
بسم الله الرحمن الرحيم

EVALUATION OF HEAT TOLERANCE POTENTIAL AND ITS ENHANCEMENT IN OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH).

BY

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2006-ag-1947

M.Sc. (Hons.) Agriculture-Horticulture

A thesis submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

INSTITUTE OF HORTICULTURAL SCIENCES

FACULTY OF AGRICULTURE
UNIVERSITY OF AGRICULTURE,
FAISALABAD, PAKISTAN

2016

DECLERATION

I hereby declare that the contents of this thesis **"Evaluation of heat tolerance potential and its enhancement in okra (*Abelmoschus esculentus* L. Moench)"** are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/ equations/ formulas/ protocols etc.). I further declare that this work has not been sent for the award of any other degree/ diploma. The University may take action if the information provided found inaccurate at any stage, (in case of any default, the scholar will be proceeded against as per HEC plagiarism policy).

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To,

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We, the Supervisory Committee, certify that after necessary revision, in accordance to the suggestions made by external reviewers, the contents and form of thesis of **Mr. Rashid Hussain, (Regd. No. 2006-ag-1947)** have been found satisfactory. The final thesis is therefore may kindly be submitted for final notification of the PhD degree.

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To

My beloved parents (Late)

&

The sweet memories of my spiritual father

Prof. Dr. Muhammad Aslam Pervez (Late)

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ACKNOWLEDGEMENTS

I have only pearls of my eyes to admire the blessing of the compassionate and omnipotent because words are bound, knowledge is limited and time is short to express His dignity. **ALMIGHTY ALLAH,** the Propitious, the Benevolent and Sovereign Whose Blessing and Glory flourished my thoughts and thrived my ambitions, giving me affectionate parents, talented teachers, sweet brothers and unique friends.

Trembling lips and wet eyes praise for **HOLY PROPHET HAZRAT MUHAMMAD (P.B.U.H.)** for enlightening our conscience with the essence of faith in ALLAH, converging all His kindness and mercy upon him.

The work presented in this manuscript was accomplished under the sympathetic attitude, fatherly behavior and enlightened supervision of **PROF. DR. MUHAMMAD ASLAM PERVEZ (LATE)** who left for his heavenly journey about one and half year before the submission of this dissertation. May Allah Almighty rest his soul in eternal peace.

The work presented in this manuscript was accomplished under the sympathetic attitude, scholarly criticism, cheering perspective and enlightened supervision of **Dr. Choudhary Muhammad Ayyub,** Associate Professor, Institute of Horticultural Sciences, University of Agriculture Faisalabad.

I am really indebted to **Dr. Muhammad Amjad**, Professor, Institute of Horticultural Sciences University of Agriculture, Faisalabad, **Dr. Ejaz Ahmad Waraich,** Assistant Professor, Department of Agronomy, University of Agriculture, Faisalabad and **Dr. Muhammad Javeed Akhtar,** Associate Professor, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, for their scholastic guidance and help in the planning, execution and completion of this dissertation too.

Friends are the comrades of the battle, the battle to generate knowledge, sift myths and facts and to remove ambiguity. They were co–sharer of my struggle and my work. Company includes: **Dr. Muhammad Rashid Shaheen, Dr. Madeha Butt, Dr. Zaid Mustafa, Mubeen Sarwar, Saif Ali Bhatti, Syed Saddet Hussain and Asif Ghafoor** who always encouraged me to work hard and get forward. I can never forget the sweet memories of the time passed with them. Extreme love, utmost sincerity and caring behavior of my childhood fellows, **Waseem Ahsin, Anam ul Haq and Imran Munir.**

I will be missing my duties if I do not thank my loving sisters, my cute and sweet brothers, nephews and nieces and especially my respected father **Muhammad Siddique (Late)** and my loving mother **Shahmeem Akhtar (Late)** who have stood by me mentally and spiritually during all the years away from home. They always helped me in solving all of my troubles with their love, affection, inspiration, and boosted my moral to fly high to accomplish my goal and every time wished to see me glittering up on the skies of success.

(Rashid Hussain)

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ABSTRACT

Lady's finger or Okra is a member of family malvacae. It is multipurpose crop; its fibrous tender fruits called pods are used as vegetable; seeds, stem and roots used for industrials purposes. In past it was considered as minor crop and no care was taken for its improvement at national and international level in research programs. It grows well at temperature ranges of 18 to 35ºC and gives highest yield. Heat and drought events will be intensified due change in climate, activating modifications in the ecosystem and failure or low productivity of crops which are prone to abiotic stresses and same case is with okra. Major constraint which affects vegetative and reproductive phase of okra is heat stress. Heat stress damage quality as well as yield of okra. The research was conducted with the aim to screen out genetically diverse and improved germplasm while eradicating physiological and genetic basis of better adaptation under thermal stress and enhancement of heat tolerance by foliar application of proline.

In first experiment comparative performance of one hundred okra genotypes was investigated under heat stress condition. For this purpose one hundred (100) okra genotypes were grown under control environmental conditions of high temperature 45/35°C (day/night). Data for morphological attributes (root length, shoot length, shoot and root fresh weight, number of leaves and root and shoot dry weight) and physiological attributes (transpiration rate, stomatal conductance to water, photosynthetic rate, leaf surface temperature, chlorophyll contents, sub-stomatal CO_2 and water use efficiency) was recorded. According to recorded data genotypes were classified on the basis of their performance against heat stress conditions. Under heat stress conditions genotypes showed significant different response and genotypes were divided into heat sensitive and heat tolerant ones. VI051062 and VI060131 were categorized as most heat tolerant and VI046554, while VI048594 were categorized as most heat sensitive ones.

In second experiment twenty five okra genotypes screened out from one hundred okra genotypes in experiment # 1 in growth room, (twenty heat tolerant and five heat sensitive genotypes) were sown in summer 2014, in the field conditions at vegetable research area of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Genotypes were sown at three different sowing dates (02, April, 12, April and 22, April) to check the effect of heat stress on different morpho-physiological and yield attributes. All the cultural practices were kept same for all sowing dates and for all genotypes. There were four replications and each replication contained five plants. In this experiment genotypes VI051062 and VI060131 proved to be most heat tolerant while VI046554 and VI048594 proved to be most heat sensitive under field conditions on the basis of morpho-physiological and yield parameters

In third experiment four okra genotypes, two tolerant (VI051062 and VI060131) and two sensitive (VI046554 and VI048594) selected from experiment # 2, were exposed to heat stress (45/35°C day/night temperature) under controlled environmental conditions, two weeks after exposure to heat stress plants were sprayed with proline (control, 1.0, 1.5, 2.0, 2.5 and 3.0mM) to optimize best dose of proline for enhancement the heat tolerance in okra

genotypes. Morphological and physiological were studied to optimize the best dose of proline for enhancement of heat stress tolerance in okra genotypes. Results revealed that proline application @ 2.5 mM is best for enhancing the heat tolerance potential of okra.

Fourth experiment was carried out to check the effect of optimized dose of proline (in experiment # 3) on the morphological, physiological and biochemical attributes of four okra genotypes two heat tolerant and two heat sensitive, under controlled environmental conditions at high temperature 45/35°C (day/night). It was noted that exogenous application of proline @ 2.5 mM significantly affected morphological, physiological, biochemical, water related and enzymatic attributeswhich in turn enhanced the heat tolerance potential of okra genotypes.

It can be concluded from the study that by sowing the heat tolerant genotypes, identified in research, the growth period of okra can be extended. Exofenous application of proline @ 2.5 mM can further alleviate the drastic effects of high temperature and growing period can be extended.

CHAPTER 1 INTRODUCTION

Okra (*Abelmoschus esculentus* L.) commonly known as bhindi is very famous summer vegetable. It belongs to family malvaceae and thought to be originated from tropical Africa, also grown in the some regions of Mediterranean. Wild forms are also found in India. Now a days it is grown in tropical and temperate parts of the world in summer season (Zeb et al., 2015). According to Kumar et al., (2013) tender fruit production for vegetable proposes; its production is 6 million tons per year worldwide. The area and production for okra in Pakistan is approximately 14147 ha and 1084726 tons, respectively (MNFSR, 2015). Because of its robust nature, dietary fiber and diverse seed protein with the balancing amount of both lysine and tryptophan amino acids; it is also known as "a perfect villagers vegetable" (Gemede et al., 2015). Okra is multipurpose crop; its fibrous tender fruits also called pods, used as vegetable for culinary purpose, for the sugar cane juice cleaning, when seeds ripe; they are roasted and ground for coffee making purpose; crude fiber obtained from ripened fruits and stem are used in industry for paper making. It is documented that edible oil is being extracted from okra seeds. Okra is considered as vital source of carbohydrates, proteins, potassium, water, vitamin, calcium and minerals which are less in the diet of developing countries (Olaniyi et al., 2010; Maurya, 2013). As for as its medicinal value is concerned; it is rich in high vitamin C, folate contents and fiber. It is as good source of potassium, calcium and also has good antioxidant properties (Kumar et al., 2013). Almost every part of okra plant is useful; roots have high contents of mucilage which is strongly demulcent in action. Plasma can be replaced by mucilage. For the treatment of syphilis, the infusion extracted from its roots is used. In Nepal root juice of okra is used for treatment of cuts, boils and wounds. Catarrhal infections, gonorrhea and dysuria are also treated by okra root juice. Seeds have properties like cordial, stimulant and antispasmodic. Sudorific properties are also found in roasted seed of okra (Martin, 1982).

Okra have played vital role in mitigating food insecurity and malnutrition in the developing countries. Its use as indigenous vegetable is being promoted for this purpose. But in past; it was considered as minor crop and no care was taken for it improvement at international level in research programs (Kumar et al., 2010). On the other side vegetable oil demand is increasing rapidly with the increase in human population; also due to industrial revolution with health promoting oil components. Because of presence of some underutilized

and newer resources vegetable oil is being much concerned (Schalau, 2002). Okra seeds contain 20-40% amount of oil; therefore it has potential to grow as oil seed crop as well as vegetable crop (Benchasri, 2012).

Climatic requirements for okra cultivation are reported as it is a hot weather, lowland tropical crop and susceptible to drought or water deficit conditions. Day and night temperature requirement is 25 to 40°C and over 22°C, respectively. In tropics when there is 18^{o}C to 35^{o}C its yield was highest (Ezeakunne, 1984). Another report suggests optimum temperature for high yield of okra as 24 to 30°C. Minimum temperature of soil for good seed germination is 15°C and optimum temperature for germination is 35°C (Ossom and Kunene, 2011). According to Katung, (2007) for good production of okra crop there should be temperature of 18ºC to 35ºC. From above references a clear picture can be drawn for temperature range of okra for better crop production. According to that picture 18ºC to 35ºC is a temperature at which okra crop grows well and gives highest production.

Due to overpopulation and environmental issues the food insecurity on earth is increasing day by day (Nellemann et al. 2009). According to UN Population Division (2011), population of the earth is expected to increase up to 8 to 11 billion till 2050 as higher as 16 billion till 2100. Definitely more people will need more food. To accomplish demand for food agricultural crop production should be increased. Climate plays a vital role for crop production and is basic determinant for agriculture production. For a particular locality and crop; length of growing season is determined by climate. For photosynthesis and fixation of carbon; the amount of heat and water stress is also determined by climatic factors. Under increasing population pressure and environmental issues the anthropogenic warming of the earth is a great threat to food security on earth (Terando, 2012).

Output of global warming is abiotic (heat) stress. At various growth stages plant faces many stresses like salt stress, drought stress, cold stress, light stress and heat stress (Tester and Bacic, 2005), resultantly growth and yield of agricultural crops is reduced (Shafi et al., 2010). It is reported that among these stresses especially heat stress is injurious for plant growth and development. To combat these stresses plant adopts different contrivances like morphological changes, physiology and biochemical processes (Camara- Zapata et al., 2004). Due to climate change heat stress is becoming an alarming concern for plant scientists across the world. Due to projected impacts of climate change on agriculture, situation is becoming

worse (Watanabe and Kume, 2009; Shah et al., 2011). Peng et al., (2004) reported an increase of 0.35 to 1.13°C in minimum and maximum temperatures for the time period of 1979 to 2003. It is predicted that by the end of 21st century average earth's temperature will increase two to four degree celcious (IPCC 2007), because of natural and anthropogenic factors (Eitzinger et al., 2010). Temporary or constant high temperature stress on plant causes morpho-physiological, biochemical and anatomical changes in plant which ultimately cause economic yield reduction (Wahid et al., 2007). Metabolic processes in plant have their own optimum temperature ranges but at high temperature above the optimum these processes are being affected. According to some reports due to global warming temperature stress is becoming more critical factor for crop production; among major abiotic stresses high temperature is considered as deleterious abiotic stress (Hasanuzzaman et al., 2013). In these situations it is need of the hour to identify heat tolerant set of germplasm for ahead coming adverse situations.

Common response of plant to different abiotic stresses is the production of variety of osmoprotectant (Serraj and Sinclair, 2002). Osmoprotectants are low molecular weight orgamic compounds which are nontoxic at cellular concentrations. Usually they save plants from stress induced damages by scavenging reactive oxygen species, adjusting the cellular osmatic potential, protecting membranes structures and stabilizing enzymes and proteins (Yancey et al., 1982; Bohnert and Jensen, 1996). Some osmoprotectants save cellular compartments from dehydration. These solutes are polyol, trehalose, quaternary amines, sucrose, glycine betaine and proline (Rhodes and Hanson, 1993). To address the situation of abiotic stresses in plants under the circumstances of climate change there is need to search for short cut approach to get rid of these stresses and to enhance agricultural production.

One new pathway to alleviate the drastic effects of abiotic stress is the exogenous application of these osmoprotectants. Exogenous application of phytohormones, signaling molecules, trace elements and osmoprotectants has been reported to have positive effects on plants under elevated temperature conditions by enhancing the ability of antioxidant enzymes (Hasanuzzaman et al., 2013). Proline and glycine betaine accumulate in plants as a responsive mechanism against heat stress. Hence the heat sensitive plants are unable to produce or able to produce these osmolytes in lesser amounts; in such plants species drastic effects of abiotic stresses can be lessen by the exogenous application of these

osmoprotectants (Jones and Gorham, 1983). Proline is an amino acid, it accumulate in higher plants in large quantities in response to abiotic stresses (Kavi-Kishoren et al., 2005). Proline has multiple roles under stress conditions in plants. As an osmolyte it plays role for osmatic adjustment, protects the sub cellular structures (e.g. proteins and membranes), scavenges reactive oxygen species and buffers the redox potentials under changing environments. Proline also lessens the cytoplasmic acidosis; sustains proper $NADP^+$/NADPH ratios compatible with metabolism (Hare and Cress, 1997). Upon relief from stress rapid breakdown of proline gives ample amount of reducing agent which supports to generate ATP and oxidative phosphorylation of mitochondria for repairing and recovery of stress induced damages (Hare et al., 1998).

As mentioned above, the best growing temperature range for okra is 18 to 35°C. Like other crops in Pakistan, Okra faces a dual menace of biotic and abiotic stresses. So, there is a dire need to identify and develop heat tolerant genotypes of vegetables, necessary to feed ever growing population of Pakistan and the world. Keeping in view the importance of okra, a study was initiated to examine the morpho-physiological, biochemical and enzymatic of okra and to assess the effect of proline on the heat tolerance of okra, with the following objectives

- To screen out okra genotypes for heat tolerance on the basis of morphological, physiological and biochemical attributes.
- To optimize the best dose of proline on the basis of morphological, physiological and biochemical changes that can alleviates the drastic effects of heat stress.

CHAPTER 2 REVIEW OF LITERATURE

Due to damaging effects of high temperature on plant growth, global warming is predicted to negatively influence the plant growth and yield. Catastrophic loss of crop production with the outcome of wide spread famine will be the final product of climatological extremes including heat stress (Bita and Gerats, 2013). Abiotic stresses are interconnected, they cause physiological, morphological, molecular and biochemical changes either individually or in combination, ultimately affect the growth, productivity and yield (Rao et al., 2016). Severe cellular damage causing abiotic stresses include heat, drought, salinity and cold. They cause yield losses in crop plants (Hasanuzzaman et al., 2013).

During the growth and reproduction of plant temperature fluctuations occur naturally (Giorno et al., 2013). Intermolecular interactions for proper growth, development of crop plants and fruit set can be damaged during hot summers caused by extreme variations in temperature. The alarming threat of changing climate is having extensive impact on agriculture production throughout the world as there are significant yield losses posing threat for worldwide food security due to heat waves (Christensen and Christensen, 2007).

Additional challenges to agricultural production and food security will be faced by human population increase (Varshney et al., 2011). According to Bita and Gerats, (2013) peoples from developing areas are likely to be dramatically affected by climate change because they rely on agriculture for their livelihood. Till 2050, population of the world will grow up to level of 9 billion people among them 75 % people live in rural areas. There is a dire need to increase agricultural production up to 70 % to meet the food demand for population expansion. To cope with increasing food demand and to eradicate the crop losses caused by global warming, there is urgent need to introduce strategies for improvement of food availability. Major abiotic stresses like drought, salinity, cold and heat stress induce serious cellular damage in plant species (Hasanuzzaman et al., 2013). In the tropical and subtropical areas of the world temperature during the growing period has elevated the most extreme seasonal temperature observed till now, which will even more intensify the process of land degradation (Varshney et al., 2011).

2.1. Heat stress and its effects on phases of plant life

If heat stress effect the physiological process called direct modifications or change the development pattern called indirect modification. These modifications may differ at one phenological stage to another. Long term effects of high temperature on developing seed may be vigor loss or germination delay results in reduced emergence and establishment of seedlings (Weaich et al., 1996).

All growth stages of plant are affected by heat stress starting from seed germination till final harvesting ultimately reducing the crop yield. Delayed or low germination or failed germination has been noticed in many crop plants under elevated temperature conditions Ashraf and Hafeez (2004) and Wahid et al., (2007). At seedling stage plants are more susceptible to elevated temperature than fully grown plants. Plant growth reduction under heat stress is caused by changes in physiological mechanisms (Wollenweber et al., 2003). According to Hall, (1992) key impact of heat stress on shoot growth is severe reduction in length of internode causing the premature death of plants. For instance under heat stress growth of sugarcane plant yield smaller internodes, early senescence, increase tillering and reduced total biomass production (Ebrahim et al., 1998). Heat stress causes the changes at cellular level including reduction in meristematic activity, cell and cell wall differentiation and elongation (Salah and Tardieu, 1996; Potters et al., 2007). Scorching of stem and leaves, leaf senescence, root and shoot growth reduction, fruit damage, and decrease in crop productivity are various physiological injuries observed in elevated temperature conditions (Vollenweider and Günthardt-Goerg, 2005). Effects of heat stress on shoot net carbon assimilation and biomass production result in reduction of plant growth and reduced dry matter production (Wahid et al., 2007). Plants under heat stress, 5°C temperature above optimum growing temperature enhance the cellular and metabolic responses for survival under stress conditions (Guy, 1999). These responses include alteration in cellular structures organizations, including membranes and cytoskeleton functions(Weis and Berry, 1988), decrease in production of normal proteins and rapid production of heat shock proteins (Bray et al., 2000), production of abscisic acid , antioxidants, other protective molecules and other phytohormones (Maestri et al.,2002).

High temperature stress make changes in photosynthesis and respiration leading to shorten the plant life span and reduces the productivity (Barnabas et al., 2008). Reduction in

enzymatic activity and changes in chloroplast protein complexes are the early effects of high thermal stress (Ahmad et al., 2009), in addition causing serious damages to the organization of microtubules, cell membrane and resultantly to the cytoskeleton, high temperature alters the permeability of the membranes, cell expansion and differentiation (Smertenko et al., 1997; Potters et al., 2007). Primary sites for heat stress injuries are carbon flux of the stroma and membranes of the thylakoid (Wise et al., 2004), ribulose 1, 5-bisphosphate (Rubisco), enzyme of photosynthesis and Calvin-Benson cycle is highly sensitive to elevated temperature and its activity is inhibited even at low levels of thermal stress (Maestri et al., 2002; Morales et al., 2003). Aberrant stacking and swelling of grana is a very specific effect of high temperature stress on photosynthetic membranes. Ion leakage from cells of leaves exposed to heat stress is due to damage to the photosynthetic membranes, it changes the allocation of energy to the photosystem (Wahid and Shabbir, 2005). According to Chen and Jiang, (2009) for sustainable photosynthesis and respiration, the preservation of cell membrane function is crucial. Lethal effects of high temperature on photosynthetic apparatus and chlorophyll are also linked with excessive production of active oxygen species (Camejo et al., 2005; Guo et al., 2006). Plant photosynthetic and respiration activity reduces due to decrease amount of photosynthetic pigments and increase in function of chlorophyllase (Todorov et al., 2003).

Thermal stress negatively affects the growth, developmental and physiological process. When thermal stress occurs at particular stage of development like reproduction stage, this poses a major constraint for plant adaptation to changing environment (Hall, 2001). For instance thermal stress during reproductive stage of wheat speed up the decline in photosynthesis and leaf area, which cause decrease in shoot and grain weight as well as sugar amount and weight of kernels, water use efficiency also reduced (Shah and Paulsen, 2003). It has been long recognized that flowering and sexual reproduction are highly sensitive to thermal stress, ultimately results in reduction in plant productivity (Hedhly et al., 2009). Some studies on the thermal stress on grasses report that most sensitive growth stage is flower bud initiation (Hedhly et al., 2009). Other reports on legumes and cereals showed that flowering stage is highly sensitive to temperature stress and cause great damage to the yield. (Wahid et al., 2007; Saha et al., 2010). Particular developmental stage sensitive to high temperature is male gametophyte, comparatively tolerant is female gametophyte and pistil

(Hedhly, 2011). Thermal stress often fastens the anthesis rather than delay; it means that before the accumulation of sufficient food reserves reproductive development will starts (Zinn et al., 2010).

In the sensitive crop plants male sterility is widely caused by the heat stress and pollen impairment has been observed as major factor for reduction in yield under thermal stress (Sakata and Higashitani, 2008). For instance in Arabidopsis and barley under thermal stress there is alteration in chloroplast, mitochondrial abnormalities occur, cell proliferation affected and vacuoles swollen. In the pollen grains carbohydrates accumulation reduces and alteration in assimilate partitioning is caused due to heat stress and alterations in apoplastic and symplastic loading of phloem occur (Taiz and Zeiger, 2006).

Thermal stress down regulates several enzymes like vacuolar and cell wall invertase and sucrose synthase in nourishing pollen grains, resultantly starch and sucrose production reduce and also the level of soluble sugars declines (Sato et al., 2006). Under moderately high temperature stress depletion available carbohydrates occur in tomato at critical growth stage, ultimately reduction in fruit set and yield occur (Sato et al., 2006). Reduction in tomato anthers under thermal stress is accompanied by closure of locules and reduction in pollen dispersal of several crops (Pressman et al., 2002). After fruitful fertilization thermal stress can halt the embryo development. During seed development if temperature stress occur it reduces the germination potential and loss of seed vigor, reduction in seedling emergence and germination occur (Akman, 2009; Ren et al., 2009). For instance thermal stress during reproductive development of wheat speed up the decline in photosynthesis and area of the leaf, ultimately decrease all growth parameters (Shah and Paulsen, 2003), ultimately changes the nutritional quality of the flour (Hedhly et al., 2009)

In general homeostasis, biosynthesis and metabolites compartmentalization is altered under elevated temperature conditions (Maestri et al., 2002). Thermal stress alters the functions of enzymes involved in the carbon metabolism, sucrose synthesis and starch accumulation (Ruan et al., 2010). Accumulation of metabolites (soluble sugars, glycine betaine and proline) under stress environment is the primary response of plant to changing environment (Wahid, 2007). Other osmoprotectants like sugar alcohols, tertiary or quaternary ammonium compounds also produce under stress condition (Sairam and Tyagi, 2004). Protein stability and structural stabilization of membrane bilayer increase due to production of osmolytes

(Sung et al., 2003; Mirzaei et al., 2012). According to (Wahid, 2007) secondary metabolites like phenolics, flavonoids, plant steroids and anthocyanins are off significance importance under stress environments and specifically associated with heat tolerance. For instance high temperature stress in summer vegetables cause accumulation of these osmolytes; increase the antioxidant enzyme activity (Rivero et al., 2001). Under changing environmental conditions phytohormones production increase like salicylic acid, ABA, ethylene etc, while production of some others decreases like auxin, gibberellic acids and cytokinin (Talanova et al., 2003; Larkindale and Huang, 2004; Larkindale et al., 2005).

Production of reactive oxygen species under thermal stress is symbol of cellular damage, in which pigment peroxidation and membrane lipids permeability and function. Reactive oxygen species damage variety of cell components like photosynthetic apparatus and several other apparatuses therefor limits the other activities of the metabolism affecting the growth and development of plants (Sairam and Tyagi, 2004; Xu et al., 2006). Reduction in energy and power generation occurs due to active oxygen species impairment to chloroplast electron transport chain and mitochondria (Foyer and Noctor, 2009). ROS not only cause damage to cellular membranes but sometime they play positive role in linking plant responses to pathogenic infection, abiotic stresses, molecular signals, developmental stimuli and programmed cell death (Gechev et al., 2006). For plant's adaptation to abiotic stresses under changing environmental conditions ROS signaling network in the present in the mitochondria and chloroplast play a vital role. These signals offer to complex interplay between organelles homeostasis under stressful environment and varied cell components and monitoring essential processes like transcription, metabolism of energy, translation and phosphorylation of proteins (Mittler et al., 2011).

In short we can conclude that heat stress negatively affects crop plants at various stages starting from seed germination till harvest and postharvest of crops. Heat stress also badly influence the ongoing processes in plants such as physiological processes including primary and secondary metabolism, photosynthesis and hormonal and lipid signaling. Heat stress significantly affects the plant growth and development by disturbing the membranes, cytoskeleton and protein structures. Most serious damage of heat stress to plant's life is when plant is at reproductive stage; at this stage heat stress affects pollen grain development. Heat stress also encourages the accumulation of heat shock proteins which protects the protein

structures from denaturation.

2.2. How plant respond under elevated temperature conditions

To safeguard existence under thermal stress plants have developed several mechanisms. These mechanisms can be classified into short term strategies like stress avoidance or acclimation and long term mechanisms include morphological and phenological evolutionary revisions like change in orientation of leaf, cooling through transpiration and change in composition of membrane lipid (Wang et al., 2004). Production of economical and significant yield under thermal stress condition is dependent on various plant physiological parameters and mechanisms that add to tolerance in field conditions. In many situations heat tolerant genotype is recognize by higher photosynthetic rate, increased membrane thermostability and avoidance from heat (Nagarajanet al.,2010; Scafaro et al., 2010).

Heat tolerance is directly correlated with the ability of plants to sustain gaseous exchange attributes under heat stress conditions (Salvucci and Crafts-Brandner, 2004). Tissue senescence is one of the typical symbols of thermal stress, characterized by damage to membranes attached with increment in membrane's fluidity, peroxidation of lipids and degradation of proteins in various processes of metabolism (Savchenko et al., 2002). Saturation of membrane's lipid is thought to be important element in heat stress tolerance. Due higher amount of saturated fatty acids membrane's lipids increases the melting temperature and stops the thermal induced membranes fluidity. For the purpose of maintaining membrane fluidity, plants produce saturated and mono unsaturated fatty acids in large amounts and also modulate the metabolism in response to heat stress (Zhang et al., 2005). According to Larkindale and Huang (2004) for maintaining stability of membranes and enhancing thermal tolerance, the increase in fatty acids level seems to be critical.

Sakamoto and Murata (2000) stated that one of the most important adoptive responses to thermal stress in plants is accumulation of osmoprotectants and osmoprotectants play a vital role in the osmotic adjustment. For example, accumulation of glycine betaine, soluble sugars and proline is essential for regulation of osmotic activities and safeguard cellular structures from thermal stress by establishing cell water balance, cellular redox buffering and membrane stability (Farooq et al., 2008). Transgenic strategies have confirmed the positive role of proline overproduction under thermal stress, in transformed plants excessive accumulation of proline correlates with the negative osmotic pressure of leave and larger

production of xanthophyllic pigments for protection against thermal stress (Dobra et al., 2010). Under elevated temperature conditions glycine betaine play a vital role as osmoprotectant (Sakamoto and Murata, 2002). According to Allakhverdiev et al. (2008) activation of Rubisco is maintained by glycine betaine produced in the chloroplast and stops it inactivation caused by heat stress. Liu and Huang, (2000) reported that availability of carbohydrates in large amounts under thermal stress represent physiological trait of plant which shows its tolerance potential. Photosynthesis has principal product sucrose, it translocate to sing organs from source through phloem. Signaling of sugar and allocation of carbon under thermal stress regulate plant growth and development (Roitsch and González, 2004). Li et al. (2012) reported that in summer vegetables higher activities of invertase in vacuolar and cell wall and higher import of sucrose into young fruit of tomato enhance the heat tolerance through signaling of sugars and increase in the strength of sink. Firon et al. (2006) says that pollen quality may be determined by contents of carbohydrates in the growing and grown pollen grains. There is a mechanism in summer crops (tomato) to maintain higher amount of carbohydrates under thermal stress. This is specially observed in heat tolerant genotypes. In plants sugars are supposed to play a vital role as antioxidants (Lang-Mladek et al., 2010). Sucrose is reported to act as molecule for signaling whereas at higher concentrations it acts as scavenger of reactive oxygen species (Sugio et al., 2009).

Plants produce secondary metabolites in response to thermal stress which protect plants from the oxidative stress. Production of phenolics has been reported in many summer vegetables under heat stress by activation of their biosynthesis and suppression of oxidation. This could be considered as mechanism of plant for survival under heat stress (Rivero et al., 2001). Plants accumulate anthocyanin under thermal stress, anthocyanin has multiple role in plants, it works as UV screen, lower the osmotic potential of leaf, regulate water uptake and reduce the water loss through transpiration. These functions may enable the plant's leaves to cope quickly in changing environment (Wahid et al., 2007). In plant species production of carotenoids save plants from stresses. For instance xanthphylls and terpenoids like tocopherol and isoprene stabilize and save lipid phase of the membranes of thylakoid (Velikova et al., 2005; Camejo et al., 2006).

According to Kotak et al. (2007) plant growth regulators, like ET, AUX, SA, ABA and CK are supposed to play vital role in heat stress tolerance of plants. In the field

environment, ABA production is an essential part of heat stress tolerance because of its interference in survival under thermal stress (Maestri et al., 2002). Ding et al. (2010) reported that ability of plants to produce ABA under thermal stress is a key attributes for heat tolerance. It has seen that ABA induces heat tolerance in maize (Musatenko et al., 2003). Seed treatment with salicylic acid enhances the heat tolerance in Arabidopsis mutant having defect in salicylic acid signaling. There was 40 % reduction in thermal tolerance potential of transgenic plants which were unable to accumulate salicylic acid (Kaya et al.,2001; Clarke et al.,2004). Cytokinins are known for their role in oxidative stress alleviation in plants. Higher levels of cytokinins appear to enhance themo-tolerance and increase the yield in crop plants under heat stress (Hare et al., 1997; Hsu et al., 2010). Exogenous application cytokinins based seaweed extract enhance leaf cytokinin contents and postponed senescence under thermal and drought stress (Zhang and Ervin, 2008). In Arabidopsis and barley thermal stress suppress auxin signaling in anther specified way, resulting in pollen abortion. When auxin sprayed exogenously, it restores the development of pollens under thermal stress conditions (Jaggard et al., 2010). It has been reported that auxin application enhances fertility under temperature stress.

Plants try to acclimatize wide range of thermal stress by process of reprogramming i.e. they change proteome, metabolism and transcriptome and even by proceeding early cell death process leading to abortion of organ or whole plant death (Qi et al., 2011; Sánchez-Rodríguez et al., 2011). The capability to survive under higher than normal temperatures results from renovation of sensitive components and reduction of more thermal injuries and stabilization of metabolic homeostasis under thermal stress conditions. Massive generation of heat shock proteins is also referred as thermos-tolerance (Vierling, 1991). Heat tolerance is supposed to be multi-genic trait, several metabolic and biochemical traits are included in the maintenance and development of heat tolerance. To cope with heat stress plant activate antioxidant enzymes, gather compatible solutes, express stress related genes, start translation of proteins (Kaya et al., 2001). We can conclude that plant response to heat stress is totally dependent on the genotypic makeup of the plant (Prasad et al., 2006; Challinor et al., 2007). Some genotypes are more tolerant than other within same species.

2.3. Okra and heat stress

Plants exposed to environmental stresses may show adverse effects of stresses on growth, yield and metabolism (Lawlor, 2002). Heat stress, drought, air pollution, salinity pesticides, heavy metals and pH of soil are key limiting issues for production in crop plants. All these factors adversely affect all functions of plant life (Hern-ndez et al. 2001). Heat stress is a major stress among all these stresses because it leads towards other stresses. In the field conditions heat stress affects the summer vegetables throughout the growing seasons. Okra is a moderately heat tolerant crop. Its production is affected throughout period, also almost all growth stages are affected by heat stress (Gunawardhana and Silva, 2011; Hasanuzzaman et al., 2013).

According to Dilruba et al. (2009) several external and internal factors influenced growth of okra genotypes. This could be used as tool to identify crop production potential in different crops. Ghanti et al. (1991) reported there were significant effects on the fresh weight of fruits and dry matter partitioning when okra was grown under different sowing dates with hormonal application to check its response to heat stress. Number of pods per plant is important because it contributes for final crop yield. It was greatly influenced by the thermal stress in okra crop. Maximum number of fruits per plant was harvested when crop was sown at 06, April followed by the sowing time when crop was sown on 21, April. Least number of pods per plant were harvested when okra sown on 22nd of March (Pandita et al., 1991). From these reports it is clear that best okra production is temperature dependent. At proper sowing time it gives maximum yield compared to other sowing times. Gunawardhana and Silva, (2011) also reported the importance of optimum temperature for the higher yield of okra. According to them plant height is increased due to intermodal elongation under higher optimum temperature. They observed larger number of flowers per plant, increased number of fruits per pant at 34^{o}C when there was no water stress. At 34^{o}C all growth attributes were at higher values in terms of production. As reported by Ketsa and Chutichudet (1994) showed that soluble solid in the pericarp of okra fruit increased and go to its peak at 32^{o}C within 7th day and at 34^{o}C they were at peak in only 6th day. But in contrast at optimum temperature soluble solids reached go to maximum value on the 8th day then show steady decrease. In case of fiber content; higher pericarp fiber contents were observed at higher temperature compared to the optimum temperature. Fiber contents development caused

toughness of the fruits and deteriorates the cooking quality of the fruits. According to the Gunawardhana and Silva, (2011) high temperature shorten harvest span of okra. After full bloom pods can be harvested at 7-8 days interval at optimum temperature. When there are temperature stress pods matures within 5-6 days after full bloom. It is clear that time to harvest pod for fresh consumption of okra is negatively affected by thermal stress. Dubey, (1997) reported that abiotic stresses disturb photosynthesis in almost all plants and reduce productivity of plants. In case of okra genotypes abiotic stresses reduce rate of photosynthesis by badly affecting all photosynthesis related attributes like transpiration rate, sub-stomatal conductance of CO_2, reduce chlorophyll index, degrade the ultra-structure of chloroplast and inhibition of arboxylation and photochemical reactions (Dubey, 1997). Experimentation on okra cultivars grown under abiotic stress (salt stress) showed that there was reduction in stomatal conductance to carbon dioxide absorption in tolerant cultivars as compared to sensitive ones (Dubey, 2005). Previous study showed that under stress environment tolerant okra genotypes showed higher transpiration rate and leaf turgor pressure and water use efficiency as compared to sensitive ones. He also showed positive correlation of CO_2 assimilation with water use efficiency. Abiotic stresses are major problem affecting the growth (shoot length, root length, shoot and root fresh and dry weights, net biomass production), physiology, biochemistry and development of okra Akhtar et al. (2012).

2.4. Ways to alleviate heat stress in plants

There are numerous ways to alleviate the drastic impact of heat stress. A number of them are reported in this section.

2.4.1. Seed priming

Plants resistance against biotic stresses has been induced by a mechanism known as priming (pre-treatment of plants or seeds via chemical or stress exposure to make them resistant to stress) (Borges et al., 2014). To face the existing requirement of high standard in agricultural market it is necessary to increase the high quality seed. A key factor for crop performance is to achieve uniform and rapid seedlings emergence as lower rates of germination oftenely rendering plantlets to various soil-borne diseases and drastic environmental conditions (Osburn and Schroth, 1989).

Seed priming enhanced germination rate which further results in high level of abiotic /biotic stress tolerance as well as crop yields. All these factors that enhance the

competitiveness of product is directly associated with seed vigor, an important trait controlled through various environmental and genetic factors (Rajjou et al., 2012). It is reported that under stress environment several pretreatments such as 'drying and imbibing' as well as 'advancing or hardening' showed good impacts on germination of seed in various cereal and horticultural species (Austin et al., 1969; Berrie and Drennan,1971).

Seeds are faced drastic environmental stresses during maturation and post-dispersal storage as well as during early stage of germination, these conditions ultimately lead to broad oxidative damage of nucleic acids, lipids and proteins (Kranner et al., 2010; Ventura et al., 2012). Various bioactive compounds and valuable microorganisms can also incorporate with priming mixture. Is it identified that the relationship of plants with specific bacteria or fungi results into tremendously convenient results, as these beneficial microorganisms are capable of establishing endophytic associations with the plant, which enhanced plant growth, increases phytohormones production and enhancement of abiotic/biotic stress tolerance (Waller et al., 2005). Moreover, another distinguishing aspect of stress tolerance is to enhanced stomatal conductance in chickpea plants obtained from primed seeds (Ghassemi-Golezani et al., 2012). The treatment of SA was found more effectual in enhancing performance of seedlings in stress conditions, leading to enhancement of Chl a and Chl b ratio, photosynthetic pigments and triggering enzyme activities such as APX and SOD. In addition enhancement of proline level in plants obtained from SA treated primed seeds were evident (Li and Zhang, 2012).

2.4.2. Grafting

Root-shoot connections play significant role in plant stress conditions. In horticultural industry grafting technique has been employed extensively to enhance plant resistance against salts and pathogens. Several rootstocks reveal tolerance to pathogens and restricted transport or uptake of salts from roots towards shoots. There are several mechanisms which gave positive effects of rootstocks and scion by means of different chemical alterations or root-shoot hydraulic signals (Gregory et al., 2013). Rootstock grafting with CTKs synthesis enhanced the shoot growth of tomato plants (Ghanem et al., 2011). ABA was found to be a long-distance signal molecule moves from the root to other parts of shoot through xylem and activates various defense mechanism and leaf growth from salinity and drought conditions (Aloni et al., 2010; Ntatsi et al., 2013). Additionally, (ABAGE) ABA glucose ester (the

conjugate) has also been recommended as a long-distance signal molecule from root-shoot (Jiang and Hartung, 2008). Researches on cucumber and Arabidopsis have revealed the fact that the constant apoplastic H_2O_2 generation is indispensable for the stress response (Miller et al., 2009). However, it remains unclear that HSP70 protein present in xylem sap plays role as a long-distance signal molecule or not (Dafoe and Constabel, 2009).

It is reported that grafting of rootstocks having stress tolerance can improve stress resistance. On the other hand, researches on rootstock-induced resistance have been restricted to examinations of salinity or soil-borne pathogens in which the special rootstocks are resistant to the pathogen or have or restricted transport of salt from roots-shoots (Martinez-Ballesta et al., 2010; Schwarz et al., 2010). For instance, stress tolerance against salinity has been increased by grafting watermelon onto salt-resistance bottle gourd rootstock (Yang et al., 2013).

2.4.3. Genetic engineering

A considerable active research on plants focuses on the plant genetic, molecular and physiological responses to stress conditions as well as development of techniques for improvement of resistance and acclimation. Conventional biotechnological techniques to enhance abiotic stress resistance focuses on increasing plant defense mechanisms. The conventional molecular approach for induction of stress tolerance in plants involves intensification the endogenous systems through sensors and signaling elements (i.e kinases, transcription factors), to direct action effectors or genes (i.e antioxidative enzymes, enzymes for osmo-protectants, heat shock proteins) (Reguera et al., 2012; Zurbriggen, 2010). However, this approach shows comparative levels of success with an ever-growing number of resistant plants reported (Agarwal, 2013). An alternative approach consisting of cyanobacterial isofunctional expression by replacement of decaying endogenous components in stress condition. The protein ferredoxin [2Fe-2S] is well known electron acceptor of electron transport chain delivers electrons for vital regulatory and metabolic pathways in chloroplasts. However, under drastic environmental circumstances Ferredoxin levels declines. To compensate the decline level of ferredoxin, a cyanobacterial flavodoxin was introduce in tobacco plants which results in better electron distribution, cellular homeostasis, good antioxidant effect and ultimately generalized stress resistance (Reguera et al., 2012; Lodeyro et al., 2012). Various soluble sugars, polyamines and amino acids are well

recognized solutes synthesized in relation to maintain cell turgidity and stabilize cellular membranes. It was reported from recent studies that at higher concentration soluble sugars act as ROS scavengers (Bolouri-Moghaddam et al., 2010). It is observed that miRNAs play important role in metbolic and biological process, as well as plant development (Sunkar and Jagadeeswaran, 2012). Recent studies identified several stress regulated miRNAs mostly targeting TFs, depicting their role as master regulators at the core of stress gene-regulatory networks (Sunkar and Jagadeeswaran, 2012; Zhou et al., 2010). Transgenic strategies have been applied recently to knock down or overexpress particular miRNAs as well as their targets (Khraiwesh et al., 2012; Zhou et al., 2010). For example tomato plants were produced against drought stress by over-expression of miR319 and miR169c and rice with improved drought, salt and cold tolerance (Zhang et al., 2011; Zhou et al., 2013).

2.4.4. Plant nutrition and abiotic stresses

Recent studies revealed that soil fertility and productivity are decreasing worldwide due to deterioration of soils without considering appropriate soil-management practices (Cakmak, 2005). Insufficient supply of nutrients and lack of soil fertility are common problems leading to diminish food production, particularly in developing countries. Environmental hazards like extreme temperature, salinity, soil acidity, water deficiency, flooding, and pathogenic infections are increasing with growing world population and severe wastage of natural resources. These stresses drastically reduce crop yields below possible maximum yield. There is intense need of employing different biotechnological approaches and breeding strategies as well as with balanced and proper supply of nutrients for sustain food security and minimize the damaging effects of these stresses on crop productiveness. Among the various mineral nutrients, potassium plays a vital role in survival of plants in stress conditions. It is necessary for various physiological processes like photosynthesis, enzymes activation, turgescence maintenance and minimized excess transport or uptake of ions like sodium and iron in flooded and saline soils (Marschner, 1995; Mengel and Kirkby, 2001).

The studies on Se role in alleviating environmental stress have been widely investigated in humans, animals and in plants. Several stresses like drought, water excess, cold, salinity and light intensity can accumulate ROS in plants. These drastic environmental stresses can be minimized by slight mixing of Se in growth substrates which reduce the

excess ROS accumulation particularly, O^{2-} and H_2O_2. At lower concentration of Se (1.5 M Na_2 SeO_3) declined the O^{2-} level in Viciafaba L roots exposed to Pb (Mroczek-Zdyrska and Wójcik, 2011). Under drastic environmental stress conditions, plants chloroplasts damaged resulting disrupted photosynthesis process. However addition of suitable Se levels can reduce chloroplasts damage to some extent and enhance chlorophyll contents (Yao et al., 2011; Malik et al., 2012).

2.4.5. Application of osmoprotectants for improvement of heat stress

Significant consideration has been dedicated to the introduction of stress tolerance in accessible high yielding genotypes. Of the several approaches to attain this target, signaling molecules, foliar application, seed treatment, osmoprotectants and oxidants (H_2O_2) are well known approaches. Organic compounds such as glycinebetaine, polyamines and proline have been effectively applied for induction of heat tolerance in a variety of plant species. It was observed that salt stress harshly changed the gas exchange and biochemical attributes of salt tolerant as well as sensitive genotypes of okra. Silicon application minimized the drastic salt stress effects with improvement of biochemical as well as gas exchange parameters in sensitive genotypes (Abbas, 2015). A similar type of Si effects is identified for stressed cotton plants (Farooq et al., 2013) and in tomatoes (Haghighi and Pessarakli, 2013). Preconditioning particularly at slightly higher temperature has been found to minimize the drastic damage of heat induction in black spruce seedlings. Preconditioned plants of tomato exhibited comparatively better stomatal conductance, osmotic potential and growth as compared to non-conditioned tomato plants (Morales et al., 2003). Similarly comparison between heat acclimated and non-acclimated leaves of turf grass exhibited lower chloroplast damage, higher thermo stability and lower malondialdehyde (a product of lipid peroxidation) in heat stress condition (Xu et al., 2006).

2.4.6. Genetic improvement for heat-stress tolerance

Plant may respond to abiotic stress in a general to specific manner. For specific response plant may require to show gene expression involved for special process to specific stress (Yang et al., 2009). To combat stress environment plant adopts some sort of molecular control mechanism. This molecular control mechanism can be used as genetic engineering strategy to engineer genetically modified plants to enhance ability of abiotic stress tolerance. This technique is requiring overexpression of stress related gene. Under stress environment

plant must adopt some tolerance mechanism. Changes in plant physiological mechanism which enable plants to grow under stress environment are dependent on genes which play vital role to safeguard cellular structures and maintain functions. Mostly monogenic trait is easy to engineer than multi-genic traits for abiotic stresses. Presently strategies for genetic engineering are based on transfer of one or several genes which are engaged in signaling or regulatory pathways enzyme activation or other protective functions

2.5. Exogenous application of proline

Abiotic stress tolerance is complicate at cell level and whole plant level (Foolad, 1999; Foolad et al., 2003; Ashraf and Harris, 2004). This complication is part of stress factors and several phenomena like physiological, biochemical and molecular (Zhu, 2002). Biotechnological research in past two decades has given considerable view of mechanism of stress tolerance at molecular level in plants (Holmberg and Bulow, 1998; Kasuga et al., 1999; Prabhavathi et al., 2002; Rontein et al., 2002).

Most common response to abiotic stresses is overproduction of several compatible solutes which are organic in nature (Serraj and Sinclair, 2002). These organic molecules have low molecular weight; solubility is very high and normally nontoxic at higher cell concentrations. Their roles are identified as protection of plants from different abiotic stresses, osmotic adjustment at cellular level, scavenging of active oxygen species, enzymes and protein structure stability and protection of integrity of membranes (Yancey et al., 1982; Bohnert and Jensen, 1996). There is one class of these solutes which protect cell components from the injuries occurring due to dehydration, they are known as osmoprotectants. These osmoprotectants include quaternary ammonium compounds, polyols, sucrose, trehalose, prolinebetaine, choline sulfate, pipecolatebetaine, alaninebetaine, hydroxyprolinebetaine, glycine betaine and proline (Rhodes and Hanson, 1993).

Many attempts has been made for production of plants through genetic engineering techniques to insert gene in plant to overproduce these compounds. But there has been very little success. Then scientists think for alternate approaches to induce stress tolerance in crop plants in the current scenario of climate change. As alternate strategy to alleviate drastic effects of abiotic stresses; exogenous application of these organic molecules has been proved to be effective tool.

2.5.1. Proline Biosynthesis in Plants

Key enzyme for synthesis of proline and ornithine is Pyrroline-5-carboxylate synthase (P5CS). The end product of this biosynthesis process is proline. Proline and its intermediate substrates notable properties related to osmotic stress and redox balance. Network cycle for proline biosynthesis is given in the following figure.

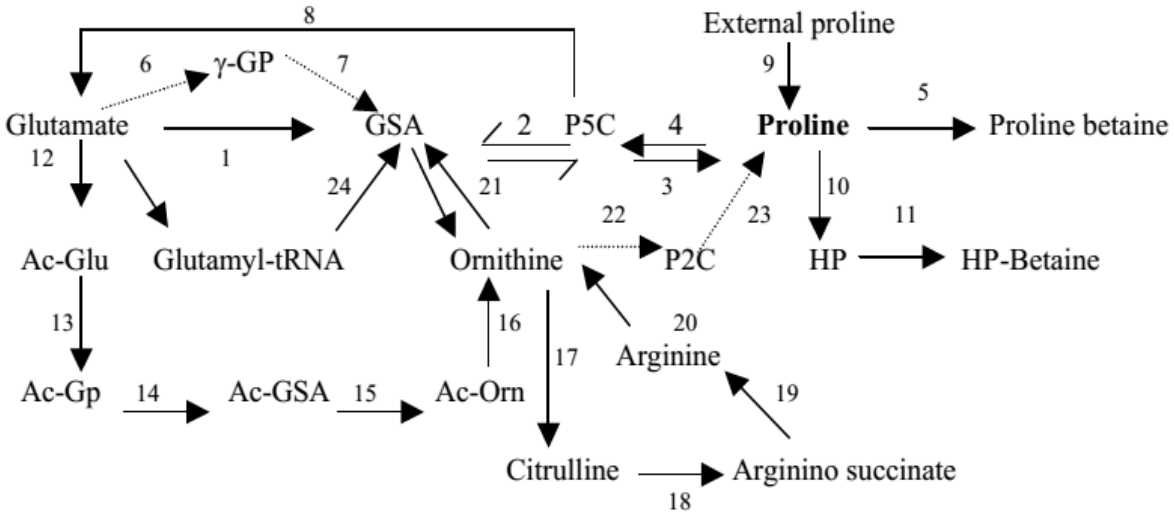


Figure 1. Network of proline biosynthesis and degradation pathways in plants and lower organisms (Kishor et al., 2005).

2.5.2. Proline accumulation in plants

Under osmotic stress conditions free proline accumulation has been recorded in number of taxa for over forty five years. Amount of proline accumulated in plants depend upon the degree of stress and plant species under stress (Baumgartne et al., 2005). Proline production and accumulation in plant has been reported in response to many abiotic stress (Haudecoeur et al., 2009) such as heat stress (Saradhi et al., 1995), salinity (Yoshiba et al. (1995), drought (Choudhary et al., 2005), UV irradiation (Saradhi et al., 1995), heavy metals and oxidative stress (Yang et al., 2009).

In okra crop proline accumulate in plant leaves under abiotic stress condition. In different okra cultivars different amount of proline accumulate in leaves. According to available reports okra cultivar Haritha accumulate proline contents in leaves up to 35 %, 42 %, under abiotic stress condition. Mahyco cultivar showed lower level of proline accumulation in leaves as it was 19 % and 29 % under drought stress condition. In case of proline accumulation in the root zone, there has been significant increase in the proline

contents in the roots. Very high concentrations (80 %) of proline have been observed at cellular level in response to abiotic stresses because of decrease in degradation and increase in the production under stress (Csonka, 1991).

In Arabidopsis (model plant for study) it has been noticed that proline accumulate up to 20 % under sodium stress. Role of proline under stress condition is crystal clear but its role in growth of plant is not clear (Csonka 1991; Delauney 1993). Genes associated with enzymes responsible for proline biosynthesis and degradation has been cloned and partially categorized. Factors involved in regulation of gene expression for these enzymes are still unidentified (Roosens 2002).

2.5.3. Exogenous application of proline and abiotic stresses

Protection of protein integrity and improvement in enzymatic activities are performed by molecular chaperone known as proline. Examples of other such roles of proline include protein aggregation prevention and protection of enzyme M4 lactate dehydrogenase under thermal stress (Rajendrakumar et al., 1994; Szabados and Savoure 2010). Proline application through exogenous way can play vital role in improving plant stress tolerance. This role of proline for alleviation can be in the form of cryoprotection (Songstad et al., 1990; Santarius, 1992) or osmoprotection (Jones and Gorham, 1983; Handa et al., 1986). Proline has role as osmolyte for adjustment of osmotic potential, eradicate free radicals, and stabilize structures at sub-cellular level (e.g. proteins and membranes) under stressful environment (Srinivas and Balasubramanian, 1995). According to Hare and Cress, (1997) proline maintains proper $NADP^+$/NADPH ratios for metabolic process. After exposure to stress proline provide numerous agents that provide help for production of ATP and upkeep mitochondrial phosphorylation oxidation for rescue from stress and restore stress caused damages (Hare and Cress, 1997; Hare et al., 1998).

Much work has been reported on exogenous application of proline for induction of abiotic stress tolerance with great success. Talat et al. (2013) reported role of exogenous application of proline for stress alleviation in wheat. On the behalf of findings of study it was concluded that abiotic stress badly affects the germination %, chlorophyll contents and growth in wheat. But when proline was applied by foliar method it significantly reduce harmful effects of stress and accelerate growth, improve germination and enhance chlorophyll contents. Overall proline enhances stress tolerance in wheat. In several other

plants exogenous application of proline paly role as osmoprotectant and enhance growth and development (Csonka and Hanson, 1991). In many halophytes proline application under stress enhance growth and stops excessive production of ethylene caused by salt or drought stress (Chrominski et al., 1989). Okuma et al. (2000) reported role of proline in tobacco plant where tobacco growth was suppressed due to abiotic stress and proline application @ 10 mM enhance growth by protecting membranes and enzymes. Same role of proline is reported by Yan et al. (2000).

In case of cell cultures proline application proved to be good for stress alleviation e.g. proline application by exogenous method in soybean cell culture enhance function of peroxidase and superoxide dismutase. These enzymes normally play role for enhancement of abiotic stresses (Yan et al., 2000; Hua and Guo, 2002).

Foliar application of proline in vegetables has vital role for enhancement of abiotic stress tolerance. When proline was sprayed on melon cultivars under abiotic stress conditions it enhances biomass production, chlorophyll index, rate of photosynthesis, scavenge reactive oxygen species and enhance antioxidant enzymes activities (Yan et al., 2011). Zouari et al. (2016) conducted study to check the effect of exogenously applied proline under cadmium stress and found that cadmium negatively affects date palm growth and physiological attributes. But with foliar application of proline there was significant enhancement in growth and physiological attributes and significantly enhance enzymatic activities.

Foolad and Jones (1993) reported usefulness of proline application for seed germination. Seed germination was affected by salt stress in tomato a summer vegetable but deleterious effects were eradicated by foliar application of proline. Reduced seed germination and seedlings growth may be caused by water shortage and deactivation of some enzymes. Proline seems to compensate for such bad effects (Gul and Khan, 2008).

Ben et al. (2010) found wonderful results for plant water relations, mineral nutrition, physiological and growth attributes under salt stress by foliar application of proline. All these parameters were significantly affected by salt stress and proline improve all these parameters. Singh et al. (2015) showed positive role of proline application in Solanum melongena seedling under heavy metal stress. With the application of arsenate in Solanum melongena there was oxidative stress. Proline application alleviates drastic effects of arsenate by reduced accumulation of arsenate in seedling.

Proline play vital role in flower transition if several plant species. *Arabidopsis* show earlier flower when there was proline accumulation. With some mutants reduced proline accumulation was obtained for late flowering (Mattioli et al. 2008). For flower transition proline may play role for signaling.

In case of cellular studies proline show a positive correlation with cell elongation. This positive correlation might be summarized in term of synthesis of protein; proteins are important constituents of plant cell wall and have vital role in cell division regulation, self-assembly of cell wall and extension of cell (Munoz et al. 1988; Showalter 1993; Majewska-Sawka 2000). Increased amount of proline production could affect biosynthesis rate of hydroxyprolinerich glycoproteins and maintain physiological process attached to elongation of cell, elongation of pollen tube (Zhang et al. 1982), elongation of primary root (Verslues and Sharp 1999), elongation of pollen tube (Zhang et al. 1982).

Gene expression induction has been considered as vital role of proline application (Hellmann et al. 2000). Proline like other amino acids play role for signal transduction for gene expression (Fafournoux et al. 2000). Proline may perform role as coupling of signals for metabolic status to cell growth (Beck and Hall 1999; Dann and Thomas 2006).

Proline accumulation in cytoplasm is linked with reduced concentrations of toxic ions and surge in volume of cytosolic volume (Cayley et al., 1992). Reduction in water potential to extreme low levels driving water to inward cell is thought to be vital role of compatible osmolytes. This maintains high turgor pressure to maintain higher growth (Chiang and Dandekar 1995).

Through literature it has been clear that these organic compounds are vital for plant life under stress environments. For the purpose to increase concentrations of these compounds attempts had been made in past. First it was tried to produce plant through conventional breeding that are capable of producing higher amounts of these compound. Second approach was to genetically engineer plants that will be able to produce sufficient amount of these organic compounds to combat stress. A short cut method used as third approach is exogenous application of these osmolytes to induce tolerance against environmental stresses. In conclusion we can say exogenous application of proline to plants before, during and after stress has shown to increase endogenous levels of proline.

CHAPTER 3 MATERIALS AND METHODS

Abiotic stresses profoundly affect the crops by restraining yield. Plant faces many distinctive abiotic stresses at different stages of growth and development. It is documented that water, light, heat and salt stress are major abiotic stresses, which reduce plant growth and ultimately affect the yield. Among these stresses heat stress seems to causing serious losses in okra crop by reducing growth period, as the plant experiences wilting, lack of fruit set and forced maturity from May to onward in Punjab province. Like other crops in Pakistan okra faces dual natured problem of abiotic and biotic stresses. To feed the growing population of Pakistan it is need of the hour to screen the heat tolerant genotypes of the vegetables especially of okra. For this purpose a comprehensive study was planned to investigate the morphological, physiological, biochemical and enzymatic attributes of okra in response to heat stress and on this basis genotypes were categorized into heat tolerant and heat sensitive ones. The other part of this study was to determine the effect of proline in alleviating the drastic effects of heat stress. The studies were carried out at Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. The study comprised of the following experiments.

3.1. Experiment # 1

Screening of okra genotypes against heat stress.

- **Location**: Growth room, Institute of Horticultural Sciences
- **No. of genotypes**: 100
- **Replications**: 4
- **Growth medium**: Sand culture

3.1.1. Experimental details:

This was a single factor study in which comparative response of selected okra genotypes against heat stress was studied. Seeds of 100 okra genotypes imported from The World Vegetable Centre (AVRDC) were sown in plastic pots in growth room, situated in the Institute of Horticultural Sciences, University of Agriculture, and Faisalabad. Four seeds per pot were sown and each replication comprised of four pots. The seeds were watered according to the need of plants by observing moisture of sand. Hoagland's solution was used as nutrient medium. Plants were grown at 28/22°C (day and night) for four weeks. After four weeks temperature of growth room was increased to 45/35°C (day and night) by increasing

2°C every day to avoid the osmotic shock. Plants were kept at 45/35°C (day and night) for one week to check the effect of heat stress on plants. One week after exposure to high temperature, plants were harvested to analyze the effects of heat stress. The experimental units were arranged in a CRD with four replications. Data for the following parameters was recorded.

3.1.2. Morphological parameters

3.1.2.1. Seedling shoot length (cm)

Shoot length was recorded by using scale in centimeters (cm), from the base of stem to the tip of the shoot and average value for replicates was computed.

3.1.2.2. Seedling root length (cm)

Plants were uprooted and roots were washed with tap water. Root length was measured using scale in centimeters (cm) from the base of stem to the tip of the root and average value for replicates was computed.

3.1.2.3. Seedling shoot fresh weight (g) and root fresh weight (g)

After measuring shoot and root lengths, seedlings were wiped out with filter paper in order to eliminate any water present on their leaves and shoots. A digital balance was used to obtain the shoot and root fresh weights and average fresh weight of each replicate was recorded.

3.1.2.4. Seedling shoot dry weight (g) and root dry weight (g)

After calculating fresh weights, five randomly selected plants from each replicate were taken in paper bags which were placed in oven (Memmert-110, Schawabach, Germany) and were dried at 70°C for 72 hours. The dry weights were obtained by using digital balance and average dry weight of each replicate was taken.

3.1.2.5. Number of leaves

The number of leaves was counted for three seedlings and average was recorded for each replication.

3.1.3 Physiological parameters

3.1.3.1 Transpiration rate, photosynthetic rate, sub-stomatal CO_2 and leaf temperature

For the measurement of physiological attributes such as transpiration rate (mmol m^{-2} s^{-1}), photosynthetic rate (μmol m^{-2} s^{-1}), sub-stomatal CO_2 (vpm) and leaf temperature (°C), three young fully developed and healthy leaves $plant^{-1}$ (two plants in each replication

treatment^{-1}) were selected. These selected leaves were placed one by one in the chamber of portable apparatus termed as Infra-Red Gas Analyzer (IRGA) (LCi-SD, ADC Bio-scientific UK). All the readings of above mentioned physiological attributes were taken at day time from 10.00 a.m. to 12.00 a.m. at atmospheric pressure 99.9 kPa, molar flow of air per unit leaf area 403.3 mmol $m^{-2}s^{-1}$, PAR (photosynthetically active radiation) at leaf surface was maximum up to 1711µmol m^{-2} s^{-1}, water vapor pressure in the chamber ranged from 6.0 to 8.9 mbar, surrounding CO_2 concentration was 352µmol mol^{-1} and atmospheric temperature ranged from 22.4 to 27.9°C (Zekri, 1991; Moya et al., 2003).

3.1.3.2. Water use efficiency (µmol CO_2 mmol1 H_2O)

Water use efficiency (WUE) (µmol CO_2 $mmol^{-1}$ H_2O) is the ratio between photosynthesis (Pn) and the amount of water transpired (E) which was measured as:

9 =PANQOAABBE?EAJ?U(9 7') = 2DKPKOUJDAPEN=PA#; 6N=JOLEN=PEKNJ=PA';

3.1.3.3. Chlorophyll contents (SPAD value)

Chlorophyll contents were measured by using chlorophyll meter (CCM-200plus Bio-Scientific USA).

3.1.4. Experimental design and statistical analysis

Experiment was designed following Complete Randomized Design (CRD) with single factor. Collected data was subjected to statistical analysis by using the Fisher's analysis of variance technique and significance of treatments were essayed by using HSD (Tukey Test). Statistical analysis and correlations between variables were also estimated by using statistics 8.1.

Table 3.1: List of okra genotypes investigated in this study

Sr. #	Name	Country of origin	Sr. #	Name	Country of origin
1	VI039622	Bangladesh	51	VI049632	Bangladesh
2	VI039638	Bangladesh	52	VI049954	Taiwan
3	VI039643	Bangladesh	53	VI049961	Thailand
4	VI039651	Bangladesh	54	VI050150	Thailand
5	VI039652	Bangladesh	55	VI050170	Thailand
6	VI040649	Thailand	56	VI050549	India
7	VI040770	Thailand	57	VI051038	Taiwan
8	VI040865	Thailand	58	VI051039	Taiwan
9	VI041139	Thailand	59	VI051042	Taiwan
10	VI041215	Philippines	60	VI051047	Thailand
11	VI041461	Philippines	61	VI051048	Philippines
12	VI041763	Philippines	62	VI051062	Philippines
13	VI044233	India	63	VI051114	Philippines
14	VI044241	Philippines	64	VI054562	Philippines
15	VI044244	Philippines	65	VI054565	Philippines
16	VI037997	Philippines	66	VI054566	Philippines
17	VI037995	Thailand	67	VI054568	Philippines
18	VI037994	Thailand	68	VI055017	Philippines
19	VI033773	Thailand	69	VI055018	Philippines
20	VI033775	Malaysia	70	VI055110	Philippines
21	VI033781-A	Malaysia	71	VI055119	Philippines
22	VI033781-B	Malaysia	72	VI055219	Philippines
23	VI033784	Malaysia	73	VI055220	Philippines
24	VI033785	Malaysia	74	VI055996	Philippines
25	VI033786	Malaysia	75	VI056069	Philippines
26	VI033791	Malaysia	76	VI056079	Philippines
27	VI033803	Malaysia	77	VI056402	Malaysia
28	VI033805	Philippines	78	VI056404	Myanmar
29	VI033810	Philippines	79	VI056407	Malaysia
30	VI033824	Philippines	80	VI056447	Malaysia
31	VI036201	Philippines	81	VI056448	Lao people's democratic republic
32	VI036203	Philippines	82	VI056449	Lao people 's democratic republic
33	VI036211	Philippines	83	VI056450	Lao people 's democratic republic
34	VI036212	Philippines	84	VI056451	Lao people 's democratic republic

35	VI036213	Philippines	85	VI056452	Cambodia
36	VI036215	Philippines	86	VI056453	Cambodia
37	VI046536	Philippines	87	VI056454	Cambodia
38	VI046537	Thailand	88	VI056455	Philippines
39	VI046544	Thailand	89	VI056456	Philippines
40	VI046554	Thailand	90	VI057245	Philippines
41	VI046556	Thailand	91	VI057249	Philippines
42	VI046559	Thailand	92	VI060131	Mali
43	VI046562	Thailand	93	VI060132	United states of America
44	VI046566	Thailand	94	VI060133	United states of America
45	VI047672	Thailand	95	VI060206	United states of America
46	VI047751	Thailand	96	VI060313	United states of America
47	VI047808	Bangladesh	97	VI060314	United states of America
48	VI048154	Bangladesh	98	VI060315	United states of America
49	VI048291	Bangladesh	99	VI060316	Guatemala
50	VI048596	Bangladesh	100	VI060317	United states of America

Source: AVRDC-The World Vegetable Center, Taiwan, China.

Table 3.2: Recipe of Hoagland's stock solution (used as nutrient medium)

Reagents	**Stock(g/L)**	**Ml of stock soln. for 10L ½ conc.**	**Ml of stock soln. for 200L ½ conc.**
Macro nutrients			
KNO_3	101	25	500
KH_2PO_4	136	5	100
$Ca(NO_3)_2.4H_2O$	236	25	500
$MgSO_4.7H_2O$	246	10	200
Micro nutrients			
H_3BO_3	2.86	5	100
$MnCL_2.4H_2O$	1.81	5	100
$ZnSO_4.7H_2O$	0.22	5	100
$CuSO_4.5H_2O$	0.08	5	100
$H_2MoO_4.H_2O$	0.02	5	100
Fe-EDTA	37.33	5	100

3.2. Experiment # 2.

Screening of different okra genotypes for heat tolerance under field conditions

- **Genotypes:** Total 25 screened out from 100 genotypes (Twenty percent of tolerant and five percent of sensitive genotypes screened out from experiment # 1)
- **Replications:** 4
- **Treatments:** 3
- **Treatments**

  Sowing $date_1$ = 02 April

  Sowing $date_2$ = 12 April

  Sowing $date_3$ = 22 April

3.2.1. Experimental details

Seeds were sown on ridges. Sowing was done on three different sowing dates i.e. 02 April, 12 April and 22 April to check the effect of different sowing dates on okra genotypes. All the cultural practices were kept same for all the genotypes. Data was collected for analyzing the effects of heat stress on following morphological and physiological attributes.

3.2.2. Morphological parameters

3.2.2.1. Number of plants survived

Number of plants survived for each sowing date were counted for each replication in all three sowing dates.

3.2.2.2. Number of leaves per plant

The number of leaves were counted for three plants and average was computed for each replication

3.2.2.3. Plant height (cm)

Plant height was measured at the end of the experiment with the help of a scale in centimeters (cm) from the base of stem to the tip of the shoot and average value for replicates was computed.

3.2.2.4. Pod length (cm)

Pod length was measured for three full grown pods with the help of scale in centimeters (cm) and average value for replicates was computed.

3.2.2.5. Number of pods per plant

The number of pods were counted for three plants and average was computed for each replication

3.2.2.6. Pod diameter (cm)

The pod diameter was measured for three fully grown pods with the help of vernier caliper in centimeters (cm) and average value for replicates was computed.

3.2.2.7. Leaf area per plant (cm^2)

Randomly selected leaves from each replication were separated from plants and sampled leaves were placed on electronic leaf area meter (LI-3100; LI-COR, Inc., Lincoln, Nebr.) to calculate leaf area (LA) in centimeters. Average area per leaf was worked out according to Micheal et al. (2002).

3.2.3. Physiological parameters

3.2.3.1. Chlorophyll contents (SPAD value)

Chlorophyll contents were measured by using chlorophyll meter (CCM-200plus Bio-Scientific USA).

3.2.3.2. Transpiration rate, photosynthetic rate sub-stomatal CO2 and leaf temperature

Described in section 3.1.3.1

3.2.3.3. Water use efficiency (µmol CO_2 mmol1 H_2O)

Described in section 3.1.3.2

3.2.4. Experimental design and statistical analysis

Randomized Complete Block Design (RCBD) with two factors factorial arrangements was applied to the experiment. Collected data were subjected to statistical analysis by using the Fisher's analysis of variance technique and significance of treatments were essayed by using HSD (Tukey Test). Statistical analysis and correlations between variables were also estimated by using statistics 8.1.

3.3. Experiment # 3

Optimization of proline dose for the enhancement of heat tolerance in okra

- **Genotypes:** 4 (two tolerant and two sensitive screened out from experiment experiment # 2)
- **Replications:** 4
- **Treatments:** 6 (Proline dose)
- **Treatments:**

  T_0 = Control

  T_1 = 1 mM

  T_2 = 1.5 mM

  T_3 = 02 mM

  T_4 = 2.5 mM

  T_5 = 03 mM

3.3.1 Experimental details

An optimization experiment was carried out to find the best proline dose that can enhance the heat tolerance potential under heat stress conditions. Seeds of two heat tolerant and two heat sensitive genotypes were sown in plastic pots and kept at 28/22°C (day and night) for four weeks. Four weeks after germination temperature was increased to 45/35°C (day and night) temperature. After achieving 45/35°C (day and night) temperature proline was sprayed. One week after proline application data was collected for studying the effect of proline on following parameters:

3.3.2. Morphological parameters

The parameters studied were same as described in experiment # 1 section 3.1.2.

3.3.3. Physiological parameters

3.3.3.1. Leaf temperature, transpiration rate, photosynthetic rate and sub-stomatal CO_2

Described in section 3.1.3.1

3.3.3.2. Water use efficiency (µmol CO_2 mmol1 H_2O)

Described in section 3.1.3.2

3.3.3.3. Chlorophyll contents (SPAD value)

Chlorophyll contents were measured by using chlorophyll meter (CCM-200plus Bio-Scientific USA).

3.3.4. Experimental design and statistical analysis

Experiment was designed following Complete Randomized Design (CRD) two factors factorial arrangements. Collected data was subjected to statistical analysis by using the Fisher's analysis of variance technique and significance of treatments was essayed by using HSD (Tukey Test). Statistical analysis and correlations between variables were also estimated by using statistics 8.1.

3.4. Experiment # 4

Effects of proline (optimized dose in experiment # 3) on morphological, physiological and biochemical attributes of heat tolerant and heat sensitive okra genotypes

- **Genotypes:** 4 (two tolerant and two sensitive screened out fromvexperiment # 2)
- **Replications:** 4
- **Treatments:** 2
- **Treatments:**

  T_1 = Control

  T_2 = Proline dose (Optimized dose in experiment # 3)

3.4.1. Experimental details

Seeds of 04 okra genotypes screened from 1st and 2nd experiment were sown in plastic pots in growth room situated in the Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Four seeds per pot were sown and each replication comprised of four pots. The pots were watered according to the need of plants by observing the moisture of sand. Plants were grown at 28/22°C (day and night) for four weeks. After four weeks of germination temperature of growth room increased to 45/35°C (day and night) by increasing 2°C every day to avoid the osmotic shock. After achieving 45/35°C (day and night) temperature proline (dose optimized in experiment # 3) was sprayed. One week after proline application data was collected for studying the effect of proline on heat tolerant and heat sensitive genotypes and following parameters were recorded.

3.4.2. Morphological attributes

The parameters studied were same as described in experiment # 1 section 3.1.2.

3.4.3. Physiological attributes

The parameters studied were same as mentioned in experiments # 1 section 3.1.3.

3.4.4. Biochemical attributes

3.4.4.1. Chlorophyll contents (SPAD value)

Chlorophyll contents were measured as described in section 3.1.3.3.

3.4.4.2. Superoxide dismutase (SOD) (U mg^{-1} protein)

The activity of SOD was analyzed according to the protocol of Giannopolitis and Ries (1977) by calculating its potential to hinder the photo reduction of nitroblue tetrazolium (NBT). The reaction solution (3 mL) contained 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 mM EDTA, 50 mM phosphate buffer (pH 7.8) and 20-50 mL of enzyme extract. The test tubes having the reaction solution were irradiated under light (15 fluorescent lamps) at 78 mmol m-2 s-1 for 15 min. The absorbance of the irradiated solution was noted at 560 nm by using a Spectrophotometer (Hitachi-650, Japan). One unit of SOD activity was explained as the amount of enzyme that restrained 50% of NBT photo decline.

3.4.4.3. Peroxidase (POD) (U mg^{-1} protein)

The peroxidase (POD) activity was measured by the procedure of Chance and Maehly (1955) with some alteration. The POD reaction solution (3 mL) comprised of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL of enzyme extract. Variations in absorbance of the reaction solution at 470 nm were calculated after every 20 seconds. One unit POD activity was assigned as an absorbance change of 0.01 units per min. The activity of each enzyme was expressed on the basis of protein content.

3.4.4.4. Catalase (CAT) (U mg^{-1} protein)

Catalase (CAT) activity was measured by the procedure of Chance and Maehly (1955) with some alteration. The CAT reaction solution (3 mL) comprised of 50 mM phosphate buffer (pH 7.0), 5.9 mM H_2O_2 and 0.1 mL of enzyme extract. Changes in absorbance of the reaction solution were recorded after every 20s at 240 nm. One unit CAT activity was specified as an absorbance change of 0.01 units per min.

3.4.4.5. Proline contents

Reagents

Three compounds (acetic acid, ninhydrine and toluene) were used for the estimation of proline contents. In the first phase a solution comprising of glacial acetic acid (20 mL) and 6M orthophosphoric (20 mL) was prepared and then ninhydrine (1.25 g) was dissolved in this solution. This solution was shaken softly and mixed well (it is stable for 24 hours).

However, acetic acid was used in making the ninhydrine solution with orthophosphoric and 2 mL was utilized in test tubes containing filtrate before heating. Whereas toluene (10 mL) was used for the proline extraction from reaction mixture.

Procedure

The proline was calculated according to the method of the Bates et al., 1973 from homogenized fresh leaf tissue (0.5 g) in 10 mL of 3% sulfo-salicylic acid. A solution containing 2 mL of filtered homogenate of fresh okra leaf samples, 2 mL of ninhydrin solution (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M orthophosphoric acid) and 2 mL of glacial acetic acid was heated at 100ºC for 60 minutes. The test tubes containing this mixture were then shifted in ice bath to terminate the reaction. Reaction mixture was then extracted with 10 mL toluene, mixed robustly by passing a continuous air stream for 1-2 minutes. Toluene was aspirated from chromophore. Aqueous phase was separated, warmed at room temperature and absorbance was noted at 520 nm using double beam spectrophotometer (Hitachi-120, Japan) and toluene was used as a blank. Proline concentration was determined from a standard curve and calculated on fresh weight basis.

Calculations

(Mole proline g^{-1} fresh weight = (g proline ml^{-1} × ml of toluene/115.5) / (g of sample/5)

3.4.4.6. Glycine betaine contents

Reagents

Various reagents like hydrochloric acid (2N), potassium tri-iodide and 1-2, dichloromethane were used for the determination of glycinebetaine. The potassium tri-iodide solution was made by dissolving potassium iodide (10 g) and iodine (7.5 g) in 100 mL of hydrochloric acid (1 N).

Procedure

Glycine-betaine was estimated by grinding the 1.0 g of fresh leaf material in 10 mL of distilled water. The mixture was filtered and 1 mL of it was mixed with 1 mL of HCl (2N) and then 0.5 mL of this solution was taken in test tubes having 0.2 ml of potassium tri-iodide solution. The final mixture was cooled in an ice bath for 1.5 hours with random shaking and then 2.0 mL of ice cooled distilled water along with 20 mL of 12- dichloroethane (cooled at -10^{o}C) were added in the mixture. Two layers were formed in the mixtures which were mixed

by passing a continuous stream of air for 1-2 minutes while the tubes were still in the ice bath (4oC). The upper aqueous layer was redundant and optical density of organic layer was measured at 365 nm, using double beam Spectrophotometer (Hitachi-120, Japan) (Grieve and Gratan, 1983). The concentrations of the glycinebetaine were calculated against the standard curve. The blank was developed as above with distilled water.

3.4.5. Water relations

3.4.5.1. Leaf water potential (Ψw) (-MPa)

At the end of experiment, a razor was used to cut the fully expanded leaves and was placed in the gasket of pressure chamber (Model, 615, USA) to compute leaf water potential (Ψw). The data were computed in the morning before 12.00 am (10.00 am to 12.00 a.m.).

3.4.5.2. Leaf osmotic potential (Ψs) (-MPa) by Osmometer

The same leaf that was used in pressure chamber for Ψs and was placed in a plastic bag and kept at low temperature (-20°C) in a freezer for a week. The frozen leaf material was then thawed at room temperature for half an hour and cell sap was extracted with the help of a disposable syringe. The 10 µL of extracted sap was placed on osmometer (Wescor, Model-5500) with the help of plastic syringe and Ψs measurement was taken.

3.4.5.3. Leaf turgor potential (Ψp) = (Ψw) - (Ψs) (MPa)

Turgor potential *(*Ψ*p*) signifies the difference between Ψ*w* and Ψ*s*, therefore Ψ*p* was calculated, following the below mentioned equation:

$$\Psi \quad = \Psi \quad - \Psi$$

3.4.5.4. Leaf relative water contents (RWC)

Three mature leaves were detached randomly from ten plants (two plants from each replication per treatment). After tagging, leaves were washed under tap water for at least five minutes and then blotted with tissue paper. After blotting the leaves were weighed and dipped in tap water for 24 hours and their turgid weight was taken. After measuring the turgid weight, leaves were oven dried at 72^{o}C for 48 hours and their dry weight was measured with the help of digital electrical balance (Bosch AE-160, Germany). The method reported by Weathearly and Barrs, (1962) was used to calculate the average RWC per replicate.

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100.$$

FW = fresh weight

DW = dry weight

TW = turgid weight

3.4.6. Experimental design and statistical analysis

Complete Randomized Design (CRD) with factorial arrangements was applied to the experiment in control conditions. Collected data was subjected to statistical analysis by using the Fisher's analysis of variance technique and significance of treatments were essayed by using HSD (Tukey Test). Statistical analysis and correlations between variables were also estimated by using statistics 8.1.

CHAPTER 4 RESULTS

Experiment # 1:

Screening of different okra genotypes for heat tolerance

4.1.1. Effect of heat stress on shoot length (cm)

The results for effect of heat stress on shoot length of okra genotypes are presented in table 4.1.1. All genotypes showed significantly ($p \leq 0.05$) varied response for shoot length towards high temperature. On the basis of shoot length, genotypes were divided into different groups. 14 genotypes (14%) of okra exhibited shoot length less than 10 cm while 47 genotypes (47% of total genotype under study) had shoot length within the range of 10-15cm. A shoot length range of 15.1-20 cm was recorded in 37 genotypes (37%) while only 2 genotypes (2%) exhibited shoot length of more than 20 cm. The maximum shoot length was recorded in VI056448 (23.20 cm) whereas, minimum shoot length was observed in VI033803 (5.5 cm). The complete results of all genotypes (Mean ± SE) regarding shoot length are presented in table 4.1.2.

Table 4.1.1: Effect of heat stress on shoot length of okra

Shoot length (cm)	Genotypes
< 10	14(14)*
10-15	47(47)
15.1-20	37(37)
> 20	02(02)
Minimum value	5.5
Maximum value	23.20
Mean	13.98
SD	3.476
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** S^1 0.05 (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.2: Shoot length of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	16.55 ± 0.49	51. VI049632	12.30 ± 0.49
2. VI039638	13.60 ± 0.87	52. VI049954	14.20 ± 0.78
3. VI039643	16.95 ± 0.90	53. VI049961	15.55 ± 0.54
4. VI039651	16.30 ± 0.56	54. VI050150	18.25 ± 0.49
5. VI039652	18.55 ± 3.16	55. VI050170	8.50 ± 0.56
6. VI040649	13.25 ± 0.60	56. VI050549	16.25 ± 0.95
7. VI040770	16.05 ± 0.67	57. VI051038	10.25 ± 1.00
8. VI040865	12.75 ± 1.00	58. VI051039	15.00 ± 1.23
9. VI041139	13.55 ± 1.10	59. VI051042	13.75 ± 0.67
10. VI041215	17.10 ± 0.74	60. VI051047	7.65 ± 0.67
11. VI041461	16.05 ± 0.57	61. VI051048	14.30 ± 1.33
12. VI041763	22.40 ± 0.49	62. VI051062	15.95 ± 1.00
13. VI044233	16.60 ± 1.23	63. VI051114	10.90 ± 0.51
14. VI044241	17.45 ± 0.90	64. VI054562	16.85 ± 0.90
15. VI044244	18.25 ± 0.60	65. VI054565	13.75 ± 0.67
16. VI037997	13.65 ± 0.64	66. VI054566	17.70 ± 3.30
17. VI037995	15.25 ± 0.76	67. VI054568	6.60 ± 2.34
18. VI037994	19.00 ± 0.51	68. VI055017	7.10 ± 1.39
19. VI033773	14.95 ± 0.49	69. VI055018	14.25 ± 1.00
20. VI033775	17.40 ± 0.62	70. VI055110	15.65 ± 0.49
21. VI033781-A	15.10 ± 0.51	71. VI055119	11.75 ± 1.00
22. VI033781-B	16.30 ± 0.87	72. VI055219	18.75 ± 1.05
23. VI033784	14.55 ± 0.49	73. VI055220	17.90 ± 1.55
24. VI033785	19.85 ± 0.60	74. VI055996	12.45 ± 1.05
25. VI033786	18.25 ± 0.49	75. VI056069	13.75 ± 1.47
26. VI033791	14.70 ± 1.02	76. VI056079	9.20 ± 0.49
27. VI033803	5.55 ± 1.86	77. VI056402	10.80 ± 0.56
28. VI033805	7.95 ± 0.60	78. VI056404	11.05 ± 0.95
29. VI033810	9.35 ± 0.57	79. VI056407	11.65 ± 1.05
30. VI033824	13.50 ± 0.97	80. VI056447	15.25 ± 1.05
31. VI036201	17.25 ± 1.05	81. VI056448	23.20 ± 1.44
32. VI036203	13.95 ± 0.54	82. VI056449	18.85 ± 1.63
33. VI036211	11.35 ± 1.00	83. VI056450	19.55 ± 0.57
34. VI036212	8.90 ± 0.92	84. VI056451	13.05 ± 0.49
35. VI036213	8.30 ± 1.23	85. VI056452	15.25 ± 0.85
36. VI036215	12.40 ± 0.92	86. VI056453	14.45 ± 0.52
37. VI046536	12.35 ± 1.52	87. VI056454	15.70 ± 1.28
38. VI046537	11.75 ± 1.31	88. VI056455	13.75 ± 0.80
39. VI046544	9.95 ± 0.90	89. VI056456	14.45 ± 0.71
40. VI046554	14.10 ± 0.97	90. VI057245	14.45 ± 0.50
41. VI046556	12.10 ± 1.12	91. VI057249	13.95 ± 0.50
42. VI046559	17.50 ± 0.53	92. VI060131	17.35 ± 0.60
43. VI046562	8.70 ± 0.58	93. VI060132	13.35 ± 1.31
44. VI046566	15.40 ± 2.05	94. VI060133	11.30 ± 0.78
45. VI047672	15.35 ± 1.52	95. VI060206	18.85 ± 1.31
46. VI047751	11.75 ± 0.60	96. VI060313	12.80 ± 1.28
47. VI047808	13.55 ± 0.54	97. VI060314	11.30 ± 0.92
48. VI048154	13.30 ± 1.18	98. VI060315	9.85 ± 0.76
49. VI048291	12.80 ± 0.51	99. VI060316	11.45 ± 0.49
50. VI048596	6.10 ± 1.23	100. VI060317	10.90 ± 0.56

4.1.2. Effect of heat stress on root length (cm)

Results regarding root length indicated that all okra genotypes behaved significantly different *($p \leq 0.05$)* under elevated temperature (Table 4.1.3). From 100 genotypes studied, only 3 genotypes (3%) exhibited less than 3 cm root length while a good quantity of 53 genotypes (53%) showed root length in the range of 3.1-5 cm and 39 genotypes (39%) gave root length ranged from 5.1-7.9 cm. The remaining 5 genotypes expressed root length more than 8 cm (Table 4.1.2). The minimum root length (2.80 cm) was recorded in VI033803 and maximum root length (9.65 cm) was observed in VI051062. (Mean ± SE) regarding root length are given in table 4.1.4.

Table 4.1.3: Effect of heat stress on root length of okra

Root length (cm)	Genotypes
< 3	3(3)
3.1-5	53(53)
5.1-7.1	39(39)
8> 8	5(5)
Minimum value	2.80
Maximum value	9.65
Mean	5.20
SD	5.386
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** S^1 0.05 (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.4: Root length of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	2.85 ± 0.35	51. VI049632	4.40 ± 0.33
2. VI039638	3.95 ± 0.31	52. VI049954	4.05 ± 0.40
3. VI039643	5.00 ± 0.29	53. VI049961	4.60 ± 0.58
4. VI039651	4.00 ± 0.53	54. VI050150	5.70 ± 1.26
5. VI039652	5.35 ± 1.74	55. VI050170	3.10 ± 0.22
6. VI040649	6.85 ± 1.06	56. VI050549	4.50 ± 0.43
7. VI040770	3.45 ± 0.21	57. VI051038	4.25 ± 1.34
8. VI040865	4.50 ± 0.22	58. VI051039	7.50 ± 0.75
9. VI041139	6.95 ± 0.27	59. VI051042	4.90 ± 0.20
10. VI041215	4.80 ± 0.38	60. VI051047	4.15 ± 1.29
11. VI041461	5.25 ± 0.72	61. VI051048	7.40 ± 0.38
12. VI041763	6.65 ± 0.78	62. VI051062	9.65 ± 0.95
13. VI044233	6.75 ± 1.63	63. VI051114	4.65 ± 0.78
14. VI044241	6.10 ± 0.22	64. VI054562	5.00 ± 0.53
15. VI044244	6.55 ± 0.83	65. VI054565	4.35 ± 0.61
16. VI037997	5.60 ± 0.38	66. VI054566	5.10 ± 1.26
17. VI037995	5.70 ± 0.97	67. VI054568	4.30 ± 0.33
18. VI037994	4.50 ± 0.22	68. VI055017	5.40 ± 0.38
19. VI033773	4.90 ± 0.58	69. VI055018	8.85 ± 0.35
20. VI033775	4.95 ± 0.61	70. VI055110	4.40 ± 0.22
21. VI033781-A	4.35 ± 0.20	71. VI055119	3.80 ± 0.38
22. VI033781-B	4.05 ± 0.23	72. VI055219	4.00 ± 0.22
23. VI033784	6.15 ± 0.67	73. VI055220	5.75 ± 0.56
24. VI033785	4.10 ± 0.25	74. VI055996	6.00 ± 1.66
25. VI033786	4.70 ± 0.33	75. VI056069	4.60 ± 0.25
26. VI033791	4.40 ± 0.58	76. VI056079	3.30 ± 0.22
27. VI033803	2.80 ± 0.63	77. VI056402	9.50 ± 0.43
28. VI033805	5.45 ± 1.34	78. VI056404	6.40 ± 1.14
29. VI033810	3.75 ± 0.72	79. VI056407	6.15 ± 0.56
30. VI033824	4.20 ± 0.43	80. VI056447	4.60 ± 0.58
31. VI036201	5.70 ± 0.92	81. VI056448	6.90 ± 0.38
32. VI036203	3.50 ± 0.29	82. VI056449	5.45 ± 0.23
33. VI036211	7.35 ± 0.95	83. VI056450	5.55 ± 0.21
34. VI036212	4.05 ± 0.20	84. VI056451	8.00 ± 0.22
35. VI036213	4.90 ± 1.31	85. VI056452	5.45 ± 0.23
36. VI036215	7.35 ± 0.20	86. VI056453	7.15 ± 1.00
37. VI046536	3.85 ± 0.67	87. VI056454	6.05 ± 0.89
38. VI046537	4.35 ± 0.20	88. VI056455	5.00 ± 0.25
39. VI046544	3.95 ± 0.50	89. VI056456	4.90 ± 0.33
40. VI046554	4.50 ± 0.20	90. VI057245	4.30 ± 0.38
41. VI046556	4.35 ± 0.61	91. VI057249	7.40 ± 1.03
42. VI046559	6.60 ± 0.20	92. VI060131	5.25 ± 0.45
43. VI046562	4.60 ± 0.25	93. VI060132	4.85 ± 0.89
44. VI046566	5.40 ± 0.58	94. VI060133	5.20 ± 0.20
45. VI047672	8.05 ± 0.40	95. VI060206	5.10 ± 0.48
46. VI047751	4.50 ± 0.22	96. VI060313	4.60 ± 0.20
47. VI047808	4.55 ± 0.21	97. VI060314	5.40 ± 0.48
48. VI048154	3.85 ± 0.61	98. VI060315	2.90 ± 0.33
49. VI048291	5.30 ± 0.86	99. VI060316	7.00 ± 0.97
50. VI048596	3.65 ± 0.50	100. VI060317	4.85 ± 0.35

4.1.3. Effect of heat stress on shoot fresh weight (g)

Results revealed that shoot fresh weight significantly ($p \leq 0.05$) varied under high temperature for all genotypes. Shoot fresh weight of 64 genotypes was noted < 2 g, while it ranged 2-2.5 g for 19 genotypes and 2.5-4 g for 16 genotypes. The shoot fresh weight of remaining 1 genotype exceeded than 4 g. VI051114 expressed the maximum shoot fresh weight (4.24 g), on the other hand minimum shoot fresh weight (0.45 g) was recorded in VI049961 (Table 4.1.5). The complete results of all genotypes (Mean ± SE) regarding shoot fresh weight can be seen in table 4.1.6.

Table 4.1.5: Effect of heat stress on shoot fresh weight of okra

Shoot fresh weight (g)	Genotypes
< 2	64(64)*
2- 2.5	19(19)
2.5-04	16(16)
04>	1(1)
Minimum value	0.38
Maximum value	4.24
Mean	1.80
SD	0.745
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** S^1 0.05 (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.6: Shoot fresh weight (g) length of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	1.27 ± 0.46	51. VI049632	0.78 ± 0.20
2. VI039638	1.00 ± 0.38	52. VI049954	1.43 ± 0.34
3. VI039643	2.21 ± 0.12	53. VI049961	0.45 ± 0.11
4. VI039651	1.39 ± 0.29	54. VI050150	2.91 ± 0.39
5. VI039652	2.83 ± 0.98	55. VI050170	2.01 ± 0.36
6. VI040649	0.99 ± 0.16	56. VI050549	2.22 ± 0.28
7. VI040770	1.04 ± 0.11	57. VI051038	1.85 ± 0.44
8. VI040865	1.66 ± 0.39	58. VI051039	2.92 ± 0.65
9. VI041139	2.03 ± 0.18	59. VI051042	1.50 ± 0.11
10. VI041215	1.84 ± 0.13	60. VI051047	1.21 ± 0.19
11. VI041461	2.15 ± 0.43	61. VI051048	1.05 ± 0.32
12. VI041763	2.60 ± 0.12	62. VI051062	2.88 ± 0.22
13. VI044233	1.51 ± 0.11	63. VI051114	4.24 ± 0.40
14. VI044241	1.81 ± 0.24	64. VI054562	2.32 ± 0.11
15. VI044244	1.68 ± 0.61	65. VI054565	2.54 ± 0.38
16. VI037997	2.55 ± 0.16	66. VI054566	3.08 ± 0.98
17. VI037995	2.60 ± 0.38	67. VI054568	1.35 ± 0.10
18. VI037994	1.62 ± 0.12	68. VI055017	1.54 ± 0.19
19. VI033773	2.04 ± 0.12	69. VI055018	3.48 ± 0.12
20. VI033775	2.25 ± 0.37	70. VI055110	1.37 ± 0.24
21. VI033781-A	1.43 ± 0.35	71. VI055119	1.44 ± 0.29
22. VI033781-B	1.02 ± 0.11	72. VI055219	1.10 ± 0.18
23. VI033784	1.40 ± 0.11	73. VI055220	0.91 ± 0.12
24. VI033785	0.88 ± 0.24	74. VI055996	1.63 ± 0.64
25. VI033786	2.13 ± 0.26	75. VI056069	1.55 ± 0.15
26. VI033791	1.73 ± 0.22	76. VI056079	0.74 ± 0.23
27. VI033803	1.43 ± 0.11	77. VI056402	2.54 ± 0.38
28. VI033805	1.67 ± 0.75	78. VI056404	2.40 ± 0.36
29. VI033810	0.93 ± 0.19	79. VI056407	0.65 ± 0.11
30. VI033824	1.92 ± 0.13	80. VI056447	1.19 ± 0.16
31. VI036201	1.07 ± 0.17	81. VI056448	3.77 ± 0.11
32. VI036203	1.23 ± 0.17	82. VI056449	1.74 ± 0.11
33. VI036211	1.51 ± 0.21	83. VI056450	3.11 ± 0.23
34. VI036212	0.97 ± 0.23	84. VI056451	2.29 ± 0.81
35. VI036213	1.08 ± 0.40	85. VI056452	2.66 ± 0.21
36. VI036215	2.72 ± 0.20	86. VI056453	2.37 ± 0.40
37. VI046536	1.54 ± 0.20	87. VI056454	2.14 ± 1.13
38. VI046537	1.37 ± 0.24	88. VI056455	1.86 ± 0.24
39. VI046544	1.40 ± 0.37	89. VI056456	1.90 ± 0.15
40. VI046554	1.31 ± 0.13	90. VI057245	1.71 ± 0.21
41. VI046556	1.99 ± 0.39	91. VI057249	2.11 ± 0.46
42. VI046559	2.65 ± 0.48	92. VI060131	3.25 ± 0.24
43. VI046562	1.65 ± 0.31	93. VI060132	2.42 ± 0.49
44. VI046566	1.26 ± 0.18	94. VI060133	1.77 ± 0.12
45. VI047672	1.48 ± 0.28	95. VI060206	2.40 ± 0.34
46. VI047751	1.38 ± 0.19	96. VI060313	2.69 ± 0.26
47. VI047808	1.15 ± 0.13	97. VI060314	2.18 ± 0.37
48. VI048154	0.70 ± 0.19	98. VI060315	1.51 ± 0.11
49. VI048291	0.91 ± 0.13	99. VI060316	2.63 ± 0.42
50. VI048596	0.38 ± 0.09	100. VI060317	1.29 ± 0.29

4.1.4. Effect of heat stress on root fresh weight (g)

The analyzed data related to root fresh weight showed significant ($p \leq 0.05$) different response toward stressed environment (Table 4.1.7). Results revealed that major proportion of 95 genotypes (95%) exhibited less than 0.5 g weight while it ranged 0.5-0.99g for remaining 5 genotypes (5% total genotypes under study). VI0054568 had minimum root fresh weight (0.08 g) while VI055018 possessed the highest root fresh weight (1.06 g). Complete results of all genotypes (Mean ± SE) regarding root fresh weight are expressed in table 4.1.8.

Table 4.1.7: Effect of heat stress on root fresh weight of okra

Root fresh weight (gm)	Genotypes
< 0.5	95(95)
0.5-0.99	5(5)
1-1.5	00(00)
> 1.5	00(00)
Minimum value	0.10
Maximum value	0.96
Mean	0.26
SD	0.173
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** S^1 0.05 (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.8: Root fresh weight (g) length of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	0.12 ± 0.056	51. VI049632	0.18 ± 0.051
2. VI039638	0.16 ± 0.064	52. VI049954	0.23 ± 0.076
3. VI039643	0.13 ± 0.067	53. VI049961	0.32 ± 0.053
4. VI039651	0.48 ± 0.245	54. VI050150	0.30 ± 0.043
5. VI039652	0.96 ± 0.465	55. VI050170	0.50 ± 0.071
6. VI040649	0.10 ± 0.057	56. VI050549	0.75 ± 0.067
7. VI040770	0.11 ± 0.052	57. VI051038	0.86 ± 0.085
8. VI040865	0.11 ± 0.051	58. VI051039	0.14 ± 0.056
9. VI041139	0.29 ± 0.062	59. VI051042	0.13 ± 0.062
10. VI041215	0.25 ± 0.060	60. VI051047	0.21 ± 0.069
11. VI041461	0.19 ± 0.045	61. VI051048	0.15 ± 0.046
12. VI041763	0.28 ± 0.053	62. VI051062	0.30 ± 0.045
13. VI044233	0.18 ± 0.064	63. VI051114	0.31 ± 0.050
14. VI044241	0.22 ± 0.060	64. VI054562	0.25 ± 0.058
15. VI044244	0.21 ± 0.069	65. VI054565	0.39 ± 0.117
16. VI037997	0.32 ± 0.062	66. VI054566	0.45 ± 0.208
17. VI037995	0.17 ± 0.047	67. VI054568	0.08 ± 0.080
18. VI037994	0.12 ± 0.067	68. VI055017	0.28 ± 0.088
19. VI033773	0.22 ± 0.043	69. VI055018	1.06 ± 0.133
20. VI033775	0.21 ± 0.078	70. VI055110	0.19 ± 0.060
21. VI033781-A	0.11 ± 0.048	71. VI055119	0.12 ± 0.060
22. VI033781-B	0.12 ± 0.055	72. VI055219	0.15 ± 0.062
23. VI033784	0.21 ± 0.044	73. VI055220	0.16 ± 0.058
24. VI033785	0.40 ± 0.114	74. VI055996	0.39 ± 0.144
25. VI033786	0.17 ± 0.044	75. VI056069	0.20 ± 0.067
26. VI033791	0.23 ± 0.056	76. VI056079	0.20 ± 0.051
27. VI033803	0.79 ± 0.250	77. VI056402	0.48 ± 0.083
28. VI033805	0.36 ± 0.083	78. VI056404	0.39 ± 0.119
29. VI033810	0.12 ± 0.056	79. VI056407	0.16 ± 0.071
30. VI033824	0.22 ± 0.062	80. VI056447	0.20 ± 0.078
31. VI036201	0.24 ± 0.062	81. VI056448	0.35 ± 0.056
32. VI036203	0.16 ± 0.047	82. VI056449	0.21 ± 0.047
33. VI036211	0.18 ± 0.045	83. VI056450	0.26 ± 0.078
34. VI036212	0.20 ± 0.051	84. VI056451	0.28 ± 0.098
35. VI036213	0.13 ± 0.058	85. VI056452	0.32 ± 0.050
36. VI036215	0.29 ± 0.067	86. VI056453	0.27 ± 0.064
37. VI046536	0.16 ± 0.043	87. VI056454	0.41 ± 0.262
38. VI046537	0.32 ± 0.090	88. VI056455	0.22 ± 0.047
39. VI046544	0.25 ± 0.080	89. VI056456	0.22 ± 0.056
40. VI046554	0.20 ± 0.053	90. VI057245	0.13 ± 0.064
41. VI046556	0.28 ± 0.056	91. VI057249	0.27 ± 0.085
42. VI046559	0.40 ± 0.048	92. VI060131	0.24 ± 0.064
43. VI046562	0.18 ± 0.045	93. VI060132	0.24 ± 0.055
44. VI046566	0.22 ± 0.048	94. VI060133	0.21 ± 0.058
45. VI047672	0.27 ± 0.064	95. VI060206	0.25 ± 0.067
46. VI047751	0.12 ± 0.067	96. VI060313	0.18 ± 0.047
47. VI047808	0.09 ± 0.055	97. VI060314	0.22 ± 0.073
48. VI048154	0.11 ± 0.060	98. VI060315	0.13 ± 0.067
49. VI048291	0.20 ± 0.090	99. VI060316	0.30 ± 0.083
50. VI048596	0.13 ± 0.060	100. VI060317	0.13 ± 0.071

4.1.5. Effect of heat stress on shoot dry weight (g)

Shoot dry weight differed significantly ($p \leq 0.05$) for okra genotypes when grown under high temperature condition (Table 4.1.9). Results showed that 9 genotypes (9% of total genotype under study) exhibited less than 0.1 g shoot dry weight, 31 genotypes (31%) had 0.1-0.2 g shoot dry weight and 30 genotypes (30%) showed 0.2-0.3 g shoot dry weight. A shoot dry weight of more than 0.3 g was recorded in 25 genotypes (25%) of okra. The minimum shoot dry weight (0.06 g) was recorded in VI056079 while maximum shoot dry weight (0.51 g) was noted in VI051114. The complete results of all genotypes (Mean ± SE) for shoot dry weight are available in table 4.1.10.

Table 4.1.9: Effect of heat stress on shoot dry weight of okra

Shoot dry weight (gm)	Genotypes
< 0.1	09(09)*
0.1-0.2	31(31)
0.2-0.3	30(30)
> 0.3	25(25)
minimum value	0.06
Maximum value	0.51
Mean	0.24
SD	0.093
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** S^1 0.05 (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.10: Shoot dry weight (g) length of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	0.18 ± 0.042	51. VI049632	0.15 ± 0.023
2. VI039638	0.25 ± 0.012	52. VI049954	0.32 ± 0.067
3. VI039643	0.36 ± 0.026	53. VI049961	0.23 ± 0.056
4. VI039651	0.18 ± 0.012	54. VI050150	0.37 ± 0.073
5. VI039652	0.28 ± 0.145	55. VI050170	0.20 ± 0.065
6. VI040649	0.15 ± 0.023	56. VI050549	0.29 ± 0.012
7. VI040770	0.15 ± 0.053	57. VI051038	0.25 ± 0.125
8. VI040865	0.30 ± 0.013	58. VI051039	0.39 ± 0.012
9. VI041139	0.22 ± 0.018	59. VI051042	0.22 ± 0.031
10. VI041215	0.25 ± 0.021	60. VI051047	0.14 ± 0.023
11. VI041461	0.23 ± 0.059	61. VI051048	0.13 ± 0.039
12. VI041763	0.35 ± 0.026	62. VI051062	0.46 ± 0.012
13. VI044233	0.25 ± 0.028	63. VI051114	0.51 ± 0.023
14. VI044241	0.31 ± 0.018	64. VI054562	0.31 ± 0.028
15. VI044244	0.28 ± 0.053	65. VI054565	0.24 ± 0.026
16. VI037997	0.29 ± 0.012	66. VI054566	0.33 ± 0.153
17. VI037995	0.43 ± 0.039	67. VI054568	0.10 ± 0.012
18. VI037994	0.19 ± 0.037	68. VI055017	0.16 ± 0.026
19. VI033773	0.33 ± 0.016	69. VI055018	0.44 ± 0.026
20. VI033775	0.39 ± 0.037	70. VI055110	0.17 ± 0.023
21. VI033781-A	0.26 ± 0.012	71. VI055119	0.25 ± 0.018
22. VI033781-B	0.28 ± 0.023	72. VI055219	0.15 ± 0.014
23. VI033784	0.33 ± 0.039	73. VI055220	0.13 ± 0.016
24. VI033785	0.14 ± 0.012	74. VI055996	0.17 ± 0.087
25. VI033786	0.29 ± 0.076	75. VI056069	0.18 ± 0.034
26. VI033791	0.21 ± 0.014	76. VI056079	0.06 ± 0.018
27. VI033803	0.09 ± 0.010	77. VI056402	0.30 ± 0.034
28. VI033805	0.17 ± 0.070	78. VI056404	0.28 ± 0.023
29. VI033810	0.07 ± 0.026	79. VI056407	0.12 ± 0.031
30. VI033824	0.26 ± 0.018	80. VI056447	0.21 ± 0.021
31. VI036201	0.14 ± 0.031	81. VI056448	0.46 ± 0.012
32. VI036203	0.12 ± 0.021	82. VI056449	0.27 ± 0.053
33. VI036211	0.21 ± 0.012	83. VI056450	0.38 ± 0.034
34. VI036212	0.12 ± 0.023	84. VI056451	0.22 ± 0.090
35. VI036213	0.17 ± 0.048	85. VI056452	0.29 ± 0.023
36. VI036215	0.34 ± 0.059	86. VI056453	0.30 ± 0.056
37. VI046536	0.16 ± 0.053	87. VI056454	0.31 ± 0.093
38. VI046537	0.13 ± 0.042	88. VI056455	0.31 ± 0.034
39. VI046544	0.14 ± 0.037	89. VI056456	0.23 ± 0.013
40. VI046554	0.12 ± 0.021	90. VI057245	0.18 ± 0.031
41. VI046556	0.25 ± 0.090	91. VI057249	0.28 ± 0.026
42. VI046559	0.34 ± 0.013	92. VI060131	0.24 ± 0.056
43. VI046562	0.17 ± 0.059	93. VI060132	0.36 ± 0.013
44. VI046566	0.18 ± 0.016	94. VI060133	0.24 ± 0.023
45. VI047672	0.27 ± 0.067	95. VI060206	0.35 ± 0.031
46. VI047751	0.19 ± 0.014	96. VI060313	0.26 ± 0.034
47. VI047808	0.20 ± 0.012	97. VI060314	0.29 ± 0.039
48. VI048154	0.17 ± 0.023	98. VI060315	0.14 ± 0.021
49. VI048291	0.25 ± 0.034	99. VI060316	0.23 ± 0.012
50. VI048596	0.06 ± 0.021	100. VI060317	0.27 ± 0.056

4.1.6. Effect of heat stress on root dry weight (g)

All genotypes revealed significant ($p \leq 0.05$) different response in respect of root dry weight under temperature stress. According to results in table 4.1.11 a major amount of 90 genotypes (90% of total genotypes under study) had root dry weight of less than 0.1 g while remaining 10 genotypes (10%) showed root dry weight in the range of 0.1-0.2 g. VI033785 genotype showed highest root dry weight (0.160 g) while VI056079 and VI047751 gave lowest root dry weight (0.030 g). The complete results of all genotypes (Mean ± SE) regarding root dry weight are presented in table 4.1.12.

Table 4.1.11: Effect of heat stress on root dry weight of okra

Root dry weight (gm)	Genotypes
< 0.1	90(90)
0.1-0.2	10(10)
0.2-0.9	00(00)
> 0.91	00(00)
Minimum value	0.030
Maximum value	0.160
Mean	0.061
SD	0.028
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.12: Root dry weight (g) length of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	0.040 ± 0.009	51. VI049632	0.045 ± 0.003
2. VI039638	0.060 ± 0.009	52. VI049954	0.045 ± 0.006
3. VI039643	0.070 ± 0.009	53. VI049961	0.035 ± 0.003
4. VI039651	0.045 ± 0.006	54. VI050150	0.055 ± 0.006
5. VI039652	0.045 ± 0.006	55. VI050170	0.055 ± 0.006
6. VI040649	0.085 ± 0.012	56. VI050549	0.050 ± 0.015
7. VI040770	0.030 ± 0.004	57. VI051038	0.065 ± 0.018
8. VI040865	0.035 ± 0.006	58. VI051039	0.075 ± 0.012
9. VI041139	0.105 ± 0.006	59. VI051042	0.035 ± 0.006
10. VI041215	0.055 ± 0.006	60. VI051047	0.035 ± 0.003
11. VI041461	0.060 ± 0.009	61. VI051048	0.065 ± 0.006
12. VI041763	0.100 ± 0.004	62. VI051062	0.070 ± 0.015
13. VI044233	0.045 ± 0.006	63. VI051114	0.080 ± 0.004
14. VI044241	0.090 ± 0.009	64. VI054562	0.060 ± 0.009
15. VI044244	0.070 ± 0.009	65. VI054565	0.095 ± 0.023
16. VI037997	0.110 ± 0.026	66. VI054566	0.110 ± 0.038
17. VI037995	0.055 ± 0.012	67. VI054568	0.035 ± 0.006
18. VI037994	0.045 ± 0.003	68. VI055017	0.050 ± 0.009
19. VI033773	0.115 ± 0.029	69. VI055018	0.140 ± 0.006
20. VI033775	0.095 ± 0.018	70. VI055110	0.045 ± 0.003
21. VI033781-A	0.040 ± 0.004	71. VI055119	0.035 ± 0.003
22. VI033781-B	0.040 ± 0.004	72. VI055219	0.065 ± 0.006
23. VI033784	0.120 ± 0.026	73. VI055220	0.040 ± 0.009
24. VI033785	0.160 ± 0.026	74. VI055996	0.120 ± 0.020
25. VI033786	0.045 ± 0.012	75. VI056069	0.035 ± 0.006
26. VI033791	0.045 ± 0.006	76. VI056079	0.030 ± 0.004
27. VI033803	0.045 ± 0.006	77. VI056402	0.065 ± 0.006
28. VI033805	0.090 ± 0.032	78. VI056404	0.095 ± 0.018
29. VI033810	0.035 ± 0.006	79. VI056407	0.035 ± 0.006
30. VI033824	0.060 ± 0.009	80. VI056447	0.045 ± 0.006
31. VI036201	0.090 ± 0.004	81. VI056448	0.070 ± 0.004
32. VI036203	0.040 ± 0.004	82. VI056449	0.060 ± 0.009
33. VI036211	0.075 ± 0.006	83. VI056450	0.030 ± 0.004
34. VI036212	0.045 ± 0.006	84. VI056451	0.060 ± 0.009
35. VI036213	0.065 ± 0.018	85. VI056452	0.085 ± 0.012
36. VI036215	0.065 ± 0.012	86. VI056453	0.045 ± 0.006
37. VI046536	0.045 ± 0.003	87. VI056454	0.125 ± 0.018
38. VI046537	0.075 ± 0.018	88. VI056455	0.045 ± 0.003
39. VI046544	0.045 ± 0.006	89. VI056456	0.050 ± 0.004
40. VI046554	0.060 ± 0.004	90. VI057245	0.035 ± 0.006
41. VI046556	0.050 ± 0.009	91. VI057249	0.065 ± 0.006
42. VI046559	0.140 ± 0.009	92. VI060131	0.060 ± 0.020
43. VI046562	0.095 ± 0.003	93. VI060132	0.060 ± 0.015
44. VI046566	0.065 ± 0.006	94. VI060133	0.045 ± 0.006
45. VI047672	0.040 ± 0.004	95. VI060206	0.055 ± 0.006
46. VI047751	0.030 ± 0.004	96. VI060313	0.035 ± 0.003
47. VI047808	0.035 ± 0.006	97. VI060314	0.035 ± 0.003
48. VI048154	0.030 ± 0.004	98. VI060315	0.035 ± 0.003
49. VI048291	0.040 ± 0.004	99. VI060316	0.035 ± 0.003
50. VI048596	0.035 ± 0.006	100. VI060317	0.060 ± 0.009

4.1.7. Effect of heat stress on number of leaves per plant

All genotypes gave significantly ($p \leq 0.05$) varied response regarding number of leaves under heat stress (Table 4.1.13). Results revealed that 17 genotypes (17% of total genotypes under study) produced less than 3 leaves per plant, on the other hand somewhat large number of genotypes i.e. 66 (66%) possessed leaves per plant in the range of 3-5, while 17 genotypes (17%) produced more than 5 number of leaves per plant. VI037994 possessed the highest number of leaves (6.50), while lowest number of leaves (1.50) was given by VI048291. Means ± SE regarding number of leaves per plant are shown in table 4.1.14.

Table 4.1.13: Effect of heat stress on number of leaves of okra

Number of leaves	**Genotypes**
< 3	17(17)
03-05	66(66)
> 05	17(17)
Minimum value	2
Maximum value	6.5
Mean	4.28
SD	1.062
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.14: Number of leaves of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	3.00 ± 0.41	51. VI049632	5.00 ± 0.41
2. VI039638	3.50 ± 0.29	52. VI049954	4.50 ± 0.29
3. VI039643	2.50 ± 0.65	53. VI049961	2.50 ± 0.29
4. VI039651	2.50 ± 0.65	54. VI050150	4.50 ± 0.65
5. VI039652	3.50 ± 0.65	55. VI050170	5.50 ± 0.65
6. VI040649	5.00 ± 0.41	56. VI050549	2.50 ± 0.29
7. VI040770	2.50 ± 0.65	57. VI051038	3.00 ± 0.91
8. VI040865	6.00 ± 0.91	58. VI051039	3.00 ± 0.41
9. VI041139	5.00 ± 0.41	59. VI051042	3.50 ± 0.65
10. VI041215	5.50 ± 0.29	60. VI051047	4.50 ± 0.29
11. VI041461	5.00 ± 0.41	61. VI051048	5.00 ± 0.41
12. VI041763	4.50 ± 0.29	62. VI051062	5.50 ± 0.65
13. VI044233	2.50 ± 0.65	63. VI051114	5.00 ± 0.41
14. VI044241	4.50 ± 0.29	64. VI054562	5.00 ± 0.41
15. VI044244	3.00 ± 0.41	65. VI054565	5.50 ± 0.29
16. VI037997	3.50 ± 0.65	66. VI054566	4.50 ± 0.29
17. VI037995	5.00 ± 0.91	67. VI054568	5.00 ± 0.41
18. VI037994	6.50 ± 0.29	68. VI055017	5.50 ± 0.29
19. VI033773	4.00 ± 0.41	69. VI055018	5.00 ± 0.41
20. VI033775	5.00 ± 0.41	70. VI055110	3.00 ± 0.41
21. VI033781-A	5.00 ± 0.41	71. VI055119	3.50 ± 0.65
22. VI033781-B	4.50 ± 1.19	72. VI055219	4.00 ± 0.41
23. VI033784	5.50 ± 0.29	73. VI055220	3.00 ± 0.41
24. VI033785	5.50 ± 0.65	74. VI055996	4.00 ± 0.41
25. VI033786	2.50 ± 0.29	75. VI056069	4.50 ± 0.29
26. VI033791	3.50 ± 0.65	76. VI056079	3.50 ± 0.29
27. VI033803	6.00 ± 0.41	77. VI056402	5.50 ± 0.29
28. VI033805	5.00 ± 0.41	78. VI056404	5.50 ± 0.65
29. VI033810	5.00 ± 0.41	79. VI056407	5.50 ± 0.29
30. VI033824	5.00 ± 0.41	80. VI056447	3.50 ± 0.65
31. VI036201	3.50 ± 0.65	81. VI056448	3.00 ± 0.41
32. VI036203	2.50 ± 0.65	82. VI056449	4.00 ± 0.41
33. VI036211	5.00 ± 0.91	83. VI056450	5.00 ± 0.41
34. VI036212	5.50 ± 0.29	84. VI056451	3.00 ± 0.41
35. VI036213	3.50 ± 0.65	85. VI056452	4.50 ± 0.65
36. VI036215	4.50 ± 0.29	86. VI056453	3.50 ± 0.29
37. VI046536	4.50 ± 0.29	87. VI056454	4.25 ± 1.11
38. VI046537	4.00 ± 0.91	88. VI056455	3.00 ± 0.41
39. VI046544	4.50 ± 1.76	89. VI056456	4.00 ± 0.91
40. VI046554	3.00 ± 0.41	90. VI057245	4.50 ± 0.65
41. VI046556	5.00 ± 0.41	91. VI057249	5.00 ± 0.41
42. VI046559	4.00 ± 0.41	92. VI060131	4.50 ± 0.29
43. VI046562	5.00 ± 0.41	93. VI060132	4.00 ± 0.41
44. VI046566	3.50 ± 0.29	94. VI060133	5.00 ± 0.41
45. VI047672	2.00 ± 0.41	95. VI060206	4.00 ± 0.41
46. VI047751	5.00 ± 0.41	96. VI060313	6.50 ± 1.19
47. VI047808	5.50 ± 0.29	97. VI060314	5.50 ± 0.29
48. VI048154	4.50 ± 0.65	98. VI060315	4.75 ± 0.48
49. VI048291	1.50 ± 0.65	99. VI060316	5.00 ± 0.91
50. VI048596	3.50 ± 0.29	100. VI060317	5.00 ± 0.41

4.1.8. Effect of heat stress on chlorophyll contents (SPAD value)

All genotypes of okra differed significantly ($p \leq 0.05$) in response to elevated temperature conditions for chlorophyll contents. On the basis of chlorophyll contents different groups of genotypes were made. According to the data expressed in table 4.1.15 it is observed that 10 genotypes (10% of total genotypes under study) gave chlorophyll contents < 10, while 52 genotypes (52%) gave chlorophyll contents in the range of 10.1-20 and 34 genotypes (34%) showed 20.1-30 chlorophyll contents. The remaining 4 genotypes exhibited greater than 30 chlorophyll contents. VI055119 gave the maximum chlorophyll contents (41.25) while minimum chlorophyll contents (6.75) were observed in VI048596. Means ± SE regarding chlorophyll contents are given in table 4.1.16.

Table 4.1.15: Effect of heat stress on chlorophyll contents of okra

Chlorophyll contents (SPAD value)	Genotypes
< 10	10(10)*
10.1-20	52(52)
20.1-30	34(34)
> 30	04(04)
Minimum value	6.75
Maximum value	41.25
Mean	18.49
SD	6.660
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.16: Chlorophyll contents of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	28.60 ± 1.12	51. VI049632	15.95 ± 2.99
2. VI039638	26.10 ± 0.87	52. VI049954	29.75 ± 0.67
3. VI039643	23.45 ± 1.74	53. VI049961	28.45 ± 2.14
4. VI039651	29.55 ± 3.33	54. VI050150	14.10 ± 0.97
5. VI039652	27.65 ± 3.33	55. VI050170	30.35 ± 4.82
6. VI040649	17.35 ± 1.20	56. VI050549	13.60 ± 2.22
7. VI040770	14.15 ± 1.00	57. VI051038	23.80 ± 7.90
8. VI040865	9.15 ± 1.20	58. VI051039	18.10 ± 0.50
9. VI041139	20.60 ± 0.97	59. VI051042	17.80 ± 0.83
10. VI041215	9.75 ± 2.25	60. VI051047	23.15 ± 0.64
11. VI041461	14.45 ± 0.85	61. VI051048	21.10 ± 0.53
12. VI041763	10.05 ± 0.67	62. VI051062	22.20 ± 0.87
13. VI044233	10.05 ± 0.49	63. VI051114	21.65 ± 2.59
14. VI044241	13.95 ± 1.25	64. VI054562	13.35 ± 0.80
15. VI044244	12.50 ± 1.23	65. VI054565	13.60 ± 0.74
16. VI037997	15.90 ± 0.65	66. VI054566	14.90 ± 1.33
17. VI037995	16.75 ± 1.10	67. VI054568	17.55 ± 2.08
18. VI037994	4.60 ± 0.97	68. VI055017	23.15 ± 1.58
19. VI033773	9.40 ± 1.28	69. VI055018	18.40 ± 0.49
20. VI033775	16.25 ± 1.36	70. VI055110	14.95 ± 1.31
21. VI033781-A	15.60 ± 1.55	71. VI055119	41.25 ± 1.80
22. VI033781-B	15.55 ± 3.04	72. VI055219	20.50 ± 3.87
23. VI033784	24.75 ± 1.10	73. VI055220	12.50 ± 1.07
24. VI033785	11.55 ± 0.90	74. VI055996	14.15 ± 1.52
25. VI033786	17.30 ± 2.05	75. VI056069	19.30 ± 0.69
26. VI033791	24.90 ± 0.74	76. VI056079	20.60 ± 0.56
27. VI033803	21.90 ± 0.65	77. VI056402	20.70 ± 0.49
28. VI033805	18.80 ± 1.07	78. VI056404	22.00 ± 0.62
29. VI033810	19.50 ± 3.24	79. VI056407	16.45 ± 0.80
30. VI033824	18.15 ± 1.36	80. VI056447	26.15 ± 1.05
31. VI036201	9.65 ± 1.91	81. VI056448	11.20 ± 1.50
32. VI036203	14.05 ± 1.69	82. VI056449	26.40 ± 0.53
33. VI036211	20.55 ± 0.80	83. VI056450	18.80 ± 3.70
34. VI036212	22.25 ± 1.42	84. VI056451	14.65 ± 2.99
35. VI036213	23.25 ± 0.49	85. VI056452	13.05 ± 0.95
36. VI036215	21.10 ± 1.44	86. VI056453	16.10 ± 1.28
37. VI046536	20.40 ± 1.77	87. VI056454	14.60 ± 2.96
38. VI046537	13.35 ± 0.90	88. VI056455	24.60 ± 0.58
39. VI046544	16.50 ± 0.87	89. VI056456	10.65 ± 1.58
40. VI046554	8.80 ± 0.50	90. VI057245	25.80 ± 2.33
41. VI046556	11.45 ± 0.90	91. VI057249	14.50 ± 0.69
42. VI046559	15.00 ± 2.39	92. VI060131	38.30 ± 1.12
43. VI046562	18.80 ± 1.33	93. VI060132	12.75 ± 0.76
44. VI046566	14.60 ± 0.78	94. VI060133	23.95 ± 1.20
45. VI047672	27.15 ± 0.57	95. VI060206	14.30 ± 1.72
46. VI047751	23.00 ± 0.50	96. VI060313	12.35 ± 0.52
47. VI047808	25.35 ± 2.02	97. VI060314	14.20 ± 1.02
48. VI048154	17.35 ± 1.47	98. VI060315	13.25 ± 0.71
49. VI048291	28.70 ± 2.56	99. VI060316	11.85 ± 1.52
50. VI048596	6.75 ± 1.47	100. VI060317	32.75 ± 1.63

4.1.9. Effect of heat stress on leaf temperature (ºC)

Results revealed that all genotypes exhibited significantly ($p \leq 0.05$) variable response under high temperature regime for leaf temperature (Table 4.1.17). Results revealed that 4 genotypes (4 %) remained cooler as compared to other groups with temperature less than 25ºC, while 8 genotypes (8%) showed a variable temperature range of 25.1-29.9ºC. A slightly higher temperature within the range of 30-33ºC was exhibited by 19 genotypes (19%) whereas, a comparatively large number consisting of 69 genotypes (69%) possessed leaf temperature above 33ºC. Highest leaf temperature (36.15ºC) recorded in VI033775 while, lowest leaf temperature (21.95ºC) recorded in VI056451. Means ± SE regarding leaf temperature are given in table 4.1.18.

Table 4.1.17: Effect of heat stress on leaf temperature of okra

Leaf temperature (°C)	Genotypes
< 25	04(04)
25.1-29.9	08(08)
30-33	19(19)
>33	69(69)
Minimum value	21.95
Maximum value	36.15
Mean	33.17
SD	2.900
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.18: Leaf temperature of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	24.90 ± 0.44	51. VI049632	35.50 ± 0.20
2. VI039638	26.55 ± 0.17	52. VI049954	35.35 ± 0.14
3. VI039643	28.20 ± 0.29	53. VI049961	35.35 ± 0.06
4. VI039651	29.65 ± 0.25	54. VI050150	35.45 ± 0.18
5. VI039652	30.77 ± 0.20	55. VI050170	35.15 ± 0.06
6. VI040649	30.75 ± 0.32	56. VI050549	35.50 ± 0.20
7. VI040770	31.75 ± 0.12	57. VI051038	32.80 ± 0.04
8. VI040865	32.35 ± 0.06	58. VI051039	33.05 ± 0.39
9. VI041139	31.50 ± 0.09	59. VI051042	33.15 ± 0.80
10. VI041215	33.29 ± 0.10	60. VI051047	33.35 ± 0.06
11. VI041461	33.73 ± 0.09	61. VI051048	33.60 ± 0.09
12. VI041763	34.35 ± 0.02	62. VI051062	33.75 ± 0.06
13. VI044233	34.68 ± 0.07	63. VI051114	33.95 ± 0.39
14. VI044241	34.40 ± 0.47	64. VI054562	34.00 ± 0.45
15. VI044244	35.10 ± 0.37	65. VI054565	34.25 ± 0.06
16. VI037997	32.33 ± 0.23	66. VI054566	32.58 ± 0.17
17. VI037995	35.35 ± 0.14	67. VI054568	32.90 ± 0.41
18. VI037994	35.50 ± 0.02	68. VI055017	32.95 ± 0.39
19. VI033773	35.75 ± 0.06	69. VI055018	33.10 ± 0.82
20. VI033775	36.15 ± 0.02	70. VI055110	33.45 ± 0.43
21. VI033781-A	36.00 ± 0.41	71. VI055119	33.50 ± 0.16
22. VI033781-B	35.90 ± 0.41	72. VI055219	33.80 ± 0.04
23. VI033784	35.70 ± 0.08	73. VI055220	33.95 ± 0.39
24. VI033785	35.70 ± 0.04	74. VI055996	34.15 ± 0.84
25. VI033786	35.55 ± 0.06	75. VI056069	34.15 ± 0.43
26. VI033791	32.65 ± 0.59	76. VI056079	34.25 ± 0.06
27. VI033803	33.15 ± 0.05	77. VI056402	34.50 ± 0.17
28. VI033805	33.08 ± 0.37	78. VI056404	34.50 ± 0.08
29. VI033810	33.40 ± 0.17	79. VI056407	34.50 ± 0.12
30. VI033824	35.40 ± 0.12	80. VI056447	34.45 ± 0.43
31. VI036201	35.25 ± 0.31	81. VI056448	34.70 ± 0.41
32. VI036203	35.30 ± 0.12	82. VI056449	34.58 ± 0.17
33. VI036211	33.48 ± 0.16	83. VI056450	34.55 ± 0.16
34. VI036212	33.70 ± 0.16	84. VI056451	21.95 ± 0.21
35. VI036213	34.30 ± 0.13	85. VI056452	22.75 ± 0.32
36. VI036215	34.55 ± 0.18	86. VI056453	24.10 ± 0.31
37. VI046536	34.80 ± 0.06	87. VI056454	26.00 ± 0.65
38. VI046537	35.05 ± 0.23	88. VI056455	27.40 ± 0.15
39. VI046544	35.25 ± 0.10	89. VI056456	28.35 ± 0.35
40. VI046554	35.30 ± 0.12	90. VI057245	29.05 ± 0.39
41. VI046556	35.40 ± 0.17	91. VI057249	29.60 ± 0.45
42. VI046559	35.85 ± 0.47	92. VI060131	30.40 ± 0.34
43. VI046562	35.95 ± 0.43	93. VI060132	30.90 ± 0.41
44. VI046566	35.15 ± 0.06	94. VI060133	31.95 ± 0.43
45. VI047672	35.05 ± 0.39	95. VI060206	32.25 ± 0.39
46. VI047751	34.75 ± 0.25	96. VI060313	32.40 ± 0.12
47. VI047808	35.25 ± 0.10	97. VI060314	32.55 ± 0.06
48. VI048154	35.30 ± 0.12	98. VI060315	32.70 ± 0.04
49. VI048291	35.40 ± 0.16	99. VI060316	32.55 ± 0.16
50. VI048596	35.85 ± 0.06	100. VI060317	33.45 ± 0.29

4.1.10. Effect of heat stress on photosynthetic rate (µmol m^{-2} s^{-1})

All genotypes responded significantly ($p \leq 0.05$) different for photosynthetic rate under high temperature regimes (Table 4.1.19). Results showed that 24 genotypes (24% of total genotype under study) showed photosynthetic rate less than 1 µmol m^{-2} s^{-}, while those of 25 genotypes (25%) gave photosynthetic rate in the range of 1-1.75 µmol m^{-2} $^{s-1}$ and it ranged 1.76-2.99 µmol m^{-2} $^{s-1}$ for 23 genotypes (23%). A greater than 3 µmol m-^{2} s- photosynthetic rate was possessed by remaining 28 genotypes (28%). The minimum photosynthetic rate (0.06 µmol m-^{2} s-) recorded in VI036212 and maximum photosynthetic rate (7.46 µmol m^{-2} s^{-}) was observed in VI040770. The complete results of all genotypes (Mean ± SE) regarding photosynthetic rate are shown in table 4.1.20.

Table 4.1.19: Effect of heat stress on photosynthetic rate of okra

Photosynthetic rate (µmol m-^{2} s-^{1})	**Genotypes**
< 01	24(24)*
1-1.75	25(25)
1.76-2.99	23(23)
> 3	28(28)
Minimum value	0.06
Maximum value	7.43
Mean	2.20
SD	1.544
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.20: Photosynthetic rate of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	5.96 ± 0.39	51. VI049632	1.29 ± 0.66
2. VI039638	3.10 ± 0.25	52. VI049954	1.45 ± 0.35
3. VI039643	1.39 ± 0.03	53. VI049961	5.05 ± 1.52
4. VI039651	2.39 ± 0.46	54. VI050150	1.09 ± 0.39
5. VI039652	0.93 ± 0.22	55. VI050170	0.76 ± 0.37
6. VI040649	4.94 ± 0.25	56. VI050549	0.29 ± 0.08
7. VI040770	7.43 ± 1.86	57. VI051038	2.51 ± 0.57
8. VI040865	2.97 ± 0.19	58. VI051039	1.27 ± 0.44
9. VI041139	2.23 ± 0.51	59. VI051042	0.48 ± 0.09
10. VI041215	1.04 ± 0.41	60. VI051047	0.47 ± 0.03
11. VI041461	3.21 ± 1.73	61. VI051048	0.85 ± 0.27
12. VI041763	2.62 ± 0.13	62. VI051062	3.15 ± 1.32
13. VI044233	3.90 ± 1.48	63. VI051114	3.19 ± 0.93
14. VI044241	3.44 ± 1.12	64. VI054562	0.81 ± 0.04
15. VI044244	5.68 ± 0.09	65. VI054565	4.72 ± 1.95
16. VI037997	0.82 ± 0.29	66. VI054566	2.98 ± 0.20
17. VI037995	1.41 ± 0.56	67. VI054568	2.35 ± 0.06
18. VI037994	2.69 ± 0.14	68. VI055017	4.26 ± 0.65
19. VI033773	1.78 ± 0.21	69. VI055018	1.43 ± 0.21
20. VI033775	1.55 ± 0.49	70. VI055110	4.31 ± 1.31
21. VI033781-A	3.08 ± 0.29	71. VI055119	4.55 ± 0.14
22. VI033781-B	1.31 ± 0.13	72. VI055219	1.59 ± 0.16
23. VI033784	0.90 ± 0.09	73. VI055220	2.16 ± 0.40
24. VI033785	2.13 ± 0.82	74. VI055996	1.06 ± 0.01
25. VI033786	1.20 ± 0.17	75. VI056069	3.72 ± 1.35
26. VI033791	4.54 ± 0.85	76. VI056079	3.34 ± 0.29
27. VI033803	0.83 ± 0.38	77. VI056402	2.37 ± 0.97
28. VI033805	1.04 ± 0.10	78. VI056404	2.67 ± 1.16
29. VI033810	0.66 ± 0.02	79. VI056407	2.98 ± 0.27
30. VI033824	1.13 ± 0.24	80. VI056447	5.17 ± 0.63
31. VI036201	1.76 ± 0.11	81. VI056448	1.78 ± 0.01
32. VI036203	3.41 ± 0.65	82. VI056449	1.69 ± 0.28
33. VI036211	0.78 ± 0.27	83. VI056450	2.74 ± 0.74
34. VI036212	0.35 ± 0.13	84. VI056451	1.83 ± 0.36
35. VI036213	0.08 ± 0.03	85. VI056452	2.10 ± 0.02
36. VI036215	0.06 ± 0.02	86. VI056453	1.73 ± 0.38
37. VI046536	0.32 ± 0.10	87. VI056454	1.12 ± 0.17
38. VI046537	1.09 ± 0.31	88. VI056455	1.30 ± 0.06
39. VI046544	0.28 ± 0.06	89. VI056456	0.97 ± 0.18
40. VI046554	1.32 ± 0.52	90. VI057245	2.60 ± 0.80
41. VI046556	0.12 ± 0.02	91. VI057249	1.16 ± 0.11
42. VI046559	0.29 ± 0.07	92. VI060131	3.79 ± 0.44
43. VI046562	0.61 ± 0.33	93. VI060132	2.68 ± 0.72
44. VI046566	0.78 ± 0.21	94. VI060133	4.12 ± 1.16
45. VI047672	2.12 ± 0.02	95. VI060206	4.69 ± 0.44
46. VI047751	1.99 ± 0.03	96. VI060313	1.49 ± 0.41
47. VI047808	3.04 ± 0.50	97. VI060314	0.94 ± 0.37
48. VI048154	5.91 ± 1.38	98. VI060315	3.68 ± 0.48
49. VI048291	1.59 ± 0.28	99. VI060316	1.83 ± 0.81
50. VI048596	0.76 ± 0.40	100. VI060317	3.14 ± 0.65

4.1.11. Effect of heat stress on transpiration rate (mmol m^{-2} s^{-1})

The observations regarding transpiration rate showed that all genotypes behaved significantly ($P \leq 0.05$) under elevated temperature (Table 4.1.21). Results showed that 32 genotypes (32% of total genotypes under study) had transpiration rate greater than 1 mmol m^{-2} s^{-1}, on the other hand each of 51 genotypes (51%) fell in the range of 01-02 mmol m^{-2} s^{-1} and 02-03 mmol m^{-2} s^{-1} by other 13 genotypes (13%). A transpiration rate of less than 3 mmol m^{-2} s^{-1} was recorded by remaining 4 genotypes (4%). VI033810 showed lowest transpiration rate (0.31 mmol m^{-2} s^{-1}) while highest transpiration rate (3.47 mmol m^{-2} s^{-1}) was noted in VI044244. Means ± SE regarding transpiration rate are given in table 4.1.22.

Table 4.1.21: Effect of heat stress transpiration rate of okra

Transpiration rate (mmol m^{-2} s^{-1})	**Genotypes**
< 01	32(32)
01-02	51(51)
02-03	13(13)
> 03	04(04)
Minimum value	0.31
Maximum value	3.47
Mean	1.38
SD	0.687
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.22: Transpiration rate of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	0.85 ± 0.039	51. VI049632	1.08 ± 0.113
2. VI039638	0.82 ± 0.039	52. VI049954	0.96 ± 0.102
3. VI039643	1.09 ± 0.071	53. VI049961	0.71 ± 0.044
4. VI039651	1.47 ± 0.060	54. VI050150	1.08 ± 0.021
5. VI039652	1.59 ± 0.168	55. VI050170	1.19 ± 0.430
6. VI040649	0.80 ± 0.074	56. VI050549	0.96 ± 0.105
7. VI040770	1.84 ± 0.171	57. VI051038	0.58 ± 0.043
8. VI040865	2.73 ± 0.202	58. VI051039	1.00 ± 0.026
9. VI041139	1.03 ± 0.094	59. VI051042	1.37 ± 0.061
10. VI041215	1.79 ± 0.310	60. VI051047	0.72 ± 0.103
11. VI041461	2.41 ± 0.422	61. VI051048	0.79 ± 0.087
12. VI041763	1.86 ± 0.038	62. VI051062	0.56 ± 0.103
13. VI044233	2.07 ± 0.410	63. VI051114	1.05 ± 0.023
14. VI044241	2.70 ± 0.156	64. VI054562	1.85 ± 0.166
15. VI044244	3.47 ± 0.404	65. VI054565	1.51 ± 0.018
16. VI037997	1.70 ± 0.442	66. VI054566	1.35 ± 0.139
17. VI037995	3.14 ± 0.018	67. VI054568	0.80 ± 0.088
18. VI037994	3.35 ± 0.076	68. VI055017	0.95 ± 0.034
19. VI033773	2.66 ± 0.335	69. VI055018	1.00 ± 0.016
20. VI033775	3.15 ± 0.138	70. VI055110	1.45 ± 0.018
21. VI033781-A	1.80 ± 0.093	71. VI055119	0.71 ± 0.134
22. VI033781-B	1.90 ± 0.018	72. VI055219	1.10 ± 0.042
23. VI033784	2.16 ± 0.176	73. VI055220	1.08 ± 0.166
24. VI033785	3.00 ± 0.236	74. VI055996	1.27 ± 0.289
25. VI033786	1.87 ± 0.113	75. VI056069	0.79 ± 0.050
26. VI033791	1.22 ± 0.171	76. VI056079	0.49 ± 0.025
27. VI033803	0.94 ± 0.034	77. VI056402	1.23 ± 0.133
28. VI033805	0.87 ± 0.125	78. VI056404	1.05 ± 0.165
29. VI033810	0.31 ± 0.100	79. VI056407	0.80 ± 0.067
30. VI033824	2.20 ± 0.052	80. VI056447	0.89 ± 0.165
31. VI036201	2.44 ± 0.287	81. VI056448	0.86 ± 0.122
32. VI036203	2.69 ± 0.181	82. VI056449	0.65 ± 0.016
33. VI036211	0.72 ± 0.019	83. VI056450	0.99 ± 0.041
34. VI036212	0.86 ± 0.113	84. VI056451	0.78 ± 0.082
35. VI036213	0.78 ± 0.032	85. VI056452	0.72 ± 0.056
36. VI036215	0.93 ± 0.119	86. VI056453	0.86 ± 0.051
37. VI046536	1.21 ± 0.157	87. VI056454	1.00 ± 0.102
38. VI046537	1.63 ± 0.340	88. VI056455	1.15 ± 0.010
39. VI046544	1.04 ± 0.063	89. VI056456	1.40 ± 0.079
40. VI046554	2.22 ± 0.171	90. VI057245	1.10 ± 0.143
41. VI046556	1.29 ± 0.116	91. VI057249	1.36 ± 0.006
42. VI046559	1.13 ± 0.208	92. VI060131	0.94 ± 0.038
43. VI046562	1.24 ± 0.032	93. VI060132	1.67 ± 0.027
44. VI046566	2.21 ± 0.067	94. VI060133	1.00 ± 0.128
45. VI047672	1.03 ± 0.142	95. VI060206	1.69 ± 0.059
46. VI047751	1.62 ± 0.136	96. VI060313	1.54 ± 0.036
47. VI047808	1.49 ± 0.079	97. VI060314	1.41 ± 0.079
48. VI048154	1.21 ± 0.017	98. VI060315	2.29 ± 0.359
49. VI048291	1.21 ± 0.160	99. VI060316	1.43 ± 0.234
50. VI048596	0.42 ± 0.022	100. VI060317	1.49 ± 0.143

4.1.12. Effect of heat stress on water use efficiency (µmol CO_2 $mmol^{-1}$ H_2O)

All genotypes showed significantly ($P \leq 0.05$) diverse response to water use efficiency under elevated temperature (Table 4.1.23). Results decipited that a vast proportion of 64 genotypes (64% of total genotypes under study) had water use efficiency less than 2 µmol CO_2 $mmol^{-1}$ H_2O. A water use efficiency of 23 genotypes (23 %) was in the range of 02-04 µmol CO_2 $mmol^{-1}$ H_2O and those of 05 genotypes (5 %) had water use efficiency ranged from 04-05 µmol CO_2 $mmol^{-1}$ H_2O. The remaining 08 genotypes (8 %) exhibited above than 5 µmol CO_2 $mmol^{-1}$ H_2O water use efficiency. The maximum water use efficiency (7.60 µmol CO_2 $mmol^{-1}$ H_2O) was noted in VI049961 and VI036215 gave the minimum value (0.07 µmol CO_2 $mmol^{-1}$ H_2O) of water use efficiency. The complete results of all genotypes (Mean ± SE) for water use efficiency are presented in table 4.1.24.

Table 4.1.23: Effect of heat stress water use efficiency of okra

Water use efficiency (µmol $CO_{2\ mmol}^{-1}{}_{H2O}$)	**Genotypes**
< 2	64(64)
02-04	23(23)
4.1-05	05(05)
> 05	08(08)
Minimum value	0.10
Maximum value	7.60
Mean	2.02
SD	1.778
p -values**	≤ 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.24: Water use efficiency of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	7.10 ± 0.75	51. VI049632	1.44 ± 0.76
2. VI039638	3.76 ± 0.14	52. VI049954	1.69 ± 0.54
3. VI039643	1.29 ± 0.09	53. VI049961	7.60 ± 2.61
4. VI039651	1.60 ± 0.25	54. VI050150	1.04 ± 0.39
5. VI039652	0.65 ± 0.21	55. VI050170	0.50 ± 0.13
6. VI040649	6.47 ± 0.92	56. VI050549	0.34 ± 0.12
7. VI040770	3.87 ± 0.66	57. VI051038	4.61 ± 1.29
8. VI040865	1.12 ± 0.15	58. VI051039	1.30 ± 0.47
9. VI041139	2.09 ± 0.31	59. VI051042	0.35 ± 0.06
10. VI041215	0.58 ± 0.22	60. VI051047	0.71 ± 0.12
11. VI041461	1.05 ± 0.53	61. VI051048	1.23 ± 0.47
12. VI041763	1.42 ± 0.10	62. VI051062	5.75 ± 2.20
13. VI044233	1.65 ± 0.39	63. VI051114	3.10 ± 0.94
14. VI044241	1.36 ± 0.49	64. VI054562	0.45 ± 0.05
15. VI044244	1.70 ± 0.17	65. VI054565	3.18 ± 1.33
16. VI037997	0.44 ± 0.06	66. VI054566	2.34 ± 0.39
17. VI037995	0.45 ± 0.18	67. VI054568	3.02 ± 0.27
18. VI037994	0.80 ± 0.03	68. VI055017	4.60 ± 0.84
19. VI033773	0.73 ± 0.17	69. VI055018	1.45 ± 0.23
20. VI033775	0.52 ± 0.18	70. VI055110	2.99 ± 0.91
21. VI033781-A	1.70 ± 0.07	71. VI055119	7.28 ± 1.56
22. VI033781-B	0.69 ± 0.08	72. VI055219	1.46 ± 0.20
23. VI033784	0.44 ± 0.08	73. VI055220	1.98 ± 0.09
24. VI033785	0.66 ± 0.22	74. VI055996	0.99 ± 0.23
25. VI033786	0.66 ± 0.13	75. VI056069	5.12 ± 2.04
26. VI033791	4.26 ± 1.29	76. VI056079	7.03 ± 0.95
27. VI033803	0.93 ± 0.43	77. VI056402	2.26 ± 1.03
28. VI033805	1.28 ± 0.22	78. VI056404	3.17 ± 1.53
29. VI033810	3.30 ± 1.25	79. VI056407	3.78 ± 0.34
30. VI033824	0.51 ± 0.10	80. VI056447	6.04 ± 0.44
31. VI036201	0.77 ± 0.13	81. VI056448	2.21 ± 0.32
32. VI036203	1.34 ± 0.33	82. VI056449	2.64 ± 0.47
33. VI036211	1.08 ± 0.37	83. VI056450	2.89 ± 0.87
34. VI036212	0.49 ± 0.22	84. VI056451	2.57 ± 0.73
35. VI036213	0.10 ± 0.04	85. VI056452	3.00 ± 0.26
36. VI036215	0.07 ± 0.03	86. VI056453	1.95 ± 0.34
37. VI046536	0.31 ± 0.13	87. VI056454	1.22 ± 0.30
38. VI046537	0.90 ± 0.37	88. VI056455	1.14 ± 0.06
39. VI046544	0.26 ± 0.05	89. VI056456	0.73 ± 0.17
40. VI046554	0.66 ± 0.28	90. VI057245	2.80 ± 1.10
41. VI046556	0.09 ± 0.01	91. VI057249	0.86 ± 0.08
42. VI046559	0.32 ± 0.12	92. VI060131	4.13 ± 0.63
43. VI046562	0.47 ± 0.25	93. VI060132	1.63 ± 0.45
44. VI046566	0.35 ± 0.08	94. VI060133	3.88 ± 0.67
45. VI047672	2.18 ± 0.29	95. VI060206	2.81 ± 0.35
46. VI047751	1.25 ± 0.09	96. VI060313	0.95 ± 0.25
47. VI047808	2.11 ± 0.45	97. VI060314	0.71 ± 0.31
48. VI048154	4.86 ± 1.10	98. VI060315	1.85 ± 0.50
49. VI048291	1.29 ± 0.06	99. VI060316	1.66 ± 0.82
50. VI048596	1.98 ± 1.07	100. VI060317	2.22 ± 0.61

4.1.13. Effect of heat stress on sub-stomatal to CO_2 (vpm)

All genotypes showed significantly ($p \leq 0.05$) different response towards sub-stomatal CO_2 under elevated temperature (Table 4.1.25). Out of total genotypes, 30 genotypes (30% of total genotypes under study) expressed sub-stomatal CO_2 less than 600vpm. Sub-stomatal CO_2 of 44 genotypes (44%) was recorded in the range of 600-700 vpm while 20 genotypes showed internal leaf CO_2 in the range of 700-800 vpm. The remaining 06 genotypes (06%) expressed their sub-stomatal CO_2 greater than 800 vpm. The minimum sub-stomatal CO_2 (448 vpm) was recorded in VI055119, while VI055996 gave the maximum leaf internal CO_2 (956 vpm). The complete results of all genotypes (Mean ± SE) for sub-stomatal CO_2 are given in table 4.1.26.

Table 4.1.25: Effect of heat stress on sub-stomatal CO_2 of okra

Sub-stomatal CO_2 (vpm)	**Genotypes**
< 600	30(30)*
600-700	44(44)
700-800	20(20)
> 800	06 (06)
Minimum value	448
Maximum value	929
Mean	652.38
SD	103.72
p -values**	< 0.05

*Values in parenthesis indicate the percentage of total genotypes used in the study

** $p > 0.05$ (non-significant); $p \leq 0.05$ (significant); $p \leq 0.01$(highly significant).

Table 4.1.26: Sub-stomatal CO_2 of 100 okra genotypes as affected by heat stress (Mean ± Standard error)

Genotypes	Means±SE	Genotypes	Means±SE
1. VI039622	649.50 ± 101.92	51. VI049632	839.00 ± 51.24
2. VI039638	525.75 ± 3.42	52. VI049954	504.00 ± 26.96
3. VI039643	587.00 ± 12.46	53. VI049961	929.00 ± 288.97
4. VI039651	588.75 ± 4.66	54. VI050150	514.50 ± 23.97
5. VI039652	664.00 ± 13.59	55. VI050170	921.00 ± 2.94
6. VI040649	468.00 ± 28.44	56. VI050549	652.00 ± 34.36
7. VI040770	561.50 ± 7.24	57. VI051038	895.50 ± 140.01
8. VI040865	554.50 ± 12.18	58. VI051039	689.50 ± 29.11
9. VI041139	575.00 ± 19.27	59. VI051042	728.75 ± 10.05
10. VI041215	579.50 ± 4.73	60. VI051047	711.50 ± 5.84
11. VI041461	604.00 ± 30.16	61. VI051048	659.50 ± 23.97
12. VI041763	596.50 ± 9.74	62. VI051062	473.00 ± 162.53
13. VI044233	571.50 ± 14.44	63. VI051114	612.50 ± 51.39
14. VI044241	665.00 ± 51.47	64. VI054562	747.00 ± 3.49
15. VI044244	508.50 ± 27.58	65. VI054565	603.00 ± 75.11
16. VI037997	678.50 ± 27.00	66. VI054566	660.50 ± 15.57
17. VI037995	571.50 ± 12.99	67. VI054568	583.50 ± 13.31
18. VI037994	616.00 ± 27.86	68. VI055017	517.00 ± 52.62
19. VI033773	636.50 ± 9.40	69. VI055018	744.00 ± 9.13
20. VI033775	661.50 ± 5.20	70. VI055110	700.50 ± 65.59
21. VI033781-A	611.50 ± 2.90	71. VI055119	448.00 ± 83.77
22. VI033781-B	665.50 ± 4.73	72. VI055219	786.50 ± 11.06
23. VI033784	696.00 ± 9.13	73. VI055220	751.00 ± 25.96
24. VI033785	689.50 ± 13.31	74. VI055996	956.50 ± 24.41
25. VI033786	689.00 ± 11.90	75. VI056069	598.50 ± 129.07
26. VI033791	514.50 ± 54.65	76. VI056079	500.00 ± 46.85
27. VI033803	629.00 ± 24.42	77. VI056402	753.50 ± 45.41
28. VI033805	640.00 ± 16.99	78. VI056404	702.50 ± 86.94
29. VI033810	672.00 ± 4.51	79. VI056407	719.00 ± 27.29
30. VI033824	724.50 ± 12.29	80. VI056447	572.00 ± 19.84
31. VI036201	717.00 ± 13.96	81. VI056448	789.00 ± 20.41
32. VI036203	641.50 ± 8.86	82. VI056449	834.50 ± 25.85
33. VI036211	650.50 ± 9.95	83. VI056450	843.00 ± 41.09
34. VI036212	676.00 ± 11.90	84. VI056451	777.50 ± 3.01
35. VI036213	696.50 ± 5.69	85. VI056452	732.50 ± 21.84
36. VI036215	695.00 ± 4.97	86. VI056453	650.00 ± 15.85
37. VI046536	703.00 ± 5.45	87. VI056454	688.50 ± 25.28
38. VI046537	680.50 ± 20.13	88. VI056455	646.50 ± 5.85
39. VI046544	699.50 ± 7.24	89. VI056456	660.00 ± 3.37
40. VI046554	644.00 ± 16.99	90. VI057245	585.00 ± 42.82
41. VI046556	712.00 ± 4.97	91. VI057249	666.50 ± 6.71
42. VI046559	737.00 ± 8.58	92. VI060131	514.00 ± 29.83
43. VI046562	784.00 ± 10.14	93. VI060132	671.50 ± 15.00
44. VI046566	684.00 ± 6.45	94. VI060133	620.75 ± 30.54
45. VI047672	562.00 ± 14.72	95. VI060206	627.50 ± 7.77
46. VI047751	521.00 ± 14.15	96. VI060313	651.50 ± 15.00
47. VI047808	454.00 ± 19.27	97. VI060314	669.00 ± 25.57
48. VI048154	675.50 ± 198.04	98. VI060315	628.00 ± 20.98
49. VI048291	476.00 ± 7.51	99. VI060316	562.00 ± 51.47
50. VI048596	672.25 ± 83.29	100. VI060317	472.75 ± 42.92

4.1.14 Rankings of genotypes

Genotypes were ranked on the basis of their performance towards parameters investigated and thus presented their heat tolerance potential. The parameters in which higher values required were termed as positive parameters while parameters with lower values required were termed as negative parameters. In all positive parameters (root length, root fresh weight, root dry weight, shoot length, shoot fresh weight, shoot dry weight, water use efficiency, chlorophyll contents, photosynthetic rate and number of leaves) 100 marks were given to the genotype having highest score and 01 mark was given to the genotype having lowest record e.g. 100 marks were given to the genotype with longest shoot and 01mark got by the genotype having shortest shoot while remaining genotypes arranged accordingly between two extremes. In case of negative parameters (transpiration rate, sub-stomatal CO_2 and leaf temperature) 100 marks were given to the genotypes recorded lowest score and 01 mark given to the genotype having highest score e.g. 01 mark was given to the genotype secured highest transpiration rate and 100 marks were given to the genotype recorded lowest transpiration rate and so on. Then secured marks were summed up. The genotype which secured highest cumulative record was selected as most heat tolerance while most heat sensitive genotype was considered with lowest score. In accordance to their cumulative score the genotypes are arranged in table 4.1.27. According to results two genotypes named VI051062 and VI060131 were found to be most heat tolerant by scoring highest marks 1087 and 974, respectively while genotype VI048596 secured minimum marks (280) followed by VI046554 with 379 marks showing their heat sensitive properties . These four varieties were selected for further studies.

Table 4.1.27: Okra genotypes arranged from heat tolerant to heat sensitive ones (ranking based on cumulative score attained by each genotype on the basis of their performance in attributes studied)

Sr. #	Genotypes	CC	SL	RL	SFW	RFW	SDW	RDW	NL	SSC	TR	PR	WUE	LT	Rank Sum
1	VI051062	73	71	100	92	76	98	74	84	97	97	77	94	54	1087
2	VI060131	99	83	60	97	59	49	56	44	92	73	85	88	89	974
3	VI054566	37	87	58	95	92	83	94	44	50	42	72	71	75	900
4	VI051114	70	18	44	100	79	100	81	60	68	61	78	80	51	890
5	VI041139	65	41	88	65	74	42	92	60	79	63	59	65	84	877
6	VI056452	19	63	67	88	80	70	82	44	18	90	55	78	99	853
7	VI056404	72	19	81	78	86	65	87	84	26	60	66	81	39	844
8	VI060206	31	94	57	79	63	88	54	35	65	26	92	75	81	840
9	VI055018	56	53	98	98	99	97	29	60	16	66	41	52	67	832
10	VI055017	77	4	64	44	72	22	48	84	89	72	88	90	70	824
11	VI041763	8	99	84	84	70	88	91	44	73	20	65	50	44	820
12	VI056402	67	16	99	82	93	75	66	84	13	46	61	70	39	811
13	VI056454	34	70	77	69	91	81	98	21	35	68	30	41	96	811
14	VI040649	50	37	86	12	3	19	82	60	98	85	94	96	88	810
15	VI054565	23	45	26	81	87	51	87	84	71	32	93	82	46	808
16	VI056448	11	100	87	99	84	98	74	11	9	80	50	69	34	806
17	VI056453	44	55	90	77	67	75	30	21	56	78	48	62	98	801
18	VI056450	57	97	70	96	66	93	1	60	6	69	69	76	37	797
19	VI060133	81	20	59	56	47	49	30	60	66	66	87	87	82	790
20	VI050150	27	89	72	93	78	92	54	44	90	57	29	36	14	775
21	VI039643	79	80	54	72	14	90	74	3	75	56	39	45	93	774
22	VI056449	89	94	69	55	49	64	56	35	8	95	47	73	36	770
23	VI033791	84	59	30	54	57	39	30	21	90	47	90	89	74	764
24	VI039652	91	92	63	91	98	68	30	21	48	30	22	20	87	761
25	VI051039	54	61	95	94	21	94	78	11	32	65	34	47	69	755
26	VI056451	36	36	96	75	72	43	56	11	12	88	53	72	100	750
27	VI057249	33	49	94	67	69	68	66	60	45	41	32	31	90	745
28	VI047672	90	66	97	39	67	62	22	2	83	63	56	67	30	744
29	VI039638	87	43	13	13	25	52	56	21	87	82	75	84	95	733
30	VI051038	80	15	23	59	97	52	66	11	5	96	63	91	72	730
31	VI056447	88	63	39	21	39	40	30	21	80	76	96	95	42	730
32	VI044241	25	85	78	57	50	78	84	44	47	7	82	49	43	729

33	VI044244	16	89	82	52	44	67	74	11	93	1	97	59	29	714
34	VI033805	57	6	67	51	85	26	84	60	61	77	26	44	68	712
35	VI060316	14	23	89	86	76	45	6	60	83	37	52	57	76	704
36	VI039622	93	77	2	25	9	32	22	11	57	81	99	98	97	703
37	VI056455	82	45	54	60	50	79	42	11	58	52	36	40	94	703
38	VI060132	18	39	47	80	59	90	56	35	43	27	67	55	86	702
39	VI055119	100	25	10	38	8	52	6	21	100	93	91	99	58	701
40	VI036215	68	31	91	90	74	86	66	44	31	75	1	1	38	696
41	VI049954	96	52	17	35	58	82	30	44	94	70	42	58	18	696
42	VI056069	60	45	39	46	40	30	6	44	72	87	84	93	48	694
43	VI041461	32	72	60	70	37	47	62	60	70	11	79	37	55	692
44	VI046559	39	86	83	87	90	86	99	35	17	53	6	6	5	692
45	VI036211	64	22	91	41	33	40	78	60	55	90	16	38	59	687
46	VI039651	95	75	15	32	94	31	30	3	74	35	62	54	85	685
47	VI037997	42	44	71	83	82	70	93	21	38	25	18	11	80	678
48	VI057245	86	55	24	53	14	32	6	44	76	55	64	74	91	674
49	VI037995	48	63	72	84	29	96	51	60	81	4	40	13	18	659
50	VI033775	45	84	53	74	46	95	87	60	49	3	44	18	1	659
51	VI041215	7	81	46	58	62	58	51	84	78	24	25	19	64	657
52	VI033784	83	58	79	33	47	84	97	84	30	16	21	10	8	650
53	VI051048	68	54	93	16	22	10	66	60	52	86	20	42	57	646
54	VI049961	92	68	39	2	80	46	6	3	3	93	95	100	18	645
55	VI060317	98	17	47	26	17	62	62	60	1	33	76	68	60	627
56	VI060313	15	34	39	89	31	59	6	99	54	31	43	34	78	612
57	VI054562	21	79	54	76	63	79	62	60	15	21	17	12	50	609
58	VI047808	85	41	38	20	2	37	6	84	99	33	73	66	24	608
59	VI033773	5	60	49	66	54	85	95	35	62	9	50	28	7	605
60	VI060314	30	20	65	71	50	70	6	84	44	38	23	26	77	604
61	VI044233	8	78	85	41	31	52	30	3	81	17	86	56	35	603
62	VI033803	71	1	1	36	100	4	30	97	63	73	19	33	65	593
63	VI056456	10	55	49	61	50	47	48	35	51	39	24	27	92	588
64	VI040865	4	33	33	50	5	77	6	97	86	6	70	39	79	585
65	VI055996	29	32	76	48	87	26	96	35	2	44	27	35	48	585
66	VI056407	46	24	79	3	26	7	6	84	21	84	71	85	39	575
67	VI048291	94	34	62	9	43	57	22	1	96	48	46	45	16	573
68	VI055110	38	69	30	29	36	24	42	11	27	36	89	77	60	568
69	VI050549	23	74	33	73	96	70	47	3	53	70	5	7	11	565

70	VI056079	65	11	5	5	40	1	1	21	95	98	80	97	46	565
71	VI050170	97	8	4	64	95	38	51	84	4	51	13	16	27	552
72	VI054568	52	2	25	28	1	5	6	60	77	83	60	79	71	549
73	VI033785	13	98	20	7	89	14	100	84	32	5	57	21	8	548
74	VI055219	63	93	15	19	23	18	66	35	10	54	45	53	53	547
75	VI037994	1	96	33	47	12	36	42	99	67	2	68	30	11	544
76	VI033781-A	41	62	26	36	5	61	22	60	69	23	74	60	2	541
77	VI040770	28	73	6	15	4	21	1	3	85	22	100	86	83	527
78	VI047751	75	25	33	31	12	35	1	60	88	29	54	43	33	519
79	VI033824	55	40	22	62	55	59	62	60	20	15	31	17	15	513
80	VI033786	49	89	45	68	30	74	30	3	34	19	33	23	10	507
81	VI055220	16	88	75	8	28	11	22	11	14	58	58	64	51	504
82	VI036201	6	82	72	17	61	14	84	21	22	10	49	29	24	491
83	VI048154	50	38	12	4	7	24	1	44	40	48	98	92	21	479
84	VI036212	74	10	19	11	40	6	30	84	39	79	8	15	56	471
85	VI033810	61	12	9	10	9	3	6	60	42	100	12	83	62	469
86	VI060315	20	13	3	41	17	14	6	59	64	12	83	61	73	466
87	VI046566	34	67	65	24	55	32	66	21	36	14	15	8	27	464
88	VI046537	21	25	26	30	82	11	78	35	37	28	28	32	30	463
89	VI036213	78	7	49	18	17	29	66	21	29	89	2	3	45	453
90	VI046556	12	28	26	63	70	52	48	60	23	43	3	2	16	446
91	VI046562	57	9	39	49	33	26	87	60	11	45	11	14	3	444
92	VI051047	76	5	21	22	44	13	6	44	24	90	9	25	63	442
93	VI049632	43	29	30	6	35	19	42	60	7	58	35	51	11	426
94	VI051042	53	45	49	40	17	44	6	21	19	40	10	8	65	417
95	VI033781-B	40	75	17	14	9	65	22	44	46	18	37	24	4	415
96	VI046536	62	30	11	45	26	23	42	44	25	50	7	5	32	402
97	VI046544	47	14	13	34	63	14	30	44	28	62	4	4	24	381
98	VI036203	26	49	7	23	24	8	22	3	60	8	81	48	21	380
99	VI046554	3	51	33	27	38	8	56	11	59	13	38	21	21	379
100	VI048596	2	3	8	1	14	2	6	21	41	99	14	63	6	280

Where CC: chlorophyll contents, SL: shoot length, RL: root length, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves, SSC: sub-stomatal CO_2, TR: transpiration rate, PR: photosynthetic rate, WUE: water use efficiency, LT: leaf temperature

4.1.15 Correlation among different attributes of okra genotypes

The correlation among various attributes of okra genotypes is presented in table 4.1.28. Results showed that chlorophyll contents have a significant negative correlation with shoot length (SL), root dry weight (RDW) and transpiration rate (TR) whereas, significantly positive correlation with water use efficiency (WUE). A strongly positive correlation in case of shoot length with shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), root dry weight (RDW) and transpiration rate (TR) was observed while strongly negative correlation was noted with number of leaves (NL). Root length expressed highly significantly positive correlation with shoot fresh weight (SFW), shoot dry weight (SDW) and root dry weight (RDW). In case of shoot fresh weight (SFW), strongly positive correlation with root dry weight (RDW), root fresh weight (RFW) and shoot dry weight (SDW) was recorded. Shoot dry weight (SDW) exhibited a significantly positive correlation with root dry weight (RDW). A negative correlation with root dry weight and water use efficiency (WUE) was noted while positive correlation with transpiration rate (TR) was observed. Sub-stomatal CO_2 possessed negative correlation with photosynthetic rate (PR) and transpiration rate (TR). A highly negative correlation of transpiration rate with water use efficiency (WUE) and highly positive correlation with leaf temperature (LT) was recorded. A strongly significantly positive correlation was revealed between photosynthetic rate (PR) and water use efficiency (WUE), on the other hand negative correlation was seen between leaf temperature (LT) and water use efficiency (WUE).

Table 4.1.28: Correlation matrix among different attributes of okra genotypes

Attributes	CC	SL	RL	SFW	RFW	SDW	RDW	NL	SSC	TR	PR	WUE	LT
CC	1												
SL	-0.204*	1											
RL	-0.102	0.232*	1										
SFW	-0.090	0.316**	0.445**	1									
RFW	0.075	-0.135	0.019	0.230*	1								
SDW	-0.007	0.472**	0.477**	0.794**	0.050	1							
RDW	-0.200*	0.239*	0.295**	0.267**	0.096	0.265**	1						
NL	-0.116	-0.277**	0.027	0.046	0.090	0.008	0.103	1					
SSC	-0.079	-0.022	0.008	0.019	0.147	-0.010	0.182	0.001	1				
TR	-0.456**	0.381**	-0.092	0.019	-0.129	0.147	0.218*	0.100	-0113	1			
PR	0.148	0.183	-0.120	-0.132	-0.188	-0.049	-0.185	-0.136	-0321**	0.100	1		
WUE	0.413**	-0.053	-0.067	-0.168	-0.106	-0.123	-0.242*	-0.134	-0.220*	-0.431**	0.755**	1	
LT	-0.109	-0.014	-0.117	-0.181	-0.027	-0.060	0.016	0.169	0.067	0.298**	-0.117	-0.215*	1

Where: Values indicates Pearson's correlation coefficient, * = Significant ($p \leq 0.05$); ** = Highly significant ($p \leq 0.01$) CC: chlorophyll contents, SL: shoot length, RL: root length, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight, NL: number of leaves SSC: sub-stomatal CO_2, TR: transpiration rate, PR: photosynthetic rate, WUE: water use efficiency, LT: leaf temperature

4.2 Experiment # 2

Screening of different okra genotypes for heat tolerance under field conditions

Twenty five okra genotypes (twenty tolerant and five sensitive) screened out from experiment # 1 were used for screening under field conditions. Seeds of all genotypes were sown in field at vegetable research area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, in the summer season of 2014. There were three sowing dates to check the effect of heat stress on different morphological, physiological and yield attributes. Three sowing dates were 02 April, 12 April and 22 April. All the cultural practices were kept same for all sowing dates and for all genotypes. Experiment was replicated four times and there were five plants per replication. The results for morphological, physiological and yield attributes studied are given below.

4.2.1: Effect of sowing dates on number of plants survived of okra genotypes

Sowing date had significant ($p \leq 0.05$) effect on number of plants survival in okra genotypes (Table 4.2.1). VI051062 (heat tolerant genotype) showed maximum plant survival (05) in sowing date 1 (02 April) and 3 (22 April). The same number of plants (05) survived by second tolerant genotype VI060131 in sowing date 3 (22 April). Minimum plant survival (0.5) was observed in VI048596 (heat sensitive genotype), followed by VI046554 with plant survival (0.75) in 2nd sowing date (12 April). Tolerant genotypes VI051062 and VI060131 responded more effectively towards sowing dates with higher plant survival as compared to sensitive genotypes VI048596 and VI046554 with least number of plants survived. Means for effect of heat stress on number of plants survived in three sowing dates are presented in table 4.2.2.

4.2.2. Effect of sowing dates on plant height (cm) of okra genotypes

Plant height of okra genotypes varied significantly ($p \leq 0.05$) varied among sowing dates (Table 4.2.1). Sowing date 1 (02 April) gave maximum plant height (150 cm) and (148 cm) in VI051062 and VI060131 (heat tolerant genotypes), respectively. VI048596 and VI046554 (heat sensitive genotypes) showed minimum plant height (80 cm) and (85 cm), respectively in sowing date 2 (20 April). Tolerant genotypes VI051062 and VI060131 had more effectual response on plant height in sowing date 1 (02 April) with maximum plant height while sensitive genotypes (VI048596 and VI046554) respond less effectively in 3rd sowing date (22 April) with minimum plant height. Means for effect of heat stress on plant

height under three sowing dates are presented in table 4.2.3.

Table 4.2.1: Analysis of variance for effect of different sowing dates on number of plant survived, plant height, number of pod per plant, pod length, pod diameter, number of leaves per plant and leaf area of okra genotypes

Sr. No.	Parameter	SOV	Significance level (p - value)
1	Number of plants survived	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
2	Plant height (cm)	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
3	Number of pod plant^{-}	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
4	Pod length (cm)	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
5	Pod diameter (cm)	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
6	Number of leaves plant^{-}	Genotype (G)	*
		Sowing date (SD)	**
		G x SD	**
7	Leaf area (cm^2)	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**

NS = non-significant ($p > 0.05$); * = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$)

Table 4.2.2: Effect of heat stress on number of plants survived in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	5.00 a	4.75ab	5.00 a	4.92 A
VI060131	4.50 abc	4.50 abc	5.00 a	4.67 AB
VI054566	3.25	1.75	4.25 a-d	3.08 CDE
VI041139	3.00 a-h	2.25 d-j	4.25 a-d	3.17 CD
VI056452	3.25 a-g	3.75 a-e	3.75 a-e	3.58 BC
VI056404	3.00 a-h	2.75 b-i	2.50 c-j	2.75 CDE
VI060206	4.75 ab	1.25 g-j	3.00 a-h	3.00 CDE
VI055018	1.50 f-j	1.00 hij	3.50 a-f	2.00 EFG
VI041763	2.50 c-j	2.25 d-j	2.00 e-j	2.25 DG
VI056454	1.25 g-j	1.50 f-j	3.25 a-g	2.00 EFG
VI040649	2.25 d-j	1.75 e-j	2.75 b-i	2.25 DG
VI054565	3.50 a-f	2.50 c-j	2.00 e-j	2.67 CF
VI056448	3.50 a-f	2.75 b-i	3.25 a-g	3.17 CD
VI056453	2.50 c-j	3.50 a-f	2.50 c-j	2.83 CDE
VI050150	3.75 a-e	2.00 e-j	1.75 e-j	2.50 CG
VI046544	2.25 d-j	2.50 c-j	1.50 f-j	2.08 DG
VI036203	3.25 a-g	1.50 f-j	2.50 c-j	2.42 DG
VI046554	2.25 d-j	0.75 i-j	1.75 e-j	1.58 DG
VI048596	2.25 d-j	0.50 j	1.50 f-j	1.42 G
Mean	3.03 A	2.29 B	2.95 A	

Table 4.2.3 Effect of heat stress on plant height (cm) in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	150.00 a	145.00 ab	140.00 a-d	145.00 A
VI060131	148.50 a	141.50 abc	138.00 a-d	142.67 AB
VI054566	140.00 a-d	135.00 a-e	120.00 b-i	131.67 BCD
VI041139	130.00 a-g	130.00 a-g	115.75 d-k	125.25 CDE
VI056452	138.50 a-d	140.00 a-d	125.00 a-h	134.50 ABC
VI056404	142.75 abc	125.00 a-h	94.50 i-o	120.75 DH
VI060206	140.00 a-d	128.00 a-g	88.00 mno	118.67 EH
VI055018	147.25 a	118.00 c-j	100.00 h-o	121.75 CG
VI041763	130.00 a-g	77.50 o	105.00 g-n	104.17 I
VI056454	125.00 a-h	130.00 a-g	110.00 e-m	121.67 CG
VI040649	120.00 b-i	135.00 a-e	115.00 d-l	123.33 CF
VI054565	115.00 d-l	90.00 l-o	120.00 b-i	108.33 HI
VI056448	110.00 e-m	95.00 i-o	130.00 a-g	111.67 FI
VI056453	120.00 b-i	100.00 h-o	125.00 a-h	115.00 EI
VI050150	130.00 a-g	131.00 a-f	85.00 mno	115.33 EI
VI046544	110.00 e-m	125.00 a-h	80.00 no	105.00 I
VI036203	100.00 h-o	105.50 f-n	125.00 a-h	110.17 GHI
VI046554	95.00 i-o	93.00 j-o	85.00 mno	91.00 J
VI048596	92.00 k-o	90.00 l-o	80.00 no	87.33 J
Mean	125.47 A	117.61 B	109.54 C	

4.2.3: Effect of sowing dates on number of pods per plant of okra genotypes

Sowing dates significantly affected number of pods per plant in okra genotypes (Table 4.2.1). Highest numbers of pods per plant (40) were recorded in 1st sowing date (02 April) by VI051062, while VI060131 stood second with (38) number of pods plant^{-1} in same sowing date. VI046554 gave lowest (12) number of pods plant^{-1} in 3rd sowing date (22 April), followed by VI048596 having (13) number of pods plant^{-1} in sowing date 2 (12 April) VI051062 and VI060131 showed better response in all sowing dates having highest number of pods plant^{-1} while VI046554 and VI048596 had minimum number of pods per plant in all sowing dates. Means for effect of heat stress on number of pods per plant in three sowing dates are given in table 4.2.4.

Table 4.2.4: Effect of heat stress on number of pod per plant in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	40.00 a	36.00 abc	30.00 c-g	35.33 A
VI060131	38.00 a-b	34.50 a-d	34.00 a-d	35.50 A
VI054566	30.00 c-g	30.00 c-g	25.00 g-k	28.33 BC
VI041139	25.00 g-k	25.00 g-k	28.00 d-h	26.00 BCD
VI056452	20.00 j-n	30.00 c-g	20.00 j-n	23.33 DE
VI056404	25.00 jk	28.00 d-h	24.00 j-l	25.67 BCD
VI060206	35.00 abc	22.50 h-l	18.00 l-p	25.17 CD
VI055018	32.00 b-f	24.50 g-l	27.00 e-i	27.83 BC
VI041763	15.00 m-p	19.50 j-o	24.00 g-l	19.50 F
VI056454	25.00 g-k	30.00 c-g	23.00 h-l	26.00 BCD
VI040649	30.00 c-g	34.50 a-d	22.00 h-l	28.83 B
VI054565	33.50 a-e	22.50 h-l	26.00 f-j	27.33 BC
VI056448	28.00 d-h	27.00 e-i	24.00 g-l	26.33 BCD
VI056453	35.00 abc	30.00 c-g	18.00 l-p	27.67 BC
VI050150	20.00 j-n	33.00 b-e	12.00 p	21.67 EF
VI046544	30.00 c-g	18.00 l-p	14.00 nop	20.67 EF
VI036203	22.00 h-l	21.00 i-m	18.00 l-p	20.33 EF
VI046554	18.00 l-p	15.00 m-p	12.00 p	15.00 G
VI048596	19.00 k-o	13.00 o-p	14.00 nop	15.33 G
Mean	27.39 A	26.00 B	21.74 C	

4.2.4 Effect of sowing dates on pod length (cm) of okra genotypes

Pod length significantly ($p \leq 0.05$) varied for okra genotypes under different different sowing dates (Table 4.2.1). The maximum pod length (16 cm) was calculated in VI060131 genotype in sowing date 1 (02 April) as well as in VI051062 with pod length (14 cm) in the same sowing date. On the other hand minimum pod length (04 cm) was noted in VI046554

genotype in sowing date 3 (22 April) whereas genotype VI048596 gave same pod length (06 cm) in all sowing dates. All sowing dates expressed efficient response on tolerant genotypes with highest pod length while sensitive genotypes showed lowest pod length in all sowing dates. Means for effect of heat stress on pod length under three sowing dates are shown in table 4.2.5.

Table 4.2.5: Effect of heat stress on pod length (cm) in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	14.00 ab	13.00 abc	10.00 c-g	12.33 AB
VI060131	16.00 a	13.00 abc	9.00 d-h	12.67 A
VI054566	12.00 bcd	10.00 c-g	6.00 hij	9.33 CD
VI041139	14.00 ab	12.00 bcd	11.00 b-e	12.33 AB
VI056452	10.00 c-g	9.00 d-h	5.75 hij	8.25 DE
VI056404	8.00 e-i	7.00 g-j	8.00 e-i	7.67 DEF
VI060206	10.00 c-g	6.00 hij	7.00 g-j	7.67 DEF
VI055018	12.25 bcd	9.00 d-h	11.00 b-e	10.75 BC
VI041763	9.00 d-h	11.25 b-e	8.00 e-i	9.42 CD
VI056454	7.00 g-j	9.00 d-h	10.00 c-g	8.67 DE
VI040649	6.00 hij	6.00 hij	11.00 b-e	7.67 DEF
VI054565	9.00 d-h	5.00 ij	8.00 e-i	7.33 EF
VI056448	10.75 b-f	5.00 ij	5.00 i-j	6.92 EF
VI056453	5.00 i-j	7.25 f-j	6.00 hij	6.08 F
VI050150	8.00 e-i	11.00 b-e	4.00 j	7.67 DEF
VI046544	12.00 bcd	5.00 i-j	6.00 hij	7.67 DEF
VI036203	10.00 c-g	7.00 g-j	6.00 hij	7.67 DEF
VI046554	8.00 e-i	5.75 hij	4.00 j	5.92 F
VI048596	6.00 hij	6.00 hij	6.00 hij	6.00 F
Mean	9.84 A	8.28 B	7.46 C	

4.2.5 Effect of sowing dates on pod diameter (cm) of okra genotypes

Pod diameter of okra genotypes varied significantly (S” 0.05) in relation to sowing dates. The analysis of variance about pod diameter is given in table 4.2.1. Genotypes VI051062 and VI060131 were having highest pod diameter (02 cm, 1.90 cm, respectively) in sowing date of 1 (02 April). The least pod diameter (1.07cm) was observed in VI048596 as well as in VI046554 (1.10 cm) at third sowing date (22 April). Tolerant genotypes (VI051062, VI060131) showed better response in 1st sowing date (02 April) with maximum pod diameter, however these genotypes gave good results in all sowing dates as compared to sensitive genotypes (VI046554, VI042596) with lowest pod diameter in all sowing dates. Means for effect of heat stress on pod diameter under three sowing dates are expressed in

table 4.2.6.

Table 4.2.6 Effect of heat stress on pod diameter (cm) in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	2.00 a	1.90 b	1.80 cd	1.90 A
VI060131	1.90 b	1.85 bc	1.75 de	1.83 B
VI054566	1.70 e-f	1.65 fgh	1.65 fgh	1.67 C
VI041139	1.54 j-m	1.55 i-l	1.50 lm	1.53 E
VI056452	1.65 fgh	1.57 h-l	1.40 no	1.54 E
VI056404	1.70 e-f	1.64 f-i	1.33 opq	1.56 DE
VI060206	1.80 cd	1.67 efg	1.25 q-t	1.57 DE
VI055018	1.85 bc	1.50 lm	1.45 mn	1.60 D
VI041763	1.40 no	1.52 klm	1.36 nop	1.43 FG
VI056454	1.50 lm	1.45 mn	1.65 fgh	1.53 E
VI040649	1.63 f-j	1.40 no	1.60 g-k	1.54 E
VI054565	1.68 efg	1.33 opq	1.40 no	1.47 F
VI056448	1.75 de	1.28 p-s	1.22 r-u	1.42 G
VI056453	1.30 pqr	1.20 s-v	1.16 t-x	1.22 JK
VI050150	1.60 g-k	1.40 no	1.10 w-x	1.37 H
VI046544	1.55 i-l	1.17 t-w	1.07 x	1.26 IJ
VI036203	1.45 mn	1.20 s-v	1.16 t-x	1.27 I
VI046554	1.40 no	1.12 vwx	1.10 w-x	1.21 K
VI048596	1.35 op	1.14 u-x	1.07 x	1.19 K
Mean	1.62 A	1.45 B	1.37 C	

4.2.6 Effect of sowing dates on leaf area (cm^2) of okra genotypes

Leaf area of all okra genotypes differed significantly ($p \leq 0.05$) for all sowing dates. (Table 4.2.1). At sowing date one (02 April) maximum leaf area (500 cm^2) was counted in genotype VI051062, followed by sowing date 2 (12 April) with leaf area (485 cm^2). Genotype VI060131 also showed same leaf area (485 cm^2) at 1st sowing date (02 April). At the same time, genotype VI042596 showed lowest leaf area (300 cm^2, 315 cm^2 315 cm^2) at sowing date of two (12 April), one (02 April) and third (22 April) respectively. Results showed that sowing dates had positive effective on genotypes VI051062 and VI060131 (Tolerant) with higher leaf area in contrast with genotypes VI042596 and VI046554 (sensitive) having lower leaf area. Means for effect of heat stress on leaf area in three sowing dates are presented in table 4.2.7.

Table 4.2.7 Effect of heat stress on leaf area (cm^2) in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	500.00 a	485.00 b	450.00 e-f	478.33 A
VI060131	485.00 b	478.00 b-c	448.00 efg	470.33 B
VI054566	470.00 c	465.00 c-d	440.00 e-h	458.33 C
VI041139	450.00 ef	450.00 ef	435.00 g-j	445.00 D
VI056452	465.00 cd	422.00 j-m	430.00 h-k	439.00 DE
VI056404	452.00 de	430.00 h-k	425.00 i-l	435.67 E
VI060206	437.00 f-i	417.00 klm	420.00 klm	424.67 F
VI055018	430.00 h-k	417.00 klm	415.00 lm	420.67 FG
VI041763	400.00 no	425.00 i-l	418.00 klm	414.33 GHI
VI056454	420.00 klm	422.00 j-m	410.00 mn	417.33 FGH
VI040649	410.00 mn	415.00 lm	412.00 lmn	412.33 HI
VI054565	400.00 no	410.00 mn	417.00 klm	409.00 I
VI056448	350.00 q	408.00 mn	400.00 no	386.00 J
VI056453	340.00 qr	400.00 no	390.00 o	376.67 K
VI050150	345.00 q	425.00 i-l	370.00 p	380.00 JK
VI046544	330.00 rs	370.00 p	368.00 p	356.00 L
VI036203	325.00 st	350.00 q	365.00 p	346.67 M
VI046554	320.00 st	320.00 st	342.50 qr	327.50 N
VI048596	315.00 t	300.00 u	315.00 t	310.00 O
Mean	402.32 B	411.00 A	403.71 B	

4.2.7 Effect of sowing dates on number of leaves of okra genotypes

Sowing dates significantly ($p \leq 0.05$) affected number of leaves of okra genotypes. The summary for analysis of variance is given in table 4.2.1. Maximum number of leaves (71) were counted in heat tolerant genotype VI060131 in sowing date one (02, April), followed by (70) same genotype VI060131 in second sowing date (12, April). Minimum number of leaves (11) were counted in heat sensitive genotype VI048596 on second sowing date (02, April), followed by number of leaves (15) in the heat sensitive genotype VI048596 in sowing date 1^{st} (02, April). Both heat tolerant genotypes VI051062 and VI060131 produced highest number of leaves regardless of sowing date. On the other hand VI046554 and VI042596 both heat sensitive genotypes gave the lowest number of leaves on all the three sowing dates. Means for effect of heat stress on number of leaves at three sowing dates are given in table 4.2.8.

Table 4.2.8 Effect of heat stress on number of leaves in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	68.50 a	65.00 ab	60.50 bc	64.67 A
VI060131	71.00 a	70.00 a	58.50 bcd	66.50 A
VI054566	64.25 ab	60.50 bc	55.00 cde	59.92 B
VI041139	58.50 bcd	55.00 cde	40.00 j-o	51.17 C
VI056452	53.75 c-f	50.00 ei	41.50 j-m	48.42 C
VI056404	51.00 d-h	45.50 g-j	47.00 f-j	47.83 C
VI060206	41.00 j-n	40.00 j-o	35.00 l-q	38.67 DEF
VI055018	45.25 g-j	41.75 j-m	33.50 n-r	40.17 DE
VI041763	37.00 k-p	30.00 p-t	30.00 p-t	32.33 HI
VI056454	31.50 p-s	32.50 o-s	40.00 j-o	34.67 FGH
VI040649	55.00 cde	29.50 p-t	42.75 i-l	42.42 D
VI054565	52.50 d-g	25.50 s-w	36.00 l-q	38.00 EF
VI056448	60.50 bc	22.25 t-x	27.00 rv	36.58 EFG
VI056453	50.00 e-i	34.75 m-r	20.00 vwx	34.92 FGH
VI050150	34.75 m-r	44.50 h-k	21.50 u-x	33.58 GHI
VI046544	40.75 j-n	29.50 p-t	19.00 wx	29.75 IJ
VI036203	34.50 m-r	25.50 s-w	20.00 vwx	26.67 JK
VI046554	25.50 s-w	29.00 q-u	21.50 u-x	25.33 K
VI048596	15.50 x-y	11.00 y	19.00 wx	15.17 L
Mean	46.88 A	39.04 B	35.14 C	

4.2.8 Effect of sowing dates on photosynthetic rate (µmol m^{-2} s^{-1}) of okra genotypes

Photosynthetic rate of okra genotypes was significantly ($p \leq 0.05$) different in all sowing dates (Table 4.2.17). Maximum photosynthetic rate (10.78) was noted in VI051062 followed by (9.17) in VI060131 at sowing date of one (02 April). VI046554 gave lowest photosynthetic rate (1.25) at 3rd sowing date, while VI048596 (2.85) at sowing date of two (12 April). Tolerant genotypes behaved more efficiently with highest photosynthetic rate regardless of sowing dates as compared to sensitive ones with lowest photosynthetic rate in all sowing dates. Means for effect of heat stress on photosynthetic rate in three sowing dates are given in table 4.2.9.

4.2.9 Effect of sowing dates on transpiration rate (mmol m^{-2} s^{-1}) of okra genotypes

Sowing date significantly ($p \leq 0.05$) influenced transpiration rate of okra genotypes. The summary for analysis of variance for transpiration rate is given in table 4.2.17. The genotype VI060131was noted having lowest transpiration rate (3.22) at 1st sowing date (02 April), while genotype VI051062 gave (3.30) transpiration rate at same sowing date. Genotype VI046554 expressed highest transpiration rate (7.37) at sowing date 3 (22 April)

while VI048596 gave second highest transpiration rate (6.72) at same sowing date. Results revealed that 1st sowing date left positive effect on tolerant genotypes with lowest transpiration rate while sensitive genotypes respond less effectively toward sowing date with higher transpiration rate. Means for effect of heat stress on transpiration rate in three sowing dates are expressed in table 4.2.10.

Table 4.2.9 Effect of heat stress on photosynthetic rate in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	10.78 a	7.20 a-d	5.18 c-i	7.72 A
VI060131	9.17 ab	6.31 b-f	5.31 b-i	6.93 A
VI054566	6.33 b-f	5.05 c-k	0.78 n	4.05 BCD
VI041139	6.99 a-e	1.61h-n	1.10 lmn	3.23 CF
VI056452	1.25 j-n	5.57 b-g	3.73 d-n	3.52 CF
VI056404	3.34 d-n	3.03 f-n	1.80 g-n	2.72 CF
VI060206	2.09 g-n	2.28 g-n	0.47 n	1.61 F
VI055018	2.27 g-n	2.92 f-n	1.01mn	2.07 EF
VI041763	2.08 g-n	3.83 d-n	4.99 c-l	3.63 CDE
VI056454	2.03 g-n	1.47 i-n	1.22 k-n	1.57 F
VI040649	4.83 c-m	0.44 n	1.80 g-n	2.35 DEF
VI054565	2.92 f-n	1.95 g-n	1.08 lmn	1.98 EF
VI056448	4.24 d-n	3.07 f-n	0.96 mn	2.75 CF
VI056453	1.82 g-n	1.51 h-n	2.08 g-n	1.80 EF
VI050150	8.58 abc	5.60 b-g	3.21e-n	5.80 AB
VI046544	6.61 b-f	5.40 b-h	1.63 h-n	4.55 BC
VI036203	5.12 c-k	3.29 d-n	1.43 i-n	3.28 CF
VI046554	5.13 c-j	3.90 d-n	1.25 j-n	3.43 CF
VI048596	3.82 dn	2.85 fn	3.02 f-n	3.23 CF
Mean	4.70 A	3.54 B	2.21 C	

4.2.10 Effect of sowing dates on water use efficiency (µmol CO_2 $mmol^{-1}$ H_2O) of okra genotypes

Sowing dates had significant ($p \leq 0.05$) influence on water use efficiency of okra genotypes. The analysis of variance on the subject of water use efficiency given in table 4.2.17. The maximum water use efficiency was noted in VI051062 (3.30) and VI060131 (2.87) at sowing date one (02 April). In contrast, genotype VI046554 had lowest water use efficiency (0.17) at 3rd sowing date (22 April) whereas, (0.45) was recorded in VI048596 at same sowing date. It is observed clearly from results that tolerant genotypes (VI051062 and VI060131) respond more effectively towards all genotypes with highest water use efficiency. On the contrary, sowing dates remained less effective in case of sensitive genotypes

(VI048596 and VI046554) having lowest water use efficiency. Means for effect of heat stress on water use efficiency in three sowing dates are expressed in table 4.2.11

Table 4.2.10 Effect of heat stress on transpiration rate in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	3.30 qrs	3.44 q-s	5.08f-s	3.94 J
VI060131	3.22 rs	3.34p-s	5.42 d-r	3.99 J
VI054566	4.87 f-s	4.91 f-s	5.04 f-s	4.94 FJ
VI041139	8.63 ab	8.81 a	5.98 c-l	7.81 A
VI056452	4.07 k-s	3.60 n-s	5.67 c-o	4.45 HIJ
VI056404	6.20 c-k	6.25 c-k	5.54 c-q	6.00 CF
VI060206	6.27 c-k	6.69 a-g	5.55 c-p	6.17 BE
VI055018	4.21is	6.57 b-h	5.77 c-n	5.52 DH
VI041763	5.85 c-m	6.68 a-g	5.78 c-n	6.10 CDE
VI056454	5.76 c-n	6.03 c-k	5.92 c-l	5.90 CG
VI040649	3.12 s	3.68 m-s	5.97 c-l	4.26 IJ
VI054565	5.90 c-m	6.38 c-j	6.59 a-h	6.29 BE
VI056448	4.99 f-s	7.63 a-d	6.63 a-g	6.42 BCD
VI056453	6.58 a-h	6.89 a-f	6.87 a-f	6.78 ABC
VI050150	7.71 abc	7.71abc	6.45 b-i	7.29 AB
VI046544	4.19 j-s	4.55 g-s	5.86 c-m	4.87 GJ
VI036203	3.77 l-s	5.20 e-s	6.80 a-f	5.26 EI
VI046554	4.38 h-s	4.38 h-s	7.37 a-e	5.38 DI
VI048596	6.15 c-k	6.15 c-k	6.72 a-g	6.34 BE
Mean	5.22 C	5.73 B	6.05 A	

Table 4.2.11 Effect of heat stress on water use efficiency in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	3.30 a	2.11abc	1.02 c-j	2.14 A
VI060131	2.87 ab	1.90 bcd	0.99 c-j	1.92 A
VI054566	1.32 c-j	1.04 c-j	0.16 i-j	0.84 BC
VI041139	0.81d-j	0.18 ij	0.19 i-j	0.40 C
VI056452	0.41f-j	1.53 c-g	0.66 e-j	0.86 BC
VI056404	0.55 e-j	0.51e-j	0.32 g-j	0.46 BC
VI060206	0.38 g-j	0.34g-j	0.08 j	0.27 C
VI055018	1.04 c-j	0.44 e-j	0.18 i-j	0.55 BC
VI041763	0.33 g-j	0.57 e-j	0.86 d-j	0.59 BC
VI056454	0.35 g-j	0.25 hij	0.21i-j	0.27 C
VI040649	1.64 b-f	0.12 j	0.30 g-j	0.69 BC
VI054565	0.54 e-j	0.31g-j	0.17 i-j	0.34 C
VI056448	1.39 c-i	0.40 g-j	0.15 i-j	0.65 BC
VI056453	0.30 g-j	0.22 i-j	0.30 g-j	0.27 C
VI050150	1.11c-j	0.73 d-j	0.50 e-j	0.78 BC
VI046544	1.67 c-j	1.16 c-j	0.28 hij	1.04 B
VI036203	1.48 c-h	0.63 e-j	0.22 i-j	0.78 BC
VI046554	1.18 c-j	0.91 c-j	0.17 i-j	0.76 BC
VI048596	0.62 e-j	0.46 e-j	0.45 e-j	0.51 BC
Mean	1.12 A	0.73 B	0.38 C	

4.2.11 Effect of sowing dates on leaf temperature (°C) of okra genotypes

Leaf temperature (°C) was significantly ($p \leq 0.05$) influenced by sowing dates in okra genotypes (Table 4.2.17). At sowing date one (02 April) lowest temperature (29.59) was noted in genotype VI060131, followed by VI051062 with leaf temperature (30.30) at same sowing date. VI048596 genotype showed maximum leaf temperature (36.12) at third sowing date (22 April) while 2nd highest leaf temperature (35.85) was found at sowing date of 2 (20 April) in genotype VI046554. Both tolerant genotypes behaved efficiently in all sowing dates having lowest leaf temperature in comparison with sensitive ones. Means for effect of heat stress on leaf temperature in three sowing dates are presented in table 4.2.12.

Table 4.2.12 Effect of heat stress on leaf temperature in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	30.30rs	32.35op	32.58no	31.74I
VI060131	29.59s	32.75mno	32.88mno	31.74I
VI054566	31.46q	32.80mno	33.33j-n	32.53H
VI041139	32.31op	33.00l-o	33.27k-n	32.86GH
VI056452	33.38j-n	33.08l-o	32.98l-o	33.15EFG
VI056404	34.40fgh	33.53i-m	33.53i-m	33.82D
VI060206	31.41q	33.80h-l	33.80h-l	33.00FG
VI055018	30.50r	34.02g-k	34.02g-k	32.85GH
VI041763	31.57pq	34.25ghi	34.25ghi	33.36EF
VI056454	34.24ghi	34.43fgh	34.43fgh	34.36C
VI040649	35.20b-f	34.50fgh	34.50fgh	34.73C
VI054565	34.35ghi	34.35ghi	34.35ghi	34.35C
VI056448	34.09g-k	34.55e-h	35.48a-d	34.71C
VI056453	31.36q	34.48fgh	34.81c-g	33.55DE
VI050150	30.52r	34.80c-g	34.83c-g	33.38EF
VI046544	34.67d-g	35.55abc	35.69ab	35.30B
VI036203	36.06a	35.85ab	35.82ab	35.91A
VI046554	35.36a-e	35.85ab	35.50abc	35.57A
VI048596	34.15g-j	35.83ab	36.12a	35.36B
Mean	32.89C	34.20B	34.32A	

4.2.12 Effect of sowing dates on sub-stomatal CO_2 (vpm) of okra genotypes

Sowing dates significantly ($P \leq 0.05$) affected sub-stomatal CO_2 of all okra genotypes (Table 4.2.17). The genotype VI051062 was at top with sub-stomatal CO_2 (168.25), followed by sub-stomatal CO_2 (195.50) by genotype VI060131 in sowing date 1 (02 April). In case of highest sub-stomatal CO_2 (523.25) genotype VI050150 was noted at top while genotype VI041139 stood second with sub-stomatal CO_2 (438.25) at sowing date 01 (02 April) and

sowing date 03 (12 April) respectively. In the light of results it is observed that both tolerant genotypes (VI051062, VI060131) remained better with lowest sub-stomatal CO_2 at 1st sowing date, while genotypes (VI050150, VI041139) showed poor response towards sowing dates with highest sub-stomatal CO_2. Means for effect of heat stress on sub-stomatal CO_2 at three sowing dates are expressed in table 4.2.13.

Table 4.2.13 Effect of heat stress on sub-stomatal CO_2 in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	168.25f	296.25b-f	353.50b-e	272.67D
VI060131	195.50ef	295.75b-f	372.75a-d	288.00CD
VI054566	274.50c-f	301.25b-f	411.25abc	329.00BCD
VI041139	206.75ef	399.00a-d	438.25ab	348.00A-D
VI056452	326.75b-f	370.25a-d	427.75abc	374.92AB
VI056404	322.50b-f	329.50b-f	408.50abc	353.50A-D
VI060206	341.75b-e	292.50b-f	417.00abc	350.42A-D
VI055018	313.25b-f	376.75a-d	404.25abc	364.75ABC
VI041763	397.50a-d	304.25b-f	401.75abc	367.83ABC
VI056454	349.50b-e	393.75a-d	399.00a-d	380.75A-D
VI040649	239.25def	382.25a-d	395.75a-d	339.08A-D
VI054565	319.25b-f	348.25b-e	398.75a-d	355.42ABC
VI056448	280.50b-f	335.50b-e	400.75a-d	338.92A-D
VI056453	347.00b-e	375.00a-d	392.50a-d	371.50AB
VI050150	523.25a	323.75b-f	384.25a-d	410.42A
VI046544	286.00b-f	311.50b-f	385.50a-d	327.67BCD
VI036203	315.75b-f	373.00a-d	381.50a-d	356.75ABC
VI046554	279.00b-f	377.25a-d	387.00a-d	347.75AB
VI048596	276.50b-f	314.25b-f	383.50a-d	324.75BCD
Mean	303.30C	342.11B	397.03A	

4.2.13 Effect of sowing dates on stomatal conductance to water (mmol $m^{-2}s^{-1}$) of okra genotypes

Stomatal conductance to water was significantly ($p \leq 0.05$) influenced by three sowing dates in okra genotypes (Table 4.2.17). VI060131 genotype gave highest stomatal conductance to H_2O (5.86) at sowing date 3 (22 April) while 2nd highest stomatal conductance (4.62) was noted in VI046554 at same sowing date. The lowest stomatal conductance to water (0.04) was recorded in genotype VI060206, whereas second lowest stomatal conductance (0.07) was calculated in VI040649 at second sowing date (12 April). The genotype VI060131 (tolerant), while VI046554 (sensitive) respond less effectively towards sowing dates as compared to genotypes VI060206 and VI054565 with lowest

stomatal conductance to water. Means for effect of heat stress on stomatal conductance to water at three sowing dates are shown in table 4.2.14.

Table 4.2.14 Effect of heat stress on stomatal conductance to H_2O in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	0.26d	0.14d	2.54bcd	0.98AB
VI060131	0.21d	0.12d	5.86a	2.06A
VI054566	0.13d	0.08d	1.37cd	0.52AB
VI041139	0.29d	0.15d	3.54abc	1.32AB
VI056452	0.09d	0.08d	2.80a-d	0.99AB
VI056404	0.17d	0.08d	2.99a-d	1.08AB
VI060206	0.19d	0.04d	2.09bcd	0.77AB
VI055018	0.13d	0.12d	3.66abc	1.30AB
VI041763	0.18d	0.08d	2.94a-d	1.07AB
VI056454	0.17d	0.17d	1.77bcd	0.71AB
VI040649	0.07d	0.12d	1.48cd	0.55AB
VI054565	0.16d	0.07d	3.98abc	1.40AB
VI056448	0.14d	0.10d	3.64abc	1.29AB
VI056453	0.20d	0.10d	2.78a-d	1.03AB
VI050150	0.25d	0.14d	1.73bcd	0.71AB
VI046544	0.13d	0.13d	1.12cd	0.46B
VI036203	0.13d	0.13d	3.09a-d	1.12AB
VI046554	0.10d	0.16d	4.62ab	1.63AB
VI048596	0.12d	0.10d	2.02bcd	0.75AB
Mean	0.16B	0.11B	2.84A	

4.2.14 Effect of sowing dates on chlorophyll contents (SPAD value) of okra genotypes

Sowing dates significantly ($p \leq 0.05$) influenced chlorophyll contents of okra genotypes (Table 4.2.17). The 2nd sowing date efficiently improved the genotype VI051062 with maximum chlorophyll contents (72.58), followed by chlorophyll contents (62.63) in genotype VI041763 at same sowing date. The lowest chlorophyll contents (15.93) and (18.15) were found in genotype VI046554 at sowing date of 3rd and 2nd respectively. Results show that chlorophyll contents were efficiently improved in tolerant genotype (VI051062) regardless of sowing dates. On the other hand, sensitive genotype (VI046554) not positively affected by sowing dates with minimum chlorophyll contents. Means for effect of heat stress on chlorophyll contents at three sowing dates are shown in table 4.2.15.

Table 4.2.15 Effect of heat stress on chlorophyll contents (SPAD value) in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Means
VI051062	42.63c-f	72.58a	47.78bcd	54.33A
VI060131	20.85lmn	34.30c-m	32.88d-m	29.34DEF
VI054566	24.83g-n	36.50c-l	31.48d-n	30.93C-F
VI041139	31.18d-n	40.00c-h	39.95c-h	37.04CD
VI056452	21.50j-n	27.83f-n	21.03k-n	23.45FG
VI056404	25.38g-n	49.98bc	40.73c-g	38.69BC
VI060206	32.63d-m	41.25c-g	34.90c-l	36.26CD
VI055018	22.10i-n	31.33d-n	18.20mn	23.88FG
VI041763	33.60c-m	62.63ab	41.05c-g	45.76B
VI056454	38.35c-i	37.68c-j	37.63c-k	37.88BC
VI040649	32.88d-m	37.63c-k	26.65f-n	32.38CDE
VI054565	23.68h-n	28.68f-n	22.28i-n	24.88EFG
VI056448	34.08c-m	34.45c-m	31.03e-n	33.18CDE
VI056453	33.78c-m	39.08c-h	33.00d-m	35.28CD
VI050150	31.00e-n	45.50cde	27.98f-n	34.83CD
VI046544	41.23c-g	34.45c-m	27.08f-n	34.25CD
VI036203	31.00e-n	45.50cde	27.98f-n	34.83CD
VI046554	24.05h-n	18.15mn	15.93n	19.38G
VI048596	32.50d-n	39.28c-h	32.75d-m	34.84CD
Mean	30.38B	39.83A	31.07B	

4.2.15 Effect of sowing dates on electrolyte leakage (%) of okra genotypes

Sowing dates significantly ($p \leq 0.05$) affected electrolyte leakage in okra genotypes (Table 4.2.17). The minimum electrolyte leakage (63.70 %) was found in genotype VI051062 at sowing date 1 (02 April), while genotype VI060131 was ranked second with electrolyte leakage (64.15 %) at the same sowing date. On the other hand, at same sowing date of one (02 April) the genotype VI048596 gave highest electrolyte leakage (98.02 %). Another genotype VI056452 stood second with electrolyte leakage (97.39%) at third sowing date (22 April). The genotypes VI051062 and VI06013 (tolerant) had effective response in case of all sowing dates especially 1 (02 April) in contrary to other sensitive genotypes with higher electrolyte leakage. Means for effect of heat stress on electrolyte leakage at three sowing dates are shown in table 4.2.16.

Table 4.2.16: Effect of heat stress on electrolyte leakage (%) in various okra genotypes at three sowing dates

Genotypes	SD1	SD2	SD3	Mean
VI051062	63.70w	64.41vw	65.21v	64.44N
VI060131	64.15vw	64.86vw	64.77vw	64.59N
VI054566	72.52t	73.22rst	73.58rst	73.11L
VI041139	91.22i	92.13ghi	92.45f-i	91.93CD
VI056452	79.42lmn	97.12ab	97.39ab	91.31D
VI056404	96.16b	97.01ab	97.28ab	96.82A
VI060206	73.32rst	74.23rs	74.55r	74.03K
VI055018	87.28j	88.18j	88.50j	87.99E
VI041763	76.12q	77.02pq	77.34opq	76.82J
VI056454	92.81d-h	93.66c-f	93.93cde	93.47B
VI040649	78.50mno	79.41lmn	79.73k-n	79.21H
VI054565	79.79klm	80.50kl	80.85k	80.38G
VI056448	69.14u	69.99u	70.26u	69.79M
VI056453	93.23c-g	94.13cd	94.45c	93.94B
VI050150	73.05st	73.95rs	74.27rs	73.76KL
VI046544	77.37opq	78.08op	78.43no	77.96I
VI036203	73.30rst	74.15rs	74.42r	73.96K
VI046554	91.48hi	92.38f-i	92.71e-h	92.19C
VI048596	98.02a	80.32kl	80.64kl	86.33F
Mean	80.56C	81.30B	81.62A	

Table 4.2.17: Analysis of variance for the effect of different sowing dates on photosynthetic rate, transpiration rate, water use efficiency, leaf temperature, sub-stomatal conductance to CO2, sub-stomatal conductance to H2O, chlorophyll contents and electrolyte leakage of various okra genotypes

Sr. No.	Parameter	SOV	Significance level (p - value)
1	Photosynthetic rate	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
2	Transpiration rate	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
3	Water use efficiency	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
4	Leaf temperature	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
5	Sub-stomatal conductance to CO_2	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
6	Sub-stomatal conductance to H_2O	Genotype (G)	*
		Sowing date (SD)	**
		G x SD	**
7	Chlorophyll contents	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**
8	Electrolyte leakage	Genotype (G)	**
		Sowing date (SD)	**
		G x SD	**

NS = non-significant ($p > 0.05$); * = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$)

4.3. Experiment # 3

Optimization of proline levels for the enhancement of heat tolerance in okra

Four okra genotypes screened out from experiment # 2 consisting of two tolerant genotypes i.e. VI051062 and VI060131 and two sensitive genotypes i.e. VI046554 and VI048594 were planted to check the effectiveness of proline in improving tolerance to elevated temperature stress. Seeds of tolerant and sensitive genotypes were sown in plastic pots consisting of sand as growth medium and Hoagland's solution as nutrient medium. There were four replications in experiment and five plants per replication. Plants were kept at 28/22°C (day and night) for four weeks. Temperature increased to 45/35°C (day and night) after four weeks of germination. After obtaining the required temperature proline treatments i.e., 0, 1.0, 1.5, 2.0, 2.5 and 3.0 mM were applied and plants were kept at the same day and night temperature. All proline treatments were applied as foliar spray. After two weeks plants were harvested and analyzed for the effects of proline on heat tolerance potential of okra to choose optimized dose for further study. The results of different parameters studied are as follow:

4.3.1. Effect of proline on shoot length (cm) of heat tolerant and heat sensitive okra genotypes

Foliar application of proline significantly ($p \leq 0.05$) increased the shoot length of okra genotypes as compared to control. The interaction (G x T) was significant in terms of shoot length (Table 4.3.1). Results revealed that highest shoot length (17.17±0.21cm) was measured in VI051062 (heat tolerant variety), followed by VI060131 (heat tolerant variety) recording 16.07±0.06 cm shoot length when proline applied @ 2.5 mM. On the other hand lowest shoot length (10.42±0.18 cm) was measured in VI048596 (heat sensitive variety) where proline applied at concentration of 01mM as well as in VI046554 at the same proline level with shoot length of 11.12±0.36 cm. Mean ± SE for effect of proline on shoot length of heat tolerant and heat sensitive of okra genotypes are presented in Appendix 1.

4.3.2 Effect of proline on root length (cm) of heat tolerant and heat sensitive okra genotypes

Results decipited a significant ($p \leq 0.05$) impact of proline on root length of okra genotypes was noted as compared to control. The interaction (G x T) was highly significant in case of root length (Table 4.3.1). According to results (Figure 4.3.1) genotype VI051062

(heat tolerant cultivar) was found to have maximum root length (10.91±0.12 cm) with foliar application of proline at 2.5 mM concentration, followed by VI060131 (heat tolerant cultivar) having root length (10.48±0.22 cm) when proline applied at rate of 3 mM. Minimum root length (5.11±0.07 cm) was recorded in VI048596 (heat sensitive cultivar) at control while VI046554 (heat sensitive cultivar) gave root length 5.93± 0.10 cm with same level of proline. Mean ± SE for effect of proline on root length of heat sensitive and heat tolerant okra genotypes are given in Appendix 2.

4.3.3 Effect of proline on number of leaves of heat tolerant and heat sensitive okra genotypes

The results of table 4.3.1 depicted that number of leaves in okra genotypes significantly ($p \leq 0.05$) increased with foliar application of proline as compared to comtrol. The results are illustrated in figure 4.3.1. Genotype V1051062 gave higher number of leaves per plant (12.00±0.40) while genotype VI060131 stood second with 11.50±0.64 number of leaves @ 2.5 mM proline level. The minimum number of leaves (4.00±0.40) was given by VI048596 as well as another sensitive cultivar VI046554 with number of leaves (4.25±0.75) when proline applied @1.5 mM and 02 mM, respectively. In case of number of leaves the interaction (G x T) was non-significant (4.3.2). Mean ± SE for effect of proline on number of leaves of heat sensitive and heat tolerant okra genotypes are presented in Appendix 3.

4.3.4 Effect of proline on shoot fresh weight (g) of heat tolerant and heat sensitive okra genotypes

The results revealed that shoot fresh weight of all okra genotypes significantly ($p \leq 0.05$) increased with foliar application of proline compared to control (Table 4.3.1). The interaction (G x T) was significant for shoot fresh weight. Shoot fresh weight was highest (3.46±0.01gm) in VI051062 (heat tolerant genotype) when proline applied foliarly @ 2.5mM while VI060131 (heat tolerant genotype) stood 2nd having shoot fresh weight of 3.35±0.01gm at same proline concentration. The lowest value of shoot fresh weight was observed in VI048596 (2.13±0.12gm) and in VI046554 (3.12±0.03) with control (Figure 4.3.2). Mean ± SE for effect of proline on shoot fresh weight of heat sensitive and heat tolerant okra genotypes are given in Appendix 4.

Table 4.3.1: Analysis of variance for effect of proline on shoot length, root length, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of heat tolerant and heat sensitive okra genotypes

Sr. No.	Parameter	SOV	Significance level (*P*-value)
1	Shoot length	Genotype (G)	**
		Treatment (T)	**
		G x T	*
2	Root length	Genotype (G)	**
		Treatment (T)	**
		G x T	**
3	Number of leaves	Genotype (G)	**
		Treatment (T)	**
		G x T	NS
4	Shoot fresh weight	Genotype (G)	**
		Treatment (T)	**
		G x T	**
5	Shoot dry weight	Genotype (G)	**
		Treatment (T)	**
		G x T	NS
6	Root fresh weight	Genotype (G)	**
		Treatment (T)	**
		G x T	**
7	Root dry weight	Genotype (G)	**
		Treatment (T)	**
		G x T	*

NS = non-significant ($p > 0.05$); * = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$)

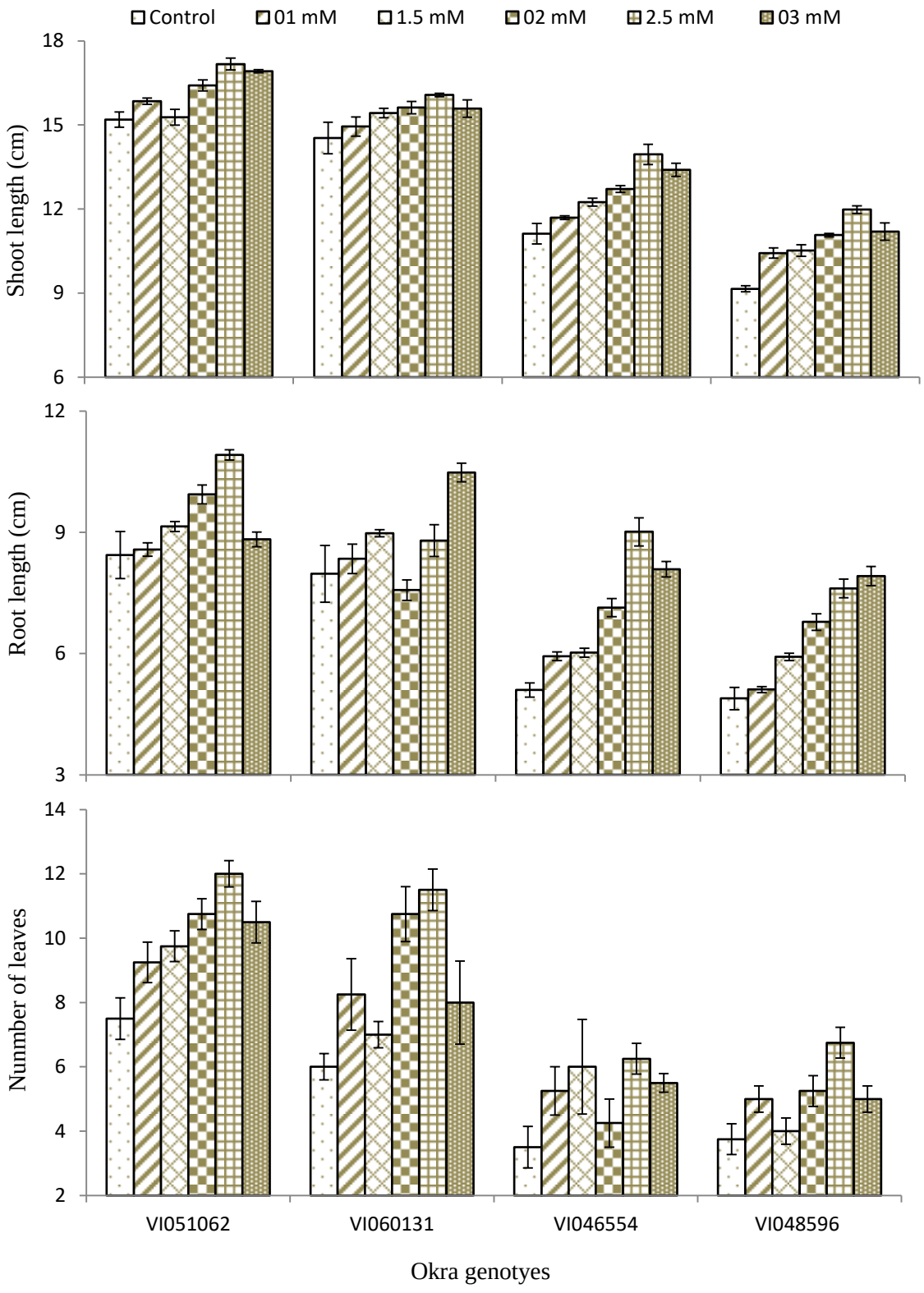


Figure 4.3.1: Effect of proline on shoot length, root length and number of leaves of heat tolerant and heat sensitive okra genotypes.

4.3.5. Effect of proline on shoot dry weight (g) of heat tolerant and heat sensitive okra genotypes

The foliar application of proline significantly ($p \leq 0.05$) enhanced the shoot dry weight as compared to control (Table 4.3.1). The interaction (G x T) of root length was non-significant. The results presented in figure 4.3.2 revealed that highest shoot dry weight (0.63±0.01gm) was recorded in VI051062, followed by VI060131 with shoot dry weight of 0.47±0.01 gm at the same proline concentration of 2.5 mM. The lowest shoot dry weight (0.2±0.01 gm) was calculated in VI048596 as well as in VI046554 having 0.20±0.02 gm shoot dry weight with foliar application of proline at 01 mM concentration. Mean ± SE for effect of proline on shoot dry weight of heat tolerant and heat sensitive okra genotypes are given in Appendix 5.

4.3.6. Effect of proline on root fresh weight (g) of heat tolerant and heat sensitive okra genotypes

Proline has ($p \leq 0.05$) significant effect on root fresh weight of okra genotypes compared tocontrol (Table 4.3.1). The interaction (G x T) for root fresh weight found to be highly significant. Results illustrated in figure 4.3.2 depicted higher root fresh weight (0.37±0.01gm) in genotype VI060131at proline level of 1.5 mM while VI051062 gave 0.32±0.01 gm root fresh weight with proline application @ 2.5 mM. The minimum root fresh weight (0.05±0.01 gm) was counted in VI048594 followed by VI046554 with root fresh weight of 0.17±0.01 when proline was applied foliarly @ 3 mM and 1 mM, respectively. Mean ± SE for effect of proline on root fresh weight of heat sensitive and heat tolerant okra genotypes are shown in Appendix 6.

4.3.7. Effect of proline on root dry weight (g) of heat tolerant and heat sensitive okra genotypes

It was observed that proline significantly ($p \leq 0.05$) increased the root dry weight of four okra genotypes in comparison with control (Table 4.3.1). The interaction (G x T) of root dry weight was significant. The results showed that cultivar VI051062 gave highest root dry weight (0.085±0.01 gm) @ 2.5 mM proline concentration while VI046554 genotype remained second with 0.0787±0.03 gm root dry weight at the same proline level. The minimum root fresh weight (0.0325±0.12 gm) was counted in genotype VI048594 and VI046554 when foliar application of proline was done @ 1 mM (Figure 4.3.2.).

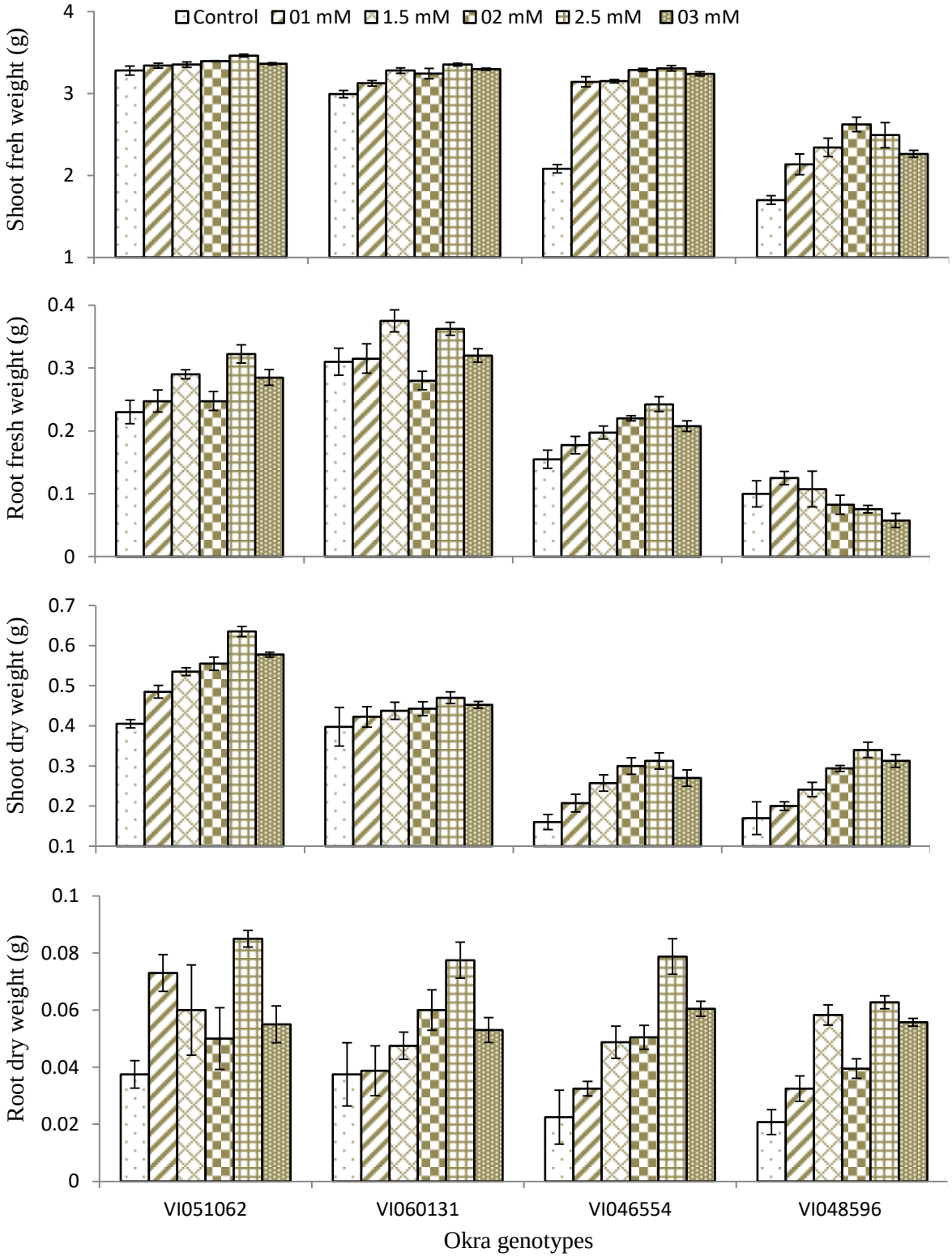


Figure 4.3.2: Effect of proline on shoot and root fresh weight, shoot and root dry weight of heat tolerant and heat sensitive okra genotypes.

Mean ± SE for effect of proline on root dry weight of heat sensitive and heat tolerant okra genotypes are presented in Appendix 7.

4.3.8. Effect of proline on leaf temperature (°C) of heat tolerant and heat sensitive okra genotypes

The foliar application of proline significantly ($p \leq 0.05$) lowered the leaf temperature of okra genotypes over control. The interaction (G x T) was observed to be highly significant (Table 4.3.2). The results presented in figure 4.3.3 showed that minimum leaf temperature (28.19±0.14 °C) by VI051062 genotype, followed by VI060131 with leaf temperature of 29.10±0.05°C when proline applied foliarly. In case of maximum leaf temperature VI048594 was observed with 33.43±0.08°C while VI046554 stood second having 33.39±0.06°C with proline application @ 1.5 mM and 01 mM respectively. Mean ± SE for effect of proline on leaf temperature of heat sensitive and heat tolerant okra genotypes are given in Appendix 8.

4.3.9. Effect of proline on sub-stomatal CO_2 (vpm) of heat tolerant and heat sensitive okra genotypes (vpm)

The sub-stomatal CO_2 significantly ($p \leq 0.05$) reduced with increasing proline concentrations as compared to control (Table (4.3.2). The interaction (G x T) was found highly significant. The results revealed that genotype VI051062 had lowest sub-stomatal CO_2 (953.5±34.5vpm) at the rate of 2.5 mM proline level and 1078.8±18.8 vpm sub-stomatal CO_2 was calculated in VI060131cultivar at the same proline concentration. The highest sub-stomatal CO_2 1793.0±31.0vpm was given by VI048596 genotype, followed by VI046554 with sub-stomatal CO_2 1681.5±37.7 vpm at the same rate of 1mM (Figure 4.3.3). Mean ± SE comparisons for effect of proline on sub-stomatal CO_2 of heat sensitive and heat tolerant okra genotypes are given in Appendix 9.

4.3.10. Effect of proline on stomatal conductance to water (mmol $m^{-2}s^{-1}$) of heat tolerant and heat sensitive okra genotypes

The exogenous application of proline significantly ($p \leq 0.05$) influenced stomatal conductance to water in okra genotypes (Table 4.3.2). The significant interaction (G x T) was noted in this case. It is clear from the figure 4.3.3 that (0.57±0.02 mmol $m^{-2}s^{-1}$) stomatal conductance to water was recorded in VI051062 genotype, followed by VI060131 with stomatal conductance (0.61±0.03 mmol $m^{-2}s^{-1}$) at 2.5mM proline level. On the other hand, the maximum stomatal conductance (1.89±0.03 mmol $m^{-2}s^{-1}$) recorded in VI048596 as well

as in VI046554 (1.80±0.01 mmol $m^{-2}s^{-1}$) at the proline level of 01 mM and 1.5 mM respectively. Mean ± SE for effect of proline on stomatal conductance of heat tolerant and heat sensitive okra genotypes are given in Appendix 10.

Table 4.3.2: Analysis of variance for effect of proline on leaf temperature, Stomatal conductance to CO_2 and H_2O, photosynthetic and transpiration rate, water use efficiency and chlorophyll contents of heat tolerant and heat sensitive okra genotypes

Sr. No.	Parameter	SOV	Significance level (*P*-value)
1	Leaf temperature	Genotype (G)	**
		Treatment (T)	**
		G x T	**
2	Stomatal conductance to CO_2	Genotype (G)	**
		Treatment (T)	**
		G x T	**
3	Stomatal conductance to water	Genotype (G)	**
		Treatment (T)	**
		G x T	*
4	Photosynthetic rate	Genotype (G)	**
		Treatment (T)	**
		G x T	**
5	Transpiration rate	Genotype (G)	**
		Treatment (T)	**
		G x T	**
6	Water use efficiency	Genotype (G)	**
		Treatment (T)	**
		G x T	**
7	Chlorophyll content	Genotype (G)	**
		Treatment (T)	**
		G x T	**

NS = non-significant ($p > 0.05$); * = significant ($p \leq 0.05$); ** = highly significant ($p \leq 0.01$)

4.3.11. Effect of proline on transpiration rate (mmol m^{-2} s^{-1}) of heat tolerant and heat sensitive okra genotypes

The results in table 4.4.2 showed that transpiration rate influenced significantly ($p \leq 0.05$) with foliar application of proline in all okra genotypes as compared to control. In case of transpiration rate highly significant interaction (G x T) was noted. The results given in figure 4.3.3 showed that minimum transpiration rate (3.65±0.22 mmol m^{-2} s^{-1}) was calculated in VI051062 and transpiration rate of 4.43±0.08 mmol m^{-2} s^{-1} was observed in VI046554 at the same proline level of 2.5 mM. Genotype VI048596 was found to having maximum transpiration rate 5.78±0.09 mmol m^{-2} s^{-1} at @ of 1.5 mM while second highest transpiration rate 5.51±0.17mmol m^{-2} s^{-1} was noted in VI046554 @ 01 mM. Mean ± SE for effect of proline on transpiration rate of heat sensitive and heat tolerant okra genotypes are expressed in Appendix 12.

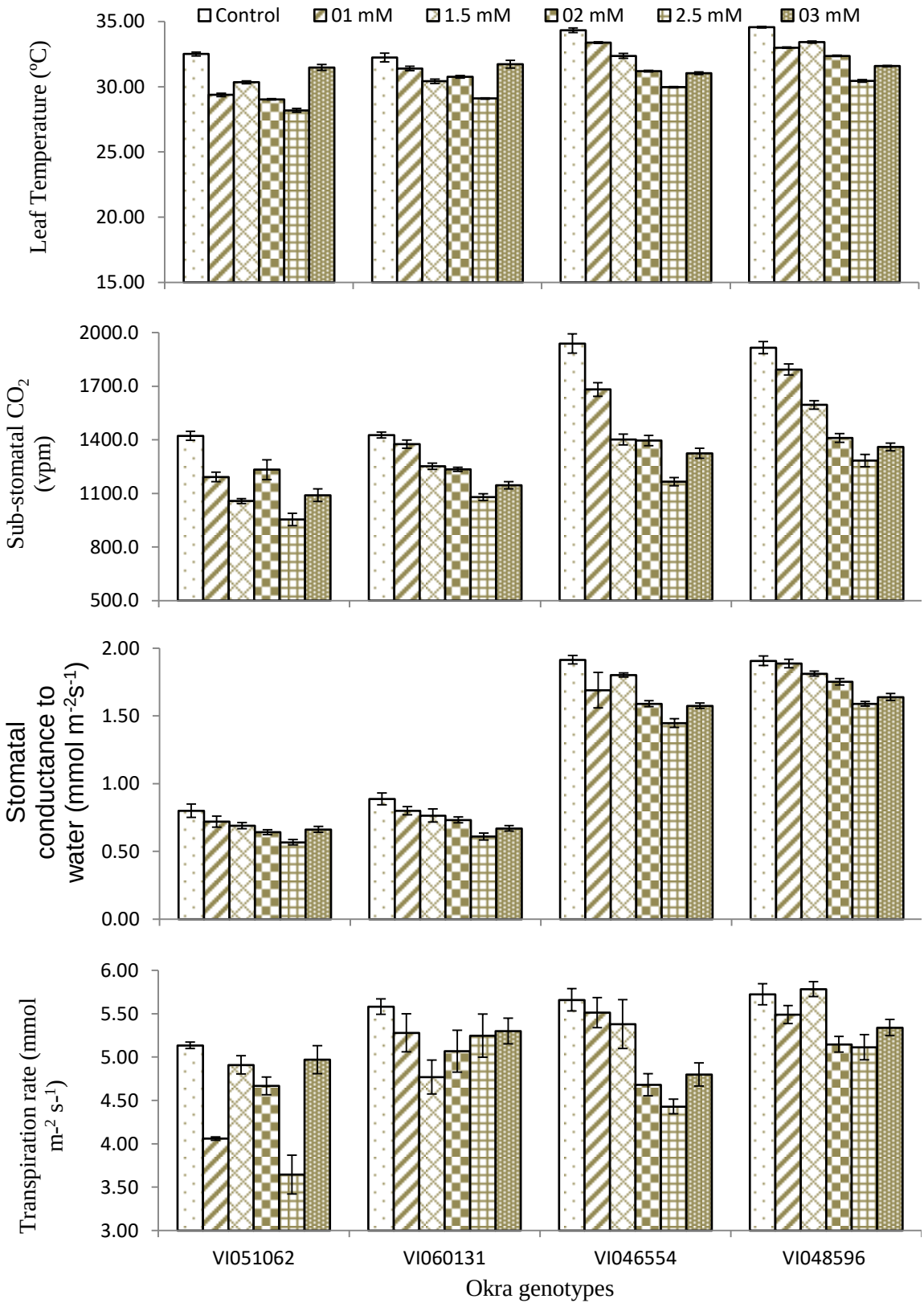


Figure 4.3.3: Effect of proline on leaf temperature, sub-stomatal conductance to CO_2 and H_2O and transpiration rate of heat tolerant and heat sensitive okra genotypes.

4.3.12. Effect of proline on photosynthetic rate (μmol m^{-2} s^{-1})of heat tolerant and heat sensitive okra genotypes

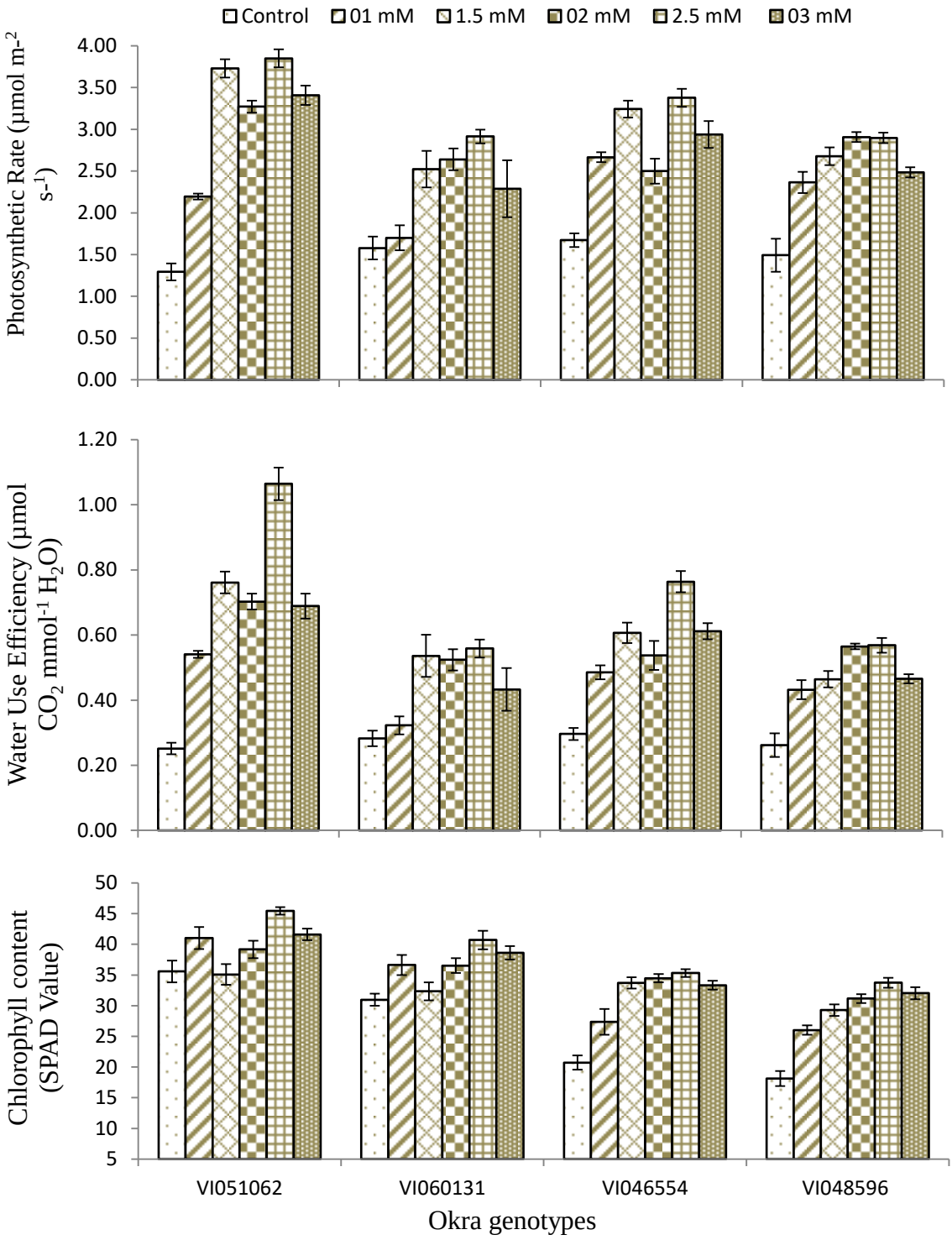
Photosynthetic rate of okra genotypes enhanced significantly ($p \leq 0.05$) with proline application. The interaction (G x T) found to be highly significant in this regard (Table 4.3.2). In accordance with the figure 4.3.4 the highest photosynthetic rate 3.85±0.11 μmol $m^{-2\ s-1}$ was found in VI051062 followed by VI046554 with photosynthetic rate of 3.38±0.11 μmol m^{-2} s^{-1} at 2.5mM concentration of proline. In case of lowest value VI060131was observed with photosynthetic rate of 1.70±0.15 μmol m^{-2} s^{-1} as well as VI051062 with photosynthetic rate of 2.20±0.04μmol $m^{-2\ s-1}$ at 1.5mM and 01mM respectively. Mean ± SE for effect of proline on photosynthetic rate of heat sensitive and heat tolerant okra genotypes are shown in Appendix 11.

4.3.13. Effect of proline on water use efficiency (μmol CO_2 $mmol^{-1}$ H_2O) of heat tolerant and heat sensitive okra genotypes

The foliar application of proline significantly ($p \leq 0.05$) improved water use in efficiency of okra genotypes (Table 4.3.2). The interaction (G x T) was highly significant terms of water use efficiency. The figure 4.3.4 showed that maximum water use efficiency (1.06±0.05 μmol CO_2 $mmol^{-1}$ H_2O) was calculated in VI051062 and 0.76±0.03 μmol CO_2 $mmol^{-1}$ H_2O in VI046554 at proline level of 2.5 mM. The lower water use efficiency 0.32±0.03 μmol CO_2 $mmol^{-1}$ H_2O and 0.43±0.03 μmol CO_2 $mmol^{-1}$ H_2O was observed in VI060131 and VI048594 at level of 01Mm. Mean ± SE for effect of proline on water use efficiency of heat sensitive and heat tolerant okra genotypes are presented in Appendix 13.

4.3.14. Effect of proline on chlorophyll contents (SPAD value) of heat tolerant and heat sensitive okra genotypes

Chlorophyll contents of okra genotypes increased significantly ($p \leq 0.05$) with foliar application of proline. The interaction (G x T) was observed to be highly significant in this regard (Table 4.3.2). The results showed that highest chlorophyll contents 45.425±0.61 were recorded in VI051062 with proline application @ 2.5 mM while VI060131 gave 40.7±0.51chlorophyll contents at same level of proline application. The lowest number of chlorophyll contents 26.025±0.75 and 27.352±2.09 were calculated by genotype VI048596 and VI046554 respectively (Figure 4.3.4). Mean ± SE for effect of proline on chlorophyll contents of heat sensitive and heat tolerant okra genotypes are indicated in Appendix 14.



4.3.4: Effect of proline on photosynthetic rate, water use efficiency and chlorophyll contents of heat tolerant and heat sensitive okra genotypes.

Experiment # 4

Four okra genotypes, two heat tolerant i.e. VI051062 and VI061131, while two heat sensitive i.e. VI046554 and VI048596, screened out in experiment # 2 were planted to check the effect of foliar application of proline dose (optimized in experiment # 3). The results for morphological, physiological, biochemical, water related and enzymztic attributes are given below.

4.4.1. Morphological traits

4.4.1.1. Effect of proline on shoot length (cm) of heat tolerant and heat sensitive okra genotypes

Shoot length was significantly influenced by the application of proline in okra under heat stress. All the genotypes responded well with respect to control (Table 4.4.1). Longest shoot length was recorded in VI051062, followed by VI061131, VI046554 and VI048596. Heat tolerant genotypes produce longer shoots as compared to heat sensitive okra genotype under both, proline application and control treatment as shown in figure 4.1.1. Similarly, in control treatment VI051062 and VI061131 and in proline application VI046554 were at par for shoot length.

4.4.1.2. Effect of proline on root length (cm) of heat tolerant and heat sensitive okra genotypes

Proline application significantly increased root length in okra genotype and its interaction with okra genotypes was observed as significant as shown in table 4.4.1. Results showed increased root length by proline application in VI051062 but statistically similar with its control treatment. Other all genotype was significantly different for root length under proline application and shown higher value as compared to control (Figure 4.4.1). Over all, heat tolerant okra genotypes (VI051062 and VI061131) were better to establish a deeper root than heat sensitive okra genotypes (VI046554 and VI048596).

4.4.1.3. Effect of proline on number of leaves per plant of heat tolerant and heat sensitive okra genotypes

In the current study exogenous proline application significantly enhanced number of leaves per plant and genotypes responded positively to produce leaves. Interaction effect of proline and genotype was significant as represented in of analysis of variance (ANOVA) in table 4.4.1. Highest number of leaves per plant was produced in heat tolerant okra genotypes

VI051062 and VI061131 (heat tolerant genotype) as shown in figure 4.1.1 and they were stastically at par. Heat sensitive okra genotypes (VI046554 and VI048596), followed the heat tolerant genotypes under both, proline application and control treatment. Overall, both heat tolerant and sensitive okra genotypes responded actively to proline application for enhancing the number of leaves per plant as compared to control treatment.

4.4.1.4. Effect of proline on shoot fresh weight (g) of heat tolerant and heat sensitive okra genotypes

The exogenous application of proline significantly (S ” 0.05) increased the shoot fresh weight in okra genotypes. Interaction effect of genotype and proline application was recorded as significant (Table 4.4.1). The highest shoot fresh weight was observed in VI051062, followed by VI061131 when proline was applied @ 2.5mM. The lowest shoot fresh weight was recorded in VI046554, followed the VI048596 heat sensitive genotype (Figure 4.4.2). In short, the proline application had more effect on heat tolerant genotypes as compared to heat sensitive genotypes for shoot fresh weight accumulation than in control treatment.

4.4.1.5. Effect of proline on shoot dry weight (g) of heat tolerant and heat sensitive okra genotypes

The exogenous application of proline significantly ($p \leq 0.05$) improved the shoot dry weight of okra genotypes. Interaction effect of genotype and proline was recorded as significant (Table 4.4.1). The results for shoot dry weight are shown in Figure 4.4.2. The results depicted that VI051062 gave the highest shoot dry weight followed by VI061131 when proline was applied @ 2.5mM. The lowest shoot dry weight was observed in VI048596 with no application of proline (control treatment, followed by the same genotype (Figure 4.4.2). In general proline application had more effect on heat tolerant genotypes as compared to heat sensitive genotypes for shoot dry weight accumulation than in control treatment.

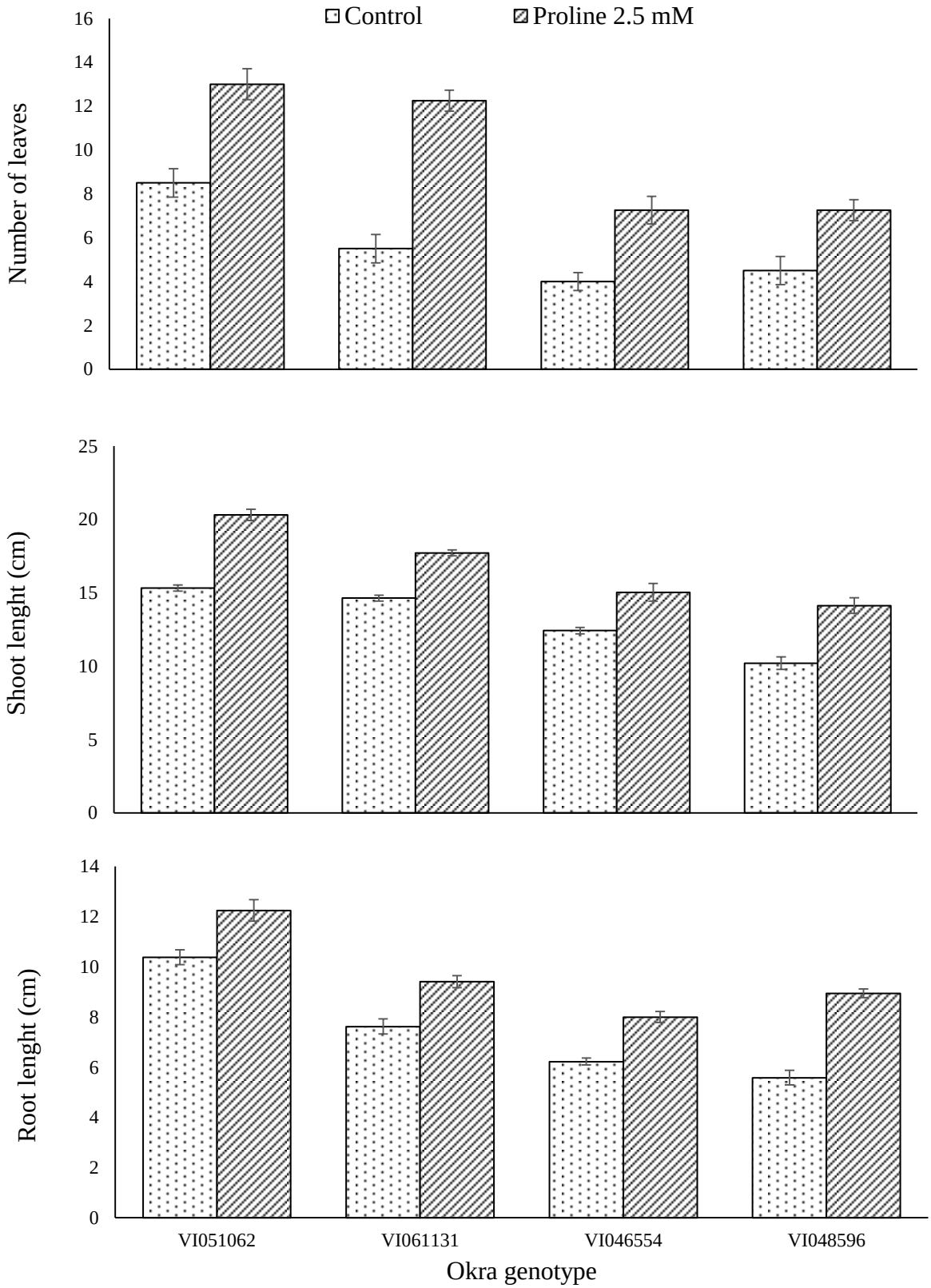


Figure 4.4.1. Effect of proline on number of leaves per plant, shoot length and root length of heat tolerant and heat sensitive okra genotypes.

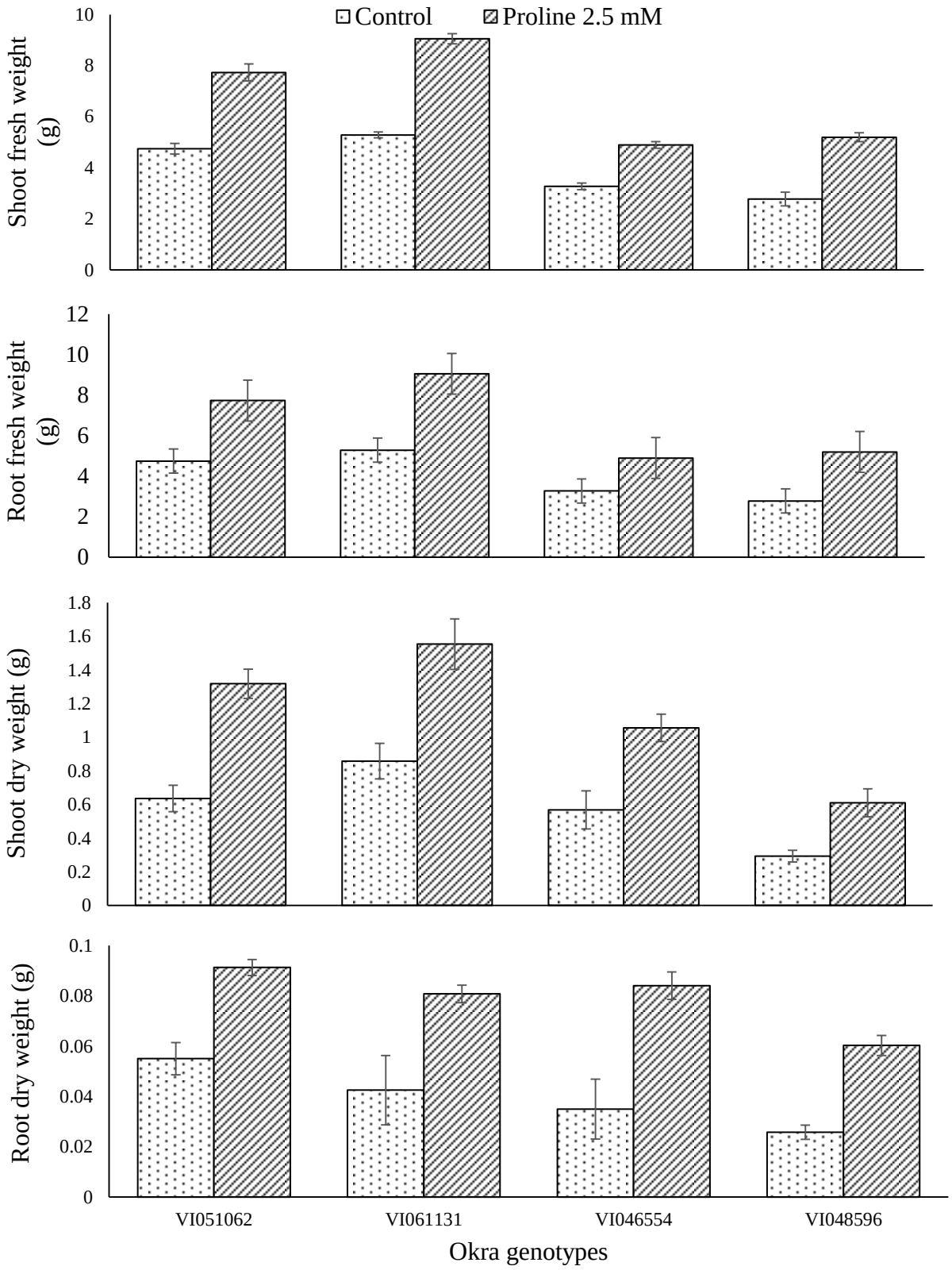


Figure 4.4.2. Effect of proline on shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of heat tolerant and heat sensitive okra genotypes.

4.4.1.6. Effect of proline on root fresh weight (g) of heat tolerant and heat sensitive okra genotypes

In this study, root fresh weight was significant between proline application and control treatment for all the genotypes as shown in analysis of variance (table 4.4.1). The interaction effect of proline and okra genotype was also significant (table 4.4.1). Results depicted that more root fresh weight was gathered by heat tolerant genotype VI051062, followed by VI061131, the same type of genotype. Lowest root fresh weight was gathered by VI048596 that was a heat sensitive genotype (Figure 4.4.2). In short exogenous proline application was effectively increased root fresh weight either heat tolerant or sensitive okra genotypes than control treatment. But its effect was more pronounced in heat tolerant genotype (VI051062 and VI061131) than heat sensitive okra genotype.

4.4.1.7. Effect of proline on root dry weight (g) of heat tolerant and heat sensitive okra genotypes

Root dry weight significantly varied with proline application and control treatment for all the genotypes as shown in analysis of variance (table 4.4.1). The interaction effect of proline and okra genotype was observed as non-significant. Results depicted that more dry weight was gathered by heat tolerant genotype VI051062, followed by VI061131 that was at par with VI046554. Lowest root dry weight was attained by VI048596 that was a heat sensitive genotype (Figure 4.4.2). Exogenous proline application effectively increased root dry weight in both heat tolerant or sensitive okra genotypes than control treatment (Figure 4.4.3).

Table. 4.4.1. Analysis of variance for the effect of proline on shoot length, root length, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of heat tolerant and heat sensitive okra genotypes

Serial no.	Parameter	Source of variance	Significance level ($p \leq 0.05$)
1	Number of leaves	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
2	Shoot fresh weight	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
3	Shoot dry weight	Proline	*
		Okra genotype	*
		Proline×okra genotypes	NS
4	Root fresh weight	Proline	*
		Okra genotypes	*
		Proline×okra genotype	*
5	Root dry weight	Proline	*
		Okra genotypes	*
		Proline×okra genotype	NS
6	Root length	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
7	Shoot length	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
* = Significant NS = Non-significant			

4.4.2. Physiological traits

4.4.2.1. Effect of proline on leaf temperature (°C) of heat tolerant and heat sensitive okra genotypes

Leaf temperature was significantly lowered by the application of proline on okra under heat stress. All the genotypes significantly lowered their leaf temperature with respect to control (table 4.4.2). Highest leaf temperature lowering was recorded in VI061131, followed by VI051062, VI046554 and VI048596. VI046554 showed highest leaf temperature but was at par with VI048596 under same control treatment as shown in figure 4.4.3. Similarly, in control treatment VI051062 and VI061131 and in Proline application were at par for leaf temperature.

4.4.2.2. Effect of proline on stomatal conductance to carbon dioxide (µmol m^{-2} s^{-1}) of heat tolerant and heat sensitive okra genotypes

Proline application significantly increased the stomatal conductance carbon dioxide (SSC) in both heat tolerant and heat sensitive okra genotypes. Interaction effect of genotype and proline was observed as non-significant as depicted in table 4.4.2. Results showed that there were more SSC were found in heat sensitive okra genotypes as compared to heat tolerant genotypes. All sensitive genotypes (VI046554 and VI048596) and heat tolerant genotype (VI051062 and VI061131) were mutually at par for SSC as depicted in figure 4.4.3.

4.4.2.3. Effect of proline on stomatal conductance to water (mmol m^{-2} s^{-1}) of heat tolerant and heat sensitive okra genotypes

Stomatal conductance to the water was significantly affected by proline application. Interaction of proline and genotype was observed as significant as represented in table 4.4.2. Results depicted that highest stomatal conductance to water was recorded in heat sensitive genotypes (VI046554 and VI048596) under control treatment as compared to proline application. Okra genotypes which were heat tolerant conducted low water through stomata as compared to sensitive okra genotypes. However, heat tolerant genotypes (VI051062 and VI061131) lost less water with proline (2.5 mM) application as compared to control (Figure 4.4.3).

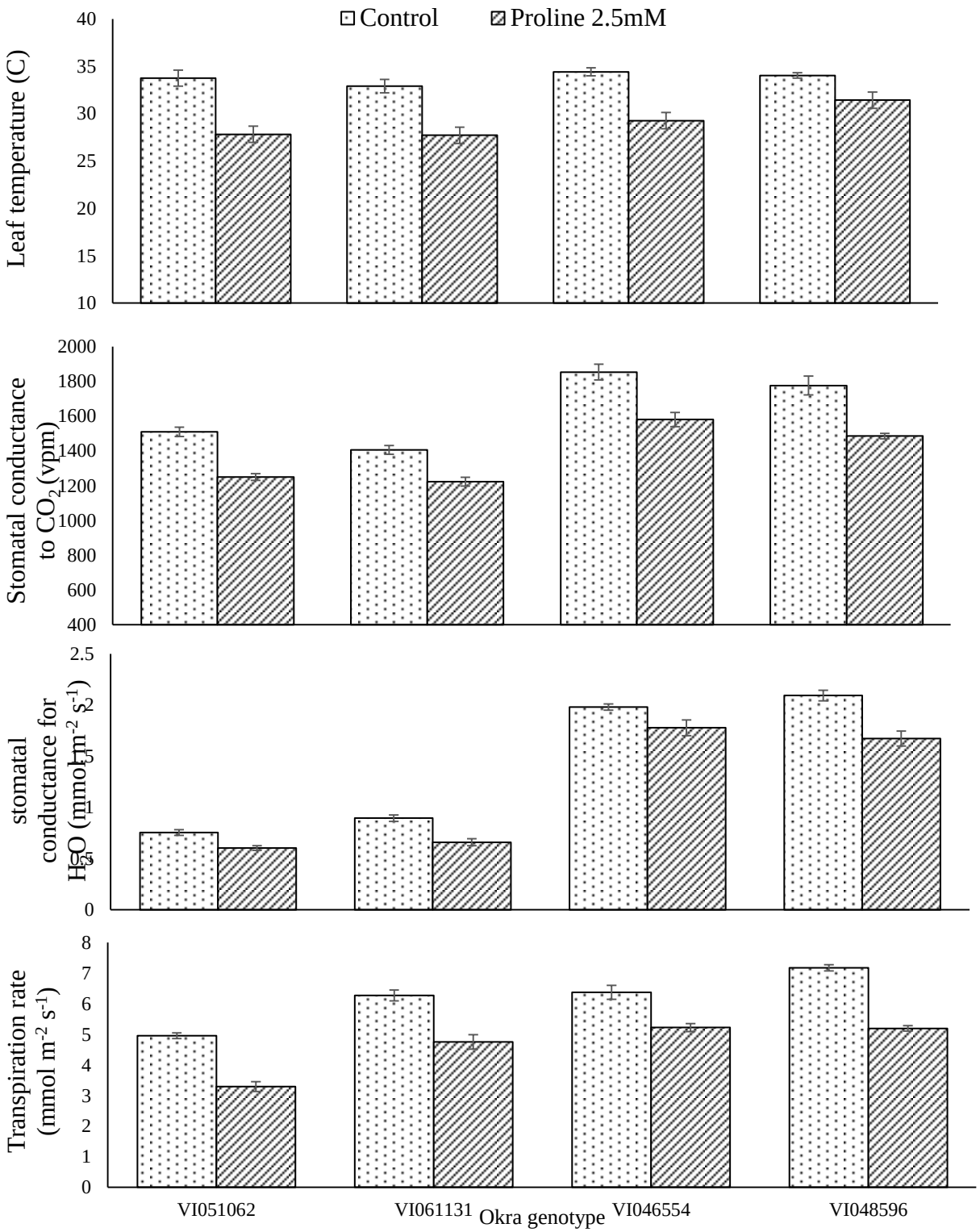


Figure 4.4.3. Effect of proline on leaf temperature, sub-stomatal conductance to CO_2, sub-stomatal conductance to H_2O and Transpiration rate of heat tolerant and heat sensitive genotypes

4.4.2.4. Effect of proline on transpiration rate (mmol m^{-2} s^{-1}) of heat tolerant and heat sensitive okra genotypes

Exogenous application of proline left a significant effect on transpiration rate and similarly all the okra genotype showed significantly variable response for transpiration rate. Interaction effect (Proline x Genotype) was observed as significant as shown in analysis of variance (Table 4.4.2). The highest transpiration rate was recorded in case of heat okra genotype (VI046554 and VI048596) in control as compared to proline application. Similarly, heat tolerant okra genotype showed low transpiration rate than sensitive genotype under proline application (Figure 4.4.3). Overall, proline application in all okra genotype decreased the transpiration rate with respect to their control.

4.4.2.5. Effect of proline on photosynthetic (µmol m^{-2} s^{-1}) rate of heat tolerant and heat sensitive okra genotypes

Proline application enhanced the photosynthesis in all the okra genotype as compared to control (table 4.4.2). Interaction effect of genotype and proline was significant. VI051062 (tolerant genotype) and VI046554 (sensitive genotype) showed maximum photosynthesis but these both were at par (Figure 4.4.4). Lowest photosynthesis rate was recorded in case of VI048596 under both proline and control condition (Figure 4.4.4.)

4.4.2.6. Effect of proline on water use efficiency (WUE) (µmol CO_2 $mmol^{-1}$ H_2O) of heat tolerant and heat sensitive okra genotypes

Proline application had a significant positive effect on WUE and okra genotypes were also significantly different for WUE as presented in table 4.4.2. Figure 4.4.4 showed that there was high WUE in heat tolerant okra genotype (VI051062 and VI061131) than heat sensitive genotypes. However the proline application and control treatment showed a huge difference both in heat tolerant and sensitive okra genotypes.

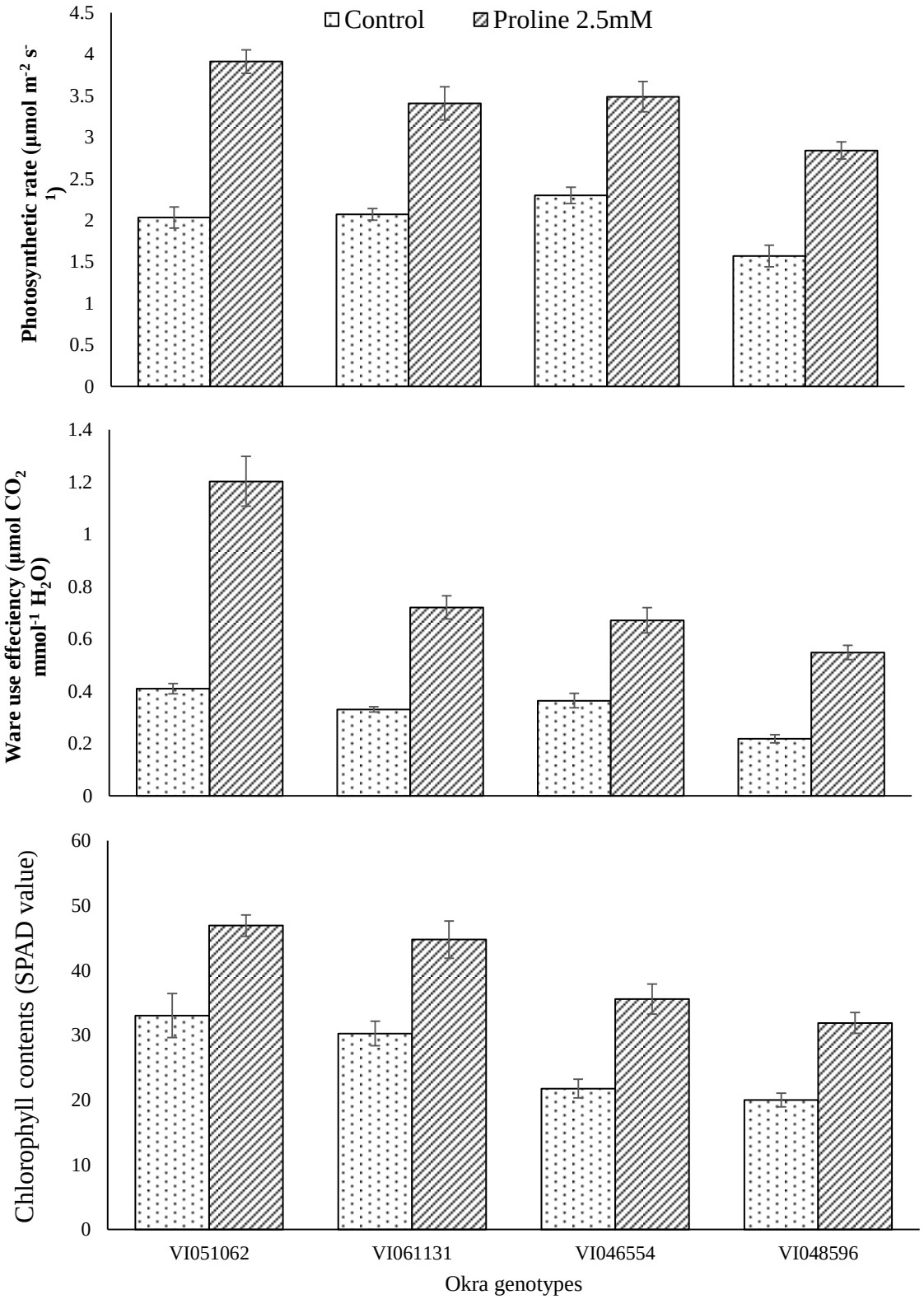


Figure 4.4.4: Effect of proline on photosynthetic rate, water use efficiency and chlorophyll contents of heat tolerant and heat sensitive okra genotypes.

4.4.2.7. Effect of proline on chlorophyll contents (SPAD value) of heat tolerant and heat sensitive okra genotypes

Table 4.4.2 showed that there was significant difference between proline treatment and control for all heat tolerant and sensitive okra genotypes. However, the interaction effect of genotype and proline application was observed as non-significant. Results for chlorophyll contents depicted that highest chlorophyll concents was recorded in VI051062 that was statistically at par with VI061131 under proline application rather control treatment. Heat sensitive genotypes (VI046554 and VI048596) were lagged by heat tolerant okra genotypes for chlorophyll contents. Overall, proline application enhanced chlorophyll production in heat tolerant as well as heat sensitive okra genotype as compared to control (Figure 4.4.4).

Table 4.4.2: Analysis of variance for the effect of proline on leaf temperature, photosynthetic rate, stomatal conductance to carbon dioxide, stomatal conductance to water and transpiration rate, water use efficiency and chlorophyll contents of heat tolerant and heat sensitive okra genotypes

Sr. #	Parameter	Source of variance	Significance level ($p \leq 0.05$)
1	Leaf temperature	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
2	Stomatal conductance for carbon dioxide	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	NS
3	Stomatal conductance for water	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
4	Transpiration rate	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	NS
5	Photosynthetic rate	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
6	Water use efficiency	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
7	Chlorophyll contents	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	NS

* = Significant NS = Non-significant

4.4.3. Biochemical traits

4.4.3.1. Effect of proline on glycine betaine (GB) (µmol g^{-1} F. wt.) contents of heat tolerant and heat sensitive okra genotypes

Application of proline significantly ($p \leq 0.05$) increased the glycine betaine (GB) contents of both heat tolerant and susceptible okra genotypes as compared to the control. Interaction effect of genotype and proline was significant (table 4.4.3). Highest GB contents were produced in VI061131 compared to control, while lowest GB contents were recorded in VI048596 (Figure 4.4.5) as compared to its control. Proline application under heat stress was useful for all the okra genotypes and increased the GB contents.

4.4.3.2. Effect of proline on proline contents (µmol g^{-1} F. wt.) of heat tolerant and heat sensitive okra genotypes

Exogenous application of proline significantly increased the leaf proline contents of okra genotype (Table 4.4.3). Genotypes VI051062 and VI061131 were at the top for proline contents followed by sensitive genotype VI046554 and VI048596 as represented in figure 4.4.5. While in the comparison of proline application (@ 2.5 mM) and control, all the genotypes showed higher proline contents but the extent was relatively more in heat tolerant genotypes as compared heat sensitive okra genotypes.

4.4.3.4. Effect of proline on carotenoid contents (mg g^{-1} F. wt.) of heat tolerant and heat sensitive okra genotypes

A significant difference among the proline treatment and control was recorded for carotinoids contents in all the genotypes (Table 4.4.3). Results in the figure 4.4.5 depicted that carotenoids contents were significantly variable ($p \leq 0.05$) among the okra genotypes and highest content (0.96) were produced in VI061131 that was statistically similar to (0.83) in genotype VI051062. In the same way VI046554 and VI048596 showed no mutual difference with carotenoid contents of 0.69 and 0.57, respectively. However, the interaction of genotype and proline was non-significant.

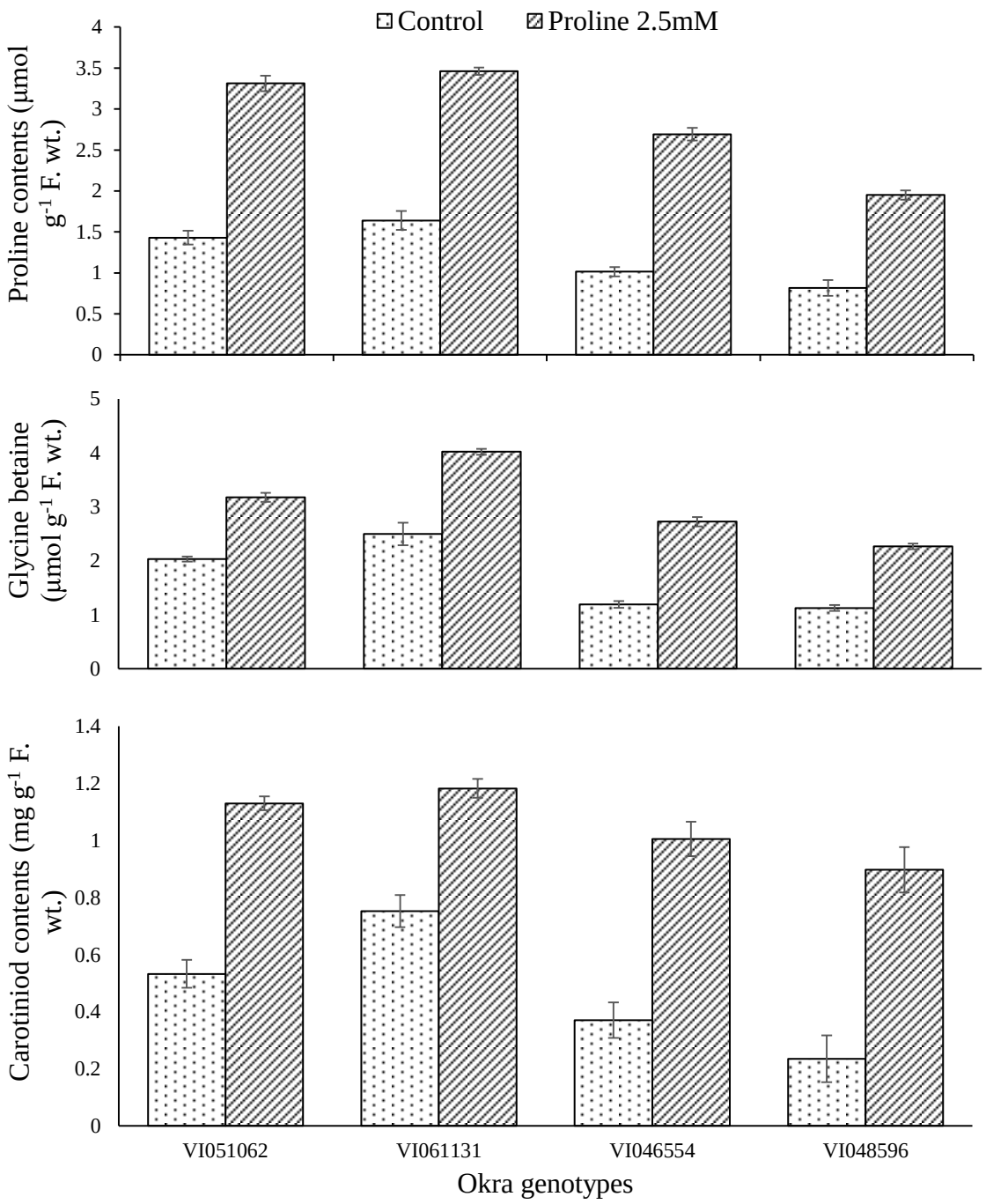


Figure 4.4.5. Effect of proline on proline contents, glycine betaine contents and carotenoid contents of heat tolerant and heat sensitive okra genotypes.

Table 4.4.3: Analysis of variance for the effect of proline on glycine betaine contents, proline contents, total free amino acids and carcinoid contents of heat tolerant and heat sensitive okra genotypes

S #	Parameter	Source of Variance	Significance level ($p \leq 0.05$)
1	Glycine betaine contents	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
2	Proline contents	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
4	Carotenoid contents	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	NS
* = Significant NS = Non-significant			

4.4.4. Water relation

4.4.4.1. Effect of proline on turgor potential (MPa) of heat tolerant and heat sensitive okra genotypes

Exogenous application of proline significantly affected on turgor potential and all the okra genotypes were significantly variable for turgor potential. Interaction effect (proline x genotype) was observed as significant as shown in table 4.4.4. It was observed that highest turgor potential was in heat tolerant okra genotypes (VI051062 and VI061131) under proline application as compared to control treatment. Moreover heat sensitive okra genotype showed low turgor potential than tolerant genotype but under proline application (Figure 4.4.6). Overall, proline application in all okra genotype increased the turgor potential with respect to their control.

4.4.4.2. Effect of proline on osmotic potential (-MPa) of heat tolerant and heat sensitive okra genotypes

Analysis of variance (table 4.4.4) showed that there was significant difference between proline treatment and control in all heat tolerant and sensitive okra genotypes for osmotic potential. However, the interaction effect of genotype and proline application was observed as non-significant. Results for osmotic potential depicted that highest osmotic potential was recorded in VI051062 that was, followed by VI061131, VI046554 and VI048596 which were statistically different with proline application as compared to their control treatment. Overall, proline application enhanced osmotic potential in heat tolerant as well as heat sensitive okra genotype as compared to control (Figure 4.4.6). But tolerant genotypes performed better than sensitive ones.

4.4.4.3. Effect of proline on relative water contents (%) of heat tolerant and heat sensitive okra genotypes

Analysis of variance (table 4.4) showed that there was significant difference between proline treatment and control in all heat tolerant and sensitive okra genotypes for relative water contents (RWC). However the interaction effect of genotype and proline application was observed as significant. Results for RWC depicted that highest relative values were recorded in VI051062 that was followed by VI061131 respectively. VI046554 and VI048596 were statistically at par under proline application rather control and their values were than the control treatment of VI046554 and VI048596. Heat sensitive genotypes (VI046554 and

VI048596) were lagged by heat tolerant okra genotypes for RWC. Over all proline application enhanced RWC in heat tolerant as well as heat sensitive okra genotype as compared to no proline application (Figure 4.4.6).

4.4.4.5. Effect of proline on water potential (-MPa) of heat tolerant and heat sensitive okra genotypes

The results for water potential indicated that proline application significantly ($p \leq 0.05$) enhanced water potential of okra genotypes (Table 4.4.4). The results for water potential in figure 4.4.6 depicted that VI051062 (heat tolerant genotype) showed the highest water potential and was followed by VI051062 and in heat sensitive genotype VI046554 followed the VI051062 and VI061131 in for water potential and was followed by VI048596 (heat sensitive genotype). Overall, proline application enhanced the water potential in both heat tolerant as well as heat sensitive okra genotypes as compared their respective control.

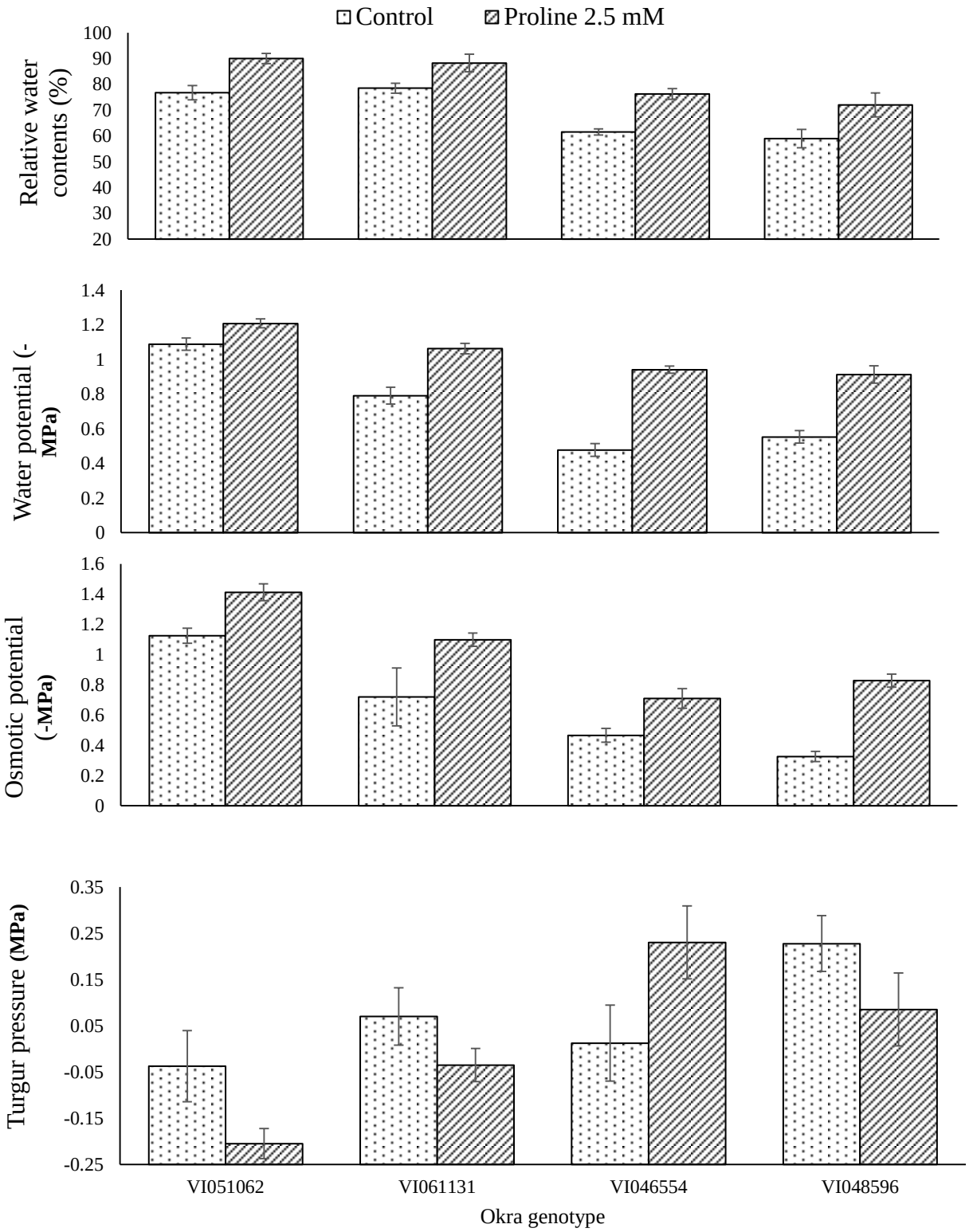


Figure4.4.6: Effect of proline on relative water contents, leaf water, osmotic potential and turgor pressure of heat tolerant and heat sensitive okra genotypes.

Table 4.4.4: Analysis of variance for the effect of proline on turgor potential, osmotic potential, water potential, and relative water contents of heat tolerant and heat sensitive okra genotypes

S #	Parameter	Source of variance	Significance level ($p \leq 0.05$)
1	Turgor potential	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
2	Osmotic potential	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	NS
3	Water potential	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
4	Relative water contents	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
* = Significant NS = Non-significant			

4.4.5. Enzymatic activities

4.4.5.1. Effect of proline on superoxide dismutase (SOD) (U mg^{-1} protein) concentration of heat tolerant and heat sensitive okra genotypes

The results for superoxide dismutase (SOD) indicated that proline application significantly ($p \leq 0.05$) enhanced its activity and genotypes and treatment interaction effect was also significant (Table 4.4.5). VI061131 (heat tolerant genotype) showed the highest SOD concentration and was at par with VI051062. In heat sensitive genotype VI046554 showed high SOD concentration compared to VI048596. Over all proline application enhanced the SOD concentration in both heat tolerant as well as heat sensitive okra genotype as compared their respective control (Figure 4.4.7).

4.4.5.2. Effect of proline on peroxidase (POD) (U mg^{-1} protein) concentration of heat tolerant and heat sensitive okra genotypes

Peroxidase (POD) concentration was significantly enhanced by proline application and okra genotypes behave differently. Interaction of proline and genotype was observed as significant (Table 4.4.5). Results depicted that highest POD concentration was recorded in heat tolerant genotypes (VI051062 and VI061131) under proline application as compared to their control (Figure 4.4.7). Okra genotypes which were heat sensitive produced low POD as compared to tolerant genotype under both with proline application and control treatment.

4.4.5.3. Effect of proline on catalase (CAT) (U mg^{-1} protein) concentration of heat tolerant and heat sensitive okra genotypes

Proline application significantly enhanced the catalase (CAT) concentration in heat tolerant as well as heat sensitive okra genotypes. The interaction effects of proline and okra genotype recorded as significant. Results showed that highest CAT concentration was obtained in heat tolerant okra genotype but VI051062 produced slightly more concentration than VI061131. These were, followed by VI046554 and VI048596 (heat sensitive genotype) however these heat sensitive genotypes were mutually at par. All the genotypes heat tolerant or heat sensitive produced relatively higher concentration of CAT with proline application than their respective control treatment (Figure 4.4.7).

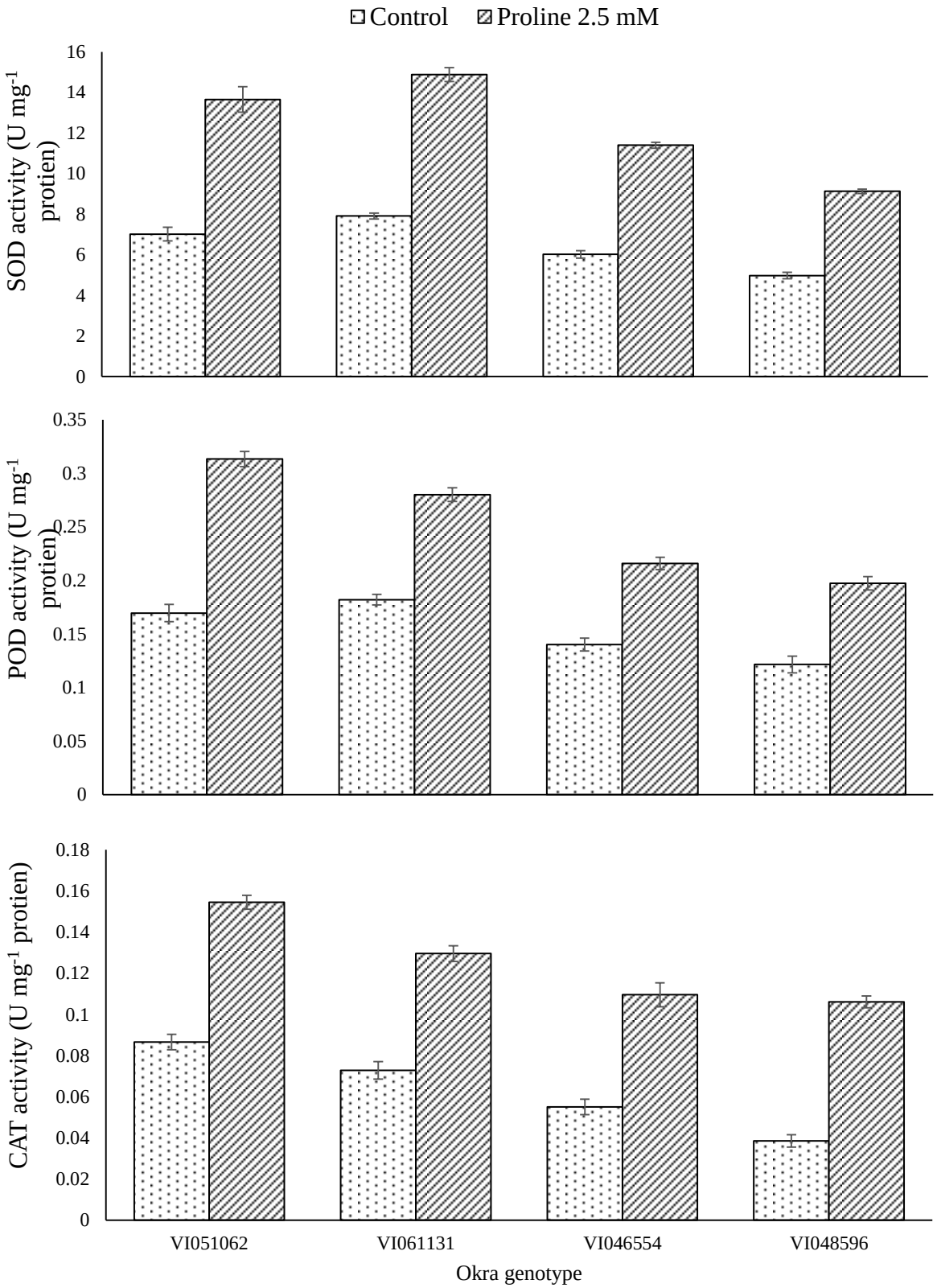


Figure 4.4.7: Effect of proline on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of heat tolerant and heat sensitive okra genotypes.

Table 4.4.5: Analysis of variance for the effect of proline on superoxide dismutase (SOD) contents, peroxidase (POD) contents and catalase (CAT) contents of heat tolerant and heat sensitive okra genotypes

Sr. #	Parameter	Source of Variance	Significance level ($p \leq 0.05$)
1	Superoxide dimutase	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
2	Peroxidase	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	*
3	Catalase	Proline	*
		Okra genotypes	*
		Proline×okra genotypes	NS
* = Significant NS = Non-significant			

CHAPTER 5 DISCUSSION

Experiment # I

Under stressful atmospheric conditions like drought, salt stress and heat stress (high temperature) there may be alterations in morphological, molecular, physiological and biochemical processes in plants (Wahid et al., 2007). In present study same results were found in okra genotypes under heat stress conditions. In current study okra genotypes showed different behavior for all parameters like morphological, physiological and biochemical under heat stress, which showed that potential of okra genotypes to tolerate heat stress, is genotype dependent. This is also confirmed by pervious reports in which they conducted same kinds of experiment in tomato under control environmental conditions (Ashraf and Harris, 2013). According to previous work on okra under salt stress conditions which confirm that genotypes may show different behavior under different environmental conditions (Shaheen et al., 2015). At different temperatures number of genotypes did not show positive growth response compared to others (Ali et al., 2014). A prior study in summer vegetables gave maximum temperature range for different growth stages for germination, seedling growth, fruit set and fruit color development (Naika et al., 2005).

Significant difference was observed in genotypes response to elevated temperature in the present study. In this experiment growth attributes showed significant varied response to elevated temperature in different genotypes. With vigorous growth under raised temperature conditions, tolerant genotypes showed their ability to withstand under adverse environmental conditions compared to sensitive ones with significantly less growth reduction. Shoot length showed positive significant correlation with root length, root dry weight, shoot fresh weight, shoot dry weight and transpiration; which proved that growth attributes are interconnected parameters (Saleem et al., 2011). Under stress environmental conditions okra genotypes are able to give sustainable yield due to their higher ability of net carbon assimilation (Gunawardhana, and Silva 2011). In case of root length it was observed that root length has positive correlation with shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and number of leaves. Long and ramified root system provides an array of network for uptake of nutrients and water from soil to produce high biomass through enhanced photosynthetic rate. This is in agreement with the findings of former reports (Jaleel et al., 2009). It is also reported that at young seedling stage plants are more prone to abiotic stresses

than fully grown or on later growth stages. Some reports suggested that plant leaves are more affected by high temperature at early growth stage than mature leaf stage; leaf ability to tolerate heat stress at early stage is less as compared to old stage of plant life (Chen et al., 1982). Temperature effects the growth and development of plants. Plant processes like enzyme activities, transpiration rate and photosynthetic rate are temperature dependent (Porter and Gawith 1999). Reduction of enzyme activity and modifications in chloroplast protein complexes listed as early effects of high temperature on plants (Ahmad et al., 2009). High temperature causes injuries to cellular parts like microtubules organization, cytoskeleton and cell membrane. It alters membrane permeability, elongation, cell expansion and cell differentiation (Bita and Gerats 2013). Shoot fresh weight has highly positive significant correlation with root fresh weight, root dry weight and shoot dry weight and positive correlation with number of leaves and transpiration rate. Higher photosynthetic rate improves all growth parameters of okra seedling under heat stress due to high carbon assimilation; all this is due to higher number of leaves. Findings are in line with the previous studies in which summer vegetable (tomato) seedlings were grown under elevated temperature (Ashraf and Harris, 2013). Shoot dry weight had positive significant positive correlation with root dry weight and number of leaves. Root fresh weight and root dry weight are also positively correlated with root dry weight, shoot fresh weight, shoot dry weight and number of leaves. In case of number of leaves; it has significant positive correlation with shoot length, root length, shoot and root fresh weight, shoot and root dry weight. Positive correlation of number of leaves with other growth attributes means higher photosynthetic rate and higher photosynthetic rate means enhanced growth and biomass production. Results are confirmed by previous reports (Nkansah and Ito 1994).

Chlorophyll contents showed positive significant correlation with water use efficiency and positive correlation with photosynthetic rate. Significant variation was observed in chlorophyll contents of heat tolerant and heat sensitive genotypes. This may be due to transformation of microscopic structures under elevated temperatures. This structural transformation was more in sensitive genotypes and tolerant genotypes which showed resistance to such transformations in microscopic strctures (Ashraf and Arfan 2005; Semenova 2004; Kreslavski et al., 2008). Positive correlation with photosynthetic rate showed that greater number of chlorophyll contents, greater the photosynthetic rate; higher

photosynthetic rate lead to vigorous growth and ultimately increase ability to with stand odd environmental conditions (Shaheen et al., 2015)

In all green plants the most fundamental and complicated physiological process is photosynthesis and all of its components are sensitive to stress conditions such as photosynthetic pigments, electron transport chain, carbon dioxide reduction pathways and photosystems; any type of stress at any stage of life affects overall photosynthetic efficiency of green plants (Ashraf and Harris, 2013). In present study the photosynthetic rate varied significantly in different genotypes. There was a positive correlation of photosynthetic rate with chlorophyll contents, shoot length and transpiration rate and highly negative significant correlation with sub-stomatal carbon dioxide. Under abiotic stress environments photosynthetic pigments degradation occur e.g. under salt stress condition breakdown of chlorophyll pigments occur. Previous reports showed plants exposed to high temperature showed reduced chlorophyll contents biosynthesis (Reda et al., 2011). All these evidences of modification in photosynthetic apparatus showed any damage to above mentioned photosynthetic processes ultimately reduces the plant growth and hinders the tolerance against stress condition. Positive correlation of chlorophyll contents with photosynthetic rate showed that in sensitive genotypes there was greater damage to chlorophyll than tolerant ones. This is in agreement with previous studies where they reported that in leaf tissues high temperature reduces the solubility of CO_2 relative to O_2 as a result availability of CO_2 for substrate reduces (Li et al., 2007). In case of transpiration rate it was significant negatively correlated with water use efficiency and positive significant correlation observed between leaf temperature and transpiration rate. In the past studies it has been reported that in rice seedlings there was greater biomass production due to high water use efficiency and reduced transpiration rate, ultimately higher photosynthetic rate (Karaba et al 2007). In present study the genotypes with higher transpiration rate showed less water use efficiency, hence proved sensitive genotypes. Tolerant genotypes exhibit less transpiration and greater water use efficiency resultantly higher photosynthetic rate and biomass production and withstand under stress conditions. Organ dehydration and restricted growth occured due to excessive loss of water from plants through transpiration. Osmotic stress is caused due to reduced tissue water status and root hydraulic conductance under heat stress conditions (Shaheen et al., 2015). Disturbance in the uptake and translocation of ions, organic solutes and water and restriction

in respiration and photosynthetic rate, increment in evapotranspiration, chlorophyll fluorescence increased and decrease the leaf osmotic potential are all outcomes of heat stress (Huve et al., 2005; Taiz and Zeiger 2006), stomatal closure and reduced tissue water occur due to all the above described factors (Wahid et al., 2007). In present study there was a highly negative significant correlation between transpiration and water use efficiency. High transpiration rate evaporates more water from plant body resulting in less water use efficiency.

Leaf temperature is an important parameter in physiological life of crop plants. It directly affects photosynthesis and water use efficiency; ultimately controls all growth stages (Brooks and Farquhar 1985). In this experiment genotypes varied significantly in leaf temperature. Leaf temperature showed highly negative significant correlation with photosynthetic rate and water use efficiency and significant positive correlation with transpiration and negative correlation with growth attributes like shoot length, root length, shoot and root fresh weight, shoot dry weight and chlorophyll contents was observed. Beyond the optimum limit leaf temperature inhibits the photosynthetic rate by stimulating photorespiration and cause damages to photosynthetic apparatus (Schrader et al., 2004). Rubisco activity is reduced at moderate elevation in leaf temperature resultantly reduce photosynthetic rate (Salvucci et al., 2001). All these studies showed that photosynthesis and water use efficiency are leaf temperature dependent attributes. High leaf temperature means less photosynthesis and water use efficiency, ultimately reduced growth and biomass production.

Experiment # 2

Twenty five okra genotypes (twenty heat tolerant, while five heat sensitive genotypes screened out from experiment # 1) were sown in field at vegetable research area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. There were three sowing dates (02 April, 12 April and 22 April) to check the effect of heat stress on different morphological, physiological and yield attributes. All the cultural practices were kept same for all sowing dates and for all genotypes. Experiment was replicated four times and there were five plants per replication. The data for morphological, physiological and yield attributes was recorded. The results obtained are discussed here.

Okra fruit yield, seed yield and quality are considerably affected by sowing. Every

cultivar requires different climatic conditions as well as proper sowing time to produce higher yield (Ghannad et al., 2014). In currentstudy sowing date significantly affected the number of plant survived. Maximum number of plants survived on 1st sowing date i.e. 02, April while minimum number of plants survived on 2nd sowing date i.e. 12, April. Possible reason for number of plant survival is the temperature at germination time. It is reported that germination is the most sensitive stage prone to heat stress. If there is improper temperature at germination stage, it exerts negative impacts on germination resulting in abnormal seedling growth, reduced germination percentage and poor seedling vigor to withstand surrounding environmental conditions (Toh et al., 2008; Kumar et al., 2011; Johkan et al., 2011; Hasanuzzaman et al., 2013). Results are similar with Dilruba et al., (2009) who found the highest performance of okra cultivar grown at different sowing date but 06, April proved to be best sowing time which is nearest to current 1st sowing date. In the present study there was highly significant interaction between sowing date and genotypes in case of number of leaves, leaf area and plant height. Maximum plant height was recorded in VI051062 (150cm) and VI060131 (148cm) in plants sown on 02, April, minimum was recorded in VI048596 (80cm) and VI046554 (85cm) on third sowing date i.e. 22, April. Maximum number of leaves was counted in heat tolerant genotype VI060131 in 1st sowing date and minimum number of leaves (11) was counted in heat sensitive genotype VI048596 on 2nd sowing date. Maximum leaf area (500 cm2) was computed in genotype VI051062 on 1st sowing date and minimum leaf area (300cm2) was computed in genotype VI042596 on 2nd sowing date. In this case 02, April seems to be appropriate sowing date because at this sowing date the growth was fast in case of plant height, number of leaves and leaf area. Possible reason could be the proper growing environment for the crop. 2nd April sowing date could be optimum for plants to use natural resources, water and nutrients etc. These results are at par with the Muhammad et al. (2001); Dash et al. (2013); Chattopadhyay et al. (2011). All these reported that plant growth significantly varied by sowing dates. There were more number of leaves and leaf area in case of 1st sowing date. More number of leaves and more leaf area mean more net photosynthetic rate which results in higher carbon assimilation which improves the growth attributes. Results are at par with the findings of (Shaheen et al., 2015).

Sowing dates significantly affected number of pods per plant. Highest number of pods per plant (40) was recorded in 1st sowing date (02 April) by genotype VI051062.

VI046554 gave lowest (12) number of pods per plant in 3rd sowing date (22 April). Tolerant genotypes showed better response in all sowing dates having highest number of pods per plant while sensitive genotypes had minimum number of pods per plant in all sowing dates. The findings are at par with (Al-Harbi, 2008; Asadipour and Hamid 2014) where they found the effect of temperature on the pod production in okra. Dilruba, (2009) also supported results that this trait is highly depends upon the sowing time. According to Wahid et al. (2007) high temperature affects the plant differently at different growth stages. Reproductive stage is highly sensitive to temperature stress and ultimately reduction in crop yield occurs due to adverse effects of high temperature on fertilization and post fertilization processes. This is the reason behind lower pod production in later sowing dates as temperature increases gradually in summer season.

Heat stress significantly affects the pod length and pod diameter under all three sowing dates. The maximum pod length (16cm) was calculated in genotype VI060131 in sowing date 1 (02 April). On the other hand minimum pod length (04cm) was noted in VI046554 genotype in sowing date 3 (22 April). Genotype VI051062 and VI060131 were found to be having highest pod diameter (02cm, 1.90cm), respectively in sowing date of 1 (02 April). The least pod diameter (1.07cm) was observed in VI048596 as well as in VI046554 (1.10cm) at third sowing date (22 April). Results are supported by the findings of Ezeakunne (2004) where he states that yield related attributes like pod size, pod length, number of pod per plant and pod weight are higher when crop is sown earlier. Similar findings were recorded when okra was sown on 02 April instead of 12 or 22, April. Hussain, (2006) also had similar findings when he sow okra on two different sowing times.

In case of leaf gaseous exchange parameters maximum photosynthetic rate (10.78) was noted in VI051062 sowing date of one (02 April). VI046554 gave lowest photosynthetic rate (1.25) at 3rd sowing date (22, April). Lowest transpiration rate (3.22) was recorded in genotype VI060131 at 1st sowing date (02 April), while genotype VI046554 expressed highest transpiration rate (7.37) at sowing date 3rd (22 April). The maximum water use efficiency was noted in VI051062 (3.30) and VI060131 (2.87) at sowing date one (02 April). In contrast, genotype VI046554 had lowest water use efficiency (0.17) at 3rd sowing date. At sowing date one (02 April) lowest temperature (29.59) was noted in genotype VI060131, followed by VI051062 with leaf temperature (30.30) at same sowing date. VI048596

genotype showed maximum leaf temperature (36.12) at third sowing date (22 April). The genotype VI051062 was at top with lowest sub-stomatal CO_2 (168.25) on 1st sowing date (02 April). Highest sub-stomatal CO_2 (523.25) was recorded genotype VI050150 at same sowing date 02, April. The 2nd sowing date efficiently improved genotype VI051062 with maximum chlorophyll contents (72.58), followed by chlorophyll contents (62.63) in genotype VI041763 at the same sowing date. The lowest chlorophyll contents (15.93) (18.15) were found in genotype VI046554 at sowing date of 3rd and 2nd respectively.

Genotypes performing well for growth parameters also showed good results in case of physiological parameters. Genotypes with higher number of leaves, leaf area and higher plant heights showed higher photosynthetic rate. The possible reason could be the availability of leaf area for capturing more sun light for photosynthesis ultimately more carbon assimilates for growth and production. These results are confirmed by (Amjad, 2001; Ekwu and Nwokwu, 2012 and Asadipour and Hamid 2014). Furthermore, genotypes having less leaf temperature and higher chlorophyll contents showed higher photosynthetic rate. This can be supported by Shaheen et al. (2015) where he described that at low leaf temperature and higher chlorophyll contents enhanced the photosynthetic activity of heat sensitive and heat tolerant tomato genotypes. In current bstudy heat tolerant genotypes produced more chlorophyll contents as compared to sensitive ones, confirmed by Shaheen et al. (2015). Possible reason could be transformation of microscopic structures under higher temperature stress conditions. This change might be more in sensitive genotypes as compared to tolerant ones because tolerant genotypes had ability to withstand such transformations under temperature stress conditions (Kreslavski et al., 2008; Semenova 2004). Balouchi (2010), Reda and Mandoura (2011) reported that plants exposed to heat stress showed reduced biosynthesis of chlorophyll contents.

The minimum electrolyte leakage (63.70 %) was found at sowing date 1 (02 April) in VI051062 genotype. On the other hand, at same sowing date (02 April) the heat sensitive genotype VI048596 gave highest electrolyte leakage (98.02). Electrolyte leakage was more pronounced in heat sensitive genotypes than heat tolerant genotypes. Camejo et al. (2005) reported the same results in tomato genotypes under heat stress conditions. Chemical bonds within the biological membranes were loosening due to heat stress which accelerates the kinetic energy and movement of molecules across membranes (Savchenko et al., 2002).

Increase in electrolyte leakage is direct indication of decreased membrane thermostability which can be used as direct measurement tool for high temperature stress tolerance in variety of plants including potato and tomato (Chen et al., 1982), cowpea (Ismail and Hall, 1999), cotton (Ashraf et al., 1994), soybean (Martineau et al., 1979), barley (Wahid and Shabbir, 2005) and wheat (Blum, 1988).

Experiment # 3

Four okra genotypes, two tolerant (VI051062 and VI060131) and two sensitive (VI046554 and VI048594) screened out from a large number of okra genotypes in experiment # 2 were exposed to heat stress (45/35°C day/night temperature). One week after exposure to heat stress under controlled environmental conditions plants were sprayed with proline (0.00, 1.0, 1.5, 2.0, 2.5 and 3.0mM) to optimize the best dose of proline for enhancing the heat tolerance potential of okra genotypes. Following morphological parameters (shoot length, root length, numbers of leaves, shoot fresh weight root fresh weight, shoot dry weight and root dry weight), physiological attributes (photosynthetic rate, transpiration rate, sub-stomatal to CO_2, sub-stomatal to water, leaf surface temperature, water use efficiency and chlorophyll contents) were studied to optimize the best dose of proline for enhancement of heat tolerance potential of okra genotypes.

The result showed that exogenous application of proline significantly increases the shoot length of okra genotypes. Results were more prominent in heat tolerant genotypes than the sensitive ones. Tolerant genotype VI051062 gives the highest shoot length at 2.5 mM proline concentration, followed by VI060131 at the same proline level. The lowest shoot length was noticed in heat sensitive genotype VI060131 at 01 mM proline application, followed by VI046554 at the same proline concentration. The results are in line with the previous reports where exogenous application of proline under abiotic stress condition enhanced the growth parameters like root length, shoot length, shoot and root fresh and dry mass and leaf area (Wani et al., 2016). Under stressful environment, changes in growth patterns are the best measurement of plant performance (Vollenweider and Gunthardt-Goerg 2005). Previous reports explain the role of exogenous applied proline in enhancing stress tolerance in plants. This role may be as cryoprotection (Songstad et al., 1990; Santarius, 1992) or osmoprotection (Jones and Gorham, 1983; Handa et al., 1986). Exogenous application of proline @ 30 mM at early growth stages alleviates the drastic effects of abiotic

stresses in rice plants (Roy et al., 1993).

The findings of current experiment showed that proline @ 2.5 mM gave the highest root length in heat tolerant genotype VI051062, followed by heat tolerant genotype VI060131 at proline concentration of 03 mM. Minimum root length was recorded in heat sensitive genotype VI048596 at proline level of 01 mM while genotype VI046554 gave minimum root length at same proline concentration. The heat tolerant genotypes responded well to proline application mean exogenously applied proline enhanced the stress tolerance in okra genotypes. During abiotic stress conditions reduced plant growth and reduced photosynthesis was observed by Chen et al. (2009). When there was enhancement in stress tolerance due to proline application there could be possible reason for shoot and root length increase that proline increase the photosynthetic ability of plant. More photosynthetic rate means more assimilation of carbohydrates; more carbohydrates production ultimately increases the growth and development. This is in agreement with the finding of Wani et al., (2016); Arbona et al. (2005).

In present study maximum number of leaves (12.00±0.40) was found in heat tolerant genotype VI051062 with the application of 2.5 mM proline, followed by VI060131 stood second with 11.50±0.64 number of leaves at same concentration. Highest shoot fresh weight and dry weight was found in genotype VI051062, followed by genotype VI060131 at proline concentration of 2.5 mM. Highest root dry weight recorded in genotype VI051062, followed by VI046554 at proline level of 2.5 mM. In case of chlorophyll contents highest chlorophyll contents were recorded in VI051062, followed by VI060131at proline level of 2.5. Highest photosynthesis was recorded in VI051062, followed by VI046554 at 2.5 mM proline application lowest was found in VI060131 at 1.5 mM proline application. These results are similar to findings that exogenous proline application stimulate cell growth Kumar and Sharma(1989), plant growth (Fedina 1993; Hamed and Wakeel 1994) and improve plant metabolic activity under stress environment (Alia et al., 1991; Rana and Rana 1996). Higher number of leaves means higher photosynthesis. Further the photosynthesis under stress environment is enhanced with the application of proline this in agreement with (Shahbaz et al., 2013).

Heat stress is a major limiting factor for plant growth and final yield. Due to high temperature sunburns on leaves, branches, stem, scorching of leaves and twigs, reduction in

root and shoot growth and reduced yield are considered as pre and post-harvest damages (Vollenweider and GunthardtGoerg, 2005). Heat stress is important factor for reduced dry matter production and yield.

The primary sites for heat stress injuries are the photochemical reactions in thylakoid lamellae and carbon metabolism in the chloroplast (Wise et al., 2004). Any change in photosynthetic apparatus is a good indicator of heat stress and there is a strong correlation of it with plant growth and yield (Wahid et al., 2007). Chlorophyll contents also have strong positive significant correlation with photosynthetic rate (Shaheen et al., 2015). In this study chlorophyll varied significantly among genotypes under heat stress environment. Heat exposure might have transformed microscopic structures (Semenova, 2004; Kreslavski et al., 2008). Tolerant genotypes withstand against such type of transformation and produced higher amount of chlorophyll contents as compared to sensitive genotypes. This concedes with the previous reports (Shaheen et al., 2015). Now it can be concluded that in heat tolerant genotypes showed increase in heat tolerance potential with proline application through the enhanced photosynthetic rate and chlorophyll production. Ultimately there was increase in seedlings growth and biomass production. Same was reported by Chrominski et al., (1989).

Minimum leaf temperature (28.19±0.14 °C) was recorded by VI051062 genotype, followed by VI060131 with leaf temperature of 29.10±0.05°C when foliar application of proline @ 2.5 mM was done. Maximum leaf temperature was found in VI048594 which was 33.43±0.08°C and in genotype VI046554 which was 33.39±0.06°C when proline was applied @ 1.5mM and 01mM respectively. Same trend was observed in case of transpiration rate, it was minimum in VI051062 and VI060131 and maximum in VI048594 and VI046554. In case of photosynthetic rate and water use efficiency maximum were recorded in VI051062 and VI060131 with proline application @ 2.5 mM. Minimum was recorded in heat sensitive genotypes VI048594 and VI046554 at proline concentration of 01 mM.

In heat stress studies leaf temperature is of great significance. It represents the ability of genotypes to sustain optimum temperature which is required for normal metabolic process (Shaheen et al., 2015). Thermotolerance adjustments of photo system II is influenced by photosynthetic photon flux densities and leaf temperature. It gives the indication of optimized photosynthesis under stress conditions as long as the upper thermal limit does not exceed (Salvucci and CraftsBrandner, 2004; March and et al., 2005). Furthermore leaf

temperature is negatively correlated with photosynthesis and water use efficiency (Shaheen et al., 2015). Same trend was found in current study genotypes with lower leaf temperature shoed higher photosynthetic rate and vice versa.

Photosystem II is chiefly thermolabile and under heat stress its activity is reduced or stopped (Camejo et al., 2005), this may be the property of its location in thylakoid where it is located (Mcdonald and Paulsen, 1997). Due to heat stress enzymes inactivation and denaturation take place resultantly it decreases the rate of biochemical reactions and photosynthesis (Nakamoto and Hiyama, 1999). Such alterations differ in different genotypes in response to heat stress (Mcdonald and Paulsen, 1997). Similar response was observed in current study that different genotypes behaved differently at elevated temperature in respect of photosynthesis.

Under any stressful environment degree of distortion relies on the equality between repair and damage during stress. Proline application significantly affects the photosynthesis, leaf temperature and transpiration rate under elevated temperature. It is reported that foliarly applied proline mitigate the reduction in photosynthesis by stabilizing the mitochondrial electron transport complex II (Hamilton and Heckathorn 2001), enzymes like Rubisco (Allen et al., 1997) and protecting the structures of proteins and membranes (Holmstrom et al., 2000). The similar results were reported in *Olea europaea* L (Ben et al., 2010) and tobacco. Transpiration rate has reported to have negative correlation with water use efficiency (Shaheen et al., 2015). Higher water loss from plant due to high transpiration rate results in low water use efficiency. Physiological drought is a term caused by heat stress when transpiration rate exceed the hydraulic conductance capacity of root and it result in water deficit for plants. The proline application significantly enhanced water use efficiency and reduced the transpiration rate in heat tolerant and heat sensitive genotypes. Foliar application of proline and other osmolytes reduce stomatal opening and reduce the transpiration rate and enhance the water use efficiency. These findings are supported with the results of Raghavendra and Reddy (1987).

Experiment # 4

Several environmental stresses effect the plant growth and development in heat stress is one of the major and basic stresses. This effect sometimes, is so severe that plant cannot with stand against it which ultimately leads the plant to death. Exogenous application of organic compounds increase plant's ability to tolerate heat stress is a present focus in Agriculture. Different organic compound being used in stress tolerance in plant and reduced the adverse effect of stress, proline has promising effects. In plants, proline is involved in the response to numerous environmental stresses and in different developmental processes. The accumulation of proline in response to different stresses is a well-established fact and different roles for proline as osmolyte, as an energy source or as an ROS scavenger have been proposed. In current study exogenous application of proline played a vital role in improving morphological, physiological and biochemical function in okra genotypes. In experiment # 4 effect of optimized dose (in experiment # 3) of proline was investigated on morphological, physiological, biochemical, water related and enzymatic attributes of okra genotypes (two heat tolerant and two heats sensitive) was tested.

Number of leaves per plant was more in heat tolerant genotypes as compared to heat sensitive genotype because under heat stress tolerant okra genotype retained their leaves and accumulated high proline in the leave while most of the leaves in heat sensitive okra genotypes were dropped as discussed in the results section (Figure 4.1.1). Root and shoot fresh weight was relatively high in heat tolerant okra genotype because under stress condition they carry on their activities such as photo synthesis and then the transfer of these photosynthetic products to root and shoot. Low water loss was also a reason to gain high root and shoot fresh weight while sensitive genotype could not with stand against heat stress and reduced fresh weight. Dry matter accumulation was also more in tolerant genotype as compared heat sensitive genotypes and proline application significantly enhanced it. Reason behind it was that the proline application increased morphological parameters and these findings have already been reported (Szabados and Savoure, 2010). The high root and shoot, weight (fresh and dry) are positively related to number of leaves that contain the photosynthetic apparatus and convert the solar energy to biomass. Similarly, deep and long root are helpful in uptake of water and nutrient from the soil and light from sun, respectively. In heat tolerant genotypes, all these characters helped to achieved high values of

morphological parameter and they perform batter as compared to heat sensitive okra genotypes.

Proline application was favorable for production of biochemical substances such as glycine betaine contents, proline contents, total free amino acids and carotenoid and chlorophyll contents in heat tolerant okra genotype. Under heat stress sensitive genotype all these parameters were low because in exposure to heat stress, sensitive genotypes reduced number of leaves, fresh and dry weight and loosed more water which affected the above mentioned parameters. Heat affects directly the photosynthetic and metabolic activities and reduced the efficiency which reduced the production of photosynthetic product and their conversion to amino acids and protein. Proline contents in heat tolerant genotypes favored them to cope with high temperature. Free amino acids contents were also positively correlated with more number of leaves, dry matter and chlorophyll contents. Chlorophyll is heat sensitive protein in plant leaves which is an essential part of photosystem and light absorbing system. More chlorophyll contents are directly related to more number of leaves. Similarly, carotenoids are directly correlated with leaves number and chlorophyll. Heat tolerant okra genotypes contained more above mentioned contents.

Physiological functions of plant are affected by heat stress. In current study leaf temperature of sensitive genotypes was high and they loosed more water as transpiration as compared to tolerant genotypes. Transpiration rate was more in heat sensitive genotypes which reduced the osmotic and turgor potential. Lower water contents and high transpiration through stomata were the reasons for low photosynthetic rate in sensitive genotypes. These physiological functions might include positive effects on stomatal opening, a necessary prerequisite of enhanced evapotranspiration. Stimulation of evapotranspiration by heat stress was recently described by Zhang et al. (2008). The reduction in photosynthetic rate observed under high temperature stress might be due to decreased stomatal conductance, as observed in this study. Similarly, a higher CO_2 assimilation rate in heat tolerant cultivars as compared to heat-sensitive ones under high temperature stress was observed in previous studies and was attributed to their efficient photosynthetic apparatus (Camejo et al., 2005). Heat stress in sensitive genotypes caused more stomatal conductance for both water and carbon dioxide. While reduction in stomatal conduction was more in heat tolerant genotype with the application of proline @ 2.5 mM. Stomatal conductance was linearly correlated with

transpiration rate, photosynthetic rate and ultimately water use efficiency in okra genotypes. Heat stress causes the rapid loss of water from the plant surface, which results in tissue and organ dehydration and restricts growth in plants. High temperature causes osmotic stress to the plant tissues owing to reduced root hydraulic conductance and tissue water status. Turgor potential, osmotic potential and water potential was high in heat tolerant okra genotypes compared with sensitive okra genotypes. High value of the above mentioned parameters resulted in higher relative water contents and furthermore proline application decreased stomatal loss of water. Collectively they favored the dry matter production under heat stress and increased water use efficiency in heat tolerant genotypes. One of the mechanisms involved in acclimation seems to be an accumulation of compatible solutes, especially of proline (Pro) or glycine betaine. Apart from its osmolyte functions, proline exhibits many other protective effects, including maintenance of redox balance and radical scavenging (Szabados and Savoure, 2010; Hong-Bo et al., 2008). Transfer of plants to an elevated temperature (40 ˚C) represents a fast, acute stress. In our study we found a decrease of leaf water potential in heat-stressed sensitive genotype. This decrease was postponed in the heat tolerant okra genotypes which might indicated their enhanced stress tolerance. Enzymatic activities under heat stress were more in heat tolerant genotypes. The enhanced activity and concentration of superoxide dimutase, peroxidase and catalase favored heat tolerant okra genotypes to establish under stress condition while sensitive genotype might not cope with the situation and reduced all critical processes.

In conclusion both heat tolerant genotypes performed batter and efficiently with proline application @ 2.5 mM for all morphological, physiological, biochemical, water related and enzymatic attributes as compared to control treatment. The study proved that the proline application is a good strategy to mitigate the effect of heat stress in okra.

CHAPTER 6 SUMMARY

The present study was designed to investigate the comparative performance of okra genotypes under high temperature stress. 100 okra genotypes were exposed to the controlled conditions of high temperature (45/35°C day and night). Different morphological, physiological and biochemical attributes were recorded and genotypes were categorized accordingly for their performance under conditions of elevated temperature. On the basis of shoot length genotypes were divided into different groups. Results revealed that 14 genotypes (14%) of okra exhibited shoot length fewer than 10 cm while 47 genotypes (47% of total genotype under study) had shoot length within the range of 10-15cm. A shoot length range of 15.1-20 cm was recorded in 37 genotypes (37%) while only 2 genotypes (2%) exhibited shoot length of more than 20 cm. The maximum shoot length was recorded in VI056448 (23.40 cm) whereas, minimum shoot length was observed in I033803V (5.5 cm). Results regarding root length of 100 genotypes showed that only 3 genotypes (3%) exhibited less than 3 cm root length whilst a good quantity of 53 genotypes (53%) showed root length in the range of 3.1-5 cm and 39 genotypes (79%) gave root length ranged from 5.1-7.9 cm. The remaining 5 genotypes expressed root length more than 8 cm. The minimum root length (2.80 cm) was recorded in VI033803 and maximum root length (9.65 cm) was observed in VI051062. Shoot fresh weight of 64 genotypes (64% of the total genotype under study) was noted < 2 g, while it ranged 2-2.5 g for 19 genotypes (19%) and 2.5-4 g for 16 genotypes (16%). The shoot fresh weight of remaining 1 genotype (1%) exceeds more than 4 g. VI051114 expressed the maximum shoot fresh weight (4.24), on the other hand minimum shoot fresh weight (0.38 g) was recorded in VI037995. According to the results of root fresh weight a major proportion of 95 genotypes (95%) exhibited less than 0.5 g weight while it ranged 0.5-0.99g for remaining 5 genotypes (5% total genotypes under study). It is notable that no okra genotype fell within the range of 1-1.5g and greater than 1.5g in terms of root fresh weight. VI040649 had smallest root fresh weight (0.10 g) while VI039652 possessed the highest root fresh weight (0.96g). In case of shoot dry weight is expressed in table 4.1.9. Results showed that 9 genotypes (9% of total genotype under study) exhibited less than 0.1 g shoot dry weight, 31 genotypes (31%) had 0.1-0.2g shoot dry weight and 30 genotypes (30%) showed 0.2-0.3g shoot dry weight. A shoot dry weight of more than 0.3g was recorded in 25 genotypes (25%) of okra. The minimum shoot dry weight (0.06g) was recorded in

VI051114 while maximum shoot dry weight (0.51 g) was noted in VI048596. About root dry weight it is clear that from 100 genotypes studied, major amount of 90 genotypes (90% of total genotypes under study) had root dry weight of < 0.1g while remaining 10 genotypes (10%) showed root dry weight in the range of 0.1-0.2g. VI033785 genotype showed highest root dry weight (0.160g) while VI056079 gave lowest root dry weight (0.030g). According to data 17 genotypes (17% of total genotypes under study) produced less than 3 leaves per plant, on the other hand somewhat large number of genotypes i.e. 66 (66%) possessed leaves per plant in the range of 3-5, while 17 genotypes (17%) produced more than 5 number of leaves per plant.VI037994 possessed the highest number of leaves (6.50), while lowest number of leaves (02) were given by VI047672. Chlorophyll contents of 10 genotypes (10% of total genotypes under study) gave chlorophyll contents < 10, while next 52 genotypes (52%) gave chlorophyll contents in the range of 10.1-20 and 34 genotypes (34%) showed 20.1-30 chlorophyll contents. The remaining 4 genotypes exhibited greater than 30 chlorophyll contents. VI055119 gave the maximum chlorophyll contents (41.25) while minimum chlorophyll contents (6.75) were gave by VI048596.).

Data regarding leaf temperature showed that only 4 genotypes (4%) remained cooler as compared to other groups with temperature less than 25ºC, while 8 genotypes (8%) showed a variable temperature range of 25.1-29.9ºC. A slightly higher temperature within the range of 30-33ºC was exhibited by 19 genotypes (19%), whereas, a comparatively large number consisting of 69 genotypes (69%) possessed leaf temperature above than 33ºC. The highest leaf temperature (36.15ºC) was observed in VI033775 while, the coolest leaf temperature (21.95ºC) was noted in VI056451. According to results 24 genotypes (24% of total genotype under study) showed photosynthetic rate fewer than 1 µmol m^{-2} s^{-1}, while those of 25 genotypes (25%) gave photosynthetic rate in the range of 1-1.75 µmol m^{-2} s^{-1} and it ranged 1.76-2.99 µmol m^{-2} s^{-1} for 23 genotypes (23%). A greater than 3 µmol m^{-2} s^{-1} photosynthetic rate was possessed by remaining 28 genotypes (28%). The minimum photosynthetic rate (0.06 µmol m^{-2} s^{-1}) was recorded in VI054568 and maximum photosynthetic rate (7.46 µmol m^{-2} s^{-1}) was observed in VI040770. According to data 32 genotypes (32% of total genotypes under study) had transpiration rate > 1 mmol m^{-2} s^{-1}, on the other hand each of 25 genotypes (25%) fell in the range of 01-02 mmol m^{-2} s^{-1} and 2-3 mmol m^{-2} s^{-1} by other 13 genotypes (13%). A transpiration rate 0f < 3 mmol m^{-2} $s^{-1}$was

recorded by remaining 4 genotypes (4%). VI033810 showed lowest transpiration rate (0.31 mmol m^{-2} s^{-1}) while highest transpiration rate (3.47 mmol m^{-2} s^{-1}) was noted in VI044244. Vast proportion of 64 genotypes (64% of total genotypes under study) showed water use efficiency less than 2 μmol CO_2 $mmol^{-1}$ H_2O. A water use efficiency of 23 genotypes (23%) was in the range of 02-04 μmol CO_2 $mmol^{-1}$ H_2O and those of 05 genotypes (5%) had water use efficiency ranged from 04-05 μmol CO_2 $mmol^{-1}$ H_2O. The remaining 08 genotypes (08%) exhibited above than 5 μmol CO_2 $mmol^{-1}$ H_2O water use efficiency. In case of sub-stomatal CO_2 30 genotypes (30% of total genotypes under study) expressed sub-stomatal CO_2 less than 600vpm. Sub-stomatal CO_2 of 44 genotypes (44%) was recorded in the range of 600-700vpm while 20 genotypes showed internal leaf CO_2 in the range of 700-800vpm. The remaining 06 genotypes (06%) expressed their sub-stomatal CO_2 greater than 800vpm. The minimum sub-stomatal CO_2 (448vpm) was recorded in VI055119, while VI049961 gave the maximum leaf internal CO_2 (929vpm). All the genotypes showed significantly variable response to elevated temperature. According to results two genotypes named VI051062 and VI060131 found to be most heat tolerant while genotype VI048596 and VI046554 proved to be most heat sensitive properties.

In 2nd experiment twenty five okra genotypes (twenty tolerant, while five sensitive) screened out from experiment # 01 were sown under field conditions. There were three sowing dates to check the effect of heat stress on different morphological, physiological and yield attributes. Three sowing dates were 02, April, 12, April and 22, April. All the cultural practices were kept same for all sowing dates and for all genotypes. Sowing date had significant effect on number of plants survival in okra genotypes. VI051062 (Heat tolerant genotype) showed maximum plant survival (05) in sowing dates 1 (02 April) and 3 (22 April). The same number of plants (05) survived by second tolerant genotype VI060131 in sowing date of 3 (22 April). Minimum plant survival (0.5) was observed in VI048596 (Heat sensitive genotype) followed by VI046554 with plant survival (0.75) in 2nd sowing date (12 April). Sowing date 1 (02 April) gave maximum plant height (150 cm) and (148 cm) in VI051062 and VI060131 (heat tolerant genotypes) respectively. VI048596 and VI046554 (Heat sensitive genotypes) showed minimum plant height (80 cm) and (85 cm), respectively in sowing date 2 (20 April). Tolerant genotypes VI051062 and VI060131 had more effectual response on plant height in sowing date 1 (02 April) with maximum plant height while

sensitive genotypes (VI048596 and VI046554) responds less effectively in 3rd sowing date (22 April) with minimum plant height.

Highest number of pods plant^{-1} (40) were recorded in 1st sowing date (02 April) by VI051062, while VI060131 stood second with (38) number of pods plant^{-1} in same sowing date. VI046554 gave lowest (12) number of pods plant^{-1} in 3rd sowing date (22 April) followed by VI048596 having (13) number of pods plant^{-1} in sowing date 2 (12 April). VI051062 and VI060131 (tolerant genotypes) showed better response in all sowing dates having highest number of pods plant^{-1} while VI046554 and VI048596 (sensitive genotypes) had minimum number of pods plant^{-1} in all sowing dates. The maximum pod length (16 cm) was calculated in VI060131 genotype in sowing date 1 (02 April) as well as in VI051062 with pod length (14 cm) in same sowing date. On the other hand minimum pod length (04 cm) was noted in VI046554 genotype in sowing date 3 (22 April) whereas genotype VI048596 gave same pod length (06 cm) in all sowing dates. All sowing dates expressed efficient response on tolerant genotypes with highest pod length while sensitive genotypes showed lowest pod length in all sowing dates. Genotype VI051062 and VI060131 were found to having highest pod diameter (02 cm, 1.90 cm), respectively in sowing date of 1 (02 April). The least pod diameter (1.07cm) was observed in VI048596 as well as in VI046554 (1.10 cm) at third sowing date (22 April). Tolerant genotypes (VI051062, VI060131) showed better response in 1st sowing date (02 April) with maximum pod diameter, however these genotypes gave good results in all sowing dates as compared to sensitive genotypes (VI046554, VI042596) with lowest pod diameter in all sowing dates. At sowing date one (02 April) maximum leaf area (500 cm^2) was counted in genotype VI051062, followed by sowing date 2 (12 April) with leaf area (485 cm^2). Genotype VI060131 also showed same leaf area (485 cm^2) at 1st sowing date (02 April). At the same time, genotype VI042596 showed lowest leaf area (300 cm^2, 315 cm^2 315 cm^2) at sowing date of two (12 April), one (02 April) and third (22 April), respectively. Result shows that sowing dates had positive effective on genotypes VI051062 and VI060131 (Tolerant) with higher leaf area in contrast with genotypes VI042596 and VI046554 (sensitive) having lower leaf area. Maximum number of leaves (71) were counted in heat tolerant genotype VI060131 in sowing date one (02, April) followed by (70) same genotype VI060131 in second sowing date (12, April). Minimum number of leaves (11) were counted in heat sensitive genotype VI048596 on

second sowing date (02, April), followed by number of leaves (15) in the heat sensitive genotype VI048596 in sowing date 1st (02, April). Both heat tolerant genotypes VI051062 and VI060131 produced highest number of leaves regardless of sowing date. On the other hand VI046554 and VI042596 both heat sensitive genotypes give the lowest number of leaves on all the three sowing dates. Maximum photosynthetic rate (10.78) was noted in VI051062, followed by (9.17) in VI060131 at sowing date of one (02 April). VI046554 gave lowest photosynthetic rate (1.25) at 3rd sowing date, while VI048596 (2.85) at sowing date of two (12 April). Tolerant genotypes behaved more efficiently with highest photosynthetic rate regardless of sowing dates as compared to sensitive ones with lowest photosynthetic rate in all sowing dates. The genotype VI060131was noted having lowest transpiration rate (3.22) at 1st sowing date (02 April), while genotype VI051062 gave (3.30) transpiration rate at same sowing date. Genotype VI046554 expressed highest transpiration rate (7.37) at sowing date 3 (22 April) while VI048596 gave second highest transpiration rate (6.72) at same sowing date.

Results revealed that 1st sowing date left positive effect on tolerant genotypes with lowest transpiration rate while sensitive genotypes respond less effectively toward sowing date with higher transpiration rate. The maximum water use efficiency was noted in VI051062 (3.30) and VI060131 (2.87) at sowing date one (02 April). In contrast, genotype VI046554 had lowest water use efficiency (0.17) at 3rd sowing date (22 April) whereas, (0.45) was recorded in VI048596 at same sowing date. It is observed clearly from results that tolerant genotypes (VI051062 and VI060131) respond more effectively towards all genotypes with highest water use efficiency. On the contrary, sowing dates remained less effective in case of sensitive genotypes (VI048596 and VI046554) having lowest water use efficiency. At sowing date one (02 April) lowest temperature (29.59) was noted in genotype VI060131 followed by VI051062 with leaf temperature (30.30) at same sowing date. VI048596 genotype showed maximum leaf temperature (36.12) at third sowing date (22 April) while 2nd highest leaf temperature (35.85) was found at sowing date of 2 (20 April) in genotype VI046554. Both tolerant genotypes behaved efficiently in all sowing dates having lowest leaf temperature in comparison with sensitive ones. The genotype VI051062 was at top with sub-stomatal CO_2 (168.25), followed by sub-stomatal CO_2 (195.50) by genotype VI060131 in sowing date 1 (02 April). In case of highest sub-stomatal CO_2 (523.25) genotype VI050150 was noted at top while genotype VI041139 stood second with sub-

stomatal CO_2 (438.25) at sowing date 01 (02 April) and sowing date 03 (12 April), respectively. In the light of results it is observed that both tolerant genotypes (VI051062, VI060131) remained better with lowest sub-stomatal CO_2 at 1st sowing date, while genotypes (VI050150, VI041139) showed poor response towards sowing dates with highest sub-stomatal CO_2. VI060131 genotype gave highest stomatal conductance to H_2O (5.86) at sowing date 3 (22 April) while 2nd highest stomatal conductance (4.62) was noted in VI046554 at same sowing date. The lowest stomatal conductance to water (0.04) was recorded in genotype VI060206 whereas second lowest stomatal conductance (0.07) was calculated in VI040649 at second sowing date (12 April). The genotype VI060131 (tolerant), while VI046554 (sensitive) respond less effectively towards sowing dates as compared to genotypes VI060206 and VI054565 with lowest stomatal conductance to water. Chlorophyll contents were significantly influenced by sowing dates in all okra genotypes. The 2nd sowing date efficiently improved the genotype VI051062 with maximum chlorophyll contents (72.58), followed by chlorophyll contents (62.63) in genotype VI041763 at same sowing date. The lowest chlorophyll contents (15.93) and (18.15) were found in genotype VI046554 at sowing date of 3rd and 2nd respectively. Results showed that chlorophyll contents were efficiently improved in tolerant genotype (VI051062) regardless of sowing dates. On the other hand, sensitive genotype (VI046554) not positively affected by sowing dates with minimum chlorophyll contents. Sowing dates significantly affected electrolyte leakage in okra genotypes. The minimum electrolyte leakage (63.70) was found at sowing date 1 (02 April) in VI051062 genotype while VI060131 genotype ranked second with electrolyte leakage (64.15) at same sowing date. On the other hand, at same sowing date of one (02 April) the genotype VI048596 gave highest electrolyte leakage (98.02). Another genotype VI056452 stood second with electrolyte leakage (97.39) at third sowing date (22 April).

Third experiment was designed for optimization of best proline dose that can alleviate the drastic effect of heat stress in okra. For this purpose four okra genotypes, two tolerant (VI051062 and VI060131) and two sensitive (VI048596 and VI046554) screened out from a large number of okra genotypes from experiment # 2 were exposed to heat stress (45/35°C day/night temperature) two weeks after exposure to heat stress under controlled environmental conditions and were sprayed with proline (0.00, 1.0, 1.5, 2.0, 2.5 and 3.0mM) to optimize the best dose of proline. Following morphological parameters like shoot length,

root length, numbers of leaves, shoot fresh weight root fresh weight, shoot dry weight and root dry weight, physiological attributes like photosynthetic rate, transpiration rate, sub-stomatal to CO_2, sub-stomatal to water, leaf surface temperature, water use efficiency and chlorophyll contents were studied to optimize the best dose of proline for enhancement of heat stress tolerance in okra genotypes.

Results revealed that highest shoot length (17.17±0.21cm) was counted in VI051062 (heat tolerant variety) followed by VI060131 (heat tolerant variety) recording 16.07±0.06 cm shoot length when proline applied @ 2.5mM. On the other hand lowest shoot length (10.42±0.18cm) was noted in VI048596 (heat sensitive variety) where proline applied at concentration of 01mM as well as in VI046554 at the same proline level with shoot length of 11.12±0.36cm. Genotype VI051062 (Heat tolerant cultivar) was found to having maximum root length (10.91±0.12 cm) with foliar application of proline at 2.5mM concentration, followed by VI060131 (heat tolerant cultivar) having root length (10.48±0.22 cm) when proline applied @ 3mM. Minimum root length (5.11±0.07cm) was recorded in VI048596 (heat sensitive cultivar) at proline level of 1mM while VI046554 (heat sensitive cultivar) gave root length 5.93± 0.10cm with same level of proline. Genotype VI051062 gave higher number of leaves per plant (12.00±0.40) while VI060131 stood second with 11.50±0.64 number of leaves @ 2.5 mM proline level. The minimum number of leaves (4.00±0.40) was given by VI048596 as well as another sensitive cultivar VI046554 with number of leaves (4.25±0.75) when proline applied @ 1.5mM and 02mM, respectively. Shoot fresh weight was calculated in highest quantity (3.46±0.01gm) by VI051062 (Heat tolerant variety) when proline applied foliarly @ 2.5mM while VI060131 (heat tolerant cultivar) stood 2nd having shoot fresh weight of 3.35±0.01gm at same proline concentration. The lowest value of shoot fresh weight was observed in VI048596 (2.13±0.12gm) and in VI046554 (3.12±0.03) with foliar application of proline @ 01 mM. Highest shoot dry weight (0.63±0.01gm) was recorded in VI051062 followed by VI060131 with shoot dry weight of 0.47±0.01gm at the same proline concentration of 2.5mM. The lowest shoot dry weight (0.2±0.01gm) was calculated in VI048596 as well as in VI046554 having 0.20±0.02gm shoot dry weight with foliar application of proline at 01mM concentration. Higher root fresh weight (0.37±0.01gm) in genotype VI060131at proline level of 1.5mM while VI051062 gave 0.32±0.01gm root fresh weight with proline application @ 2.5mM. The minimum root fresh weight

(0.05±0.01gm) was counted in VI048594 followed by VI046554 with root fresh weight of 0.17±0.01 when proline applied foliarly at the rate of 3mM and 1mM respectively. The cultivar VI051062 gave highest root dry weight (0.085±0.01gm) @ 2.5mM proline concentration while VI046554 genotype remained second with 0.0787±0.03gm root dry weight at same proline level. The minimum root fresh weight (0.0325±0.12gm) was counted in genotype VI048594 and VI046554 when foliar application of proline was done @ 1mM.

Leaf temperature (28.19±0.14 °C) was recorded in VI051062 genotype, followed by VI060131 with leaf temperature of 29.10±0.05°C when proline applied foliarly. In case of maximum leaf temperature VI048594 was observed with 33.43±0.08°C while VI046554 stood second having 33.39±0.06°C with proline application @ 1.5 mM and 01mM, respectively. The cultivar VI051062 had lowest sub-stomatal CO_2 (953.5±34.5vpm) at the rate of 2.5 mM proline level and 1078.8±18.8 vpm sub-stomatal CO_2 was calculated in VI060131cultivar at same proline concentration. The highest sub-stomatal CO_2 1793.0±31.0vpm was given by VI048596 genotype, followed by VI046554 with sub-stomatal CO_2 1681.5±37.7 vpm at the same rate of 1mM. It is clear from results that (0.57±0.02 mmol $m^{-2\ s-1}$) stomatal conductance to water was recorded in VI051062 genotype followed by VI060131 with stomatal conductance (0.61±0.03 mmol $m^{-2\ s-1}$)) at 2.5mM proline level. On the other hand, the maximum stomatal conductance (1.89±0.03 mmol m^{-2s-1}) was occurred in VI048596 as well as in VI046554 (1.80±0.01 mmol m^{-2s-1}) at the proline level of 01mM and 1.5 mM respectively. The highest photosynthetic rate 3.85±0.11 µmol $m^{-2\ s-1}$ was found in VI051062 followed by VI046554 with photosynthetic rate of 3.38±0.11 µmol m^{-2} s^{-1} at 2.5 mM concentration of proline. In case of lowest value VI060131was observed with photosynthetic rate of 1.70±0.15 µmol m^{-2} s^{-1} as well as VI051062 with photosynthetic rate of 2.20±0.04µmol m^{-2} s^{-1}, at the rate of 1.5mM and 01mM respectively. The results showed that minimum transpiration rate (3.65±0.22 mmol m^{-2} s^{-1}) was calculated in VI051062 and transpiration rate of 4.43±0.08 mmol m^{-2} s^{-1} was observed in VI046554 at same proline level of 2.5mM. Genotype VI048596 was found to having maximum transpiration rate 5.78±0.09 mmol $m^{-2\ s-1}$ at @ of 1.5mM while second highest transpiration rate 5.51±0.17mmol $m^{-2\ s-1}$ was noted in VI046554 at the rate of 01 mM. The maximum water use efficiency (1.06±0.05 µmol CO_2 $mmol^{-1}$ H_2O) was calculated in VI051062 and 0.76±0.03 µmol CO_2 $mmol^{-1}$ H_2O in VI046554 at proline level of 2.5mM. The lower water use

efficiency 0.32±0.03 μmol CO_2 $mmol^{-1}$ H_2O and 0.43±0.03 μmol CO_2 $mmol^{-1}$ H_2O was observed in VI060131 and VI048594 at level of 01Mm. The highest chlorophyll contents 45.425±0.61 were recorded in VI051062 with proline application @ 2.5 mM while VI060131 gave 40.7±0.51chlorophyll contents at same level of proline application. The lowest number of chlorophyll contents 26.025±0.75 and 27.352±2.09 were calculated by genotype VI048596 and VI046554 respectively. On the basis of results it was concluded that exogenous application of proline effectively enhanced the heat tolerance capacity in okra genotypes mainly at 2.5 mM. The proline application enhanced the tolerance potential of tolerant genotype as well as sensitive ones. However, tolerant genotypes responded more effectively to proline application as compared to sensitive genotypes.

Fourth experiment was conducted to explore the effect of optimized dose of proline in experiment # 3 on morphological, physiological, water related, biochemical and enzymatic attributes of heat tolerant and heat sensitive genotypes. Shoot length was significantly influenced by the application of proline in okra under heat stress. Longest shoot length was recorded in VI051062, followed by VI061131, VI046554 and VI048596. Heat tolerant genotypes established longer shoot as compared to heat sensitive okra genotype under both, proline application and control treatment. Proline application significantly enhanced root length in okra genotype. Results showed increased root length by proline application was recorded in VI051062 but statistically similar with its control treatment. All other genotypes were significantly different for root length under proline application and shown higher value as compared to control. Over all heat tolerant okra genotypes (VI051062 and VI061131) were better to establish a deeper root than heat sensitive okra genotypes (VI046554 and VI048596). Leaves are the basic part of plant which contain photosynthetic apparatus and convert solar energy into chemical food energy. Leaves play an important role in capturing sun light and direct toward photosystem. In our study exogenous proline application significantly enhanced number of leaves per plant and genotypes responded positively to produce leaves. Highest number of leaves per plant was produced in heat tolerant okra genotype VI051062 and VI061131. Overall, both heat tolerant and sensitive okra genotypes responded actively to proline application for enhancing the number of leaves per plant as compared to control treatment. The highest shoot fresh weight was observed in VI051062 followed by VI061131 when proline was applied @ 2.5mM. The lowest shoot fresh weight

was recorded in VI046554 which followed the VI048596 heat sensitive genotype. In short, the proline application had more effect on heat tolerant genotypes as compared to heat sensitive genotypes for shoot fresh weight accumulation than in control treatment. The results depicted that VI051062 gave the highest shoot dry weight, followed by VI061131 when proline was applied @ 2.5mM. The lowest shoot dry weight was observed in VI048596 with control treatment. Generally speaking the proline application had more effect on heat tolerant genotypes as compared to heat sensitive genotypes for shoot dry weight accumulation than in control treatment. More fresh weight was gathered by heat tolerant genotype VI051062, followed by VI061131. Lowest root fresh weight was gathered by VI048596 that was a heat sensitive genotype. In short exogenous proline application was effective to increase root dry weight either heat tolerant or sensitive okra genotypes as compared control treatment. Results depicted that more dry weight was gathered by heat tolerant genotype VI051062, followed by VI061131, which was at par with VI046554. Lowest root dry weight was attained by VI048596 that was a heat sensitive genotype. Highest leaf temperature lowering was recorded in VI061131, followed by VI051062, VI046554 and VI048596. VI046554 showed highest leaf temperature but was at par with VI048596 under same control treatment. Proline application significantly increased the stomatal conductance (SSC) for carbon dioxide (CO_2) gas in both heat tolerant and heat sensitive okra genotypes. Results indicated that there were more SSC were found in heat sensitive okra genotypes as compared to heat tolerant genotypes. All sensitive genotypes (VI046554 and VI048596) and heat tolerant genotype (VI051062 and VI061131) were mutually at par for SSC. Stomatal conductance for the water was significantly affected by both proline application and okra genotypes. Results depicted that highest stomatal conductance was recorded in heat sensitive genotypes (VI046554 and VI048596) under control treatment as compared to proline application. Okra genotypes which were heat tolerant conducted low water through stomata as compared to sensitive genotype under both proline application and control treatment. However heat tolerant genotypes (VI051062 and VI061131) lost low water with proline (2.5 mM) application as compared to control (Figure 4.4.3). The highest transpiration rate was noted in case of heat okra genotype (VI046554 and VI048596) under control as compared to proline application treatment. Similarly, heat tolerant okra genotype showed low transpiration rate than sensitive genotype under proline

application. Overall, proline application in all okra genotype decreased the transpiration rate with respect to control. Proline application enhanced the photosynthesis in all okra genotype as compared to control. VI051062 (tolerant genotype) and VI046554 (sensitive genotype) showed maximum photosynthesis but these both were at par. Lowest photosynthesis rate was recorded in case of VI048596 under both proline and control condition. Results showed that there was high WUE in heat tolerant okra genotype (VI051062 and VI061131) than heat sensitive genotypes. However the proline application and control treatment showed a huge difference both in heat tolerant and sensitive okra genotypes. Results for chlorophyll contents depicted that highest chlorophyll concentration was recorded in VI051062 that was statistically at par with VI061131 under proline application rather control treatment. Heat sensitive genotypes (VI046554 and VI048596) were lagged by heat tolerant okra genotypes for chlorophyll contents. Overall, proline application enhanced chlorophyll production in heat tolerant as well as heat sensitive okra genotype as compared to no proline application. Highest GB content were produced in VI061131 compared with control while lowest GB contents were recorded in VI048596 with 1.14 more as compared to its control. Proline application under stress was useful for all okra genotypes and increased the GB contents. Proline is an important amino acid which is produced under stress condition in crop plants. Heat tolerant genotypes (VI051062 and VI061131) were at the top for proline contents, followed by sensitive genotype (VI046554 and VI048596). While in the comparison of proline application (@ 2.5 mM) and control, all the genotypes showed higher proline contents but the extent was relatively more in heat tolerant genotypes as compared heat sensitive okra genotypes. Total free amino acids (TFAA) were non-significant in all heat tolerant and sensitive okra genotypes and proline application had no significant effect on production of TFAA. TFAA were slightly more in heat sensitive okra genotype than heat tolerant but values were not statistically different. Highest carotenoid contents (0.96) were produced in VI061131 that was statistically similar to (0.83) in VI051062. In the same way VI046554 and VI048596 showed no mutual difference with carotenoid content of 0.69 and 0.57. Exogenous application of proline significantly affected on turgor potential and all the okra genotype were significantly variable for turgor potential. It was observed that highest that highest turgor potential was in heat tolerant okra genotype (VI051062 and VI061131) under proline application as compared to control treatment. Moreover, heat sensitive okra

genotype showed low turgor potential than tolerant genotype but under proline application. Over all proline application in all okra genotype increased the turgor potential with respect to control. There was significant difference between proline treatment and control in all heat tolerant and sensitive okra genotypes for osmotic potential. Results of osmotic potential depicted that highest osmotic potential was recorded in VI051062 that was followed by VI061131. VI046554 and VI048596 were statistically different with proline application compared to control treatment. Overall, proline application enhanced osmotic potential in heat tolerant as well as heat sensitive okra genotype as compared to control. But tolerant genotypes performed better than sensitive ones. Summary of results of RWC depicted that highest relative values were recorded in VI051062, followed by VI061131. VI046554 and VI048596 were statistically at par under proline application rather control and their values were than the control treatment of VI046554 and VI048596. Heat sensitive genotypes (VI046554 and VI048596) were lagged by heat tolerant okra genotypes for RWC. Overall, proline application enhanced RWC in heat tolerant as well as heat sensitive okra genotype as compared to no proline application. The results for water potential depicted that VI051062 (heat tolerant genotype) showed the highest water potential and was followed by VI051062. In heat sensitive genotype VI046554, followed the VI051062 and VI061131 in for water potential and was followed by VI048596 (heat sensitive genotype). Overall, proline application enhanced the water potential in both heat tolerant as well as heat sensitive okra genotypes as compared their respective control.

The results for supper oxide (SOD) indicated that proline application significantly ($p \leq 0.05$) enhanced its activity and genotypes effect was also significant. VI061131 (heat tolerant genotype) showed the highest SOD concentration and was at par with VI051062. In heat sensitive genotype VI046554, followed the VI051062 and VI061131 in SOD concentration and was followed by VI048596 (heat sensitive genotype).

Peroxidase (POD) concentration was significantly enhanced by proline application and okra genotypes behave differently. Results depicted that highest POD concentration was recorded in heat tolerant genotypes (VI051062 and VI061131) under proline treatment as compared to control. Proline application significantly enhanced the catalase (CAT) concentration in heat tolerant as well as heat sensitive okra genotypes. Results showed that highest CAT concentration was obtained in heat tolerant okra genotype but VI051062

produced slightly more concentration than VI061131. These were followed by VI046554 and VI048596 (heat sensitive genotype), however these heat sensitive genotypes were mutually at par. All the genotypes heat tolerant or heat sensitive produced relatively higher concentration of CAT with proline application than their respective control treatment.

We can conclude that okra genotypes vary in their ability to tolerate heat stress. Heat stress tolerance is genotype dependent character. In this study okra genotypes showed varied response to heat stress under controlled environmental conditions. Genotypes VI051062 and VI061131 showed their highest potential to tolerate heat stress under controlled conditions; while genotypes VI046554 and VI048596 proved to be most heat sensitive under same environment. In the field conditions at different sowing dates again genotypes VI051062 and VI061131 proved to be most heat tolerant and genotypes VI046554 and VI048596 proved to be most heat sensitive genotypes. In case of proline application for enhancementof heat stress tolerance in okra genotypes; proline @ 2.5 mM enhances the heat tolerance of both heat tolerant and heat sensitive genotypes. Proline application @ 2.5 mM significantly affected growth, physiological, biochemical, water related and enzymatic attributes to alleviate drastic effects of heat stress in okra genotypes. Further more heat tolerant genotypes responded well to exogenous application of proline than heat sensitive genotypes.

RECOMMENDATIONS

- Farmer should sow heat tolerant genotypes (VI051062 and VI061131) for higher yield and enhanced period of production.
- Proline @2.5mM concentration should be sprayed to mitigate the drastic effects of heat stress in okra.
- Genotypes identified as heat tolerant (VI051062 and VI061131) should be used in breeding programs.

FUTURE THRUSTS

- Marker assisted selection should be done to investigate the molecular basis of heat tolerance.
- Proline application through means other than foliar should be checked.
- Signal transduction pathways, translational control of gene expression regarding proline should be explored to better understand the phenomenon of stress tolerance achieved by proline

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APPENDICES

Experiment # 3

Appendix 1: Impact of proline application on the shoot length of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	15.19±0.27	14.53±0.55	11.12±0.36	9.15±0.11
01	15.85±0.10	14.94±0.34	11.69±0.06	10.42±0.18
1.5	15.27±0.27	15.43±0.16	12.24±0.14	10.51±0.20
2.0	16.41±0.19	15.62±0.21	12.71±0.12	11.07±0.06
2.5	17.17±0.21	16.07±0.06	13.95±0.36	11.98±0.13
3.0	16.92±0.05	15.58±0.30	13.4±0.23	11.91±0.30

Appendix 2: Impact of proline application on the root length of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	8.43±0.58	7.97±70	5.09±17	4.88±27
01	8.57±0.16	8.34±36	5.93± 0.10	5.11±0.07
1.5	9.14±0.12	8.97±0.08	6.02±0.11	5.91±0.09
02	9.93±0.23	7.57±0.25	7.13±0.22	6.78±0.20
2.5	10.91±0.12	8.79±0.39	9.01±0.34	7.61±0.23
03	8.82±0.18	10.48±0.22	8.08±0.19	7.91±0.23

Appendix 3: Impact of proline application on the shoot fresh weight of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
control	3.28±0.05	2.99±0.04	2.08±0.05	1.7±0.05
01	3.34±0.02	3.12±0.03	3.14±0.06	2.13±0.12
1.5	3.35±0.03	3.28±0.03	3.15±0.01	2.34±0.11
02	3.39±0.006	3.24±0.06	3.28±0.01	2.62±0.08
2.5	3.46±0.01	3.35±0.01	3.30±0.03	2.49±0.15
03	3.36±0.01	3.29±0.01	3.24±0.02	2.26±0.04

Appendix 4: Impact of proline application on the root fresh weight of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
control	0.23±0.01	0.31±0.02	0.15±0.01	0.1±0.02
01	0.24±0.01	0.31±0.02	0.17±0.01	0.12±0.01
1.5	0.29±0.00	0.37±0.01	0.19±0.01	0.10±0.02
02	0.24±0.01	0.28±0.01	0.22±0.00	0.08±0.01
2.5	0.32±0.01	0.36±0.01	0.24±0.01	0.07±0.00
03	0.28±0.01	0.32±0.01	0.20±0.00	0.05±0.01

Appendix 5: Impact of proline application on the soot dry weight of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
control	0.40±0.01	0.39±0.04	0.16±0.01	0.17±0.04
01	0.48±0.01	0.42±0.02	0.20±0.02	0.2±0.01
1.5	0.53±0.00	0.43±0.02	0.25±0.02	0.24±0.01
02	0.55±0.01	0.44±0.01	0.3±0.02	0.29±0.00
2.5	0.63±0.01	0.47±0.01	0.31±0.02	0.34±0.01
03	0.57±0.00	0.45±0.00	0.27±0.02	0.31±0.01

Appendix 6: Impact of proline application on the root dry weight of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
control	0.0375±0.05	0.037±0.04	0.022±0.05	0.0207±0.05
01	0.073±0.02	0.0387±0.03	0.0325±0.06	0.0325±0.12
1.5	0.06±0.03	0.047±0.03	0.0487±0.01	0.0582±0.11
02	0.05±0.00	0.06±0.06	0.0505±0.01	0.0395±0.08
2.5	0.085±0.01	0.0775±0.01	0.0787±0.03	0.0627±0.15
03	0.055±0.01	0.053±0.01	0.0605±0.02	0.0557±0.04

Appendix 7: Impact of proline application on the number of leaves of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
control	7.50±0.64	6.00±0.40	3.50±0.64	3.75±0.47
01	9.25±0.62	8.25±1.10	5.25±0.75	5.00±0.40
1.5	9.75±0.47	7.00±0.40	6.00±1.47	4.00±0.40
02	10.75±0.47	10.75±0.85	4.25±0.75	5.25±0.47
2.5	12.00±0.40	11.50±0.64	6.25±0.47	6.75±0.47
03	10.50±0.64	8.00±1.29	5.50±0.28	5.00±0.40

Appendix 8: Impact of proline application on leaf temperature of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
control	32.51±0.15	32.25±0.34	34.33±0.17	34.57±0.05
01	29.38±0.12	31.40±0.17	33.39±0.06	33.00±0.04
1.5	30.35±0.11	30.43±0.17	32.37±0.20	33.43±0.08
02	29.04±0.04	30.77±0.10	31.20±0.06	32.37±0.06
2.5	28.19±0.14	29.10±0.05	29.97±0.05	30.45±0.10
03	31.48±0.22	31.73±0.29	31.05±0.09	31.59±0.05

Appendix 9: Impact of proline application on sub-stomatal CO_2 of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	1422.0±25.0	1426.5±16.3	1938.5±53.7	1915.8±33.2
01	1192.0±26.7	1375.3±23.2	1681.5±37.7	1793.0±31.0
1.5	1057.3±13.6	1251.5±17.5	1401.8±30.1	1595.3±23.4
02	1232.8±55.4	1234.5±10.9	1396.0±28.5	1410.3±24.1
2.5	953.5±34.5	1078.8±18.8	1165.5±23.0	1283.8±34.4
03	1089.8±34.8	1145.3±20.5	1324.0±28.2	1360.0±22.0

Appendix 10: Impact of proline application on transpiration rate of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	5.14±0.04	5.58±0.09	5.66±0.13	5.73±0.12
01	4.06±0.02	5.28±0.22	5.51±0.17	5.49±0.10
1.5	4.91±0.11	4.77±0.20	5.38±0.28	5.78±0.09
02	4.67±0.10	5.07±0.24	4.68±0.13	5.15±0.09
2.5	3.65±0.22	5.25±0.25	4.43±0.08	5.11±0.15
03	4.97±0.16	5.30±0.15	4.80±0.13	5.34±0.09

Appendix 11: Impact of proline application on photosynthetic rate of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	1.29±0.10	1.58±0.14	1.67±0.08	1.49±0.20
01	2.20±0.04	1.70±0.15	2.67±0.06	2.37±0.13
1.5	3.73±0.11	2.52±0.22	3.24±0.10	2.68±0.11
02	3.27±0.07	2.64±0.13	2.50±0.15	2.91±0.06
2.5	3.85±0.11	2.92±0.08	3.38±0.11	2.90±0.06
03	3.41±0.12	2.29±0.34	2.94±0.16	2.49±0.06

Appendix 12: Impact of proline application on water use efficiency of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	0.25±0.02	0.28±0.02	0.30±0.02	0.26±0.04
01	0.54±0.01	0.32±0.03	0.49±0.02	0.43±0.03
1.5	0.76±0.03	0.54±0.06	0.61±0.03	0.46±0.03
02	0.70±0.02	0.52±0.03	0.54±0.04	0.56±0.01
2.5	1.06±0.05	0.56±0.03	0.76±0.03	0.57±0.02
03	0.69±0.04	0.43±0.07	0.61±0.02	0.47±0.01

Appendix 13: Impact of proline application on stomatal conductance to water of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	0.80±0.05	0.89±0.04	1.92±0.03	1.91±0.03
01	0.72±0.04	0.80±0.03	1.69±0.13	1.89±0.03
1.5	0.69±0.02	0.77±0.05	1.80±0.01	1.81±0.02
02	0.64±0.02	0.73±0.02	1.59±0.02	1.75±0.02
2.5	0.57±0.02	0.61±0.03	1.45±0.03	1.59±0.02
03	0.66±0.02	0.67±0.02	1.58±0.02	1.64±0.02

Appendix 14: Impact of proline application on chlorophyll contents of various okra genotypes (Mean ± Standard Error)

Proline (mM)	VI051062	VI060131	VI046554	VI048594
Control	35.585±1.77	30.975±0.96	20.72±1.16	18.132±1.22
01	41.017±1.79	36.625±1.62	27.352±2.09	26.025±0.75
1.5	35.077±1.68	32.35±1.46	33.712±0.92	29.275±0.92
02	39.165±1.41	36.522±1.20	34.462±0.67	31.152±0.73
2.5	45.425±0.61	40.7±0.51	35.325±0.63	33.75±0.81
03	41.61±0.95	38.612±0.08	33.332±0.73	32.03±0.96