genotypes were more in number than contig two. At sequence position 148 insertion and at 519-520, 523 substitution with ambiguous nucleotide bases in IIHR-3 bases was observed. At sequence position 71, 143, 147 and 594 variation in IIHR-81 genotype sequence were observed.

Genotype specific SNPs and indels were observed for *C. sativus* (VR101, SPP-58 and SPP63); VR-101 and SPP-58 genotype showed similar pattern in sequence while SPP-63 followed a different pattern. In contig two at sequence position 263, 295, 324, 637-642, 680-681 variation between VR-101 and SPP-63 were observed. In contig 3 substitution with ambiguous nucleotide bases were observed in these three genotypes. At sequence position 18, 19, 20, 54, 439, 528, 531, 533 transitions, transversion with ambiguous and unambiguous nucleotide bases were observed in SPP-58 sequence. At sequence position 107, 212, 261, 289, 406, 433, 538 and 564 variations in VR-101 was observed. Maximum number of variations in sequence was observed for SPP-63 compare to VR-101 and SPP-58. At sequence position 28, 37, 46, 186, 228, 230, 236, 264, 265, 276,281, 282, 284, 291, 292, 304, 356, 359, 362, 377, 384, 398, 408, 412, 413, 418, 422, 455, 464, 469, 480, 490, 503, 507, 557, 559, 579, 600 and 630 variation in the form of substitution and deletions in SPP-63 was observed.

Least variation were found between the two genotypes i.e. SKY/DR/RS-91 and SKY/DR/RS-79 of *C. melo var callosus*. insertion of ambiguous nucleotide base was observed in SKY/DR/RS-91 at sequence position 406 while in SKY/DR/RS-79 nucleotide base “A” was present (Fig 4.7).

In *C.melo var. momrdica*, various transition and transversions were found in IC-415531 and IC-415555 along with a number of deletions. Transitions and transversions were observed at sequence position 28, 37, 46, 136, 186, 228, 230, 236, 264, 265, 276,281, 282, 284, 291, 292, 304, 356, 359, 362, 377, 384, 398, 406, 408, 412, 413, 418, 422, 455, 464, 469, 480, 490, 503, 507, 519, 520, 527, 528-531 557, 559, 579, 600 and 630, were observed between 415531and 415555 genotypes sequences (Fig 4.7).





```
Position: 1                                                                                         712 bp
                     640       650       660       670       680       690       700       710       720       730       740       750       760       770       780       790
Translate Consensus  CATCTCTTTTCACTTTTTTTTATTTTTGTGTGCATTTTTAAACCTCATATTTATTTTATTCTTTGCCACACGTCSKAGTTT
cont1a(1>700)   ←    CATCTCTTTTCACTTTTTTTTATTTTTGTGTGCATTTTTAAACCTCATATTTATTTTATTCTTTGCCACA
cont3a(1>704)   →    CATCTCTTTTCACTTTTTTTTATTTTTGTGTGCATTTTTAAACCTCATATTTATTTTATTCTTTGCCACACGTCGGAGTTT
cont2a(1>698)   →    CATCTCTTTTCACTTTTTTTTATTTTTGTGTGCATTTTTAAACCTCATATTTATTTTATTCTTTGCCACACGTCCT
```

Fig 4.5 (a-e): contig one showing variations between the genotypes IIHR-3(1a), IIHR-81 (2a) and VR-101 (3a).

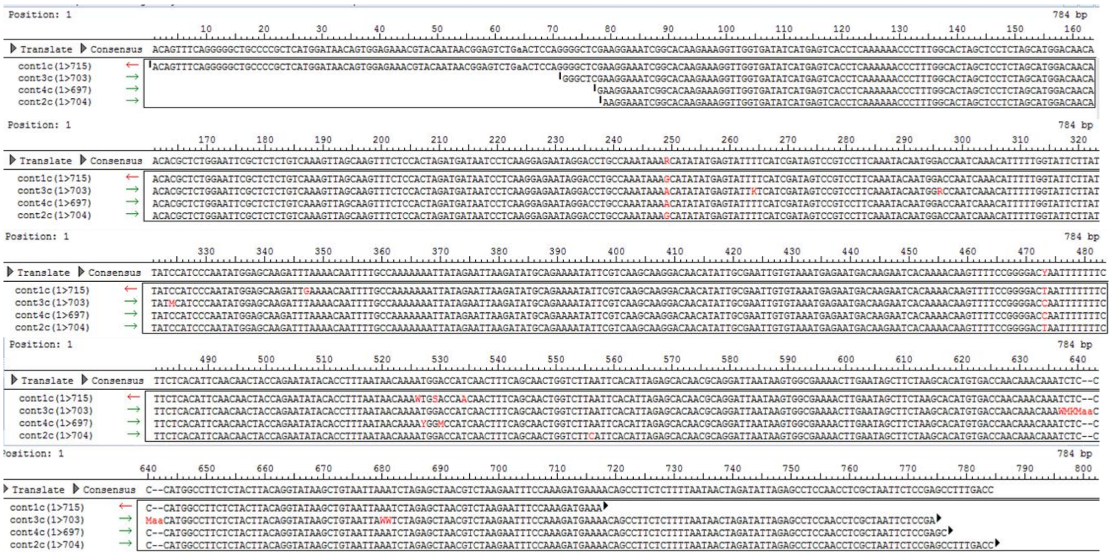


Fig 4.6 (a-e): Sequences showing SNPs between the genotypes IIHR-3(1c), IIHR-81(2c) ,VR-101(3c), SPP-63(4c).

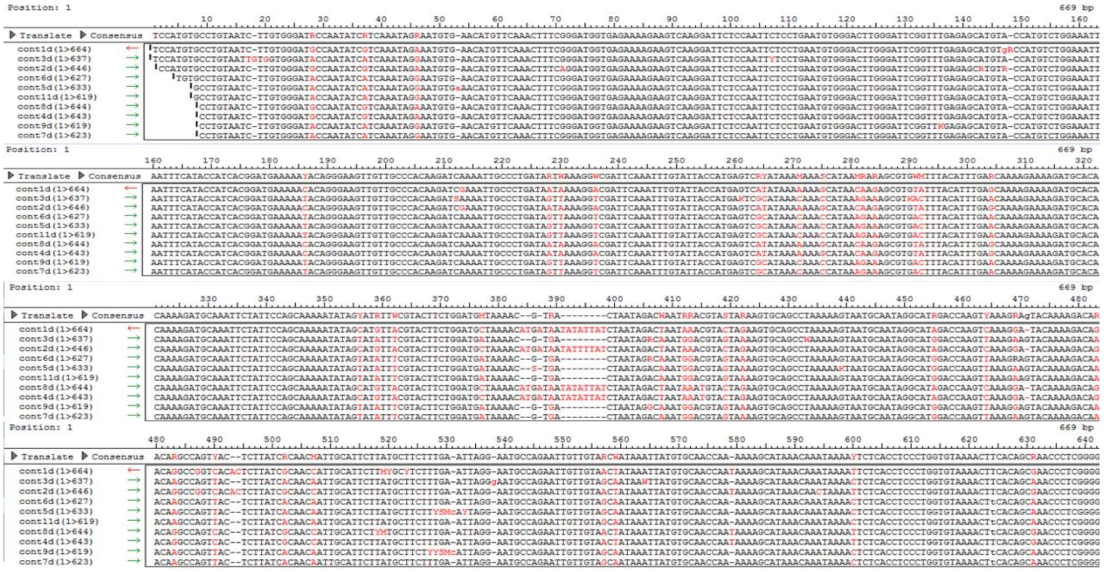


Fig 4.7 (a-d): sequences showing SNPs between the various genotypes of *Cucumis spp.* in contig three*.*

Variation between the different *spp.* of cucumis was also has been observed. Maximum variation between the species was observed in our experimentation between *C.melo* and *C.sativus spp.* At sequence position 249, 473 in contig two and 213 in contig three variation in nucleotide bases was observed between these two *spp.* At sequence position 213 variation between *C.melo* and other species was also observed. Variation between *C.melo* and *C.callosus was* observed at sequence position 28, 37, 46, 136, 186, 228, 230, 236, 264, 265, 276,281, 282, 284, 291, 292, 304, 356, 359, 362, 377, 384, 398, 406, 408, 412, 413, 418, 422, 455, 464, 469, 480, 490, 503, 507, 519, 520, 527, 528-531 557, 559, 579, 600 and 630 (Fig 4.6). SNPs between different genotypes of *Cucumis spp.* summarised in Table no. 4.4.

Table4.4: SNPs detected between different genotypes of *Cucumis spp.*(1- IIHR-3, 2-IIHR-81, 3-VR1-01, 4-SPP-58, 5-SPP-63, 6-SKY/DR/RS-91, 7-SKY/DR/RS-79) 8-IC-415531, 9-IC415555)

Genotype	Mismatches	C/T	A/G	Transition	A/T	A/C	C/G	T/G	Transversion	Indel
1c-2c	5	0	0	0	1	1		1	3	2
3c-4c	13	0	0	0	0	0	0	0	0	13
1d-2d	9		1	1		1			1	7
3d-4d-5d	64	7	18	25	5	3	2	1	10	29
6d-7d	1	0	0	0	0	0	0	0	0	1
8d-9d	55	5	19	24	6	5	2	0	13	18

5. DISCUSSION

Drought stress is one of the most serious abiotic stresses that causes reduction in plant growth, development, and yield in many parts of the world (Gong *et al*., 2005; Martinez *et al*., 2007; Kusvuran *et al*., 2011), due to this the productivity of the agriculture is also being seriously affected. Plants adopt a number of strategies to survive such adverse conditions in this regard adaptive inducible mechanism has the importance for its amenability to manipulation. However, constitutive mechanism inbuilt with the plant structure could also be used to develop tolerant plant type.

The present study has shown that species and genotypes differ for their tolerance level to water stress. Among five tolerant genotypes three genotypes were of *C.prophetarum* while in others species tolerant types were rare in number. Overall 44 genotypes 11.36% were tolerant, 29.54% were moderately tolerant and 38.63% were found moderately susceptible and 20.45% were susceptible. This classification is based on stress symptoms developed by the plant under water deficit.

Under water deficit condition reduction in plant growth was observed in term of reduced RL, SL, RFW, SFW, RDW, SDW, loss in metabolic activities occur during water stress, this severely affect the cell division, enlargement and differentiation of plants. Reduction in plant growth under water stress was also observed in pearl millet (Kusaka *et al.,* 2005) and *Abelmoschus esculentus* (Bhatt and Srinivasa Rao, 2005) supporting the present outcome of the study.

Root length is considered to be one of the most important characters for drought stress tolerance because roots are in direct contact with the soil and enhance the ability of the plant to capture water. It is the main supplier of nutrients all over the plant. However, in our study a decrease in root length and shoot length was observed under water deficit condition. This decrease in root and shoot length under water stress may be due to reduction in mitotic cell division during water stress condition.Decreased root length due to water stress was also noted in wheat and maize (Nayar and Gupta, 2006), decrease shoot length was also noted in *Erythrina* seedlings (Muthuchelian *et al.,* 1986) under water stress. Moreover, root length was not associated with tolerance level and hence may not be important for drought tolerance level per se in this *spp.* This could be because of evolutionary reasons. At the same time correlation studies further show that root length was significantly correlated with root dry weight and shoot length in treated plants supporting the earlier observations that root length is important for water and nutrient supply to plant for growth under stress.

In our study reduction in fresh weight and dry weight of root and shoot was also observed. This decrease in FW under drought condition may be a consequence of suppression of cell expansion and cell growth due to the low turgor pressure. Reduction in fresh weight under drought stress in wheat was also observed (Rane *et al*., 2001). Similar results were also observed in pearl millet (Kusaka *et al*., 2005) and *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005). Root dry weight and shoot fresh weight was significantly correlated with GPOX activity in control plants. Probably increased activity of antioxidents could support metabolic activity of plant thereby accumulation of more dry matter.

The drought leads to increased the formation of reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), O_2 – (superoxide) and OH (hydroxyl) radicals, through enhanced leakage of electrons to molecular oxygen (Arora *et al*., 2002), excessive ROS production can cause oxidative stress, which damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Yordanov *et al*., 2000), these by inducing protective mechanism against these ROS.

The balance between reactive oxygen species production and activities of antioxidant enzymes determines whether the oxidative signalling and/or damage will occur (Moller *et al*., 2007). The reactions of the plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Chaves *et al*., 2003; Dacosta & Huang, 2007).

Detoxification of reactive oxygen species in plants, induces enzymatic mechanisms such as superoxide dismutase, catalase, ascorbate Peroxidase and Peroxidase. An overall increase in the activity of CAT and GPOX was observed in the present study.

There were significant variations in catalase activity among different genotypes under both stressed and non-stressed conditions. Increased catalase activity was found in 40 genotypes while it was decreased in 4 genotypes under water deficit condition. Increase in catalase activity could be because of high rate of H_2O_2 production as a result of water stress. Catalase eliminates H_2O_2 by decomposing it directly to water and oxygen. However, its activity was not associated with genotype tolerance level. Catalase is essential for the removal of H_2O_2 produced in the peroxisomes by photorespiration (Noctor *et al*., 2000; Yasar *et al*., 2006; Amirjani, 2010). Increase in catalase activity is related with increase in stress tolerance (Upadhyay *et al.,*1990; Olmos *et al.,*1994; Kraus, 1995; Kumutha *et al.,* 2009). The observation of catalase activities may increase, decrease or remain unchanged under drought stress by Zhang and Kirkham, (1996) supports our findings. This could probably happen due to accumulation of mutations in promoter or structural genes in the genotypes bred or maintained for higher yields under proper management.

The APOX activity was found to be increased in 19 genotypes while decreased in 25 genotypes. APOX reduces H_2O_2 to water by ascorbate as a specific electron donor. Increased APOX activity has been reported to play a role in resistance to oxidative stress in *Radix Astragali* (Yong *et al*., 2006), tomato (Sanchez-Rodriguez *et al*., 2010) and almod (Sorkheh *et al.*, 2010), *Zea mays* (Jiang and Zhang, 2002), wheat (Dalmia and Sawhney, 2004), *P. accutifolius* (Turkan *et al.,* 2005) and soybean (Riekert van Heerden and Kruger, 2002) under water stress. Average decrease in APOX activity in *Cucumis spp.* could be an indication that this mechanism might be controlled by metabolic conditions. However, its level is correlated with growth parameters in most of the spp. This difference could be attributed to evolutionary history of different spp.

The GPOX activity was found to increase in 40 genotypes and decrease in 4 genotypes. Increased in activity of GPOX may be due to high ROS formation due to drought stress. Higher peroxidase activity was also observed in soybean (Zheng and Han, 1997) and ground nut (Reddy *et al*., 2003) under drought stress.

In order to find out allelic differences for catalase activity level, the gene sequence was obtained for two genotypes (higher and low catalase) each from 5 species. Since some SNPs in the coding region (cSNPs) may have functional significance if the resulting amino acid change causes the altered phenotype and are assumed to be primarily responsible for phenotypic differences between individual of the same species (Collins *et al.,* 1998; Brookes, 1999; Marth *et al*., 1999; Picoult-Newberg *et al*., 1999).

In the present study species specific SNPs and indels were observed for *C.prophetarum, C.utilissimus, C.momordica, C.melo and C. sativus*. SNPs in the form of transitions, transversion and indels were observed between the genotypes of same species and between different *Cucumis spp*. These SNPs could be the key factors in variations of catalase enzyme activity in the genotypes under water stress because SNP in coding region may directly impact a relevant protein, and can influence gene expression (Krawezak,1992; Drazen, 1999). The uneven distribution of SNP in coding and non-coding regions was also identified in other crops (Kanazin *et al*., 2002; Zhu *et al*., 2003; Salmaso *et al*., 2004).

The failure of amplification of two transcription factors (WRKY and ERF) using *C.melo* sequence based primers is suggestive of considerable evolutionary diversification in different species of cucumis. However, allele mining is an important tool to find out the single nucleotide variation in coding and non-coding region of a genome and their proficient role in stress tolerance. SNP mining is useful for producing high yield of agriculturally improved varieties, high resolution genetic map construction, diversity analysis, cultivar identification and characterization of genetic resources and also in genetic diagnosis.

It can be concluded from the present study that none of the morpho-physiological traits define drought tolerance in a species or genotype even. It is rather evolution of number of factors governing various traits provide tolerance to specific genotype. Moreover, different antioxidant enzymes may evolve at different rates in various species hence it is difficult to locate SNPs or haplotypes associated with enzyme efficiency over the species. It would be appropriate to include large number of genotypes from the same species to define haplotypes and associate them with enzyme activity.

6. SUMMARY AND CONCLUSION

The present investigation was carried out to understand the role of morpho-physiological parameters and allelic variation in catalase gene for drought stress tolerance. 62 genotypes were grown in control condition of green house at 30ºC, and one set of this were subjected to water stress.

1. Amongst 44 genotypes accessed for their drought tolerance level 11.36% were tolerant genotypes, 29.54% were moderately tolerant and 38.63% were found moderately susceptible and 20.45% were susceptible.
2. Decrease in root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight was observed under water stress.
3. Antioxidant enzymes Catalase, APOX and GPOX activity was measured under control and stressed condition. Increased activity of these enzyme were observed.
4. Increased catalase activity compared to control was observed in stressed plants. In 40 genotypes increase in catalase activity was noted while in four genotypes decreased activity of catalase was observed.
5. Activity of APOX enzyme was also measured in control as well as in stress condition. Increased activity of APOX was observed in 19 genotypes while in 25 genotypes APOX activity was decreased.
6. GPOX activity was also observed for control as well as stressed condition. In stress condition increased in GPOX activity was observed in 40 genotypes while in four genotypes decreased activity was noted.

7. In our study overall correlation of all the species of *cucumis* together did not show correlation with tolerance level however; significant correlations among a number of traits were observed.

8. 5 overlapped specific primers for catalase gene was designed with primer 3 tool from NCBI home. Temperature gradient was set to obtained specific annealing temperature for each set of primers.

9. In our study transcriptional factors were also undertaken viz. ERF1 and WRKY TF’s. these factors showed genotype specificity in amplification.

10. SNPs were observed between the genotypes of same species and between the different *Cucumis* species in the coding region of the sequence. A number of transitions, transversion and indels at different sequence positions were observed for different genotypes of *cucumis* species. These variations may be responsible for variation in catalase enzyme activity of *Cucumis* genotypes. Between the different species variation was noted between *C.melo* and *C.sativus* and *C.melo* and *C.*callosus species.

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Allele mining for drought related antioxidant genes in *Cucumis spp.*

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ABSTRACT

A study was conducted to evaluate 44 genotypes of Ten *Cucumis spp.* with respect to drought tolerance and to determine its effect on various morpho-physiological (root length, shoot length, root and shoot fresh and dry weight) and biochemical (Catalase, Ascorbate peroxidase and Guiaicol peroxidase) parameters. Of these 44 genotypes 11.36% were tolerant, 29.54% were moderately tolerant and 38.63% were moderately susceptible and 20.45% were susceptible. Decrease in root and shoot length, root and shoot fresh weight, root and shoot dry weight was observed in most of the genotypes. The activity of Catalase and GPOX was increased in water deficit conditions while the activity of APOX decreased most of the genotypes. Though none of the growth parameters and enzyme activity was correlated with tolerance level, various morpho-physiological and biochemical parameters, were associated under control and stress conditions. Allelic variations in the form of SNPs were observed between and within various *Cucumis spp.* studied. Transition, transversion and indels were obtained at different positions in the sequences. Variation between genotypes were observed at various sequence positions while in between the species variations was observed for *C.melo* and *C.sativus* and *C.callosus*. These variation could be important for the dissimilarity of catalase enzyme activity in different genotypes of *Cucumis spp.* under water stress condition.

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APPENDIX

List of solution used for experimental analysis

1. **TBE 5x Stock**

   Tris base – 54 gms

   Boric Acid – 27.5 gms

   0.5M EDTA – 20 ml

   Volume was made upto 1 litre and Autoclaved

2. **Chloroform : Isoamyl alcohol (24: 1)**

   To make 500 ml 480 ml of Chloroform and add 20 ml of Isoamyl alcohol was added.

3. **70% Alcohol**

   70 ml of Absolute alcohol with 30 ml of ddH_2O, Mixed properly.

4. **0.5 M EDTA (pH – 8.0)**

   186.1 gms of Disodium Ethylene Diaminetetra Acetate. 2 H_2O to 800 ml of H_2O. Shaked vigoursly on a magnetic stirrer for several hr. Adjusted the pH to 8.0 with NaOH. Dispensed into aliquots and sterilized by autoclaving.

5. **1 M Tris**

   Dissolved 121.1 gms of Tris in 800 ml of H_2O. Adjusted the pH to desired value by adding concentrated HCl.

PH	HCl
7.4	70 ml
7.6	60 ml
8.0	42 ml

   Adjusted the volume of the solution to 1 litre with ddH_2O. Dispensed into aliquots and sterilized by autoclaving.

6. **4 M NaCl**

233 gms of NaCl in 800 ml of H_2O. Adjusted the volume to 1 litre with ddH_2O. Dispensed into aliquots and sterilized by autoclaving.

7. **T_{10} E_1 pH – 8.0**

1 ml of 1M Tris (pH – 8.0) added to the 200µl 0.5 M EDTA – pH – 8.0 and made the volume 100 ml.

8. **ß – mercaptoethanol (BME)**

It is obtained as 14.4 M solution. Stored in a dark bottle at 4°C.

9. **Ethidium Bromide (10 mg/ml)**

Added 1 gm of Ethidium Bromide to 100 ml of H_2O. Stirred on magnetic stirrer for several hr to ensure that the dye has dissolved. Wrap the container in aluminum foil or transferred the solution in dark bottle and stored at room temperature.

10.

Agarose 0.8 % and 1.5 %

0.8 gms in 100 ml of 1 x TBE

1.5 gms in 150 ml of 1 x TBE

11.

Gel loading dye (10X)

0.25 gms of Bromophenol Blue

0.25 gms of Xylene cyanol

50% glycerol

Made 100 ml final volume with 50% glycerol

12.

Assay buffer for Taq DNA polymerase (10 X)

100mM Tris- HCl (pH – 9.0)

15 mM $MgCl_2$

500mM KCl

0.1 % Gelatin

13. **TE buffer**

Tris-Cl pH 8.0

EDTA