Breeding for drought tolerance in cocoa (*Theobroma cacao* L.)

By

JUBY BABY

2016-11-042

THESIS

Submitted in partial fulfillment of the
requirement for the degree of

Master of Science in Agriculture

(Plant Breeding and Genetics)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA
2019

DECLARATION

I, hereby declare that this thesis entitled **'Breeding for drought tolerance in cocoa (*Theobroma cacao* L.)'**, is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
22/02/2019

Juby Baby
(2016-11-042)

CERTIFICATE

Certified that this thesis entitled **'Breeding for drought tolerance in cocoa (*Theobroma cacao* L.)',** is a record of research work done independently by **Ms. Juby Baby,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara
22/02/2019

Dr. Minimol J. S.
(Major Advisor)
Associate Professor
(Plant Breeding and Genetics)
Cocoa Research Centre,
Vellanikkara, Thrissur

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Juby Baby**, (2016-11-042), a candidate for the degree of Master of Science in Agriculture, with major field in Plant Breeding and Genetics, agree that the thesis entitled "**Breeding for drought tolerance in cocoa (*Theobroma cacao* L.)**" may be submitted by Ms. Juby Baby, in partial fulfilment of the requirement for the degree.

Dr. Minimol J. S
(Major Advisor)
Associate Professor
(Plant Breeding and Genetics)
Cocoa Research Centre, Vellanikkara, KAU

Dr. Jiji Joseph
Professor and Head
Plant Breeding and Genetics
College of Horticulture
Vellanikkara, KAU

Dr. A. V. Santhoshkumar
Professor and Head
Forest Biology and Tree Improvement
College of Forestry, Vellanikkara, KAU

Dr. B. Suma
Professor and Head
Cocoa Research Centre
Vellanikkara, KAU

22/2/19

Dr. C. Mohan
(External examiner)
Principal Scientist
Division of Crop Improvement
ICAR-CTCRI, Trivandrum

ACKNOWLEDGEMENT

I bow my head before the Lord Almighty whose grace and enlightment had endowed me the inner strength and confidence to complete my thesis work successfully.

With immense pleasure and gratitude, I grab this opportunity to express my deep sense of gratitude and indebtness to my major advisor **Dr. Minimol J.S.,** *Associate Professor, Cocoa Research Centre, Vellanikkara for her valuable suggestions, ever - willing help, inspiring guidance, constructive criticisms, constant encouragement and above all, understanding and whole hearted co-operation rendered to me during the course of my research work.*

I consider it as my privilege to express my deep felt gratitude to **Dr. Jiji Joseph**, *Professor and Head, Plant Breeding and Genetics, College of Horticulture, Vellanikkara and member of my advisory committee for her constant support, whole hearted co-operation and encouragement provided throughout the course of the M.Sc. work.*

With deep reverence, I express my heartfelt thanks to **Dr. A. V. Santhosh Kumar**, *Professor and Head, Forest Biology and Tree Improvement, College of Forestry, Vellanikkara for his timely co-operation, constant support and valuable suggestions related to my work and for providing me with an ambient working place.*

I express my gratitude to **Dr. B. Suma**, *Professor and Head, Cocoa Research Centre, Vellanikkara for her assistance and encouragement provided throughout the course of this study.*

Words fall short as I place on record my indebtness to **Dr. S. Biju**., **Dr. P. Sindhumole**, **Dr. Dijee Bastin**, **Dr. Rose Mary Francies** *and* **Dr. K.T. Presanna Kumari (Retd.)** *for their valuable guidance and suggestion, advice and help rendered during the period of work.*

I am deeply obliged to ***Dr. S. Krishnan****, Professor and Head, Department of Agricultural Statistics, for his valuable guidance and suggestions made in this study.*

I owe a special thanks to all the research associates of Cocoa Research Centre especially ***Chithira****,* ***Jayasree****,* ***Simya****,* ***Ajisha****,* ***Litta****,* ***Sruthy****,* ***Priya*** *and* ***Sudha*** *and all the labourers for their affection, care and immense help during my research work. I would also like to give a special thanks to* ***Ms. Suma****, Field Officer of Cocoa Research Centre for her immense help and encouragement in carrying out the field work during the course of my study.*

I would like to give thanks to all the non- teaching staffs at Plant Breeding and Genetics department and College of Horticulture for the help they extended during the course of my study.

I duly acknowledge the encouragement, moral and unconditional support, precious suggestions and timely persuasions by my dear seniors especially ***Ajinkya****,* ***Suma****,* ***Asna****,* ***Riya****,* ***Manjunatha****,* ***Neeraja****,* ***Veeresh****,* ***Sunil****,* ***Reshma*** *and* ***Nikhil*** *not only in my research work but also throughout my PG programme. I express my sincere thanks to my classmates* ***Megha****,* ***Anju*** *and* ***Chakravarthi*** *and friends* ***Athmaja****,* ***Aruna****,* ***Jeen****,* ***Sreelekshmi****,* ***Midhuna*** *and* ***Shibana*** *and juniors especially* ***Shilpa****,* ***Sarga****, whose prayers, love, kind help and affection which gave me enough mental strength and perseverance to get through all odds and tedious circumstances.*

A word of apology to those I have not mentioned in person and a note of thanks to one and all who worked for the successful completion of this endeavour.

Finally, I take this opportunity to express my never ending gratitude to my beloved parents, and my sister for their prayers, moral support, constant encouragement, love and warm blessings.

May the Almighty God richly bless all of you.

Juby Baby

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CONTENTS

LIST OF ABBREVIATIONS

%	Per cent
CO_2	Carbon dioxide
cm	Centimetre
COH	College of Horticulture
CMS	Cell membrane stability
CSI	Chlorophyll stability index
^0C	Degree celsius
HD^2	$(\text{Height x Diameter})^2$
GCA	General combining ability
GCV	Genotypic co-efficient of variation
σ^2g/Vg	Genotypic variance
g	Gram
GB	Glycine betaine
hr	Hour
µg	Microgram
µmol	Micromole
mg	Milligram
mmol	Millimole
mM	Millimolar
ml	Millilitre
mm	Millimetre
M	Molar
nm	Nanometre
NRA	Nitrate reductase activity
No.	Number
PCV	Phenotypic co-efficient of variation

σ^2p/Vp	Phenotypic variance
RWC	Relative water content
rpm	Rotations per minute
s	Second
SCA	Specific combining ability
SOD	Superoxide dismutase
H_2O	Water
W	Watts

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Introduction

I. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a perennial crop plant belonging to the family Malvaceae and which is native of Amazon river basin. It is mainly grown in humid tropical regions which is ideal for it's development.

Cocoa, has been consumed as a beverage crop even before the introduction of tea and coffee and has been a part of many South American and Egyptian cultures. The literal meaning of cocoa is the "Food of Gods" as the plant was worshipped in many cultures and the people thought this plant as a gift from the heavens.

The original commercial cultivation of cocoa started after it's discovery by Sir Hernen Cortez and gradually it spreaded to American and European countries. However now, the highest production of cocoa is reported from West African countries. Cocoa was introduced to India during the British Raj and after independence the Government took initiative to collaborate with western countries for introducing cocoa and growing it as a commercial crop. The first cultivation of cocoa was done in Wayanad district of Kerala which soon gained it's popularity in nearby states of Tamil Nadu, Karnataka and Andhra Pradesh.

As this crop is having the centre of origin in tropical humid rain forests of Amazon, it is obvious that it requires an ample amount of water for its cultivation and cannot withstand long periods of drought. The main reason behind this is that it has a very shallow tap root system which enables it to absorb water from surface layers only. Hence, it has to be sufficiently irrigated. It requires an average rainfall ranging between 1500-3000 mm with a dry season of not more than three months with less than 100 mm rainfall per month and mean maximum temperature varies between 30°C to 32°C and a mean minimum between 18-21°C with an absolute minimum of 10°C and is mainly recommended as an irrigated crop. When cultivated as an irrigated crop, it requires irrigation once in 4 days and 24 litres of water per plant. It is mainly cultivated as a shade tolerant crop and intercropped between tall plantation crops.

The growth and yield of cocoa is influenced by a number of environmental factors, particularly rainfall, temperature and water stress. The harvest of cocoa pods is spread over the year but peak harvesting is normally done during July-August and November-December.

Drought is considered as one of the major factors affecting cocoa cultivation. Water stress affects the most important determinants of yield-canopy architecture, photosynthesis and partitioning of assimilates. Being a perennial crop, the requirement for water is fairly high. Hence, efforts should be made to adopt such measures so as to increase the water use efficiency. The recent reports says that there is day to day decrease in the potential water sources for day to day basic activities and in such a condition, it is necessary to evolve genotypes which can withstand long periods of scarcity in water table. Recent studies have been indicated that near extinction of this crop may happen due to the rising climatic change within 40 years which the crop cannot withstand and hence, efforts have to be made to evolve such genotypes or to find such genotypes which are tolerant to this stress.

Cocoa Research Centre, Kerala Agricultural University is on the move to evolve drought tolerant genotypes. Earlier studies helped to identify some drought tolerant genotypes which formed the basis for this study. In the present study, an attempt was done to exploit the drought tolerant nature of genotypes for the production of drought tolerant hybrids.

Review of literature

II. REVIEW OF LITERATURE

Cocoa (*Theobroma cacao* L.) is a tree crop which was considered to be the "Food of the Gods". There are three main cocoa groups *viz*. Criollo, Forastero and Trinitario, which are distinguished by their botanical features and geographic origins (Bartley, 2005). Primary centre of origin of cocoa is South America (Motamayor *et al*., 2008). Across the years, different cocoa populations have been identified in the primary centre (Amazon forest) and distributed to various cocoa-growing areas, quarantine centres, and gene banks. Due to it's importance, cocoa is now cultivated in almost all the tropical regions, especially West Africa, which accounts for about 70 per cent of the world's cocoa production annually (ICCO, 2013).

In nature, plants are continuously exposed to several biotic and abiotic stresses. Among these stresses, drought stress is one of the most adverse factor for plant growth and productivity and it is considered as a severe threat for sustainable crop production in this era of climate change. Drought induces a wide diversity of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical and molecular responses of plants to drought, it is essential for a holistic perception of plant resistance mechanisms to water-stressed conditions.

Cocoa is a crop which originated in the humid tropics of rainforests and hence, this crop requires ample amount of water. The major concern regarding it's commercial cultivation is the scarcity of water. Water stress affects several physiological processes in cocoa which results in reduction in yield. Water scarcity is more of a problem where the crop is cultivated under rainfed conditions as inconsistency in rainfall pattern can lead to water stress in cocoa. Available literature on drought tolerance in cocoa as well as related crops is reviewed in this chapter.

2.1. Importance of hybrid production in cocoa

Cocoa has been known to exhibit strong heterosis for yield and yield contributing characters (Atlanda and Toxopeus, 1971). Introduced clones which utilizes heterosis are exploited for hybrid seed production (Warren, 1992). It is justified in saying that the history of cocoa can be divided into two, before and after the development of hybrids (Dias *et al.*, 2003). One of the advantages of hybrids is that they are early bearing and can also tolerate diseases and pests better than earlier materials (Edwin and Masters, 2005). Even though there are many studies being carried out in cocoa, the research lies very behind in exploiting the full potential of cocoa.

Many physiological and genetic investigation had exposed the inability of utilizing the full yield potential of cocoa and which is yet to be exploited (Bertus, 2004). Apart from pests and diseases, water stress is one of the major factor that needs to be attended in cocoa. Gilbert and Medina (2016) defined drought as a decrease in water inputs or precipitation in an agro/ecosystem over time that is sufficient to result in soil water deficit.

2.2. Importance for drought breeding in cocoa

Many breeding strategies have utilized the morphological and physiological selection traits to select cocoa genotypes with improved tolerance to drought stress. Frimpong *et al.* (1999), selected drought tolerant genotypes based on plant height, leaf number and stem growth under greenhouse condition. Daymond and Hadley (2004) studied the effect of temperature stress on early stem growth and chlorophyll content in four cocoa clones. They also observed a high level of genetic variability in four cocoa genotypes under temperature stress.

Efforts made earlier to identify drought tolerance characters in cocoa accessions resulted in many tolerant varieties (Balasimha *et al.*, 1985; Balasimha and Rajagopal, 1988). Drought and irrigation combination have been examined in crops such as tropical woody plants (Engelbrecht and Kurser, 2003), maize (Makumbi *et al.*, 2011) and rye (Hubner *et al.*, 2012), but not in cocoa.

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According to Laderach *et al.* (2013), there is a growing concern that the global increase in temperature and simultaneous increase in potential evapotranspiration may result in increased drought stress condition for cocoa. Padi *et al.* (2013) identified some drought-tolerant cocoa genotypes which were grown under no shade.

When compared with other tree crops, cocoa is less efficient in controlling water stress (Raja-Harun *et al.*, 1988) and cannot tolerate long periods of water scarcity (Bae *et al.*, 2008). The ability of plants for adjusting the osmotic potential during water stress differs in cocoa genotypes (Balasimha and Daniel, 1988). The ability to identify the genotypes that combine the traits for good growth and high yield with efficient Water Use Efficiency (WUE) is an essential requirement for breeding cocoa for drought affected areas (Dias *et al.*, 2007). Although across these years, only a few germplasm materials have been evaluated for this purpose (Padi *et al.*, 2013).

Hence, it is now important to study the water requirement of cocoa and breed new genotypes accordingly that are more tolerant to environmental stress which is currently being expressed in crop production area (Hadley, 2007).

2.3. Water stress on growth

Cell growth is considered as one of the most drought sensitive physiological processes due to the reduction in turgor pressure. Growth is the result of daughter-cell production by meristematic cell divisions and subsequent massive expansion of the young cells. Under severe water deficient conditions, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998).

Drought-induced reduction in leaf area is due to suppression of leaf expansion through reduction in photosynthesis (Rucker *et al.*, 1995). A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Zhao *et al.*, 2006). Drought causes impaired mitosis, cell elongation and expansion which results in reduced growth and other yield traits

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(Hussain *et al.*, 2008). Water deficit reduces the individual leaf size, number of leaves per plant and leaf longevity by decreasing the soil water potential. Leaf area expansion depends on leaf turgor, temperature and assimilate supply for growth.

Khan *et al.* (2001) conducted a study consisting of six treatments (genotypes), T_0 (control), T_1, T_2, T_3, T_4 and T_5 in maize. Six irrigations were given to the treatments. The first irrigation was applied to all the treatments equally. Second irrigation was applied to all treatments except T_5. Third irrigation was given to all treatments except T_4 and T_5. Fourth irrigation was applied to T_2, T_4 and T_0. Fifth irrigation was applied to T_1 and T_0 treatments and sixth irrigation was given only to T_0 treatment. It was observed that plant height, leaf area and stem diameter decreased noticeably with increasing water stress.

Stem length significantly reduced under water stress in potato (Heuer and Nadler, 1995). In soybean, the stem length decreased under water deficit conditions (Specht *et al.*, 2001; Zhang *et al.*, 2004), and similar cases were observed in many crops such as *Vigna unguiculata* (Manivannan *et al.*, 2007a), *Abelmoschus esculentus* (Sankar *et al.*, 2007 and 2008) and *Petroselinum crispum* (Petropoulos *et al.*, 2008). The plant height reduced up to 25 per cent when stress was imposed on citrus seedlings (Wu *et al.*, 2008).

Prabhudeva *et al.* (1998) subjected sunflower genotypes to water stress at bud initiation and seed filling stages and observed that the seed along with the biological yield reduced mostly under water stress at bud initiation than at seed filling stage. Reduced biomass has been observed in water stressed soybean plants (Specht *et al.*, 2001). Dry matter partitioning and temporal biomass distribution has been proved to be strongly correlated with plant productivity under drought stress (Kage *et al.*, 2004). Fresh and dry weights of plants under water limited conditions are desirable characters to study as these indicates the relative amount of water that the plant can hold. Mild water stress affected the shoot dry weight in sugar beet genotypes (Mohammadian *et al.*, 2005), rice (Lafitte *et al.*, 2007), *Poncirus trifoliatae* seedlings (Wu *et al.*, 2008) and *Petroselinum crispum* (Petropoulos *et* *al.*, 2008). A common negative effect for plant status seen in crop plants during water stress is the reduced fresh and dry biomass production (Farooq *et al.*, 2009).

2.4. Water stress on biochemical characters

2.4.1. Proline

Proline is the most widely studied solute because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. A study was conducted in maize where progressive drought stress induced a considerable accumulation of proline in water stressed maize plants. The proline content increased as the drought stress progressed and reached the maximum after 10 days of stress imposition, but then decreased under severe water stress as observed after 15 days of stress (Anjum *et al.*, 2011).

The main functions of proline includes influencing protein solvation and preserving the quaternary structure of complex proteins, maintaining membrane integrity under dehydration stress and reducing oxidation of lipid membranes which is also known as the process of photo-inhibition (Demiral and Turkan, 2004). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been found to be generally higher in stress-tolerant as compared to stress-sensitive plants. It also helps in stabilizing sub-cellular structures, scavenging the free radicals and buffering cellular redox potential under stress conditions in plants (Ashraf and Foolad, 2007). Proline can act as a signalling molecule for co-ordinating mitochondrial functions. It influences cell proliferation or cell death and triggers specific gene expression, which is essential for after recovery from stress conditions (Szabados and Savoure, 2009).

Proline accumulation was found to be similar in many plants under water stress, like barley (Singh *et al.*, 1972), sorghum (Jones and Turner, 1978), soyabean (Sarkar, 1992), rabi sorghum (Sathbai *et al.*, 1997), cluster bean (Garg *et al.*, 1998) and black gram (Kumari *et al.*, 2000).

In cocoa, water stress increased the proline content of seedlings from 57 to 333 μmol g^{-1} (Rajagopal and Balasimha, 1994). Proline content was found to increase in pea cultivars as well (Alexieva *et al.*, 2001). Drought tolerant petunia (*Petunia hybrida*) varieties were reported to accumulate free proline under drought that acted as an osmoprotectant and induced drought tolerance (Yamuda *et al.*, 2005).

2.4.2. Superoxide Dismutase

Abiotic stresses such as drought causes an imbalance of oxidative metabolism, changes the components of the mitochondrial membrane and also limits the transport of electrons through the respiratory chain containing cytochromes (Juszczuk *et al.*, 2001), which will result in accumulation of free radicals of oxygen (Wang *et al.*, 2009). To tackle this toxicity due to excessive accumulation of AOS (Active Oxygen Species), plants at the cellular level establish an effective anti-oxidative system, which consists of enzymes like superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) (Aroca *et al.*, 2003). Oxidative stress results from the generation of reactive oxygen species (ROS), such as superoxide ion (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) which are detrimental to the survival of plants in a stress environment (Luna *et al.*, 2004). Superoxide dismutase (SOD) enzymes are metalloenzymes, which are the first defense forms produced by catalyzing the dismutation of O^{2-} radicals to H_2O and O_2.

Martinez *et al.* (2001) studied *Curtilobum solanum* and *Solanum tuberosum* and found that these plants developed tolerance to water stress due to over production of SOD in chloroplasts.

2.4.3. Nitrate Reductase Activity

It is one of the key enzymes which catalyses the reduction of nitrate to nitrite, which is the initial step in nitrate assimilation in plants (Bhaskar, 1997).

Nitrate Reductase stability under drought was 0.59 and 0.53 in tolerant and susceptible species of cocoa respectively (Balasimha and Daniel, 1988). There was a gradual decline in nitrate reductase activity in field grown wheat plants when drought stress was imposed (Kathju *et al*., 1990). In various crop species examined, NRA had shown to be reduced during water stress condition (Dubey and Pessarakli, 1995). Garg *et al*. (1998) observed an increase in proline and a reduction in NR activity under water stress in cluster bean genotypes. Reduction in NR activity was observed in maize (Foyer *et al*., 1998) and in wheat (Yadav *et al*., 1998). Decline in NRA activity during stress is mainly caused by low NO^{3-} absorption which will result in water uptake deprivation (Ferrario-Mery *et al*., 1998). Deka and Baruah (2000) found a decrease in NRA content in rice when stress conditions were imposed.

NRase is closely associated with plant growth and development (Sinha and Nicholas, 1981). It is generally accepted that drought stress has a negative effect on plant photosynthetic activity, N concentrations, free amino acids or soluble protein contents and it is accompanied with a decline of nitrate reductase activity in many plant species, such as maize (Foyer *et al*., 1998), potato (Ghosh *et al*., 2000), winter wheat (Xu and Yu, 2006) etc. The plants subjected to water stress produces less amount of total protein which causes a decrease in the synthesis of nitrate reductase activity caused by low nitrate flux (Costa *et al*., 2008).

2.4.4. Glycine Betaine

Among the many quaternary ammonium compounds that are reported in plants, glycine betaine is the most common one. Glycine betaine effectively stabilizes the quaternary structure of enzymes and complex proteins, and it maintains the highly ordered state of membranes at non- physiological temperature and concentrations (Papageorgiou and Murata, 1995).

They are present most commonly in chloroplasts where it helps in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Robinson and Jones, 1986). Levels of accumulated

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glycine betaine are generally correlated with the extent of stress tolerance (Rhodes and Hanson, 1993).

Glycine betaine accumulation has been found in many crops like sugar beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*) (Weimberg *et al*., 1984; Fallon and Phillips, 1989; McCue and Hanson, 1990; Rhodes and Hanson., 1993 and Yang *et al.,* 2003) under stress conditions.

2.5. Water stress on physiological characters

2.5.1. Relative Water Content

Relative water content is considered as the most meaningful index for assessing dehydration tolerance and is used as a measure of plant water status, that reflects the metabolic activity in tissues. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and as the leaf matures.

RWC is related to water uptake by the roots as well as water loss by transpiration. Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a simultaneous increase in leaf temperature (Siddique *et al*., 2001). A decrease in the relative water content (RWC) in response to drought stress had been noted in wide variety of plants when leaves were subjected to drought (Nayyar and Gupta, 2006). When two poplar species were subjected to progressive drought stress, the decrease of RWC in the water-stressed cuttings was 23.3 per cent in *Populus cathayana*, whereas it was 16 per cent in *Populus kangdingensis*. RWC was affected by the interaction of severity, duration of the drought event and species (Yang and Miao, 2010).

2.5.2. Photosynthesis

Environmental stresses can have a direct impact on the photosynthetic apparatus, which disrupts all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and the stomatal control of the CO_2 supply, along with an increased accumulation of carbohydrates, peroxidative destruction of lipids and disturbance of water balance (Allen and Ort, 2001).

The ability of crop plants to acclimate to different environments is directly or indirectly related with the plant's ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and consequently, the growth and yield of the whole plant (Chandra, 2003).

Drought stress severely impeded the gas exchange parameters of crop plants and this happened due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins (Menconi *et al.*, 1995). Drought stress is known to inhibit photosynthetic activity in tissues due to an imbalance between light capture and its utilization (Foyer and Noctor, 2000). Anjum *et al.* (2011) found that drought stress in maize led to considerable decline in net photosynthesis (33.22 %), transpiration rate (37.84 %), stomatal conductance (25.54 %), water use efficiency (50.87 %), intrinsic water use efficiency (11.58 %) and intercellular CO_2 (5.86 %) as compared to well water plants used as control.

Drought stress effects on photosynthetic rate and leaf gas exchange characteristics of four wheat cultivars were studied under semi-controlled conditions. Four cultivars selected were Kanchan, Sonalika, Kalyan Sona, and C306 and they were grown in pots and were subjected to four levels of water stress. Among the cultivars, Kalyan Sona showed the highest photosynthetic rates both at vegetative and anthesis stages. Exposure of plants to drought stress led to an apparent decrease in photosynthetic rate, stomatal conductance and mesophyll conductance and a concomitant increase in intercellular CO_2 concentration. Plants subjected to drought at the early vegetative stage displayed similar physiological characters. Photosynthetic rates decreased with decrease in stomatal conductance during drought stress (Siddique *et al.*, 1999).

2.5.3. Leaf temperature

Water is one of the most essential components for plants. It serves as a solvent for different solutes and transporter of solutes between cells and organs. The greater part of water uptake from the soil is consumed by transpiration preventing temperature increases. Leaf temperature is an important factor in controlling leaf water status under water deficit conditions (Leopold *et al*., 1994). A study conducted on banana crop observed a 4°C rise in leaf temperature when drought stress was imposed as compared to non-stressed plants (Surendar *et al*., 2013).

2.5.4. Transpiration rate

Water stress has been known to reduce the transpiration rate in plants. Transpiration rate was highest (4.75 mmol $H_2O\ m^{-2}\ s^{-1}$) in cashew seedlings stressed for two days while it declined to 2.11 mmol $H_2O\ m^{-2}\ s^{-1}$ when stress period was extended for five days (Latha, 1998). Bhatt *et al*. (1998) observed a decrease in transpiration rate under water stress in oats. Transpiration rate was reduced under water stress in beech plants (Peuke *et al*., 2002).

Three accessions of cocoa (NC 23, NC 29 and NC 39) had shown 54 to 59 percent decrease in transpiration rate under stress conditions as compared to plants under well watered conditions (Balasimha *et al*., 1988). He also concluded that effective stomatal regulation is a key drought tolerance response of cacao that can result in decreased transpirational water loss. Studies conducted have observed correlations between stomatal closure and decreased water potentials or increased evaporative demand (Balasimha *et al*., 1991). The stomatal opening in cacao had found to be very sensitive to water deficit and relative humidity, with proven genetic variation in the level of sensitivity (Acheampong *et al*., 2013; Acheampong *et al*., 2015).

In another field study carried out by Central Plantation Crop Research Institute (CPCRI), Vittal, India, eleven, three-year old cocoa genotypes from five different countries were evaluated under drought conditions. They were selected

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from Colombia, Brazil, Peru, Costa Rica and Ecuador. All accessions showed a decreasing trend in photosynthetic parameters, three accessions presented greater resilience to water deficit by reducing transpirational water loss through greater stomatal sensitivity and induced stomatal closure (Apshara *et al.*, 2013).

2.5.5. Membrane stability

Cell membrane stability is a major physiological index used for the evaluation of drought tolerance (Premachandra *et al.*, 1991). It can be called as a genetically related phenomena as quantitative trait loci (QTL) for this character have been mapped in drought stressed rice plants at different growth stages (Tripathy *et al.*, 2000). Biological membranes are the first target of many abiotic stresses and it is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance with respect to plants (Bajji *et al.*, 2002). Dhanda *et al.* (2004), in their work displayed that membrane stability of leaves was the most important trait to screen the germplasm for drought tolerance.

In a study conducted on maize plants, K nutrient improved the drought tolerance, and it was mainly due to the improved membrane stability (Gnanasiri *et al.*, 1991). Tolerance to drought was evaluated as increase in cell membrane stability under water deficit conditions and this was used to differentiate between cultivars. A good correlation was observed between membrane stability and relative growth rate (Premachandra *et al.*, 1991). Cell membrane stability declined rapidly in Kentucky blue- grass when exposed to drought and heat stress simultaneously (Wang and Huang, 2004).

The drought tolerant cocoa accessions had comparatively lower electrolytic leakage because of increased wax and lipid fractions in water stress conditions as compared to susceptible ones (Bhat *et al.*, 1990). Rajagopal and Balasimha (1994) observed that the electrolyte leakage of drought tolerant coconut genotypes was lower than in susceptible ones, due to water stress. Membrane damage generally increased with water stress.

2.5.6. Chlorophyll content

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. The chlorophyll content decreased to a significant level at higher water stress conditions in *Vaccinium myrtillus* (Tahkokorpi *et al.*, 2007), sunflower (Kiani *et al.*, 2008), cotton (Massacci *et al.*, 2008) and *Catharanthus roseus* (Jaleel *et al.*, 2008). Both the chlorophyll 'a' and 'b' are sensitive to soil drying conditions (Farooq *et al.*, 2009). Drought stress caused changes in chlorophyll 'a' and 'b' ratios and carotenoids (Farooq *et al.*, 2009).

Loss of chlorophyll contents under water stress is considered as a main cause of inactivation of photosynthetic pigments. Furthermore, water stress induced reduction in chlorophyll content has resulted in loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.*, 1981). Low concentrations of photosynthetic pigments can directly lower photosynthetic potential and hence it's primary production. From the physiological point of view, leaf chlorophyll content is a parameter of significant interest in its own way. Studies conducted have testified the loss of more chlorophyll from mesophyll cells rather than from bundle sheath cells.

2.5.7. Chlorophyll stability

This is also one of the factors that contribute for assessing the drought tolerant conditions in plants. Ravindran and Menon (1981) used chlorophyll stability index for *in vitro* screening for drought tolerance in cocoa. In cashew, chlorophyll stability index of drought tolerant accessions were higher than sensitive varieties (Latha, 1998). High chlorophyll stability index helps the plant to withstand stress conditions through better availability of chlorophyll. This results in increased photosynthetic rate and high dry matter production (Mohan *et al.*, 2000).

2.6. Correlation and path analysis studies

Matthews and Boyer (1984) found that the photosynthesis process during drought is possible due to the osmoregulation which affects the state of the leaf stomata and adaptation of the photosynthetic apparatus to drought conditions. Similar results had been obtained earlier in other studies (Gupta and Berkowitz, 1988). Ludlow (1987) and Weng (1993) reported a positive correlation between photosynthesis and osmoregulation. Decrease in RWC is known to induce stomatal closure and thus a parallel decrease in photosynthetic rate (Cornic, 2000).

A study was carried out to classify five triticale genotypes (‘Piano’, ‘Timbo’,‘Lamberto’, ‘Babor’ and ‘Boreas’) as drought-tolerant and drought-sensitive types based on field performance trials and to study their correlation with a classification based on measurements of some physiological and biochemical parameters in greenhouse conditions. A positive correlation between the photosynthesis rate and osmotic potential was found for the evaluated genotypes. Under drought conditions, the highest photosynthesis rates were observed for cultivars ‘Piano’, ‘Timbo’ and ‘Lamberto’. A significant correlation was also seen between the transpiration rate and the osmotic potential. The transpiration rate was found to be the highest in cultivars ‘Piano’, ‘Timbo’ and ‘Lamberto’. The lowest values of stomatal conductance were reported for the drought-sensitive genotypes ‘Babor’ and ‘Boreas’. This indicated severe disturbances in stomatal movement and lack of complete closure in drought conditions. For cultivars tolerant to water deficit, such as ‘Timbo’ and ‘Piano’, the stomatal conductance was high or close to that of the control (Hura *et al.*, 2007).

A set of 18 wheat genotypes collected using focused identification germplasm strategy (FIGS) were evaluated for drought tolerance at seedling stage. Stress was imposed by keeping the plants at 40 per cent field capacity for one week followed by watering to allow recovery. Correlation was studied among the traits to find out the feasibility of parameters, which can be used on for phenotyping. Under control conditions, shoot dry weight was positively correlated with the shoot length and root length showed a positive correlation with Membrane Stability Index (MSI). Under stress conditions, more relationships between the parameters became evident. Root dry weight was positively correlated to shoot dry weight. Seedling survival had moderate positively correlation with shoot length and relative water content (RWC). Strong positive correlation was observed between seedling survival and shoot dry weight and root dry weight. RWC was proposed to be closely linked to drought tolerance. Though a consistent reduction in RWC was observed in all the studied lines (Dharwar Dry, C 306 and KP 1876) it was correlated with seedling survival under stress. A significant negative correlation was found between drought susceptibility index and relative water content. These results were in agreement with the fact that maintenance of tissue water status under stress condition led to drought tolerance in maize and triticale genotypes. The trait membrane stability was positively correlated with the thousand-grain weight under drought as well as heat stress (Bansal *et al*., 2016).

Correlation and path analysis studies were conducted to know the relationship among morphological traits and their contribution towards yield under normal and drought stress in twenty diverse rice genotypes. Twenty rice genotypes (Basmati 122, Harandi 379, Hansraj 62, Sonfine 43, Begmi 51A, Toga 286A, Mushkan 312-2, Basmati 242, Basmati 140, Basmati 376, Basmati 388, Begumi, Munji 78B-1, RB2, Sufaida 20, Jhona 109, Dagar 303, Begumi 302, Kala Bunda 50, Jhona 86) were kept under irrigated (control) and water stress condition using completely randomized design (CRD) with three replications. Correlation and path analysis when conducted, it was observed that growth (Plant height = 0.17**) and yield attributes such as panicle length (0.49**), grains/ panicle (0.69**), grain weight/ panicle (0.99**), tillers/ plant (0.14) and 1000-grain weight (0.11*) were positively correlated in all genotypes under normal or drought stress conditions. Among genotypes, highest plant height was observed in Basmati-140 (43.13cm) and the lowest was found in Sufaida 20 (26.27cm) under drought condition. Plant height was significantly reduced under drought stress than control condition in genotype Munji 78 B-1 from 64.71cm to 35.30cm. Drought drastically affected the yield/plant in different genotypes. Under drought stress, genotypes Harandi-379, Munji-78B-1 and Basmati-242 performed well for yield/plant with values of 7.54g, 7.69g and 9.28g, respectively. Grain weight/panicle showed highest positive effect (0.914 and 0.788) on yield/plant and followed by spikelet fertility (0.022 and 0.056) under both drought and normal conditions, respectively. These results indicated that grain weight/panicle, 1000 seed weight and plant height can be used as selection indices for drought resistance in rice (Bhutta *et al.*, 2017)

Path analysis study was carried out of phenotypic traits in young cocoa plans that were kept under drought conditions. The aim was to investigate the phenotypic correlation among morphological characteristics of cacao progenies subjected to irrigation and drought conditions and their division into direct and indirect effects. Mating design followed was complete diallel design and the seedlings were kept under two water regimes (control and drought) with six replications. When path analysis was carried out, it was found that stem diameter (SD) was positively correlated with root biomass (0.66), stem dry biomass (0.74), leaf dry biomass (0.77) and total dry biomass (0.82) under controlled condition. Under drought, stem diameter was additionally related to root volume (0.46). Total leaf area (TLA) was positively correlated with stem biomass, leaf dry biomass, total dry biomass and root mean diameter (RD) [<1mm], both under control and drought stress conditions. Stem biomass (SB) was positively correlated with leaf dry biomass (0.68), total dry biomass (0.73), root length (0.54) and root volume (0.45) in the control condition. Under soil water limitation, stem biomass was correlated only with leaf dry biomass and total biomass. The root volume (RV) was positively correlated with total biomass (0.45) under drought conditions. The increase in root volume was associated with root length (0.52), especially with medium diameter RD [1-2mm] roots. A breakdown of phenotypic correlations into direct and indirect effects, through path analysis, indicated that total leaf area (0.11), leaf dry biomass (0.27), and root mean diameter [1-2mm] (0.42) showed the largest direct effects on the development of the root system (root volume) of the cacao progenies under control conditions. On the other hand, in the drought conditions, stem dry biomass (0.38), leaf dry biomass (0.21) and RD (0.93) were the major direct effects on the increase of the progenies root volume. The stem dry biomass, root length and rot diameter showed a positive and significant correlation with root volume (RV) under drought conditions. Despite the direct effect of root diameter [1-2mm] on root volume in the drought conditions, the correlation, although positive, was not significant (Santos *et al.*, 2018).

2.7. Combining ability studies

Estimate of combining ability using diallel-mating design has widely been used to provide information about the performance of parental populations and their heterotic pattern in crosses, identifying heterotic groups and predicting performance of new populations (composites) derived from such crosses (Filho, 1985).

Drought studies were conducted in tea and diallel mating design was followed to find out the combining ability of the crosses. It was found that by maintaining biclonal gardens, specific crosses involving parents with positive SCA effects for characters like yield, fermentability and pubescence followed by prudent clonal selection may further result in marked progress in these traits (Zobel and Talbert, 1984; Cotterrill *et al.*, 1987).

The choice of efficient breeding program depends on a large knowledge of type of gene action involved in expression of character. Dominance gene action action favours production of hybrids, whereas additive gene action indicates that the selection procedure will be effective in breeding (Edwards and Smith, 1976). Eariler studies have shown that both additive and non- additive gene effects are important for controlling yield related attributes (Malvar *et al.*, 1996). Different types of gene action under drought have been reprted. They concluded that additive effects are more important under drought conditions (Betran *et al.*, 2003).

Drought studies were done in coconut seedlings and coconut cultivars (two dwarf cultivars- CGD and MYD; four tall cultivars- ECT, PHOT, LCT and FMST) with desirable characters. A 2 x 4 Line x Tester mating design was designed to study the combining ability and gene action with respect to physiological traits under drought conditions. Physiological parameters like leaf water potential, transpiration rate, net photosynthetic rate (Pn) and lipid peroxidation were recorded in seedlings under three conditions: non-stress, water stress and recovery conditions. The studies clearly showed different responses by the seedlings of various cross combinations to drought stress. Analysis of variance of the parameters indicated that the stress sensitive traits i.e., transpiration rates, lipid peroxidation, photosynthetic rates and water potentials were governed by genetic control. The transpiration rate had higher SCA indicating heterosis for this character. The photosynthetic rate on the other hand, was governed by the non-additive gene action, and therefore, can be exploited for heterosis breeding. The nature of gene action governing some of these drought related traits could be used in selective breeding for drought tolerance (Rajagopal *et al.*, 2007).

Adewale *et al*. (2014) carried out a study to determine the breeding value of cocoa for pod and bean characters and for this, fourteen genotypes were evaluated for pod length, number of beans per pod, weight, bean length, width and thickness using line x tester mating design. They observed that hybrids from same female parent differed significantly for all the traits studied. The GCA and SCA differs for all the traits. The ratio of GCA/SCA revealed that all characters were having additive inheritance except for number of beans/pod and bean length which were non-additive in nature. Heterosis was found to be in the range of -17.82 per cent for bean thickness to 52.40 per cent for pod weight.

The effect of the specific combining ability is interpreted as the deviation of a cross compared to what would be expected based on the GCA of their parents (Griffing, 1956). Generally non-additive effects action contributed by high SCA effects is not used for hybridisation due to lack of gene flow for the trait in question. However, a study conducted in cowpea under drought stress showed that both additive and non-additive genetic effects were responsible for the inheritance of drought adaptation traits. Non-additive genetic effects were having comparative importance along with additive genetic effects implying that the performance of progeny were better in specific crossing combinations but could not be predicted for a wide range of crosses. Therefore, improvement of drought adaptation traits through selection of crosses with high positive SCA effects and advancing them to later generation would be effective (Mwale *et al.*, 2017).

A study was conducted to find out the combining ability of the genotypes for the physiological parameters as well as morphological parameters. Relation between GCA and SCA estimates indicated that for some parameters, additive genic effects were more expressive whereas for others non-additive effects were reported. A balance between additive and non-additive gene effects were more important. The magnitudes of additive and non-additive gene effects showed that both, selection among parents and within progenies and even a combination of these two strategies, would be useful for breeding purposes (Pereira *et al.*, 2017).

2.8. Binary Regression studies

It is mainly used when independent variables do not satisfy the multivariate normality assumption. Cox (1950) developed this model which processes producing sigmoidal/elongated S-shaped curves which are quite common in agriculture. They are useful when a non-linear relationship can be established between response variable and qualitative and quantitative factors affecting it.

Sinclair (1986) had used binary regression model for description of leaf expansion and transpiration responses to soil water deficit in several grain legumes. In cotton, a particular screening method was developed to screen the drought tolerant seedlings using the binary regression model. The genotypes differed in their per cent survival following drought condition and drought tolerant genotypes were also identified (Longenberger *et al.*, 2006).

Materials & Methods

III. MATERIALS AND METHODS

The present study "Breeding for drought tolerance in Cocoa (*Theobroma cacao* L.)" was conducted in the Department of Plant Breeding and Genetics, College of Horticulture (CoH), Cocoa Research Centre, Vellanikkara and College of Forestry, Vellanikkara during the period between 2016-2018.

3.1. Experiment I: Crossing between the clones

Four genotypes identified to be tolerant to drought in preliminary studies conducted at Cocoa Research Centre (CRC), by screening existing germplasm (Binimol, 2005), listed below (Table 1) were used as parents in the hybridisation programme (Plate 1).

Table 1. List of parents used for hybridisation

Sl. No.	Accession No.	Source
1	M 13.12	Progeny of pods from Vittal
2	G I 5.9	T76/1224/1201 (Amazon)
3	G II 19.5	Progeny of pods from Nileshwar
4	G VI 55	Progeny of pods from Cadbury farm, Chundale

The selected genotypes were hand pollinated in all possible combinations following the diallel method by a manual technique described by Mallika *et al.* (2002). The mature buds were selected a day before the pollination and they were covered with a hood plastered by using clay onto the trunk. This prevents the pollination with undesirable pollen. The next day, preferably in the morning between 7:00 am to 10:00 am, the desired pollen is collected and then the hood is removed from the female flowers (Plate 2).

(a). M 13.12 (Progeny from Vittal)

(b). G I 5.9 (Amazon type)

(c). G II 19.5 (Progeny from Nileswar)

(d). G VI 55 (Progeny from Chundale)

Plate 1. Parents selected for hybridisation

(a) Hand pollination of the selected parents

(b). Removal of staminodes and placing pollen onto stigma

Plate 2. Hand pollination of the selected genotypes

(a) One month old seedlings

(b) Three month old seedlings

Plate 3. Seedlings raised in the nursery

One or more staminodes are removed and the anther is placed onto the stigma of the female flower. The pollinated flower is then covered back with the hood and this covering is removed during next day morning.

The pods matured approximately within 5-6 months; mature pods so obtained were further raised in the nursery (Plate 3). Germination percentage of each cross was evaluated by counting the number of seeds germinated to the total number of seeds sown.

3.2. Experiment II: Screening for initial vigour

Seedlings were evaluated for their initial vigour at the third month of germination based on the HD^2 value obtained by measuring the height and diameter of the individual plants (Enriquez, 1981). The total number of leaves and the chlorophyll content were also observed (Plate 5).

3.3. Experiment III: Screening for drought tolerance

Superior hybrids based on HD^2 value were shifted to drought screening structure. Drought was imposed based on gravimetric method. Initial trials indicated that cocoa cannot tolerate drought less than 40 per cent field capacity (CCRP report, 2015). Hence, 40 per cent field capacity was maintained using the formula:

Weight of cover + Dry soil weight = y

Weight of soil at 100 per cent field capacity = z

Amount of water present = z-y = a

At 40 per cent field capacity = a/100 x 40 = b

To maintain the 40 per cent field capacity = y + b

The plants were maintained at 40 per cent field capacity for two weeks. This was achieved by supplementing water lost by evaporation and transpiration twice daily (morning and evening) (Plate 6). Out of 1505 hybrids evaluated, 120 hybrids were selected. Based on the percentage of leaves retained, the hybrids were classified following a score chart.

3.3.1. Score Chart for screening

This was prepared according to the number of leaves retained. The plants were classified into 4 categories (Table 2).

Table 2. The score chart depicting the leaves retained in the hybrids

Sl No.	Percentage of leaves retained	Classification
1	0-10	Highly susceptible
2	10.1-40	Susceptible
3	40.1-70	Tolerant
4	More than 70	Highly tolerant

The humidity and the temperature of the mist chamber (Plate 7) was recorded using Berlin's psychrometer every day. The instrument is whirled at a vigorous speed inside the chamber at four corners and at the centre so as to get a stable value.

The hybrids were screened based on number of leaves retained. The number of leaves retained after stress imposition (Plate 8) was found out through visual observation and percentage of leaves retained was calculated using the formula:

$$\text{Percentage of leaves retained} = \frac{\text{Number of leaves retained}}{\text{Total number of leaves}} \times 100$$

The biochemical and physiological analysis (Plate 9) were carried out.

(a). Labelling the sown seeds

(b). Plants growing in the nursery

Plate 4. After care in the nursery

(a). Height and girth of the seedlings

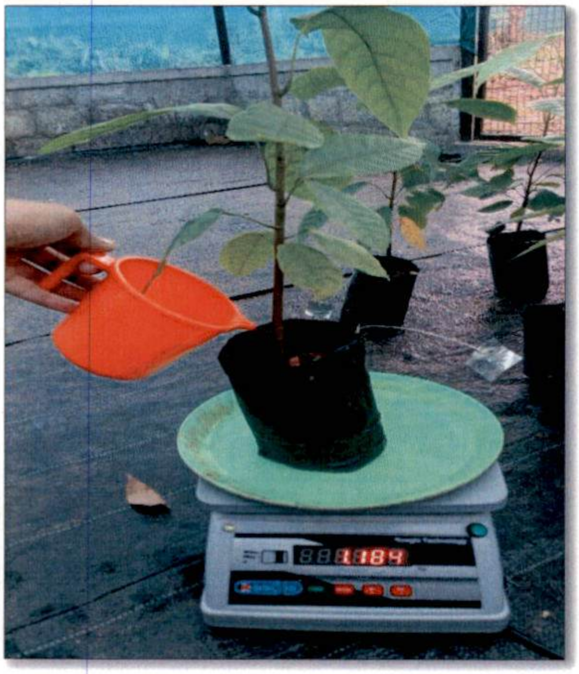
(b). Chlorophyll content using SPAD meter

Plate 5. Observation on height, diameter and chlorophyll content through SPAD meter

Plate 6. Screening test at 40 per cent field capacity

Plate 7. Drought screening structure

(a). Measuring the weight



(b). Maintaining the weight at 40 per cent field capacity by watering

Plate 8. Gravimetric method followed for inducing drought condition

3.3.2. Biochemical parameters

3.3.2.1. Proline (µg/g)

Proline is an important amino acid found in proteins. In plants, proline is synthesized from glutamic acid through a pathway catalysed by pyrroline -5- carboxylate synthetase and pyrroline - 5 - carboxylate reductase.

Reagents Used:

Sulphosalicylic acid (3 %) – 3 g in 100 ml distilled water.

Acid ninhydrin - 1.25g ninhydrin in 30 ml glacial acetic acid. This solution was then warmed for complete solubility till the colour become greenish blue and then 20 ml of 6M phosphoric acid was added and the solution changed to yellow colour. The mixture was used within 24 hours of preparation.

Phosphoric acid (6M) – Dissolve 41.176 ml of Ortho- phosphoric acid in 58.824 ml distilled water.

Procedure:

Leaf sample of 0.5 g was grinded in 10 ml of three per cent sulphosalicylic acid (3g in 100 ml distilled water). The grounded sample was centrifuged at 3000 rpm for 10 minutes. The filtrate was separated and in a test tube, two ml of the filtrate was taken along with two ml of acid ninhydrin solution and two ml of glacial acetic acid. The mixture was kept in water bath at 100°C for one hour. After one hour, the test tube was taken out and kept on ice bath for 10 -15 minutes for the sample to cool down. To this, 4 ml of toluene was added and vortexed for 15 – 20 seconds. Two distinct layers were formed wherein, the toluene acquired the colour. The intensity of the colour was measured in a spectrophotometer at 520 nm (Bates *et al.*, 1973).

$$\mu\text{moles/g tissue of proline} = \frac{\mu\text{g of proline x ml toluene x 5}}{\text{115.5 x g sample}}$$

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3.3.2.2. Nitrate Reductase Activity (NRA) (mmol nitrate/g/hr)

NRA was analysed by a method given by Evans and Nason, (1953).

Reagents:

Reaction mixture – Five percent propanol (5 ml) along with 0.02 per cent potassium nitrate (0.02 g) dissolved in 0.1 M potassium phosphate buffer (100 ml).

One per cent sulphanilamide – one g sulphanilamide dissolved in 100 ml of 3N HCl (prepared by dissolving 9 ml of HCl in 91 ml of water).

0.2 percent N- naphthyl ethylene diamene dihydrochloride (NEDA) – 0.2 g NEDA in 100 ml distilled water.

0.1 M phosphate buffer

Procedure:

The sample leaf was cleaned thoroughly with distilled water. One gram leaf discs were taken and suspended in five ml reaction mixture and incubated for two hours at 30°C. 0.4 ml of the reaction mixture was taken from the sample and 0.2 ml of one per cent sulphanilamide and 0.2 ml of 0.2 per cent N- naphthyl ethylene diamene dihydrochloride were added. After 20 minutes, 4 ml of distilled water was added and the intensity of the pink colour so developed was measured at 570 nm.

3.3.2.3. Superoxide dismutase (SOD) (units/mg protein/g)

Reagents:

Potassium dihydrogen phosphate (Solution A): 6.80 g dissolved in 500 ml of double distilled water.

Di-potassium hydrogen phosphate (Solution B): 8.71 g dissolved in 500 ml double distilled water.

Preparation of phosphate buffer (0.1 M): 16 ml of solution A and 84 ml of solution B was mixed. The solution pH is adjusted to 7.5, to get 100 ml of 0.1M phosphate buffer.

For preparing the grinding media, 0.0186 g of EDTA was added to 100 ml phosphate buffer.

Enzyme assay:

13.33 mM methionine (0.2 ml of 200 mM)

75 μM nitroblue tetrazolium chloride (NBT) (0.1 ml of 2.25 mM)

0.1 mM ethylene diamene tetra-acetic acid (EDTA) (0.1 ml of 3 mM)

50 mM phosphate buffer (1.5 ml of 100 mM)

50 mM sodium carbonate (0.1 ml of 1.5 M)

0.05 to 0.1 ml SOD enzyme

0.8 ml to 0.85 water (to make the final volume up to 3 ml)

Preparation of enzyme extract:

Leaf sample (0.2 g) was ground with two ml extraction buffer and then centrifuged at 10,000 rpm for ten minutes at 4°C. The supernatant was used as an enzyme source within 12 hours of extraction. This supernatant was then added to the three ml reaction mixture and then 0.1 ml of riboflavin was added. These tubes were kept under fluorescent lamps (15 W) for 15 minutes and in the dark to stop the reaction. The reading was taken at 560 nm. One unit of enzyme activity is the amount of enzyme which reduced the absorbency reading to 50 per cent in comparison with tubes lacking the enzyme (Dhindsa *et al.*, 1981).

$$\text{Unit (of enzyme)} = \frac{\text{Blank} - \text{Sample}}{\text{Blank}/2}$$

Enzyme activity is expressed as: units/mg of protein.

50 per cent inhibition = one unit of SOD

Percentage inhibition = "Z" units

Total volume of the enzyme extract = three ml = 3000 μl = Z/100 x 3000

0.1ml extract have 0.2 g tissue

So, SOD units/ mg = Total volume /200

3.3.2.4. Glycine Betaine (μmol/g)

Quarternary amines (QAMs), in particular, glycine betaine are accumulated during the stress conditions in stress tolerant plants. They serve multiple purposes related to both osmotic adjustment and osmo–protection damage. Glycine betaine can protect enzymes from heat and drought damage. This indicates it's capability to protect enzymes from stress damages.

Reagents:

Potassium tri- iodide solution: Dissolve 7.5 g iodine and 10 g potassium iodide (KI) in 100 ml of 1N HCl.

1,2 – Dichloroethane, 1N H_2SO_4

Procedure:

The samples were extracted using the method of Grieve and Grattan (1983).

500 mg of finely ground dry leaf samples were mechanically shaken with 20 ml distilled water for 24 hours at 25°C. The samples were then filtered and the filtrate was made up to 20 ml with deionised water and used for estimation immediately. One ml of this extract was diluted with 1 ml of 2N H_2SO_4 and 0.5 ml of this acidified extract was cooled in ice water for one hour. Later 0.2 ml of cold potassium tri iodide solution was added and mixed gently with a vortex mixture and the tubes were stored at 0°C for 15 minutes at 10,000 rpm. The supernatant was aspirated with a fine tipped glass tube. The per iodide crystals were dissolved in 9

ml of 1, 2 – Dichloroethane with vigorous vortexing. After 2.5 hours, the absorbance was measured at 365 nm and was expressed in µmol/g dry weight.

3.3.3. Physiological analysis

3.3.3.1. Relative Water Content (RWC) (%)

Relative Water Content (RWC) is the appropriate measure of plant water stress in terms of the physiological consequence of cellular water deficit. It estimates the current water content of the sampled leaf tissue relative to the maximal water content it can hold at full turgidity.

Twenty leaf discs of one centimetre diameter were taken from the youngest matured leaf and fresh weight was recorded. The discs were then floated in water taken in a petri-plate and then covered with another petri dish, for four hours at room temperature and ambient light. The tissues were then gently bloated with tissue paper and the turgid weight was recorded. The leaf discs were oven dried for 80°C for 24 hours and the dry weight was recorded (Barrs, 1968).

$$\text{Relative Water Content} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.3.3.2. Cell Membrane Stability (%)

The cell membrane stability was studied by observing the leakage of the membrane under stress. Leaf discs of 0.1g were taken in a test tube and 15 ml of distilled water was added and kept for three hours. The leaf discs were removed and the electrical conductivity of the solution was measured (C_1). After the initial measurements, leaf discs were returned to the original solution and boiled for 10 minutes. Leaf discs were removed and the solution was cooled. The electrical conductivity of the solution was observed again (C_2) (Dexter *et al.*, 1932).

$$\text{Membrane stability} = \frac{\text{Initial conductivity}}{\text{Final conductivity}}$$

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3.3.3.3. Chlorophyll Stability Index (CSI) (%)

Two fresh leaf samples of 0.1g were weighed separately and kept in two test tubes containing seven ml dimethyl sulfoxide (DMSO). One sample was subjected to a temperature of 55°C for 30 minutes by keeping on hot water bath (treated) and the other sample was kept at room temperature (control). The samples were removed after 30 minutes. The control should be kept for some days so as to extract the complete chlorophyll content out of the leaves which took approximately two weeks for cocoa. After the incubation period, three ml of DMSO was added to make it up to 10 ml (V). The absorbance at 652nm (A_{652}) was recorded (Kaloyereas, 1958). The chlorophyll content (mg g^{-1} of fresh tissue) of the two samples (control and treated) were estimated as shown below:

$$\text{Chlorophyll content} = \frac{A_{652} / 34.2 \times 1000 \times V}{1000 \times W}$$

The Chlorophyll Stability Index was worked out using the following formulae:

$$\text{CSI} = \frac{(\text{Chlorophyll in control} - \text{Chlorophyll in treated})}{\text{Chlorophyll in control}}$$

3.3.3.4. Leaf temperature (°C)

The leaf temperature was measured using the infrared gas analyser (IRGA). The reading was recorded during morning and evening hours. It is measured in °C (Plate 10).

3.3.3.5. Transpiration rate (mmol H_2O m^{-2} s^{-1})

The transpiration rate of leaves was measured using the infrared gas analyser (IRGA). The reading was recorded during morning and evening hours (Plate 10).

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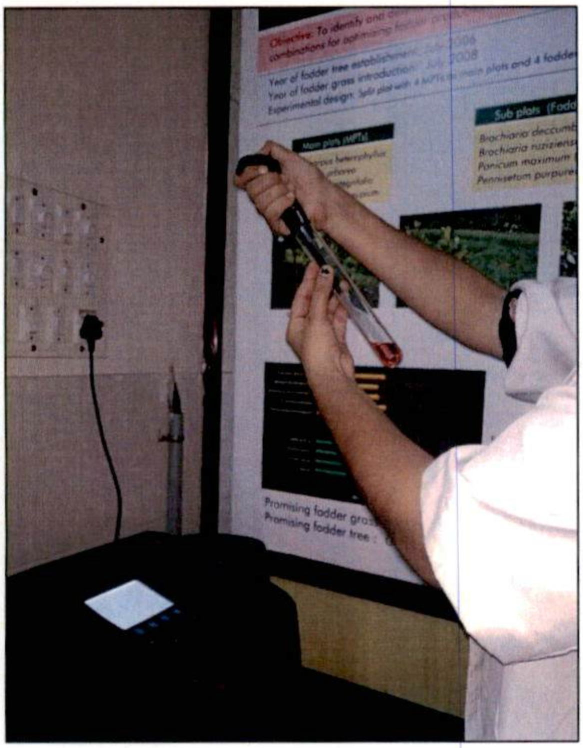
3.3.3.6. Photosynthesis rate (μmol CO_2 m^{-2} s^{-1})

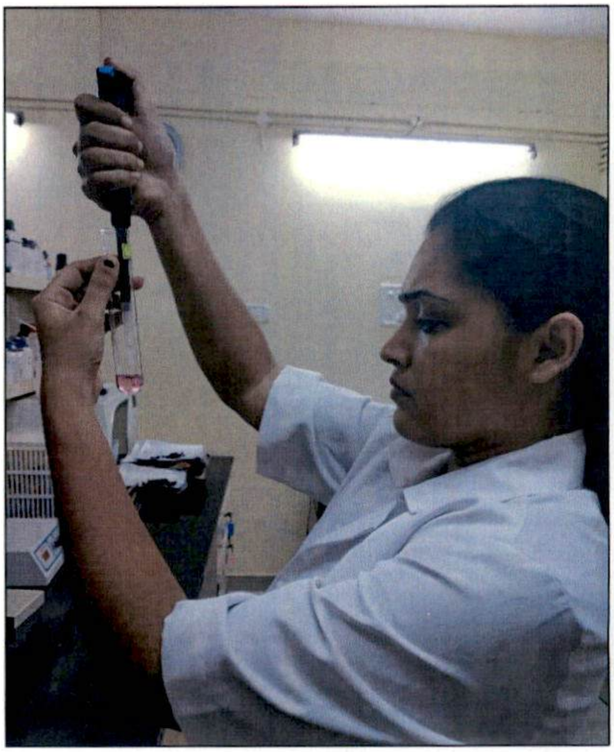
The photosynthetic rate of leaves was measured using the infrared gas analyser (IRGA). The reading was recorded during morning and evening hours (Plate 10).

3.3.3.7. Chlorophyll content (SPAD units)

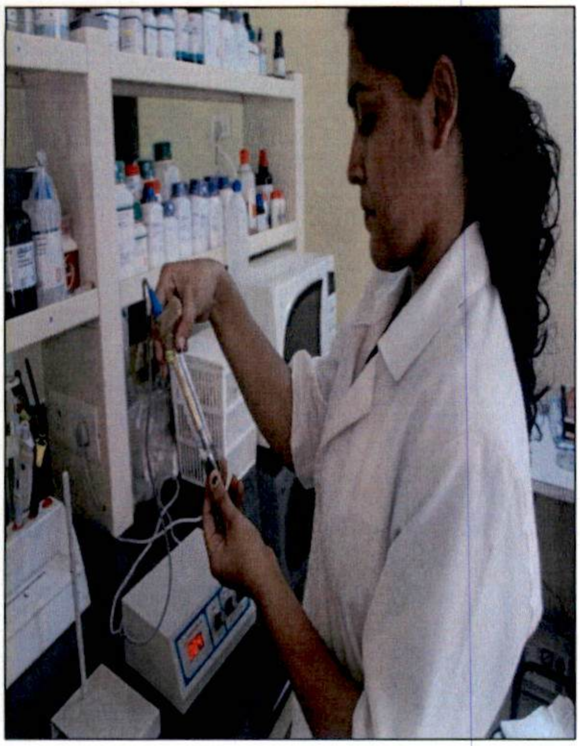
The chlorophyll content was taken using spadmeter.



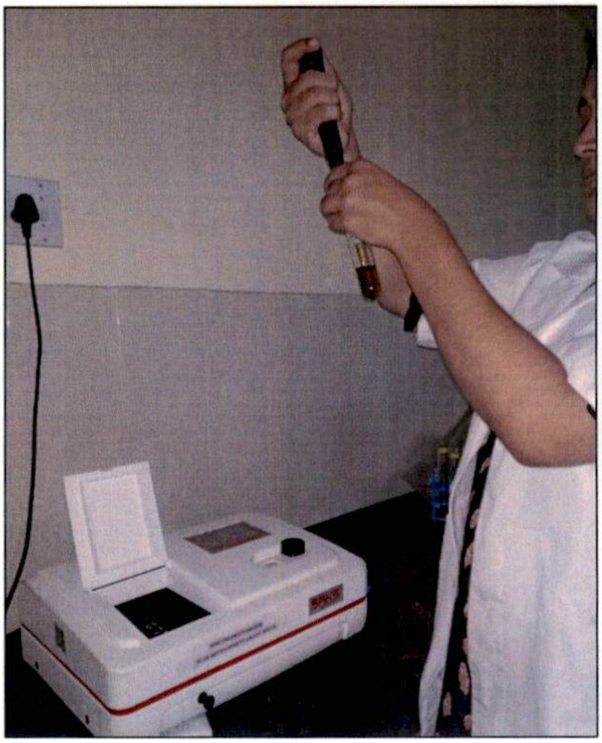
(a). Analysis of glycine betaine



(b). Analysis of nitrate reductase activity



(c). Analysis of membrane stability



(d). Analysis of chlorophyll stability index

Plate 9. Biochemical and physiological analysis

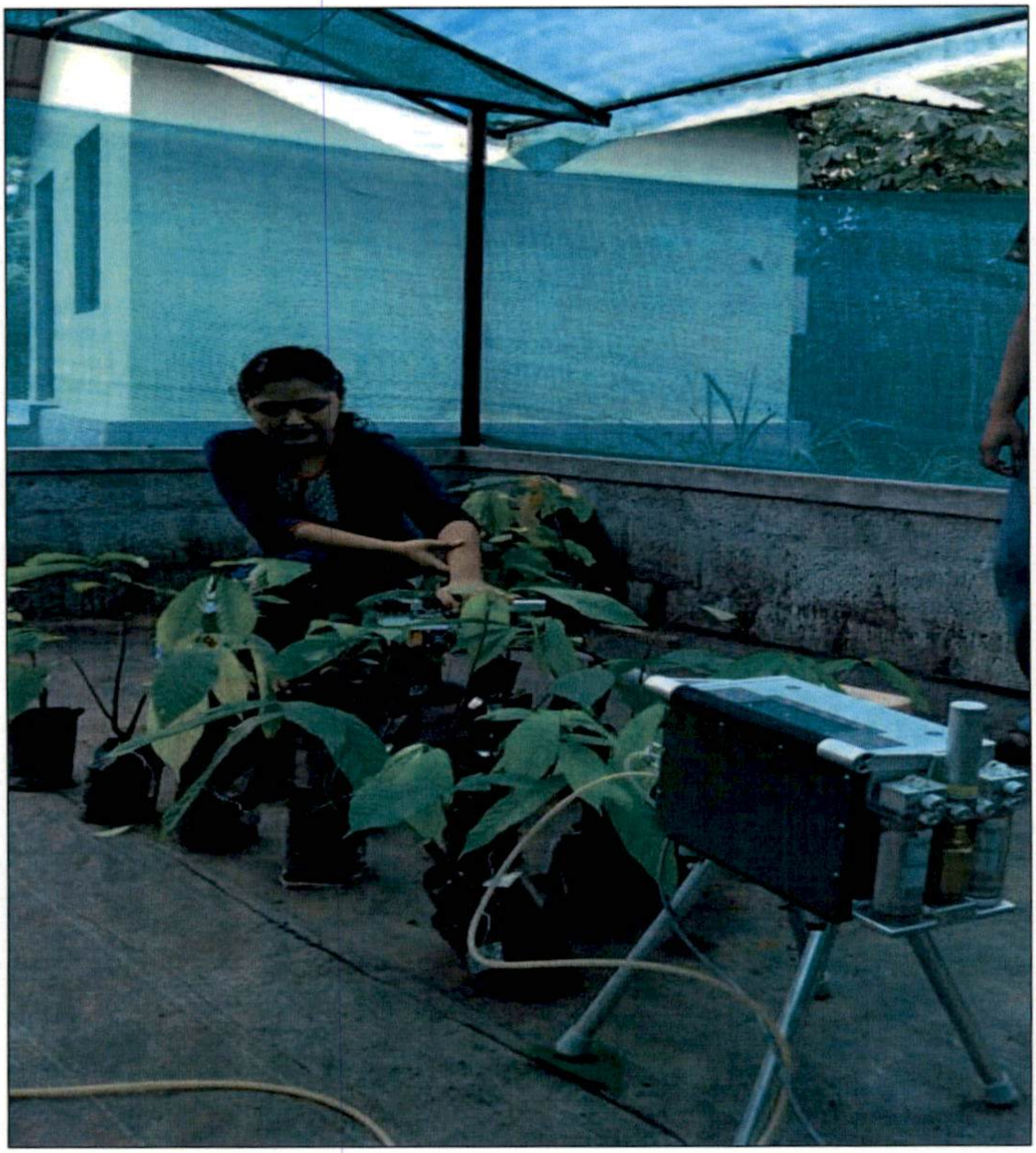


Plate 10. Recording photosynthesis, transpiration rate and leaf temperature using IRGA

3.4. Statistical analysis of the hybrids selected

Analysis of variance was done for all biochemical and physiological characters for all selected hybrids following **completely randomised design (CRD).**

Table 3. Skeleton of ANOVA for completely randomised design

Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F value**
Treatments	t-1	TrtSS	TrtMS = TrtSS/(t-1)	TrtMS/EMS
Error	t-n	ESS	EMS = ESS/(t-n)	
Total	n-1	TSS		

Where,

t= no. of treatments

n=total no. of observations

3.5. Correlation studies

The correlation coefficients were calculated to determine the degree of association of characters with percentage of leaves retained. The genotypic coefficients of correlation between character pairs were determined by using the variance and covariance components as given by Al-Jabouri *et al.* (1958).

$$\text{Genotypic correlation, } r_g(x,y) = \frac{COV(x, y)}{\sqrt{\sigma^2_g(x)} \, . \, \sqrt{\sigma^2_g(y)}}$$

Where,

Cov_g = Genotypic covariance of character x and y

$\sigma^2_g(x)$ = Genotypic variance of character of x

$\sigma^2_g(y)$ = Genotypic variance of character y

3.6. Path coefficient analysis

The direct and indirect effects of yield components was estimated by path coefficient analysis by using the simple correlation coefficient. The method was developed by Wright (1921) and used by Dewey and Lu (1959). The path coefficient is the standard partial regression coefficient, which is estimated by setting up simultaneous equation and solving by elimination method or metric inversion method.

$$Po_1 + Po_2\, r_{12} + \text{----------------} + Pop\; r_1p = ro_1$$
$$Po_1 + r_{12} + Po_2 + \text{------------} + Pop\; r_2p = ro_2$$
$$Po_1 + r_1p + Po_2\, r_2p + \text{-----------} + Pop = rop$$

Where,

Po_1, Po_2 --------------- Pop = Direct path coefficient of variable 1, 2………P on the dependent variables.

r_{12}, r_{13} ---------------------- r_1p --------- rp (p – 1) = possible correlation coefficients between various independent variables.

ro_1, ro_2 ---------------------- rop = the correlation coefficients between dependent variable and independent variables.

The direct effect of i^th^ variable via j^th^ variable was estimated as $(Poj \times r^{ij})$. It is clear that the correlation coefficients is the sum of direct and indirect effect on dependent variable, from the simultaneous equation. Residual effect of P^2 ox was calculated as under:

$$P_2ox = 1\ (P^2\, 01 + 2\, Po_2\, r_{12} + 2\, Po_1\, Po_3\, r_{13}\ \text{---------}\ 2\, Po_2\, Po_3\, r_{23} + \text{--------}\ P2\ op)$$

3.7. Genetic components

3.7.1. Variance

3.7.1.1. Genotypic variance $(\sigma^2 g) = \dfrac{\text{Mean sum of treatments - Mean sum of error}}{\text{No. of replication}}$

3.7.1.2. Phenotypic variance ($\sigma^2 p$) = Genotypic variance ($\sigma^2 g$) + Environmental variance ($\sigma^2 e$)

3.7.1.3. Environmental variance ($\sigma^2 e$) = Mean sum of errors (MSe)

3.7.2. Co-efficient of variation:

3.7.2.1. Phenotypic coefficient of variation = $\frac{\text{Phenotypic variance}}{\text{Mean}} \times 100$

3.7.2.2. Genotypic co-efficient of variation = $\frac{\text{Genotypic variance}}{\text{Mean}} \times 100$

3.7.2.3. Environmental co-efficient of variation = $\frac{\text{Environmental variance}}{\text{Mean}} \times 100$

PCV and GCV were classified as low (0-10 %); moderate (10-20 %) and high (>20 %) as per Sivasubramanian and Menon (1973).

3.7.3. Heritability (H^2)

$$\text{Heritability (\%)} = \frac{\text{Genotypic variance (Vg)}}{\text{Phenotypic variance (Vp)}} \times 100$$

The heritability estimates were categorized as low (0 – 30 %), medium (31 – 60 %) and high (>60 %) as suggested by Johnson *et al*. (1955).

3.7.4. Genetic Advance (GA)

It was first given by Johnson *et al.* (1955).

$$GA = H^2 \times \sigma p \times k$$

Where,

H^2 = heritability estimate in broad sense

σp = Phenotypic standard deviation of the trait

k = Standard selection differential which is 2.06 at 5 per cent selection intensity

Further, the Genetic gain was calculated by computing the formula:

3.7.5. Genetic gain

$$\text{Genetic gain (\%)} = \frac{\text{Genetic Advance}}{\text{Grand mean}} \times 100$$

Genetic gain is low (< 10 %); moderate (10 – 20 %) and high (> 20 %) as suggested by Johnson *et al*. (1955).

3.8. Combining ability studies

Combining ability analysis of the traits with significant genotypic difference was done according to the model I (fixed genotypic effects) and method II (half diallel) of Griffing (1956). In this method, only the GCA and SCA can be calculated. The reciprocal variance cannot be estimated (Table 4 and 5).

The direct crosses were selected and the analysis of variance was done on all the biochemical and physiological characters. The significance of F value for genotypes indicated the significant difference among the genotypes studied and they were further continued for combining ability analysis. Griffing's analysis indicates the performance of the parents and their relative contribution to the F_1 expressed as general and specific combining ability. The method to work out the sum of squares due to various source of variation for combining ability regarding method II [p (p-1)/2] is given as:

Table 4. Skeleton of ANOVA for GCA and SCA

Source	df	Sum of squares
GCA	$p - 1$	$\frac{1}{(p+2)} [\Sigma (Yi + Yii)^2 - \frac{4}{p} Y^2..]$
SCA	$\frac{p(p-1)}{2}$	$\Sigma\Sigma Yij^2 - \frac{1}{(p+2)} \Sigma (Yi + Y_{ii})^2. + \frac{2}{(p+1)(p+2)} Y^2..$

Error	--	---
Total	$\frac{p(p-1)}{2} - 1$	---

Where p = number of parents

Yii = Mean value of the i^th^ parent, Yij = Mean value of the j^th^ parent

Table 5. Skeleton of ANOVA for mean sum of squares

Source	Mean sum of squares	Expectations of mean squares
GCA	Mg	$\sigma^2 e + (p+2)\left(\frac{1}{(p-1)}\right)\Sigma gi^2$
SCA	Ms	$\sigma^2 e + \left(\frac{2}{(p(p-1))}\right)\Sigma sij^2$
Error	Me′	$\sigma^2 e$

$Me' = \frac{EMS}{r}$. The EMS is taken from ANOVA table of CRD.

Mg = Mean sum of squares due to GCA

Ms = Mean sum of squares due to SCA

Me′= Mean sum of squares due to error

r = No. of replication

The significant differences within each of the component effects were tested by F-test:

Fg = Mg/Me′ and Fs = Ms/Me′, for *m* degrees of freedom

3.8.1. Genetic components

3.8.1.1. Variance due to GCA = $\Sigma gi^2 = \frac{Mg - M_s}{(p + 2)}$

3.8.1.2. Variance due to SCA = $\Sigma\Sigma sij^2 = Ms - Me'$

The ratio of GCA variance/SCA variance suggests the relative significance of additive versus non- additive genetic variance.

When the mean square of GCA and SCA are significant, the estimates of GCA and SCA effects were calculated using the formula:

3.8.1.3. GCA effect of parents (gi) = $\frac{1}{(p + 2)} \left[(Yi + Y_{ii}) - \frac{2}{p} Y..\right]$

3.8.1.4. SCA effect of hybrids (sij) = $Yij - \frac{1}{(p + 2)} (Yi. + Y_{ii} + Y_j + Y_{jj}) + \frac{2}{(p + 1)(p + 2)} Y..$

Significance of effects can be evaluated by ‘t’ test.

3.8.1.5. SE for GCA effect of parents = $\frac{\sqrt{(p - 1)}}{\sqrt{p}\,(p + 2)} Me'$

3.8.1.6. SE for SCA effect of hybrids = $\frac{\sqrt{2(p + 1)}}{\sqrt{(p + 2)}} Me'$

The‘t’ value obtained from the table is then compared with the‘t’ value obtained from parents and hybrids and the GCA and SCA effects are determined.

3.8.2. Narrow sense heritability (h^2) from SCA and GCA variance

$$h^2 = \frac{2V_{gca}}{2(V_{gca} + V_{sca} + V_e)} \times 100$$

3.9. Binary logistic regression model

It is a uni/multivariate technique that is used to estimate the probability that a character is present by predicting a binary dependent outcome from a set of explanatory variables and it is used for model binary response data. When response is binary, it takes the value zero and one which indicates resistant/susceptible variety. In this model, the independent variable is categorical.

A logistic model is used to predict the effect of change in the independent variable on the probability of belonging to a group when the dependent variable are dichotomous (Mafini and Omoruyi, 2013).

$$Pi = E(Y = 1/Xi) = \frac{1}{1+ e^{-(\alpha+\beta iXi)}}$$

Where,

Pi is the probability

Xi is the vector of independent variables

Bi are the co-efficient to be estimated

$$Pi = \frac{1}{1 + e^{-zi}} = \frac{e^{zi}}{1+ e^{zi}}$$

Where,

$$Zi = \alpha + \beta iXi$$

$$1 - Pi = \frac{1}{1 + e^{zi}}$$

It is the probability of characteristics to be grouped as those which follow the score for drought tolerance and susceptibility for given set of independent variables.

$$\frac{Pi}{1 - Pi} = e^{zi}$$

Taking algorithm on both the sides, the model will be,

$$Li = In (Pi / 1- Pi) = Zi = \alpha + \beta iXi$$

The logistic model in this study is $Y = \alpha + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X4 + \ldots\ldots\ldots\ldots$

Where, α is the intercept and β is the co-efficient of the corresponding variables.

Here,

Y = one for the characters influencing for drought tolerance

Y = zero for the characters influencing for drought susceptibility

$$\text{Per cent improvement over base population} = \frac{\text{Exp (B)}}{1+\text{Exp (B)}} \times 100$$

Results & Discussion

IV. RESULTS AND DISCUSSION

The present study was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Cocoa Research Centre, Vellanikkara and College of Forestry, Vellanikkara during the year 2016- 2018 with the objective to evolve drought tolerant cocoa hybrids and to select the superior hybrid among them.

The results of the study are presented below:

4.1. Hybridization programme

The parents were selected based on the study conducted by Binimol (2005). Four drought tolerant genotypes were identified and these were crossed in 12 different cross combinations.

Table 6. Nursery and pollination observations

Cross	Flowers pollinated	Fruit set	No. of seeds	Germinated seeds	Days to 50% Germination	Germination %
M 13.12 x G I 5.9	136	11	405	389	7-8	96.04
M 13.12 x G II 19.5	269	3	138	126	7-8	91.30
M 13.12 x G VI 55	197	5	165	151	9-10	91.50
G I 5.9 x M 13.12	189	1	35	35	9-10	100.00
G I 5.9 x G II 19.5	261	5	151	133	7-8	88.07
G I 5.9 x G VI 55	121	1	46	40	8	86.95
G II 19.5 x M 13.12	284	1	34	32	10	94.11
G II 19.5 x G I 5.9	326	7	224	220	9-10	98.21
G II 19.5 x G VI 55	172	2	53	53	7-8	100.00
G VI 55 x M 13.12	640	Nil	Nil	Nil	Nil	Nil
G VI 55 x G I 5.9	533	4	171	168	8-9	98.24
G VI 55 x G II 19.5	188	4	171	156	7-8	91.20

The highest number of pods were obtained by the cross between M 13.12 and G I 5.9 which comprised of 11 pods, followed by the cross between G II 19.5

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and G I 5.9. G I 5.9 x M 13.12, G I 5.9 x G VI 55 and G II 19.5 x M 13.12 gave only one pod whereas the cross G VI 55 x M 13.12 did not yield any pods (Table 6).

The variation in pod set was due to the difference in cross compatibility between genotypes. The cross G VI 55 x M 13.12 did not yield any pod even after pollinating 640 flowers (Table 6). This was due to cross incompatibility between the genotypes. In cocoa, cross-incompatibility was also reported like self-incompatibility, in which one of the reasons cited was that the two genotypes will be having similar genetic make- up (Richards, 1996; Mallika *et al.*, 2002).

When germination per cent was accounted, 100 per cent germination was found in cross G I 5.9 x M 13.12 and G II 19.5 x G VI 55 and the least germination per cent was found in G I 5.9 x G VI 55 cross, which was about 86.96 per cent (Table 6).

A minimum of 7-8 days were taken by crosses M 13.12 x G I 5.9, M 13.12 x G II 19.5, G I 5.9 x G II 19.5, G II 19.5 x G VI 55 and G VI 55 x G II 19.5 for 50 per cent germination of seeds and a maximum of 10 days by the cross G II 19.5 x M 13.12. The trend followed already reported results in cocoa germination (Mayer and Mayber, 1982).

4.2. Screening for the initial vigour

At the third month stage, the plants were screened for initial vigour by a method suggested by Enriquez (1981). According to his findings, plants with higher HD^2 (Height x Diameter^2) value at early stage were found to be high yielder at stable yielding stage.

The seedling's height and diameter were recorded which is presented in Appendix I along with the number of leaves and the chlorophyll content and superior ones were selected based on these observations. The range of HD^2 value used for selection differed between crosses. The selected hybrids are depicted in Table 7. A total of 120 hybrids from various crosses were selected.

After the hybrids were selected, they were subjected to drought screening by gravimetric method for two weeks after which they were analysed for biochemical and physiological parameters.

After two weeks of preliminary screening, the main symptom shown by drought stressed plants was the withering of leaves and based on which the plants were divided into 4 categories (Table 2). The reaction of hybrid to stress screening is presented in table 7.

Table 7. Reaction of hybrids to drought stress.

Hybrid number	**Hybrid**	**Total number of leaves**	**Number of leaves withered**	**Percentage leaves retained (%)**	**Reaction to drought**
H1	M 13.12 x G I 5.9 (i) 13	17	12	29.42	Susceptible
H2	M 13.12 x G I 5.9 (i) 14	25	9	64	Tolerant
H3	M 13.12 x G I 5.9 (i) 17	26	21	19.24	Susceptible
H4	M 13.12 x G I 5.9 (ii) 13	20	15	25	Susceptible
H5	M 13.12 x G I 5.9 (ii) 17	30	22	26.67	Susceptible
H6	M 13.12 x G I 5.9 (ii) 20	31	22	29.04	Susceptible
H7	M 13.12 x G I 5.9 (ii) 23	38	26	31.58	Susceptible
H8	M 13.12 x G I 5.9 (ii) 26	41	27	34.15	Susceptible
H9	M 13.12 x G I 5.9 (ii) 28	35	11	68.58	Tolerant
H10	M 13.12 x G I 5.9 (iii) 12	22	13	40.91	Tolerant
H11	M 13.12 x G I 5.9 (iv) 18	32	17	46.88	Tolerant
H12	M 13.12 x G I 5.9 (iv) 19	19	8	57.9	Tolerant
H13	M 13.12 x G I 5.9 (iv) 24	30	16	46.67	Tolerant
H14	M 13.12 x G I 5.9 (iv) 35	32	23	28.13	Susceptible
H15	M 13.12 x G I 5.9 (v) 4	36	27	25	Susceptible
H16	M 13.12 x G I 5.9 (vi) 7	23	14	39.13	Susceptible
H17	M 13.12 x G I 5.9 (vi) 22	20	16	20	Susceptible
H18	M 13.12 x G I 5.9 (vi) 31	26	21	19.24	Susceptible
H19	M 13.12 x G I 5.9 (vi) 33	28	22	21.43	Susceptible

H20	M 13.12 x G I 5.9 (vii) 4	19	11	42.11	Tolerant
H21	M 13.12 x G I 5.9 (vii) 17	42	31	26.19	Susceptible
H22	M 13.12 x G I 5.9 (vii) 19	20	15	25	Susceptible
H23	M 13.12 x G I 5.9 (vii) 20	39	31	20.52	Susceptible
H24	M 13.12 x G I 5.9 (vii) 30	39	22	43.59	Tolerant
H25	M 13.12 x G I 5.9 (vii) 32	24	13	45.84	Tolerant
H26	M 13.12 x G I 5.9 (vii) 37	27	13	51.86	Tolerant
H27	M 13.12 x G I 5.9 (viii) 6	29	8	72.42	Highly tolerant
H28	M 13.12 x G I 5.9 (viii) 17	32	14	56.25	Tolerant
H29	M 13.12 x G I 5.9 (viii) 18	26	11	57.7	Tolerant
H30	M 13.12 x G I 5.9 (viii) 32	25	16	36	Susceptible
H31	M 13.12 x G I 5.9 (viii) 33	28	13	53.58	Tolerant
H32	M 13.12 x G I 5.9 (viii) 37	26	19	26.93	Susceptible
H33	M 13.12 x G II 19.5 (i) 29	32	11	65.63	Tolerant
H34	M 13.12 x G II 19.5 (ii) 4	63	30	52.39	Tolerant
H35	M 13.12 x G II 19.5 (ii) 11	38	24	36.85	Susceptible
H36	M 13.12 x G II 19.5 (ii) 17	36	22	38.89	Susceptible
H37	M 13.12 x G II 19.5 (ii) 24	23	10	56.53	Tolerant
H38	M 13.12 x G II 19.5 (ii) 26	43	11	74.42	Highly tolerant
H39	M 13.12 x G II 19.5 (ii) 28	35	20	42.86	Tolerant
H40	M 13.12 x G II 19.5 (ii) 39	35	26	25.72	Susceptible
H41	M 13.12 x G II 19.5 (iii) 6	33	24	27.28	Susceptible
H42	M 13.12 x G II 19.5 (iii) 21	54	18	66.67	Tolerant
H43	M 13.12 x G VI 55 (i) 12	39	9	76.93	Highly tolerant
H44	M 13.12 x G VI 55 (ii) 32	43	39	9.31	Highly susceptible
H45	M 13.12 x G VI 55 (ii) 15	32	26	18.75	Susceptible
H46	M 13.12 x G VI 55 (ii) 27	40	15	62.5	Tolerant
H47	M 13.12 x G VI 55 (iii) 4	38	32	15.79	Susceptible
H48	M 13.12 x G VI 55 (iii) 8	32	17	46.88	Tolerant
H49	M 13.12 x G VI 55 (iii) 16	31	19	38.8	Susceptible
H50	M 13.12 x G VI 55 (iv) 11	39	28	28.25	Susceptible

H51	M 13.12 x G VI 55 (iv) 12	26	21	19.24	Susceptible
H52	G I 5.9 x M13.12 (i) 2	34	15	55.89	Tolerant
H53	G I 5.9 x M13.12 (i) 6	41	28	31.71	Susceptible
H54	G I 5.9 x M13.12 (i) 9	42	33	21.43	Susceptible
H55	G I 5.9 x M13.12 (i) 13	34	23	32.36	Susceptible
H56	G I 5.9 x M13.12 (i) 21	30	21	30	Susceptible
H57	G I 5.9 x M13.12 (i) 27	30	14	53.34	Tolerant
H58	G I 5.9 x G II 19.5 (i) 5	37	27	27.03	Susceptible
H59	G I 5.9 x G II 19.5 (ii) 8	46	31	32.61	Susceptible
H60	G I 5.9 x G II 19.5 (ii) 14	28	18	35.72	Susceptible
H61	G I 5.9 x G II 19.5 (ii) 16	40	27	32.5	Susceptible
H62	G I 5.9 x G II 19.5 (ii) 17	32	14	56.25	Tolerant
H63	G I 5.9 x G II 19.5 (ii) 24	38	13	65.79	Tolerant
H64	G I 5.9 x G II 19.5 (ii) 25	28	11	60.72	Tolerant
H65	G I 5.9 x G II 19.5 (iii) 10	23	15	34.79	Susceptible
H66	G I 5.9 x G II 19.5 (iv) 11	49	44	10.21	Susceptible
H67	G I 5.9 x G II 19.5 (iv) 12	49	17	65.31	Tolerant
H68	G I 5.9 x G II 19.5 (iv) 20	36	13	63.89	Tolerant
H69	G I 5.9 x G II 19.5 (iv) 25	13	10	23.08	Susceptible
H70	G I 5.9 x G II 19.5 (v) 6	34	25	26.48	Susceptible
H71	G I 5.9 x G VI 55 (i) 3	26	7	73.08	Highly tolerant
H72	G I 5.9 x G VI 55 (i) 5	23	16	30.44	Susceptible
H73	G I 5.9 x G VI 55 (i) 8	28	18	35.72	Susceptible
H74	G I 5.9 x G VI 55 (i) 17	21	6	71.43	Highly tolerant
H75	G I 5.9 x G VI 55 (i) 18	38	22	42.11	Tolerant
H76	G I 5.9 x G VI 55 (i) 26	27	7	74.08	Highly tolerant
H77	G I 5.9 x G VI 55 (i) 28	28	25	10.72	Susceptible
H78	G I 5.9 x G VI 55 (i) 35	48	35	27.09	Susceptible
H79	G II 19.5 x M 13.12 (i) 1	24	8	66.67	Tolerant
H80	G II 19.5 x M 13.12 (i) 2	27	18	33.34	Susceptible
H81	G II 19.5 x M 13.12 (i) 4	26	12	53.85	Tolerant

H82	G II 19.5 x M 13.12 (i) 5	23	8	65.22	Tolerant
H83	G II 19.5 x M 13.12 (i) 7	23	11	52.18	Tolerant
H84	G II 19.5 x M 13.12 (i) 10	27	18	33.34	Susceptible
H85	G II 19.5 x M 13.12 (i) 12	28	7	75	Highly tolerant
H86	G II 19.5 x M 13.12 (i) 13	29	19	34.49	Susceptible
H87	G II 19.5 x M 13.12 (i) 14	29	20	31.04	Susceptible
H88	G II 19.5 x M 13.12 (i) 19	21	8	61.91	Tolerant
H89	G II 19.5 x M 13.12 (i) 21	25	17	32	Susceptible
H90	G II 19.5 x M 13.12 (i) 22	25	6	76	Highly tolerant
H91	G II 19.5 x M 13.12 (i) 23	26	22	15.39	Susceptible
H92	G II 19.5 x M 13.12 (i) 25	28	19	32.15	Susceptible
H93	G II 19.5 x M 13.12 (i) 30	28	18	35.72	Susceptible
H94	G II 19.5 x M 13.12 (i) 31	24	13	45.84	Tolerant
H95	G II 19.5 x M 13.12 (i) 33	22	16	27.28	Susceptible
H96	G II 19.5 x M 13.12 (i) 34	29	8	72.42	Highly tolerant
H97	G II 19.5 x G I 5.9 (i) 8	22	6	72.73	Highly tolerant
H98	G II 19.5 x G I 5.9 (i) 14	31	25	19.36	Susceptible
H99	G II 19.5 x G I 5.9 (i) 18	36	23	36.12	Susceptible
H100	G II 19.5 x G I 5.9 (ii) 25	32	23	28.13	Susceptible
H101	G II 19.5 x G I 5.9 (ii) 29	35	9	74.29	Highly tolerant
H102	G II 19.5 x G I 5.9 (iii) 10	44	23	47.73	Tolerant
H103	G II 19.5 x G I 5.9 (iii) 30	44	20	54.55	Tolerant
H104	G II 19.5 x G I 5.9 (iv) 10	39	17	56.41	Tolerant
H105	G II 19.5 x G I 5.9 (iv) 12	31	27	12.91	Susceptible
H106	G II 19.5 x G I 5.9 (v) 12	42	34	19.05	Susceptible
H107	G II 19.5 x G VI 55 (i) 16	37	9	75.68	Highly tolerant
H108	G II 19.5 x G VI 55 (i) 18	25	16	36	Susceptible
H109	G VI 55 x G I 5.9 (i) 2	32	22	31.25	Susceptible
H110	G VI 55 x G I 5.9 (i) 15	33	26	21.22	Susceptible
H111	G VI 55 x G I 5.9 (i) 16	42	23	45.24	Tolerant
H112	G VI 55 x G I 5.9 (i) 25	42	24	42.86	Tolerant

H113	G VI 55 x G I 5.9 (iii) 6	31	14	54.84	Tolerant
H114	G VI 55 x G I 5.9 (iv) 12	34	28	17.65	Susceptible
H115	G VI 55 x G II 19.5 (i) 7	28	12	57.15	Tolerant
H116	G VI 55 x G II 19.5 (ii) 10	34	26	23.53	Susceptible
H117	G VI 55 x G II 19.5 (ii) 11	35	13	62.86	Tolerant
H118	G VI 55 x G II 19.5 (ii) 17	35	10	71.43	Highly tolerant
H119	G VI 55 x G II 19.5 (iii) 23	47	27	42.56	Tolerant
H120	G VI 55 x G II 19.5 (iv) 40	31	14	54.84	Tolerant

Roman letters in brackets- Pod number

Numbers after the brackets- Plant number in the nursery

As per the visual observation, only one hybrid i.e., H 27 exhibited highly tolerant reaction in the cross M 13.12 x G I 5.9 followed by 13 hybrids i.e., H2, H9, H10, H11, H12, H13, H20, H24, H25, H26, H28, H29 and H31 with tolerance to drought stress. Remaining hybrids were susceptible in nature (Table 7).

In cross M 13.12 x G II 19.5, H38 was highly tolerant whereas H33, H34, H37, H39, H42 was tolerant and rest were susceptible (Table 7).

The cross M 13.12 x G VI 55 gave H43 which was highly tolerant and H44 was highly susceptible in nature. Only two hybrids i.e., H46 and H48 was tolerant and rest were susceptible (Table 7).

In the cross G I 5.9 x M 13.12, all the hybrids were found to be susceptible except H52 and H57 which expressed tolerant reaction (Table 7).

In the cross G I 5.9 x G II 19.5, H62, H63, H64, H67 and H68 were tolerant and rest were susceptible (Table 7).

In cross G I 5.9 x G VI 55, three highly tolerant hybrids were observed which were H71, H74 and H76 only one tolerant hybrid i.e., H75 was observed. Rest all were susceptible when visually observed (Table 7).

In case of cross G II 19.5 x M 13.12, H85, H90 and H96 were highly tolerant whereas H79, H81, H82, H83, H88 and H94 were tolerant in nature while all the other hybrids were susceptible (Table 7).

In hybrids of G II 19.5 x G I 5.9, only two hybrids, H97 and H101 were highly tolerant, and H102, H103 and H104 were tolerant (Table 7).

Only two hybrids were obtained from cross G II 19.5 x G VI 55 in which H107 was highly tolerant in nature and H108 was susceptible in nature (Table 7).

The cross G VI 55 x G I 5.9 were having both tolerant and susceptible hybrids with H111, H112 and H113 were tolerant and H109, H110 and H114 were susceptible in nature (Table 7).

In case of cross G VI 55 x G II 19.5, hybrid H118 was highly tolerant and H116 was susceptible in nature. H115, H117, H119 and H120 were tolerant (Table 7).

The result revealed that progeny from same cross segregated in reaction to drought. This is due to heterozygous nature of the parent. In cocoa hybrid production, this is a common phenomenon reported by many workers in various traits. Studies have been made on the F_1 and F_2 generations of crosses between the Amazon clones Nanay 32, Parinari 7, and Parinari 35. There is wide variation in size from pod to pod on a tree, and this is largely paralleled by variation in bean number, bean size remaining relatively constant. There is very considerable variation in pod size on a given tree, this being largely paralleled by variation in bean number, the bean size being relatively constant (Glendinning, 1963).

4.3. Analysis of biochemical parameters

Biochemical analysis was carried out in all 120 hybrids by collecting sample during stress-imposed period. This was to know the reaction of various biochemical parameters inside the plant when stress was imposed.

4.3.1. Proline

Proline is the primary osmolyte that accumulates in the plant cells when there is drought stress. The levels of proline differs from species to species and can be 100 times greater under water deficit compared to well-watered conditions (Verbruggen and Hermans, 2008). Proline accumulates in a diverse taxonomic group of plants in response to biotic and abiotic stresses (Szabados and Savoure, 2009) and is able to protect cells from damage by functioning as both an osmotic agent and a radical scavenger.

The analysis of proline was done on various hybrids, the result of which is indicated in the graphs and Tables 8-18.

In the cross M 13.12 x G I 5.9 (Fig. 1), the highest content was found in hybrid H27 (1105.64 μg/g) followed by hybrid H9 having 996.41 μg/g of proline. This was followed by the tolerant hybrids which were H2 (695.36 μg/g), H12 (539.50 μg/g), H26 (504.07 μg/g), H31 (490.21 μg/g), H28 (479.56 μg/g), H29 (479.54 μg/g), H24 (468.86 μg/g), H11 (452.91 μg/g), H13 (446.25 μg/g), H10 (440.93 μg/g), H25 (438.26 μg/g), H20 (418.28 μg/g). Low values were found in susceptible hybrids H8 (354.35 μg/g), H30 (340.35 μg/g), H16 (310.02 μg/g), H7 (304.19 μg/g), H6 (298.52 μg/g), H14 (293.06 μg/g), H21 (290 μg/g), H1 (269.08 μg/g), H15 (254.23 μg/g), H5 (247.51 μg/g), H4 (219.44 μg/g), H32 (209.81 μg/g), H22 (189.29 μg/g), H3 (181.96 μg/g), H19 (175.86 μg/g), H17 (174.24 μg/g), H23 (173.17 μg/g) and H18 (167.18 μg/g). The control that was kept in fully irrigated condition had the lowest value of proline of about 61.33 μg/g which indicated that proline accumulates only under drought stress conditions (Table 8).

The cross M 13.12 x G II 19.5 (Fig. 2) had ten hybrids out of which H38 showed the maximum content of proline of about 2710.82 μg/g. H33 (671.38 μg/g), H42 (619.43 μg/g), H34 (547.60 μg/g), H37 (523.52 μg/g) and H39

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(522.28 µg/g) were the tolerant hybrids having considerable amount of proline. Lowest value was found in H40 (224.46 µg/g) and other hybrids having low value of proline were H36 (459.65 µg/g), H35 (359.00 µg/g) and H41 (265.09 µg/g). However, the control plant was having only 75.73 µg/g indicating that drought resulted in production of more proline (Table 9).

In cross M 13.12 x G VI 55 (Fig. 3), the highest content was found in H43 (2817.39 µg/g). The tolerant hybrids were having the proline content as follows: H46 having a proline content of 643.40 µg/g and H48 having 494.21 µg/g indicating tolerance to drought stress. The susceptible hybrids were having lower amount of proline and they were H49 (400.96 µg/g), H50 (247.77 µg/g), H51 (170.51 µg/g), H45 (163.72 µg/g) and H47 (111.90 µg/g). The highly susceptible hybrid, H44 was having the lowest proline content of only about 85.52 µg/g of proline indicating it's vulnerability to drought stress and the control plant was having only 65.60 µg/g proline (Table 10).

The cross G I 5.9 x M 13.12 (Fig. 4) had six hybrids out of which the tolerant hybrids had the higher proline content. The well irrigated plants that were observed as control was having 85.70 µg/g proline. The hybrids H52 and H57 had proline content of 536.70 µg/g and 498.21 µg/g, respectively. The susceptible hybrids, H55 (386.31 µg/g), H53 (292.40 µg/g), H56 (235.78 µg/g) and H54 (181.17 µg/g) were having lower proline content (Table 11).

In the cross G I 5.9 x G II 19.5 (Fig. 5), out of 13 hybrids, H63 had the highest proline content of 706.01 µg/g followed byH67 (699.35 µg/g), H68 (684.66 µg/g), H64 (679.37 µg/g) and H62 (568.81 µg/g). Hybrid 70 was having the lowest proline content of 167.84 µg/g. Hybrids having lower values were H59 (333.96 µg/g), H60 (333.96 µg/g), H65 (325.70 µg/g), H58 (218.46 µg/g), H69 (196.48 µg/g) and H66 (177.26 µg/g). The control was having only 138 µg/g of proline (Table 12).

The cross G I 5.9 x G VI 55 (Fig. 6) had eight hybrids, and the highest content was found in H71 (1749.05 µg/g), followed by H74 (1555.89 µg/g) and H76 (1126.96 µg/g.). The tolerant hybrid, H75 was having a proline content of

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450.25 µg/g. Lowest values was found in Hybrid 77 (169.54 µg/g) along with other hybrids, H73 (395.63 µg/g), H72 (319.70 µg/g), and H78 (224.99 µg/g). The control was having only 101.33 µg/g proline as compared to other hybrids. The tolerant hybrids exhibited a high amount of proline content when compared to other crosses (Table 13).

In cross G II 19.5 x M 13.12 (Fig. 7), the highly tolerant hybrids were having high values of proline indicating their high level of tolerance to drought stress. The hybrids were: H85 (2293.88 µg/g), H96 (2011.47 µg/g) and H90 (1689.10 µg/g).The plants which were classified as tolerant were having the values: H79 (853.88 µg/g), H82 (692.69 µg/g), H88 (625.80 µg/g), H81 (498.21 µg/g), H83 (454.91 µg/g), and H94 (447.59 µg/g). The lowest values were found in H91 (139.87 µg/g) which was a susceptible hybrid. Other susceptible hybrids were H86 (374.99 µg/g), H87 (363.66 µg/g), H93 (338.75 µg/g), H80 (325.03 µg/g), H92 (303.72 µg/g), H89 (295.73 µg/g), H84 (269.08 µg/g) and H95 (208.47 µg/g). The control had the value lower than the susceptible hybrid of about 95.47 µg/g indicating proline is produced more under stress conditions (Table 14).

In the cross G II 19.5 x G I 5.9 (Fig. 8), high values for proline was observed in H97 (1490.62 µg/g) and H101 (2726.81 µg/g). The tolerant hybrids, 102, 103 and 104 were having values 454.25 µg/g, 500.87 µg/g and 543.50 µg/g respectively. The lowest values were observed in susceptible hybrids, H106 (162.52 µg/g). Other hybrids having lower proline values were H99 (363.66 µg/g), H100 (215.67 µg/g), H105 (171.93 µg/g) and H98 (169.18 µg/g) which were also classified as susceptible. The control was having only 90.13 µg/g of proline (Table 15).

In the cross G II 19.5 x G VI 55 (Fig. 9), only two hybrids were there, one of which was highly tolerant and the other one was susceptible. The H107 was having the proline content of 1984.83 µg/g and the susceptible one, H108 was having 339.69 µg/g of proline. The control plant was having only 69.33 µg/g of proline content which was much lower than other hybrids under stress (Table 16).

The cross G VI 55 x G I 5.9 (Fig. 10) were having six hybrids and H113 was having the highest value of proline, 520.85 µg/g followed by H111 (424.27 µg/g) and H112 (411.35 µg/g). The hybrid having lowest value was H114 (128.55 µg/g). Other low values were observed in H109 (308.91 µg/g) and H110 (151.99 µg/g). The control was having 74.67 µg/g of proline (Table 17).

In the cross G VI 55 x G II 19.5 (Fig. 11), highest value was observed in H118 (1354.74 µg/g) followed by some tolerant hybrids, H117 (743.17 µg/g), H115 (520.85 µg/g), H120 (507.53 µg/g) and H119 (412.95 µg/g). The lowest value was observed in H116 which was susceptible to drought having only 182.50 µg/g of proline. The control plant was having only 94 µg/g of proline (Table 18).

When the progenies of all crosses were compared, it was seen that the content ranged from 85.52 µg/g to 2817.39 µg/g. A clear cut difference was observed between highly tolerant and susceptible genotypes indicating that proline plays an important role in drought tolerance of cocoa seedlings.

The correlation between drought tolerance ability and proline content in response to osmotic stress had been documented in early studies (Hien *et al.*, 2003; Kishor and Sreenivasulu, 2014). High levels of proline enabled the plant to maintain low water potentials.

Apart from acting as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (eg., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007).

A study was conducted on sunflower plants grown in greenhouse conditions where drought stress was induced on these plants and the proline content was calculated. It was found that the young stressed leaves synthesised nearly seven times more proline than non-stressed leaves while the mature stressed leaves synthesised only four times more. After re-watering, the synthesis of proline in both young and mature leaves returned to the initial content. These findings support a positive role of proline as an osmoregulator, particularly in

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young leaves, which seems to act as a survival mechanism for the plants under water stress (Cechin *et al*., 2006).

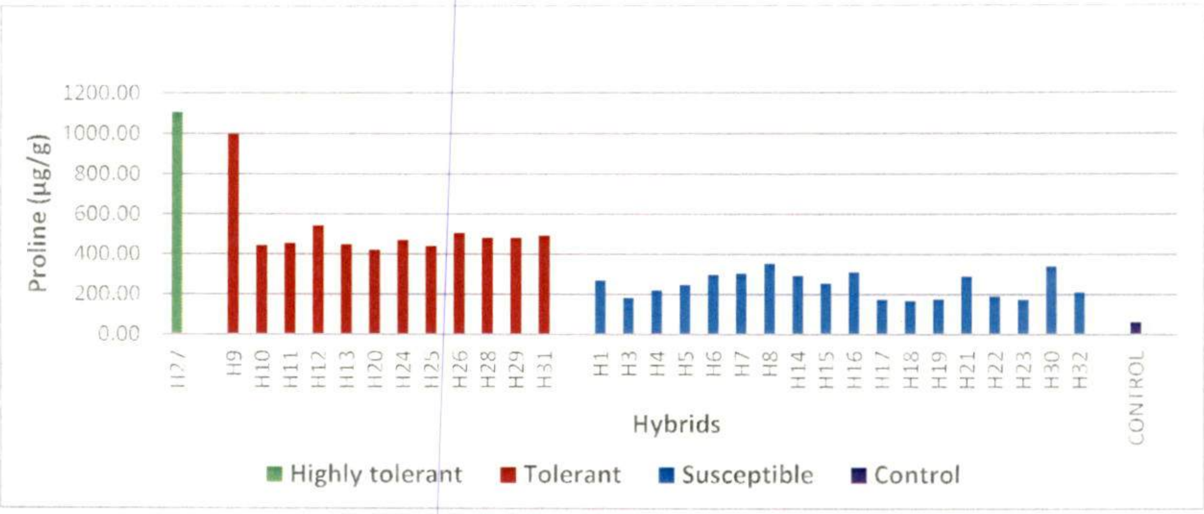

Fig. 1. Proline content of M 13.12 x G I 5.9

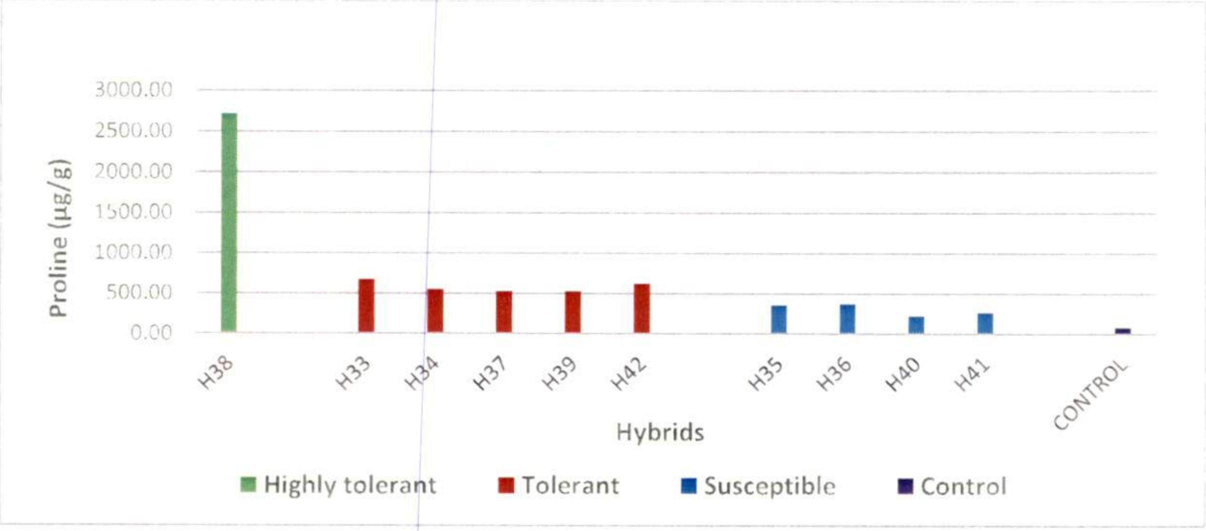

Fig. 2. Proline content of M 13.12 x G II 19.5

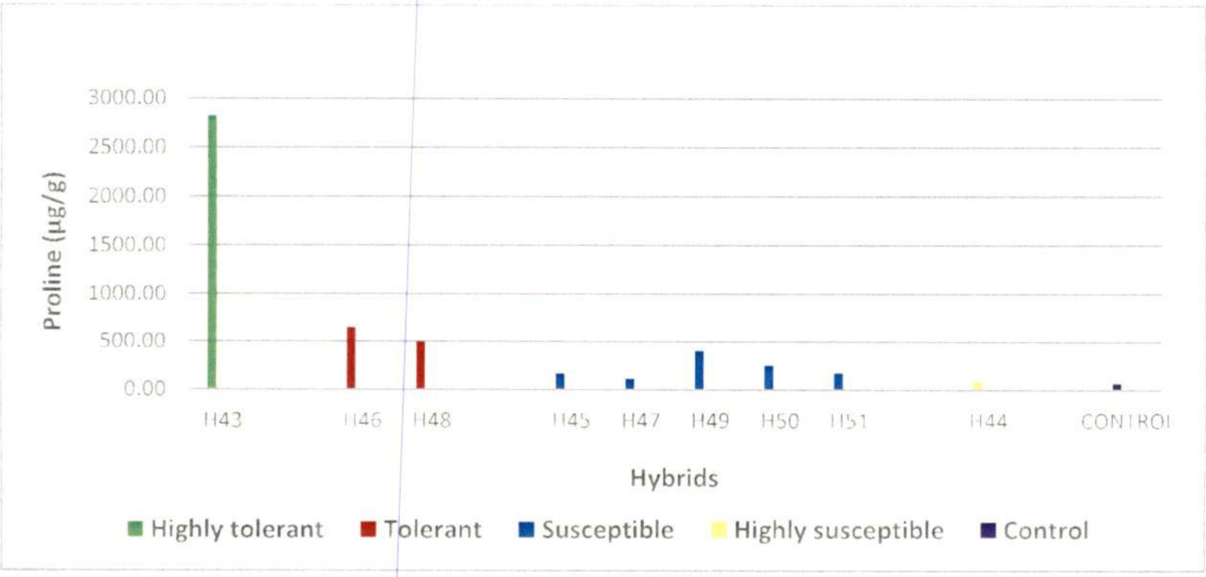

Fig. 3. Proline content of M 13.12 x G VI 55

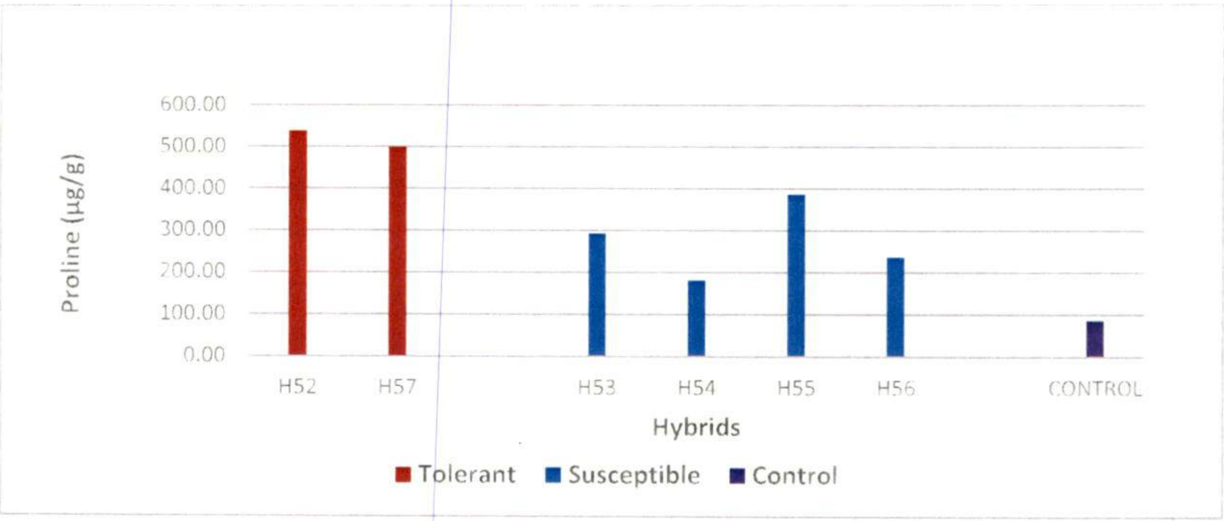

Fig. 4. Proline content of G I 5.9 x M 13.12

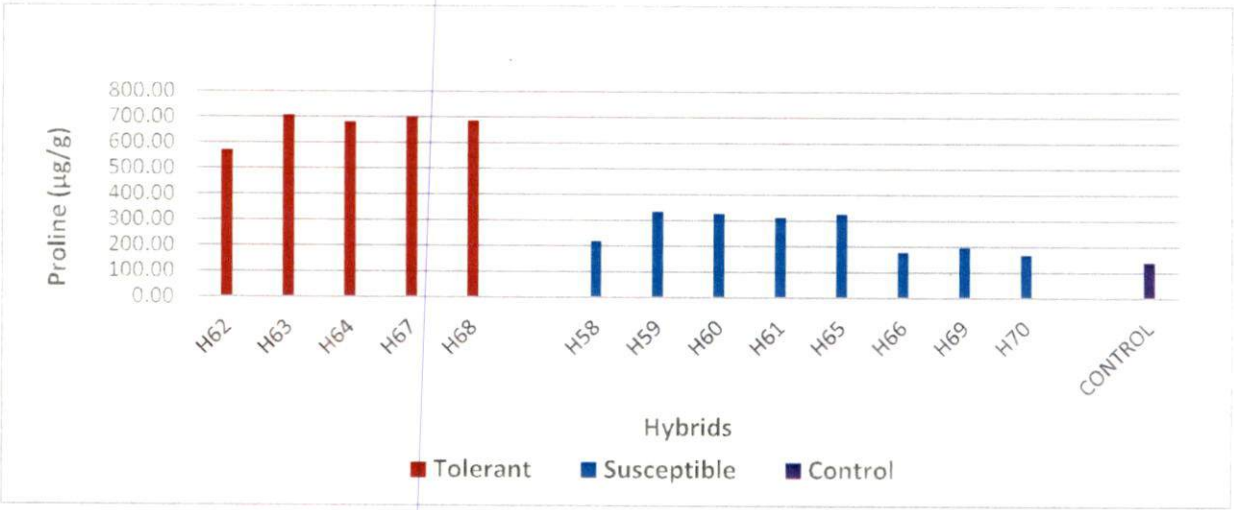

Fig. 5. Proline content of G I 5.9 x G II 19.5

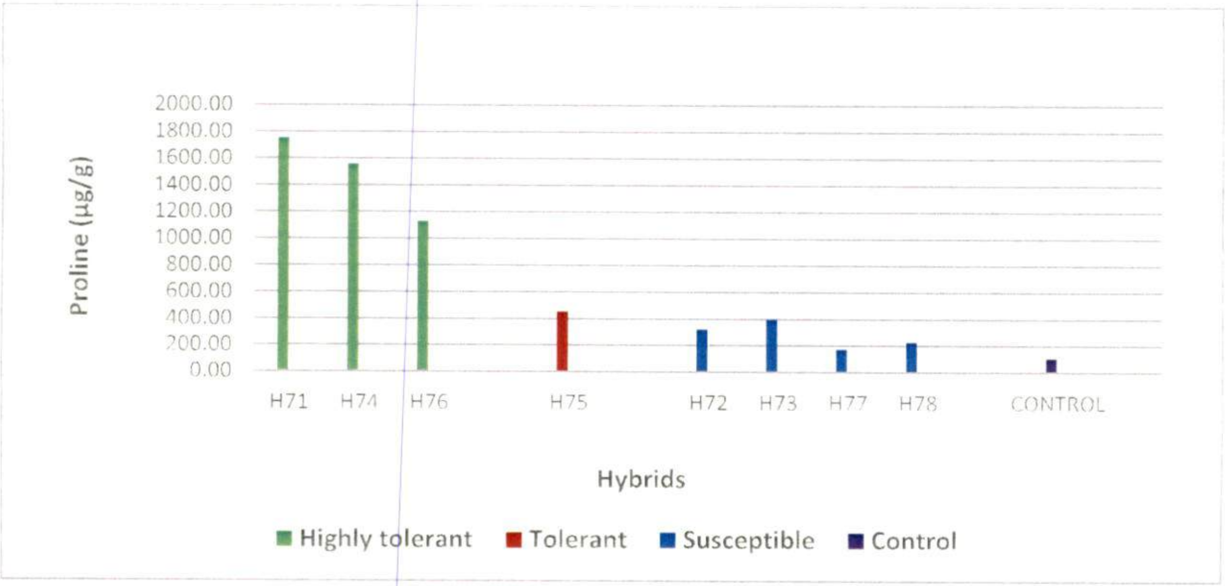

Fig. 6. Proline content of G I 5.9 x G VI 55

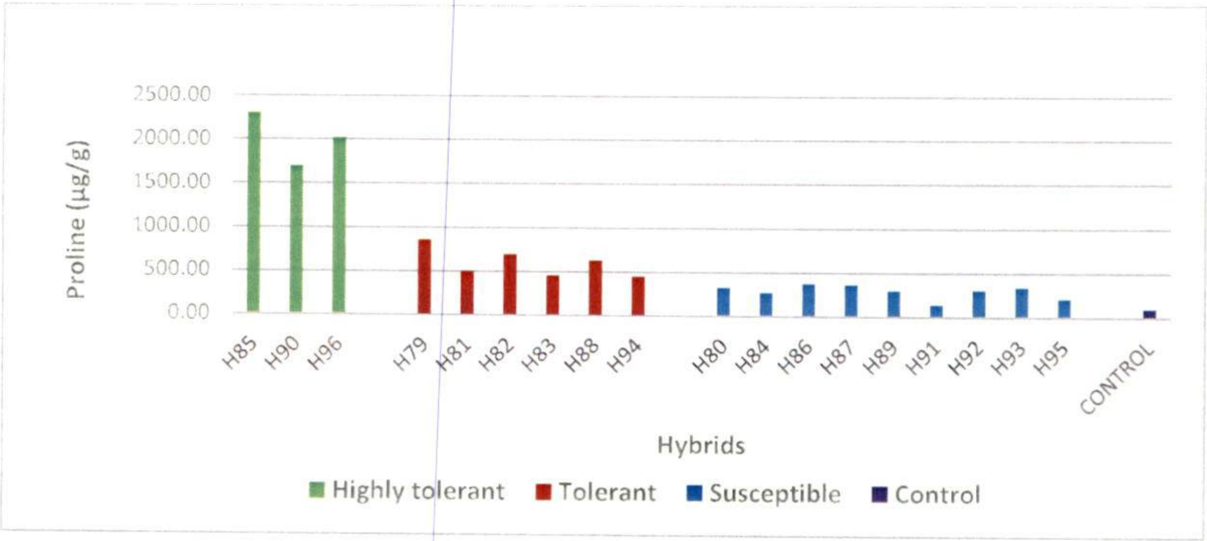

Fig. 7. Proline content of G II 19.5 x M13.12

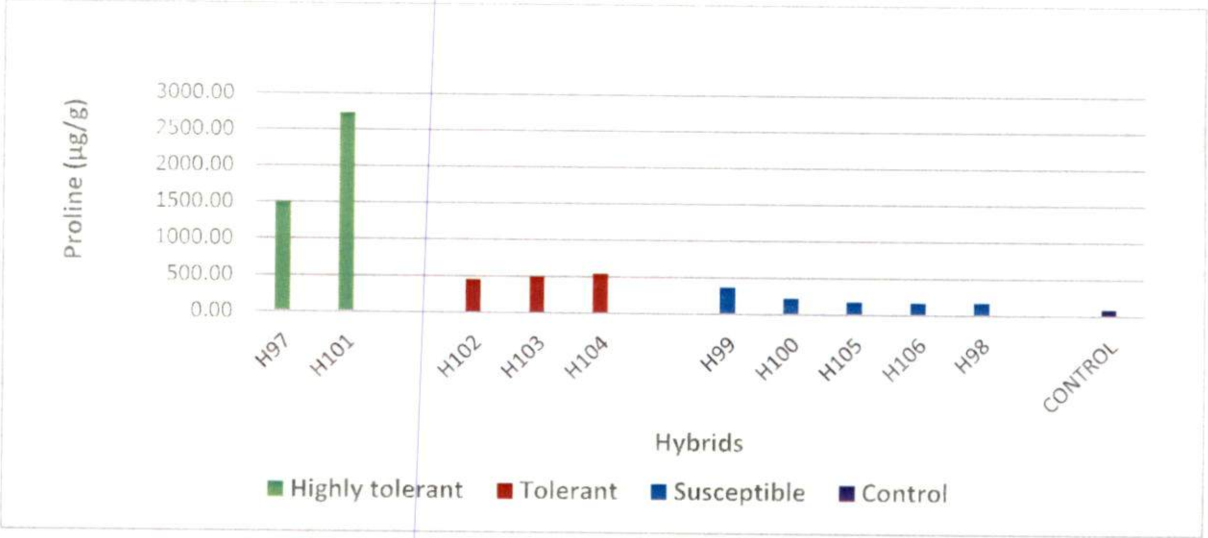

Fig. 8. Proline content of G II 19.5 x G I 5.9

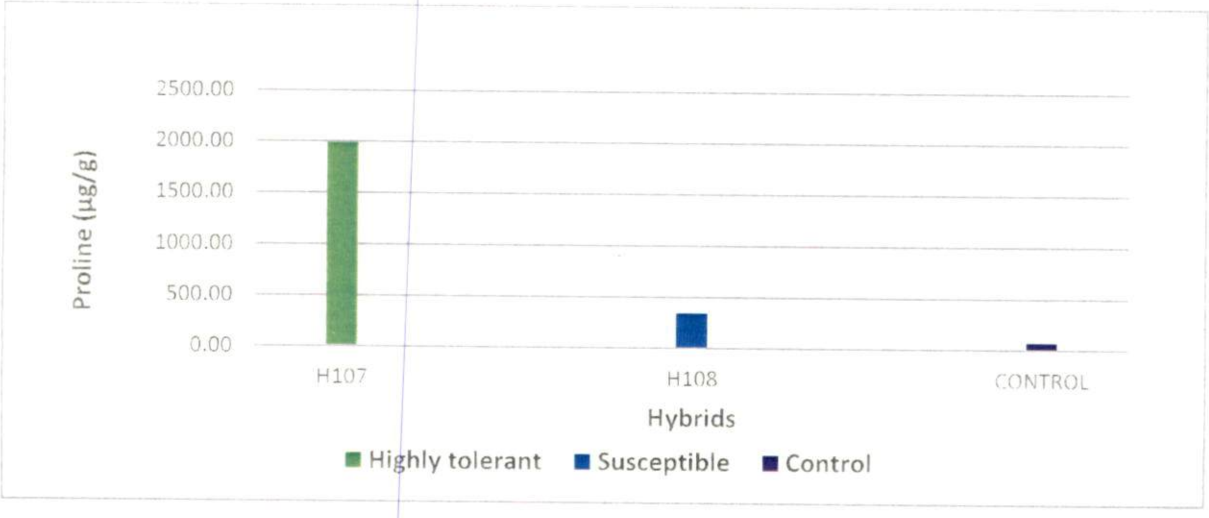

Fig. 9. Proline content of G II 19.5 x G VI 55

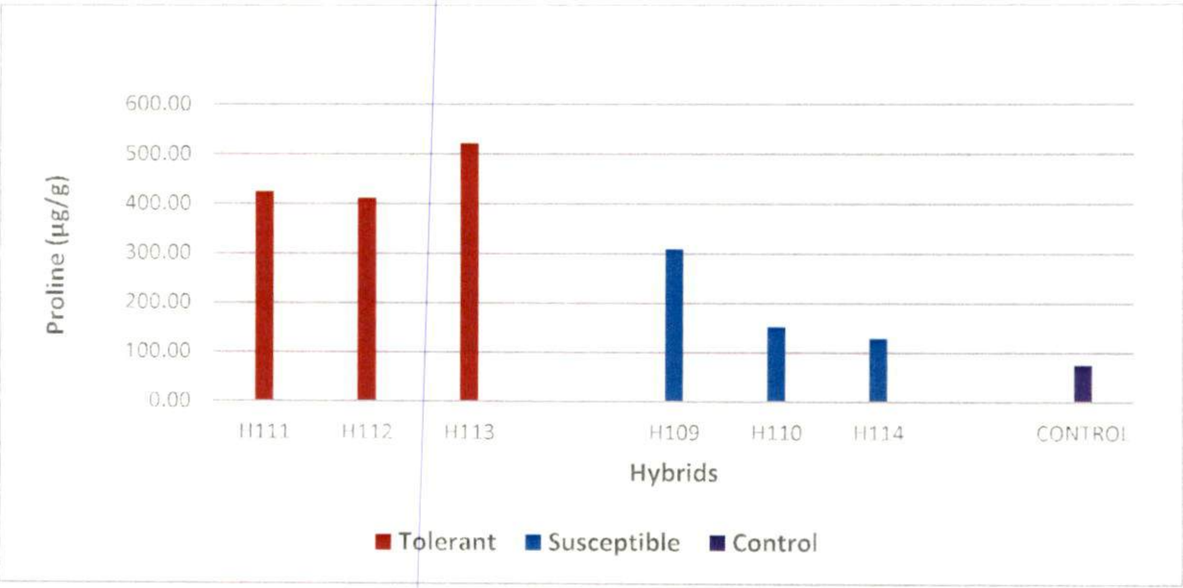

Fig. 10. Proline content of G VI 55 x G I 5.9

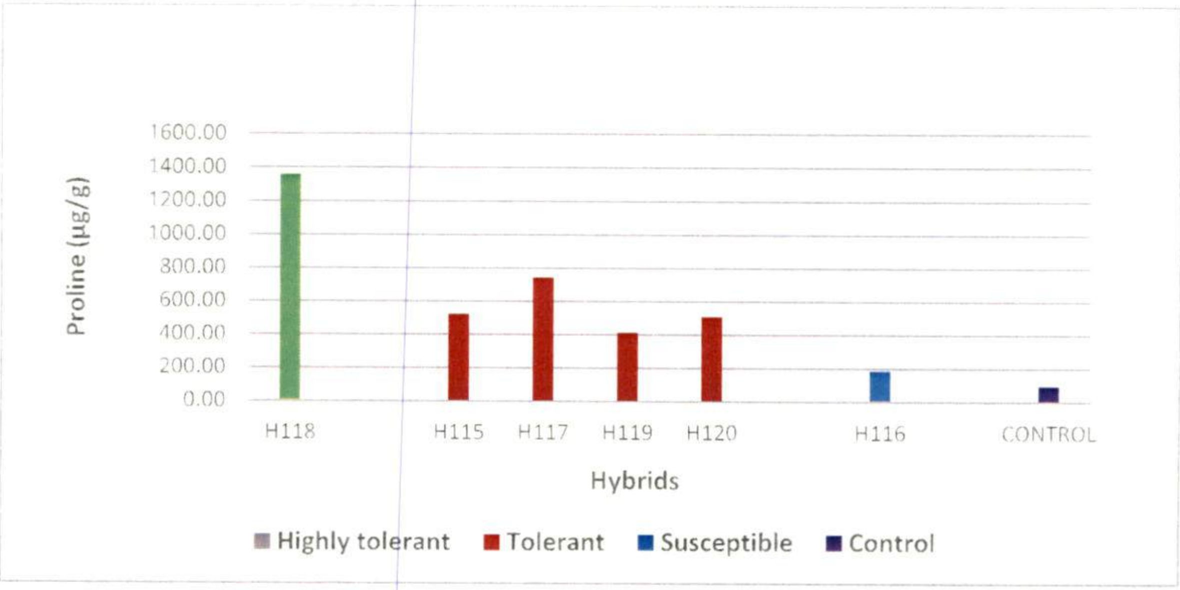

Fig. 11. Proline content of G VI 55 x G II 19.5

Table 8. Biochemical parameters of M 13.12 x G I 5.9

Sl. No.	Hybrids	Reaction to drought	Proline(µg/g)	NRA(mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H1	Susceptible	269.08	1.34	0.208	6.30
2	H2	Tolerant	695.36	5.60	0.373	7.48
3	H3	Susceptible	181.96	4.16	0.186	4.42
4	H4	Susceptible	219.44	2.28	0.205	6.03
5	H5	Susceptible	247.51	2.14	0.209	5.28
6	H6	Susceptible	298.52	3.82	0.210	6.22
7	H7	Susceptible	304.19	3.24	0.207	6.21
8	H8	Susceptible	354.34	4.04	0.188	6.15
9	H9	Tolerant	996.41	8.59	0.327	6.35
10	H10	Tolerant	440.93	11.92	0.381	6.77
11	H11	Tolerant	452.91	12.59	0.317	7.03
12	H12	Tolerant	539.50	6.94	0.351	7.09
13	H13	Tolerant	446.25	6.25	0.318	7.43
14	H14	Susceptible	293.06	4.03	0.210	5.33
15	H15	Susceptible	254.43	2.43	0.159	6.27
16	H16	Susceptible	310.02	2.19	0.128	5.25
17	H17	Susceptible	174.24	0.51	0.210	5.39
18	H18	Susceptible	167.18	2.56	0.209	6.04
19	H19	Susceptible	175.86	3.48	0.211	6.26
20	H20	Tolerant	418.28	7.12	0.318	6.78
21	H21	Susceptible	290.00	4.22	0.164	5.71
22	H22	Susceptible	189.29	3.35	0.212	5.94
23	H23	Susceptible	173.17	4.03	0.147	5.32

24	H24	Tolerant	468.86	5.85	0.351	7.99
25	H25	Tolerant	438.26	8.39	0.325	7.43
26	H26	Tolerant	504.07	5.08	0.321	11.64
27	H27	Highly tolerant	1105.64	10.93	0.364	10.43
28	H28	Tolerant	479.56	7.13	0.343	7.81
29	H29	Tolerant	479.54	5.16	0.319	6.43
30	H30	Susceptible	340.35	1.02	0.191	5.36
31	H31	Tolerant	490.21	5.11	0.345	8.77
32	H32	Susceptible	209.81	2.65	0.164	5.32
	Control		61.33	17.03	0.023	3.22
	CV (%)		**4.83**	**14.60**	**16.93**	**10.36**
	CD	0.05%	30.54	1.12	0.07	1.12
		0.01%	40.58	1.58	0.09	1.49

Table 9. Biochemical parameters of M 13.12 x G II 19.5

Sl No.	Hybrids	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H33	Tolerant	671.38	6.36	0.316	9.46
2	H34	Tolerant	547.57	6.79	0.315	9.68
3	H35	Susceptible	359.00	2.70	0.195	6.73
4	H36	Susceptible	459.65	2.07	0.203	6.06
5	H37	Tolerant	523.52	11.74	0.319	8.79
6	H38	Highly tolerant	2710.82	7.16	0.332	9.52
7	H39	Tolerant	522.28	6.59	0.322	8.66
8	H40	Susceptible	224.46	3.62	0.197	7.34
9	H41	Susceptible	265.09	4.30	0.184	6.02
10	H42	Tolerant	619.43	8.52	0.311	9.35
	Control		75.73	19.98	0.064	2.84
	CV (%)		**2.59**	**15.25**	**13.65**	**9.34**
	CD	0.05%	34.72	1.55	0.06	1.30
		0.01%	47.35	2.12	0.08	1.77

Table 10. Biochemical parameters of M 13.12 x G VI 55

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g/hr)	Glycine betaine (µmol/g)
1	H43	Highly tolerant	2817.39	9.75	0.337	8.90
2	H44	Highly susceptible	85.52	3.39	0.145	6.70
3	H45	Susceptible	163.72	3.02	0.166	5.29
4	H46	Tolerant	643.40	6.40	0.362	9.66
5	H47	Susceptible	111.90	3.49	0.215	6.98
6	H48	Tolerant	494.21	10.89	0.334	9.87
7	H49	Susceptible	400.96	4.12	0.193	6.87
8	H50	Susceptible	247.77	1.88	0.215	7.06
9	H51	Susceptible	170.51	4.05	0.215	7.13
	Control		65.60	17.27	0.035	2.82
	CV (%)		**2.65**	**13.85**	**18.88**	**11.74**
	CD	0.05%	25.98	1.24	0.08	1.53
		0.01%	35.59	1.70	0.11	2.10

4.3.2. Nitrate Reductase Activity

Water deficit induces an abrupt reduction in the uptake and nitrate flux rates from roots to leaves, preventing the mechanisms of NR protein synthesis-induction and NR activity (Foyer *et al.*, 1998). The NR activity decline during water stress is mainly attributed to low NO^{3-} absorption and availability resulting from water uptake deprivation (Ferrario-Mery *et al.*, 1998). The graphical representation of the NR content is depicted in Fig. 12-22 and the value are presented in Tables 8-18 depicted below.

In the cross between M 13.12 x G I 5.9 (Fig. 12), 32 hybrids were obtained out of which the H11 showed the maximum nitrate reductase activity of about 12.59 mmol nitrate/g/hr followed by H10 which had 11.92 mmol nitrate/g/hr. This was followed by H27 which was a highly tolerant hybrid with 10.93 mmol nitrate/g/hr. Other values of tolerant hybrids were H9 (8.59 mmol nitrate/g/hr), H25 (8.39 mmol nitrate/g/hr), H28 (7.13 mmol nitrate/g/hr), H20 (7.12 mmol nitrate/g/hr), H12 (6.94 mmol nitrate/g/hr), H13 (6.25 mmol nitrate/g/hr), H24 (5.85 mmol nitrate/g/hr), H2 (5.60 mmol nitrate/g/hr), H29 (5.16 mmol nitrate/g/hr), H31 (5.11 mmol nitrate/g/hr) and H26 (5.08 mmol nitrate/g/hr). The lowest values of nitrate reductase activity were observed in susceptible hybrids, the lowest being H17 having only 0.51 mmol nitrate/g/hr. The low values in NRA were also found in H21 (4.22 mmol nitrate/g/hr), H3 (4.16 mmol nitrate/g/hr), H23 (4.03 mmol nitrate/g/hr), H8 (4.04 mmol nitrate/g/hr), H14 (4.03 mmol nitrate/g/hr), H6 (3.82 mmol nitrate/g/hr), H19 (3.48 mmol nitrate/g/hr), H22 (3.35 mmol nitrate/g/hr), H7 (3.24 mmol nitrate/g/hr), H32 (2.65 mmol nitrate/g/hr), H18 (2.56 mmol nitrate/g/hr), H15 (2.43 mmol nitrate/g/hr), H4 (2.28 mmol nitrate/g/hr), H16 (2.19 mmol nitrate/g/hr), H5 (2.14 mmol nitrate/g/hr), H1 (1.34 mmol nitrate/g/hr) and H30 (1.02 mmol nitrate/g/hr). The control had 17.03 mmol nitrate/g/hr of the enzyme under full watered condition. The nitrate reductase enzyme is highest in plants grown in watered condition and when the plants are having water scarcity, this enzyme is produced in very less amount due to low nitrate assimilation (Larsson *et al.*, 1989; Kenis *et al.*, 1994). The hybrids having high NR values were more tolerant to drought (Table 8).

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The cross M 13.12 x G II 19.5 (Fig. 13) had hybrids having values as high as 11.74 mmol nitrate/g/hr in H37 to as low as 2.07 mmol nitrate/g/hr in H36. The tolerant hybrids in this cross were having the values, H42 (8.52 mmol nitrate/g/hr), H34 (6.79 mmol nitrate/g/hr), H39 (6.59 mmol nitrate/g/hr) and H33 (6.36 mmol nitrate/g/hr). The highly tolerant hybrid (H38) was having 7.16 mmol nitrate/g/hr. Low NRA values were found in susceptible hybrids H41 (4.30 mmol nitrate/g/hr), H40 (3.62 mmol nitrate/g/hr) and H35 (2.70 mmol nitrate/g/hr). The control was having 19.98 mmol nitrate/g/hr of the enzyme (Table 9).

In cross M 13.12 x G VI 55 (Fig. 14), H48 was having the highest NRA content of about 10.89 mmol nitrate/g/hr followed by the highly tolerant hybrid H43 having 9.75 mmol nitrate/g/hr and H46 (6.40 mmol nitrate/g/hr). The lowest value was found in H50 with only 1.88 mmol nitrate/g/hr. The highly susceptible hybrid H44 had NRA content of 3.39 mmol nitrate/g/hr. Other susceptible hybrids were H49 (4.12 mmol nitrate/g/hr), H51 (4.05 mmol nitrate/g/hr), H47 (3.49 mmol nitrate/g/hr) and H45 (3.02 mmol nitrate/g/hr). The control in this cross was having 17.27 mmol nitrate/g/hr of reductase enzyme which was much higher than the susceptible ones indicating the inability of H50 and H44 to cope up with drought stress (Table 10).

In the cross between G I 5.9 x M 13.12 (Fig. 15), highest NRA value was found in H52 (8.45 mmol nitrate/g/hr) followed by H57 having 4.25 mmol nitrate/g/hr. The lowest value was found in H54 (1.81 mmol nitrate/g/hr). Other hybrids having low NRA values were H55 (3.92 mmol nitrate/g/hr) and H56 (3.88 mmol nitrate/g/hr) and H53 (2.96 mmol nitrate/g/hr). The control was having 12.95 mmol nitrate/g/hr of the enzyme (Table 11).

The cross G I 5.9 x G II 19.5 (Fig. 16) had H64 with the highest NRA value of 9.48 mmol nitrate/g/hr followed by H67 (9.05 mmol nitrate/g/hr) and H63 (8.66 mmol nitrate/g/hr), H68 (8.56 mmol nitrate/g/hr) and H62 (8.39 mmol nitrate/g/hr). The lowest value for NRA was 1.41 mmol nitrate/g/hr found in H59. Other hybrids having low values in NRA were H69 (4.03 mmol nitrate/g/hr), H65 (4.00 mmol nitrate/g/hr), H58 (3.91 mmol nitrate/g/hr), H60 (3.62 mmol nitrate/g/hr), H70 (3.32 mmol nitrate/g/hr), H61 (2.09 mmol nitrate/g/hr) and H66

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(1.42 mmol nitrate/g/hr). This indicated that the tolerant hybrids had high resistance to drought stress and were able to regulate the nitrate reduction activity even with less water. Usually, drought stress reduces the enzyme activity and that is the reason the amount of reductase enzyme was low in hybrids whereas in the control, it was as high as15.78 mmol nitrate/g/hr (Table 12).

In the cross between G I 5.9 x G VI 55 (Fig. 17), the highest NR activity was found in H76 with 10.41 mmol nitrate/g/hr which is a highly tolerant hybrid followed by H75 having 10.26 mmol nitrate/g/hr of NR activity. Hybrids having high values were H71 (9.94 mmol nitrate/g/hr) and H74 (9.83 mmol nitrate/g/hr). The lowest NR activity was found in H73 having 3.65 mmol nitrate/g/hr which is susceptible hybrids. Other hybrids having low NR activity were H72 (4.53 mmol nitrate/g/hr), H77 (4.50 mmol nitrate/g/hr) and H78 (4.28 mmol nitrate/g/hr). The control was having 16.61 mmol nitrate/g/hr of nitrate reductase enzyme (Table 13).

In cross G II 19.5 x M 13.12 (Fig. 18), H81 showed the maximum NR activity of 14.27 mmol nitrate/g/hr followed by H88 with NR activity of 11.82 mmol nitrate/g/hr. The highly tolerant hybrids, H90, H96 and H85 had NR values 10.63 mmol nitrate/g/hr, 7.80 mmol nitrate/g/hr and 7.72 mmol nitrate/g/hr respectively. The lowest values were observed from the susceptible hybrids: 3.98 mmol nitrate/g/hr in H89 and 2.80 mmol nitrate/g/hr in H91,and the control was having the value of 15.49 mmol nitrate/g/hr which indicated that tolerant hybrids H81 behaved almost same as that of hybrid in unstressed condition (Table 14).

The cross G II 19.5 x G I 5.9 (Fig. 19) had hybrids having the highest value in NR activity found in H97 (15.45 mmol nitrate/g/hr) which is a highly tolerant hybrid followed by H102 (9.90 mmol nitrate/g/hr). Other hybrids having high NR activity were H101 (9.80 mmol nitrate/g/hr), and H104 (8.86 mmol nitrate/g/hr) and H103 (8.09 mmol nitrate/g/hr). The low NRA values were shown by hybrids H105 (5.92 mmol nitrate/g/hr), H106 (5.49 mmol nitrate/g/hr), H100 (5.13 mmol nitrate/g/hr), H99 (4.80 mmol nitrate/g/hr) and H98 (4.76 mmol nitrate/g/hr). The control was having 18.81 mmol nitrate/g/hr activity (Table 15).

The cross G II 19.5 x G VI 55 (Fig. 20) had two hybrids, the high NR value was observed in the tolerant hybrid H107 (12.98 mmol nitrate/g/hr) and the low value of NR activity was observed in H108 (6.65 mmol nitrate/g/hr). The control was having 16.66 m mol nitrate/g/hr of the activity (Table 16).

In the cross between G VI 55 x G I 5.9 (Fig. 21), the tolerant hybrids were having the high NR activity which were, H111 (6.68 mmol nitrate/g/hr), H112 (6.38 mmol nitrate/g/hr) and H113 (7.24 mmol nitrate/g/hr). The low NR values were observed in H114 (4.63 mmol nitrate/g/hr), H109 (4.12 mmol nitrate/g/hr) and H110 (3.57 mmol nitrate/g/hr). The control plant was having 14.76 mmol nitrate/g/hr of the NR activity (Table 17).

The cross G VI 55 x G II 19.5 (Fig. 22) had values as high as 17.06 mmol nitrate/g/hr in H115 followed by H117 having 11.35 mmol nitrate/g/hr NR value. Other hybrids having high NR values were H118 (8.76 mmol nitrate/g/hr), H119 (7.56 mmol nitrate/g/hr) and H120 (6.98 mmol nitrate/g/hr). The lowest value was observed in H116 having 4.10 mmol nitrate/g/hr nitrate activity. The control was having 23.05 mmol nitrate/g/hr of reductase activity (Table 18).

NRase is closely associated with plant growth and development (Sinha and Nicholas, 1981).It is generally accepted that drought stress has a negative impact on plant's photosynthetic activity, N concentrations, free amino acids or soluble protein contents accompanied with a decline of nitrate reductase activity in many plant species, such as maize (Foyer *et al.*, 1998), potato (Ghosh *et al.*, 2000), winter wheat (Xu and Yu, 2006), etc. The plants subjected to water stress produces less amount of total protein which results in a decrease in the synthesis of nitrate reductase activity caused by low nitrate flux (Costa *et al.*, 2008). In this experiment, the highly tolerant and tolerant hybrids had a considerable amount of enzyme present in them but the susceptible hybrids had very low content of NRA. The control plant on the other hand, had the highest value of NRA which was kept under 100 per cent field capacity which indicated that NRA can be used as a parameter for screening drought stress.

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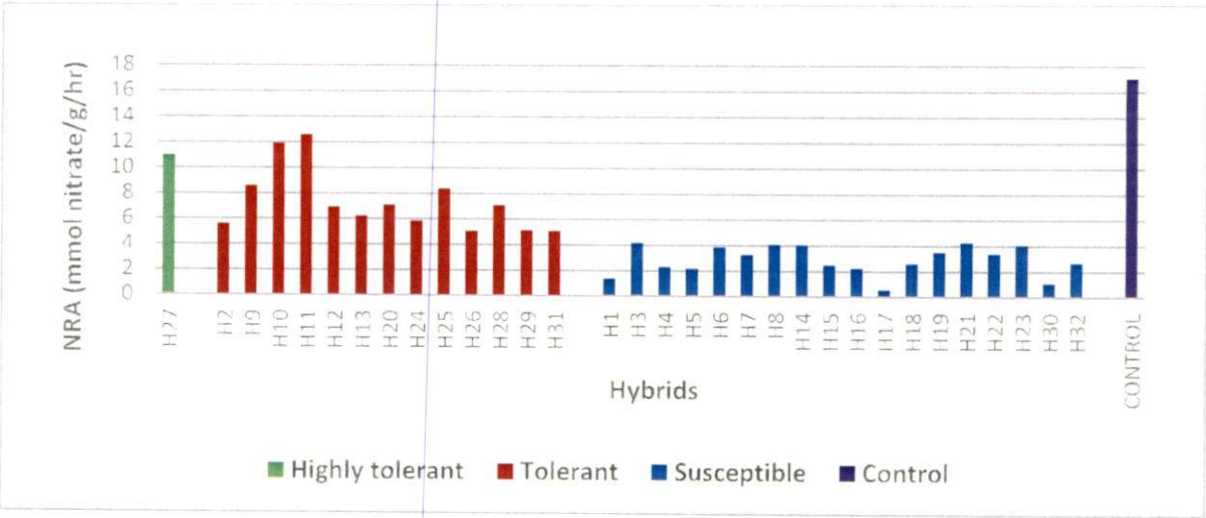

Fig. 12. NRA content of M 13.12 x G I 5.9

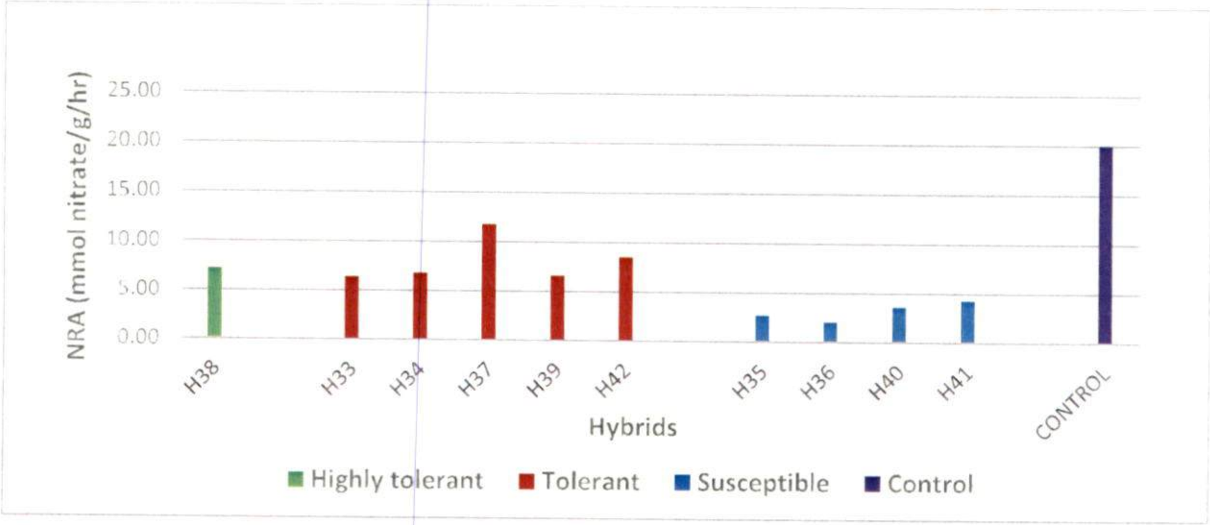

Fig. 13. NRA content of M 13.12 x G II 19.5

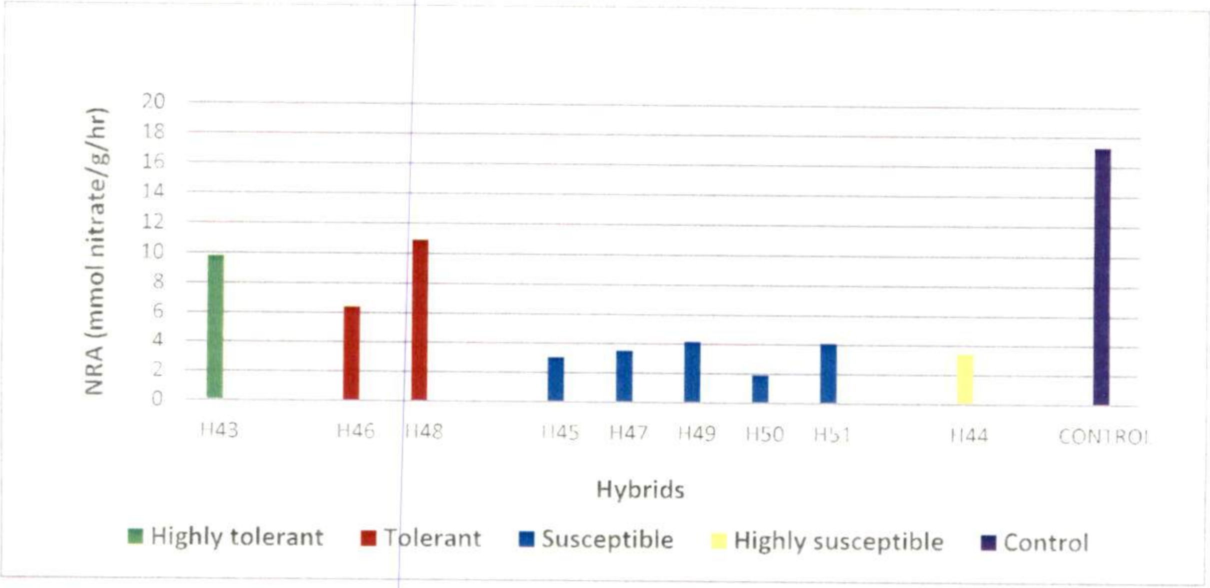

Fig. 14. NRA content of M 13.12 x G VI 55

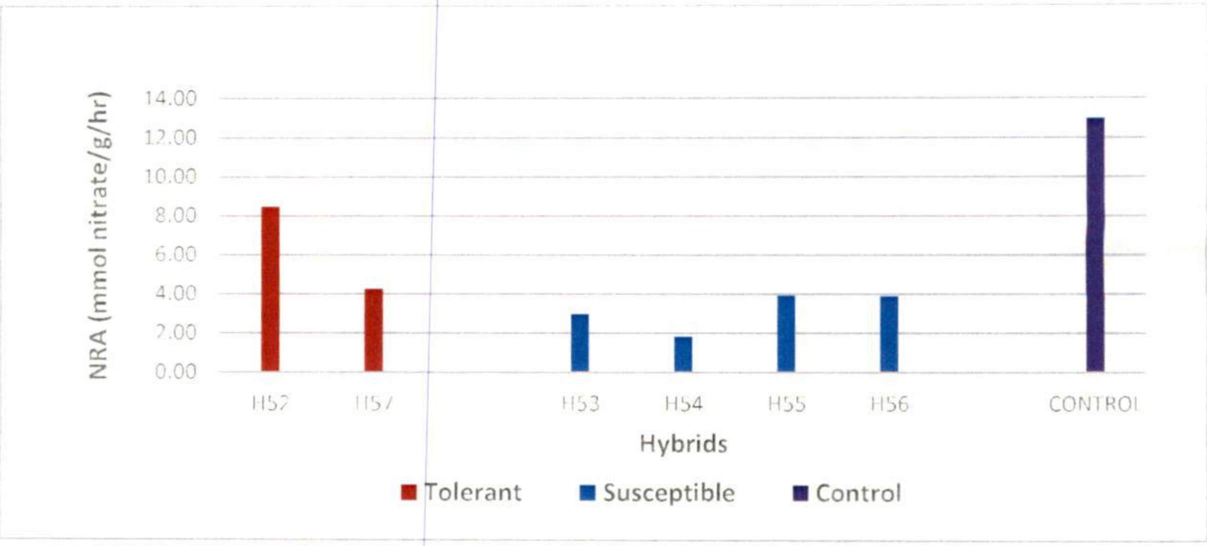

Fig. 15. NRA content of G I 5.9 x M 13.12

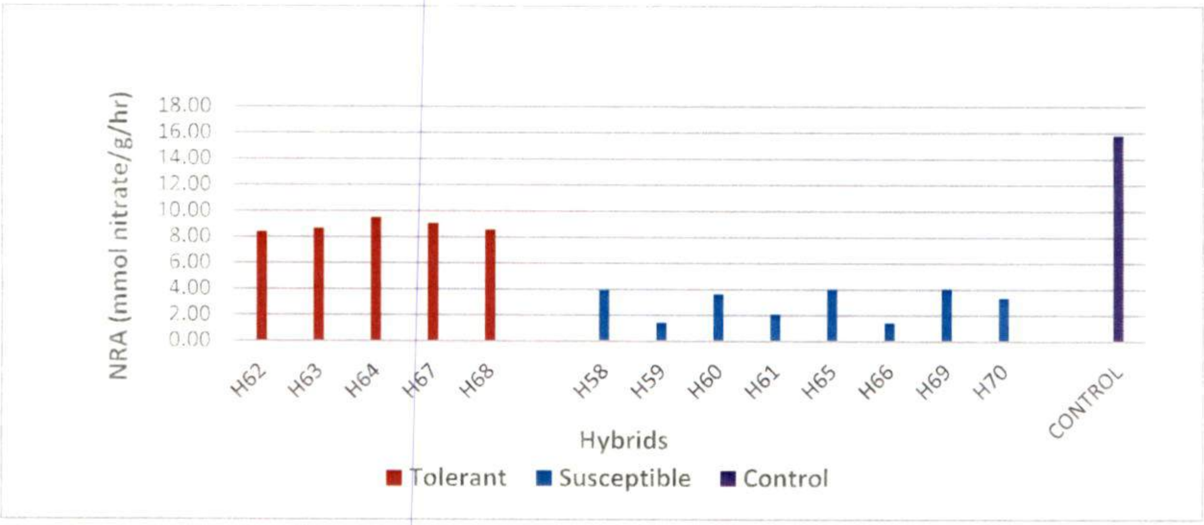

Fig. 16. NRA content of G I 5.9 x G II 19.5

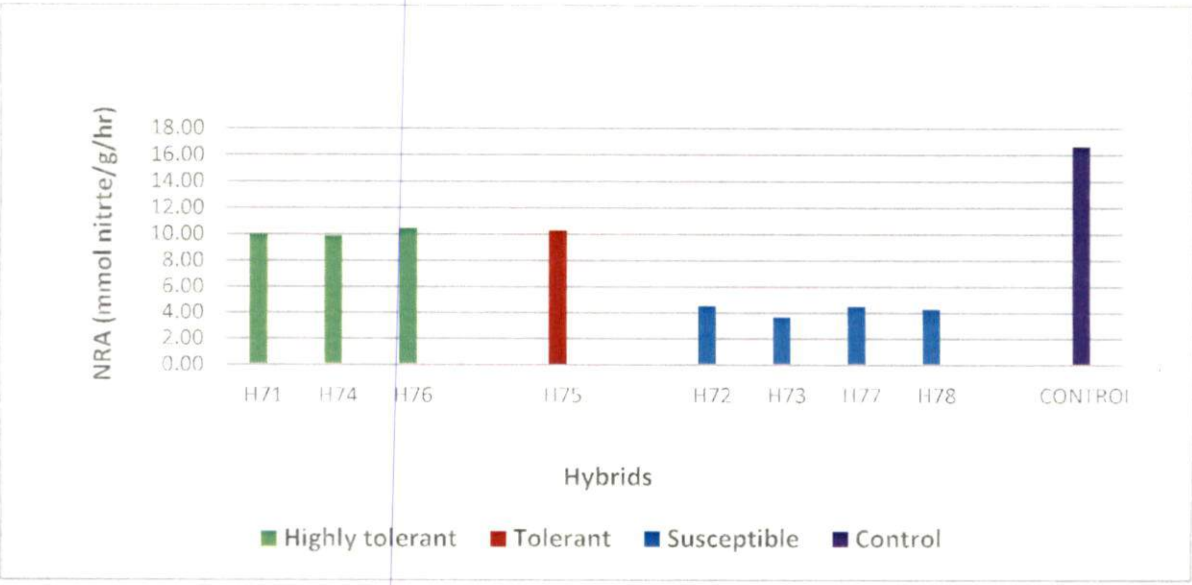

Fig.17. NRA content of G I 5.9 x G VI 55

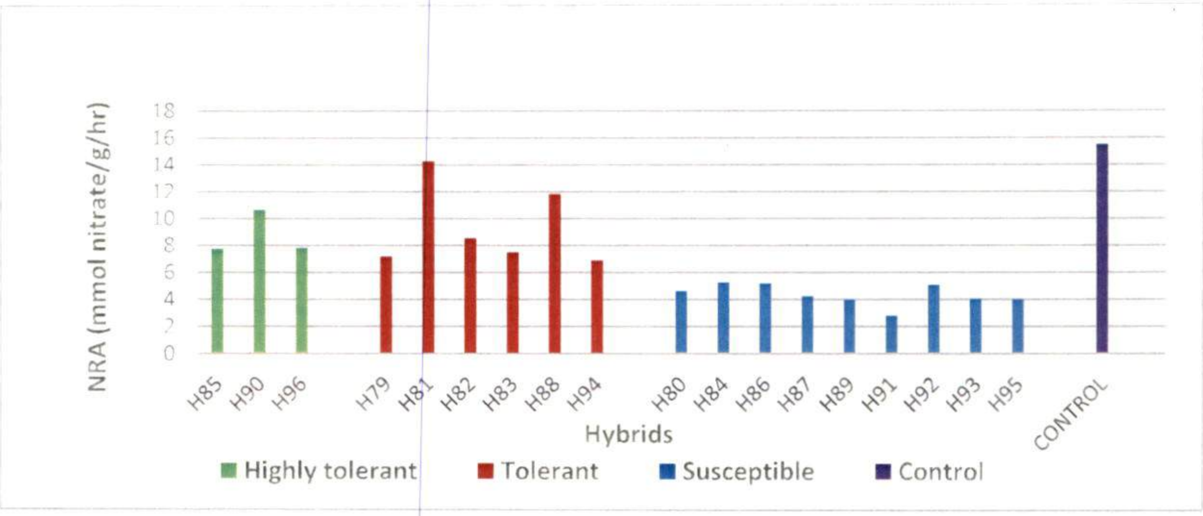

Fig. 18. NRA content of G II 19.5 x M 13.12

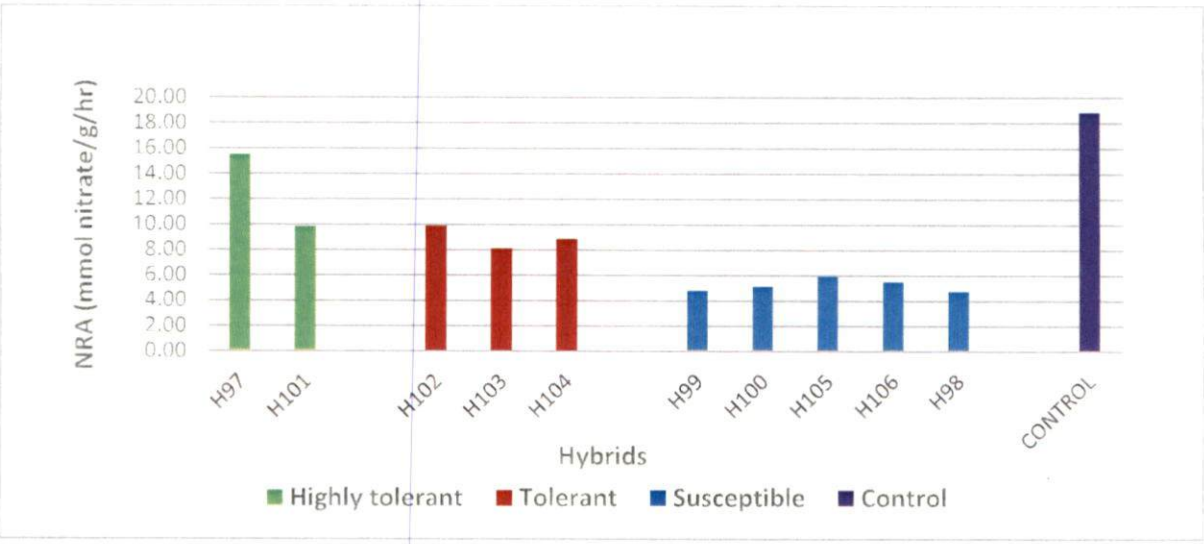

Fig. 19. NRA content of G II 19.5 x G I 5.9

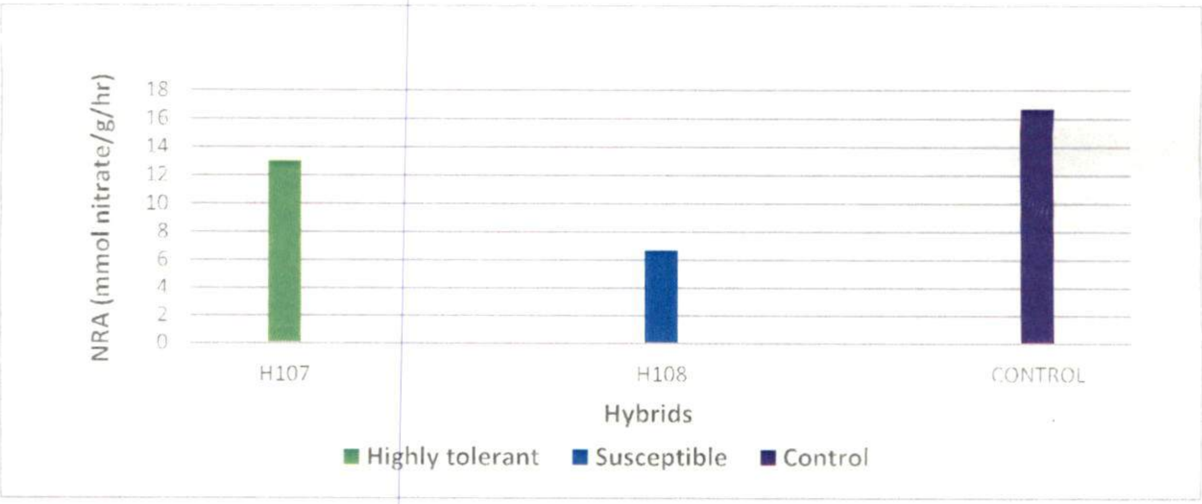

Fig. 20. NRA content of G II 19.5 x G VI 55

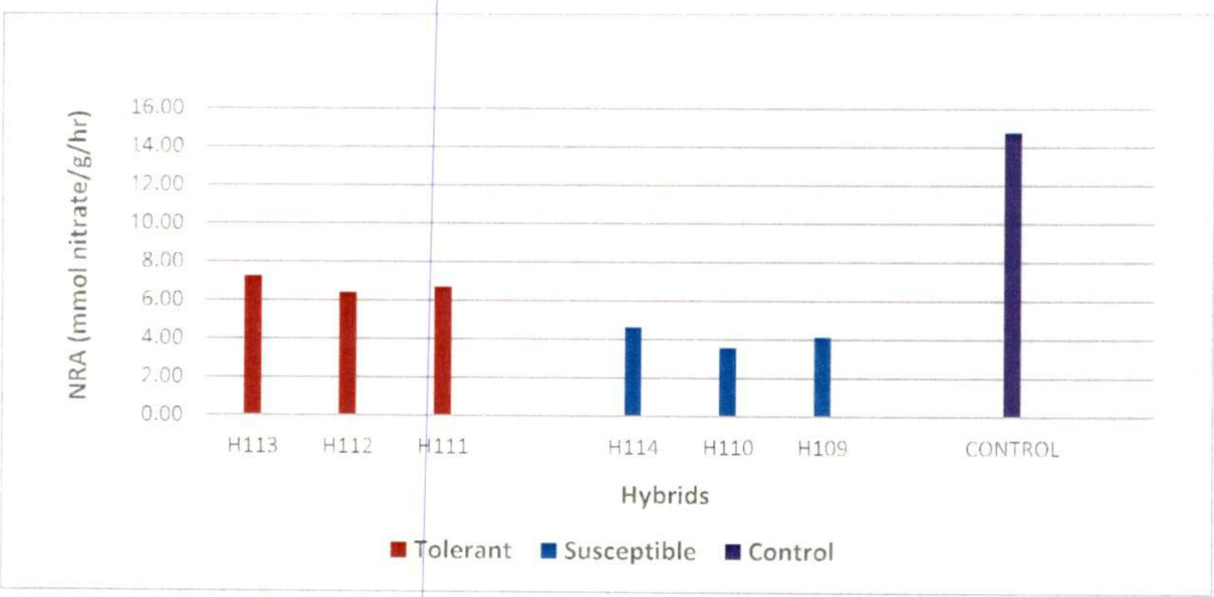

Fig. 21. NRA content of G VI 55 x G I 5.9

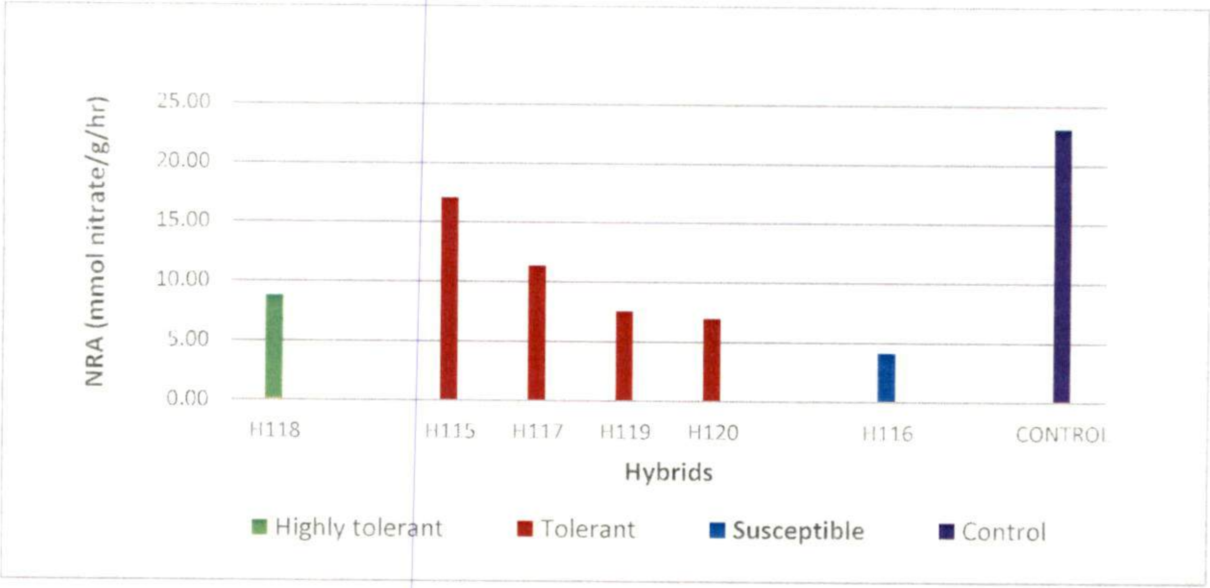

Fig. 22. NRA content of G VI 55 x G II 19.5

Table 11. Biochemical parameters of G I 5.9 x M 13.12

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H52	Tolerant	536.70	8.45	0.259	8.10
2	H53	Susceptible	292.40	2.96	0.167	6.43
3	H54	Susceptible	181.17	1.81	0.172	5.88
4	H55	Susceptible	386.31	3.92	0.156	5.36
5	H56	Susceptible	235.78	3.88	0.173	6.57
6	H57	Tolerant	498.21	4.25	0.222	7.97
	Control		85.70	12.95	0.027	3.16
	CV (%)		**6.01**	**15.02**	**18.31**	**10.21**
	CD	0.05%	38.00	1.13	0.06	1.22
		0.01%	53.28	1.58	NS	1.71

Table 12. Biochemical parameters of G I 5.9 x G II 19.5

Sl No.	Hybrids	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H58	Susceptible	218.46	3.91	0.218	6.83
2	H59	Susceptible	333.96	1.41	0.217	7.18
3	H60	Susceptible	326.36	3.62	0.162	7.34
4	H61	Susceptible	311.71	2.09	0.166	7.32
5	H62	Tolerant	568.81	8.39	0.248	8.93
6	H63	Tolerant	706.01	8.66	0.241	9.52
7	H64	Tolerant	679.37	9.48	0.243	8.61
8	H65	Susceptible	325.70	4.00	0.213	7.11
9	H66	Susceptible	177.26	1.42	0.222	7.17
10	H67	Tolerant	699.35	9.05	0.237	8.32
11	H68	Tolerant	684.66	8.56	0.276	9.38
12	H69	Susceptible	196.48	4.03	0.173	7.28
13	H70	Susceptible	167.84	3.32	0.204	6.45
	Control		138.00	15.78	0.050	3.34
	CV (%)		**6.84**	**8.68**	**18.23**	**9.82**
	CD	0.05%	47.62	0.76	0.07	1.29
		0.01%	64.37	1.03	NS	1.74

4.3.3. Superoxide Dismutase

Superoxide dismutase is one among the three antioxidant systems operating in plants that provide resistance to plants against the oxidative damage due to oxide radicals (Larson, 1988; Burke and Mahan, 1991).

The SOD values varied considerably between different hybrids in different crosses. In cross M13.12 x G I 5.9 (Fig. 23), the highest value was observed in H10 (0.381 units/mg protein/g), followed by H2 (0.373 units/mg protein/g), H27 (0.364 units/mg protein/g) which is a highly tolerant hybrid. Other tolerant hybrids had the SOD values in between 0.310 - 0.352 units/mg protein/g. The lowest values observed were in H30 (0.191 units/mg protein/g), H8 (0.188 units/mg protein/g), H3 (0.186 units/mg protein/g), H21 (0.164 units/mg protein/g), H32 (0.164 units/mg protein/g), H15 (0.159 units/mg protein/g), H23 (0.147 units/mg protein/g) and H16 (0.128 units/mg protein/g). The control plant which was under well irrigated condition was having only 0.023 units/mg protein/g of the SOD activity (Table 8).

In the cross M 13.12 x G II 19.5 (Fig. 24), highest SOD value was found in H38 (0.332 units/mg protein/g) which is a highly tolerant hybrid. Other hybrids showing high SOD values were H39 (0.322 units/mg protein/g), H37 (0.319 units/mg protein/g), H33 (0.316 units/mg protein/g), H34 (0.315 units/mg protein/g) and H42 (0.311 units/mg protein/g). The low SOD values were found in hybridsH36 (0.207 units/mg protein/g), H40 (0.197 units/mg protein/g), H35 (0.195 units/mg protein/g) and H41 (0.184 units/mg protein/g). The control plant kept under fully irrigated condition had only 0.064 units/mg protein/g of dismutase activity because it's accumulation takes place only under stress conditions (Table 9).

In the cross M 13.12 x G VI 55 (Fig. 25), the highest SOD value was found in H46 (0.362 unit/mg protein/g) followed by H43 (0.337 units/mg protein/g) and H48 (0.334 units/mg protein/g). The lowest SOD value was in hybrid H45 (0.166 units/mg protein/g) which was highly susceptible. Low SOD values were found in susceptible hybrids H47 (0.215 units/mg protein/g), H50

(0.215 units/mg protein/g), H51 (0.215 units/mg protein/g), H49 (0.193 units/mg protein/g) and H44 (0.145 units/mg protein/g). The control was having only 0.035 units/mg protein/g of SOD activity (Table 10).

The cross G I 5.9 x M 13.12 (Fig. 26) had the highest SOD values of about 0.259 units/mg protein/g expressed in hybrid H52 followed by H57 having 0.222 units/mg protein/g. The lowest values were found in H56 (0.173 units/mg protein/g), H54 (0.172 units/ mg protein /g), H53 (0.167 units/mg protein/g) and H55 (0.156 units/mg protein/g) and the plant kept as control was having 0.027 units/mg protein/g of the enzymatic activity (Table 11).

In cross G I 5.9 x G II 19.5 (Fig. 27), the highest value was present in hybrid H68 (0.276 units/mg protein/g) followed by H62 (0.248 units/mg protein/g), H64 (0.243 units/mg protein/g), and H67 (0.237 units/mg protein/g). The lowest value obtained was in hybrid H60 (0.162 units/mg protein/g). Hybrids H70 (0.204 units/mg protein/g), H69 (0.173 units/mg protein/g) and H61 (0.166 units/mg protein/g) also showed lower values of SOD. The control was having 0.050 units/mg protein/g of SOD activity (Table 12).

The cross G I 5.9 x G VI 55 (Fig. 28) had high SOD activity in hybrids H75 (0.272 units/mg protein/g) followed by H76 (0.266 units/mg protein/g), H71 (0.256 units/mg protein/g) and H74 (0.254 units/mg protein/g). Lowest SOD value was found in H77 (0.191 units/mg protein/g). Other hybrids having low SOD values were H73 (0.234 units/mg protein/g), H72 (0.227 units/mg protein/g) and H78 (0.218 units/mg protein/g). The control was having only 0.066 units/mg protein/g of the enzymatic activity (Table 13).

In cross G II 19.5 x M 13.12 (Fig. 29), SOD values were highest in H85 (0.227 units/mg protein/g). Hybrids having high SOD values were H79 (0.225 units/mg protein/g), H83 (0.224 units/mg protein/g), H94 (0.217 units/mg protein/g), H96 (0.215 units/mg protein/g), H81 (0.209 units/mg protein/g), H88 (0.199 units/mg protein/g) and H82 (0.197 units/mg protein/g). The lowest SOD value was found in H93 (0.133 units/mg protein/g) followed by H80 (0.177 units/mg protein/g), H87 (0.170 units/mg protein/g) and H95 (0.162 units/mg

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protein/g). The control was having the SOD activity of 0.042 units/mg protein/g (Table 14).

In the cross G II 19.5 x G I 5.9 (Fig. 30), the highest value was observed in hybrid H101 (0.270 units/mg protein/g) followed by H97 (0.265 units/mg protein/g), H102 (0.248 units/mg protein/g), H103 (0.247 units/mg protein/g) and H104 (0.239 units/mg protein/g). The low values were found in H99 (0.213 units/mg protein/g), H98 (0.209 units/mg protein/g), H105 (0.207 units/mg protein/g), H100 (0.199 units/mg protein/g) and H106 (0.154 units/mg protein/g). The control was having 0.017 units/mg protein/g of the enzymatic activity (Table 15).

In the cross G II 19.5 x G VI 55 (Fig. 31), two hybrids were obtained in which the hybrid 107 was highly tolerant hybrid having high SOD content of 0.163 units/mg protein/g whereas the susceptible hybrid was having 0.106 units/mg protein/g of SOD activity. The control was having the SOD value of 0.065 units/mg protein/g (Table 16).

In the cross G VI 55 x G I 5.9 (Fig. 32), high SOD values were found in hybrids H112 (0.221 units/mg protein/g) followed by H111 and H113 each having an SOD value of 0.207 units/mg protein/g. The low SOD values were found in hybrids H109 (0.185 units/mg protein/g), H114 (0.149 units/mg protein/g) and H110 (0.136 units/mg protein/g). The control was having the enzymatic activity of 0.025 units/mg protein/g (Table 17).

In cross G VI 55 x G II 19.5 (Fig. 33), the highest value was found in hybrid H118 (0.227 units/mg protein/g) followed by H115 (0.209 units/mg protein/g), H117 (0.202 units/mg protein/g), H120 (0.173 units/mg protein/g) and H119 (0.163 units/mg protein/g). The lowest value was found in hybrid H116 (0.140 units/mg protein/g) which is a susceptible hybrid. The control hybrid was having 0.032 units/mg protein/g of the SOD value which was less than the hybrids under stress conditions (Table 18).

Reactive Oxygen Species (ROS) accumulation during stress greatly depends on the balance between ROS production and ROS scavenging (Mittler *et* *al*., 2004). When plants are subjected to any kind of stress, the cells have an increased production of reactive oxygen species (ROS) which in normal cases, is removed from time to time. Under stress, these become high in number and results in oxidative damage. These are removed by anti-oxidant systems which forms the first line of defence which is superoxide dismutase. ROS-scavenging mechanisms were shown to have an important role in protecting plants against osmotic stresses (Rizhsky *et al*., 2004; Wang *et al*. 2005; Leshem *et al*., 2006; Abbasi *et al*., 2007; Koussevitzky *et al*., 2008).

When plants were subjected to analysis, the tolerant and highly tolerant hybrids showed more amount of superoxide dismutase enzyme as compared to the susceptible hybrids and the control which was kept under fully irrigated condition had the least amount of SOD in it indicated that SOD highly accumulate under drought stress condition and forms a defence system against the stress.

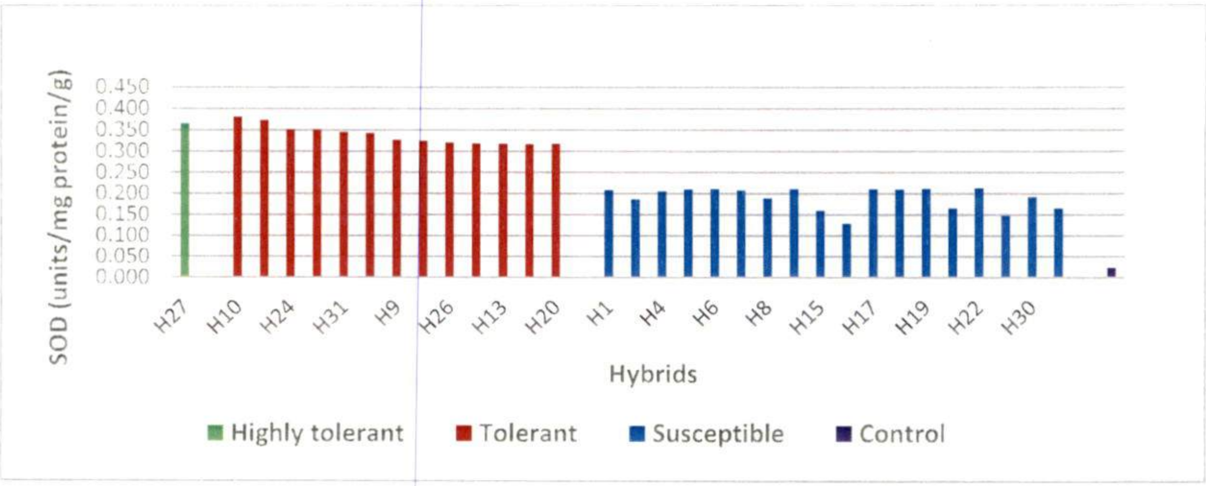

Fig. 23. SOD value of M 13.12 x G I 5.9

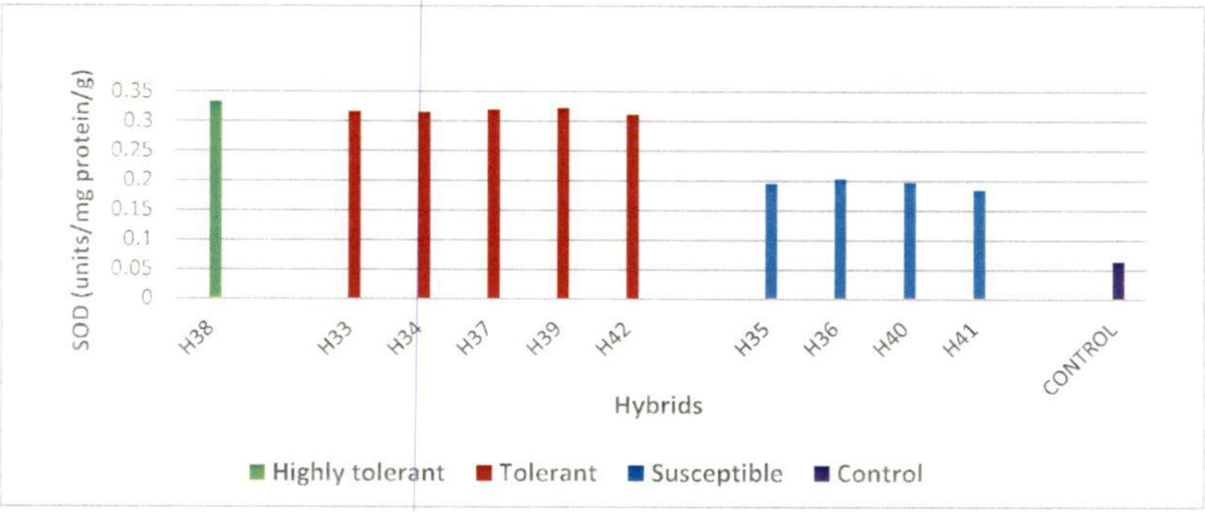

Fig. 24. SOD content of M 13.12 x G II 19.5

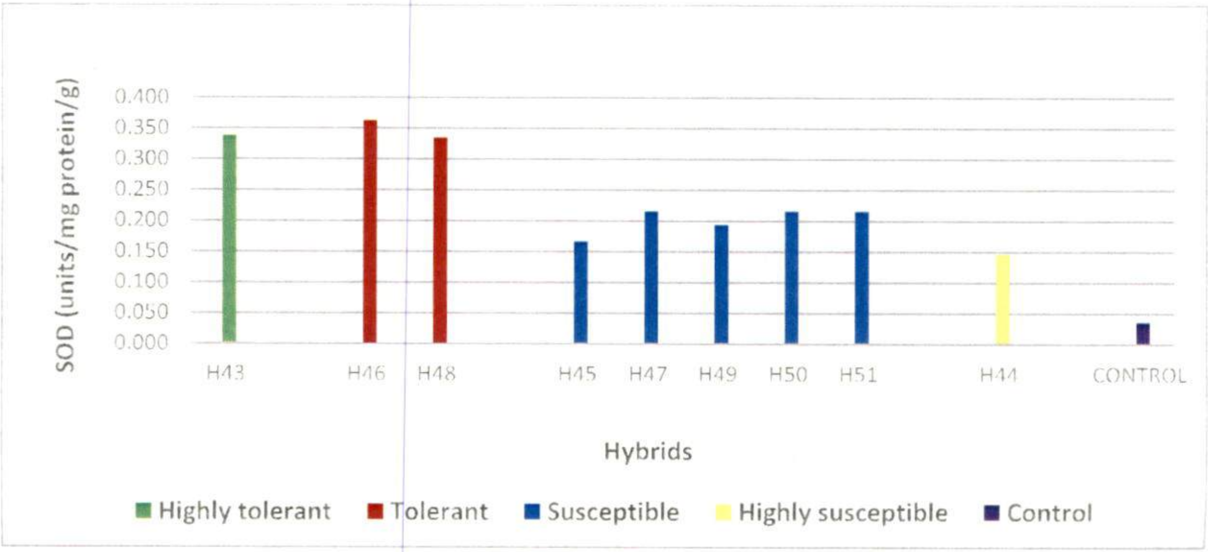

Fig. 25. SOD content of M 13.12 x G VI 55

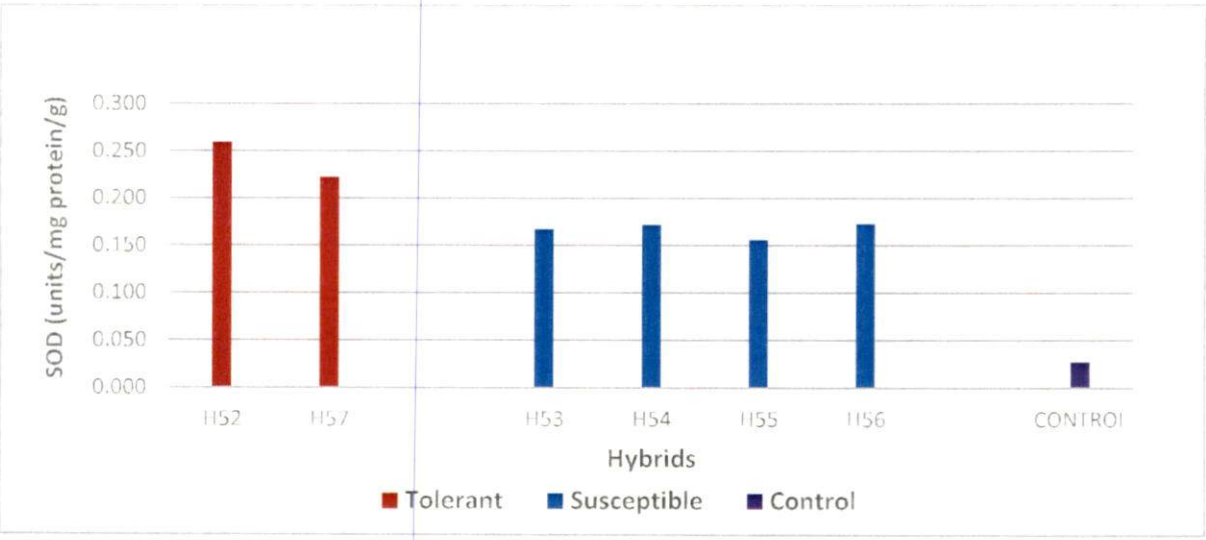

Fig. 26. SOD content of G I 5.9 x M 13.12

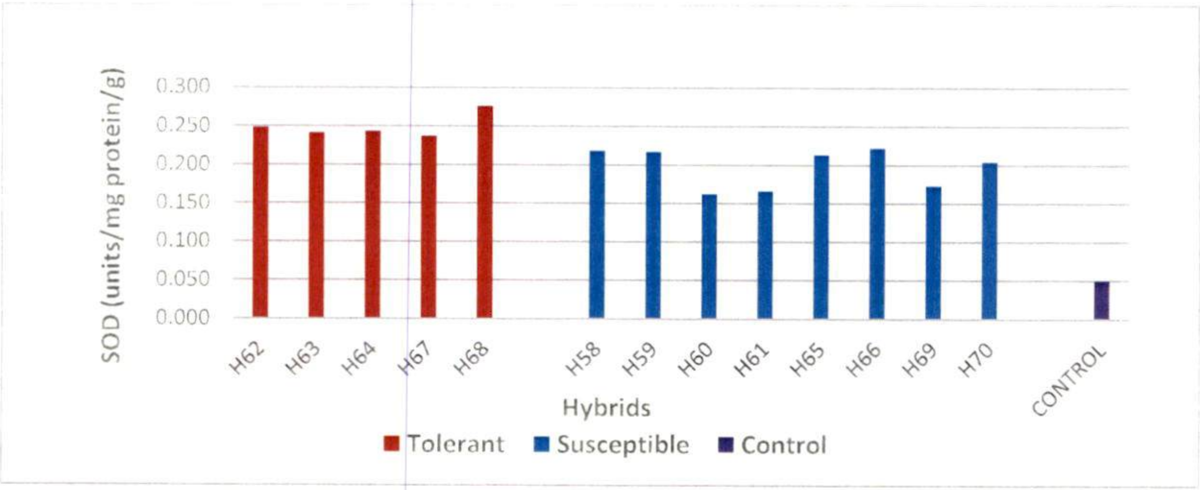

Fig. 27. SOD content of G I 5.9 x G II 19.5

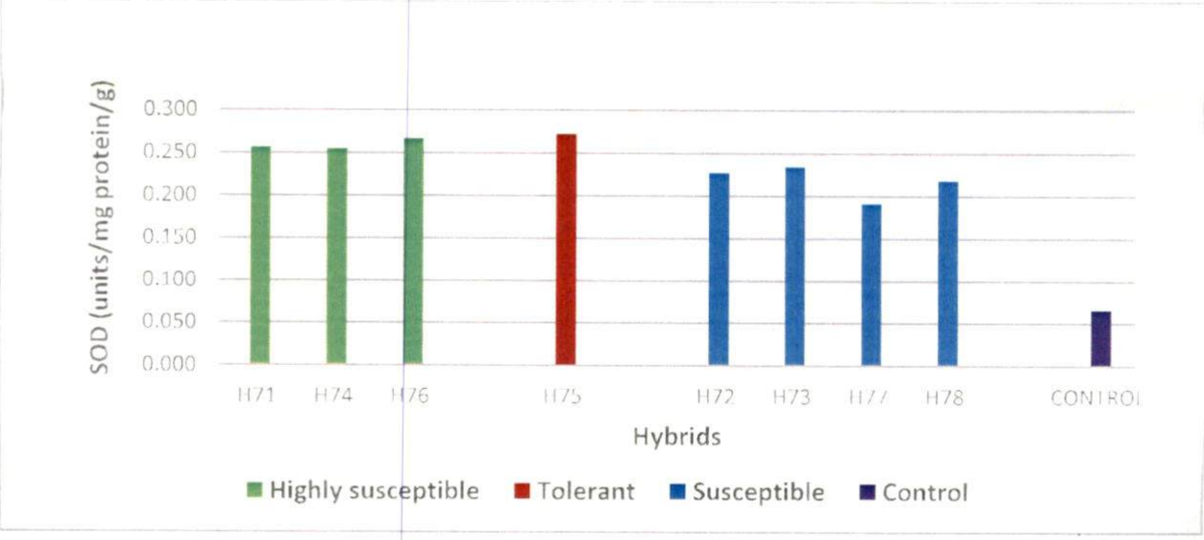

Fig. 28. SOD content of G I 5.9 x G VI 55

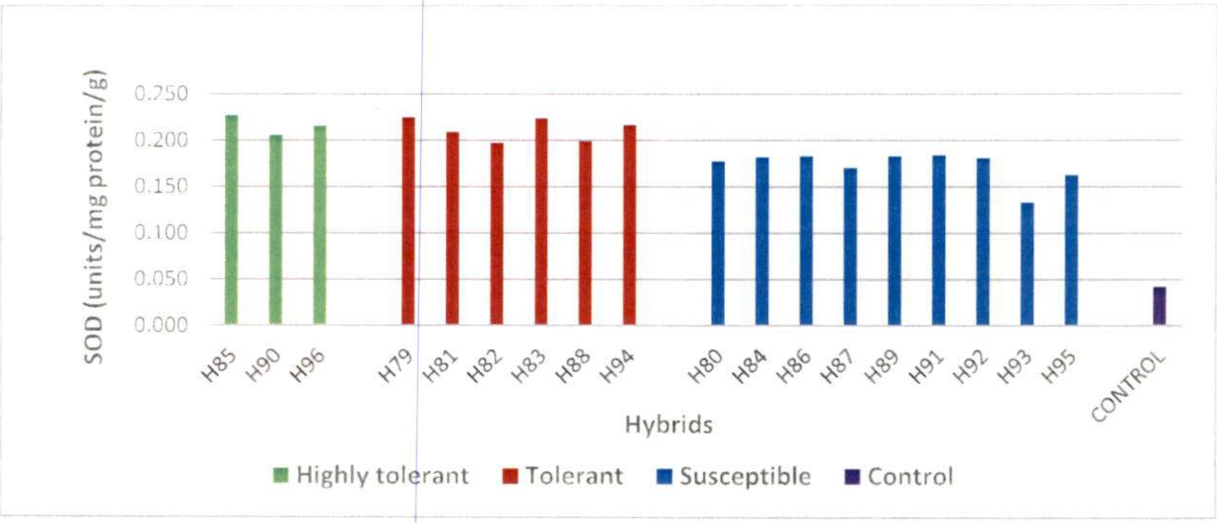

Fig. 29. SOD content of G II 19.5 x M 13.12

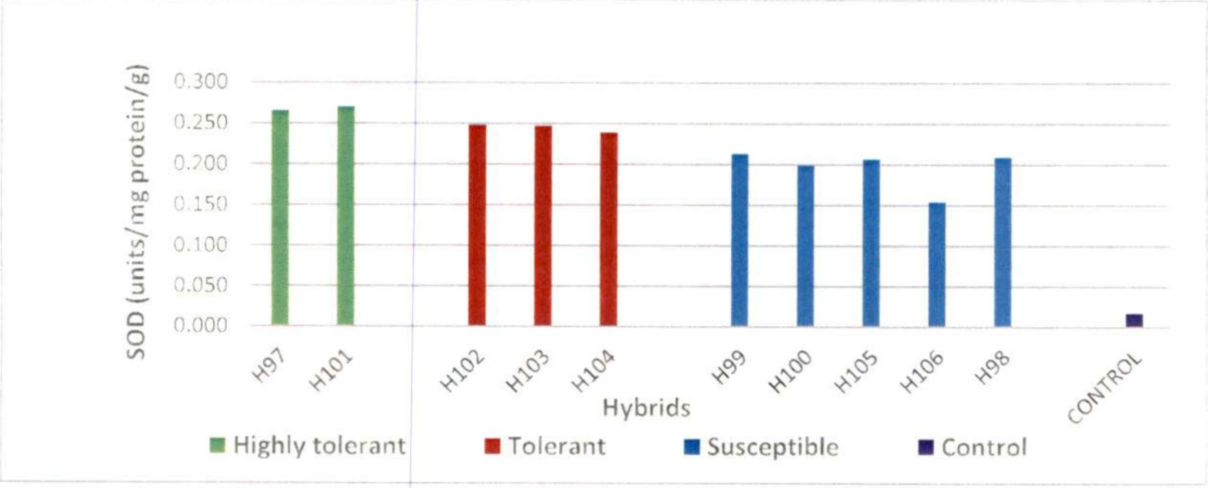

Fig. 30. SOD content of G II 19.5 x G I 5.9

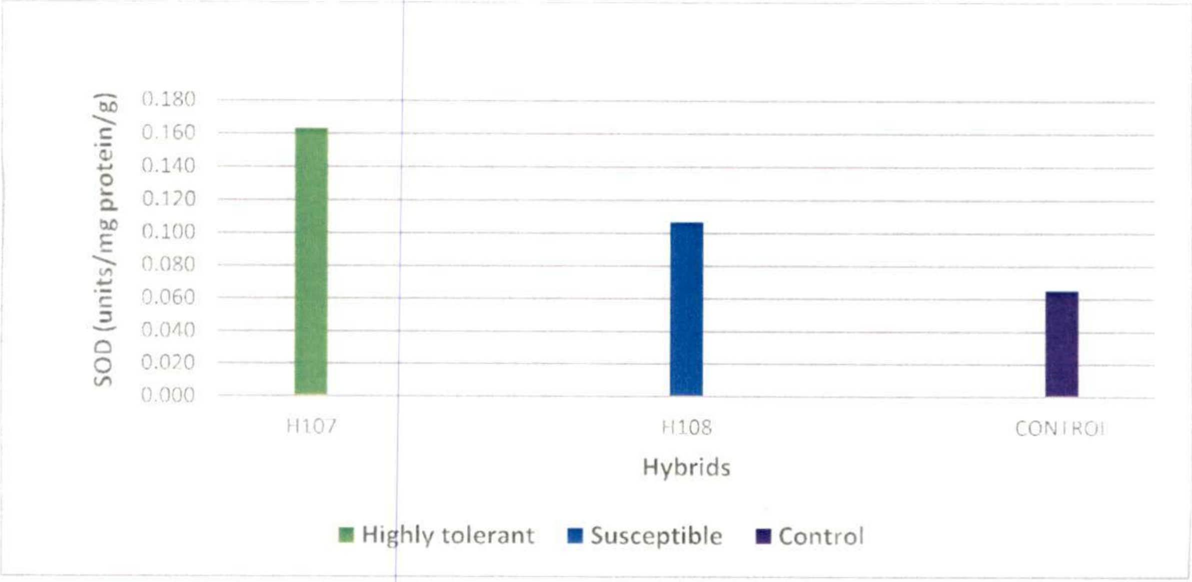

Fig. 31. SOD content of G II 19.5 x G VI 55

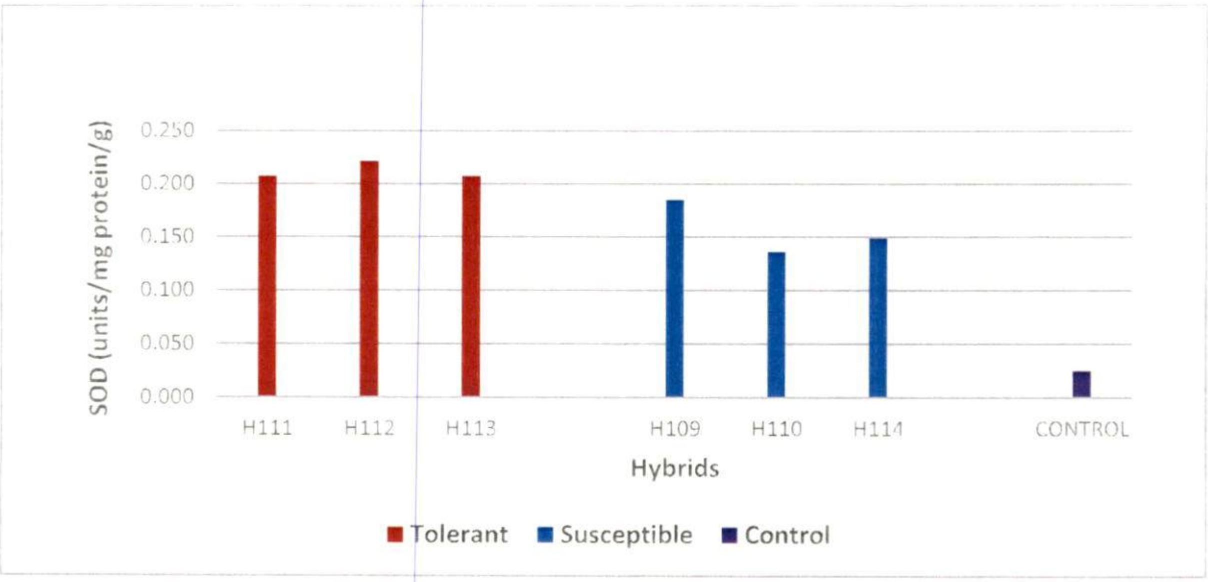

Fig. 32. SOD content of G VI 55 x G I 5.9

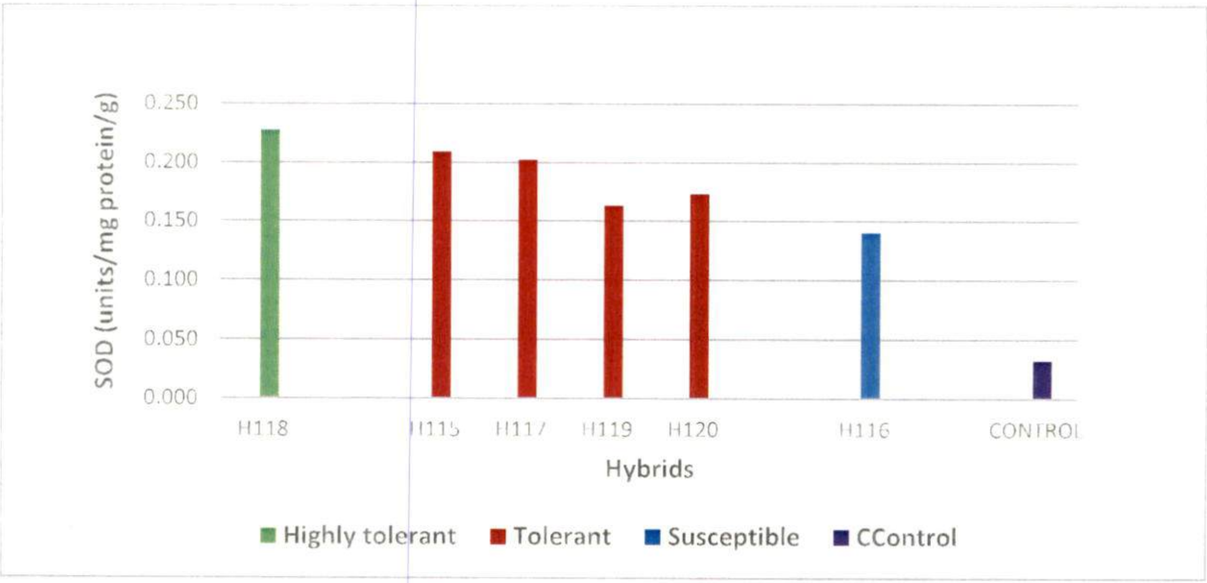

Fig. 33. SOD content of G VI 55 x G II 19.5

Table 13. Biochemical parameters of G I 5.9 x G VI 55

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H71	Highly tolerant	1749.05	9.94	0.256	9.56
2	H72	Susceptible	319.70	4.53	0.227	7.05
3	H73	Susceptible	395.63	3.65	0.234	7.07
4	H74	Highly tolerant	1555.89	9.83	0.254	8.85
5	H75	Tolerant	450.25	10.26	0.272	9.38
6	H76	Highly tolerant	1126.96	10.41	0.266	8.63
7	H77	Susceptible	169.54	4.50	0.191	6.34
8	H78	Susceptible	224.99	4.28	0.218	6.30
	Control		101.33	16.61	0.066	3.18
	CV (%)		**4.54**	**6.58**	**12.65**	**9.90**
	CD	0.05%	35.13	0.82	NS	1.35
		0.01%	47.47	1.12	NS	1.86

Table 14. Biochemical parameters of G II 19.5 x M 13.12

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H79	Tolerant	853.88	7.18	0.225	8.87
2	H80	Susceptible	325.03	4.62	0.177	6.78
3	H81	Tolerant	498.21	14.27	0.209	8.91
4	H82	Tolerant	692.69	8.54	0.197	8.70
5	H83	Tolerant	454.91	7.48	0.224	8.66
6	H84	Susceptible	269.08	5.27	0.181	7.07
7	H85	Highly tolerant	2293.88	7.72	0.227	8.43
8	H86	Susceptible	374.99	5.20	0.182	7.06
9	H87	Susceptible	363.66	4.24	0.170	7.51
10	H88	Tolerant	625.80	11.82	0.199	10.08
11	H89	Susceptible	295.73	3.98	0.183	7.31
12	H90	Highly tolerant	1689.10	10.63	0.205	8.89
13	H91	Susceptible	139.87	2.80	0.184	7.55
14	H92	Susceptible	303.72	5.09	0.180	7.20
15	H93	Susceptible	338.75	4.03	0.133	6.93
16	H94	Tolerant	447.59	6.90	0.217	9.17
17	H95	Susceptible	208.47	4.02	0.162	6.84
18	H96	Highly tolerant	2011.47	7.80	0.215	9.55
	Control		95.47	15.49	0.042	3.51
	CV (%)		**4.52**	**7.95**	**19.43**	**10.15**
	CD	0.05%	43.84	0.89	NS	1.36
		0.01%	58.78	1.19	NS	1.82

Table 15. Biochemical parameters of G II 19.5 x G I 5.9

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µ mol/g)
1	H97	Highly tolerant	1490.62	15.45	0.265	9.46
2	H98	Susceptible	169.18	4.76	0.209	7.71
3	H99	Susceptible	363.66	4.80	0.213	7.20
4	H100	Susceptible	215.67	5.13	0.199	7.90
5	H101	Highly tolerant	2726.81	9.80	0.270	9.70
6	H102	Tolerant	454.25	9.90	0.248	11.31
7	H103	Tolerant	500.87	8.09	0.247	13.79
8	H104	Tolerant	543.50	8.86	0.239	9.80
9	H105	Susceptible	171.93	5.92	0.207	8.02
10	H106	Susceptible	162.52	5.49	0.154	7.57
	Control		90.13	18.81	0.017	3.33
	CV (%)		**4.70**	**8.73**	**18.63**	**9.74**
	CD	0.05%	54.47	1.16	NS	1.53
		0.01%	74.30	1.59	NS	2.09

4.3.4. Glycine Betaine (GB)

Glycine Betaine not only acts as an osmoregulator, but also stabilizes the structures as well as activities of enzymes and protein complexes, and maintains the integrity of membranes against the damaging effects of excessive salt, cold, heat and freezing (Gorham, 1995; Sakamoto and Murata, 2002). The results are explained as graphs Fig. 34-44 and values in Tables 8-18 depicted below.

In cross M 13.12 x G I 5.9 (Fig. 34), the highest glycine betaine value was found in H26 (11.64 μmol/g) followed by hybrid H27 (10.43 μmol/g). Other hybrids having high GB values were H31 (8.77 μmol/g), H24 (7.99 μmol/g), H28 (7.81 μmol/g), H2 (7.48 μmol/g), H13 (7.43 μmol/g), H25 (7.43 μmol/g), H12 (7.09 μmol/g), H11 (7.03 μmol/g), H20 (6.78 μmol/g), H10 (6.77 μmol/g), H29 (6.43 μmol/g) and H9 (6.35 μmol/g). The lowest value was observed in H3 (4.42 μmol/g). All the other hybrids ranged between 5-6 μmol/g betaine values. The control was having 3.22 μmol/g of glycine betaine (Table 8).

In cross M 13.12 x G II 19.5 (Fig. 35), the highest value was found in hybrid H34 (9.68 μmol/g) followed by hybrid H38 (9.52 μmol/g), H33 (9.46 μmol/g), H42 (9.35 μmol/g), H37 (8.79 μmol/g) and H39 (8.66 μmol/g). The lowest value was observed in H35 (6.73 μmol/g) followed by H40 (7.34 μmol/g). Other low value hybrids were H36 (6.06 μmol/g) and H41 (6.02 μmol/g). The control was having 2.84 μmol/g of the glycine betaine content under non-stressed conditions (Table 9).

In cross M13.12 x G VI 55 (Fig. 36), the highest value was observed in H48 (9.87 μmol/g) followed by H46 (9.66 μmol/g) and H43 (8.90 μmol/g). The lowest value was observed in H45 (5.29 μmol/g) followed by H44 (6.70 μmol/g). Other low value hybrids were H51 (7.13 μmol/g), H50 (7.06 μmol/g) , H47 (6.98 μmol/g), and H49 (6.87 μmol/g), whereas the control was having only 2.82 μmol/g of the amino acid indicating it's occurrence only during drought stress (Table 10).

In cross G I 5.9 x M 13.12 (Fig. 37), high GB values were observed in H52 (8.10 μmol/g) and H57 (7.97 μmol/g). The low values were observed in H56

(6.57 µmol/g), H53 (6.43 µmol/g), H54 (5.88 µmol/g) and H55 (5.36 µmol/g). The control was having only 3.16 µmol/g of the osmolyte under watered condition (Table 11).

In cross G I 5.9 x G II 19.5 (Fig. 38), the highest value was observed in H63 (9.52 µmol/g) followed by H68 (9.38 µmol/g). Other tolerant hybrids were H62 (8.93 µmol/g), H64 (8.61 µmol/g) and H67 (8.32 µmol/g). The lowest value was observed in H70 (6.45 µmol/g). Other hybrids having low values were H60 (7.34 µmol/g), H61 (7.32 µmol/g), H69 (7.28 µmol/g), H59 (7.18 µmol/g), H66 (7.17 µmol/g), H65 (7.11 µmol/g), H58 (6.83 µmol/g). The control had 3.34 µmol/g of glycine betaine (Table 12).

In cross G I 5.9 x G VI 55 (Fig. 39), the highest value was observed in hybrid H71 (9.56 µmol/g) followed by H75 (9.38 µmol/g) which is a tolerant hybrid. Other tolerant hybrids having high GB values were H74 (8.85 µmol/g) and H76 (8.63 µmol/g). Lowest values were observed in H73 (7.07 µmol/g). Other hybrids having low GB content were H72 (7.05 µmol/g), H77 (6.34 µmol/g) and H78 (6.30 µmol/g). The control had 3.18 µmol/g of the glycine betaine content (Table 13).

In cross G II 19.5 x M 13.12 (Fig. 40), highest GB value was observed in H88 (10.08 µmol/g), some other hybrids having high GB values were H96 (9.55 µmol/g), H94 (9.17 µmol/g), H90 (8.89 µmol/g), H79 (8.87 µmol/g), H82 (8.70 µmol/g), H83 (8.66 µmol/g) and H85 (8.43 µmol/g). Lowest value was observed in H80 (6.78 µmol/g) and H95 (6.84 µmol/g) which are the susceptible hybrids. However, the control had only 3.51 µmol/g (Table 14).

In the cross between G II 19.5 x G I 5.9 (Fig. 41), the highest value was observed in H103 (13.79 µmol/g) followed by H102 (11.31 µmol/g). Other hybrids having high values were H104 (9.80 µmol/g), H101 (9.70 µmol/g) and H97 (9.46 µmol/g). The lowest value was observed in H99 (7.20 µmol/g). Some other susceptible hybrids having less GB values were H105 (8.02 µmol/g), H100 (7.90 µmol/g), H98 (7.71 µmol/g) and H106 (7.57 µmol/g). The control plant had 3.33 µmol/g of glycine betaine content (Table 15).

The cross G II 19.5 x G VI 55 (Fig. 42) had two hybrids, the hybrid H107 had high GB value of 9.34 µmol/g and the other hybrid, H108 which is the susceptible one, had 7.18 µmol/g of GB. Control had 2.43 µmol/g of glycine betaine (Table 16).

In cross G VI 55 x G I 5.9 (Fig.43), H112 was having the highest GB content of about 10.61 µmol/g followed by H113 (9.93 µmol/g) and H111 (8.94 µmol/g). The lowest value was observed in H109 (6.93 µmol/g). Other hybrids having low GB values were H110 (7.66 µmol/g), H114 (7.42 µmol/g) and H109 (6.93 µmol/g). The control had only 3.03 µmol/g of the glycine betaine content under full watered condition (Table 17).

In the cross G VI 55 x G II 19.5 (Fig. 44), the highest value was observed in hybrid H119 (9.23 µmol/g), followed by H118 (8.50 µmol/g), H115 (6.52 µmol/g), H117 (6.43 µmol/g) and H120 (5.67 µmol/g). The lowest value was observed in H116 (4.42 µmol/g). The control was observed to have 3.18 µmol/g of the glycine betaine under the non-stressed condition (Table 18).

Many plants accumulate compounds, termed compatible solutes, to cope with stress conditions. One of the most extensively studied compatible solutes is glycine betaine (Rhodes and Hanson, 1993; Rathinasabapathi, 2000; Sakamoto and Murata, 2000; Rontein *et al.*, 2002). Not only GB acts as an osmoregulator, but also stabilizes the structures and activities of enzymes and protein complexes, and maintains the integrity of membranes against the damaging effects of stress (Sakamoto and Murata, 2002).

Genes associated with glycine betaine synthesis in higher plants and microbes have been transferred into plants which do not accumulate glycine betaine, such as *Arabidopsis thaliana* (Hayashi *et al.*, 1997; Alia *et al.*, 1998), *Brassica napus* (Huang *et al.*, 2000), Persimmon (Gao *et al.*, 2000), tobacco (Holmstrom *et al.*, 2000; Shen *et al.*, 2002) and rice (Mohanty *et al.*, 2002). The metabolic engineering of glycine betaine biosynthesis in these plants improved the tolerance of transgenic plants to salt, drought and extreme temperature stresses (Sulpice *et al.*, 2003).

This indicated the importance of glycine betaine in managing stress conditions and when the hybrids of present study were analysed, the tolerant hybrids were having high amount of glycine betaine as compared to susceptible hybrids. However, the control which was kept under fully irrigated condition had least amount of glycine betaine indicating the accumulation of glycine betaine under drought stress conditions.

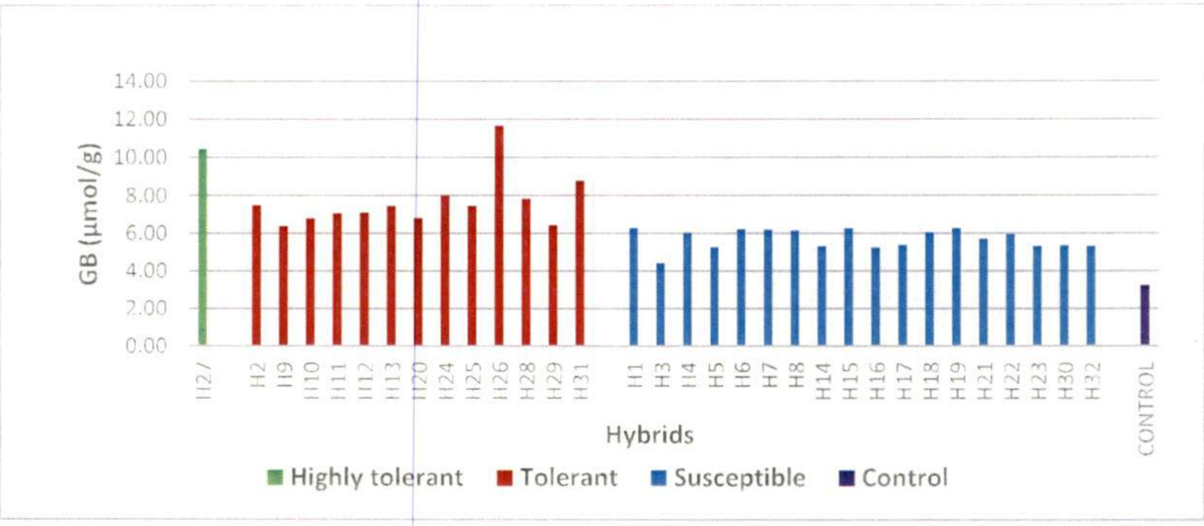

Fig. 34. GB content of M 13.12 x G I 5.9

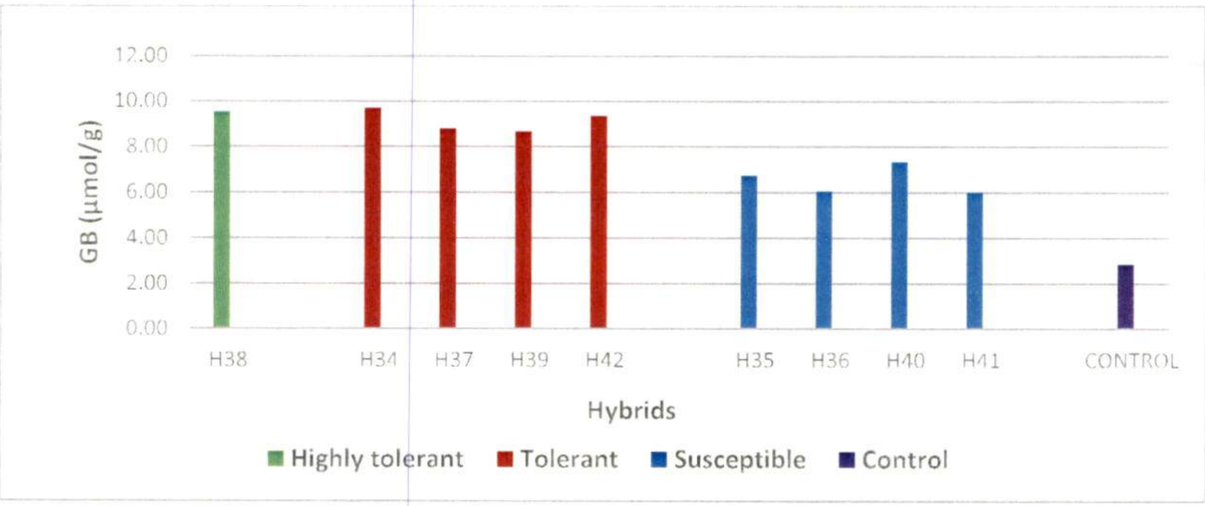

Fig. 35. Glycine betaine content of M 13.12 x G II 19.5

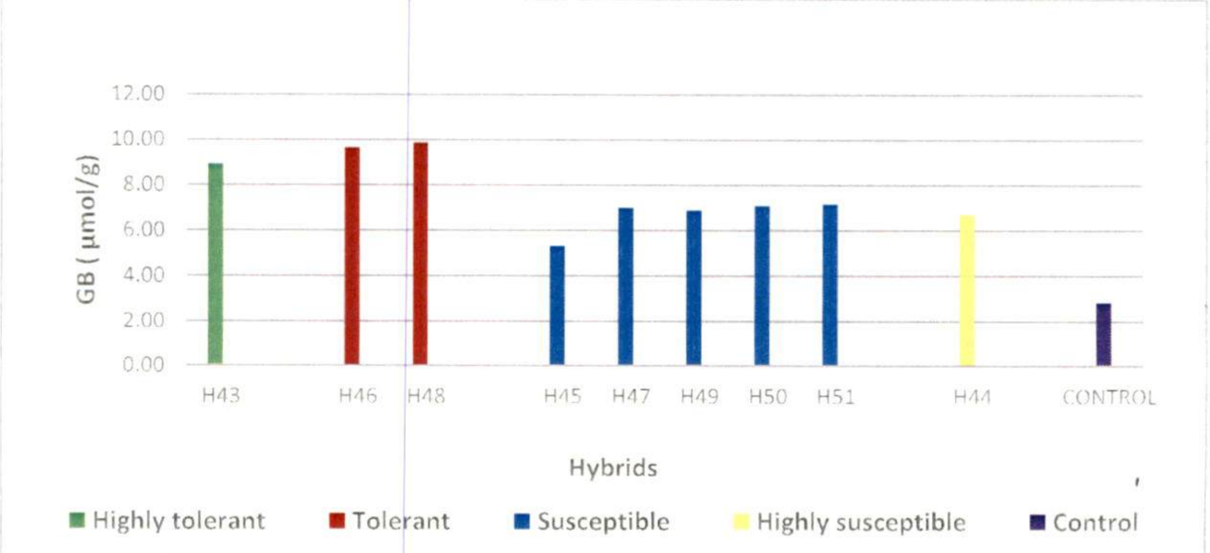

Fig. 36. GB content of M 13.12 x G VI 55

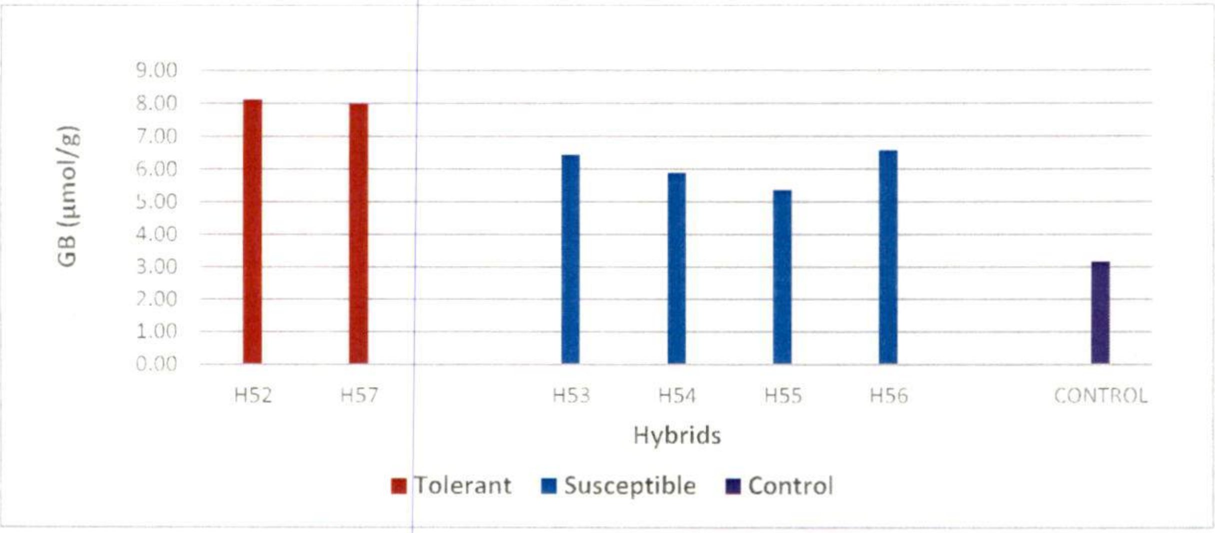

Fig. 37. GB content of G I 5.9 x M 13.12

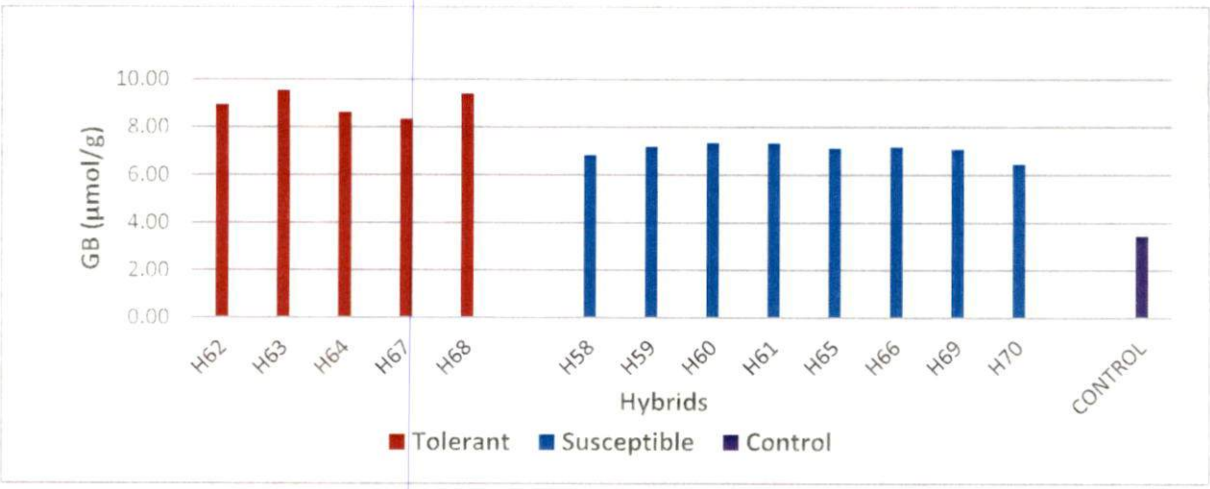

Fig. 38. GB content of G I 5.9 x G II 19.5

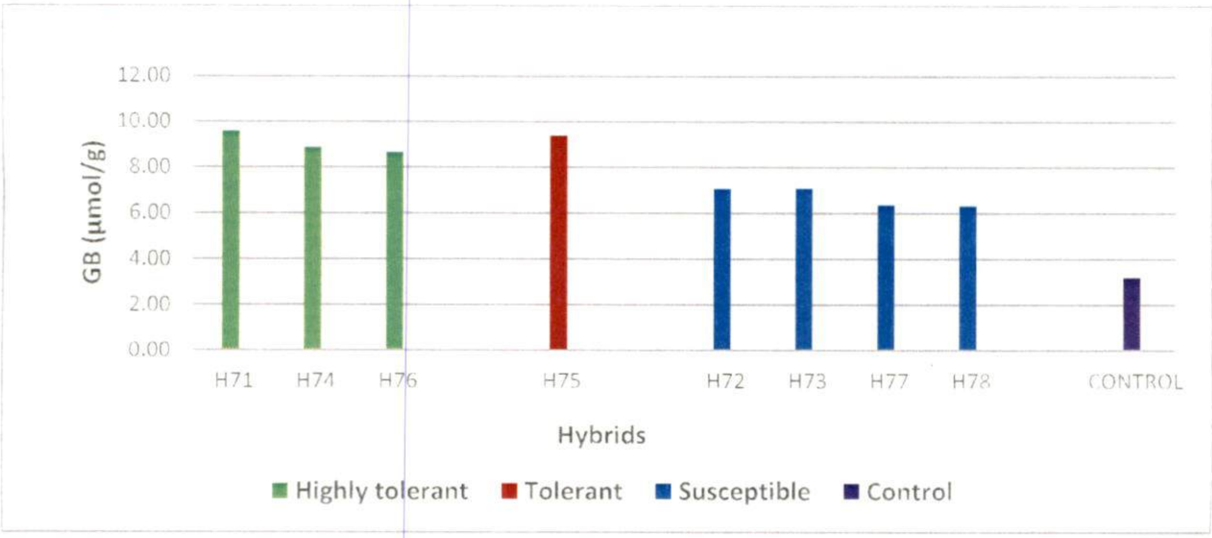

Fig. 39. GB content of G I 5.9 x G VI 55

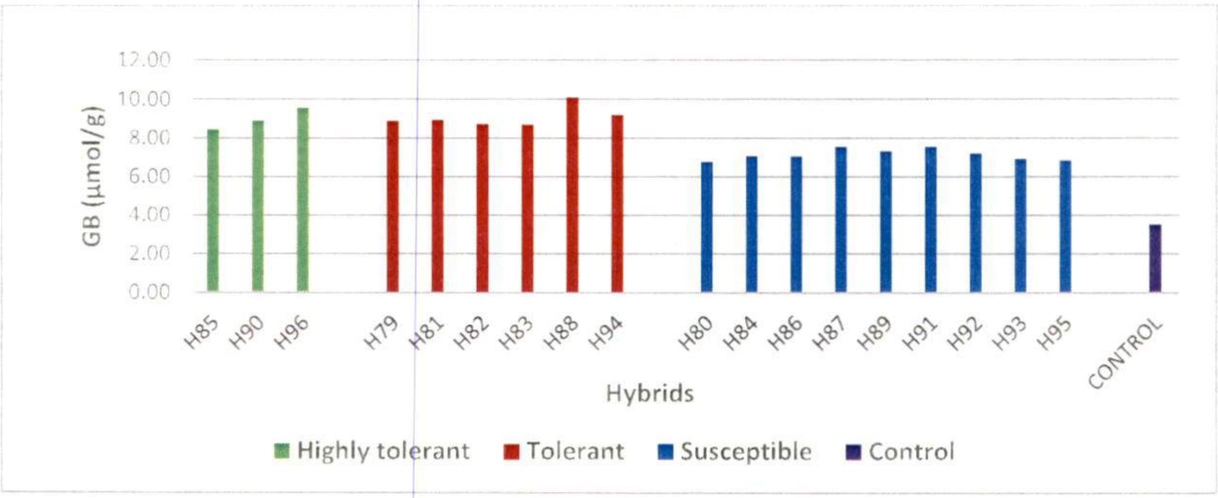

Fig. 40. GB content of G II 19.5 x M 13.12

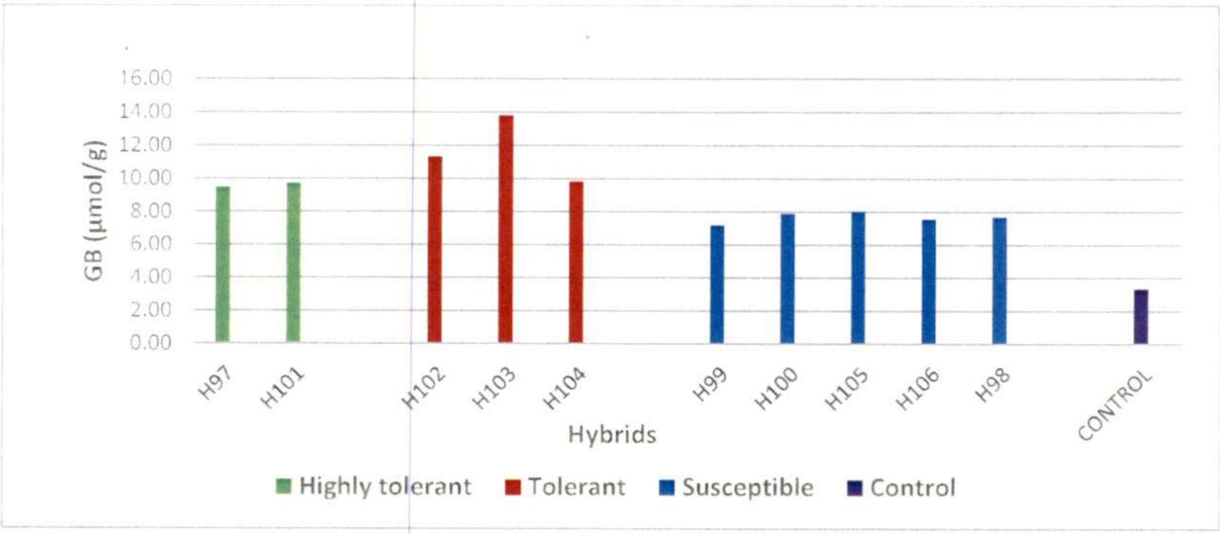

Fig. 41. GB content of G II 19.5 x G I 5.9

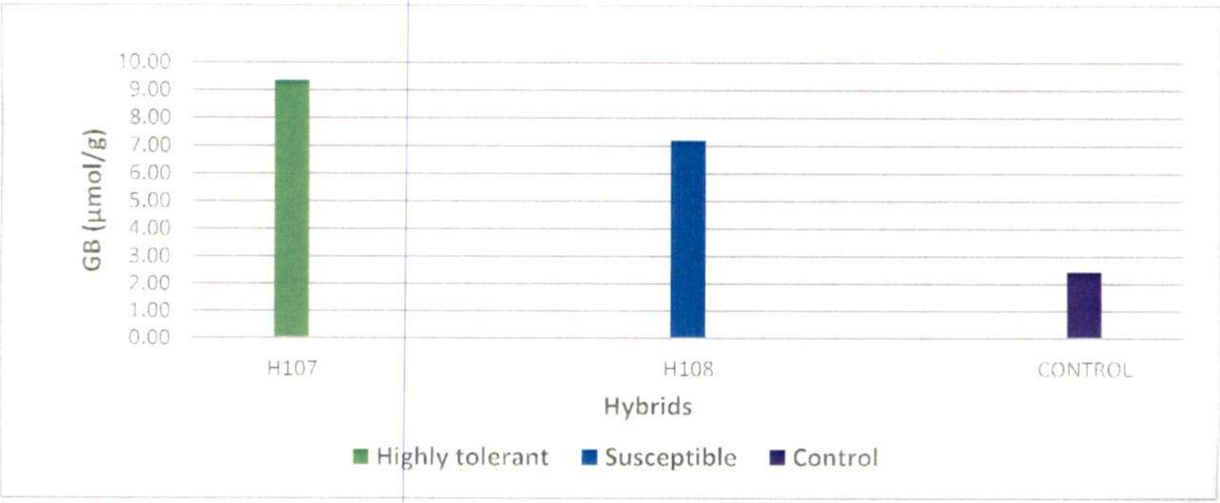

Fig. 42. GB content of G II 19.5 x G VI 55

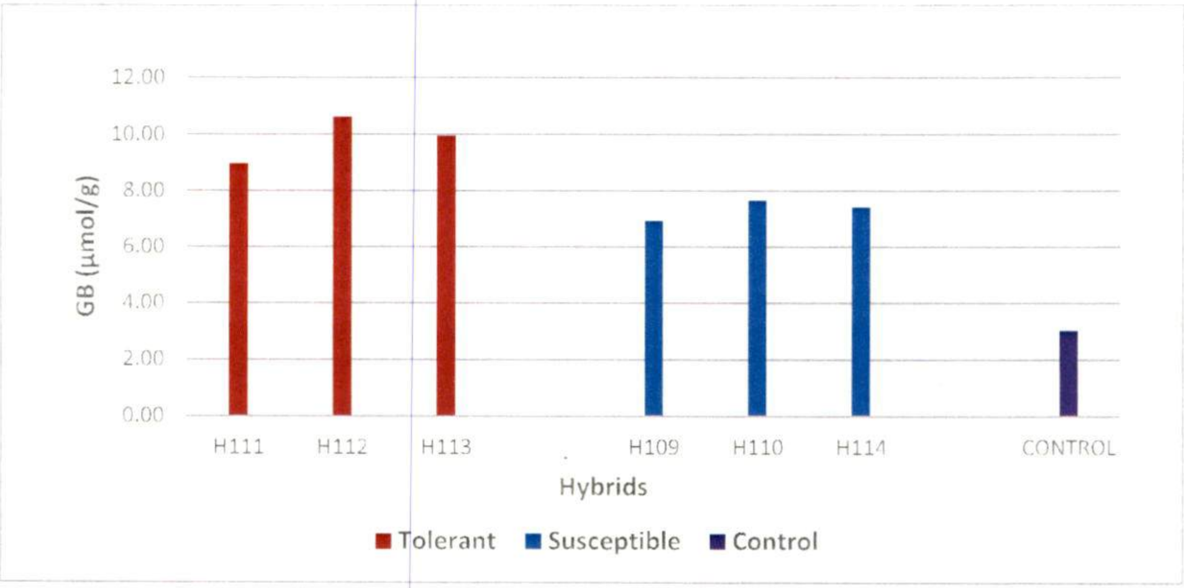

Fig. 43. GB content of G VI 55 x G I 5.9

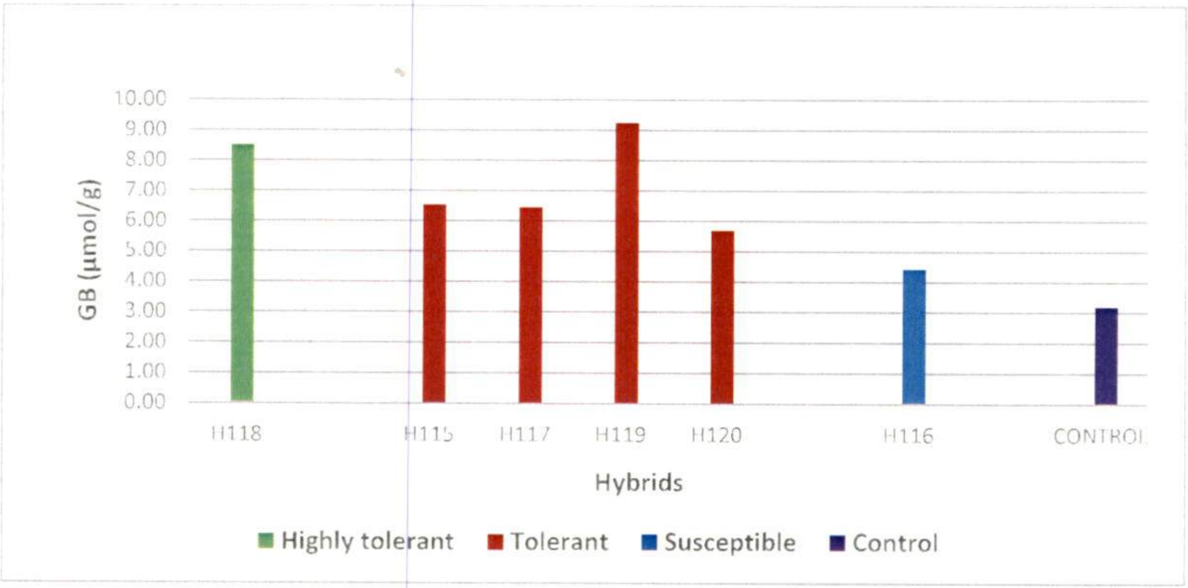

Fig. 44. GB content of G VI 55 x G II 19.5

Table 16. Biochemical parameters of G II 19.5 x G VI 55

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H107	Highly tolerant	1984.83	12.98	0.163	9.34
2	H108	Susceptible	339.69	6.65	0.106	7.18
	Control		69.33	16.66	0.065	2.43
	CV (%)		**2.75**	**4.97**	**18.99**	**5.58**
	CD	0.05%	72.38	1.11	NS	1.04
		0.01%	120.05	1.83	NS	1.73

Table 17. Biochemical parameters of G VI 55 x G I 5.9

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H109	Susceptible	308.91	4.12	0.185	6.93
2	H110	Susceptible	151.99	3.57	0.136	7.66
3	H111	Tolerant	424.27	6.68	0.207	8.94
4	H112	Tolerant	411.35	6.38	0.221	10.61
5	H113	Tolerant	520.85	7.24	0.207	9.93
6	H114	Susceptible	128.55	4.63	0.149	7.42
	Control		74.67	14.76	0.025	3.03
	CV (%)		**7.23**	**6.15**	**17.34**	**11.23**
	CD	0.05%	41.74	0.59	0.06	1.17
		0.01%	58.52	0.83	NS	2.40

Table 18. Biochemical parameters of G VI 55 x G II 19.5

Sl No.	Hybrid	Reaction to drought	Proline (µg/g)	NRA (mmol nitrate/g/hr)	SOD (units/mg protein/g)	Glycine betaine (µmol/g)
1	H115	Tolerant	520.85	17.06	0.209	6.52
2	H116	Susceptible	182.50	4.10	0.140	4.42
3	H117	Tolerant	743.18	11.35	0.202	6.43
4	H118	Highly tolerant	1354.75	8.76	0.227	8.50
5	H119	Tolerant	412.95	7.56	0.163	9.23
6	H120	Tolerant	507.53	6.98	0.173	5.67
	Control		94.00	23.05	0.032	3.18
	CV (%)		**5.21**	**10.22**	**18.49**	**8.92**
	CD	0.05%	57.48	1.74	NS	1.08
		0.01%	80.58	2.45	NS	1.51

4.4. Analysis of physiological parameters

4.4.1. Chlorophyll Stability Index

The chlorophyll stability index (CSI) is an indication of the stress tolerance capacity of plants. A high CSI value means that the stress did not have much effect on chlorophyll content of plants. A higher CSI helps plants to withstand stress through better availability of chlorophyll. This leads to increased photosynthetic rate, more dry matter production, and higher productivity. This indicates how well chlorophyll can perform under stress. The chlorophyll stability index of hybrids were represented in Fig. 45-55 and in table 19-29.

The CSI values varied among the crosses and also within the crosses. In the cross M 13.12 x G I 5.9 (Fig. 45), the highest value was observed in H26 (89.50 %) followed by H27 (86.73 %). Some other hybrids having high CSI values were H29 (83.70 %) and H20 (80.80 %). The lowest CSI was observed in H1 (48.23 %). Some other hybrids high CSI values were H9 (69.18 %), H24 (69.17 %), H12 (67.90 %), H31 (67.63 %), H28 (66.77 %), H25 (66.63 %), H11 (65.69 %), H17 (65.69 %), H2 (64.64 %), H10 (63.70 %), H13 (63.36 %), H16 (57.60 %), H4 (56.23 %), H23 (55.96 %), H19 (55.91 %), H22 (55.27 %), H7 (55.30 %), H18 (55.25 %), H5 (54.80 %), H8 (53.83 %), H6 (53.75 %), H15 (53.55 %), H32 (52.97 %), H21 (52.63 %), H30 (52.30 %) and H3 (52.00 %). The control plant kept under fully irrigated condition was having highest CSI content of about 92.65 per cent (Table 19).

In the cross M 13.12 x G II 19.5 (Fig. 46), the high CSI values were observed in H39 (81.63 %) followed by H38 (66.03 %). Other tolerant hybrids were H34 (58.27 %), H42 (54.00 %), H37 (53.13 %) and H33 (51.19 %). The lowest value was observed in H40 (43.10 %) and other hybrids having low CSI were H36 (49.90 %), H41 (49.07 %) and H35 (47.67 %). 91.07 per cent of the CSI value was found in the control kept (Table 20).

In the cross M 13.12 x G VI 55 (Fig. 47), high value was observed in H43 (74.23 %) followed by H48 (68.90 %) and H46 (66.83 %). The lowest value was observed in H47 (50.37 %). Other hybrids having low CSI values were H51

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(55.72 %), H49 (54.50 %), H44 (53.87 %), H50 (50.63 %), H45 (50.60 %) and H47 (50.37 %) whereas the control was having 90.33 per cent CSI (Table 21).

In cross G I 5.9 x M 13.12 (Fig. 48), the highest value was observed in H52 (81.43 %) followed by H57 (64.99 %). The lowest value was observed in H54 (47.17 %) followed by H55 (49.09 %), H53 (52.23 %) and H56 (55.13 %). The control was having 91.36 per cent of the chlorophyll stability under non-stressed conditions (Table 22).

In the cross G I 5.9 x GII 19.5 (Fig. 49), the highest value of CSI was observed in H64 (81.93 %). Other hybrids having high CSI values were H63 (72.10 %), H62 (71.03 %), H67 (69.21 %) and H68 (64.80 %). The lowest value was from hybrid H66 (44.64 %). Other hybrids having low CSI values were H65 (53.83 %), H59 (51.17 %), H70 (49.79 %), H69 (48.43 %), H61 (47.83 %), H58 (47.10 %) and H60 (46.48 %). The control was having 90.37 per cent of the CSI under non-stressed conditions (Table 23).

In the cross G I 5.9 x G VI 55 (Fig. 50), the highest value was observed in hybrid H74 (78.27 %) followed by H76 (77.47 %), H75 (68.60 %) and H71 (66.40 %). The lowest value observed was in H72 (35.13 %). Other hybrids having low CSI values were H77 (54.85 %), H78 (47.63 %) and H73 (42.57 %). The control plant had 91.44 per cent of the stable CSI values under watered conditions (Table 24).

In the cross G II 19.5 x M 13.12 (Fig. 51), the highest value observed was in hybrid H81 (87.31 %) followed by H88 (85.23 %), H90 (80.17 %) and H85 (74.55 %). Lowest values were observed in hybrid H89 (37.10 %). The control had 96.58 per cent stable CSI value (Table 25).

In the cross G II 19.5 x G I 5.9 (Fig. 52), the highest value was observed in hybrid H97 (88.00 %), followed by H104 (82.33 %), H101 (79.05 %), H102 (72.63 %) and H103 (71.20 %). Lowest values were observed in H98 (59.43 %), H99 (56.83 %), H100 (53.51 %), H105 (52.20 %) and H106 (48.12 %). The control had 95.83 per cent CSI value under non-stressed conditions (Table 26).

In the cross G II 19.5 x G VI 55 (Fig. 53), the hybrid H107 was having the high value of 77.77 per cent followed by H108 having about 48.63 per cent of chlorophyll stability index. The control was having a CSI value of around 91.49 per cent (Table 27).

In the cross G VI 55 x G I 5.9 (Fig. 54), the highest value was observed in H112 (89.67 %) indicating it's tolerance to drought stress followed by H111 (67.20 %) and H113 (66.52 %). Lowest value was found in hybrid H109 (53.65 %). Other hybrids having low CSI values were H114 (59.30 %) and H110 (57.30 %). The control had 94.28 per cent CSI under non-stressed condition (Table 28).

In the cross G VI 55 x G II 19.5 (Fig. 55), the highest value was observed in hybrid H115 (82.50 %) and the lowest value was observed in H116 (57.17 %). Other hybrids were H118 (76.83 %), H117 (69.17 %), H120 (67.17 %) and H119 (66.00 %). The control was having 90.14 per cent of chlorophyll stability index under non-stressed conditions (Table 29).

Chlorophyll Stability Index is a function of temperature and is found to co-relate with drought tolerance. It is a measure of integrity of membrane or heat stability of pigments under drought stress conditions (Kaloyereas, 1958). It is an important parameter used to measure drought tolerance of a plant. Sairam *et al.* (1996) reported that both drought stress and temperature stress decreased chlorophyll stability index in all wheat genotypes. High CSI help the plants to withstand drought through better availability of chlorophyll. This leads to increased photosynthetic rate and more dry matter production (Mohan *et al.*, 2000).

In the hybrids, the tolerant hybrids were having a range of 60-80 per cent of the chlorophyll stability index as compared to susceptible hybrids having less CSI value. The control on the other side, had the maximum value of CSI indicating it's reduction during drought stress.

In rice breeding programmes, chlorophyll stability index was chosen as most reliable laboratory screening indictor to screen for drought tolerance (Deivanai *et al.*, 2010).

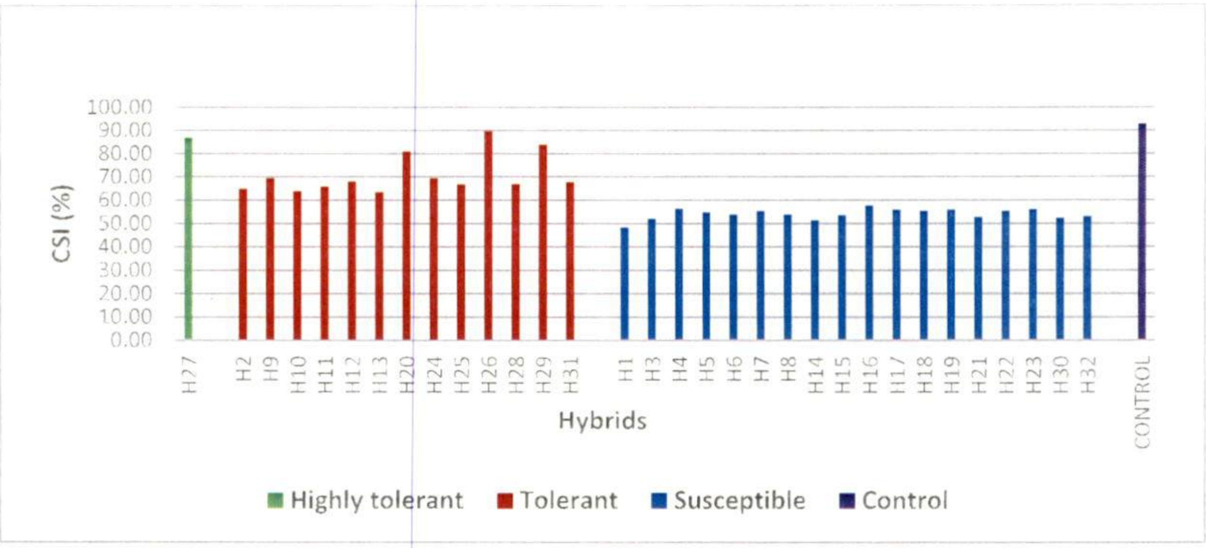

Fig. 45. CSI of M 13.12 x G I 5.9

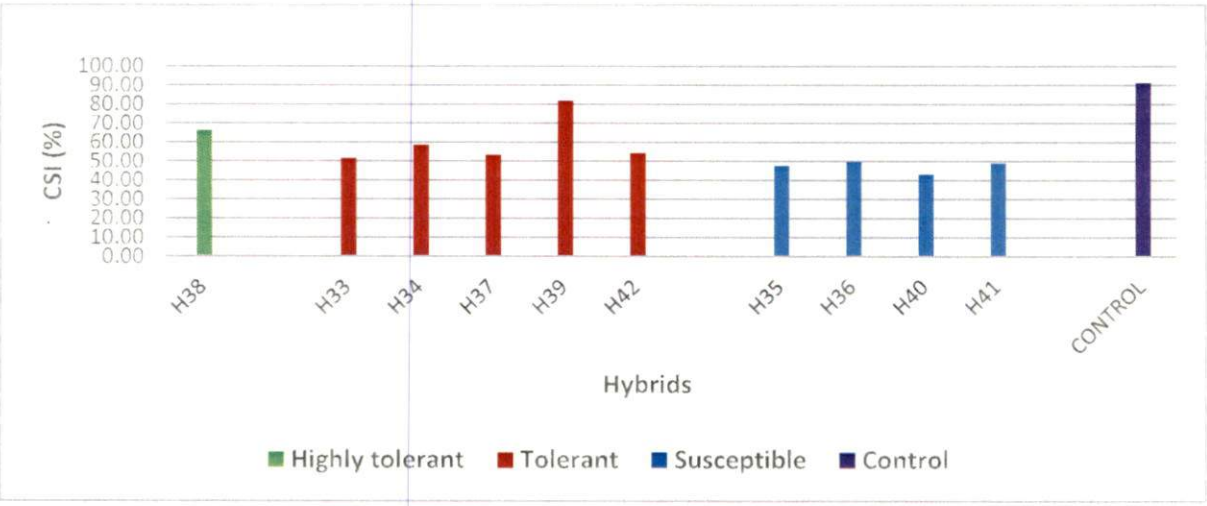

Fig. 46. CSI of M 13.12 x G II 19.5

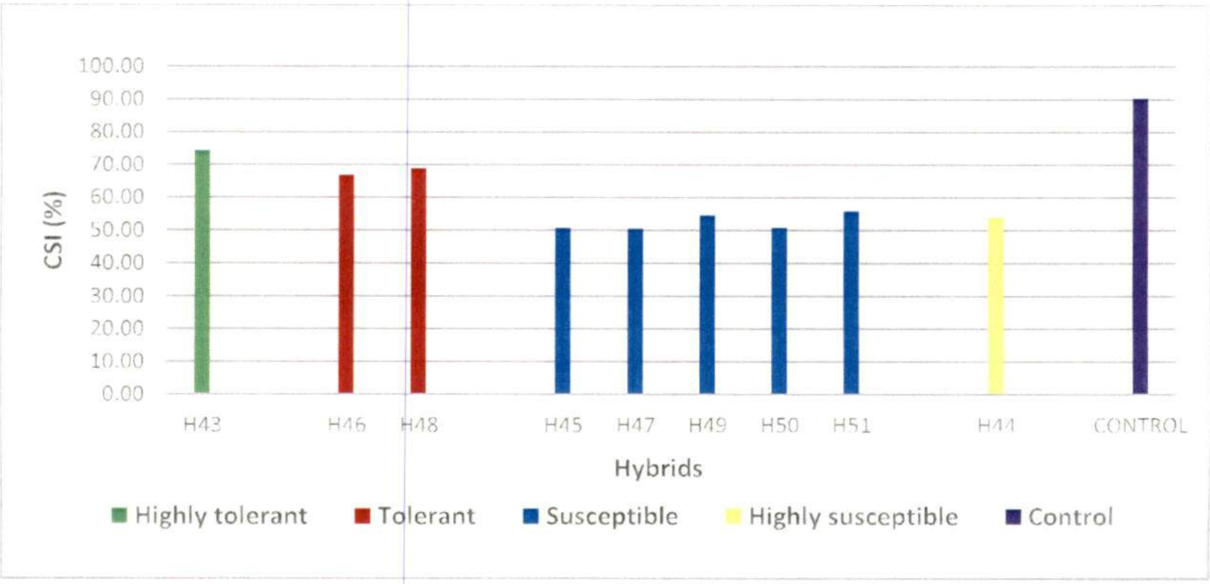

Fig. 47. CSI of M 13.12 x G VI 55

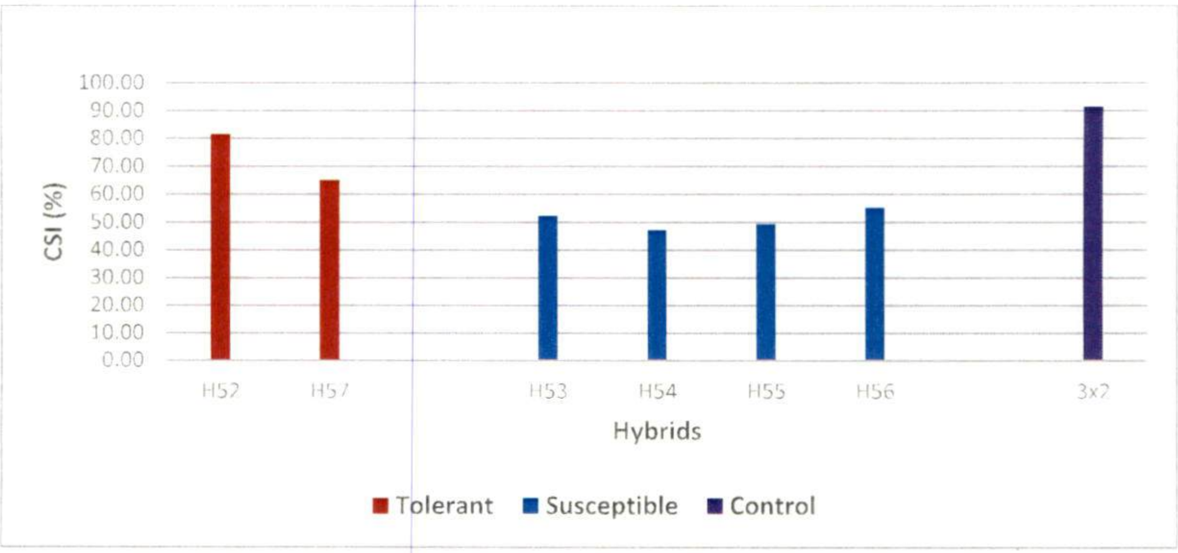

Fig. 48. CSI of G I 5.9 x M 13.12

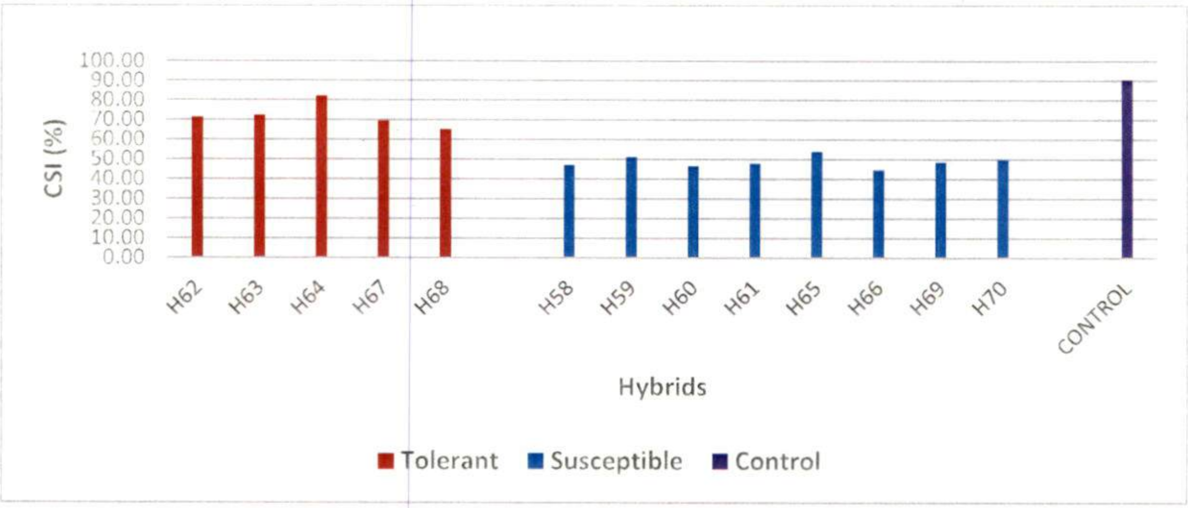

Fig. 49. CSI of G I 5.9 x G II 19.5

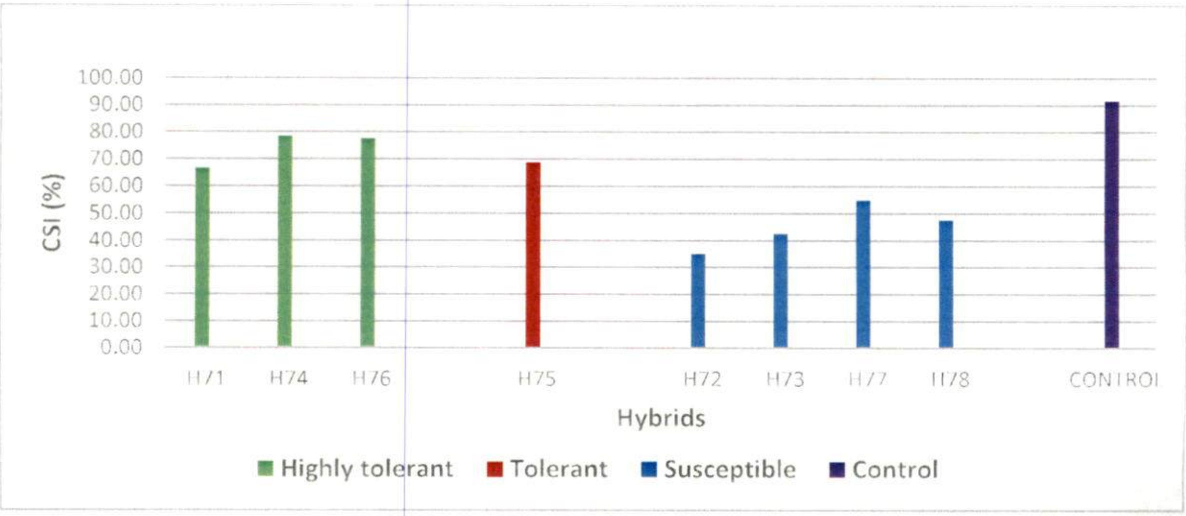

Fig. 50. CSI of G I 5.9 x G VI 55

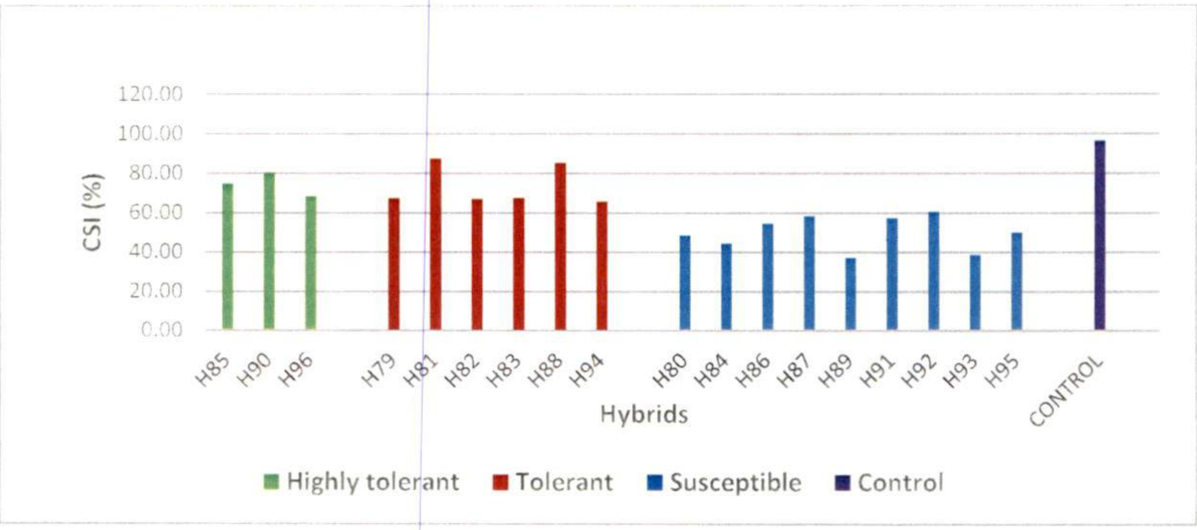

Fig. 51. CSI of G II 19.5 x M 13.12

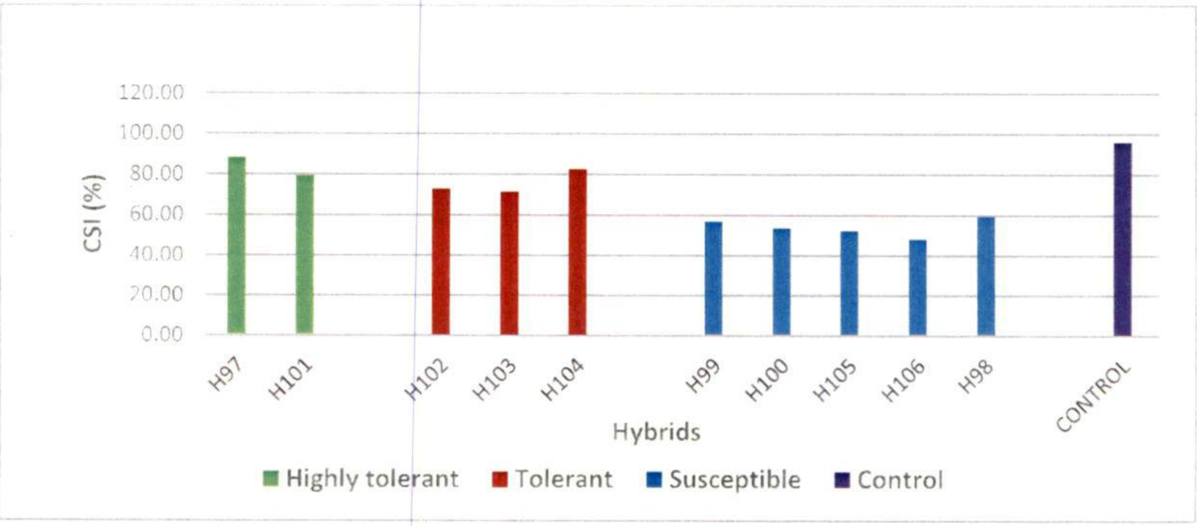

Fig. 52. CSI of G II 19.5 x G I 5.9

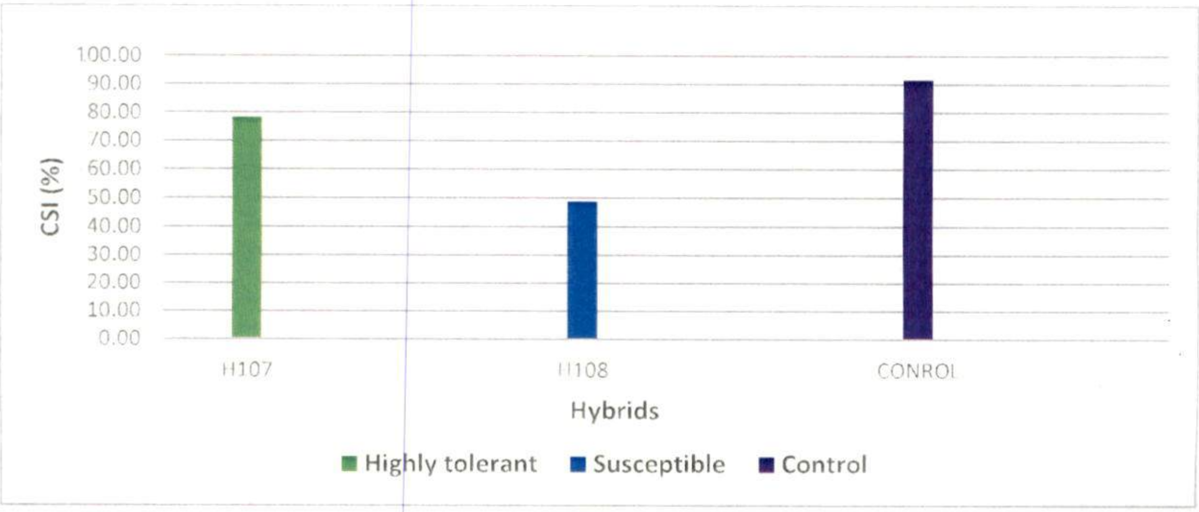

Fig. 53. CSI of G II 19.5 x G VI 55

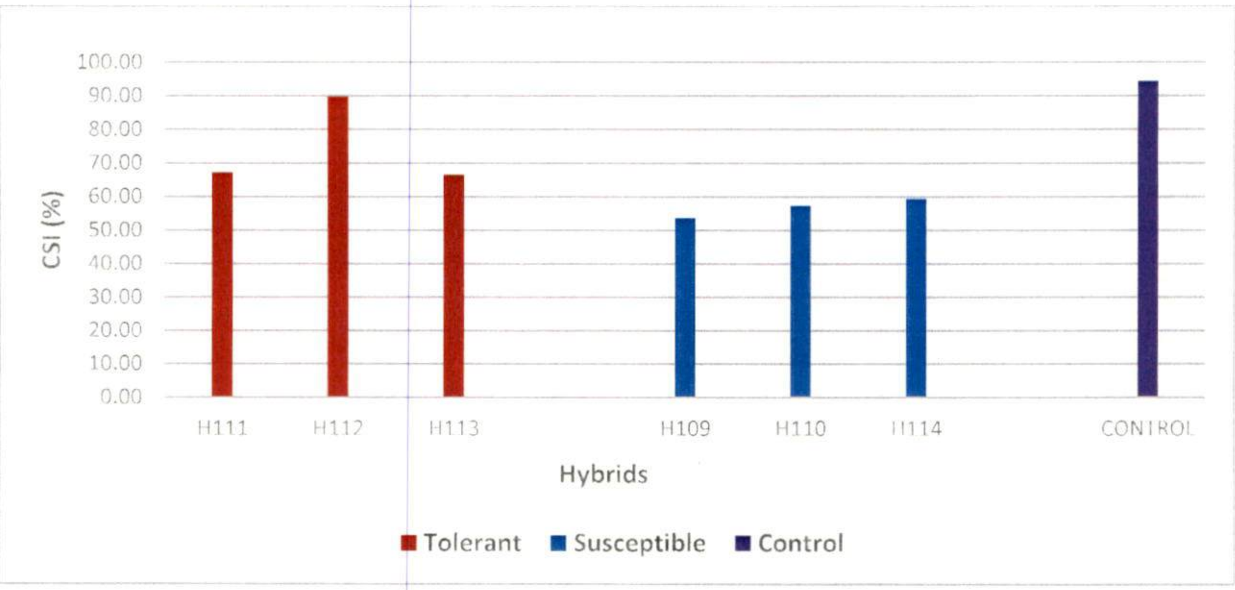

Fig. 54. CSI of G VI 55 x G I 5.9

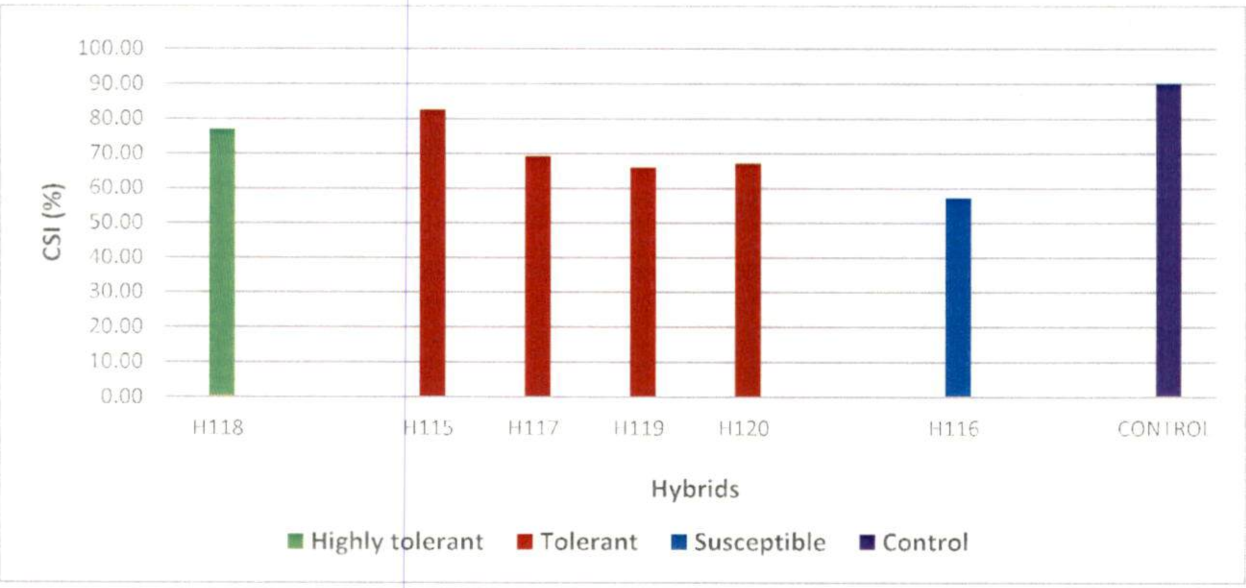

Fig. 55. CSI of G VI 55 x G II 19.5

Table 19. Physiological parameters of M 13.12 x G I 5.9

Sl. No.	Hybrids	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H1	Susceptible	48.23	50.38	23.49	35.70	1.013	1.453	31.57
2	H2	Tolerant	64.64	65.43	33.44	36.59	1.507	0.716	37.17
3	H3	Susceptible	52.00	42.93	24.69	36.48	0.770	1.850	33.53
4	H4	Susceptible	56.23	45.00	22.54	36.45	0.743	1.593	32.10
5	H5	Susceptible	54.80	46.37	25.07	33.26	0.833	2.227	32.87
6	H6	Susceptible	53.75	50.68	27.72	36.43	0.707	2.517	30.03
7	H7	Susceptible	55.30	47.20	26.21	33.43	0.795	2.030	28.77
8	H8	Susceptible	53.83	52.66	23.34	30.55	0.572	1.673	21.37
9	H9	Tolerant	69.18	67.67	34.71	37.39	1.105	0.667	36.60
10	H10	Tolerant	63.70	64.10	38.45	37.47	1.334	0.926	41.17
11	H11	Tolerant	65.69	59.34	40.44	37.37	1.643	0.525	37.03
12	H12	Tolerant	67.90	80.50	43.13	36.62	1.182	0.343	39.83
13	H13	Tolerant	63.36	66.19	70.23	36.94	1.089	0.552	41.33
14	H14	Susceptible	51.27	42.23	29.76	33.06	0.527	1.403	22.30
15	H15	Susceptible	53.55	51.86	23.46	30.66	0.647	1.400	26.57
16	H16	Susceptible	57.60	43.40	24.58	35.16	0.843	2.153	26.27
17	H17	Susceptible	65.69	45.31	27.86	36.53	1.014	2.750	28.50
18	H18	Susceptible	55.25	46.71	25.60	35.07	0.870	1.740	30.63
19	H19	Susceptible	55.91	46.95	23.77	35.32	0.770	1.940	28.40
20	H20	Tolerant	80.80	69.72	43.43	37.49	1.877	0.514	40.40

21	H21	Susceptible	52.63	48.72	20.60	36.46	0.844	1.387	31.37
22	H22	Susceptible	55.27	48.02	30.94	36.47	0.724	2.330	32.83
23	H23	Susceptible	55.96	52.54	23.54	31.38	0.635	3.417	24.70
24	H24	Tolerant	69.17	60.05	36.34	37.45	1.617	0.989	36.90
25	H25	Tolerant	66.63	57.21	36.60	37.28	1.753	0.629	38.60
26	H26	Tolerant	89.50	65.42	35.74	37.46	1.730	0.567	36.53
27	H27	Highly tolerant	86.73	86.36	60.41	37.45	1.627	0.351	40.57
28	H28	Tolerant	66.77	61.35	39.81	37.43	1.830	0.724	40.50
29	H29	Tolerant	83.70	59.06	33.31	37.44	1.519	0.471	39.63
30	H30	Susceptible	52.30	44.88	28.40	36.69	0.993	3.170	30.60
31	H31	Tolerant	67.63	81.33	37.07	37.47	1.074	0.343	37.83
32	H32	Susceptible	52.97	48.13	25.73	35.64	0.839	2.187	26.57
	Control		92.65	92.53	75.53	36.40	2.002	2.868	44.80
	CV (%)		**10.70 (8.18)**	**14.26 (9.98)**	**16.08 (9.50)**	**16.20**	**6.99**	**4.48**	**8.95**
	CD	0.05%	10.84 (6.10)	13.07 (7.94)	8.53 (5.35)	NS	0.12	0.10	4.85
		0.01%	14.40 (9.30)	17.37 (10.55)	11.34 (7.11)	NS	0.17	0.14	6.45

Table 20. Physiological parameters of M 13.12 x G II 19.5

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H33	Tolerant	51.19	76.66	71.45	31.74	0.968	0.488	32.77
2	H34	Tolerant	58.27	73.20	80.70	31.45	0.855	0.393	32.50
3	H35	Susceptible	47.67	46.67	33.47	31.17	0.697	0.703	24.53
4	H36	Susceptible	49.90	49.89	32.59	31.23	0.706	0.604	25.33
5	H37	Tolerant	53.13	90.92	48.92	31.40	0.962	0.386	35.63
6	H38	Highly tolerant	66.03	86.02	76.75	31.52	1.014	0.484	32.70
7	H39	Tolerant	81.63	81.99	36.39	31.37	0.793	0.387	41.53
8	H40	Susceptible	43.10	57.09	33.59	31.20	0.566	1.045	27.43
9	H41	Susceptible	49.07	62.58	31.69	30.53	0.648	0.623	24.87
10	H42	Tolerant	54.00	79.90	39.59	31.63	0.845	0.307	38.54
	Control		91.07	95.08	84.92	31.25	1.134	1.086	46.30
	CV (%)		**8.49 (6.01)**	**10.11 (8.50)**	**15.58 (11.06)**	**9.14**	**6.75**	**10.41**	**13.28**
	CD	0.05%	8.01 (4.94)	12.14 (8.39)	12.87 (8.36)	NS	0.09	0.10	7.14
		0.01%	10.93 (6.74)	16.55 (11.45)	17.56 (11.40)	NS	0.13	0.13	9.74

Table 21. Physiological parameters of M 13.12 x G VI 55

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H43	Highly tolerant	74.23	85.90	73.22	32.31	0.779	0.426	38.30
2	H44	Highly susceptible	53.87	48.44	34.49	30.56	0.514	0.635	26.50
3	H45	Susceptible	50.60	60.77	30.48	30.40	0.643	0.614	20.27
4	H46	Tolerant	66.83	78.07	81.29	31.03	0.737	0.446	35.37
5	H47	Susceptible	50.37	48.67	41.35	30.45	0.676	0.672	25.40
6	H48	Tolerant	68.90	71.62	48.48	31.01	0.772	0.461	33.53
7	H49	Susceptible	54.50	47.76	43.87	30.98	0.572	0.627	26.47
8	H50	Susceptible	50.63	50.30	35.32	30.36	0.536	0.665	23.43
9	H51	Susceptible	55.72	49.41	46.84	30.54	0.607	0.593	26.67
	Control		90.33	92.96	87.32	30.75	0.909	0.982	41.20
	CV (%)		**16.20 (11.30)**	**15.51 (11.32)**	**15.27 (10.77)**	**7.88**	**9.12**	**8.83**	**15.16**
	CD	0.05%	16.23 (9.69)	15.99 (9.97)	12.67 (8.18)	NS	0.10	0.09	7.40
		0.01%	NS	21.91 (13.66)	17.35 (11.20)	NS	0.14	0.19	10.13

4.4.2. Membrane stability

A major impact of plant environmental stress is cellular membrane modification, which results in its perturbed function or total dysfunction. The exact structural and functional modification caused by stress is not fully resolved. However, the cellular membrane dysfunction due to stress is well expressed in increased permeability and leakage of ions out, which can be readily measured by the efflux of electrolytes.

Hence, the estimation of membrane dysfunction under stress by measuring cellular electrolyte leakage from affected leaf tissue into an aqueous medium is finding a growing use as a measure of CMS and is a screening procedure for stress resistance.

The membrane stability varied considerably in the hybrids and also among the crosses (Fig. 56-66). In the cross M 13.12 x G I 5.9 (Fig. 56), H27 had the highest cell membrane stability of about 86.36 per cent followed by H31 having 81.33 per cent of stability. Hybrids having high membrane stability were H12 (80.50 %), H20 (69.72 %), H9 (67.67 %), H13 (66.19 %), H2 (65.43 %), H26 (65.42 %), H10 (64.10 %), H28 (61.35 %), H24 (60.05 %), H11 (59.34 %), H29 (59.06 %) and H25 (57.21 %). Lowest value was observed in H14 (42.23 %). Some other hybrids showing low membrane stability were H21 (48.72 %), H32 (48.13 %), H22 (48.02 %), H7 (47.20 %), H19 (46.95 %), H18 (46.71 %), H5 (46.37 %), H17 (45.31 %), H4 (45.00 %), H30 (44.88 %), H16 (43.40 %) and H3 (42.93 %). The control had 92.53 per cent of membrane stability under non-stressed conditions (Table 19).

In cross M 13.12 x G II 19.5 (Fig. 57), the highest value was observed in hybrid H37 (90.92 %) followed by H38 (86.02 %). Other hybrids having high CMS were H39 (81.99 %), H42 (79.90 %), H33 (76.66 %) and H34 (73.20 %). Low value was observed in H35 (46.67 %). Other susceptible hybrids having low CMS were H41 (62.58 %), H40 (57.09 %) and H36 (49.89 %), whereas the control had 95.08 per cent of the membrane stability under non-stressed conditions (Table 20).

In the cross M 13.12 x G VI 55 (Fig. 58), eight hybrids were there in which the highest value was found in hybrid H43 (85.90 %) which is a highly tolerant hybrid followed by hybrid H46 (78.07 %) and H48 (71.62 %). The lowest value was found in H49 (47.76 %). Other hybrids having low MSI values were and H45 (60.77 %), H50 (50.30 %), H51 (49.41 %), H44 (48.44 %) and H49 (47.76 %). The control had 92.96 per cent of the stability index highest under non-stressed conditions (Table 21).

In G I 5.9 x M 13.12 (Fig. 59), the highest value was observed in hybrid H57 (89.66 %) followed by H52 (72.79 %). The lowest value was found in H55 (51.15 %). Other hybrids having low values were H54 (60.11 %), H53 (52.08 %) and H56 (51.84 %). The control had 91.39 per cent of the membrane stability under non-stressed conditions (Table 22).

In the cross G I 5.9 x G II 19.5 (Fig. 60), the highest value was observed in H64 (85.65 %) and the lowest value was observed in hybrid H58 (36.03 %). Hybrids having high CMS values were H62 (76.63 %), H63 (71.24 %), H67 (70.52 %) and H68 (69.66 %). 95.88 per cent of the membrane stability was found in the control plant (Table 23).

The cross G I 5.9 x G VI 55 (Fig. 61) contained hybrids that had values as high as 89.53 per cent in hybrid H76 followed by H71 (86.01 %), H74 (73.40 %) and H75 (72.89 %). Low values were observed in hybrids H78 (61.50 %), H73 (60.84 %), H72 (58.34 %) and H77 (47.87 %). The control had 93.36 per cent of the membrane stability (Table 24).

In G II 19.5 x M 13.12 (Fig. 62), the highest value was observed in hybrid H88 (86.54 %) followed by H79 (84.32 %). All the tolerant hybrids were having membrane stability in the range of 70-80 per cent. The lowest value was observed in H84 (46.10 %). Low CMS value was also observed in H89 (46.85 %). The control had 93.50 per cent of the membrane stability under non-stressed conditions (Table 25).

In cross G II 19.5 x G I 5.9 (Fig. 63), H103 showed the highest CMS value (71.31 %) followed by H102 (71.30 %), H97 (69.88 %), H104 (67.94 %) and

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H101 (64.62 %). Lowest values were observed in H98 (40.45 %) which was a susceptible hybrid. 92.64 per cent membrane stability was found in the control plant (Table 26).

In G II 19.5 x G VI 55 (Fig. 64), two hybrids were there in which H107 had 78.31 per cent membrane stability whereas H108 had 58.94 per cent stability. The control plant had 93.07 per cent membrane stability (Table 27).

In cross G VI 55 x G I 5.9 (Fig. 65), the hybrids having the highest value were H113 having 85.41 per cent membrane stability. Other hybrids having high CMS values were H112 (78.84 %) and H111 (68.37 %). Hybrids having low CMS content were H110 (57.74 %), H109 (52.73 %) and H114 (42.25 %) whereas the control had 89.03 per cent stability under non-stressed conditions (Table 28).

In cross G VI 55 x G II 19.5 (Fig. 66), high value was found in H119 (85.72 %), followed by H117 (81.81 %), H120 (77.53 %), H118 (75.72 %) and H115 (65.71 %). Low value was found in H116 (41.99 %) which is also a tolerant hybrid. The control had 92.19 per cent of stability when kept under non-stressed conditions (Table 29).

The membrane stability of the susceptible hybrids were found to be lower than the tolerant hybrids and the control plants were having the higher membrane stability than the hybrids which was kept under fully irrigated condition.

Many studies point to cell membrane as an initial site of stress injury. The function and structure of plant cell membranes is drastically damaged by environmental stress (Liebermann *et al.*, 1958; Siminovitch *et al.*, 1964; McKersie and Tomes, 1980; McKersie *et al.*, 1982). Membrane stability had also been associated with water and high temperature stress tolerance in various crop plants (Sairam *et al.*, 1997; Sairam *et al.*, 1998). Thus, evaluation of cellular membrane integrity as a measure of environmental stress tolerance appears to be relevant criteria (Sullivan, 1972).

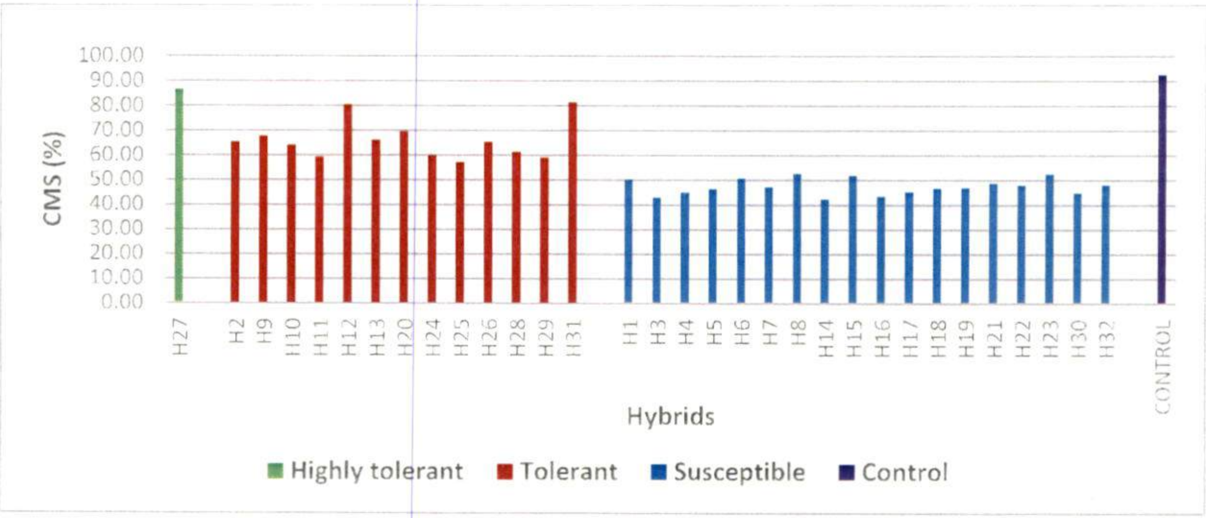

Fig. 56. CMS of M 13.12 x G I 5.9

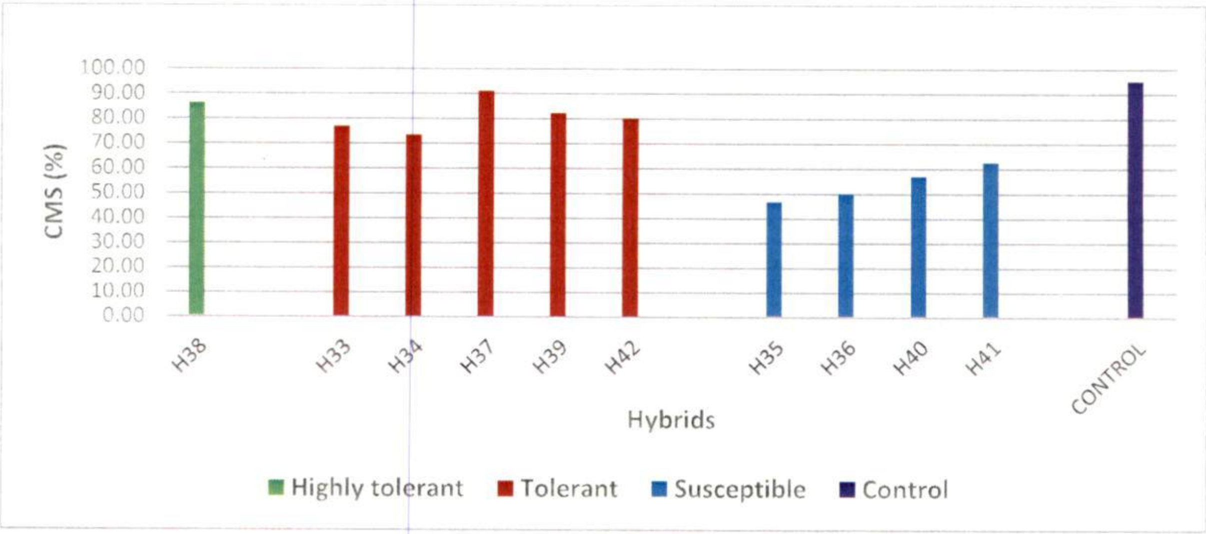

Fig. 57. CMS of M 13.12 x G II 19.5

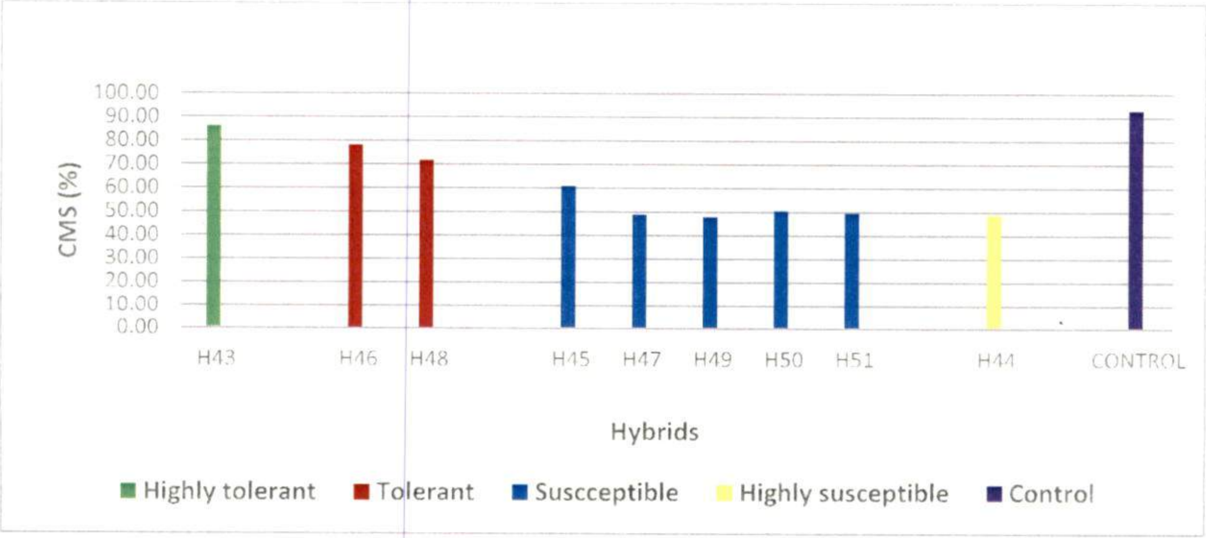

Fig. 58. CMS of M 13.12 x G VI 55

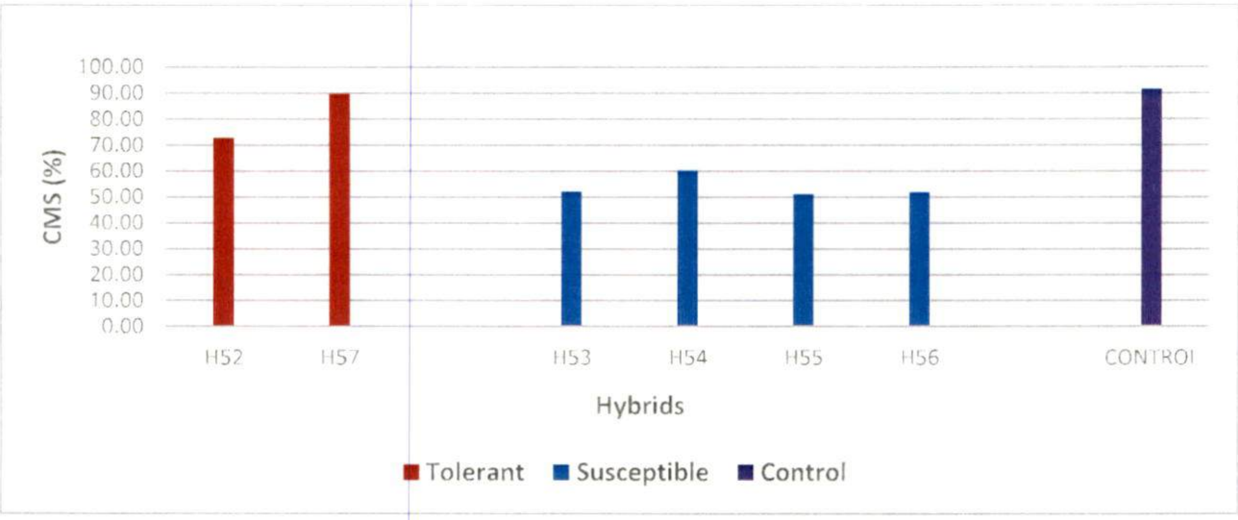

Fig. 59. CMS of G I 5.9 x M 13.12

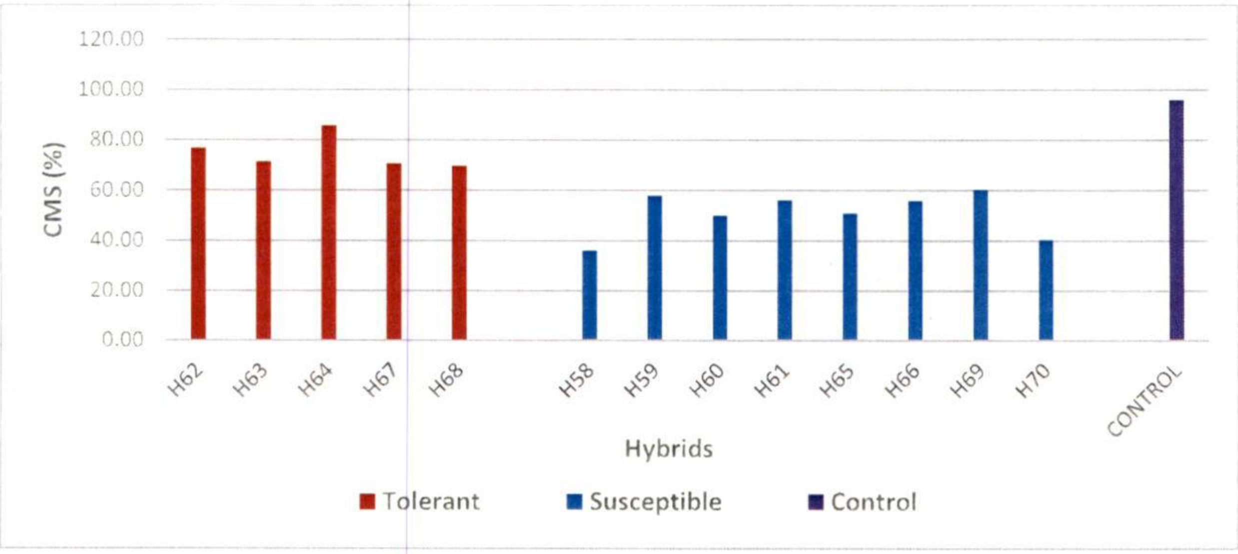

Fig. 60. CMS of G I 5.9 x G II 19.5

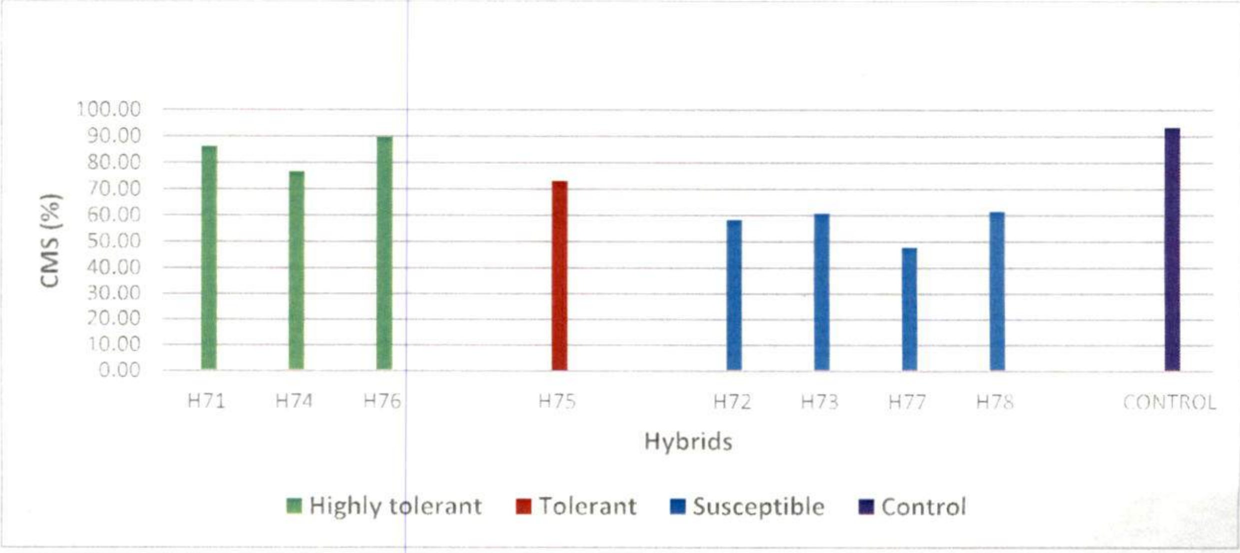

Fig. 61. CMS of G I 5.9 x G VI 55

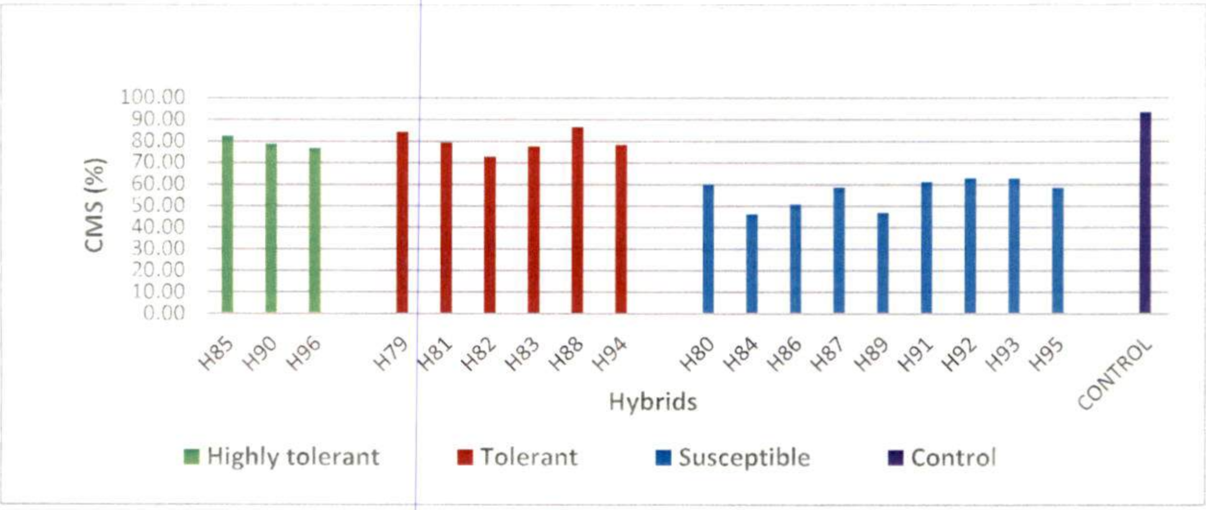

Fig. 62. CMS of G II 19.5 x M 13.12

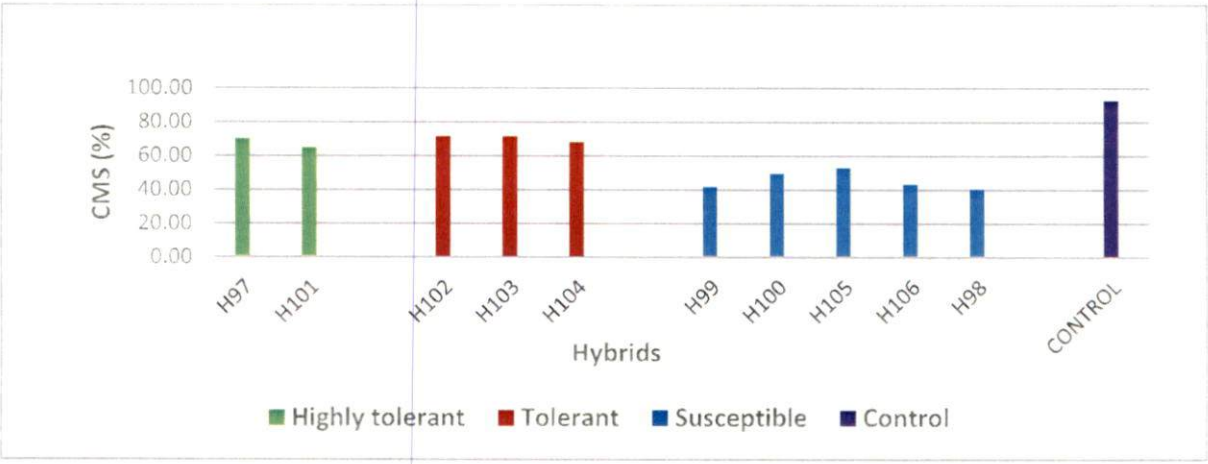

Fig. 63. CMS of G II 19.5 x G I 5.9

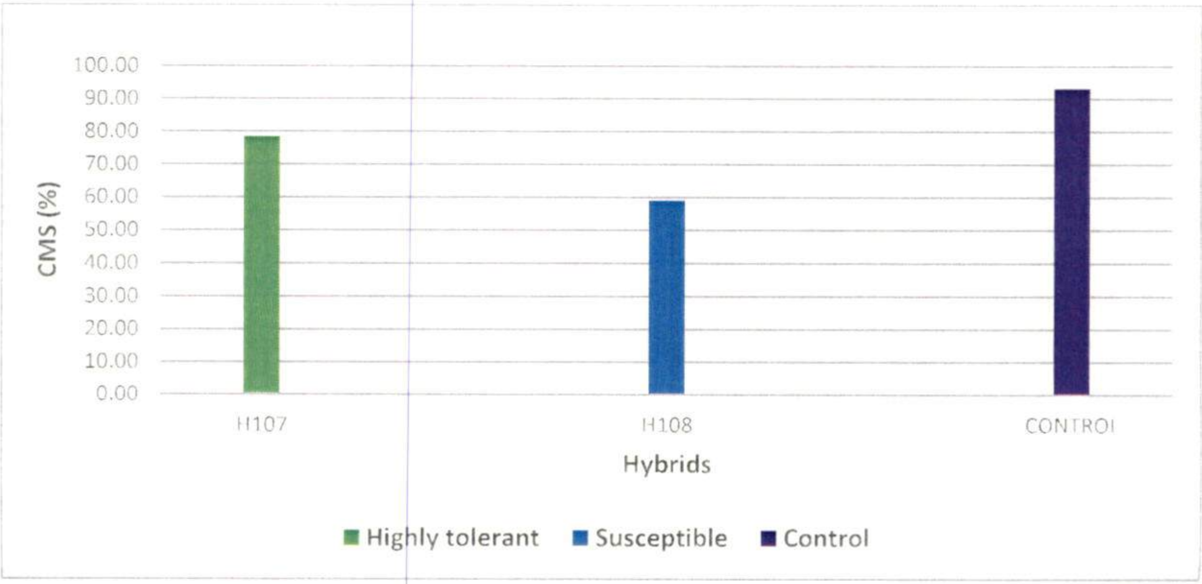

Fig. 64. CMS of G II 19.5 x G VI 55

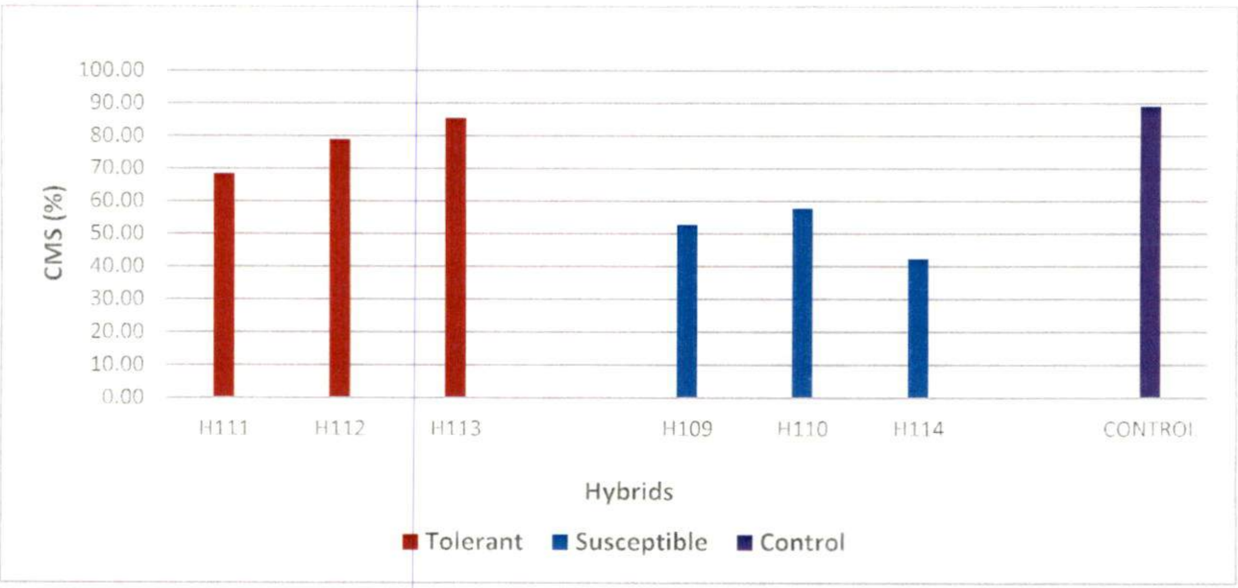

Fig. 65. CMS of G VI 55 x G I 5.9

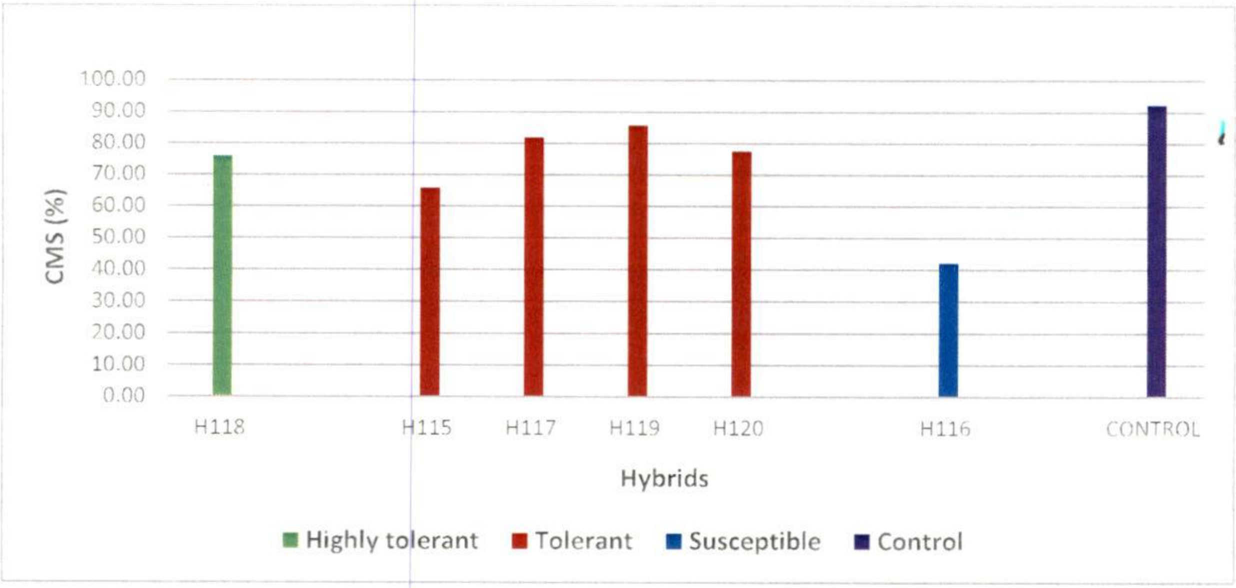

Fig. 66. CMS of G VI 55 x G II 19.5

Table 22. Physiological parameters of G I 5.9 x M 13.12

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H52	Tolerant	81.43	72.79	49.22	33.85	0.916	0.361	38.10
2	H53	Susceptible	52.23	52.08	26.57	30.57	0.670	0.617	30.87
3	H54	Susceptible	47.17	60.11	25.43	30.20	0.771	0.704	31.13
4	H55	Susceptible	49.09	51.15	29.66	30.57	0.803	0.592	28.57
5	H56	Susceptible	55.13	51.84	30.44	30.56	0.740	0.549	21.33
6	H57	Tolerant	64.99	89.66	47.76	30.74	1.283	0.402	36.00
	Control		91.36	91.39	75.01	30.55	1.430	0.950	43.70
	CV (%)		**12.70 (8.74)**	**11.84 (9.06)**	**18.17 (11.22)**	**8.10**	**7.07**	**7.74**	**12.25**
	CD	0.05%	13.18 (7.78)	13.26 (8.58)	11.26 (7.17)	NS	0.11	0.07	6.75
		0.01%	18.48 (10.91)	18.59 (12.02)	15.79 (10.05)	NS	0.15	0.10	9.47

Table 23. Physiological parameters of G I 5.9 x G II 19.5

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H58	Susceptible	47.10	36.03	28.45	30.77	0.634	0.408	21.50
2	H59	Susceptible	51.17	57.88	31.65	31.15	0.676	0.612	19.50
3	H60	Susceptible	46.48	49.93	30.75	30.82	0.634	0.445	27.43
4	H61	Susceptible	47.83	56.07	57.60	31.25	0.572	0.873	27.63
5	H62	Tolerant	71.03	76.63	76.19	31.88	0.832	0.353	35.57
6	H63	Tolerant	72.10	71.24	83.64	31.27	0.813	0.402	31.57
7	H64	Tolerant	81.93	85.65	73.39	31.67	0.920	0.324	34.77
8	H65	Susceptible	53.83	50.88	31.18	31.16	0.653	0.734	21.23
9	H66	Susceptible	44.64	55.76	39.56	31.07	0.625	0.493	27.53
10	H67	Tolerant	69.21	70.52	65.55	31.56	1.380	0.386	37.47
11	H68	Tolerant	64.80	69.66	76.25	31.36	1.047	0.345	33.70
12	H69	Susceptible	48.43	60.27	40.11	31.09	0.585	0.361	24.47
13	H70	Susceptible	49.79	40.29	38.20	30.98	0.545	0.556	23.70
	Control		90.37	95.88	87.76	31.20	1.531	1.100	45.70
	CV (%)		**15.14 (12.54)**	**15.93 (11.57)**	**13.10 (9.35)**	**9.47**	**12.69**	**7.03**	**13.74**
	CD	0.05%	14.63 (10.45)	16.06 (9.95)	11.37 (7.25)	NS	0.16	0.06	6.50
		0.01%	19.78 (14.13)	21.71 (13.44)	15.37 (9.80)	NS	0.22	0.08	8.78

Table 24. Physiological parameters of G I 9.5 x G VI 55

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H71	Highly tolerant	66.40	86.01	60.08	31.65	0.916	0.450	34.47
2	H72	Susceptible	35.13	58.34	39.16	31.28	0.747	0.621	25.37
3	H73	Susceptible	42.57	60.84	38.32	31.59	0.674	0.711	27.30
4	H74	Highly tolerant	78.27	73.40	66.70	31.84	1.292	0.473	39.13
5	H75	Tolerant	68.60	72.89	52.42	31.66	0.896	0.460	33.83
6	H76	Highly tolerant	77.47	89.53	64.62	31.61	0.952	0.419	35.37
7	H77	Susceptible	54.85	47.87	26.45	31.60	0.692	0.756	23.37
8	H78	Susceptible	47.63	61.50	37.71	31.45	0.706	0.658	20.73
	Control		91.44	93.36	74.12	31.55	1.340	1.050	42.70
	CV (%)		**14.03 (11.02)**	**10.81 (8.13)**	**14.22 (9.45)**	**8.27**	**11.85**	**8.89**	**14.95**
	CD	0.05%	14.30 (9.64)	12.87 (7.98)	11.86 (7.18)	NS	0.18	0.09	7.75
		0.01%	19.70 (13.28)	17.73 (11.00)	16.34 (9.89)	NS	0.24	0.12	10.68

4.4.3. Relative water content

High relative water content (RWC) is a resistant mechanism to drought, and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall (Ritchie *et al*., 1990). Osmotic regulation helps in cell development and plant growth in water stress (Pessarakli, 1999). It is defined that decrease of relative water content close stomata and also after blocking of stomata will reduce photosynthesis rate (Cornic, 2000).

In the cross M 13.12 x G I 5.9 (Fig. 67), the highest value was observed in H13 with 70.23 per cent of the relative water content retained during the drought stress which was followed by hybrid H27 (60.41 %). Other hybrids having moderate relative water content were H20 (43.43 %), H12 (43.13 %), H11 (40.44 %), H28 (39.81 %), H10 (38.45 %), H31 (37.07 %), H25 (36.60 %), H24 (36.34 %), H26 (35.74 %), H9 (34.71 %), H2 (33.44 %), and H29 (33.31 %). Lowest value was found in hybrid H21 (20.60 %). Some other hybrids having relatively low water content were H19 (23.77 %), H23 (23.54 %), H15 (23.46 %), H8 (23.34 %) and H4 (22.54 %). The control plant was having 75.53 per cent relative content of water under non-stressed conditions (Table 19).

In the cross M 13.12 x G II 19.5 (Fig. 68), highest value was observed in H34 (80.70 %), followed by H38 (76.75 %), H33 (71.45 %), H37 (48.92 %), H42 (39.59 %) and H39 (36.39 %). Low values were observed in H41 (31.69 %), H36 (32.59 %), H40 (33.59 %) and H35 (33.47 %). The watered plant had up to 84.92 per cent of water content (Table 20).

In the cross M 13.12 x G VI 55 (Fig. 69), H46 had 81.29 per cent of retention of water followed by H43 (73.22 %) and H48 (48.48 %). Lowest value was observed in H45 (30.48 %), H44 (34.49 %), followed by H50 (35.32 %), H47 (41.35 %), H49 (43.87 %) and H51 (46.84 %) whereas the control had 87.32 per cent water content under non-stressed conditions (Table 21).

In cross G I 5.9 x M 13.12 (Fig. 70), highest value was observed in H52 (49.22 %) followed by H57 (47.76 %). Lowest values observed were H54 (25.43

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%), H53 (26.57 %), H55 (29.66 %) and H56 (30.44 %). The control was having 75.01 per cent of water content under non-stressed conditions (Table 22).

In G I 5.9 x G II 19.5 (Fig. 71), highest values observed were H63 (83.64 %), H68 (76.25 %), H62 (76.19 %), H64 (73.39 %) and H67 (65.55 %). Lowest value observed was of hybrid H58 (28.45 %), H60 (30.75 %), H65 (31.18 %), H59 (31.65 %), H70 (38.20 %), H66 (39.56 %), H69 (40.11 %) and H61 (57.60 %). The control had 87.76 per cent of the water content under non-stressed conditions (Table 23).

In cross G I 5.9 x G VI 55 (Fig. 72), highest value was observed in H74 (66.70 %), H76 (64.62 %), H71 (60.08 %) and H75 (52.42 %). Low values were observed in the susceptible hybrids H77 (26.45 %). Other hybrids having low values were H72 (39.16 %), H73 (38.32 %) and H78 (37.71 %). The control had 74.12 per cent of water content under watered condition (Table 24).

The cross G II 19.5 x M 13.12 (Fig. 73) had H85 (83.24 %) having the highest relative water content followed by H79 (82.77 %). Some other high RWC containing hybrids were H96 (82.58 %), H94 (82.29 %) and H90 (78.40 %). Lowest value was observed in H89 (56.65 %) which was a susceptible hybrid. 86.40 per cent of water content was found in the control plant which means that the unstressed plant will be having the higher relative water content as compared to hybrids which were under drought stress (Table 25).

In cross G II 19.5 x G I 5.9 (Fig. 74), H101 (81.46 %) was having the highest value followed by H104 (72.26 %), H102 (66.21 %), H97 (62.48 %) and H103 (59.55 %). Low values were observed in H100 (32.41 %). Other hybrids were H105 (50.27 %), H98 (48.08 %), H106 (41.69 %) and H99 (35.08 %). The control was having 84.95 per cent relative water content (Table 26).

In the cross G II 19.5 x G VI 55 (Fig. 75), two hybrids were there in which H107 was having 79.34 per cent of relative water content and the other hybrid H108 was having 43.63 per cent of water content. The control had 89.40 per cent relative water content under unstressed condition (Table 27).

In the cross G VI 55 x G I 5.9 (Fig. 76), high value was observed in H112 (78.41 %) followed by H111 (64.27 %) and H113 (57.58 %). Low values were observed in H109 (33.32 %), H110 (33.35 %) and H114 (44.32 %). The control had 83.64 per cent of the relative water content under non-stressed conditions (Table 28).

In cross G VI 55 x G II 19.5 (Fig. 77), high values were observed in hybrid H118 (53.48 %) followed by H117 (50.59 %), H115 (39.28 %), H119 (33.48 %) and H120 (39.54 %). Low value was observed in H116 (23.51 %) whereas the control plant had 70.73 per cent relative water content (Table 29).

Water content and water potential (Ψw) have been widely used to quantify the water deficits in leaf tissues. Leaf water content is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues. On the other hand, water potential measures the energetic status of water inside the leaf cells (Slatyer and Taylor, 1960).

Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique *et al*., 2001). When the two poplar species were submitted to progressive drought stress, the decrease of RWC in the water-stressed cuttings was 23.3 per cent in *Populus cathayana*, whereas it was 16 per cent in *Populus kangdingensis*. RWC was affected by the interaction of severity, duration of the drought event and species (Yang and Miao, 2010).

When the hybrids were subjected to drought stress, tolerant hybrids were having high RWC as compared to susceptible hybrids and the controls had high amount of RWC.

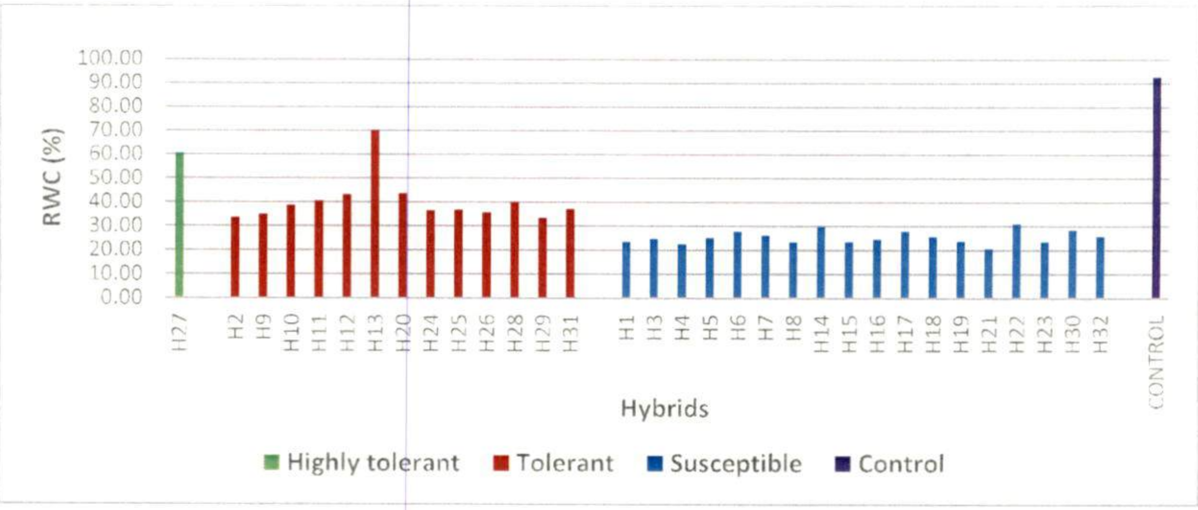

Fig. 67. RWC of M 13.12 x G I 5.9

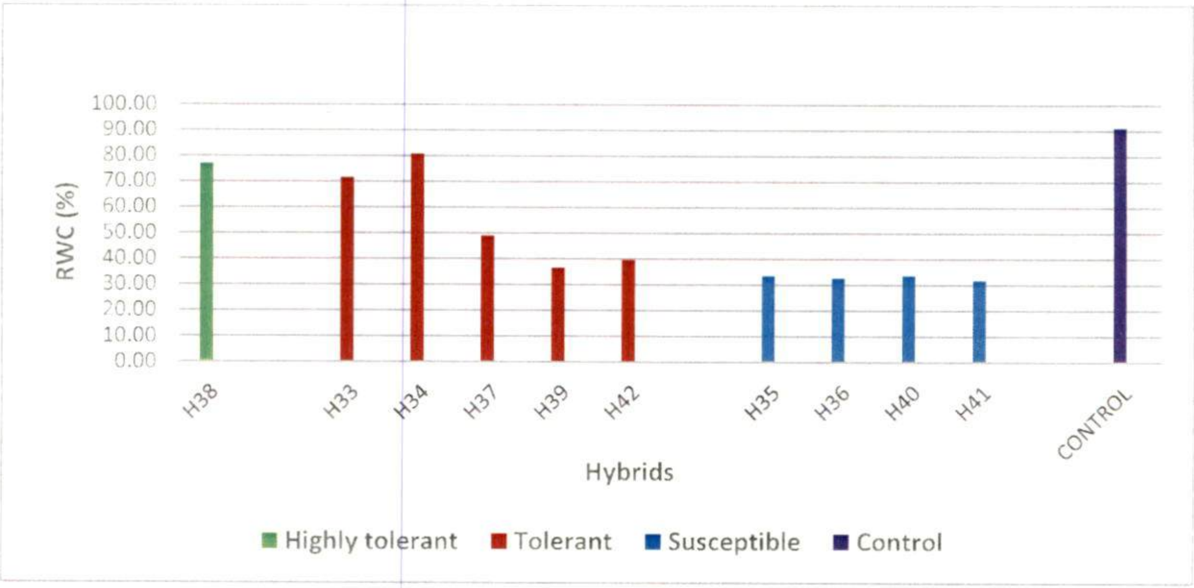

Fig. 68. RWC of M 13.12 x G II 19.5

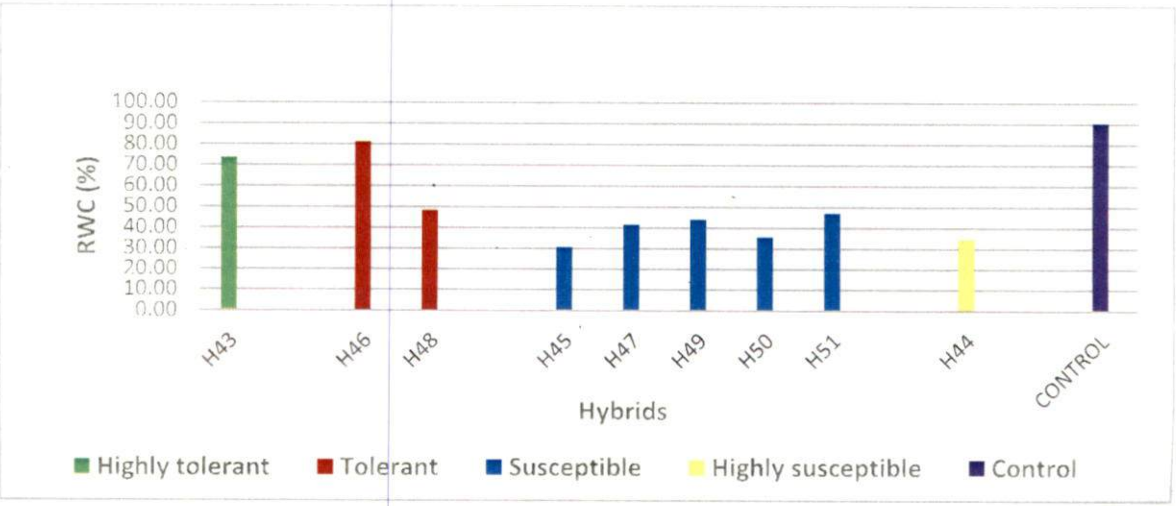

Fig. 69. RWC of M 13.12 x G VI 55

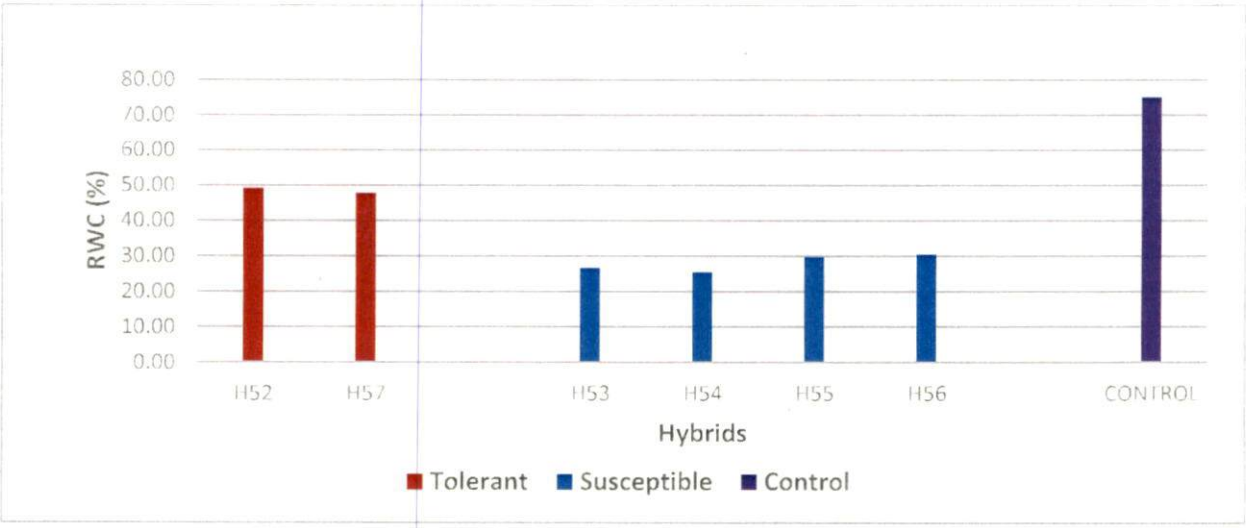

Fig. 70. RWC of G I 5.9 x M 13.12

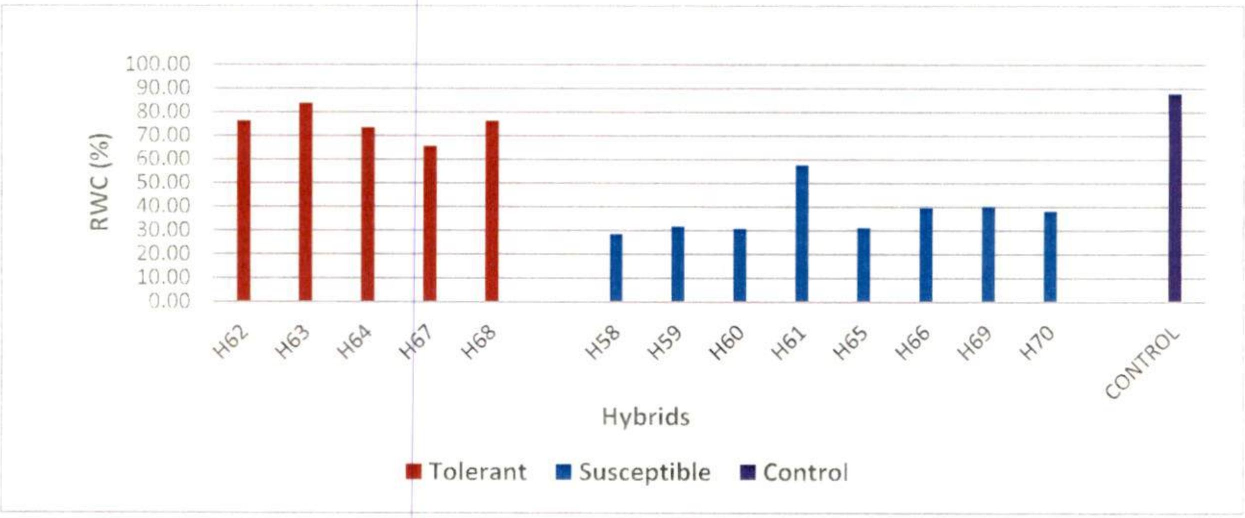

Fig. 71. RWC of G I 5.9 x G II 19.5

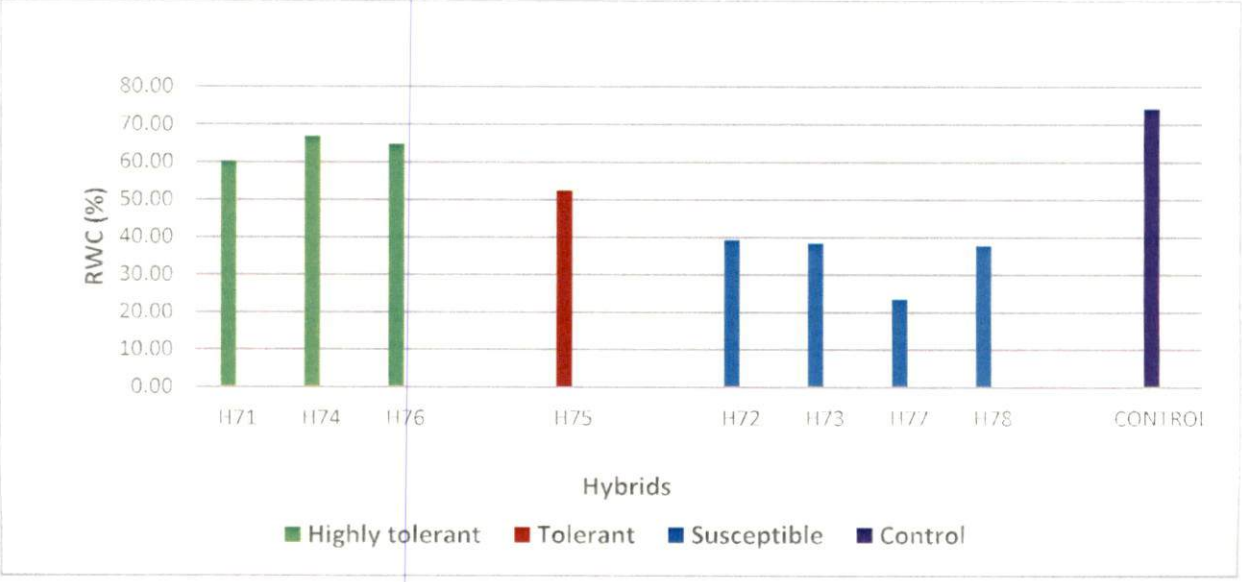

Fig. 72. RWC of G I 5.9 x G VI 55

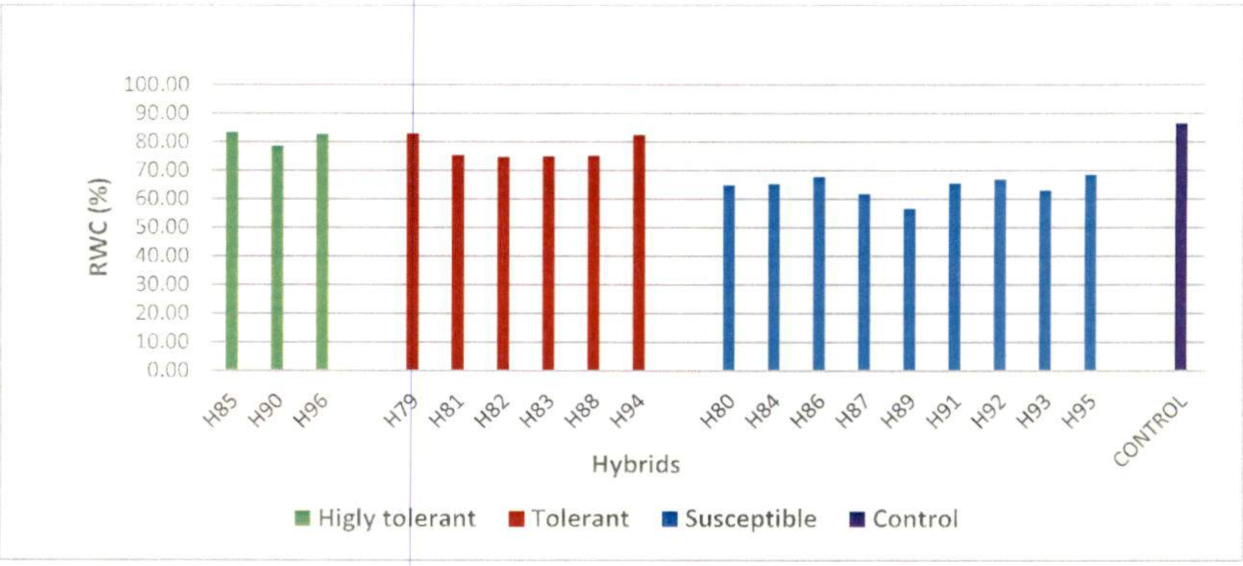

Fig. 73. RWC of G II 19.5 x M 13.12

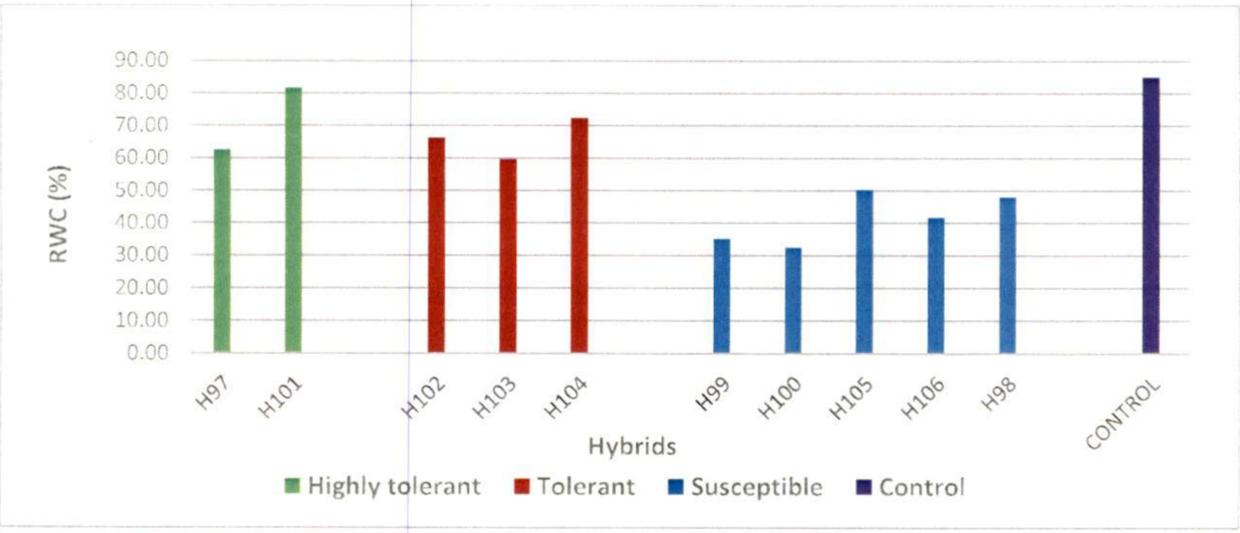

Fig. 74. RWC of G II 19.5 x G I 5.9

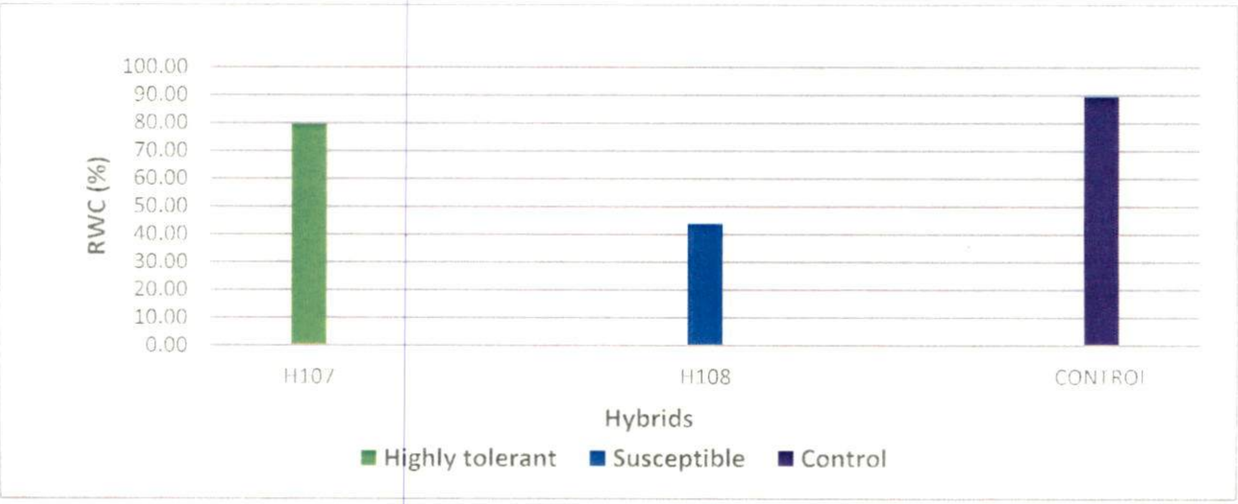

Fig. 75. RWC of G II 19.5 x G VI 55

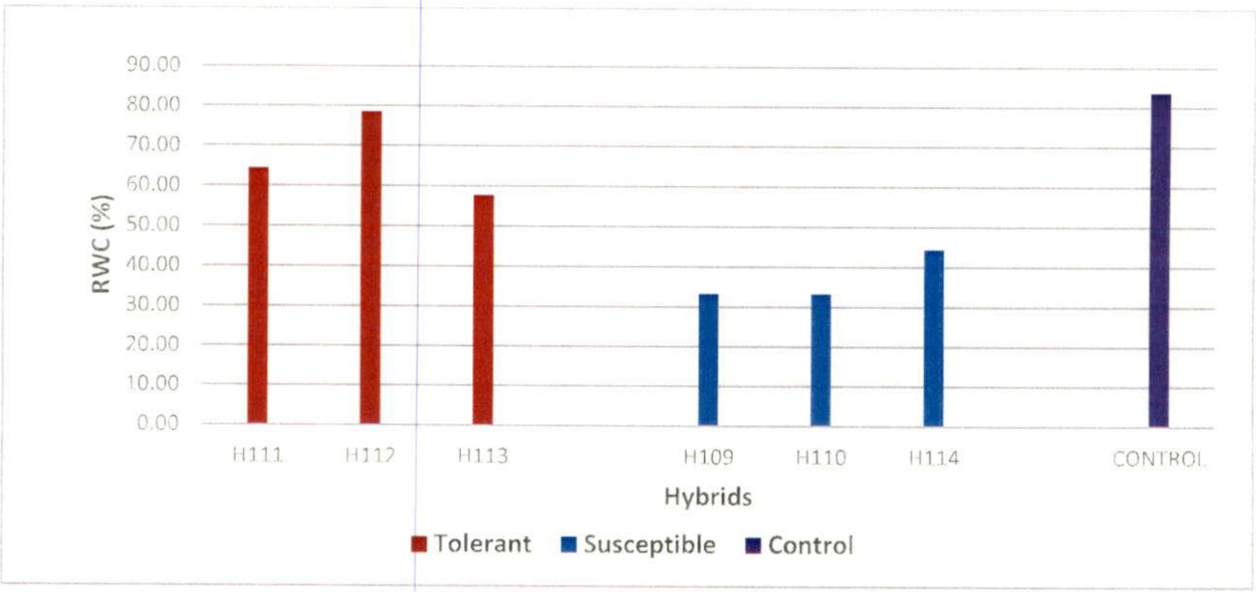

Fig. 76. RWC of G VI 55 x G I 5.9

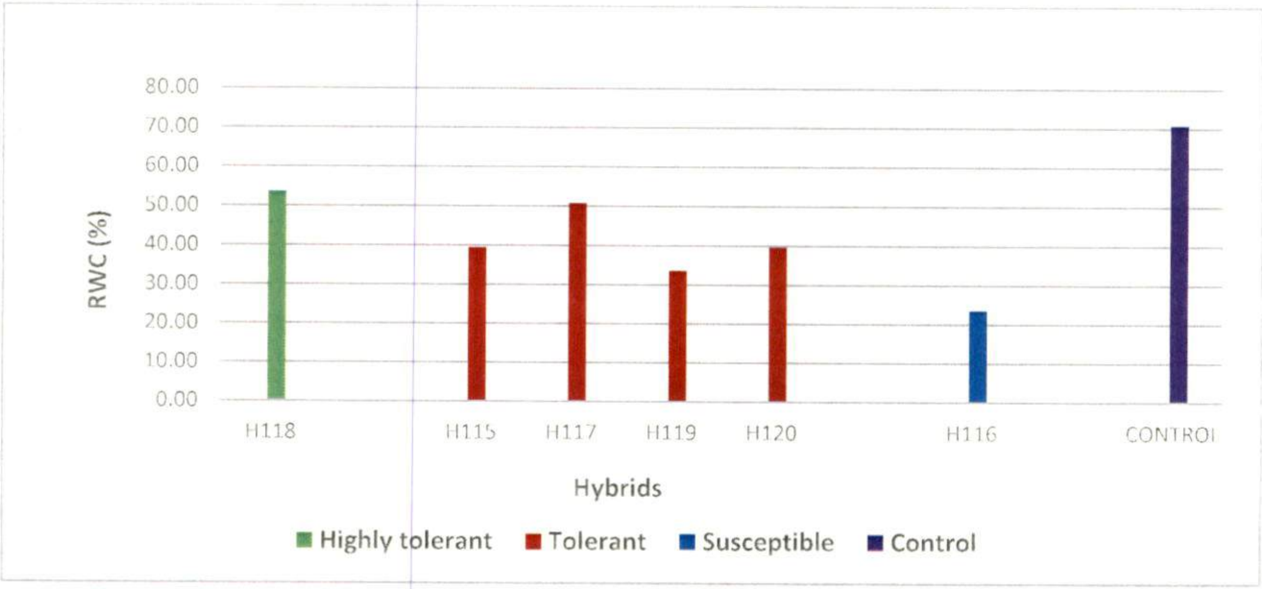

Fig. 77. RWC of G VI 55 x G II 19.5

4.4.4. Leaf temperature

Under conditions of water stress, plants are often from high temperature, which increases their vulnerability to light stress and photo inhibition (Carpentier, 1996). Plants have several mechanisms for avoiding and/or dissipating the excess excitation energy non-destructively. The results are represented in graphs (Fig. 78-88) and tables 19-29.

Temperature was almost similar in almost all hybrids. In the cross M 13.12 x G I 5.9, highest value was observed in H26 (37.46°C) and the other hybrids were between the range of 33-38°C. Lowest value was found in the susceptible hybrid H8 (30.55°C) and all the other hybrids were in the range between 30-38°C. The control plant was having the leaf temperature of about 36.40°C under non-stressed conditions (Table 19).

In the cross M 13.12 x G II 19.5, highest value was observed in H33 (31.74°C) and the lowest value by hybrid H41 (30.53°C). The control was having 31.25°C leaf temperature under non-stressed condition (Table 20).

In cross M 13.12 x G VI 55, highest value was observed in hybrid H43 (32.31°C) followed by H46 (31.03°C) and H48 (31.01°C). Lowest value was observed in hybrid H47 (30.45°C). The control plant had 30.75°C leaf temperature under non-stressed condition (Table 21).

In cross G I 5.9 x M 13.12, highest value was observed in hybrid H52 (33.85°C) followed by H57 (30.74°C). The control had 30.55°C leaf temperature under non-stressed condition. Lowest value was observed in hybrid H54 (30.20°C) (Table 22).

In cross G I 5.9 x G II 19.5, highest value was observed in hybrid H62 (31.88°C) and the values of other hybrids ranged between 30-32°C, the lowest being in H58 (30.77°C). The control had a temperature of 31.20°C (Table 23).

In cross G I 5.9 x G VI 55, the highest value was observed in hybrid H74 (31.84°C) and the lowest value observed was in hybrid H72 (31.28°C) and all other hybrids were in the range from 31-32°C but the control had a value of 31.55°C (Table 24).

In cross G II 19.5 x M 13.12, highest temperature was found in hybrid H81 (34.07°C) and the lowest value was found in hybrid H86 (33.40°C) whereas the control had 33.60°C under non-stressed conditions (Table 25).

In cross G II 19.5 x G I 5.9, the highest value was found in hybrid H102 (33.92°C) and the lowest temperature was recorded for hybrid H98 (30.28°C). All other hybrids expressed a value between 30-34°C. The control had 31.23°C leaf temperature under well irrigated condition. However, there was no significant difference (Table 26).

In the cross G II 19.5 x G VI 55, H107 had leaf temperature of about 32.79°C and H108 had 31.77°C temperature whereas the control had 32.65°C leaf temperature indicating the increase in temperature with increase in stress condition. However, there was no significant difference (Table 27).

In cross G VI 55 x G I 5.9, H112 had the highest temperature of 31.43°C while H114 had the lowest value of 30.51°C. The control had 31.14°C of leaf temperature under non-stressed condition (Table 28).

In cross G VI 55 x G II 19.5, H119 had the highest value of 33.82°C and the hybrid H116 (30.54°C) had the lowest value among other hybrids and the control plant was having the temperature of 30.60°C (Table 29).

The hybrids when subjected to drought conditions were having relatively same temperature range in case of tolerant hybrids and susceptible hybrids and the control plants were having a temperature moderate than the two classes. As they were having the same range of temperature in this experiment, it cannot be used as a reliable parameter to measure drought tolerance, although other studies revealed that drought stressed plants showed higher canopy temperature than non-stressed plants. Drought stressed plants displayed higher canopy temperatures than well-watered plants (Siddique *et al.*, 2000). From this, it can be concluded that cocoa was efficient in regulating the water stress as it regulated the temperature of the canopy even after imposing drought stress.

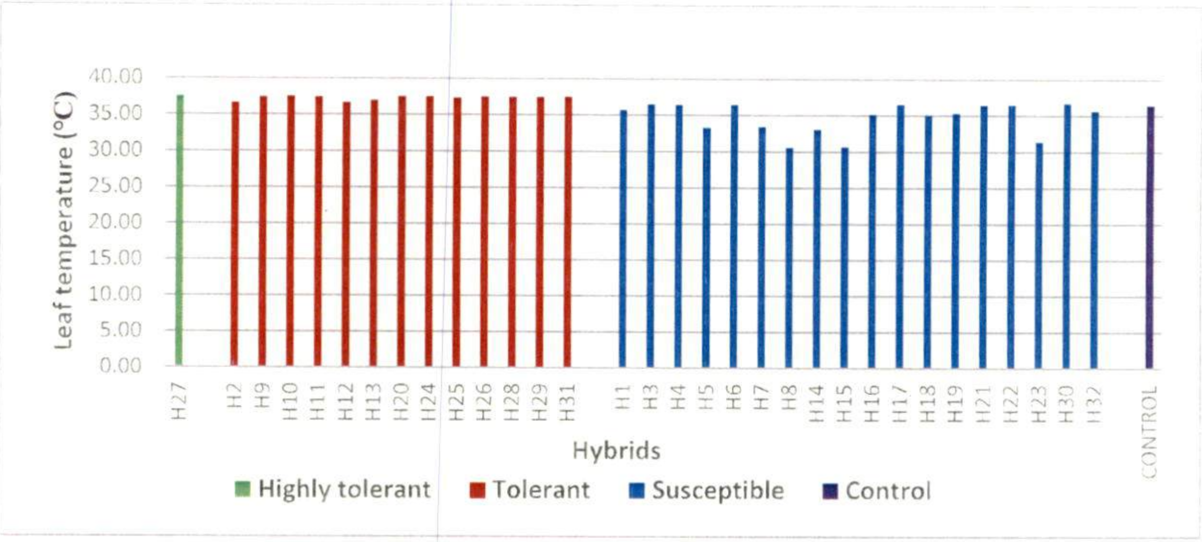

Fig. 78. Leaf temperature of M 13.12 x G I 5.9

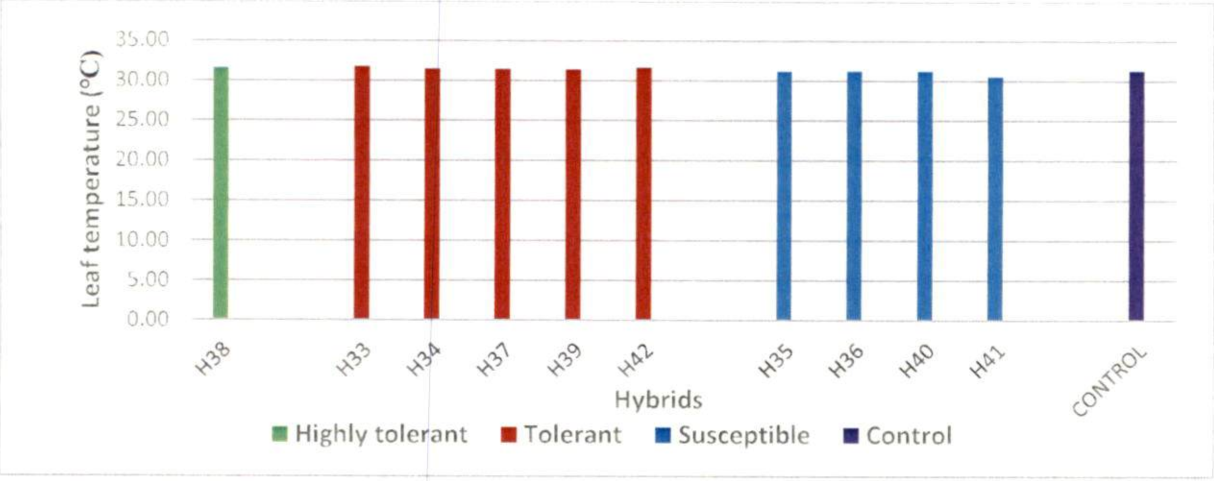

Fig. 79. Leaf temperature of M 13.12 x G II 19.5

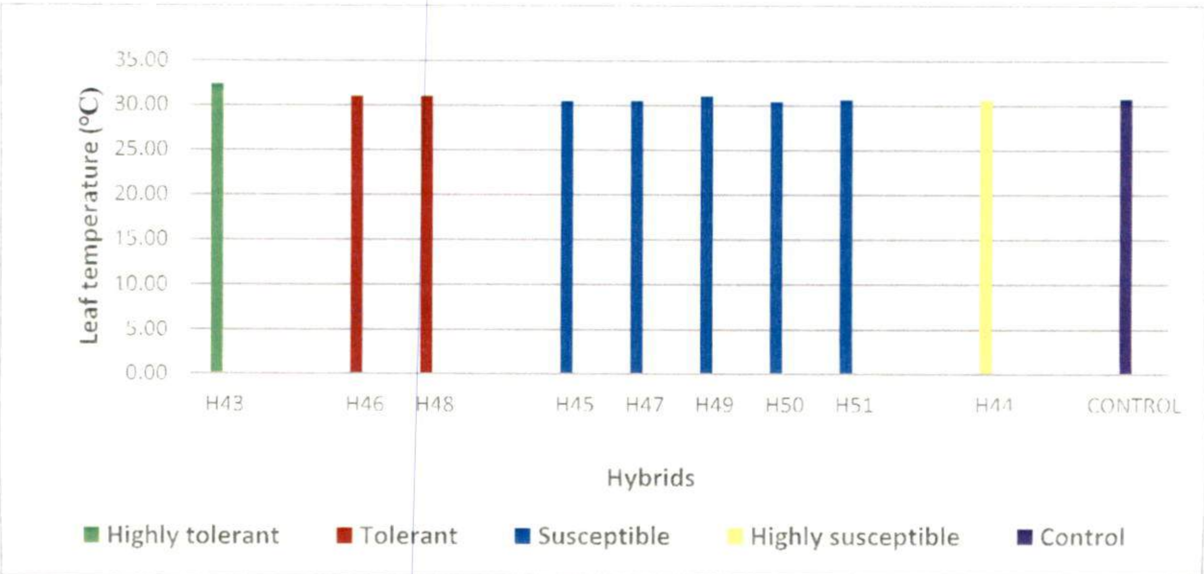

Fig. 80. Leaf temperature of M 13.12 x G VI 55

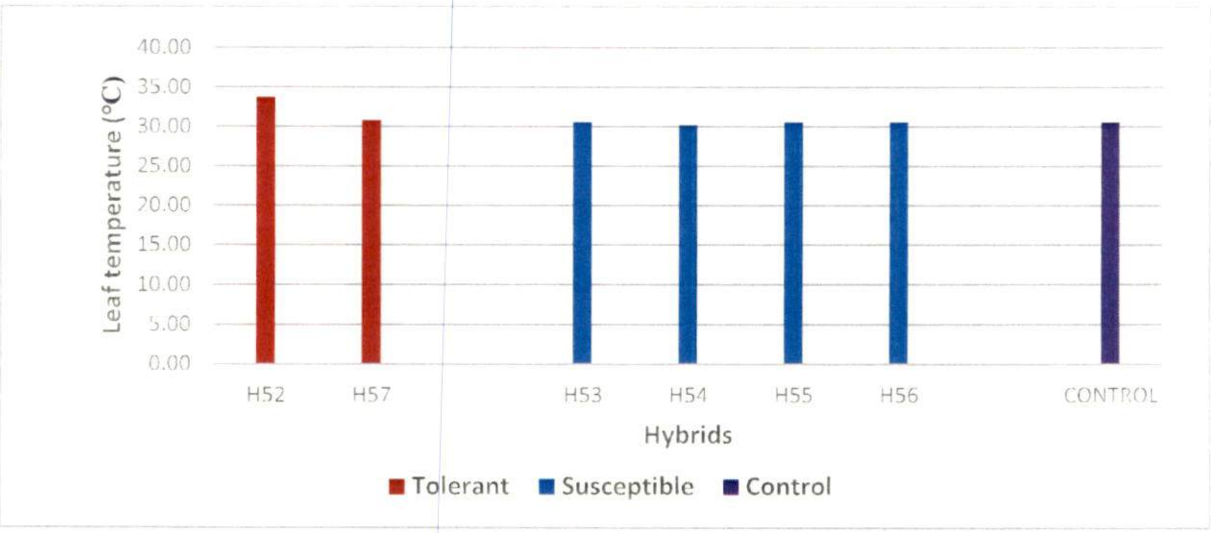

Fig. 81. Leaf temperature of G I 5.9 x M 13.12

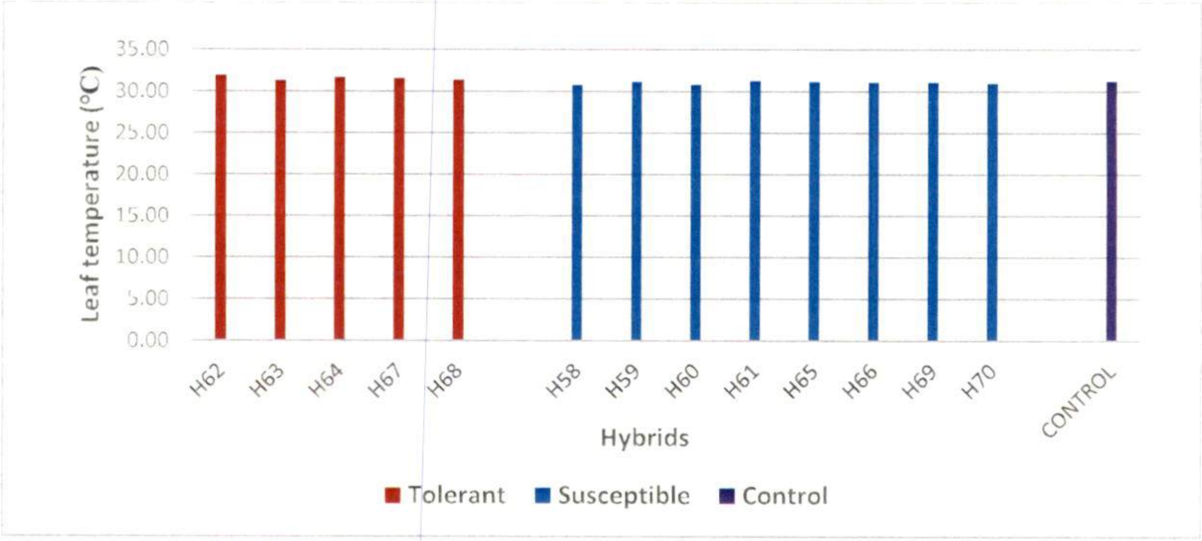

Fig. 82. Leaf temperature of G I 5.9 x G II 19.5

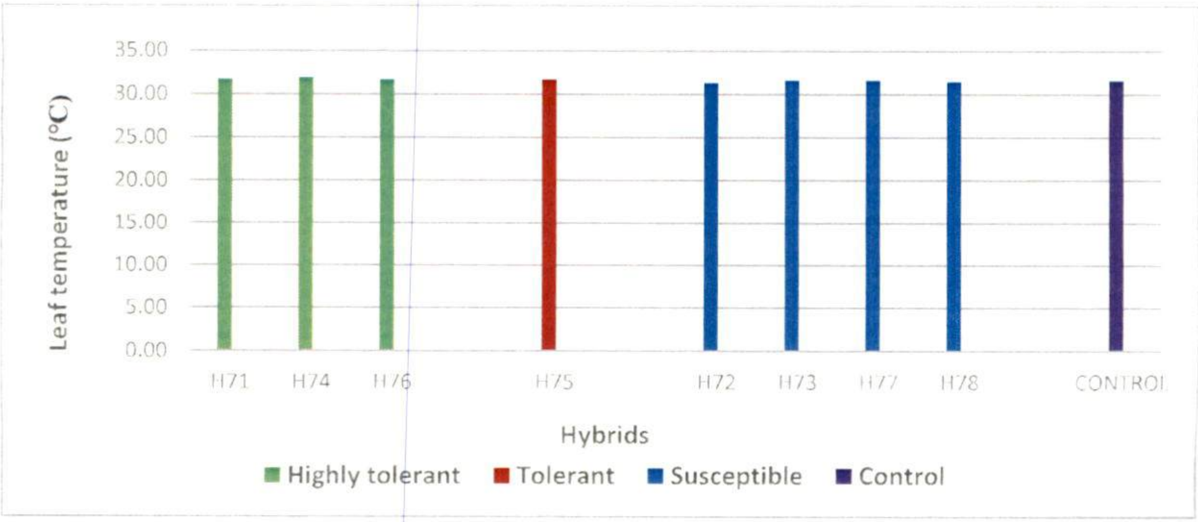

Fig. 83. Leaf temperature of G I 5.9 x G VI 55

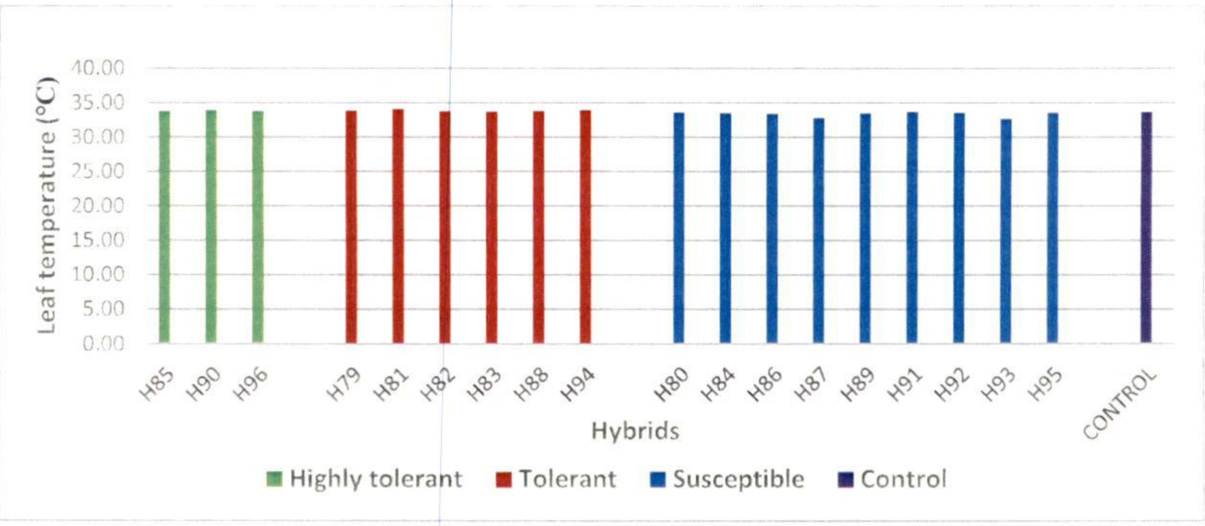

Fig. 84. Leaf temperature of G II 19.5 x M 13.12

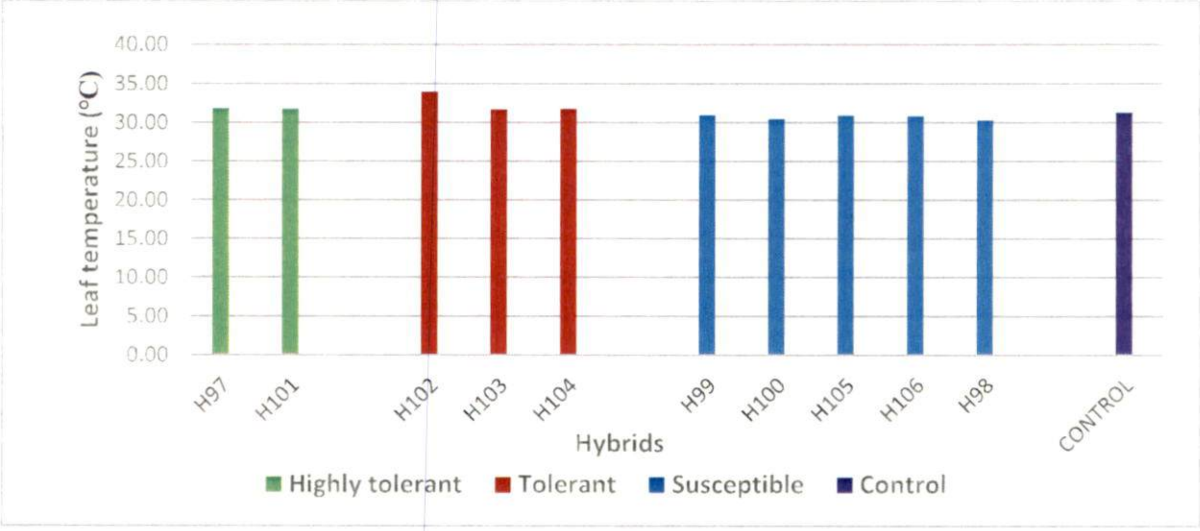

Fig. 85. Leaf temperature of G II 19.5 x G I 5.9

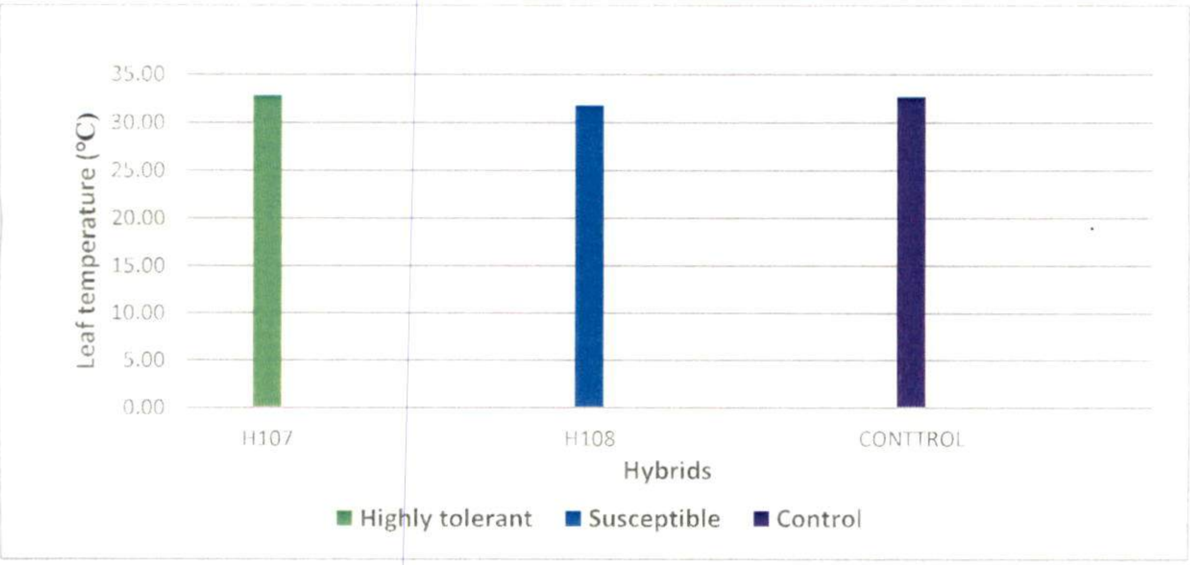

Fig. 86. Leaf temperature of G II 19.5 x G VI 55

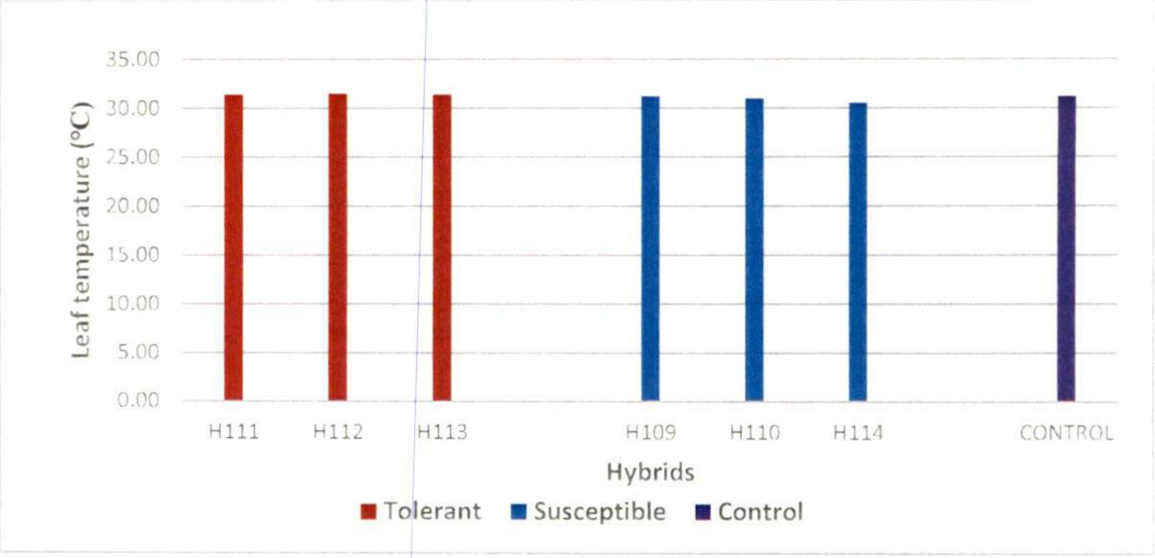

Fig. 87. Leaf temperature of G VI 55 x G I 5.9

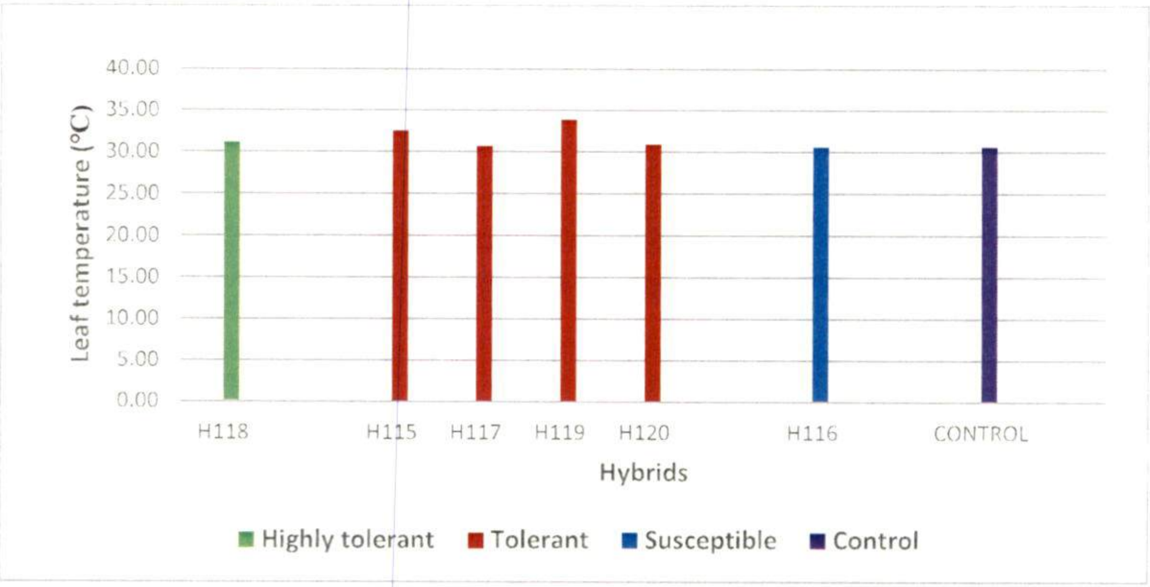

Fig. 88. Leaf temperature of G VI 55 x G II 19.5

Table 25. Physiological parameters of G II 19.5 x M 13.12

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H79	Tolerant	67.27	84.32	82.77	33.76	0.769	0.466	35.70
2	H80	Susceptible	48.53	59.86	64.77	33.57	0.598	0.731	20.17
3	H81	Tolerant	87.31	79.41	75.19	34.07	0.767	0.403	37.93
4	H82	Tolerant	66.94	72.99	74.57	33.67	0.902	0.505	40.17
5	H83	Tolerant	67.51	77.61	74.85	33.63	0.942	0.347	36.13
6	H84	Susceptible	44.44	46.10	65.20	33.50	0.623	0.611	23.70
7	H85	Highly tolerant	74.55	82.33	83.24	33.75	0.890	0.470	38.10
8	H86	Susceptible	54.57	50.73	67.71	33.40	0.644	0.685	29.10
9	H87	Susceptible	58.24	58.52	61.75	32.82	0.665	0.919	23.63
10	H88	Tolerant	85.23	86.54	74.97	33.74	0.729	0.481	34.40
11	H89	Susceptible	37.10	46.85	56.65	33.43	0.567	0.725	32.30
12	H90	Highly tolerant	80.17	78.65	78.40	33.89	1.053	0.482	34.83
13	H91	Susceptible	57.35	61.07	65.49	33.66	0.646	0.646	32.60
14	H92	Susceptible	60.67	62.94	66.78	33.53	0.548	0.970	32.10
15	H93	Susceptible	38.52	62.82	63.00	32.66	0.603	0.810	31.27
16	H94	Tolerant	65.68	78.40	82.29	33.89	0.785	0.378	41.57
17	H95	Susceptible	49.98	58.43	68.28	33.53	0.538	0.705	31.67
18	H96	Highly tolerant	68.23	76.64	82.58	33.73	1.005	0.371	36.87
	Control		96.58	93.50	86.40	33.60	1.182	1.105	42.60
	CV (%)		**13.12 (9.77)**	**13.71 (11.33)**	**11.17 (9.17)**	**10.50**	**7.97**	**7.27**	**12.06**

	CD	0.05%	13.42 (8.46)	15.44 (10.56)	13.24 (8.84)	NS	0.10	0.07	6.57
		0.01%	17.99 (1.35)	20.71 (14.15)	17.75 (11.85)	NS	0.13	0.10	8.81

Table 26. Physiological parameters of G II 19.5 x G I 5.9

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H97	Highly tolerant	88.00	69.88	62.48	31.77	1.098	0.427	44.73
2	H98	Susceptible	59.43	40.45	48.08	30.28	0.632	0.789	25.60
3	H99	Susceptible	56.83	41.64	35.08	30.95	0.644	0.987	24.27
4	H100	Susceptible	53.51	49.45	32.41	30.46	0.537	0.969	24.60
5	H101	Highly tolerant	79.05	64.62	81.46	31.70	1.168	0.507	36.57
6	H102	Tolerant	72.63	71.30	66.21	33.92	0.844	0.353	37.57
7	H103	Tolerant	71.20	71.31	59.55	31.66	0.852	0.369	32.63
8	H104	Tolerant	82.33	67.94	72.26	31.74	1.326	0.544	44.53
9	H105	Susceptible	52.20	52.97	50.27	30.91	0.701	0.816	21.47
10	H106	Susceptible	48.12	43.29	41.69	30.84	0.658	0.711	29.33
	Control		95.83	92.64	84.95	31.23	1.570	1.390	45.10
	CV (%)		**12.66 (10.16)**	**13.30 (9.49)**	**13.94 (10.01)**	**8.14**	**9.08**	**8.62**	**8.71**
	CD	0.05%	14.31 (9.55)	12.98 (7.98)	13.05 (8.20)	NS	0.13	0.10	4.77
		0.01%	19.51 (13.03)	17.70 (10.88)	17.79 (11.18)	NS	0.18	0.13	6.50

4.4.5. Photosynthesis

Cacao is native to the Amazon rainforest, where it grows in the understorey of larger trees (Toxopeus, 1985). As such, the photosynthetic characteristics of cacao are those of a shade-adapted species with a low light compensation point and leaf photosynthesis that is usually light saturated at low irradiance, typically around 20 per cent of full sunlight (Raja-Harun and Hardwick, 1988 and Mielke *et al.*, 2005). The photosynthetic rate of hybrids are depicted in graphs (Fig. 89-99) and Tables19-29 listed below.

In the cross M 13.12 x G I 5.9 (Fig. 89), the highest value was observed in hybrid H20 (1.877 µmol CO_2 m^{-2} s^{-1}) followed by H28 (1.830 µmol CO_2 m^{-2} s^{-1}), H25 (1.753 µmol CO_2 m^{-2} s^{-1}), H26 (1.730 µmol CO_2 m^{-2} s^{-1}), H11 (1.643 µmol CO_2 m^{-2} s^{-1}), H27 (1.627 µmol CO_2 m^{-2} s^{-1}), H24 (1.617 µmol CO_2 m^{-2} s^{-1}), H29 (1.519 µmol CO_2 m^{-2} s^{-1}), H2 (1.507 µmol CO_2 m^{-2} s^{-1}), H10 (1.334 µmol CO_2 m^{-2} s^{-1}), H12 (1.182 µmol CO_2 m^{-2} s^{-1}), H9 (1.105 µmol CO_2 m^{-2} s^{-1}), H13 (1.089 µmol CO_2 m^{-2} s^{-1}) and H31 (1.074 µmol CO_2 m^{-2} s^{-1}). Lowest value was observed in hybrid H14 (0.527 µmol CO_2 m^{-2} s^{-1}) followed by H8 (0.572 µmol CO_2 m^{-2} s^{-1}). Some other hybrids having low photosynthetic rate were H15 (0.647 µmol CO_2 m^{-2} s^{-1}) and H23 (0.635 µmol CO_2 m^{-2} s^{-1}). The control had 2.002 µmol CO_2 m^{-2} s^{-1} photosynthetic rate (Table 19).

In cross M 13.12 x G II 19.5 (Fig. 90), the highest value was observed in hybrid H38 (1.014 µmol CO_2 m^{-2} s^{-1}) followed by H33 (0.968 µmol CO_2 m^{-2} s^{-1}), H37 (0.962 µmol CO_2 m^{-2} s^{-1}), H34 (0.855 µmol CO_2 m^{-1} s^{-1}), H42 (0.845 µmol CO_2 m^{-2} s^{-1}) and H39 (0.793 µmol CO_2 m^{-2} s^{-1}). Lowest values was observed in H40 (0.566 µmol CO_2 m^{-2} s^{-1}) followed by H41 (0.648 µmol CO_2 m^{-2} s^{-1}), H35 (0.697 µmol CO_2 m^{-2} s^{-1}) and H36 (0.706 µmol CO_2 m^{-2} s^{-1}). The control plant had a photosynthetic rate of 1.134 µmol CO_2 m^{-2} s^{-1} which was under non-stressed condition (Table 20).

In cross M 13.12 x G VI 55 (Fig. 91), the highest photosynthetic rate was found in hybrid H43 (0.779 µmol CO_2 m^{-2} s^{-1}), H48 (0.772 µmol CO_2 m^{-2} s^{-1}) and H46 (0.737 µmol CO_2 m^{-2} s^{-1}). Low values were observed in H47 (0.676 µmol

CO_2 m^{-2} s^{-1}), H45 (0.643 μmol CO_2 m^{-2} s^{-1}), H51 (0.607 μmol CO_2 m^{-2} s^{-1}), H49 (0.572 μmol CO_2 m^{-2} s^{-1}), H50 (0.536 μmol CO_2 m^{-2} s^{-1}) and H44 (0.514 μmol CO_2 m^{-2} s^{-1}). 0.909 μmol CO_2 m^{-2} s^{-1} was the photosynthetic rate found in the control plant under well irrigated conditions (Table 21).

In the cross G I 5.9 x M 13.12 (Fig. 92), high photosynthetic rate was observed in H57 (1.283 μmol CO_2 m^{-2} s^{-1}) followed by H52 (0.916 μmol CO_2 m^{-2} s^{-1}). Low values observed were H55 (0.803 μmol CO_2 m^{-2} s^{-1}), H54 (0.771 μmol CO_2 m^{-2} s^{-1}), H56 (0.740 μmol CO_2 m^{-2} s^{-1}) and H53 (0.670 μmol CO_2 m^{-2} s^{-1}) whereas the control was having 1.430 μmol CO_2 m^{-2} s^{-1} photosynthetic rate (Table 22).

In cross G I 5.9 x G II 19.5 (Fig. 93), highest value was observed in H67 (1.380 μmol CO_2 m^{-2} s^{-1}) followed by H68 (1.047 μmol CO_2 m^{-2} s^{-1}). Other hybrids having high photosynthetic rates were H64 (0.920 μmol CO_2 m^{-2} s^{-1}), H62 (0.832 μmol CO_2 m^{-2} s^{-1}) and H63 (0.813 μmol CO_2 m^{-2} s^{-1}). Hybrid having the lowest value was H70 (0.545 μmol CO_2 m^{-2} s^{-1}) followed by H61 (0.572 μmol CO_2 m^{-2} s^{-1}). The control plant under non-stressed condition was having 1.531 μmol CO_2 m^{-2} s^{-1} photosynthetic rate (Table 23).

In the cross G I 5.9 x G VI 55 (Fig. 94), H74 had the highest photosynthetic rate of about 1.292 μmol CO_2 m^{-2} s^{-1} followed by H76 (0.952 μmol CO_2 m^{-2} s^{-1}), H71 (0.916 μmol CO_2 m^{-2} s^{-1}) and H75 (0.896 μmol CO_2 m^{-2} s^{-1}). Low values observed were in H72 (0.747 μmol CO_2 m^{-2} s^{-1}), H78 (0.706 μmol CO_2 m^{-2} s^{-1}), H73 (0.674 μmol CO_2 m^{-2} s^{-1}) and H77 (0.692 μmol CO_2 m^{-2} s^{-1}) whereas the control was having 1.340 μmol CO_2 m^{-2} s^{-1} amount of photosynthetic rate under non-stressed condition (Table 24).

In the cross G II 19.5 x M 13.12 (Fig. 95), highest value was observed in hybrid H90 (1.053 μmol CO_2 m^{-2} s^{-1}). Some hybrids having high photosynthetic values were H96 (1.005 μmol CO_2 m^{-2} s^{-1}), H83 (0.942 μmol CO_2 m^{-2} s^{-1}), H82 (0.902 μmol CO_2 m^{-2} s^{-1}) and H85 (0.890 μmol CO_2 m^{-2} s^{-1}). Hybrid having the lowest photosynthetic value was H95 (0.538 μmol CO_2 m^{-2} s^{-1}). The control

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exhibited 1.182 μmol CO_2 m^{-2} s^{-1} of photosynthetic rate under non-stressed conditions (Table 25).

In the cross G II 19.5 x G I 5.9 (Fig. 96), the highest value was observed in H104 (1.326 μmol CO_2 m^{-2} s^{-1}), followed by H101 (1.168 μmol CO_2 m^{-2} s^{-1}), H97 (1.098 μmol CO_2 m^{-2} s^{-1}), H103 (0.852 μmol CO_2 m^{-2} s^{-1}) and H102 (0.844 μmol CO_2 m^{-2} s^{-1}). Lowest value was observed in H100 (0.537 μmol CO_2 m^{-2} s^{-1}) followed by H98 (0.632 μmol CO_2 m^{-2} s^{-1}), H106 (0.658 μmol CO_2 m^{-2} s^{-1}), H99 (0.644 μmol CO_2 m^{-2} s^{-1}) and H105 (0.701 μmol CO_2 m^{-2} s^{-1}). The control was having 1.570 μmol CO_2 m^{-2} s^{-1} of photosynthetic rate (Table 26).

In the cross G II 19.5 x G VI 55 (Fig. 97), highest value was observed in H107 (0.971 μmol CO_2 m^{-2} s^{-1}) and the lowest value was observed in H108 (0.721 μmol CO_2 m^{-2} s^{-1}) whereas the control was having 1.150 μmol CO_2 m^{-2} s^{-1} photosynthetic rate under non-stressed condition (Table 27).

The cross G VI 55 x G I 5.9 (Fig. 98) had H113 which was having the highest photosynthetic rate of 0.977 μmol CO_2 m^{-2} s^{-1} followed by H111 (0.833 μmol CO_2 m^{-2} s^{-1}) and H112 (0.802 μmol CO_2 m^{-2} s^{-1}). Lowest value in the cross was of H109 (0.572 μmol CO_2 m^{-2} s^{-1}) followed by H114 (0.596 μmol CO_2 m^{-2} s^{-1}) and H110 (0.647 μmol CO_2 m^{-2} s^{-1}) and the control was having 1.084 μmol CO_2 m^{-2} s^{-1} photosynthetic rate under non-stressed condition (Table 28).

In the cross G VI 55 x G II 19.5 (Fig. 99), the highest value was observed in hybrid H119 (0.970 μmol CO_2 m^{-2} s^{-1}) and the lowest value was found in hybrid H116 (0.583 μmol CO_2 m^{-2} s^{-1}). The control had 1.061 μmol CO_2 m^{-2} s^{-1} photosynthetic rate under non-stressed conditions (Table 29).

Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (Iturbe *et al.*, 1998). Drought prevents the entering of CO_2 in leaves, influence the absorption of CO_2 by the carboxylation centre and result in the decrease of net photosynthetic rate (P_n) (Zhang, 1999). Certainly under mild or moderate drought stress stomatal closure (causing reduced

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leaf internal CO_2 concentration (Ci)) is the major reason for reduced rates of leaf photosynthetic (Cornic, 2000; Flexas *et al*., 2004).

Reduced CO_2 diffusion from the atmosphere to the site of carboxylation is the main cause for decreased photosynthesis under most water-stress conditions (Centritto *et al*., 2003; Flexas *et al*., 2004; Grassi and Magnani, 2005; Chaves *et al*., 2008; Erismann *et al*., 2008; Peeva and Cornic, 2009). Studies indicated that drought is an important factor responsible for inhibited growth of plants and reduced photosynthesis (Efeoglu *et al*., 2009).

The tolerant hybrids were found to have high photosynthetic activity as compared to the susceptible hybrids. However, the control expressed comparatively a higher rate of photosynthesis when compared to tolerant and susceptible group of hybrids.

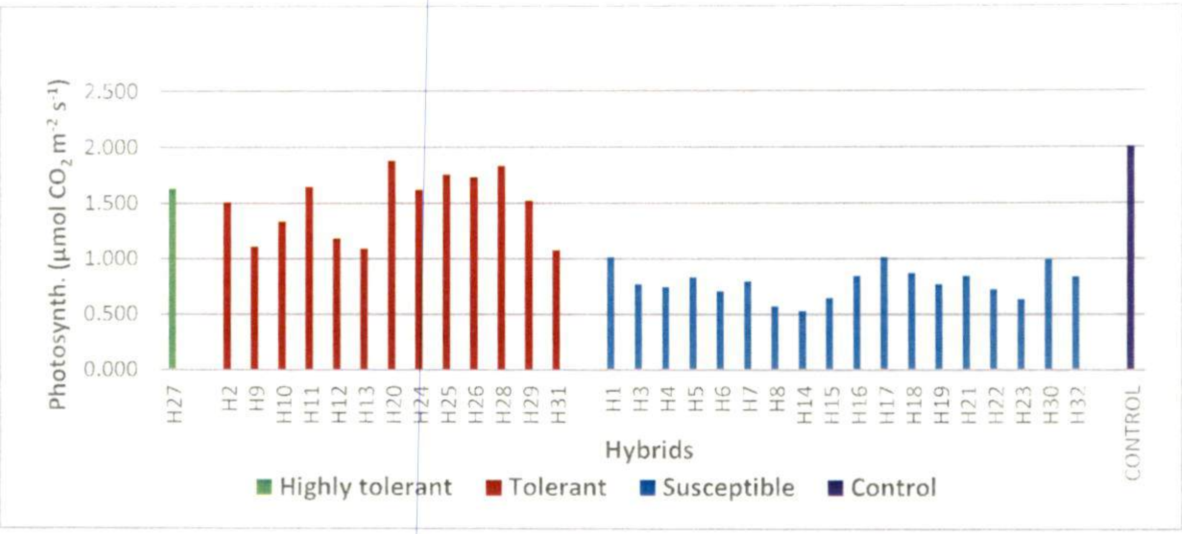

Fig. 89. Photosynthesis of M 13.12 x G I 5.9

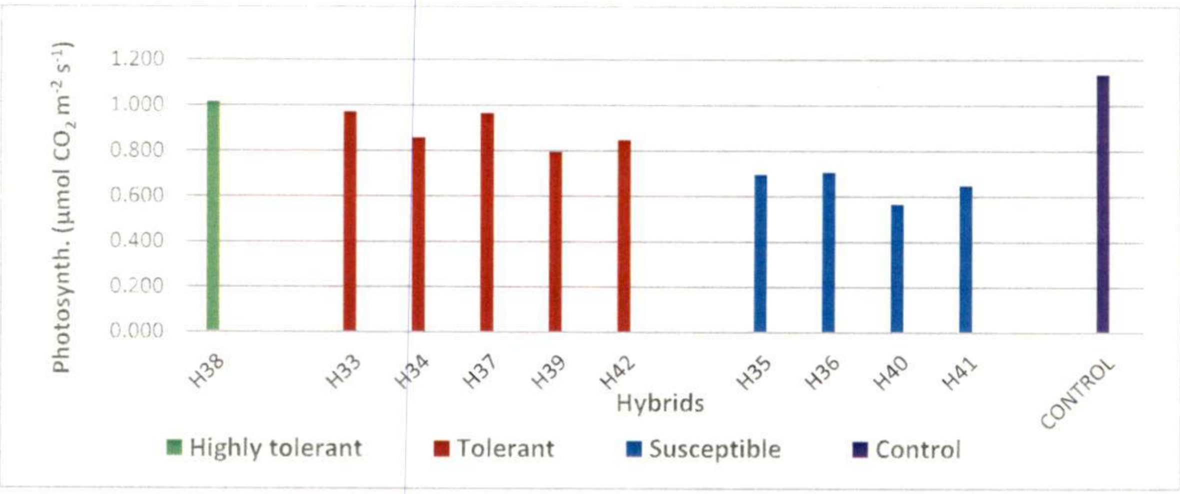

Fig. 90. Photosynthesis of M 13.12 x G II 19.5

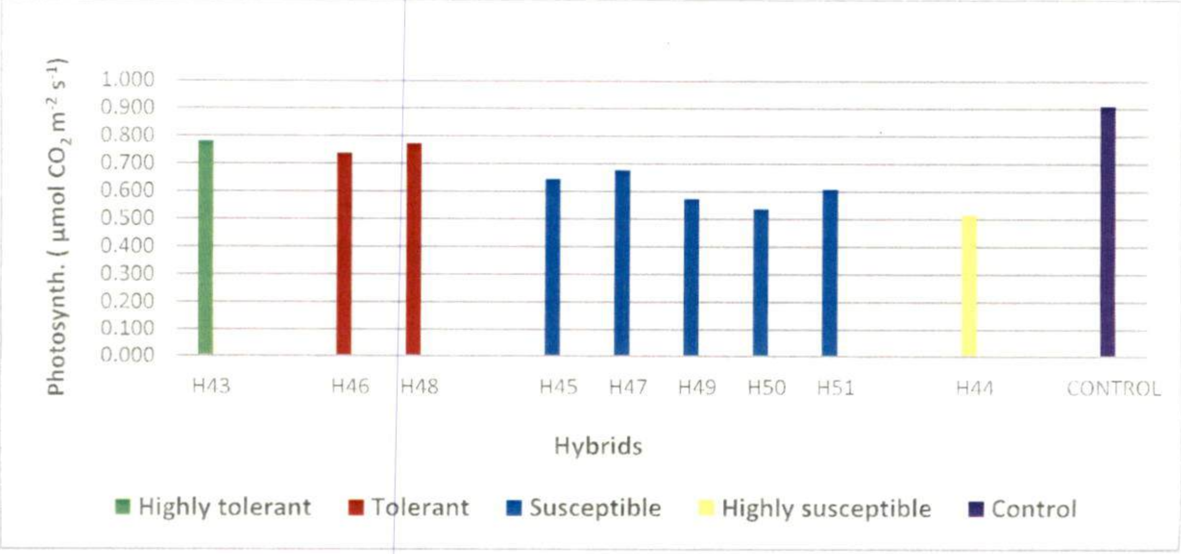

Fig. 91. Photosynthesis of M 13.12 x G VI 55

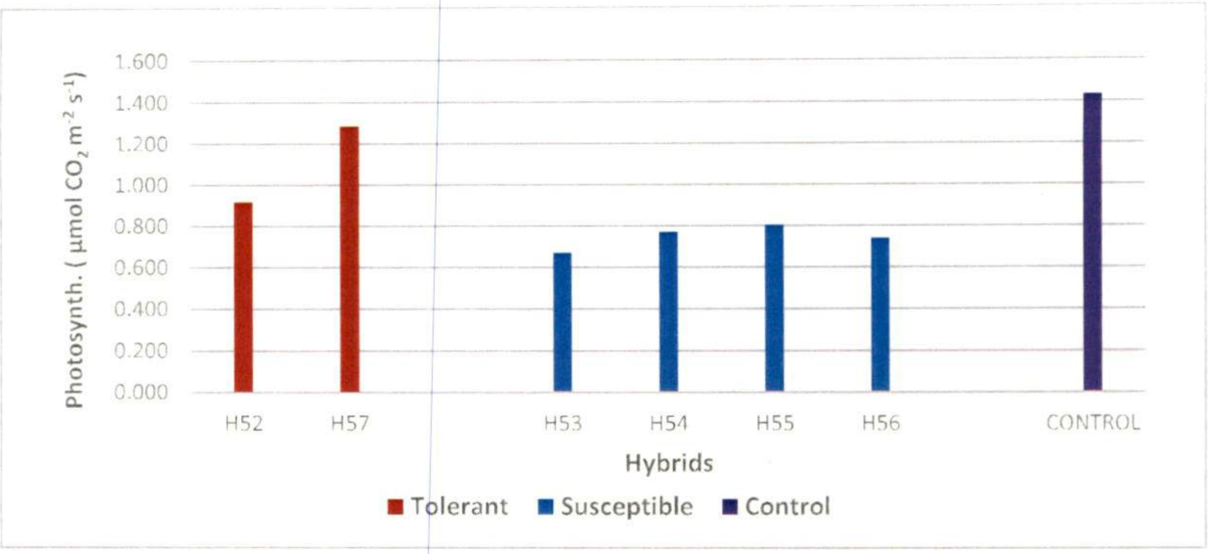

Fig. 92. Photosynthesis of G I 5.9 x M 13.12

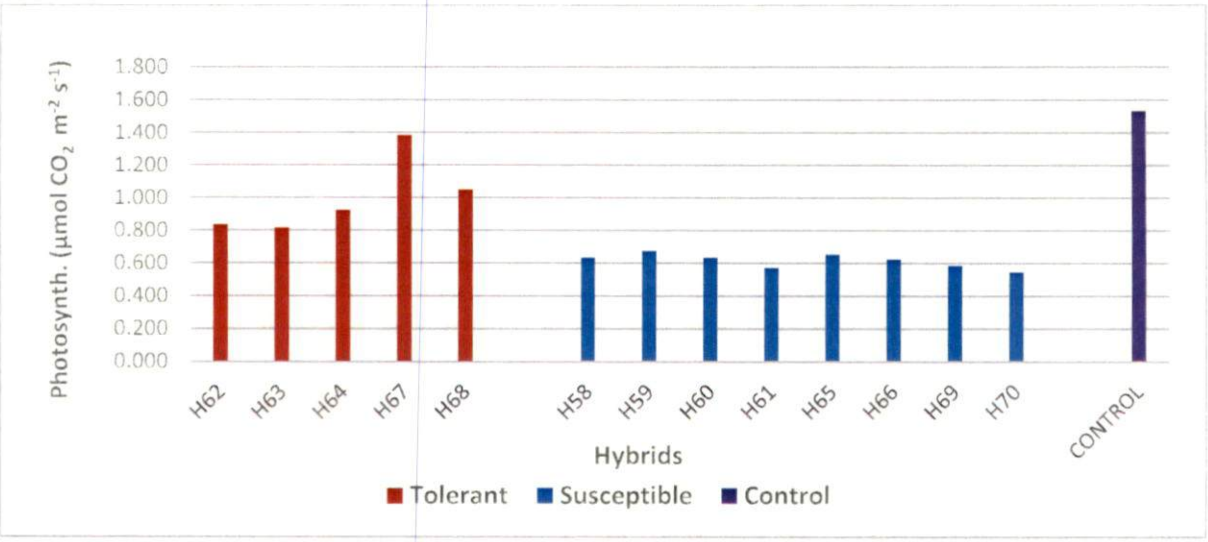

Fig. 93. Photosynthesis of G I 5.9 x G II 19.5

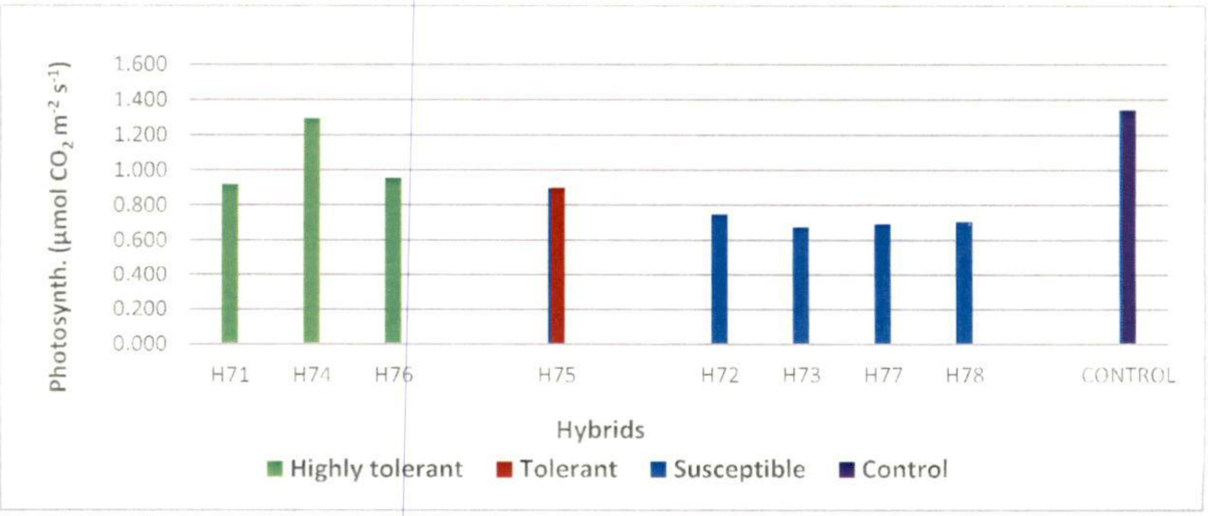

Fig. 94. Photosynthesis of G I 5.9 x G VI 55

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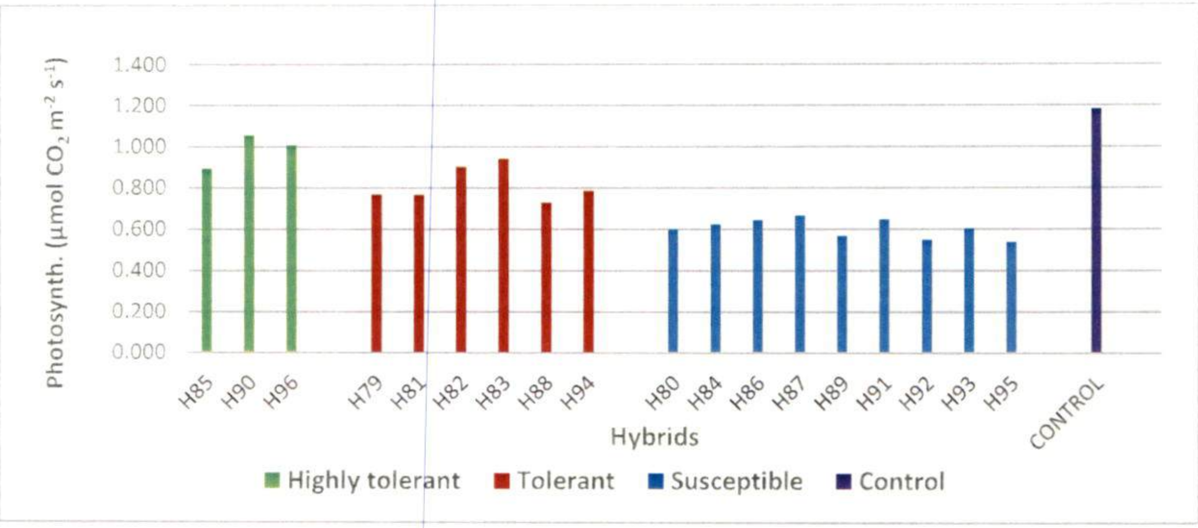

Fig. 95. Photosynthesis of G II 19.5 x M 13.12

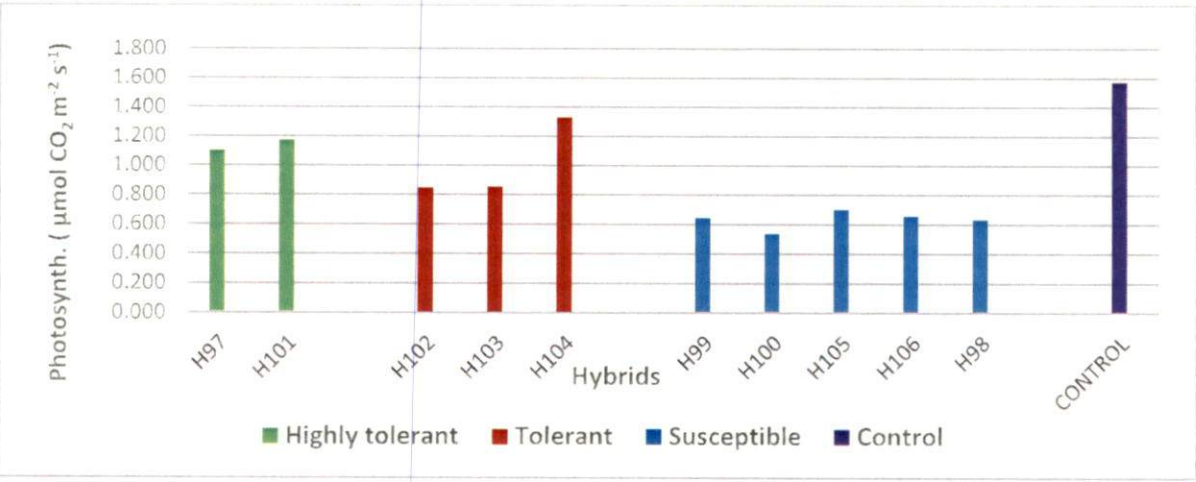

Fig. 96. Photosynthesis of G II 19.5 x G I 5.9

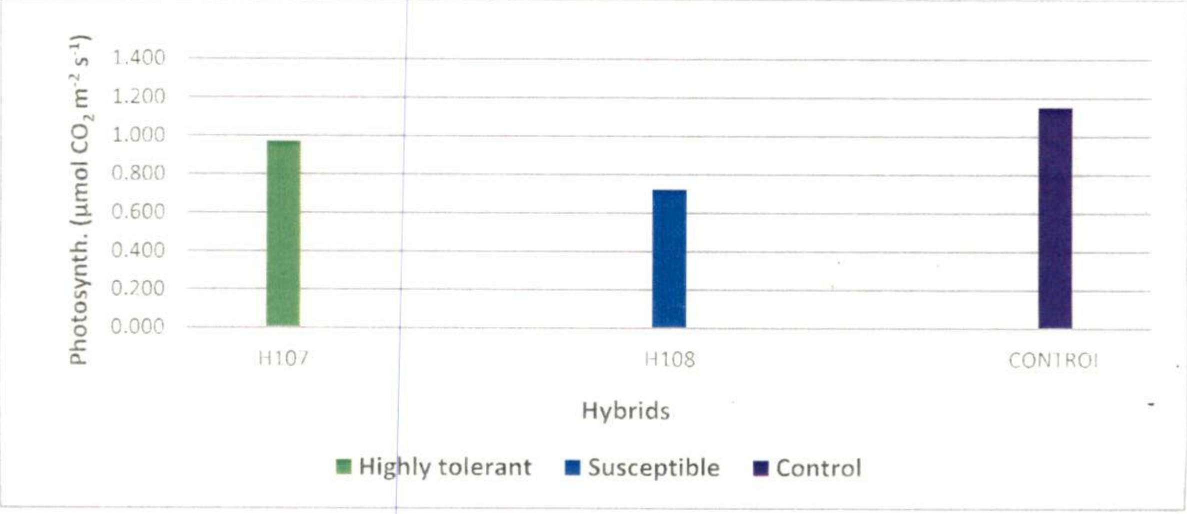

Fig. 97. Photosynthesis of G II 19.5 x G VI 55

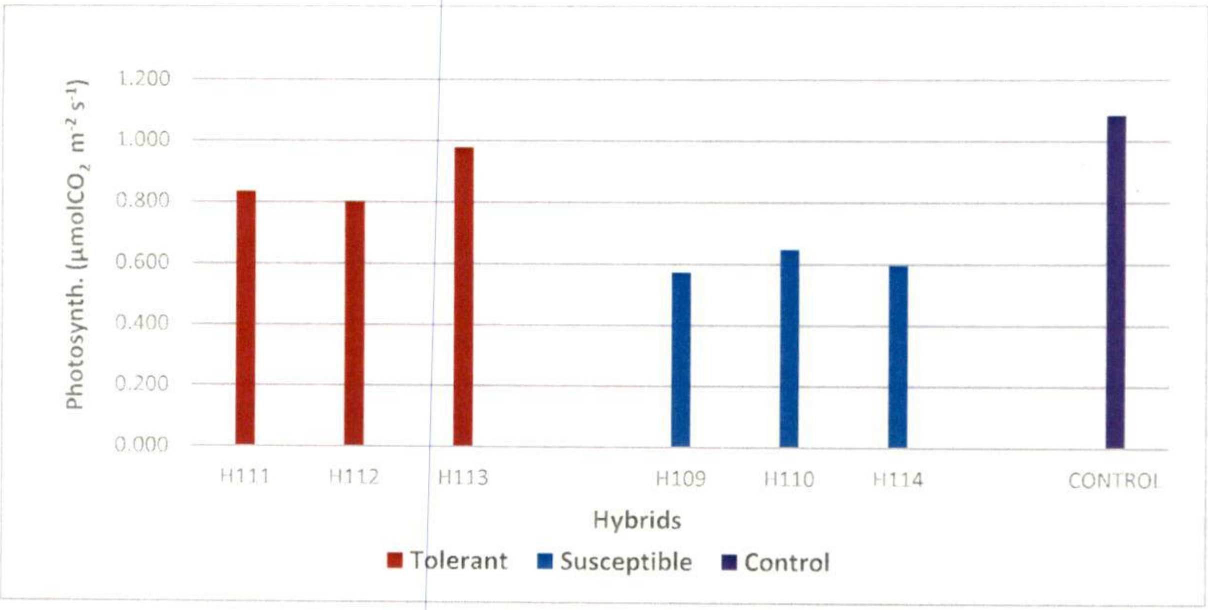

Fig. 98. Photosynthesis of G VI 55 x G I 5.9

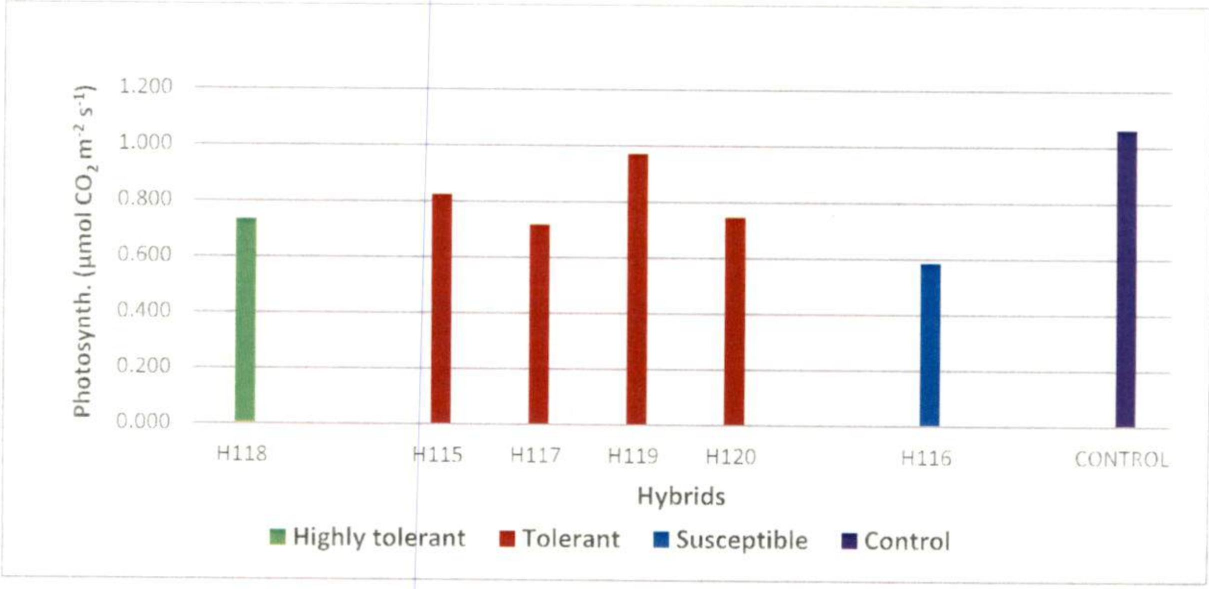

Fig. 99. Photosynthesis of G VI 55 x G II 19.5

Table 27. Physiological parameters of G II 19.5 x G VI 55

Sl No.	**Hybrid**	**Reaction to drought**	**CSI (%)**	**CMS (%)**	**RWC (%)**	**Leaf temperature (°C)**	**Photosynthesis (µmol CO_2 m^{-2} s^{-1})**	**Transpiration rate (mmol H_2O m^{-2} s^{-1})**	**Chlorophyll content (SPAD units)**
1	H107	Highly tolerant	77.77	78.31	79.34	32.79	0.971	0.451	41.27
2	H108	Susceptible	48.63	58.94	43.63	31.77	0.721	0.704	25.50
	Control		91.49	93.07	89.40	32.65	1.150	0.845	43.50
	CV (%)		**13.19 (99.95)**	**14.54 (11.86)**	**16.43 (13.76)**	**6.92**	**7.63**	**7.28**	**10.58**
	CD	0.05%	18.89 (11.99)	NS	22.89 (16.38)	NS	0.15	0.09	8.01
		0.01%	NS	NS	NS	NS	0.24	0.16	13.28

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4.4.6. Transpiration rate

Balasimha and Rajagopal (1988) found that the stomatal conductance in cocoa is reduced by photosynthetically active radiation (PAR), relative humidity (RH) and soil moisture stress, an effect which improves water conservation. Drought tolerance in cocoa is mainly attributable to effective stomatal regulation, which results in decreased transpirational water loss (Balasimha *et al.*, 1988).

In the cross M 13.12 x G I 5.9 (Fig. 100), the hybrid having the highest transpirational rate was H23 (3.417 mmol H_2O m^{-2} s^{-1}). Some other hybrids having high transpirational rate were H30 (3.170 mmol H_2O m^{-2} s^{-1}), H17 (2.750 mmol H_2O m^{-2} s^{-1}), H6 (2.517 mmol H_2O m^{-2} s^{-1}), H22 (2.330 mmol H_2O m^{-2} s^{-1}) and H5 (2.227 mmol H_2O m^{-2} s^{-1}). Lowest value was found in hybrid H12 and H31 having only 0.343 mmol H_2O m^{-2} s^{-1} transpirational rate followed by H27 (0.351 mmol H_2O m^{-2} s^{-1}). The control was having high transpirational rate of 2.868 mmol H_2O m^{-2} s^{-1} (Table 19).

In the cross M 13.12 x G II 19.5 (Fig. 101), H42 (0.307 mmol H_2O m^{-2} s^{-1}) had the lowest transpirational value followed by H37 (0.386 mmol H_2O m^{-2} s^{-1}), H39 (0.387 mmol H_2O m^{-2} s^{-1}), H34 (0.393 mmol H_2O m^{-2} s^{-1}) and H38 (0.484 mmol H_2O m^{-2} s^{-1}). High transpirational rates were found in hybrids H40 (1.045 mmol H_2O m^{-2} s^{-1}), H35 (0.703 mmol H_2O m^{-2} s^{-1}), H41 (0.623 mmol H_2O m^{-2} s^{-1}) and H36 (0.604 mmol H_2O m^{-2} s^{-1}) whereas the control was having 1.086 mmol H_2O m^{-2} s^{-1} transpirational rate under non-stressed conditions (Table 20).

In the cross M 13.12 x G VI 55 (Fig. 102), the highest value was observed in hybrid H47 (0.672 mmol H_2O m^{-2} s^{-1}) followed by H50 (0.665 mmol H_2O m^{-2} s^{-1}), H44 (0.635 mmol H_2O m^{-2} s^{-1}), H49 (0.627 mmol H_2O m^{-2} s^{-1}), H45 (0.614 mmol H_2O m^{-2} s^{-1}) and H51 (0.593 mmol H_2O 1 m^{-2} s^{-1}). Lowest values were found in hybrids H43 (0.426 mmol H_2O m^{-2} s^{-1}) followed by H46 (0.446 mmol H_2O m^{-2} s^{-1}) and H48 (0.461 mmol H_2O m^{-2} s^{-1}). The control was having transpirational rate of 0.982 mmol H_2O m^{-2} s^{-1} under non-stressed conditions (Table 21).

In cross G I 5.9 x M 13.12 (Fig. 103), highest value was seen in hybrid H54 (0.704 mmol H_2O m^{-2} s^{-1}) followed by H53 (0.617 mmol H_2O m^{-2} s^{-1}), H55 (0.592 mmol H_2O m^{-2} s^{-1}) and H56 (0.549 mmol H_2O m^{-2} s^{-1}). Lowest values were found in hybrids H52 (0.361 H_2O mmol m^{-2} s^{-1}) followed by H57 (0.402 mmol H_2O m^{-2} s^{-1}). However, the control had 0.950 mmol H_2O m^{-2} s^{-1} transpiration under well irrigated condition (Table 22).

In the cross G I 5.9 x G II 19.5 (Fig. 104), the highest value was found in hybrid H61 (0.873 mmol H_2O m^{-2} s^{-1}) and the lowest value was found in hybrid H64 (0.324 mmol H_2O m^{-2} s^{-1}) and the control had the transpirational rate of about 1.100 mmol H_2O m^{-2} s^{-1} (Table 23).

In the cross G I 5.9 x G VI 55 (Fig. 105), the highest value was found in hybrid H77 (0.756 mmol H_2O m^{-2} s^{-1}) followed by H73 (0.711 mmol H_2O m^{-2} s^{-1}), H78 (0.658 mmol H_2O m^{-2} s^{-1}) and H72 (0.621 mmol H_2O m^{-2} s^{-1}). Lowest values were found in hybrid H76 (0.419 mmol H_2O m^{-2} s^{-1}) followed by H71 (0.450 mmol H_2O m^{-2} s^{-1}), H75 (0.460 mmol H_2O m^{-2} s^{-1}) and H74 (0.473 mmol H_2O m^{-2} s^{-1}) and the control had 1.050 mmol H_2O m^{-2} s^{-1} transpirational rate under non-stressed condition (Table 24).

In the cross G II 19.5 x M 13.12 (Fig. 106), the highest value was observed in hybrid H92 (0.970 mmol H_2O m^{-2} s^{-1}) which was classified as the susceptible hybrid and the lowest value was recorded from hybrid H83 (0.347 mmol H_2O m^{-2} s^{-1}) which was a tolerant one. The control had 1.105 mmol H_2O m^{-2} s^{-1} transpirational rate when under non-stressed conditions (Table 25).

In the cross G II 19.5 x G I 5.9 (Fig. 107), the highest value was observed in hybrid H99 (0.987 mmol H_2O m^{-2} s^{-1}) and the lowest value was observed in hybrid H102 (0.353 mmol H_2O m^{-2} s^{-1}) which indicated it's tolerance to drought stress. However, the control was having 1.390 mmol H_2O m^{-2} s^{-1} amount of transpiration under non-stressed condition (Table 26).

In the cross G II 19.5 x G VI 55 (Fig. 108), the high value was in hybrid H108 (0.704 mmol H_2O m^{-2} s^{-1}) which indicated it's vulnerability to drought stress and hybrid H107 had 0.451 mmol H_2O m^{-2} s^{-1} transpiration rate. The control had

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0.845 mmol H_2O m^{-2} s^{-1} transpirational rate under well irrigated conditions (Table 27).

In the cross G VI 55 x G I 5.9 (Fig. 109), high value was observed in hybrid H110 (1.124 mmol H_2O m^{-2} s^{-1}) followed by H114 (0.759 mmol H_2O m^{-2} s^{-1}) and H109 (0.684 mmol H_2O m^{-2} s^{-1}). This indicated that these hybrids were susceptible to drought stress. The lowest value was observed in H112 (0.346 mmol H_2O m^{-2} s^{-1}), followed by H113 (0.395 mmol H_2O m^{-2} s^{-1}) and H111 (0.453 mmol H_2O m^{-2} s^{-1}). The control showed 1.250 mmol H_2O m^{-2} s^{-1} transpirational rate under non-stressed condition (Table 28).

In the cross G VI 55 x G II 19.5 (Fig. 110), H116 had the highest transpiration rate of about 0.698 mmol H_2O m^{-2} s^{-1} indicated it's susceptibility to drought stress. The low transpiration rate was recorded in hybrids H115 (0.519 mmol H_2O m^{-2} s^{-1}), H119 (0.472 mmol H_2O m^{-2} s^{-1}), H117 (0.471 mmol H_2O m^{-2} s^{-1}), H120 (0.397 mmol H_2O m^{-2} s^{-1}) and H118 (0.306 mmol H_2O m^{-2} s^{-1}) which indicated it's tolerance to drought stress. The control had 0.841 mmol H_2O m^{-2} s^{-1} of transpirational rate (Table 29).

Water stress had been known to reduce the transpiration rate in plants. Three accessions of cocoa (NC 23, NC 29 and NC 39) had shown 54 to 59 per cent decrease in transpiration rate under stress conditions as compared to plants under irrigation (Balasimha *et al.*, 1988). In other plants too, this condition was reported. Transpiration rate was highest (4.75 m mol H_2O m^{-2} s^{-1}) in cashew seedlings stressed for two days while it declined to 2.11 m mol H_2O m^{-2} s^{-1} when stress was given for five days (Latha, 1998). A decrease in transpiration rate was observed under water stress condition in oats (Bhatt *et al.*, 1998). Transpiration rate was reduced under water stress in beech (Peuke *et al.*, 2002).

The tolerant hybrids had lower transpiration rate as compared to the susceptible as well as the control plants which indicated their mechanism to cope up with drought stress.

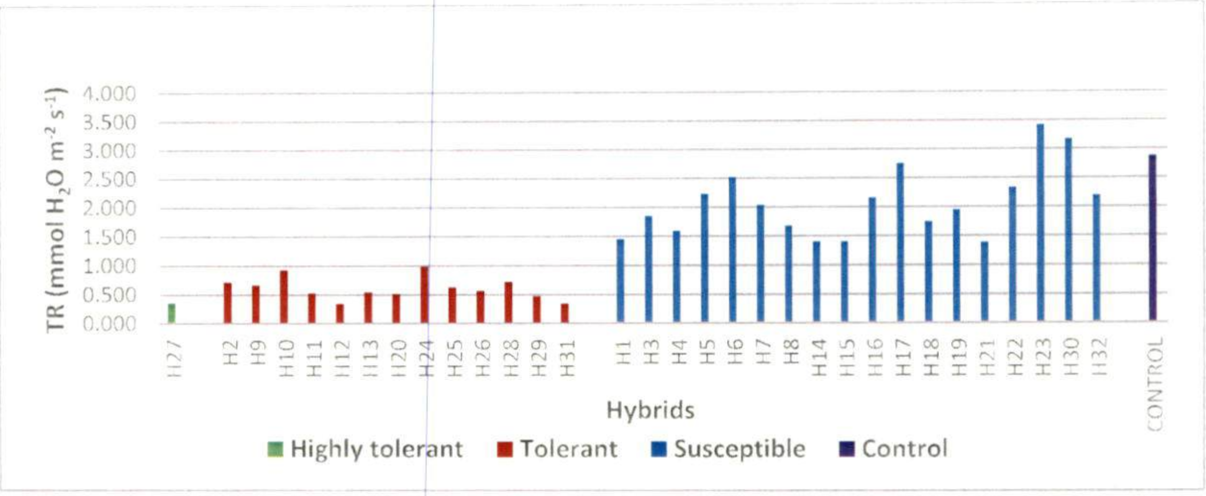

Fig. 100. Transpiration rate of M 13.12 x G I 5.9

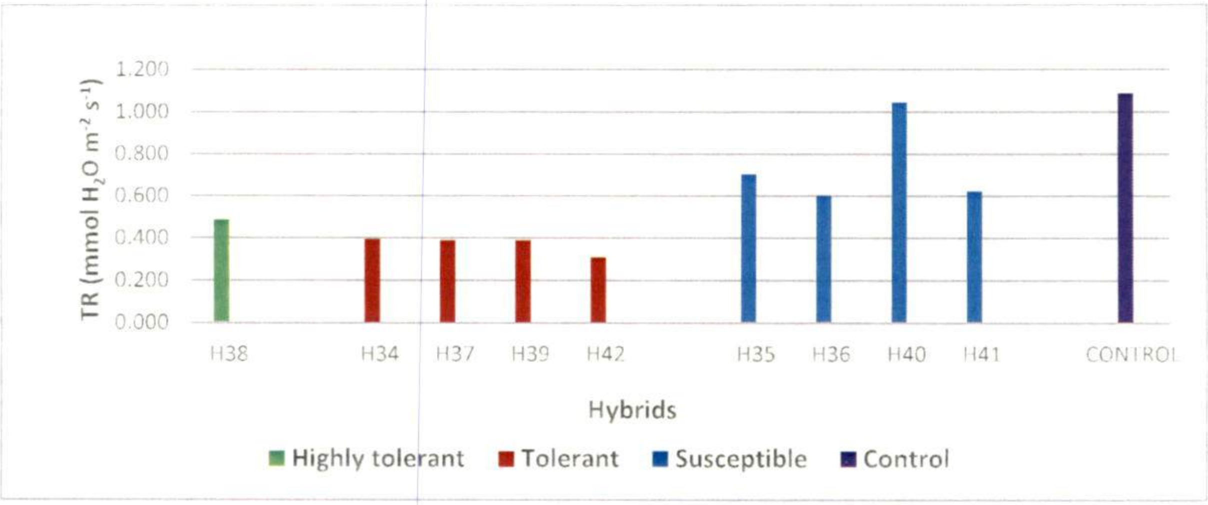

Fig. 101. Transpiration rate of M 13.12 x G II 19.5

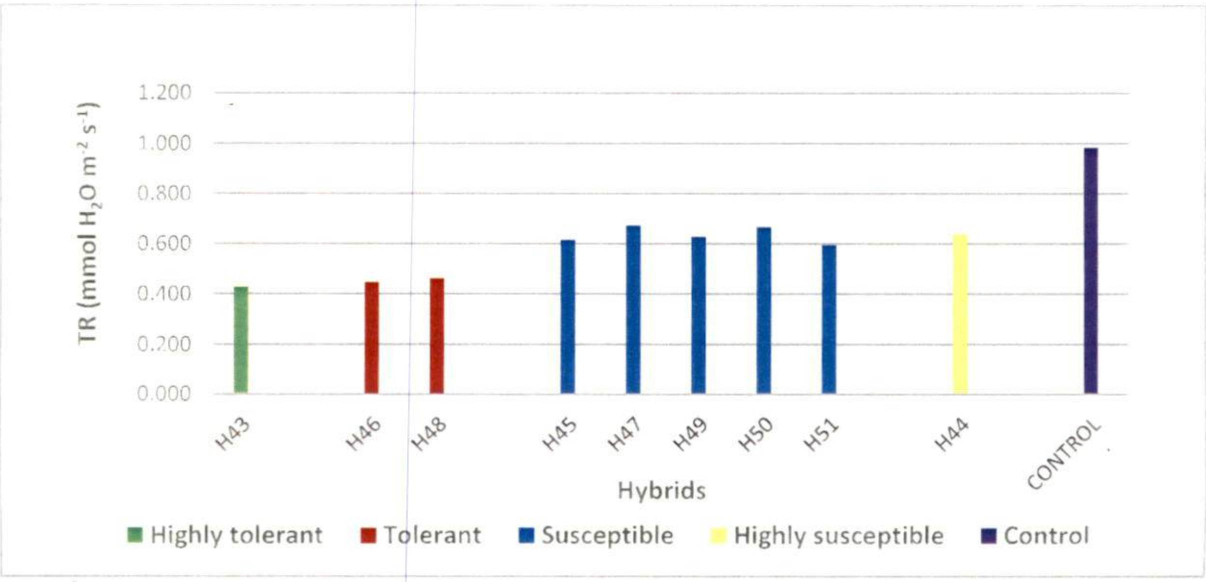

Fig. 102. Transpiration rate of M 13.12 x G VI 55

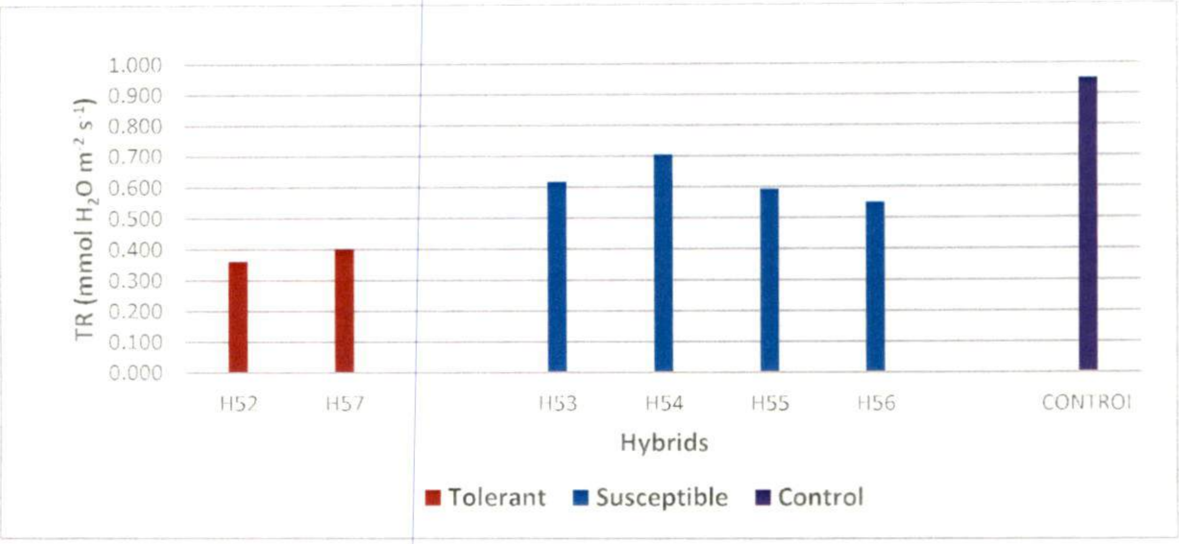

Fig. 103. Transpiration rate of G I 5.9 x M 13.12

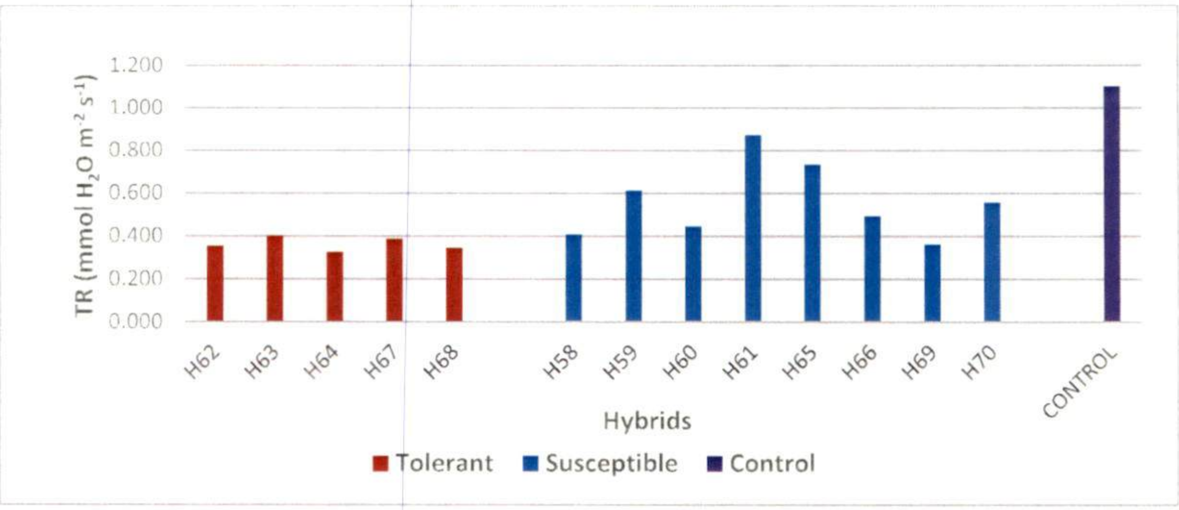

Fig. 104. Transpiration rate of G I 5.9 x G II 19.5

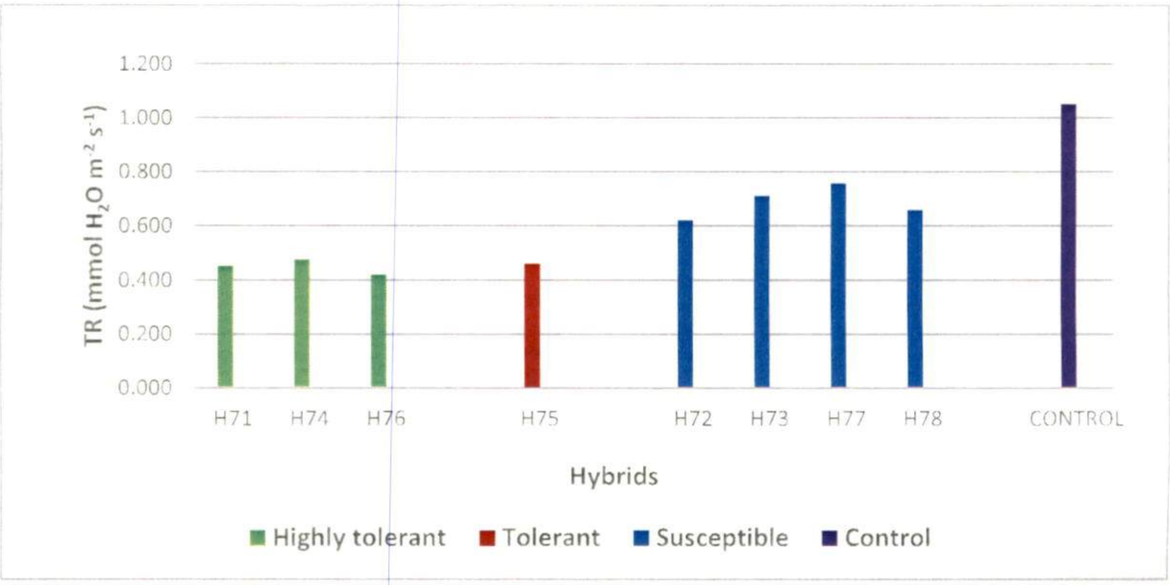

Fig. 105. Transpiration rate of G I 5.9 x G VI 55

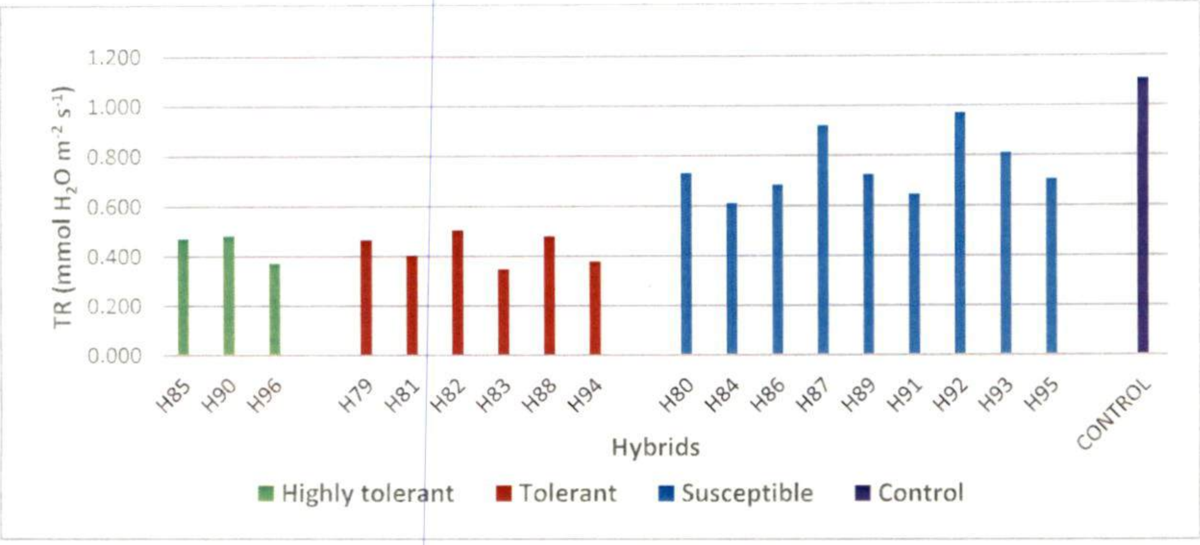

Fig. 106. Transpiration rate of G II 19.5 x M 13.12

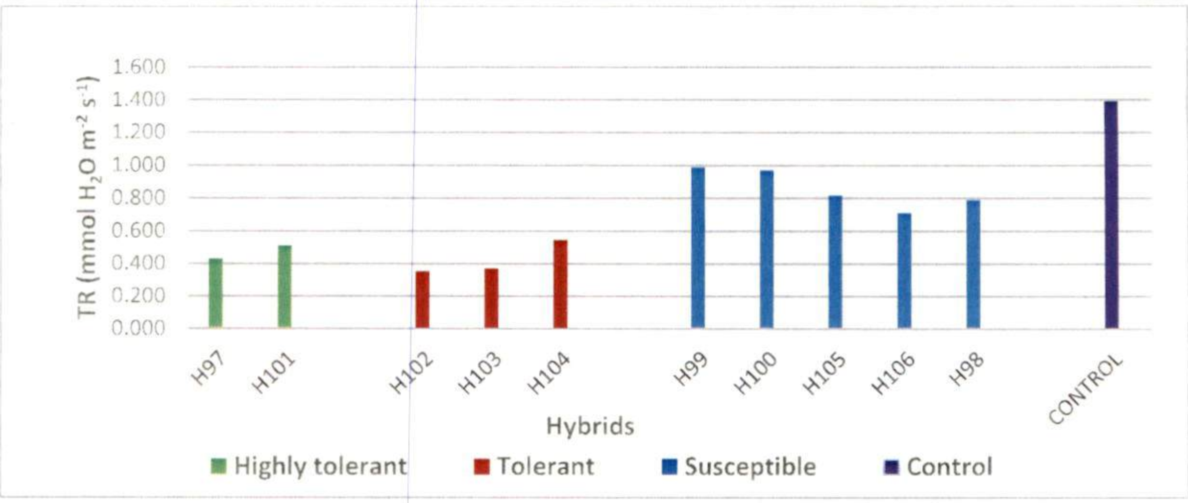

Fig. 107. Transpiration rate of G II 19.5 x G I 5.9

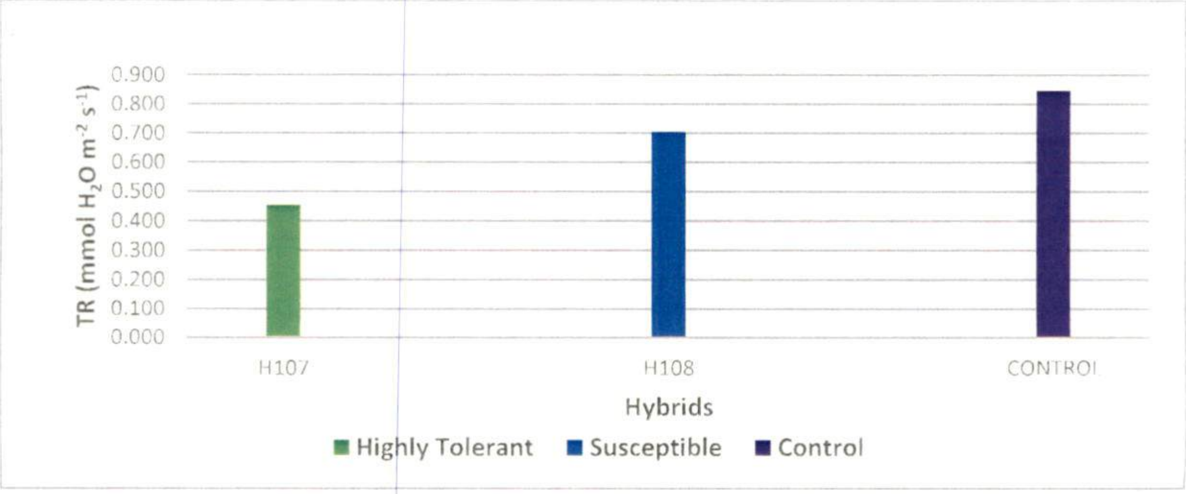

Fig. 108. Transpiration rate of G II 19.5 x G VI 55

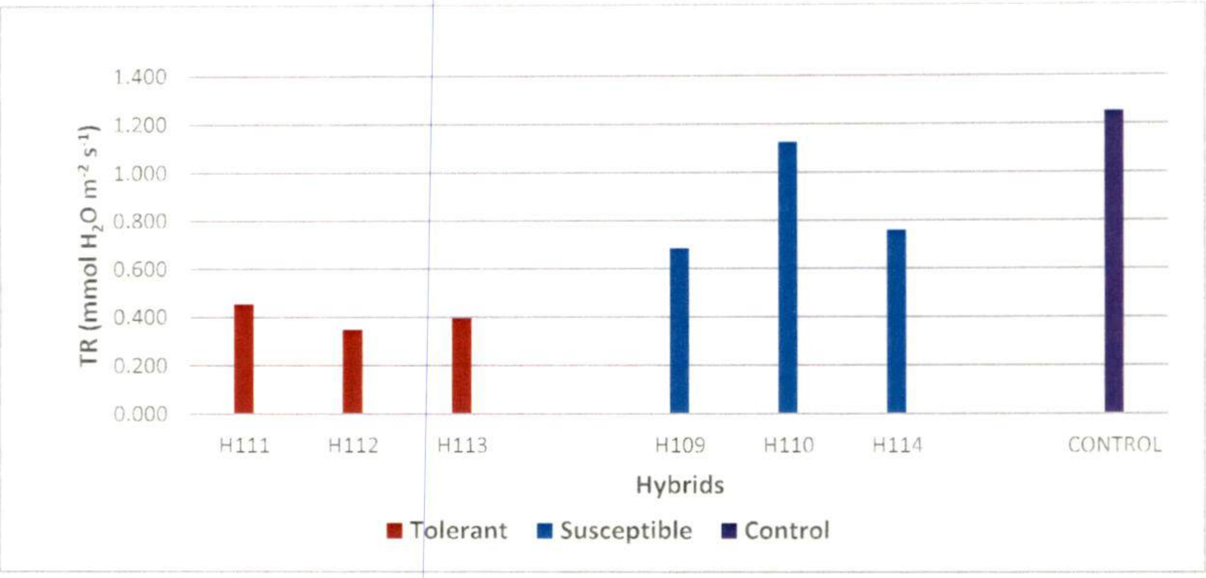

Fig. 109. Transpiration rate of G VI 55 x G I 5.9

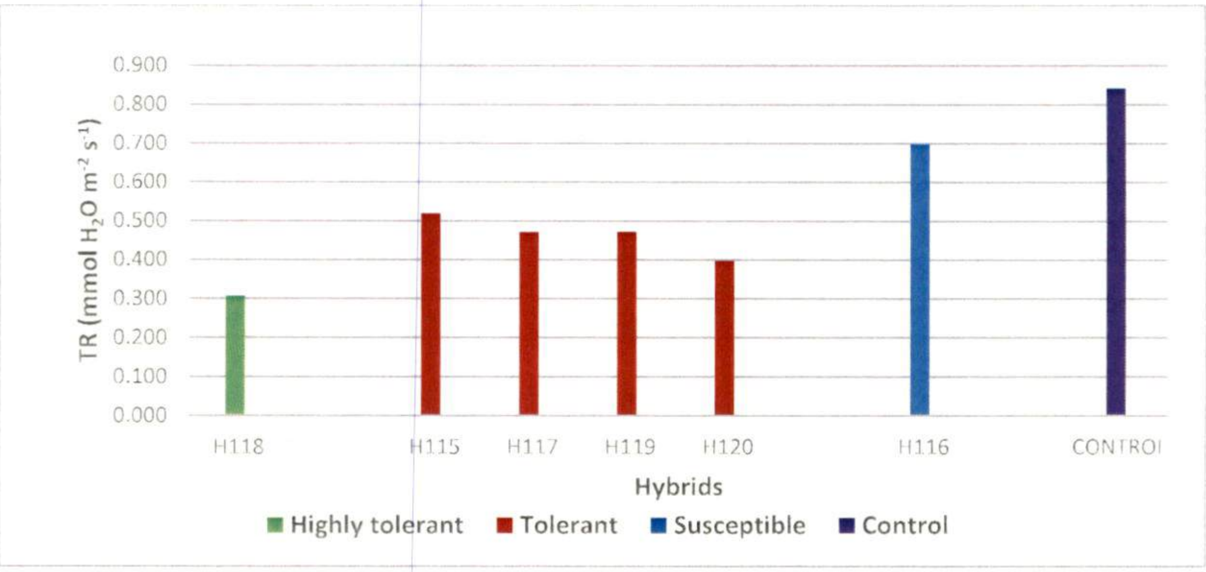

Fig. 110. Transpiration rate of G VI 55 x G II 19.5

4.4.7. Chlorophyll content

Drought stress inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (Iturbe *et al*., 1998). In this experiment, the chlorophyll content measured by spadmeter gave value between 0-50. The results are depicted in graphs (Fig. 111-121) and Tables 19-29 as indicated below.

In the cross M 13.12 x G I 5.9 (Fig. 111), the highest value observed was of hybrid H13 (41.33 SPAD units) which is a tolerant hybrid. Some other hybrids having high values in chlorophyll content were H10 (41.17 SPAD units), H27 (40.57 SPAD units), H28 (40.50 SPAD units), H20 (40.40 SPAD units), H12 (39.83 SPAD units), H29 (39.63 SPAD units), H25 (38.60 SPAD units), H31 (37.83 SPAD units), H2 (37.17 SPAD units), H11 (37.03 SPAD units), H24 (36.90 SPAD units), H9 (36.60 SPAD units) and H26 (36.53 SPAD units). Lowest value was observed in hybrid H8 (21.37 SPAD units). Some other hybrids having low chlorophyll content were H32 (26.57 SPAD units), H16 (26.27 SPAD units), H23 (24.70 SPAD units) and H14 (22.30 SPAD units). The control gave a reading of 44.80 SPAD units (Table 19).

In the cross M 13.12 x G II 19.5 (Fig. 112), the highest value was observed in hybrid H39 (41.53 SPAD units). Other hybrids having high chlorophyll content were H42 (38.54 SPAD units), H37 (35.63 SPAD units), H33 (32.77 SPAD units), H38 (32.70 SPAD units) and H34 (32.50 SPAD units). Lowest values were observed in hybrid H40 (27.43 SPAD units), H36 (25.33 SPAD units), H41 (24.87 SPAD units) and H35 (24.53 SPAD units). The control gave a reading of 46.30 SPAD units (Table 20).

In the cross M 13.12 x G VI 55 (Fig. 113), highest value was observed in hybrid H43 (38.30 SPAD units), H46 (35.37 SPAD units) and H48 (33.53 SPAD units). Lowest values were observed in hybrids H45 (20.27 SPAD units). Other hybrids were H51 (26.67 SPAD units), H44 (26.50 SPAD units), H49 (26.47 SPAD units), H47 (25.40 SPAD units) and H50 (23.43 SPAD units). The control had 41.20 SPAD units of chlorophyll under non-stressed conditions (Table 21).

In the cross G I 5.9 x M 13.12 (Fig. 114), highest value was observed in hybrid H52 (38.10 SPAD units) followed by H57 (36.00 SPAD units). Low values were observed in hybrid H56 (21.33 SPAD units) followed by H55 (28.57 SPAD units), H53 (30.87 SPAD units) and H54 (31.13 SPAD units) and the control had 43.70 SPAD units of chlorophyll content under watered condition (Table 22).

In the cross G I 5.9 x G II 19.5 (Fig. 115), H67 had the highest chlorophyll content of about 37.47 SPAD units followed by H62 (35.57 SPAD units), H64 (34.77 SPAD units), H68 (33.70 SPAD units) and H63 (31.57 SPAD units). Lowest values were observed in hybrid H59 (19.50 SPAD units). Some other hybrids having low content were H70 (23.70 SPAD units), H58 (21.50 SPAD units) and H65 (21.23 SPAD units). The control had 45.70 SPAD units of chlorophyll greenness under non-stressed conditions (Table 23).

In the cross G I 5.9 x G VI 55 (Fig. 116), the highest values were observed in hybrid H74 (39.13 SPAD units) followed by H76 (35.37 SPAD units), H71 (34.47 SPAD units) and H75 (33.83 SPAD units). The lowest values were observed in H78 (20.73 SPAD units). Other hybrids having the low chlorophyll content were H73 (27.30 SPAD units), H77 (23.37 SPAD units) and H72 (25.37 SPAD units). The control had 42.70 SPAD units of chlorophyll greenness (Table 24).

In the cross G II 19.5 x M 13.12 (Fig. 117), the hybrid having the highest value was H94 (41.57 SPAD units) and the lowest value observed in the cross was in hybrid H80 (20.17 SPAD units). The control had 42.60 SPAD units of chlorophyll content under non-stressed conditions (Table 25).

In the cross G II 19.5 x G I 5.9 (Fig. 118), the highest value was observed in hybrid H97 (44.73 SPAD units) and the lowest value was observed in hybrid H105 (21.47 SPAD units). The control had 45.10 SPAD units of chlorophyll content (Table 26).

In the cross G II 19.5 x G VI 55 (Fig. 119), the hybrid H107 was having the higher value of 41.27 SPAD units followed by hybrid H108 having chlorophyll content of 25.50 SPAD units. The control had 43.50 SPAD units under non-stressed condition (Table 27).

In the cross G VI 55 x G I 5.9 (Fig. 120), the highest value was observed in hybrid H111 (44.47 SPAD units) followed by hybrid H112 (37.47 SPAD units) and H113 (34.40 SPAD units). Lowest values were observed in hybrids H109 (22.67 SPAD units) followed by H114 (22.70 SPAD units) and H110 (25.30 SPAD units) and the control plant had 46.60 SPAD units under non-stressed conditions (Table 28).

In the cross G VI 55 x G II 19.5 (Fig. 121), the highest value was observed in hybrid H115 (36.57 SPAD units), followed by H120 (35.70 SPAD units), H119 (33.63 SPAD units), H117 (32.23 SPAD units) and H118 (32.07 SPAD units). Lowest values was observed in hybrid H116 (27.57 SPAD units). The control on the other hand, had about 40.20 SPAD units of chlorophyll under non-stressed conditions (Table 29).

Loss of chlorophyll contents under water stress is considered as a main cause of inactivation of photosynthetic pigments and also the process photosynthesis. Furthermore, water deficit induced reduction in chlorophyll content had contributed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.*, 1981). The decrease in chlorophyll under drought stress was mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). Drought stress caused a large decline in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll content in all sunflower varieties investigated (Manivannan *et al.*, 2007b). A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting.

The chlorophyll content measured showed that the susceptible hybrids were having lower content as compared to the tolerant and the control plants. This indicated it's role in drought tolerance.

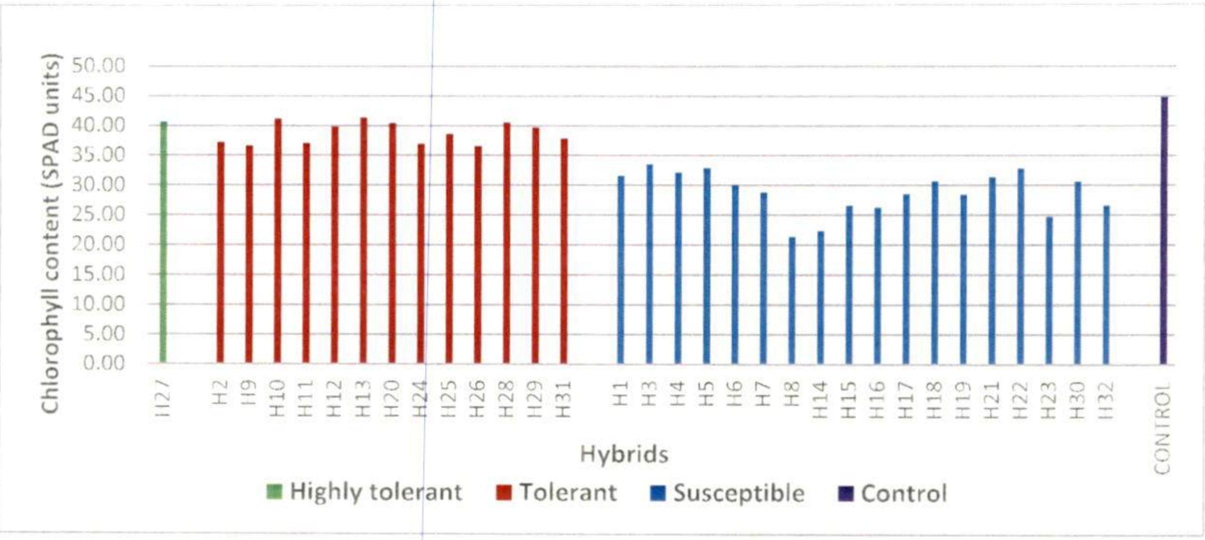

Fig. 111. Chlorophyll content of M 13.12 x G I 5.9

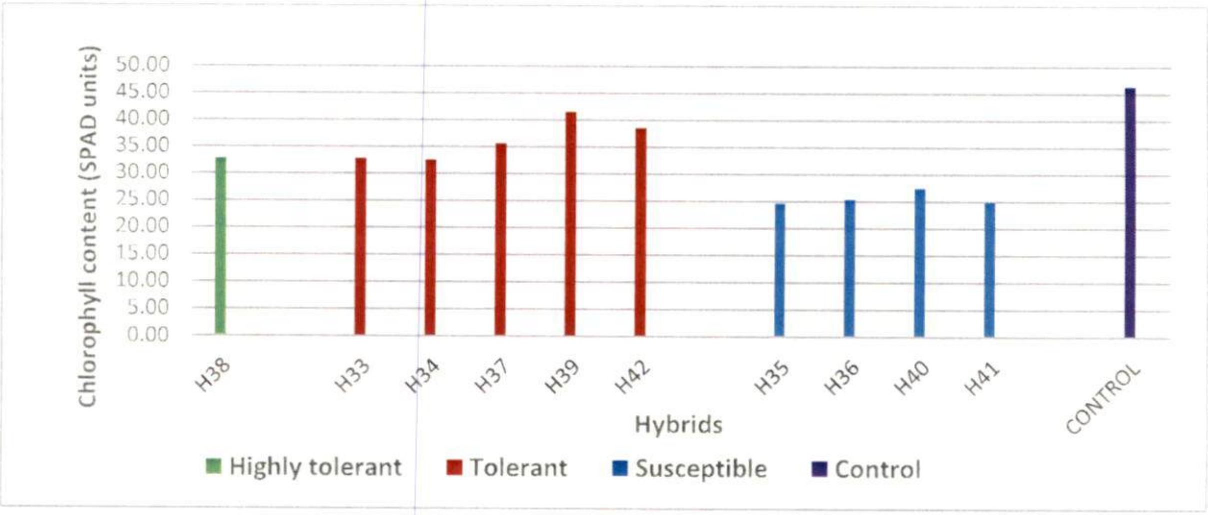

Fig. 112. Chlorophyll content of M 13.12 x G II 19.5

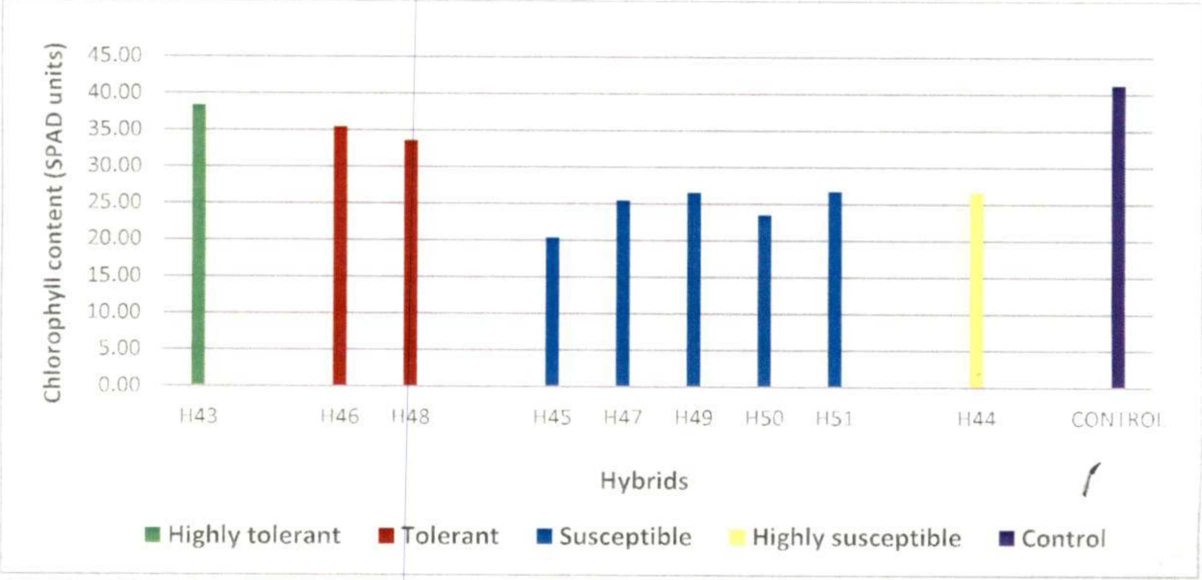

Fig. 113. Chlorophyll content of M 13.12 x G VI 55

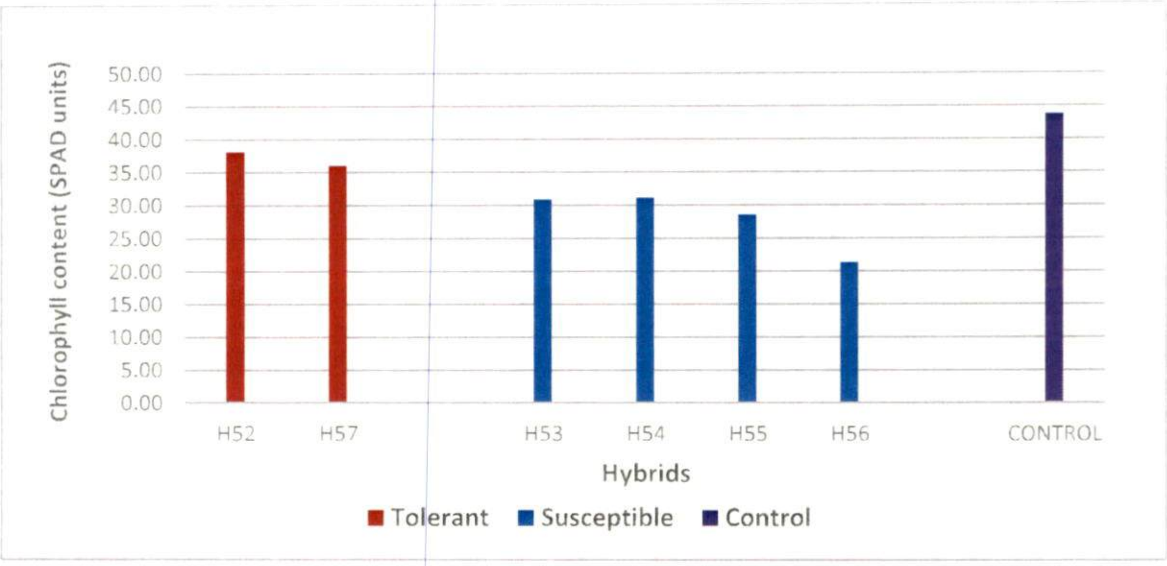

Fig. 114. Chlorophyll content of G I 5.9 x M 13.12

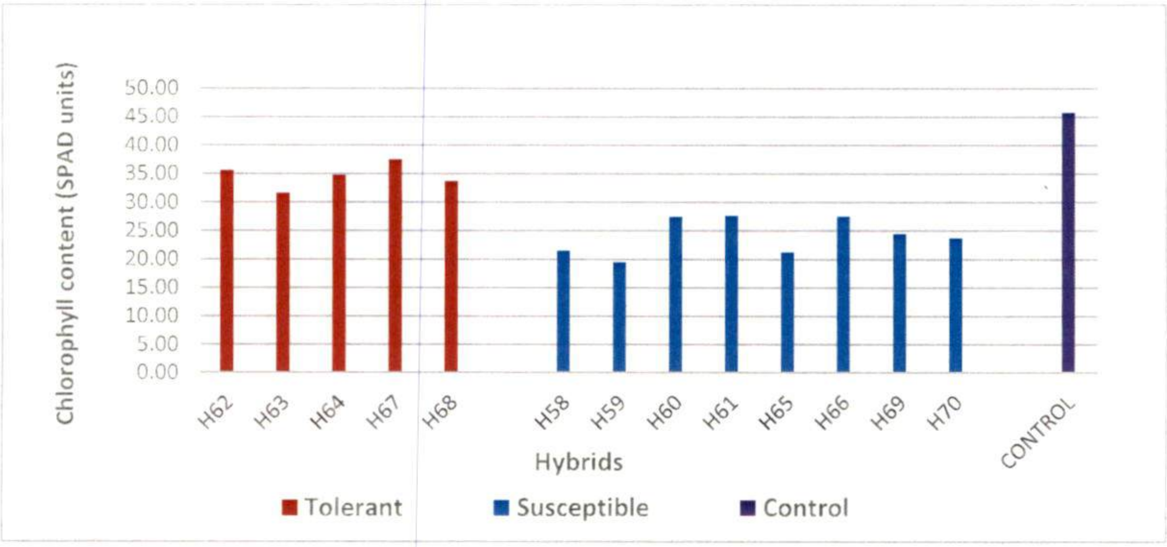

Fig. 115. Chlorophyll content of G I 5.9 x G II 19.5

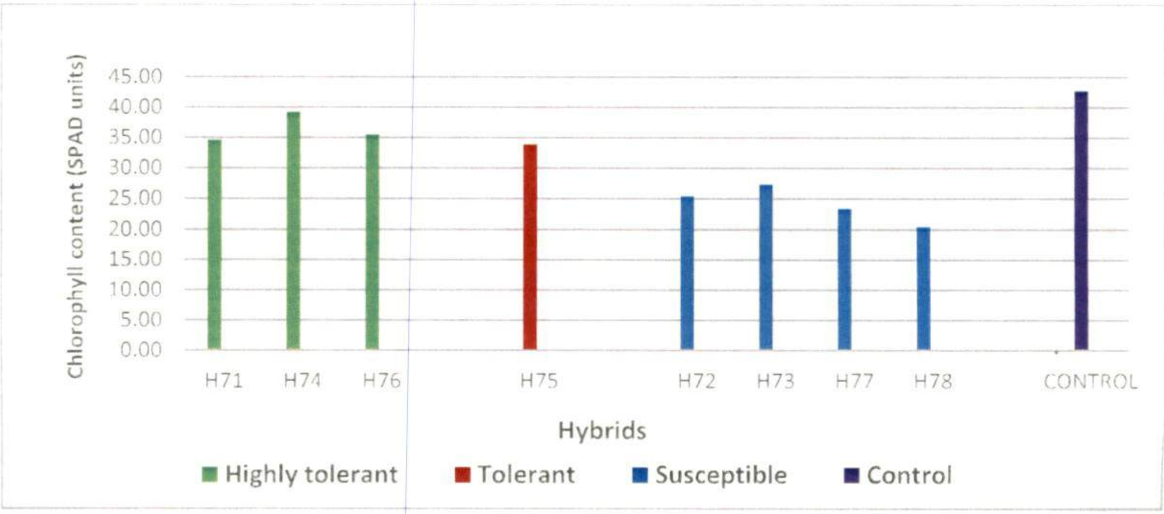

Fig. 116. Chlorophyll content of G I 5.9 x G VI 55

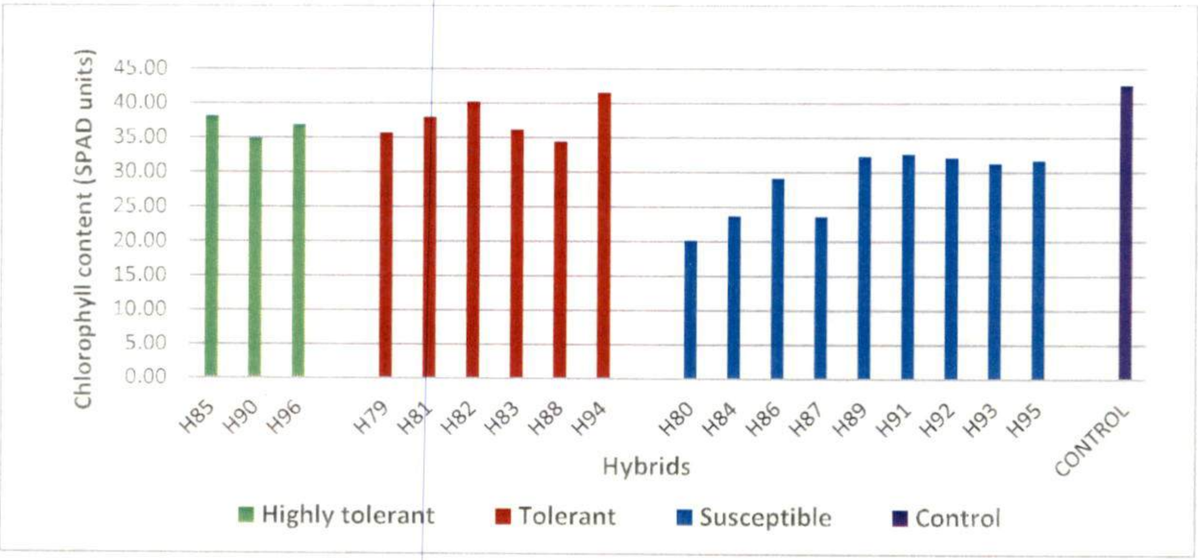

Fig. 117. Chlorophyll content of G II 19.5 x M 13.12

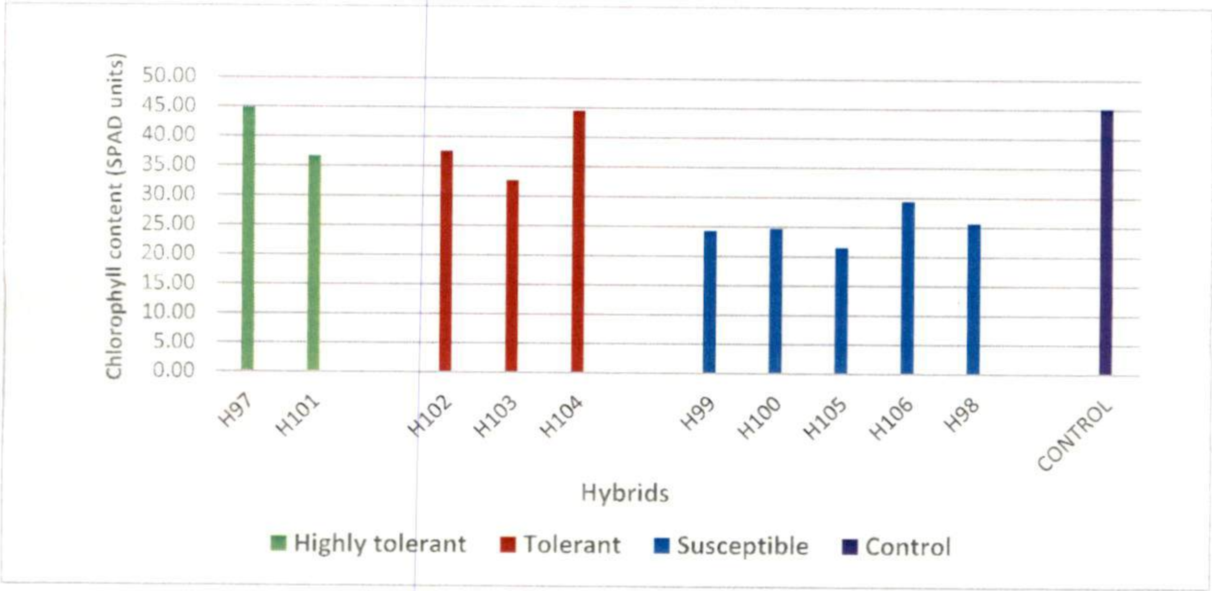

Fig. 118. Chlorophyll content of G II 19.5 x G I 5.9

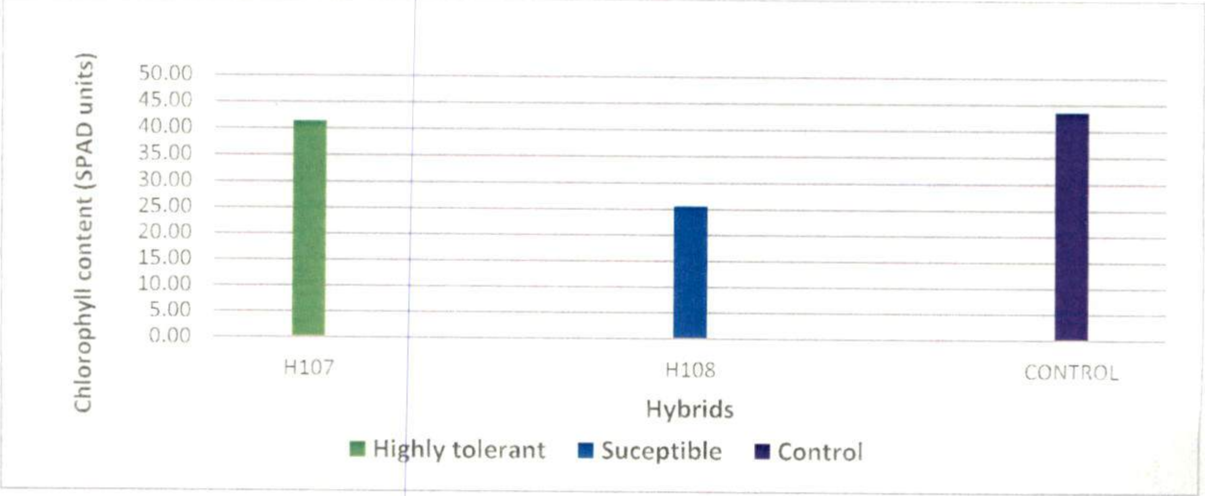

Fig. 119. Chlorophyll content of G II 19.5 x G VI 55

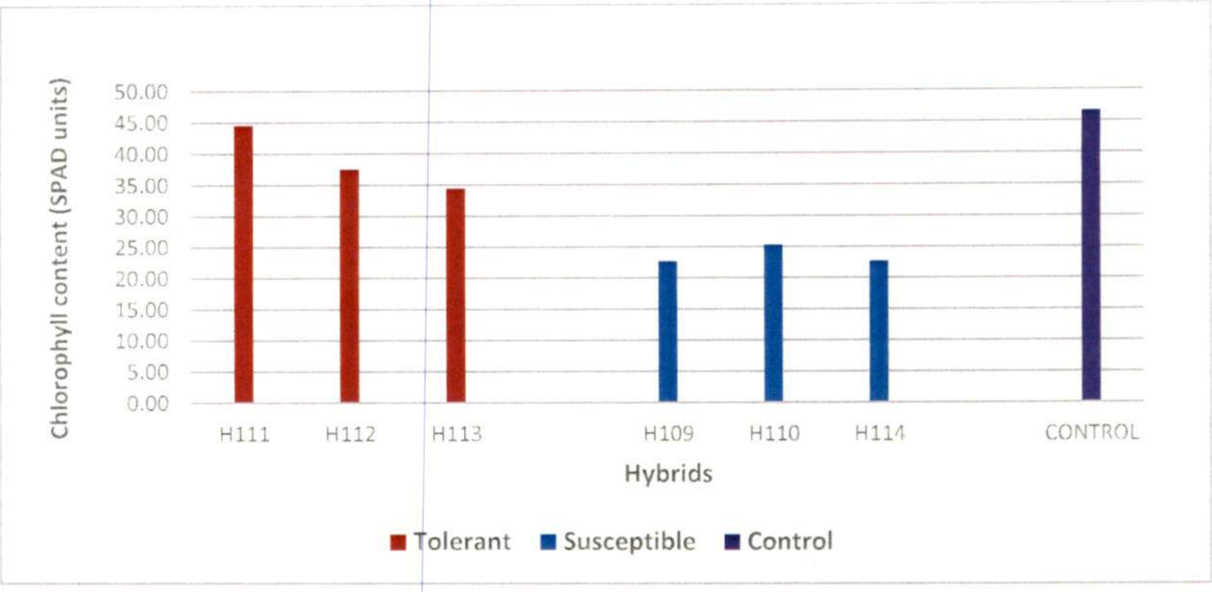

Fig. 120. Chlorophyll content of G VI 55 x G I 5.9

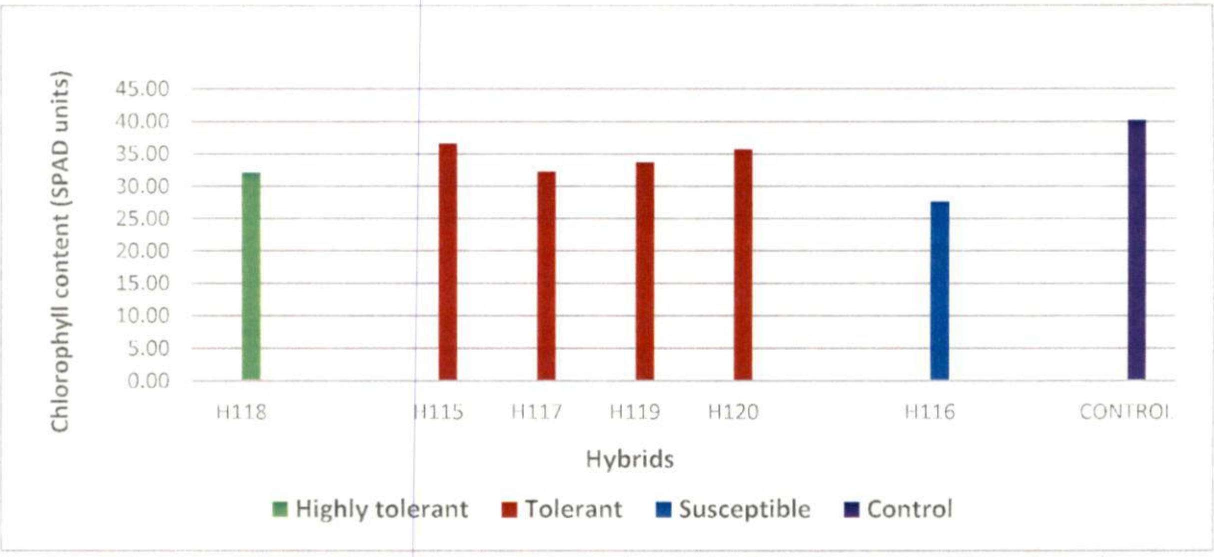

Fig. 121. Chlorophyll content of G VI 55 x G II 19.5

Table 28. Physiological parameters of G VI 55 x G I 5.9

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H109	Susceptible	53.65	52.73	33.32	31.20	0.572	0.684	22.67
2	H110	Susceptible	57.30	57.74	33.35	30.97	0.647	1.124	25.30
3	H111	Tolerant	67.20	68.37	64.27	31.35	0.833	0.453	44.47
4	H112	Tolerant	89.67	78.84	78.41	31.43	0.802	0.346	37.47
5	H113	Tolerant	66.52	85.41	57.58	31.36	0.977	0.395	34.40
6	H114	Susceptible	59.30	42.25	44.32	30.51	0.596	0.759	22.70
	Control		94.28	89.03	83.64	31.14	1.084	1.250	46.60
	CV (%)		**10.98 (8.09)**	**13.43 (10.86)**	**12.08 (8.52)**	**10.74**	**11.87**	**10.09**	**12.09**
	CD	0.05%	12.81 (7.87)	15.34 (10.43)	11.15 (7.01)	NS	0.16	0.11	6.71
		0.01%	17.96 (11.03)	21.51 (14.63)	15.63 (9.83)	NS	0.22	0.16	9.40

Table 29. Physiological parameters of G VI 55 x G II 19.5

Sl No.	Hybrid	Reaction to drought	CSI (%)	CMS (%)	RWC (%)	Leaf temperature (°C)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Chlorophyll content (SPAD units)
1	H115	Tolerant	82.50	65.71	39.28	32.47	0.824	0.519	36.57
2	H116	Susceptible	57.17	41.99	23.51	30.54	0.583	0.698	27.57
3	H117	Tolerant	69.17	81.81	50.59	30.63	0.717	0.471	32.23
4	H118	Highly tolerant	76.83	75.72	53.48	31.11	0.732	0.306	32.07
5	H119	Tolerant	66.00	85.72	33.48	33.82	0.970	0.472	33.63
6	H120	Tolerant	67.17	77.53	39.54	30.86	0.745	0.397	35.70
	Control		90.14	92.19	70.73	30.60	1.061	0.841	40.20
	CV (%)		**11.69 (9.05)**	**10.16 (8.71)**	**7.29 (4.54)**	**5.86**	**7.57**	**11.68**	**11.71**
	CD	0.05%	14.52 (9.18)	12.09 (9.05)	5.18 (3.16)	NS	0.10	0.10	NS
		0.01%	NS	18.09 (12.69)	7.27 (4.43)	NS	0.14	0.14	NS

4.5. Correlation studies on the parameters attributing to drought tolerant hybrids

Many studies have been conducted to find out how certain enzymes regulate the activity of plants during drought stress and how this affects the physiological parameters in order to give tolerance to plants (Deltoro *et al*., 1998; Yordanov *et al*., 2000). Understanding the correlation of physiological and biochemical responses to water deficit will help in breeding plant cultivars having high yield and stability under drought conditions (Yordanov *et al.*, 2000).

Among the biochemical characters, proline showed maximum correlation with the dependent variable, percentage of leaves retained (0.777). It has been already proved that proline is having direct correlation with drought stress (Singh *et al*., 1973; Mali and Mehta, 1977 and Karamanos *et al*., 1983) as indicated in the present study.

Nitrate reductase activity also expressed a significant positive correlation (0.740). The amount of NR enzyme generally decreases during drought stress and hence, the hybrids having more NR enzyme were more tolerant to drought stress and were able to regulate the nitrogen assimilation in plants (Foyer *et al*., 1998; Xu and Zhou, 2005). Therefore, in the present study NR was found to be directly related to the dependent variable.

Glycine betaine (0.628) also showed a significant correlation with percentage of leaves retained. The role of glycine betaine to drought tolerance has been reported in many cases (Robinson and Jones, 1986; Genard *et al*., 1991). When the levels of glycine betaine was correlated with the extent of increased tolerance, the accumulation of glycine betaine was found to be induced under stress conditions (Saneoka *et al*., 1995; Jagendorf and Takabe, 2001; Yang *et al*., 2003; Park *et al*., 2004).

SOD showed positive correlation (0.554) with the percentage of leaves retained which was used for distinguishing tolerant and susceptible plants. Superoxide dismutase (SOD) are the enzymes that forms the first line defense and catalyses the dismutation of O^{2-} radicals to H_2O and O_2. Hence, the amount of

SOD increases with increase in stress conditions indicating it's direct relation to drought stress (Bowler, 1992; Luna *et al*., 2004)

All the four biochemical parameters studied expressed a high correlation with the percent of leaves retained when drought stress was imposed on plants indicating their role in imparting drought tolerance (Table 30).

In case of physiological parameters, chlorophyll stability index showed a high and positive correlation (0.698) with percentage of leaves retained. Hence, it can be considered as one of the parameters for assessing the drought tolerance in plants. Chlorophyll stability index measures integrity of membrane or heat stability of the pigments under stress conditions. CSI is a single parameter which can be used to measure frost (or) drought resistance of a plant (Kaloyereas, 1958). Sairam *et al*. (1996) reported that drought stress decreased membrane stability, chlorophyll content and chlorophyll stability index in all wheat genotypes studied. High values of chlorophyll stability help the plants to withstand stress through better availability of chlorophyll.

CSI was followed by cell membrane stability (0.693) and this parameter has been widely used to differentiate stress tolerant and susceptible cultivars of many crops (Blum and Ebercon, 1981; Premachandra *et al*., 1992). At the cellular level, drought stress causes shrinkage of cells, cell membrane injury, and production of free radicals that can cause damage to the cellular apparatus (Terbea *et al*., 1995; Sgherri *et al*., 1996; Kang and Zhang, 1997). Hence, it can be cited as one of the parameters directly affected from drought stress and can be used as a parameter to assess the drought tolerance level in plants.

Chlorophyll content (0.690) also had a significant positive correlation with the dependant variable. Photosynthetic pigments absorbs energy from light and hence, the foliar chlorophyll content is an important factor affecting the performance of plant photosynthesis (Taiz and Zeiger, 2006). Several studies had showed that drought stress visibly decreases the chlorophyll a, chlorophyll b and total chlorophyll content of different crops (Mafakheri *et al*., 2010; Gholamin and Khayatnezhad, 2011), which indicated that the presence of low levels of chlorophyll in leaves was a general symptom of stress. Hence, it can be concluded that chlorophyll content was significantly correlated with drought stress in plants.

Photosynthetic rate also decreases during drought stress as the decrease in chlorophyll content can directly affect the photosynthetic machinery (Faria *et al.*, 1996). In the present study it was found to be positively correlated with the dependant variable (0.505) indicating the efficiency of drought tolerant plants.

Relative water content (0.635) was found to be positively correlated with drought tolerance. The relative water content has been used as a parameter in drought related studies in many crops. RWC is an alternative measure of plant water status which informs about the metabolic process in the tissue and lethal leaf water status (Flower and Ludlow, 1986). Upreti *et al.* (1997) noted changes in RWC under stress and normal conditions. However, the reduction was more under stress condition. Hence, it was used as a parameter to assess the water status in crops and had a direct relation with drought stress (Parida *et al.*, 2008).

However, in case of transpiration, the case is reverse. The transpiration rate had negative but significant correlation with percentage of leaves retained (-0.463) and transpiration rate of susceptible plants was found to be more than drought tolerant plants (Table 30). Water stress will reduce the transpiration rate in plants. A work was carried out in three accessions of cocoa (NC 23, NC 29 and NC 39) in which 54 to 59 per cent decrease in transpiration rate was noticed as compared to plants under irrigation (Balasimha *et al.*, 1988). This shows that plants under drought conditions, in order to maintain water balance reduce their transpiration rate. Hence, it was having a direct but opposite effect when drought stress was imposed.

Leaf temperature expressed non-significant effect (0.145) on the dependent variable. Hence, it did not had any correlation with the dependent variable.

While studying the correlation between the biochemical and physiological characters, proline had positive and significant correlation with membrane stability (0.567), relative water content (0.559), nitrate reductase (0.534), chlorophyll stability index (0.508), glycine betaine (0.458), chlorophyll content (0.451), superoxide dismutase (0.353) and photosynthetic rate (0.289). Transpiration rate had a negative but significant effect on proline (-0.307) indicating that when the amount of proline increases in a plant, the transpiration rate of that tolerant plant is low. The correlation between drought tolerance ability and proline content in response to osmotic stress had been documented. It showed an increasing trend with increasing stress conditions (Hien *et al.*, 2003; Ashraf and Foolad, 2007; Kishor and Sreenivasulu, 2014). In a study carried out in chickpea cultivars, there was an increase in proline concentration in the plants when drought stress was imposed but decrease in physiological parameters like transpiration rate (Mafakheri *et al.*, 2010). It can be concluded that proline and transpiration rate are negatively correlated with each other and tolerant plants generally have lower transpiration rate and higher proline concentration.

Matthews and Boyer, 1984 found that the photosynthesis process during drought is possible due to the osmoregulation which affects the state of the leaf stomata and adaptation of the photosynthetic apparatus to drought conditions. Similar results were obtained in studies like Gupta and Berkowitz, (1988); Shangguan *et al.*, (1999) and Verslues and Bray, (2004). Ludlow (1987) and Weng (1993) reported a positive correlation between photosynthesis and osmoregulation. During drought, proline and glycine betaine acts as osmolytes regulating the osmoregulation process in plants and hence are positively correlated with photosynthesis.

Nitrate reductase enzyme had positive and significant correlation with chlorophyll stability Index (0.713), cell membrane stability (0.678), chlorophyll content (0.639), glycine betaine (0.581), relative water content (0.558), SOD (0.440) and photosynthetic rate (0.396). However, it had negative significant correlation with transpiration rate (-0.490) (Table 30).

Sugiharto *et al*. (1990) found a significant positive correlation between the photosynthetic capacity of leaves and their leaf nitrogen concentration which suggested that most of the nitrogen used for synthesis of components of the photosynthetic apparatus. Hence, if there is a reduction in nitrate reductase activity for nitrogen assimilation, it can cause a significant reduction in photosynthetic activity and hence, reduced chlorophyll activity. It indicated the direct correlation between nitrate reductase enzyme, photosynthetic rate and chlorophyll content.

The superoxide dismutase enzyme was positively correlated with photosynthetic rate (0.679), chlorophyll content (0.584), cell membrane stability (0.480), chlorophyll stability index (0.476), glycine betaine (0.441), leaf temperature (0.414) and relative water content (0.223). The transpiration rate was negatively correlated with SOD (Table 30).

Glycine betaine had a positive and significant correlation with relative water content (0.697), cell membrane stability (0.684), chlorophyll stability Index (0.584), chlorophyll content (0.500) and photosynthetic rate (0.289). However, transpiration rate (-0.574) was negatively correlated with glycine betaine (Table 30).The percentage of ion leakage was significantly negative correlated to GB concentration in drought-stressed plants in cotton plants, suggested that GB will protect cell membrane stability under drought stress (Sulian *et al.*, 2007). To maintain membrane integrity, GB acts as an osmoprotectant (Table 30).

It was recorded that the elevated accumulation of GB in cotton helps to maintain the cell membrane stability by reducing radical oxygen species (ROS), and this was partly performed by increased activities of some antioxidant enzymes such as SOD (Mansour, 1998; Meloni *et al.*, 2003). Thus, it can be concluded that SOD is directly correlated with glycine betaine in providing tolerance to plants under drought stress.

Chlorophyll stability index was positively correlated with chlorophyll content (0.717), cell membrane stability (0.641), along with photosynthetic rate (0.564), relative water content (0.475) and negatively correlated with transpiration rate (-0.319) (Table 30).

The membrane stability was having significant and positive relation with chlorophyll content (0.652), relative water content (0.645), photosynthetic rate

(0.361), and a negative yet significant relation with transpiration rate (-0.550) (Table 30).

The relative water content was having a positive and significant relation with chlorophyll content (0.471) and negative correlation with transpiration rate (-0.528) (Table 30).

Water stress in plants was measured in terms of leaf water potential, also known as relative water content (Deivanai *et al.*, 2010). Reduction in RWC resulted in loss of turgidity which in turn led to stomatal closure and reduced photosynthetic rates (Lv *et al.*, 2007).

The photosynthetic rate showed a positive and significant correlation with chlorophyll content (0.657) and leaf temperature (0.626). The chlorophyll content (0.449) and transpiration rate (0.308) and had a positive and significant correlation with leaf temperature (Table 30).

There has been reports where photosynthetic capacity in lupins were shown to be directly dependant on leaf temperature and incident light (Chaves *et al.*, 1992). Hence, leaf temperature and photosynthetic activity are correlated with each other.

From the above result, it was found that out of the eleven characters, ten were having direct correlation with the dependant variable, which was percentage of leaves retained and hence, these were selected for further path analysis to figure out how many characters out of these ten characters were having a direct effect on the dependent variable and hence, can be used to asses drought tolerance in cocoa.

Table 30. Correlation among drought tolerant contributing characters of hybrids

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
V1	1											
V2	0.534**	1										
V3	0.353**	0.440**	1									
V4	0.458**	0.581**	0.441**	1								
V5	0.508**	0.713**	0.476**	0.584**	1							
V6	0.567**	0.678**	0.480**	0.684**	0.641**	1						
V7	0.559**	0.558**	0.223*	0.697**	0.475**	0.645**	1					
V8	0.289**	0.396**	0.679**	0.289**	0.564**	0.361**	0.103^{NS}	1				
V9	-0.307**	-0.490**	-0.307**	-0.574**	-0.319**	-0.550**	-0.528**	-0.140^{NS}	1			
V10	0.451**	0.639**	0.584**	0.500**	0.717**	0.652**	0.471**	0.657**	-0.293**	1		
V11	0.015^{NS}	0.077^{NS}	0.414**	-0.083^{NS}	0.267**	0.020^{NS}	0.096^{NS}	0.626**	0.308**	0.449**	1	
V12	0.777**	0.740**	0.554**	0.628**	0.698**	0.693**	0.635**	0.505**	-0.463**	0.690**	0.145^{NS}	1

*Correlation significant at 0.05 level

** Correlation significant at 0.01 level

V 1 - Proline (µg/g)
V 2 – Nitrate reductase activity (mmol nitrate /g/hr)
V 3 – Superoxide dismutase (units/mg protein/g)
V 4 – Glycine betaine (µ mol/g)
V 5 – Chlorophyll stability Index (%)
V 6 – Cell membrane stability (%)
V 7 – Relative water content (%)
V 8 – Photosynthesis (µmol CO_2 m^{-2} s^{-1})
V 9 – Transpiration rate (mmol H_2O m^{-2} s^{-1})
V 10 – Chlorophyll content (SPAD units)
V 11 – Leaf temperature (°C)
V 12- Number of leaves retained

4.6. Path analysis with direct and indirect effects on percentage of leaves retained after drought imposition

Path analysis with direct and indirect effects on the percentage of leaves retained is given in Table 31 and represented in Figure 122. Residual effect contribution on percentage of leaves retained was 0.148 which indicated that 85.2 of percent characters contributed to the main characters. As per Lenka and Misra (1973), the direct and indirect effects were grouped into

>1.00 - Very high

0.30 – 0.99 - High

0.20-0.29 - Medium

0.10-0.19 - Low

0.09-0.00 - Negligible

4.6.1. Direct effect

High positive effect on the percentage of leaves retained which is a direct measure of plant's tolerance to drought was expressed by proline (0.386). Proline in general was known to correlate with stress tolerance and has a direct effect on tolerance capability of plant (Ashraf and Foolad, 2007).

Medium positive effects was showed by cell membrane stability (0.284) and low direct effect was shown by parameters nitrate reductase activity (0.166), relative water content (0.121) and photosynthetic rate (0.133). It reflected the importance of these characters in deciding the parameters for assessing drought tolerance in cocoa (Table 31). A similar study was conducted in cotton to analyse effects on various physiological and biochemical parameters affecting drought stress and when path and correlation analysis were conducted, it was found that simultaneous selection based on photosynthetic rate, soluble protein, NRase, SPAD and total chlorophyll will be promising for increase in yield when drought stress was imposed (Ananthi *et al.*, 2012). Hence, these parameters had direct effect on the dependent variable.

Negligible direct effects were found in characters superoxide dismutase (0.063), chlorophyll stability Index (0.029), chlorophyll content (0.045) and negative but negligible effects were shown by glycine betaine (-0.016), transpiration rate (-0.003) and leaf temperature (-0.008) (Table 31).

This study indicated that four parameters (proline, NRA, relative water content and photosynthetic rate) had direct effect which acted as a tool to overcome the stress conditions due to drought. Hence, they can be considered as an important indicator to assess the capacity of plants to tolerate drought.

4.6.2. Indirect effect

4.6.2.1. Proline

Proline expressed positive low indirect effect on percentage of leaves retained (0.161) through medium positive direct effect of cell membrane stability (0.284). All other indirect effects were negligible which were not taken into consideration (Table 31). A study conducted in coconut represented similar results. It was found that increase in proline played a protective role by reducing the membrane damage and thus, the plants had higher membrane stability even during water stress conditions (Gomes *et al*., 2010).

4.6.2.2. Nitrate reductase activity

NRA expressed positive medium indirect effect on percentage of leaves retained (0.206) through high positive direct effect of proline (0.386) and low indirect positive effect on the retained percentage of leaves through medium positive direct effect of cell membrane stability (0.284). All other characters were having negligible indirect effects (Table 31).

4.6.2.3. Superoxide dismutase

Superoxide dismutase expressed low positive indirect effect on the percentage of leaves (0.136) retained through high positive direct effect of proline (0.386) and also had low positive indirect effect on percentage of leaves retained (0.136) through medium positive direct effect of cell membrane stability (0.284). All other characters had negligible indirect effects (Table 31). In a study conducted in sorghum, higher MSI coupled with enhanced activity of SOD enzyme in the hybrid resulted in reducing the negative impact of ROS on membrane damage indicating the presence of an efficient ant-oxidative mechanism in the hybrids and which indicated that membrane stability can be stable when the SOD enzymes keeps a check over the ROS species (Vijayalakshmi, *et al.*, 2012). Hence, it can be stated that they were directly related. Hence, it supported the result that SOD and CMS in this experiment, were found to have an indirect effect on the percentage of leaves retained and were related with each other.

4.6.2.4. Glycine betaine

Glycine betaine expressed positive low indirect effect on the total percentage of leaves retained (0.177) through high positive direct effect of proline (0.386). It had a low positive indirect effect on percentage of leaves retained (0.194) through medium positive direct effect of cell membrane stability (0.284). All other characters had negligible effects (Table 31). Many studies suggests a direct relation between proline and glycine betaine. When plants experience drought stress, proline and glycine betaine are one of the main osmolytes accumulated in tissues which helps in maintaining cell turgor pressure, stabilizing membranes by preventing electrolytic leakage, bringing the concentrations of reactive oxygen species within normal range, thus, preventing the oxidative burst in plants and many more. Hence, they also show a relation with cell membrane stability by preventing the electrolytic leakage (Murmu *et al.*, 2017).

4.6.2.5. Chlorophyll stability Index

CSI expressed positive low indirect effect on the percentage of leaves retained (0.196) through positive high direct effect of proline (0.386). It showed positive low indirect effect on the percentage of leaves (0.118) through low positive direct effect of NRA (0.166) and also showed positive low indirect effect on percentage of leaves (0.182) through positive medium direct effect of cell membrane stability (0.284). All other parameters had negligible effects (Table 31).

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4.6.2.6. Cell membrane stability

Cell membrane stability expressed medium positive indirect effect on percentage of leaves retained (0.219) through high positive direct effect of proline (0.386). It also showed low positive indirect effect on percentage of leaves (0.112) through low positive direct effect of NRA (0.166) (Table 31).

4.6.2.7. Relative water content

Relative water content expressed medium positive indirect effect on the percentage of leaves retained (0.216) through high positive direct effect of proline (0.386). Low positive indirect effect on the percentage of leaves retained (0.183) was manifested through medium positive direct effect of cell membrane stability (0.284). Rest all characters were having negligible effects (Table 31). It was reported that increase in proline was related with the RWC content. This means that when there was a decrease in relative water content, proline content increased in order to save the plant from drought stress damages (Rampino *et al*., 2006). Hence, this supported the fact that RWC was having an indirect effect on proline in regulating drought stress in plants.

4.6.2.8. Photosynthesis

Photosynthetic rate expressed low positive indirect effect on percentage of leaves retained (0.111) through high positive direct effect of proline (0.386). It also expressed low positive indirect effect on percentage of leaves retained through positive medium direct effect of cell membrane stability (0.284). All other characters were negligible in nature (Table 31).

4.6.2.9. Transpiration rate

Transpiration rate had negative low indirect effect on percentage of leaves (-0.118) through high positive direct effect of proline (0.386). It also had low negative indirect effect on percentage of retained leaves (-0.156) through medium positive direct effect of cell membrane stability (0.284). All other character had negligible effect (Table 31).

4.6.2.10. Chlorophyll content

It expressed low positive indirect effect on the percentage of leaves retained (0.174) through high positive direct effect of proline (0.386). It also had low positive indirect effect (0.106) on the percentage of leaves obtained through low positive direct effect of NRA (0.166). The chlorophyll content also expressed low positive indirect effect on percentage of leaves retained (0.185) through positive medium direct effect of cell membrane stability (0.284) (Table 31).

4.6.2.11. Leaf temperature

Leaf temperature was not able to have any indirect effect on the percentage of leaves retained aspect (Table 31).

The result indicated that all biochemical and physiological parameters are almost independent in determining drought tolerance and their interactions were negligible.

High (proline), medium (cell membrane stability) and low (NRA, relative water content and photosynthesis) direct effects and medium indirect effects such as in NRA, cell membrane stability and relative water content can be emphasised when selection procedure is carried out for drought screening in cocoa. Hence, the five parameters *viz*, proline, NRA, cell membrane stability, RWC and photosynthetic rate were considered as they were having a direct effect on the dependent variable, percentage of leaves withered.

Table 31. Path analysis with direct and indirect effects on total number of leaves retained

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
V1	**0.38675**	0.08897	0.02256	-0.00297	0.01497	0.16111	0.04507	0.03847	0.00117	0.02063	-0.00014
V2	0.20666	**0.16649**	0.02815	-0.00377	0.02098	0.19286	0.04502	0.05271	0.00187	0.02923	-0.00069
V3	0.13639	0.07327	**0.06397**	-0.00286	0.01400	0.13642	0.01798	0.09042	0.00117	0.02673	-0.00370
V4	0.17716	0.09666	0.02821	**-0.01649**	0.01721	0.19448	0.05623	0.03841	0.00219	0.02287	0.00074
V5	0.19661	0.11863	0.03043	-0.00379	**0.02900**	0.18214	0.03830	0.07504	0.00122	0.03280	-0.00238
V6	0.21919	0.11296	0.03070	-0.00444	0.01886	**0.28427**	0.05204	0.04802	0.00210	0.02984	-0.00018
V7	0.21606	0.09291	0.01426	-0.00452	0.01398	0.18336	**0.12067**	0.01377	0.00202	0.02155	0.00086
V8	0.11178	0.06594	0.04346	-0.00187	0.01660	0.10256	0.00835	**0.13309**	0.00054	0.03007	-0.0056
V9	-0.11866	-0.08163	-0.01966	0.00372	-0.0093	-0.15629	-0.04261	-0.01866	**-0.00382**	-0.01342	-0.00275
V10	0.17447	0.10638	0.03738	-0.00324	0.02111	0.18548	0.03802	0.08749	0.00112	**0.04574**	-0.00402
V11	0.00587	0.01276	0.02649	0.00054	0.00785	0.0058	-0.00774	0.08332	-0.00117	0.02055	**-0.00894**

Numbers in bold font: Direct effects

Direct effect: Number of leaves retained

V 1 - Proline (µg/g)
V 2 – Nitrate reductase activity (mmol nitrate /g/hr)
V 3 – Superoxide dismutase (units/mg protein/g)
V 4 – Glycine betaine (µ mol/g)
V 5 – Chlorophyll stability Index (%)
V 6 – Cell membrane stability (%)
V 7 – Relative water content (%)
V 8 – Photosynthesis (µmol CO_2 m^{-2} s^{-1})
V 9 – Transpiration rate (mmol H_2O m^{-2} s^{-1})
V 10 – Chlorophyll content (SPAD units)
V 11 – Leaf temperature (°C)

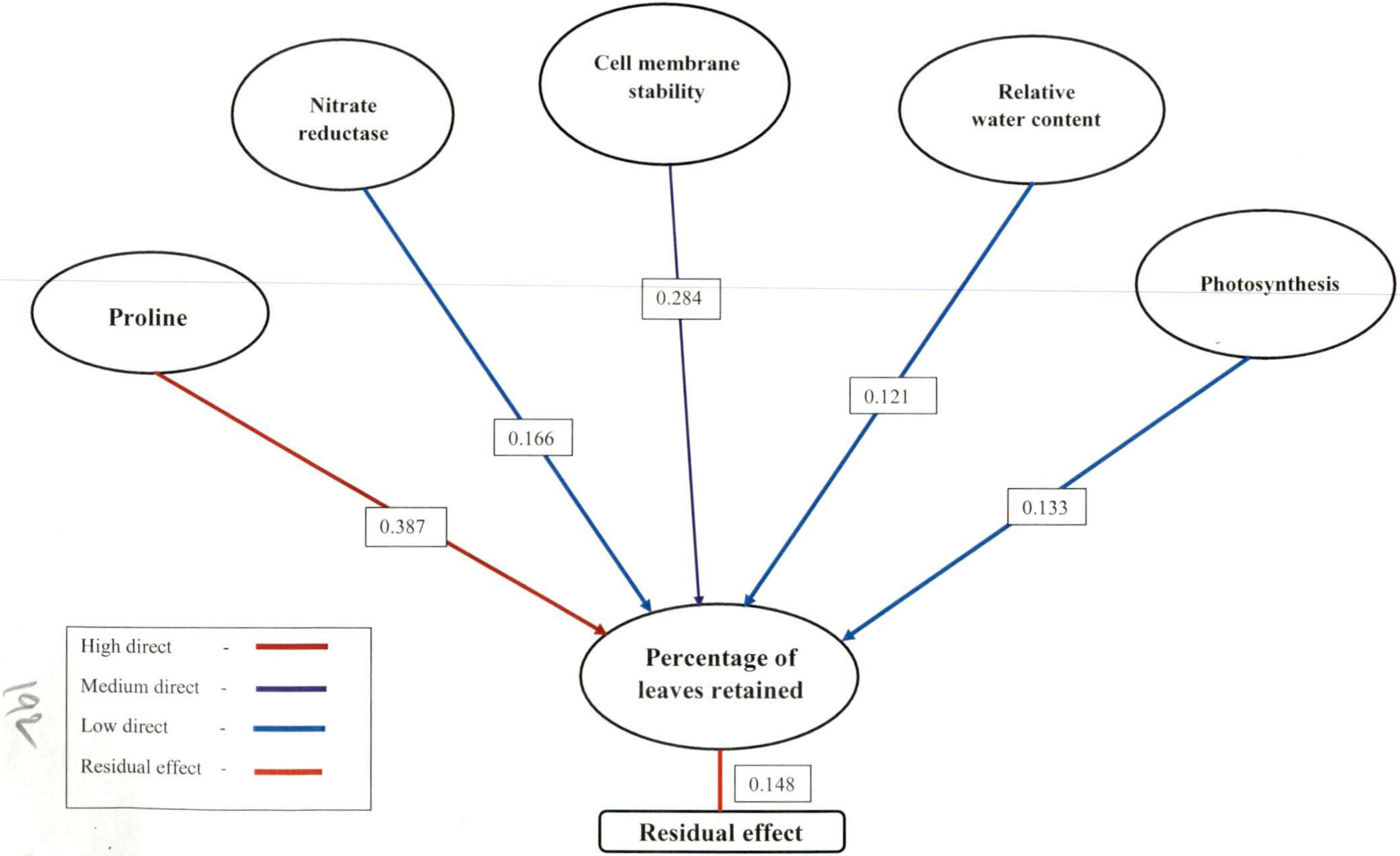

Fig. 122. Path diagram for the selected characters having direct effects on percent of leaves retained

4.7. Analysis of genetic parameters

The genetic parameters were estimated for different characters in the hybrids and proline had the highest phenotypic coefficient of variation (PCV) of about 96.10 per cent followed by transpiration rate of about 76.83 per cent and NRA had 54.90 per cent PCV. The relative water content recorded 41.62 per cent PCV, photosynthesis expressed 35.77 per cent followed by superoxide dismutase (32.27 %), cell membrane stability (25.37 %), chlorophyll stability index (22.82 %), glycine betaine (22.58 %) and chlorophyll content (22.18 %). Only character which had moderate PCV was leaf temperature having 11.90 per cent PCV (Table 32).

High genotypic coefficient of variation (GCV) values were observed in proline (96.00 %), transpiration rate (76.58 %), NRA (53.74 %), relative water content (39.20 %), photosynthesis (34.61 %), superoxide dismutase (25.20 %), cell membrane stability (21.55 %) and glycine betaine (20.22 %). Moderate GCV was found in the character chlorophyll stability index (19.10 %) and chlorophyll content (18.86 %). The low GCV value was found in leaf temperature (1.66 %) (Table 32).

With respect to heritability, high heritability was found in majority of characters according to the classification given by Johnson (1955). Among the hybrids, the highest heritability was shown by the character proline having 99.78 per cent heritability followed by transpiration rate (99.19 %), NRA (95.92 %), photosynthesis exhibited 93.71 per cent of heritability, relative water content had 88.70 per cent heritability, glycine betaine expressed 79.86 per cent heritability, chlorophyll content having 72.36 per cent heritable nature, cell membrane stability having 72.13 per cent heritability and chlorophyll stability index having 70.01 per cent heritability. Low heritability was found only for leaf temperature (1.95 %) (Table 32).

In a study conducted on wheat, heritability for RWC (90.80 %) and proline content (69.50 %) was found to be much higher than that for yield or any of the yield components in wheat under stressed condition. Phenotypic selection for

RWC and proline content may be more efficient for drought tolerance. Chaudhary *et al.* (1989) showed that osmotic adjustment and RWC, both behave as simple inherited characters. The genotypic value was found to have higher and significant value for RWC and proline along with other characters (Bayoumi *et al.*, 2008). These studies coincided with our result where proline and RWC both had high heritability.

High genetic gain was found in most of the characters except leaf temperature which was having only 0.48 per cent gain over the base population. High genetic gain among the hybrids was shown by proline indicating197.54 per cent gain if selection of this parameter was done followed by transpiration rate having 156.99 per cent gain, NRA (108.49 %), relative water content (76.05 %), photosynthesis (69.06 %), superoxide dismutase (41.55 %), cell membrane stability (37.70 %), chlorophyll content (33.06 %) and chlorophyll stability index (32.91 %) indicating that all these characters were controlled by additive gene action. The additive genes are highly heritable in nature and selection of a character is effective (Hill, 2010).

It was observed that out of these five parameters (proline, NRA, relative water content, photosynthesis and cell membrane stability) which showed direct effect on the percentage of leaves retained, only four were having high heritability and genetic gain. The parameter cell membrane stability was having comparatively low heritability and genetic gain. Hence, the four parameters i.e., proline, NRA, RWC and photosynthetic rate were selected for further analysis.

Table 32. Genetic components of the hybrids for various biochemical and physiological parameters

Characters	Phenotypic variance (σ^2p)	Genetic variance(σ^2g)	Environmental variance (σ^2e)	PCV (%)	GCV (%)	Heritability (H^2)	Genetic Gain (%)
Proline(µg/g)	270502.039	269912.251	589.788	96.10	96.00	99.78	197.54
Nitrate reductase activity (mmol nitrate/g/hr)	10.961	10.514	0.447	54.90	53.74	95.92	108.49
Superoxide dismutase (units/mg protein/g)	0.005	0.003	0.002	32.27	25.20	62.50	41.55
Glycine betaine (µmol/g)	2.974	2.375	0.599	22.58	20.22	79.86	37.15
Chlorophyll stability Index (%)	195.687	137.007	58.680	22.82	19.10	70.01	32.91
Cell membrane Stability (%)	251.663	181.522	70.141	25.37	21.55	72.13	37.70
Relative water content (%)	396.488	351.667	44.821	41.62	39.20	88.70	76.05
Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)	0.095	0.089	0.006	35.77	34.61	93.71	69.06
Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)	0.372	0.369	0.003	76.83	76.58	99.19	156.99
Chlorophyll content(SPAD units)	49.118	35.542	13.576	22.18	18.86	72.36	33.06
Leaf temperature (°C)	15.351	0.299	15.052	11.90	1.66	1.95	0.48

PCV – Phenotypic coefficient of variation (%) GCV – Genotypic coefficient of variation (%)

4.8. Combining ability studies on hybrids

Analysis of combining ability had been used in practical crop improvement programmes to determine the relative importance of general combining ability (GCA) of the parents and specific combining ability (SCA) of the crosses. Each parent for hybridization programme, differ in their ability to combine with other parent. Similarly each cross combination differs with respect to their specific combining ability to express the performance when compared to other crosses (Cockerham, 1961). Estimation of GCA and SCA variances (Table 33) and their effects helps the researchers to find which lines can be used as suitable parents in hybridization programmes, in order to develop superior hybrids. GCA accounts for additive gene action whereas SCA is the manifestation of non-additive component (Singh *et al.*, 2011).

4.8.1. General combining ability effects

4.8.1.1. Proline

Positive significant GCA effect was shown by G VI 55 (66.642) and G II 19.5 (48.39). However, negative significant GCA effect was exhibited by parent M 13.12 (-71.468) and G I 5.9 (-41.564) (Table 34).

4.8.1.2. Nitrate reductase activity

Negative significant GCA effect was shown by M 13.12 (-0.456) and rest of the parents had non-significant effects for nitrate reductase enzyme (Table 34).

4.8.1.3. Superoxide Dismutase

Positive significant GCA effect was manifested by the parent G I 5.9 (0.010) and negative significant GCA effect was shown by G II 19.5 (-0.010). The other two parents showed non-significant effects (Table 34).

4.8.1.4. Glycine betaine

In this character, only G I 5.9 showed significant yet negative GCA effect (-0.193) and rest of the other parents were non-significant regarding this parameter (Table 34).

4.8.1.5. Chlorophyll stability Index

The parent M 13.12 was having significant GCA effect (39.002) for the parameter and rest of the three parents were having non-significant effects (Table 34).

4.8.1.6. Cell membrane stability

G I 5.9 recorded positive significant GCA effect (1.444) for cell membrane stability. However, parent M 13.12 recorded negative yet significant GCA effect for the character (-2.716) (Table 34).

4.8.1.7. Relative water content

G II 19.5 (4.391) and G VI 55 (3.626) recorded positive and significant GCA effects. However, significant yet negative GC effects were shown by parents M 13.12 (-4.482) and G I 5.9 (-3.535) (Table 34).

4.8.1.8. Photosynthesis

The parent M 13.12 showed positive and significant GCA effect (0.057) whereas the parent G VI 55 showed significant yet negative GCA effect for the character (-0.071). Rest two parents G I 5.9 and G II 19.5 were having non-significant effects for this parameter (Table 34).

4.8.1.9. Transpiration rate

Parents M 13.12 (0.105) and G I 5.9 (0.048) showed positive and significant GCA effect for transpiration rate. However, the other two parents G II 19.5 (-0.115) and G VI 55 (-0.037) showed significant negative effect for the parameter (Table 34).

4.8.1.10. Chlorophyll content

M 13.12 had positive significant GCA effect (1.937) on the chlorophyll content. However, G II 19.5 had significant yet negative GCA effect (-2.394) whereas the other two parents G I 5.9 and G VI 55 had non-significant effects when chlorophyll content was considered (Table 34).

4.8.1.11. Leaf temperature

Positive significant GCA effects were found in M 13.12 (0.411) and G I 5.9 (0.389) for the leaf temperature parameter. However, significant negative GCA effects were found in G II 19.5 (-0.464) and G VI 55 (-0.336) (Table 34).

GCA effect of parents for all the parameters were scored and summarized in table 35. From this, it can be concluded that the parents G VI 55 and G II 19.5 were good combiners as both displayed significant GCA effects followed by the parent G I 5.9 (Table 35). High GCA estimates indicated about the gene flow from parents to off-springs at high frequency and gives information about the concentration of predominantly additive genes. Studies propose that when genotypes with greater estimates of GCA are used in hybridization, the resulting crosses will be superior for selection of lines in the advanced generation. According to Cruz, *et al.* (2004), the GCA estimates provide information on the concentration of predominantly additive gene in its effects and are highly useful in identifying parents to be used in breeding programs. Thus, the higher the estimated of GCA, the higher the frequency of favourable alleles, and thus the greater the increase in traits with a particular behaviour (Krause *et al.*, 2012). Many studies have been done to improve the yield parameter of crops by using the parents in recombination breeding programs to accumulate suitable genes responsible for it (Golabadi *et al.*, 2015).

Moreover, selection for GCA capitalizing on additive gene action has been advocated for improving cacao, for better adaptation to its rapidly changing production environment (Pires *et al.*, 1996).

Table 33. Analysis of variance for combining ability of hybrids for biochemical and physiological characters

Source	Degrees of freedom	Mean sum of squares										
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Due to GCA	3	313747.00**	12.619**	0.008**	2.023**	6.956	78.89**	0.039**	425.527**	0.361**	1.381**	6.409**
Due to SCA	2	134131.36**	7.132**	0.005**	1.536**	53.221	149.238**	0.105**	13.997**	0.423**	37.603**	16.048**
Error	10	18.35	0.358	0.0004	0.039	1.631	0.813	0.00033	0.592	0.00166	0.079	0.063
σ^2gca		156864.325	6.130	0.0038	0.992	2.662	39.03	0.0193	212.46	0.179	0.651	3.173
σ^2sca		134113.01	6.774	0.0046	1.497	51.59	148.425	0.104	13.405	0.421	36.952	15.98
σ^2gca/ σ^2sca		1.16	0.904	0.826	0.662	0.051	0.262	0.185	15.84	0.425	0.017	0.198

C1- Proline (µg/g)

C2- NRA (mmol nitrate/g/hr)

C3- SOD (units/mg protein/g)

C4- Glycine betaine (µmol/g)

C5- Chlorophyll stability index (%)

C11- Leaf temperature (°C)

C6- Membrane stability (%)

C7- Photosynthesis (µmol CO_2 m^{-2} s^{-1})

C8- Relative water content (%)

C9- Transpiration rate (mmol H_2O m^{-2} s^{-1})

C10- Chlorophyll content (SPAD units)

Table 34. General combining ability of parents

Characters	M 13.12	G I 5.9	G II 19.5	G VI 55
Proline (µg/g)	-71.468**	-41.564**	48.390**	66.642**
NRA (mmol nitrate/g/hr)	-0.456*	-0.082	0.399	0.139
SOD (units/mg protein/g)	0.005	0.010**	-0.010**	-0.005
Glycine betaine (µmol/g)	0.100	-0.193*	0.023	0.070
CSI (%)	39.002*	-13.161	-13.878	-11.963
CMS (%)	-2.716**	1.444**	0.789	0.483
RWC (%)	-4.482**	-3.535**	4.391**	3.626**
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	0.057**	0.029	-0.014	-0.071**
Transpiration rate (mmol H_2O m^{-2} s^{-1})	0.105**	0.048**	-0.115**	-0.037**
Chlorophyll content (SPAD units)	1.937**	0.208	-2.394**	0.248
Leaf temperature (°C)	0.411**	0.389**	-0.464**	-0.336**

**Significant at 0.05 percent level = 2.101

**Significant at 0.01 percent level = 2.878

Table 35. Score chart for general combining ability

Parents	Proline (µg/g)	NRA (mmol nitrate/g/hr)	Relative water content (%)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Score	Rank
M13.12	-1	-1	-1	1	-2	4
G I 5.9	-1	0	-1	0	-2	3
G II 19.5	1	0	1	0	2	1
G VI 55	1	0	1	-1	1	2

4.8.2. Specific combining ability effects

4.8.2.1. Proline

Positive SCA effects for proline were exhibited by crosses G II 19.5 x G VI 55 (548.370) being the highest, followed by G I 5.9 x G VI 55 (225.063), M 13.12 x G II 19.5 (212.530) and M 13.12 x G VI 55 (76.566). Negative yet significant SCA effect was shown by G I 5.9 x G II 19.5 (-92.609) whereas the cross M 13.12 x G I 5.9 had non- significant effects for the character (Table 36).

4.8.2.2. Nitrate Reductase Activity

Two crosses G II 19.5 x G VI 55 (3.158) and G I 5.9 x G VI 55 (0.995) were having positive significant SCA effects whereas the cross G I 5.9 x G II 19.5 was having significant yet negative effect (-1.215) and the other crosses were non-significant in nature indicating less combining ability with each other (Table 36).

4.8.2.3. Superoxide Dismutase

Positive significant SCA effects were found in crosses M 13.12 x G II 19.5 (0.057), M 13.12 x G VI 55 (0.026), M 13.12 x G I 5.9 (0.023) and G I 5.9 x G VI 55 (0.017).However, negative yet significant SCA effect was shown by the cross G II 10.5 x G VI 55 (-0.067) whereas the cross G I 5.9 x G II 19.5 was having no significant effect (Table 36).

4.8.2.4. Glycine betaine

Significant yet negative SCA effects were shown by crosses M 13.12 x G I 5.9 (-1.237) and M 13.12 x G VI 55 (-0.520) whereas rest of the crosses had non-significant effects (Table 36).

4.8.2.5. Chlorophyll stability Index

None of the crosses had any significant SCA effect for the parameters studied (Table 36).

4.8.2.6. Cell membrane stability

Three crosses M 13.12 x G II 19.5 (7.648), G II 19.5 x G VI 55 (2.586) and G I 5.9 x G VI 55 (2.102) were having positive significant SCA effects yet the other three crosses M 13.12 x G VI 55 (-2.432), G I 5.9 x G II 19.5 (-6.941) and M 13.12 x G I 5.9 (-7.316) were having significant but negative effects (Table 36).

4.8.2.7. Relative water content

Significant and positive SCA effects were shown by crosses G II 19.5 x G VI 55 (4.968) and G I 5.9 x G II 19 5 (2.368). Negative significant SCA effects was exhibited by cross M 13.12 x G I 5.9 (-7.796) and the other two crosses had non-significant effects (Table 36).

4.8.2.8. Photosynthesis

Positive significant SCA effects were found with cross M 13.12 x G I 5.9 (0.278) being the highest, followed by cross G II 19.5 x G VI 55 (0.201) and G I 5.9 x G VI 55 (0.171). Negative yet significant SCA effects were shown by the cross M 13.12 x G VI 55 (-0.069) (Table 36).

4.8.2.9. Transpiration rate

The crosses having positive and significant SCA effects were M 13.12 x G I 5.9 (0.592) and G II 19.5 x G VI 55 (0.051). However, significant negative SCA effects were found in crosses M 13.12 x G VI 55 (-0.175), G I 5.9 x G II 19.5 (-0.128), M 13 12 x G II 19.5 (-0.127) and G I 5.9 x G VI 55 (-0.121) (Table 36).

4.8.2.10. Chlorophyll content

Significant positive SCA effect was shown by G II 19.5 x G VI 55 (5.888) followed by cross M 13.12 x G II 19.5 (2.393). Negative yet significant SCA effect was shown by cross M 13.12 x G VI 55 (-3.390). The other two crosses has no significant effect (Table 36).

4.8.2.11. Leaf temperature

Positive significant SCA effect was observed in M 13.12 x G I 5.9 (2.694) and also G II 19.5 x G VI 55 (0.744). However, significant yet negative SCA effect was observed in M 13.12 x G VI 55 (-1.564), G I 5.9 x G II 19.5 (-1.032), M 13.12 x G II 19.5 (-0.963) and G I 5.9 x G VI 55 (-0.806) (Table 36).

The effect of the specific combining ability is interpreted as the deviation of a cross compared to what would be expected based on the GCA of their parents (Griffing, 1956).

A study conducted in cowpea under drought stress showed that both additive and non-additive genetic effects were responsible for the inheritance of drought adaptation traits. Non-additive genetic effects were having comparative importance along with additive genetic effects implying that the performance of progenies was better in specific crossing combinations but could not be predicted for a wide range of crosses. Therefore, improvement of drought adaptation traits through selection of crosses with high positive SCA effects and advancing them to later generation would be effective (Mwale *et al.*, 2017).

The result of SCA analysis was scored and summarized in Table 37. Here, the best specific combiners were the cross G II 19.5 x G VI 55 having the maximum significant positive effects for the character followed by G I 5.9 x G VI 55. The two crosses M 13.12 x G I 5.9 and M 13.12 x G II 19.5 were having the same range of effects. The crosses having least specific combining ability were M 13.12 x G VI 55 and G I 5.9 x G II 19.5 (Table 37).

The From these results, the final conclusion drawn was that parents G II 19.5 had the highest general combining ability followed by G VI 55 and G I 5.9 (Table 35). However, G VI 55 when used as a female parent and M 13.12 as male parent, the cross was incompatible and no fruits were obtained even though 640 flowers were pollinated, showing some cross incompatibility. Like self-incompatibility, cross incompatibility is also reported in cocoa (Lockwood, 1977; de Nettancourt, 1993).

Table 36. Specific combining ability of the hybrids

Characters	T1	T2	T3	T4	T5	T6
Proline (µg/g)	-0.071	212.530**	76.566**	-92.609**	225.063**	548.37**
NRA (mmol nitrate/g/hr)	-0.647	-0.085	-0.588	-1.215**	0.995*	3.158**
SOD (units/mg protein/g)	0.023**	0.057**	0.026**	0.001	0.017**	-0.067**
Glycine betaine (µmol/g)	-1.237**	0.077	-0.520**	0.016	0.062	0.206
CSI (%)	-36.687	-42.699	-41.602	11.633	11.017	16.072
CMS (%)	-7.316**	7.648**	-2.432**	-6.941**	2.102*	2.586**
RWC (%)	-7.796**	0.099	0.720	2.368**	-0.413	4.968**
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	0.278**	0.031	-0.069*	0.018	0.171**	0.201**
Transpiration rate (mmol H_2O m^{-2} s^{-1})	0.592**	-0.127**	-0.175**	-0.128**	-0.121**	0.051**
Chlorophyll content (SPAD units)	1.434	2.393*	-3.390**	0.698	-0.151	5.888**
Leaf temperature (°C)	2.694**	-0.963**	-1.564**	-1.032**	-0.806**	0.744**

**Significant at 0.05 percent level = 2.101 **Significant at 0.01 percent level = 2.878

T1- M 13.12 x G I 5.9

T2- M 13.12 x G II 19.5

T3- M 13.12 x G VI 55

T4- G I 5.9 x G II 19.5

T5- G I 5.9 x G VI 55

T6- G II 19.5 x G VI 55

Table 37.Score chart for specific combining ability

Crosses	Proline (µg/g)	NRA (mmol nitrate/g/hr)	RWC (%)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Score	Rank
M13.12 x G I 5.9	0	0	-1	1	0	4
M 13.12 x G II 19.5	1	0	0	0	1	3
M 13.12 x G VI 55	1	0	0	-1	0	5
G I 5.9 x G II 19.5	-1	-1	1	0	-1	6
G I 5.9 x G VI 55	1	1	0	1	3	2
G II 19.5 x G VI 55	1	1	1	1	4	1

4.9. Comparison between the morphological and physiological observations of the forward crosses

Six forward crosses were evaluated based on their phenotypic response to drought tolerance and result was explained in Table 38. Per cent of tolerant plants were estimated and ranked and it was seen that cross M 13.12 x G II 19.5 ranked first position followed by G I 5.9 x G VI 55 and G II 19.5 x G VI 55 in second and third position respectively (Table 38).

The same crosses (M 13.12 x G II 19.5, G I 5.9 x G VI 55 and G II 19.5 x G VI 55) exhibited high SCA (Table 37) indicated that the actual result obtained was in tune with the statistical estimate.

Table 38. Phenotypic response to drought tolerance of forward cross hybrids

Crosses	Total number of plants	Number of tolerant plants	Number of susceptible plants	Percentage of tolerant plants	Rank
M 13.12 x G I 5.9	32	14	18	43.75	4
M 13.12 x G II 19.5	10	6	4	60.00	1
M 13.12 x G VI 55	9	3	6	33.33	6
G I 5.9 x G II 19.5	13	5	8	38.46	5
G I 5.9 x G VI 55	8	4	4	50	2
G II 19.5 x G VI 55	2	1	1	50	3

Table 39. Nature of gene action by GCA to SCA variances

	Characters										
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**
σ^2gca	156864.325	6.130	0.0038	0.992	2.662	39.03	0.0193	212.46	0.179	0.651	3.173
σ^2sca	134113.01	6.774	0.0046	1.497	51.59	148.425	0.104	13.405	0.421	36.952	15.98
σ^2gca/ σ^2sca	**1.160**	**0.904**	0.826	0.662	0.051	0.262	**0.185**	**15.84**	0.425	0.017	0.198

C1- Proline (µg/g)

C2- NRA (mmol nitrate/g/hr)

C3- SOD (units/mg protein/g)

C4- Glycine betaine (µmol/g)

C5- Chlorophyll stability index (%)

C11- Leaf temperature (°C)

C6- Membrane stability (%)

C7- Photosynthesis (µmol CO_2 m^{-2} s^{-1})

C8- Relative water content (%)

C9- Transpiration rate (mmol H_2O m^{-2} s^{-1})

C10- Chlorophyll content (SPAD units)

Table 40. Selection criteria based on the selected characters

Characters	σ^2gca/σ^2sca	Direct effect (P. A.)	H^2 (%)	h^2 (%)	Genetic gain (%)	Selection criteria for
Proline (µg/g)	1.16	0.387	99.78	53.80	197.54	Population improvement and Heterosis breeding
NRA (mmol nitrate/g/hr)	0.904	0.166	95.92	45.91	108.49	Population improvement and Heterosis breeding
R.W.C (%)	15.84	0.121	88.70	78.48	76.05	Population improvement and Heterosis breeding
Photosynthesis (µmol CO_2 m^{-2} s^{-1})	0.185	0.133	93.71	14.96	69.06	Heterosis breeding

4.10. Nature of gene action

The additive and non- additive gene action was estimated by finding out the ratio of GCA and SCA variances and the highest σ^2gca/σ^2sca ratio was found in relative water content having about 15.84 indicating the predominance of additive gene action. This was followed by proline (1.160), nitrate reductase (0.904), SOD (0.826), glycine betaine (0.662), transpiration rate (0.425), membrane stability (0.262), leaf temperature (0.198), photosynthesis (0.185), chlorophyll stability index (0.051) and chlorophyll content (0.017). The four parameters selected for assessing drought tolerance in cocoa had ratios more than or near to unity (Table 39). The photosynthetic rate had a very low value of 0.185 which means there was a preponderance of non-additive gene action. However, photosynthetic rate expressed high heritability and genetic gain estimate values and hence, this parameter can be used for heterosis breeding (Singh and Narayanam, 2009). The other three characters (proline, nitrate reductase activity and relative water content) can be used both for population improvement and for heterosis breeding (Table 40).

4.11. Selection criteria for drought tolerant cocoa hybrids

A selection criteria was developed considering the four parameters (proline, nitrate reductase activity, relative water content and photosynthetic rate) based on the statistical estimate and represented in table 41. These can be used to screen plants when drought stress is imposed.

Table 41. Selection criteria for drought imposed hybrids

Sl no.	Characters	Range	Purpose
1.	Proline (μg/g)	> 410	Population improvement and Heterosis breeding
2.	NRA (mmol nitrate/g/hr)	> 6	Population improvement and Heterosis breeding
3.	RWC (%)	> 40	Population improvement and Heterosis breeding
4.	Photosynthesis (μmol CO_2 m^{-2} s^{-1})	> 0.700	Heterosis breeding

Recent progress in genetic improvement of crop drought tolerance by conventional breeding and molecular techniques have contributed in various drought breeding programmes. At the same time, it also undermined the urgent need for standard evaluation assays and selection criteria, especially when climate change and unpredictable rainfall patterns had increased worries over drought (Campos *et al.*, 2004). There have been many studies conducted in crops for selecting particular characters which will enable us to identify between drought tolerant and susceptible genotypes. Some of them are discussed below.

A study was undertaken to investigate plant traits which are associated with drought tolerance in bread wheat and to determine selection criteria for selecting genotypes tolerant to drought stress. Two wheat genotypes were evaluated under drought and irrigated conditions with three replications. Based on the correlation studies, the selection for drought tolerant plants were classified based on the characters like high biological yield, grain weight/spike, harvest index and tillers/plant for yield improvement under dry conditions (Chander and Singh, 2008).

Another study was conducted in maize in which the aim was to develop a selection criteria for identifying drought tolerant maize plants during early stages of growth and the parameters selected were root length, root fresh weight, shoot length, number of root branches, shoot dry weight, root dry weight and number of shed leaves (Akinwale *et al*., 2015).

The hybrids having a value of more than 410 μg/g proline, NRA having more than 6 mmol nitrate/g/hr, relative water content of more than 40 per cent and photosynthetic rate crossing 0.700 μmol CO_2 m^{-2} s^{-1} can be identified as drought tolerant cocoa hybrid after imposing stress. Photosynthetic rate can be used as a selection parameter for hybrid development programmes whereas the other three can be used for population improvement as well as heterosis breeding as these three characters can be transferred onto the progenies (Table 41).

4.12. Binary regression on the selected characters of the hybrids

Binomial logistic regression model was used to predict the effect of contributing characters to drought tolerance in hybrids. The characters which experienced high direct effect (the characters showing direct effect in contribution to number of leaves retained and hence, more tolerant to drought stress) along with high heritability and genetic gain were selected to perform the binary regression analysis. The main characters selected were proline, nitrate reductase activity and relative water content. Although photosynthesis was a selection criteria, regression analysis for the character was not done as the character showed less additive gene action (Table 40). Hence, this character cannot show any improvement in the next generation over the base population.

Phenes influencing drought tolerance and possible improvement for tolerance over the base population if these phenes are considered for selection is explained in Figure 123. The positive and comparable value of odds ratio Exp (B) and positive correlation indicated that proline content, NRA content and relative water content had a positive correlation with drought tolerance and also these characters expressed a significant value of less than 0.05 which is the constant indicating the 95 per cent accuracy with the results. The results are depicted in Table 42.

Based on the Exp (B) value from regression model, expressed percentage for drought tolerance over the base population was calculated and it was found that if selection is based on proline, new population formed from the base population will express 51 per cent improvement regarding the tolerance. In case of NRA, new population will show 87.48 per cent improvement and relative water content will show about 51.87 per cent improvement over the base population regarding the drought tolerance (Table 42).

Table 42. Logistic estimate of characters influencing drought tolerance in cocoa

Variables	Coefficient	Standard error	Wald	Significance	Exp(B)	Expected per cent improvement over population (%)
Proline**	0.062	0.011	31.019	0.001	1.064	51.55
Nitrate reductase activity**	1.944	0.233	69.914	0.001	6.990	87.48
Relative water content**	0.075	0.008	87.803	0.002	1.078	51.87
Photosynthesis**	12.819	1.328	84.290	0.001	1.967	66.29

**Significant value less than 0.05

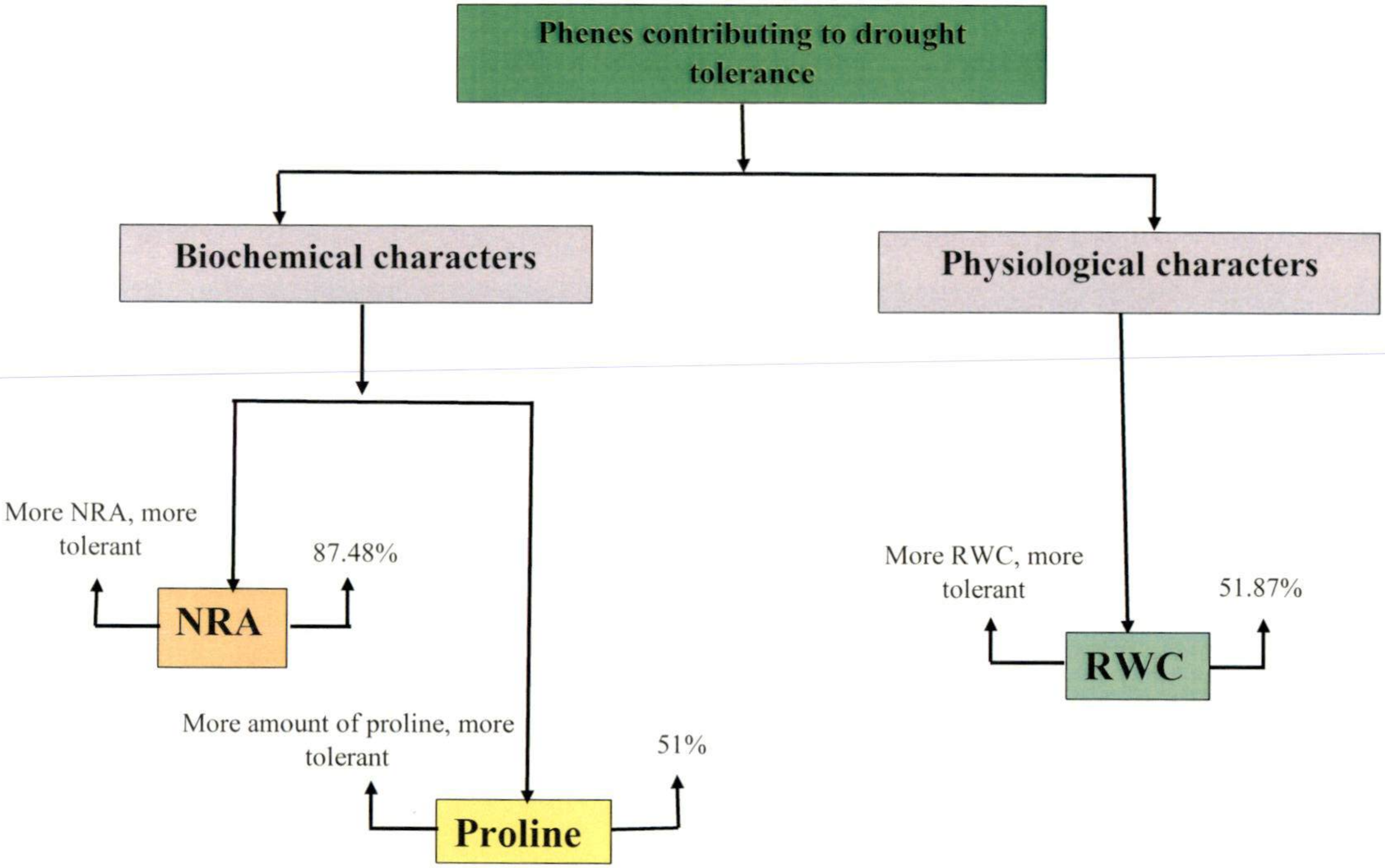

Fig. 123. Phenes and their association with drought tolerance in cocoa

Summary

V. SUMMARY

The study entitled "Breeding for drought tolerance in Cocoa (*Theobroma cacao* L.)" was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Cocoa Research Centre, Vellanikkara and College of Forestry, Vellanikkara during the period between 2016 - 2018. In the present study, an attempt was done to exploit the drought tolerant genotypes identified in KAU for the production of drought tolerant hybrids. Four genotypes identified to be tolerant to drought in preliminary studies conducted at Cocoa Research Centre (CRC), by screening existing germplasm formed the base material for the study.

The salient findings are summarized below:

Experiment I: Crossing between the clones

- The base material identified as drought tolerant genotypes from previous study (M 13.12, G I 5.9, G II 19.5 and G VI 55) were used as parents
- They were crossed manually in all possible combination by the method described by Mallika *et al*. (2002)
- Only one cross, G VI 55 x M 13.12 did not yield any pods even though 640 flowers were pollinated. More than 85 per cent germination was observed in all successful crosses and a total of 1593 seeds were sown
- In total, 1505 seedlings germinated and each cross took 7-10 days for half of the seeds to germinate. The pods matured within 5-6 months which were then sown in the nursery and each were labelled with the parentage and date of pollination

Experiment II: Screening for initial vigour

- At the third month, all the 1505 seedlings were screened for their Height x Diameter ^{2} (HD^2) value which stated that hybrids having high HD^2 value at their initial phase tend to produce high yield and vigour at their later stages
- Hence out of this 1505 seedlings, 120 hybrids were selected and these were then transferred to the drought chamber at their fifth month stage

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- Drought stress was imposed following the gravimetric method for two weeks. Field capacity was maintained at 40 per cent.

Experiment III: Screening for drought tolerance

- After two weeks, based on the number of leaves retained on the plant, they were categorised into four categories: Highly tolerant, tolerant, susceptible and highly susceptible. Various biochemical and physiological analysis were carried out in drought imposed plants
- A control representing each cross were kept at fully irrigated condition
- The biochemical parameters were proline (µg/g), superoxide dismutase (units/mg protein/g), nitrate reductase activity (mmol nitrate/g/hr) and glycine betaine (µmol/g)

  The parameters proline and glycine betaine represented osmolyte group whereas nitrate reductase and superoxide dismutase represented enzyme group. In all the crosses, the content of proline was high in highly tolerant and tolerant hybrids as compared to susceptible hybrids. The control which was fully irrigated condition was having the least amount of proline. The glycine betaine also followed the same trend, as tolerant and highly tolerant hybrids were having more amount of glycine betaine as compared to susceptible hybrids and the control indicating that these two osmolytes accumulated only during water stress. In case of superoxide dismutase, the highly tolerant and tolerant hybrids were having high amount of superoxide dismutase as compared to the susceptible hybrids whereas the control was having the least amount of superoxide dismutase enzyme. In case of nitrate reductase activity, the highly tolerant sand tolerant hybrids were having high amount of enzyme as compared to the susceptible hybrids. The control kept at fully irrigated condition was having the highest amount of nitrate reductase enzyme. The hybrids having high amount of nitrate reductase were more tolerant because generally, this enzyme reduces under drought stress

- The physiological parameters observed were chlorophyll stability index (%), membrane stability (%), relative water content (%), photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), leaf temperature (°C) and chlorophyll content (SPAD units). In case of chlorophyll stability index, highest value was observed in control under fully irrigated condition, followed by highly tolerant and tolerant hybrids. The least value was observed in susceptible hybrids indicating it's susceptibility to drought tolerance. In case of membrane stability, the highly tolerant as well as the tolerant hybrids were having high membrane stability as compared to susceptible hybrids whereas the control kept at fully irrigated condition was having highest amount of membrane stability. The highly tolerant and tolerant hybrids in case of photosynthetic rate showed higher amount as compared to susceptible hybrids whereas the control was having the highest photosynthetic rate. In case of relative water content, the control was having the highest amount of relative water content kept at fully irrigated condition followed by highly tolerant as well as tolerant hybrids whereas the susceptible hybrids were having the least amount of relative water content. The transpiration rate followed a different trend as the susceptible hybrids were having the highest amount comparable along with control which was kept at fully irrigated condition. The highly tolerant and tolerant hybrids were having the least amount of transpiration rate indicating their capacity to reduce water stress. The chlorophyll content when observed was having higher amount in case of highly tolerant as well as tolerant hybrids whereas it was comparatively lower in case of susceptible hybrids. The control at fully watered condition was having the highest chlorophyll content. The leaf temperature, although in many studies have been known to play an important role in drought assessment, expressed no significant variation among tolerant and susceptible hybrids in the present study. The highly tolerant, tolerant, susceptible hybrids as well as the control expressed similar value for leaf temperature and hence, it was not a reliable parameter in assessing drought tolerance in cocoa

- Correlation studies were conducted and it was observed that out of the eleven characters, nine characters except leaf temperature and transpiration rate were having significant and positive correlation with the morphological observation i.e., percentage of leaves retained and hence, these characters were in a way contributing to drought tolerance
- To find out whether these characters were having any direct effect on the percentage of leaves retained, path analysis was carried out and it was seen that out of these eleven characters, five characters *viz.*, proline, nitrate reductase activity, cell membrane stability, photosynthetic rate and relative water content were having direct effect on the morphological observation indicating their role in drought tolerance
- Genetic parameters when analysed, it was observed that out of these five characters having direct effects, four characters *viz.*, proline, nitrate reductase activity, relative water content and photosynthetic rate were having high heritability and high genetic gain
- Combining ability studies were conducted following the half-diallel mating system. Method II was followed as the parents and direct crosses were selected for analysis and model I was followed as the parents were fixed for mating. General combining ability for the parents were carried out by taking into consideration the four characters having high heritability and genetic gain. The best general combiners were G II 19.5 and G VI 55. When the specific combining ability for the cross combinations were analysed, it was found that G II 19.5 x G VI 55 was the best specific combiner followed by G I 5.9 x G VI 55 and M 13.12 x G II 19.5
- Phenotypic ranking was done in order to assess which cross combination resulted in more number of tolerant hybrids. The result was in tune with the cross combinations identified as best specific combiners by employing statistical tool conferring G II 19.5 x G VI 55, G I 5.9 x G VI 55 and M 13.12 x G II 19.5 as best combiners
- Further, gene action analysis was carried out for the characters and high additive gene action was showed by proline, nitrate reductase activity and

relative water content and hence, they can be chosen for population improvement and heterosis breeding whereas photosynthetic rate showed non- additive gene action indicating it's importance in heterosis breeding

- Based on these four characters, a selection criteria was designed as follows: proline (> 410 µg/g), nitrate reductase activity (> 6 mmol nitrate/g/hr), relative water content (> 40%) and photosynthetic rate (> 0.700 µmol CO_2 m^{-2} s^{-1}) and can be used for screening cocoa genotypes under drought stress
- To find out how these three characters that are having high additive gene action can bring improvement in the next generation, a binary regression analysis was done and it indicated 87.46 per cent of improvement in case of nitrate reductase, 51.87 per cent improvement in case of relative water content and 51 per cent improvement in proline over the base population
- The three best specific combiners can be further used to produce drought tolerant hybrids in cocoa and they can be further evaluated using the selection criteria identified.

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Appendix

APPENDIX I

HD^2 value of the selected hybrids

Sl no.	Cross	Height	Diameter	HD^2
1.	M 13.12 x G I 5.9 (i) 13	34	0.95	30.68
2.	M 13.12 x G I 5.9 (i) 14	37	0.85	26.73
3.	M 13.12 x G I 5.9 (i) 17	36	1.11	44.35
4.	M 13.12 x G I 5.9 (ii) 13	45	0.82	30.25
5.	M 13.12 x G I 5.9 (ii) 17	40	0.89	30.97
6.	M 13.12 x G I 5.9 (ii) 20	48	0.85	33.86
7.	M 13.12 x G I 5.9 (ii) 23	45	0.82	30.25
8.	M 13.12 x G I 5.9 (ii) 26	50	0.89	38.72
9.	M 13.12 x G I 5.9 (ii) 28	45	0.73	23.32
10.	M 13.12 x G I 5.9 (iii) 12	33	0.79	20.59
11.	M 13.12 x G I 5.9 (iii) 18	49	0.82	32.94
12.	M 13.12 x G I 5.9 (iii) 19	57	0.76	32.92
13.	M 13.12 x G I 5.9 (iii) 24	49	0.79	31.36
14.	M 13.12 x G I 5.9 (iii) 35	62	0.79	37.70

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15.	M 13.12 x G I 5.9 (iv) 4	47	0.95	41.52
16.	M 13.12 x G I 5.9 (iv) 7	45	0.95	40.61
17.	M 13.12 x G I 5.9 (iv) 22	37	0.79	23.09
18.	M 13.12 x G I 5.9 (iv) 31	39	0.78	23.72
19.	M 13.12 x G I 5.9 (iv) 33	42	0.79	26.21
20.	M 13.12 x G I 5.9 (v) 4	32	0.82	21.51
21.	M 13.12 x G I 5.9 (v) 17	29	0.92	24.50
22.	M 13.12 x G I 5.9 (v) 19	34	0.79	21.21
23.	M 13.12 x G I 5.9 (v) 20	24	0.92	20.31
24.	M 13.12 x G I 5.9 (v) 30	34.5	1.01	35.19
25.	M 13.12 x G I 5.9 (v) 32	36	0.89	28.5
26.	M 13.12 x G I 5.9 (v) 37	33	0.89	26.13
27.	M 13.12 x G I 5.9 (vi) 6	29	0.85	20.9
28.	M 13.12 x G I 5.9 (vi) 17	38	0.79	23.71
29.	M 13.12 x G I 5.9 (vi) 18	38	0.85	27.45
30.	M 13.12 x G I 5.9 (vi) 32	30	0.82	20.17

31.	M 13.12 x G I 5.9 (vi) 33	30	0.85	21.67
32.	M 13.12 x G I 5.9 (vi) 37	33	0.82	22.18
33.	M 13.12 x G II 19.5 (i) 29	29	0.82	26.22
34.	M 13.12 x G II 19.5 (ii) 4	36	0.47	7.95
35.	M 13.12 x G II 19.5 (ii) 11	60	0.31	6.06
36.	M 13.12 x G II 19.5 (ii) 17	48	0.38	6.93
37.	M 13.12 x G II 19.5 (ii) 24	44	0.47	9.31
38.	M 13.12 x G II 19.5 (ii) 26	49	0.38	7.15
39.	M 13.12 x G II 19.5 (ii) 28	49	0.37	6.70
40.	M 13.12 x G II 19.5 (ii) 39	48	0.38	6.93
41.	M 13.12 x G II 19.5 (iii) 6	52	0.47	11
42.	M 13.12 x G II 19.5 (iii) 21	55	0.47	12.14
43.	M 13.12 x G VI 55 (i) 12	36	0.79	21.90
44.	M 13.12 x G VI 55 (i) 32	43	0.76	26.16
45.	M 13.12 x G VI 55 (ii) 15	39	0.82	26.73
46.	M 13.12 x G VI 55 (ii) 27	43	0.79	27.25

47.	M 13.12 x G VI 55 (iii) 4	31	0.92	26.23
48.	M 13.12 x G VI 55 (iii) 8	36	0.85	26.60
49.	M 13.12 x G VI 55 (iii) 16	36	0.89	27.89
50.	M 13.12 x G VI 55 (iv) 11	47	0.92	40.08
51.	M 13.12 x G VI 55 (iv) 12	40	0.89	30.97
52.	G I 5.9 x M13.12 (i) 2	38	0.89	29.40
53.	G I 5.9 x M13.12 (i) 6	36	0.95	31.80
54.	G I 5.9 x M13.12 (i) 9	38	0.92	32.16
55.	G I 5.9 x M13.12 (i) 13	46	0.82	30.93
56.	G I 5.9 x M13.12 (i) 21	50	0.82	33.62
57.	G I 5.9 x M13.12 (i) 27	39	0.92	33
58.	G I 5.9 x G II 19.5 (ii) 5	39	0.89	30.20
59.	G I 5.9 x G II 19.5 (ii) 8	37	0.47	8.17
60.	G I 5.9 x G II 19.5 (ii) 14	32	0.38	12.25
61.	G I 5.9 x G II 19.5 (ii) 16	27	0.41	4.53
62.	G I 5.9 x G II 19.5 (ii) 17	33	0.47	7.53

63.	G I 5.9 x G II 19.5 (ii) 24	39	0.35	4.775
64.	G I 5.9 x G II 19.5 (ii) 25	28	0.50	7.00
65.	G I 5.9 x G II 19.5 (iii) 10	25	0.47	5.52
66.	G I 5.9 x G II 19.5 (iii) 11	42	0.98	37.11
67.	G I 5.9 x G II 19.5 (iii) 12	35	0.89	27.10
68.	G I 5.9 x G II 19.5 (iii) 20	36	0.82	24.68
69.	G I 5.9 x G II 19.5 (iii) 25	23	0.73	12.25
70.	G I 5.9 x G II 19.5 (iv) 6	29	0.85	20.46
71.	G I 5.9 x G VI 55 (i) 3	46	0.94	40.64
72.	G I 5.9 x G VI 55 (i) 5	46	0.89	36.43
73.	G I 5.9 x G VI 55 (i) 8	45	0.92	38.08
74.	G I 5.9 x G VI 55 (i) 17	40	0.75	22.50
75.	G I 5.9 x G VI 55 (i) 18	46	0.92	39.10
76.	G I 5.9 x G VI 55 (i) 26	46	1.07	52.66
77.	G I 5.9 x G VI 55 (i) 28	38	0.925	32.51
78.	G I 5.9 x G VI 55 (i) 35	54	1.29	90.69

79.	G II 19.5 x M 13.12 (i) 1	42	0.63	17.36
80.	G II 19.5 x M 13.12 (i) 2	46	0.806	29.88
81.	G II 19.5 x M 13.12 (i) 4	45	0.79	28.08
82.	G II 19.5 x M 13.12 (i) 5	39	0.641	16.02
83.	G II 19.5 x M 13.12 (i) 7	46	0.576	15.26
84.	G II 19.5 x M 13.12 (i) 10	51	0.673	23.09
85.	G II 19.5 x M 13.12 (i) 12	56	0.738	30.50
86.	G II 19.5 x M 13.12 (i) 13	55	0.66	23.95
87.	G II 19.5 x M 13.12 (i) 14	53	0.771	31.50
88.	G II 19.5 x M 13.12 (i) 19	52.5	0.710	26.46
89.	G II 19.5 x M 13.12 (i) 21	41	0.621	15.81
90.	G II 19.5 x M 13.12 (i) 22	50	0.739	27.30
91.	G II 19.5 x M 13.12 (i) 23	51.5	0.696	24.90
92.	G II 19.5 x M 13.12 (i) 25	42	0.807	27.35
93.	G II 19.5 x M 13.12 (i) 30	42	0.628	16.56
94.	G II 19.5 x M 13.12 (i) 31	42	0.71	21.17

95.	G II 19.5 x M 13.12 (i) 33	41	0.645	17.05
96.	G II 19.5 x M 13.12 (i) 34	46	0.883	35.86
97.	G II 19.5 x G I 5.9 (i) 8	55	0.826	37.52
98.	G II 19.5 x G I 5.9 (i) 14	60	0.768	35.38
99.	G II 19.5 x G I 5.9 (i) 18	50	0.93	43.24
100.	G II 19.5 x G I 5.9 (i) 25	61	0.76	35.23
101.	G II 19.5 x G I 5.9 (i) 29	57	0.768	33.61
102.	G II 19.5 x G I 5.9 (ii) 10	46	0.70	22.54
103.	G II 19.5 x G I 5.9 (ii) 30	40	0.76	23.10
104.	G II 19.5 x G I 5.9 (iii) 10	27	0.66	11.77
105.	G II 19.5 x G I 5.9 (iii) 12	29	0.92	24.73
106.	G II 19.5 x G I 5.9 (iv) 12	46	0.82	30.92
107.	G II 19.5 x G VI 55 (i) 16	68	0.897	53.61
108.	G II 19.5 x G VI 55 (i) 18	42	1.09	50.26
109.	G VI 55 x G I 5.9 (i) 2	43	0.38	6.28
110.	G VI 55 x G I 5.9 (i) 15	50	0.41	8.40

111.	G VI 55 x G I 5.9 (i) 16	43	0.38	6.20
112.	G VI 55 x G I 5.9 (i) 25	57	0.44	11.03
113.	G VI 55 x G I 5.9 (ii) 6	20	0.41	3.20
114.	G VI 55 x G I 5.9 (ii) 12	40	0.73	21.46
115.	G VI 55 x G II 19.5 (i) 7	24	0.79	26.89
116.	G VI 55 x G II 19.5 (i) 10	30	0.73	16.09
117.	G VI 55 x G II 19.5 (i) 11	33	0.70	16.17
118.	G VI 55 x G II 19.5 (i) 17	42	0.79	26.62
119.	G VI 55 x G II 19.5 (ii) 23	43	0.92	36.39
120.	G VI 55 x G II 19.5 (ii) 40	27	0.66	12.07

Roman letters in brackets- Pod number
Numbers after the brackets- Plant number

Breeding for drought tolerance in cocoa (*Theobroma cacao* L.)

By

Juby Baby

2016-11-042

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

(Plant Breeding and Genetics)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA
2019

ABSTRACT

Drought is considered to be one of the most limiting factors for cocoa production. Preliminary efforts have been made in Kerala Agricultural University to identify drought tolerant cocoa genotypes. In continuation of the same, the present study was proposed to develop superior hybrids tolerant to drought in cocoa.

The study was conducted at Department of Plant Breeding and Genetics, College of Horticulture (CoH), Cocoa Research Centre and College of Forestry, KAU. Four genotypes *viz.* M 13.12, G I 5.9, G II 19.5 and G VI 55 identified as drought tolerant in previous study were taken as parent materials. They were hand pollinated in all possible combinations. Hybrid pods were obtained in all the crosses except in G VI 55 x M 13.12 and successful crosses showed more than 85 per cent germination.

Since initial vigour was found to be correlated with final yield, the seedlings were screened at the third month stage for their Height x Diameter^2 (HD^2) value. Based on the seedling vigour, 1505 hybrids were screened representing all the crosses. During the fifth month, they were screened for drought tolerance by maintaining the soil at 40 per cent field capacity for two weeks and a total of 120 hybrids were selected. Based on the percentage of leaves retained on the hybrids, the plants were classified into four categories: highly tolerant (more than 70 % leaves retained), tolerant (40-70 % leaves retained), susceptible (10-40 % leaves retained) and highly susceptible (less than 10 % leaves retained). Hybrids maintained under full irrigation were taken as control. Various biochemical and physiological parameters related to drought were observed.

Proline, glycine betaine and superoxide dismutase were found to be high in tolerant and highly tolerant hybrids and was low in susceptible hybrids. The control was found to have very less amount of proline, glycine betaine and SOD. However, under drought conditions, plants showed reduced nitrate reductase activity. The control plants showed highest nitrate reductase activity.

In case of physiological parameters, high chlorophyll stability index, membrane stability, relative water content, photosynthetic rate and chlorophyll content were recorded in all tolerant and highly tolerant hybrids whereas these parameters were comparatively low in susceptible hybrids. The control plants showed high value for all these characters. However, in case of transpiration rate, a reverse trend was observed. The highly tolerant and tolerant hybrids showed low transpirational rate, whereas the susceptible hybrids had high transpirational rate. The control also had high transpirational rate. It indicated the ability of tolerant hybrids to conserve water during drought stress. The leaf temperature did not show any variation among the hybrids.

Correlation studies showed that all physiological and biochemical characters except transpiration rate and leaf temperature have positive correlation with percentage of leaves retained. Effect of these characters on dependent variable i.e., percentage of leaves retained was estimated by path analysis. Characters having direct effect on leaf retention were proline, nitrate reductase activity, membrane stability, photosynthetic rate and relative water content. These characters were analysed for their genetic parameters. It was found that except membrane stability, all other charcters were having high heritability and genetic gain.

Among the four characters considered, proline, nitrate reductase activity and relative water content were having additive gene action and hence, were suitable as selection parameters for population improvement and heterosis breeding. Photosynthetic rate which was having comparatively less additive gene action, can be exploited in heterosis breeding. Based on these four characters, a selection criteria was designed (Proline, > 410 μg/g, NRA, > 6 mmol nitrate/g/hr, RWC, > 40% and Photosynthetic rate, > 0.700 μmol CO_2 m^{-2} s^{-1}). Three characters which expressed high additive gene action, were subjected to binomial regression analysis and was found that if nitrate reductase activity when used as a selection criteria, population will experience 87.48 per cent improvement over base population. Similarly, 51.87 per cent and 51.55 per cent improvement will be there with the characters relative water content and proline respectively.

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Combining ability studies indicated that the best general combiners were G VI 55 and G II 19.5 and the best specific crosses were M 13.12 x G II 19.5, G I 5.9 x G VI 55 and G II 19.5 x G VI 55.

The 120 hybrids used in the study are now field planted. They have to be evaluated at their various stages of growth by subjecting to drought stress. For resistant breeding studies, 120 hybrids will not form substantial volume. Hence, more hybrids have to be evolved by using selected best combiners.