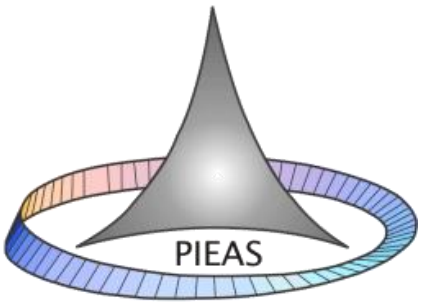
Analysis of Individual and Combined Effects of Irrigation and Heat Stress in Chickpea (*Cicer arietinum*) Through Integrated Approaches



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2016

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Thesis Submission Approval

This is to certify that the work contained in this thesis entitled **Analysis of Individual and Combined Effects of Irrigation and Heat Stress in Chickpea (*Cicer arietenum*) Through Integrated Approaches**, was carried out by **Saima Jameel**, and in my opinion, it is fully adequate, in scope and quality, for the degree of **M.Phil.** Furthermore, it is hereby approved for submission for review and thesis defense.

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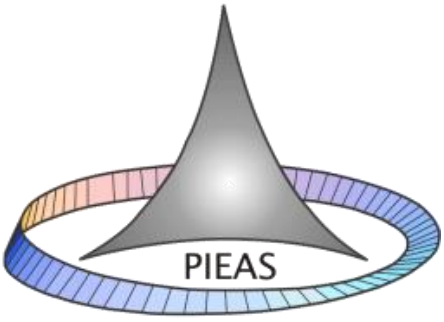
Analysis of Individual and Combined Effects of Irrigation and Heat Stress in Chickpea (*Cicer arietinum*) Through Integrated Approaches



Saima Jameel

Submitted in partial fulfillment of the requirements
for the degree of M.Phil.
2016

Department of Biological Science

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Dedicated

TO

My Mother

A strong and gentle women who taught me to trust in ALLAH, believe in hard work and that so much could be done with little.

My Father

For earning an honest living for us and for supporting & encouraging me to believe in myself**.**

Acknowledgment

All the praises and thanks are for Almighty **ALLAH,** The Compassionate, The Merciful, the source of knowledge and wisdom, Who has blessed me with good health. After that, all praises are due to His Holy Prophet **MOHAMMAD (Peace be upon him),** the most perfect and exalted among and of ever born on earth, who is forever a torch of guidance and knowledge for humanity as a whole.

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Last but not least, I offer deepest gratitude to my dear sister **Anam Jabeen** and my friend **Seher Iftikhar** for their continuous support and encouragement during my journey. Stay blessed all.

Saima Jameel

Declaration of Originality

I hereby declare that the work contained in this thesis and the intellectual content of this thesis are the product of my own work. This thesis has not been previously published in any form nor does it contain any verbatim of the published resources which could be treated as infringement of the international copyright law. I also declare that I do understand the terms ‘copyright’ and ‘plagiarism,’ and that in case of any copyright violation or plagiarism found in this work, I will be held fully responsible of the consequences of any such violation.

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(**Saima Jameel**)
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Abstract

The world climate is continuously changing. Heat stress and excessive rainfall are becoming serious threats for chickpea production. Present study was conducted to screen out kabuli (08) and desi (09) chickpea genotypes for heat stress (H), irrigation (I) and a combination of both stresses (I+H). Seedlings were grown in small plastic pots filled with autoclaved sand under completely randomized design. Irrigation stress (maintaining at 100% filed capacity) was applied after six days of emergence while control set was maintained at 50% field capacity. For heat stress, one set of fifteen days old seedlings were shifted from net house (day/night temp. 33/13^{o}C for kabuli and 32/10^{o}C for desi genotypes) to glasshouse (day/night temp. 43/20^{o}C for kabuli and 38/17^{o}C for desi genotypes). For combined stress, conditions of heat and irrigation stress were applied simultaneously. After 30 days of sowing, morphological data was recorded and leaf samples for physio-biochemical parameters were collected. After STI calculation from data under control and stressed conditions, correlation test was performed on 96 parameters. Based on associations among phenomic data (24 attributes) and physio-biochemical markers (29 for I, 41 for H and 31 for I+H for kabuli and 37 for I, 36 for H and 33 for H+I for desi genotypes) relative stress tolerance of genotypes was accessed. Among kabuli genotypes for irrigation stress, K-09015 and FG0902 were proved as highly tolerant while K0039-09 as most sensitive genotype. For heat stress, BKK 02174 and CM1235/08 were highly tolerant and 9KCC-166 was found to be most sensitive one. For combined stress, CM 1235/08 and K-09015 were found highly tolerant while FG 0902 as most sensitive genotype. Among desi genotypes, D-09027 was found to be most tolerant while NIFA-2 as most sensitive under irrigation stress while D-0913 was most tolerant and NIFA-2 was most sensitive to heat stress. For combined stress, highest tolerance was observed in D-0913 and CH 24/07 while CMC-211S was proved to be most sensitive. In conclusion, an integrated approach involving phonemics and physio-biochemical markers efficiently revealed out the relative heat, irrigation and combined stress tolerance of chickpea genotypes along with insights in mechanisms involved.

1 Introduction

1.1 Chickpea (*Cicer arietinum*)

Food is among the basic necessities of life. Without this, life is not possible. Basic requirement of human food is consummated from plant sources. Grains are the most important source for completion of human diet. But grains alone are not enough to meet the demands of our body [1]. Remaining portion of diet is gained by the legumes, which are 2nd in importance according to food requirements [2].

Legumes are member of family Leguminosae or Fabaceae, which has up to 750 genera and 20,000 species [3]. This family is categorized at third number as famous among flowering plant species. Important grain, agro-forestry and pasture species are part of legumes [4]. These are consumed by humans and animals as well. Their use as fuel wood, pulp for paper industry, ornamental plants, firebreaks and as living fences are very common.

Legumes are those flowering plants which produces seedpods. These are present in various ecosystems, which include arctic, alpine, rain forests and deserts. These are found in archaeological records early in 37 B.C. Garaham and Vance, 2003 quoted some words of Varro from 37 B.C. that legumes should be grown in light soils as these are good for subsequent crops and recognized the importance of legumes in intercrop and multiple cropping production [3].

Cereals are most important crops on earth as their total production and area is more than all others. After cereals legumes are 2nd most important crops, harvesting about 12-15% arable surface of earth. Low cost of production of legumes gets attention of farmer for planting on even marginal lands and investor for improvement of legumes [5].

Legumes are beneficial to soil as can be used as cover crop. These can be intercropped with cereals to lessen the amount of external nitrogen supply and to increase palatability for animals. Soil gets different benefits as it improves porosity of soil, structure of soil, microorganism diversity and organic matter in soil. These helps to lessen pH of soil, compaction of soil and to recycle nutrients to get maximum benefit from soil [6].

Bengal bean or Garbanzo bean is an old world pulse, now a days commonly known as chickpea. It is one of important crop among bean family and grown over fifty countries spanning India, North Africa, Middle East, America, Australia and southern Europe [7]. In production it is ranked as 3rd most important legume crop. Asia produces 87% of overall world production, while Africa, Americas, Oceania and Europe are behind respectively. Highest yielders of chickpea are China, Mainland, Israel, Moldova and Cyprus respectively.

Cicer is now classified to the tribe monogeneric, while it was included in Vicieae Alef earlier. There are nine annuals and 34 perennial herbs of which cross ability of all was checked out. It has 2n=16 chromosomes in nuclear genome. According to this crossability and fertility of their hybrids, four groups were formed of annuals [8, 9]. *C. arietinum* and *C. reticulatum* are in first group. Its plant are described as branched stems, may be erect of spreading type and sometimes shrubs like many branched, with height of 0.2-1 meter, glandular pubescent, olive, bluish green or dark green in color. Roots are vigorous and can go up to two meter but mostly up to 60 cm only. Imparipinnate leaves with 3-8 pairs of leaflets and one leaflet at the top i.e. rachis ends in a leaflet. Leaflets are elliptic to ovate with length of 0.6-2 cm and width of 0.3-1.4 cm, serrated margins, cuneate base. Flowers are single or sometime two flowers per inflorescence, axillary, length of pedicel 0.5-1.3 cm and of peduncle 0.6-3 cm, triangular bracts, 7-10 mm long calyx, corolla may be of white, pink, purplish or blue with length of 0.8-1.2 cm. ovary is sessile and diadelphous staminal column [8, 10, 11]. Pods contain up to three seeds each and is inflated [12].

Chickpea is further divided into two groups i.e. Desi type (microsperma) and Kabuli type (macrosperma). Desi type chickpea is characterized by pink flowers, anthocyanins in stem as pigments and seed coat thick and colored. Kabuli type chickpea is differentiated from Desi type by white colored flowers, no pigmentation on stems and seeds with thin seed coat, white colored and smooth surface [13]. Another type is present with pea shaped seeds but that is specific to India and is intermediate to desi and kabuli types. Desi type is mostly grown in Asian and African countries and accounts for 80-85% world chickpea production. In Europe ,West Asia, North America and North America kabuli types are mostly grown [14].

In Pakistan desi chickpea cultivars serve as potential source of amino acid, essential fatty acids and trace elements to meet needs of human diet. Different minerals found were P, Ca, K , Zn ,Cu and Mg, which constitutes micro and macro nutrients of human diet [12]. Potassium is highest and manganese is found very low in quantity. Among amino acids Sulphur containing amino acids are limiting while others are in ample quantity [15]. Some of anti-nutritional components are also part of chickpea but its benefits are far more than negative aspect [16].

Pulses especially chickpea is full of those nutritionally important components which lack in cereals. So there is more demand of its production [17]. In semi-arid areas of the world, it is most important part of human diets for those who cannot afford animal protein and also for those who are vegetarian. Proteins and carbohydrates together constitutes 80% of dry mass of chickpea seed [18]. It is good source for vitamins, minerals and dietary fiber and also it is cholesterol free pulse [19].

Chickpea is mostly eaten seed food in the world as different preparations and forms according to one's own preference, regional and ethnic values [20]. In Asia it is used as dhal, flour (basin), in soups and salads, in stews, boiled, roasted, fermented and salted form [21]. These diversified forms provide its consumers with potential nutritional health benefits [22].

Top producing countries are India, Pakistan, Turkey, Australia and Myanmar. Major share in world's production is of Asia in which India is the major contributor. Pakistan can be divided into three ecological zones according to chickpea production. Northern areas with high rainfall, central region has fertile soil but climate is semi-arid. This is also grown under rainfed condition i.e. southern Punjab, where rainfall is flimsy and agriculture is dependent upon rain [23]. Province vise chickpea is grown 82% in Punjab, 9% Khyber Pakhtunkhwa, 8% Sindh and 1% in Baluchistan. In Punjab, 90% chickpea is grown under rainfed conditions. Thal region shares 80% in country's production. Districts included in Thal region are Khushab, Mianwali, Chakwal, Bhakkar, Layyah, Faisalabad and Jhang in Punjab, Dera Ismail Khan, Bannu and Karak in Khyber Pakhtunkhwa, Shekarpur, Jacobabad, Larkana, Nawabshah and Sukkar in Sindh, Dera Allah Yar and Jafrabad in Baluchistan. In 2014 chickpea production in Pakistan was 7.5 million tones, with per hectare production of 757.3 Kg (Figure 1.1, Figure 1.2).

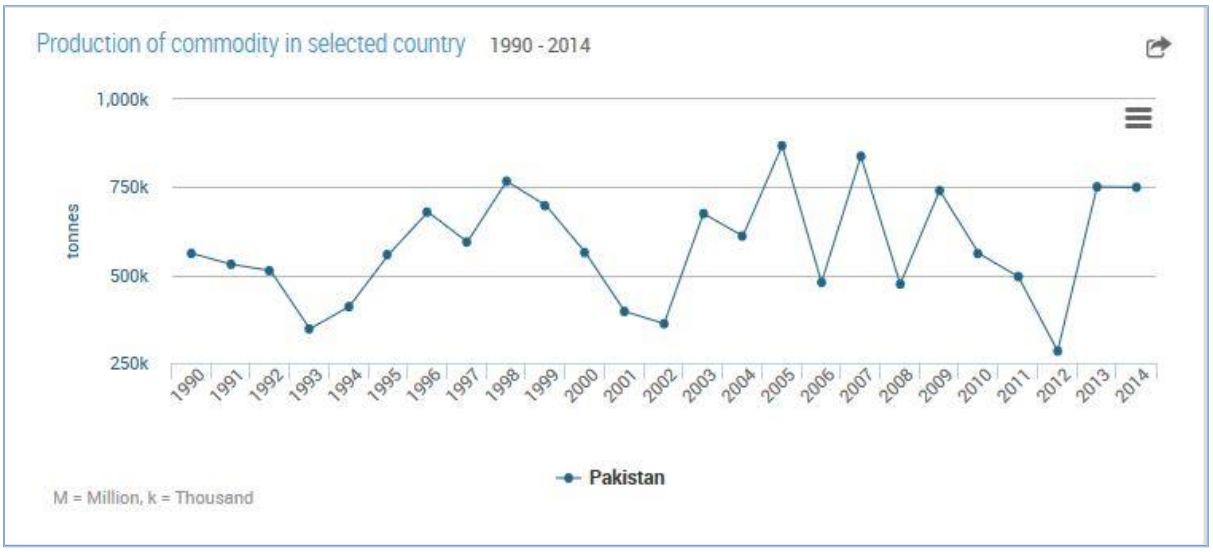

Figure 1.1: Average yield of chickpea in Pakistan from 1990 to 2014

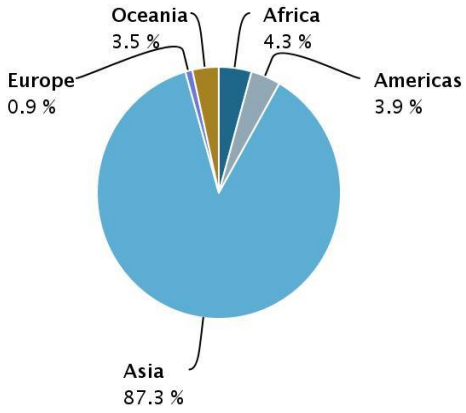

Figure 1.2: Share of different regions in world's chickpea production.

1.2 Climate Change in the World

Land, water and air are so connected on the earth that change in one thing causes a significant change in others as well and this happens through exchange of materials e.g. gasses between each other. Climate of earth is well described by changes in these factors. It is evident that from very beginning up to now earth has undergone many changes regarding climate but changes occurring in last century are so drastic that it has raised the

overall temperature of earth which is said to be warming. It was suggested in IPCC that all this is result of human activities which causes rapid global warming [24].

Now the weather conditions are changing continuously. Average temperature is increasing each year which has increased average global temperature of earth. This increase is due to many factors e.g. glaciers and sea ice is melting, waves of heat are increased, this elevated temperature cause's rise in the sea level, precipitation level is changing which causes drought in one area and heavy rainfall in other area [25]. It is evident from different experiments that these changes are due to human activities i.e. greenhouse gasses. Carbon dioxide oxide is one of the majorly causing greenhouse effect and that's why increasing global temperature. Greenhouse gas keeps earth's infrared radiations and causes an elevation in temperature which would otherwise not happen. Without this effect earth temperature may goes below zero but now it's not happening and temperature always remains above the cold one. Concentration of different greenhouse gasses has dramatically increases since 1960s. These elevation are due to modernization of humans. The thing we owe to be comfortable for us are also harmful on the other side e.g. burning of fossils, industrialization and use of land in different unlawful ways. Thus, climate change is mainly caused by human activities leading to an elevated greenhouse effect [26, 27].

The main function of biodiversity has been recognized, it conserves and increases the comfort of people by supplying life safety, health, vigor and agriculture other than facilitation of works such as biogeochemical recycling and configuration of soil, flood regulation, pest and disease control, etc. There are some hot spots places in few coral reefs, Mediterranean ecosystems and tropical forest that represent high density of species endemism and remarkably fast rates of extinction [28, 29]. Temperature and rainfall participate mainly in findings where various groups of animals and plants can survive, breed and procreate. The events about global environment modification on ecosystems and species may be dual that is direct and indirect. Therefore many direct strikes of changed climate on ecosystems and species. The cover of global change in reaction of changing the climate is expected awaited to happen. Alteration of vegetation zones or biomes will be occur there [30]. It was observed that 25% of eucalyptus in Australia had adapted themselves to 1^{o}C yearly increase in temperature [31].

It is estimated from different events which causes emission of greenhouse gasses, their pattern, our innovative ideas and many other factors as well [32]. It is the reason for them to be more in danger, even for minute alters in global environment. In Australia, huge race of frogs, plants and mammals species possibly limited to some places or under go in danger with rising in temperature of even just 0.5^0C [33]. Climate effects can regulate the ecosystem performance by decreasing biodiversity due to disappearance or changing the habitat of species as well as seagrass biotic communities may be influenced by many modes, depending on depositional methods of erosion and resulting alterations in coastline and their potential to compete. Scientific proofs reveals that these all modification are presumably going on along with the heating, changing the preceding century [34]. These elevations are found to speed up and become further evident over next ten years, while the accurate nature and changing rate of for individuals and for ecosystems is doubtful [30]. Climate change may force some plants and animal species to change because they are ineffective to live in their altering environments, which creates a problem for biodiversity hotspots conservations as listed by natural world heritage site [35].

Last century is evident of such climatic changes which caused to occur drought events and even heavy rainfall than normal, US is one of them where heavy rainfall is seen now a days. This resulted in damage to field crops and other damages caused by floods [36]. Population is growing dramatically which ultimately increases human activities. Hence rainfall increase is evident and flood related damages will definitely increase. The changes what we see now is a great evidence that in future heavy climatic changes will be seen and results will be more vigorous than now [36].

In south Asian regions, climate change is distinctively of vital concern, where change in climate has major effect on economies and societies of human. Many states are ecologically and geographically have high populations diversity living along with coastal lines and water course are at prompt danger of extinction because of climate change. Similarly, the high life reliance on agriculture maintains many communities at elevated risk. The evidence, indicated effects of environmental modifications is rising. Decrease in crop yields in major areas of the world, partly as events of temperature increase, while elevation in regularity of climatically distorted diseases have also been detected. The main dangerous warning coming from environmental modifications in Asia is availability of

clean water which is proceeded to turn down particularly in whole river and will gratingly affect near about one thousand million people in the year 2050 [37].

IPCC (intergovernmental panel on climate change) in 2007 summit discussed and published their report regarding climate change. According to them moderate increase in temperature is helpful for crop species. Pasture crops will get benefit from moderate temperature elevation, while this will cause an increase in yield of tropics and semiarid area crops. There are useful effects of rise in temperature and even by heavy rainfall in temperate regions but damages are more. Tropical areas showed complete negative response of field crops towards even mild changes in temperature. We can predict that further increase in temperature will cause more drastic effects up to end of current century. On the other hand this can be an adaptation process for crops as well because crops are mitigating slight change in temperature and are adaptive to it. Due to heat stress floods and droughts are becoming part of specified areas resulting in damage to field crops and grazing animals as well. All this is generating surprise change in climate and surprise effects on the earth [38].

1.3 Current Scenario of Climate in Pakistan

Among the south Asian countries Pakistan is the second largest country which consists of hot or cold spheres with little productivity. Change in geography all around the state, forming the immense alterations in the country climate. Climate changes from winters and hot, cold and dry summers in the arid to north and semiarid sphere in south and west. Change in climate reveals these areas to dangers of glacial move back, rise in the sea floor, temperature increase, more floods and water stress. Its most of the area is dry and partially dried, furthermore shifts in heat level and schedule of rainfall in the coming days could encroach on its food safety and millions of its inhabitant's profits. Pakistan is most convincible to the results of altering climate for the reason of its agriculture basic and mostly reliance on innate resources for people. As our economy resource is mostly agriculture, the effect of altering temperature level is tending to be dangerous in this zone. Pakistan food safety measures has been in danger and a decrease in yearly crop yield is brought about by numerous factors contributing are high environmental temperature, desertification of land, increased water logging, growing frequency of pest attacks and catastrophes has been found [39].

Change in climatic conditions tend to change the susceptibility of any crop to disease, insects, floods and storms. As a result productivity of a crop species is reduced. In Asia, productivity was found to decline due to elevated temperature, high rainfall, floods and drought. Recent records indicate drastic reduction in yield due to water logging, salinity and floods. Sensitivity of crop depends upon available temperature and humidity. It was recorded that $1^{o}C$ increase from ambient temperature of wheat causes 6-9% loss in yield. This reduction is even more in case of cash crops e.g. maize and mango [40].

In general, the climate fluctuations are now presented as "global warming." The increase of the normal temperature of atmosphere near the surface of the earth and oceans as of the mid-20^{th} century and its expected persistence is called global warming. The global warming results elevations in snowfall and melting of glacier, extreme alterations excessive events as precipitation, floods, hot and cold, sea altitude increase and departure of biodiversity [41, 42]. The impingement of global warming is major on falling winter blizzard in complete winter rainfall on the wind part and most areas of the leeward sides of the Himalayan Range since 1991, which tend to be late in beginning of wintry weather and before spring time and efficient decrease throughout the snowfall timing [43].

In Pakistan in order to find out the temperature variation, the past sheers given by various researches have been in process. In Pakistan the whole area has been divided into eight parts for demonstrating the precedent temperature sheers [44]. As explained before, the rise in environmental temperature is currently given major significance and it is viewed as global warming. The result of heat fluctuations in increasing manner on river rates of flow in Pakistan could be more radiated from region to region.

In 2012 a study was conducted to find overall condition of climate change and its impact on Pakistan's climatic conditions. Country was divided into different zones for study. Climatic data set was taken from meteorological department and was analyzed by software. It was concluded that Pakistan is facing a decline in rainfall. Major problem was due to dry season from 1998 to 2001 when there was water scarcity. If this area is excluded as dry period then rainfall trend was normal and good enough for living beings. But due to this scarcity now there is decreasing trend in average rainfall. Northern and coastal areas showed more pet decreasing trend [45].

1.4 Heat Stress in Plants

Elevated temperature beyond a specific verge level for a specific period of time which causes an irreversible damage to development and growth of plant is said to be heat stress. In usual terms, rise of 10-15^{0}C from ambient temperature is thought to be stress of heat or heat shock. This heat shock is dependent on three factors i.e. how much is intensity of temperature? What is duration for exposure to elevated temperature? And what is rate of elevation in temperature. All these factors determine the level of heat shock. It is discussed that which is best time vulnerable for heat shock, there are many school of thoughts but diurnal temperature is thought to be a better predictor for this [46].

Elevated temperature is a serious problem to worldwide crop production [47]. Different human activities are responsible for emission of gasses e.g.CO_2, chlorofluorocarbons, methane and oxides of nitrogen. These greenhouse gasses are adding up in the ambient temperature of world and temperature is rising. According to a report it is estimated that in each decade 0.3S^{o}C temperature will be increased and will reach up to 3^{0}C in 2100 [48]. This will cause alteration of crop seasons and pattern of geographical distribution of crop plants. Ultimately early crop maturity will be the effect [49].

Cell death and severe cellular injury is not very uncommon at increased temperature. If a cell is exposed at even slightly high temperature but for longer time it will be dead or injured [50]. Injuries as an effect of heat shock includes increased fluidity of all membranes, protein aggregation and denaturation. Slower injuries include inactivity of enzymes, protein degradation, lost membrane intactness and protein synthesis [51]. Heat stress also causes failure of reproductive stages due to destabilization of mitotic or meiotic stages. These all conditions eventually leads to many other stresses i.e. reactive oxygen species, reduced ion movement and starvation [52].

Whenever a plant is exposed to elevated temperature, several changes at molecular level alter the gene expression and leads to the synthesis of stress proteins as a defense system [53]. Heat shock proteins are synthesized which helps the plants to cope with such conditions by improving photosynthesis, membrane integrity, partitioning of assimilates and use of water and nutrients. These proteins range from 10 KDa to 200 KDa and work as a chaperone [54]. This is one of the adaptive strategies which plant accentuates to cope with but not alone, other mechanisms also work in accordance with this. There are many

plants as well which are unable to synthesize these proteins and may or may not have such strategies [55, 56].

1.5 Plant Responses to Heat Stress

A plant is disturbed at each level of its life stage and each level of growth and development. Different responses at all these stages are given below separately.

1.5.1 Morphological Symptoms

Temperate and tropical climates are more vulnerable to changes in temperature, especially elevated temperature. Sunburn on leaves, stems, branches, scorching of leaves, leaf drop out and abscission, stunted growth of root and shoot, fruits discoloration and ultimately reduction in economic yield are important effects due to heat stress [57, 58]. Modifications due to elevated temperature can be direct or indirect and these responses differ at each phenological stage. If developing seeds are exposed to heat stress, it causes delay in germination or loss of vigor and ultimately poor stand establishment. Dry mass of shoot, assimilation rate and relative growth rate can be reduced. It is observed that in some plants like sugarcane and maize leaf expansion is not affected by heat stress [59].

Many cereal crops are vulnerable to heat stress at anthesis and grain filling stages. In wheat, it causes loss in kernel density and weight if heat stress is for long time. Such reductions also occur in protein, oil and starch contents of maize. Number and weight of grains are susceptible to heat stress [60]. Reproductive stage in tomato is severely affected by heat stress as it causes changes in ovule viability, position of stigma and style, growth of pollen tube, pollen germination, and number of pollens, fertilization and post-fertilization stages as well. Most important effect of heat on tomato is elongated stigma and exerted style. This is proved by different studies that flowers are vulnerable to heat stress from its first day and may last for 15 days [61]. Gametogenesis and fertilization are most vulnerable stages during reproduction. Both male and female sex organs are sensitive to temperature and response is specific to each genotype. It is concluded that responses to heat stress vary with plant species and its life stage. Reproductive stage can be said as the most devastating stage during heat stress [62].

1.5.2 Anatomical Changes

Anatomical changes in response to heat stress are similar to those of drought stress. In general it is said that small cell size, closed stomata, water loss, increased Trichoderma and stomata, and larger xylem vessels are attributes of heat stress [63].

It was observed that in grapes mesophyll cells damaged and permeability of plasma membrane was increased under high temperature [64]. At subcellular level chloroplasts are affected mostly leading to reduced photosynthesis, as organization of thylakoids is disturbed. Loss of grana stacking in membranes is also studied as an attribute of elevated temperature [65]. Shape of chloroplast is changed, clumpy contents are formed in vacuoles, mitochondria becomes empty, cristae are disturbed and stroma lamella is swollen. Eventually respiratory and photosynthetic activities are disturbed. It is proved through studies that sever anatomical changes occur due to heat stress, not only at tissue or cell level but at sub cellular level as well. These changes ultimately results in reduction in plant growth and yield [66].

1.5.3 Phenological Changes

The responses of plant at each stage of its life are different to heat stress and stage of plant determines the severity of damages to plant. This study better demonstrates interaction between stress and plant. Heat stress is categorized as the major factor responsible for decline in plant development [67]. Susceptibility can be due to stage of plant but in general whole plant life is disturbed by heat stress. For example high temperature at day time damages leaf gas exchange [68]. A slight increase in temperature at reproductive stage causes floral buds and opened flower abortion, however diversity is found within and among plant species in this regard. Fruit setting is lowered due to pollen impairment [69]. Cereal crops can tolerate a small range of temperature but if heat stress is at flowering stage, it adversely affects the fertilization process. But sometimes there are many positive effects of heat as well [70]. For example in wheat crop, elevated temperature causes early heading, changes in protein content, improves bread and flour quality. Thus it is important to know that which developmental stage is more vulnerable and damaging to crop species regarding heat stress and even to know that daytime or nighttime temperature is more injurious. This will help to determine tolerance potential of a crop [71].

1.6 Physiological Responses

1.6.1 Water Relations

It is important to maintain the membrane integrity of all biological membranes. Plant always tries its best to maintain water potential and membrane integrity in any situation [72]. Those plants which can maintain these two things can survive through stress conditions and others die. During high temperature, this property of plant is reduced and water can transpire from leaf surface and leaving the plant to die [73].

It was studied in sugarcane that during heat stress, its leaf water potential was changed and its components were also altered [74]. In tomato heat stress changed root water conductivity and leaf water potential as well [75]. Generally increase in daytime temperature causes more damage to plants due to more transpiration. Eventually many other physiological processes are also stopped [76].

1.6.2 Compatible Osmolytes

Many plants have an adaptive strategy i.e. to accumulate low molecular weight molecules in the cell which prevent from cell damage and keep the membranes and organelles intact, these are said to by osmolytes or more specifically compatible osmolytes [77]. These include polyols, sugars, proline, ammonium compounds and sulphonium compounds [78]. An amphoteric and quaternary amine is glycinebetaine (GB) acts as compatible solute under various stresses. Capacity of its production varies from species to species. High level of GB was detected in maize and sugarcane during heat stress [79]. Likewise other crop species i.e. rice, Arabidopsis and tobacco do not produce GB in normal conditions but during stress this is produced [80].

Proline in widely occurring osmolyte in higher plants and is accumulated in larger quantity during stress conditions. During heat stress it acts as a buffer for redox potential of cell [81, 82]. In sugarcane it was reported to accumulate with soluble sugars as well. Soluble sugars may include fructans, mannitol or trehalose. During heat stress trehalose is more important soluble sugar conferring tolerance [82].

GABA (gama 4-aminobutyric acid) is a non-protein amino acid and acts as compatible solute. It is synthesized from glutamic acid through various pathways [83]. It causes cytosolic acidification which helps to be saved from death [84].

We can use marker assisted breeding or genetic engineering for enhanced accumulation of these compatible osmolytes so as to increase tolerance in plants.

1.6.3 Photosynthesis

Plants cannot survive without food and food is prepared by photosynthesis. Any hindrance in this process can limit plant development and growth. Elevated temperature has a direct effect on stroma of chloroplast and thylakoid lamellae so these are primary sites of injury [85]. Under high temperature it is found that chlorophyll a:b is decreased while carotenoids are increased sometimes. Degradation of chlorophyll a and b is more prominent in developed leaves as compared to new growing leaves under elevated temperature. Due to these effects reactive oxygen species are produced more in photosynthetic apparatus [86].

PSII is susceptible to heat stress and its activity is almost stopped during heat. This is due to disintegration of thylakoid membranes where this lies [87]. Heat stress leads to dissociation of OEC (oxygen evolving complex). This results in imbalance of electron transport flow. Manganese atoms are released from complex because of a protein which stabilizes this atom i.e. 33 kDa protein [88, 89]. Heat stress also disturbs other parts of reaction center i.e. D1 and D2 proteins. When PS II activity is reduced, it inhibits electron transport activity and limited reducing power generation [90]. Activity of RUBP is also reduced or diminished but not of rubisco. On the other hand PS I is thermostable and can withstand even longer period of elevated temperature [91].

C3 plants are more vulnerable to heat stress than C4 plants. In C3 rubisco activity is changed, RuBP regeneration is disrupted, electron transport chain is broken and oxygen evolving enzymes are inactivated [92]. Photosynthetic pigments are reduced along with large and small subunits of rubisco, rubisco binding proteins and soluble proteins. Other elements which are disturbed includes starch, sucrose phosphate synthase, invertase and ADP glucose pyrophosphorylase [93-96].

It is studied and proved that increased supply of CO_2 has a positive effect on photosynthetic rate and elevated temperature may increase activity of rubisco but not of photosynthesis [75, 96].

1.6.4 Assimilate Partitioning

There is always movement of water and food particles from one part to another. One part from where movement starts is source and other part where it is transported is called sink.

During heat stress this movement from source to sink is disturbed and there may be reduction in growth, yield and harvest index [97].

A very little is known about this mechanism but a case study is available in wheat. It was seen that photosynthesis has a wider range for temperature fluctuations but partitioning of assimilates is susceptible to heat shock. A slight change in temperature changes the movement and hence development processes. Movement of particle from stem was independent of temperature but flag leaf's optimum temperature was 30^0C [98].

1.6.5 Cell Membrane Thermostability (CMT)

Growth and development of plants keep on going if all biological membrane are intact and water potential is maintained [99]. Membranes are made up of lipid bilayer which is susceptible to heat and upon elevated temperature bonds of lipids are loosened and membranes become leaky. As a result cellular material is leaked and water potential is not maintained [100].

Those plant which can withstand at high temperature and can maintain their membranes intact are adaptive to heat stress. This is thought to be a parameter for measure of heat tolerance. But this is further to be investigated that for which plant species CMT can be a measure for heat tolerance because for wheat and sorghum it is not related to heat stress or economic yield [101].

1.6.6 Hormonal Changes

Throughout the plant, hormones play a vital role in signaling to each and every aspect. So it's important to maintain hormonal stability, homeostasis, contents, compartmentalization and biosynthesis [102].

Abscisic acid is one of stress tolerant hormones. It is involved in regulation of physiological processes as signal molecule. In creeping bentgrass its level was not increased during heat stress, but after recovery from stress it was accumulated suggesting that it has role in stress. ABA is also involved in gene expression and up or down regulate many genes to cope with heat stress [103].

Ethylene is a gaseous hormone involved in almost all growth and developmental stages from germination to fruiting and in stress conditions as well. Its biosynthesis is different at different times i.e. more at day time. Normally it is seen that rising temperature up to 40^0C its accumulation is increased while at 45^0C or after this its production is ceased.

Accumulated (1-amino-cyclopropane-1-carboxylic acid) ACC is also converted to ethylene at high temperature [104].

Salicylic acid (SA) is a component of signaling pathway and systemic acquired resistance (SAR). SA stabilizes the proteins and provokes transcription factors for heat shock proteins hence improving thermos tolerance. It also removes H_2O_2 i.e. helps to remove reactive oxygen species [105-107].

Role of cytokinin and gibberellins is antagonist to ABA. It was found that heat tolerant varieties stopped producing gibberellin under [108] heat stress. In creeping bentgrass, levels of different cytokinins and gibberellin was detected very low during elevated temperatures. There is another class of phytohormoes i.e. brassionsteroids. These have shown potential for conferring thermotolerance in oilseed and tomato crop but not in cereals [109].

1.6.7 Secondary Metabolites

A variety of elements synthesized during primary carbon metabolism in different pathways i.e. shikimate, methyl erythritol phosphate (MEP) and phenylpropanoid. Different phenolic compounds are responsible for thermotolerance. Principal enzyme in this process is phenyl ammonia lyase (PAL), which increases during heat stress and protects the cells by further provoking a series of reactions [108].

Carotenoids are widely distributed in plant kingdom and are responsible for their variety of roles, especially in stress conditions. For example, xanthophyll are involved in phytoprotection. Zeaxanthin is present in periphery of light harvesting complex where it gives protection from oxidative stress. Lipid phase of thylakoid membranes are also protected by xanthophyll and isoprene. These save the membranes from being leaky and keep them intact [110, 111].

Phenolics are most prevalent class of secondary metabolites e.g. flavonoids, lignins. Thermotolerance is conferred by accumulation of phenolics, increased PAL, decreased peroxidase activity and polyphenol lyase activity. Reproductive parts of plants show decreased level of anthocyanins while vegetative parts show elevated level of anthocyanins upon heat stress [112, 113].

Isoprenoids are synthesized by mevalonate pathway. As it is volatile in nature and of low molecular weight, it emits from leaf surface. By its emission photosynthesis is carried

out normally even at high temperature. So there is correlation between its emission and heat tolerance. It also prevents from oxidative stress by directly reacting with singlet oxygen [114, 115].

1.7 Mechanism of Heat Tolerance

Plants have wide range of mechanisms for their survival. Under heat stress plants can shorten their life cycle so as to escape from period of high temperature and complete its life cycle earlier. They may have acquired some morphological adaptations or resistance which transfers to its progeny as well [116]. Some plants are adapted so as during stress period they stop their growth and renew themselves when favorable conditions are available. These are overall different strategies which a plant can adopt or may have in its genes to combat with stress environment. But specifically talking about heat stress, plants have evolved specific mechanisms which are described below [117].

Whenever any fluctuation is detected from normal environment, it triggers many signaling cascades. It triggers transcription factors and stress related genes. These genes with their proteins protect the cells and maintain homeostasis [118]. Any response inadequate to a signal at any step can lead to malfunctioning of cell or cellular organelles leading to cell death. Plants growing even in their natural habitat can experience temperature fluctuations. So they need a more general mechanism for thermotolerance so as to survive [119].

Major mechanisms to counteract thermotolerance includes osmoprotectants, transporters, free radical scavengers, transcriptional controls, late embryogenesis abundant proteins and factors for signal cascades [50, 120]. Any cascade of reaction starts with perception of signals regarding elevated temperature. Heat stress is notable on membranes and biochemical reactions of plasma lemma. Initial effects are on plasma lemma due to which membrane fluidity increases. It provokes Ca^{2+} influx and reorganization of cytoskeleton, leading to upregulation of MAPK (mitogen activated proteins kinase) and CDPK (calcium dependent protein kinase) [119]. This triggers production of compatible osmolytes and antioxidants at nuclear level [102]. Reactive oxygen species are produced at cellular level to actively carrying on signaling within and between cells and for production of antioxidants as well. Antioxidant defense mechanism is of immense importance regarding thermotolerance [78].

Another important and keenly studied mechanism for heat tolerance is production of heat shock proteins (HSPs). There are many families of HSPs, each family has its own methodology for resistance and unique chaperone activity [121]. These work in a network of chaperones in which each chaperones work as in a concert. These also interact with other stress responsive mechanisms [122].

Membrane integrity is key to heat tolerance. Upon elevated temperature lipids melt and integrity is lost. To cope with this situation, plants accumulate saturated fatty acids in membranes which are resistant to heat. It is investigated in wheat that upon high temperature linolenic acid concentration is increased which confers tolerance. Low molecular weight HSPs also has protective role in electron transport of photosynthesis thus making this process normal [123, 124].

Heat stress alter the patterns of gene expression including those of HSP and inhibiting many others which are antagonist to HSP. Messenger RNA of non HSPs and non-responsive to stress are destabilized in some cases [106, 122].

1.8 Heat Stress in Chickpea

Elevated temperature or heat stress alone is not so detrimental but it is a combined effect of temperature, water status, soil type, wind and genotype of the plant [125]. Severity or occurrence of heat stress varies from time to time and all other factors as well. Depending upon these factors, stress can be categorized as acute and chronic heat stress. Acute heat stress is more prevalent and is for short time period it causing only yield loss. On the other hand chronic heat stress can occur at any stage of plant growth and is not so common. In chickpea case it is observed that along with yield loss, death of plants may occur [99,126,127].

1.8.1 Effect on Crop Establishment

Temperature at sowing time and germination is of immense importance as it is directly related to yield of chickpea. It is observed that 10-15 ^{0}C is optimum for chickpea sowing [128]. Germination optimal temperature is 22-35 ^{0}C while 31.8-33 ^{0}C is best for germination because at this temperature germination is faster [129, 130]. At more elevated temperatures, cotyledon mobilization is reserved and growth of embryo is badly affected. Response to heat stress in chickpea is genotype dependent. At temperature 45-48 ^{0}C germination percentage becomes zero [130].

Photosynthetic rate is lowered and transpiration rate is much higher at high temperature which results in poor stand establishment. Temperature also affects at flowering stage. Flowering is a linear function of mean temperature. If temperature is high then flowering is affected badly leading to lower yield [8].

1.8.2 Effect on Yield and Reproductive Stage

Chickpea has small size flowers with diadelphous stamens. It is self-pollinated crop and self-pollination takes place before opening of flower and pods are formed five to six days after pollination [131, 132]. If temperature is increased at this stage it affects pollination, flower bud opening, pod formation and ultimately yield loss. Hot and dry conditions leads to unopened flower buds [132].

If temperature is elevated after opening of flowers then yield is reduced as number of seeds and weight per seed is severely reduced. At 35 ^{0}C yield is lowered and above this pods are not formed [133]. Period of anthesis and seed set are critical stages vulnerable to heat stress. It was proved that male and female organ development is most vulnerable to heat stress. Hence some of these characters can be used as indicators of heat tolerance i.e. stigma receptivity, pollen viability and ovule viability [134, 135].

Before onset of anthesis, heat affects anther development, pollen production and sterility. Key factor for legumes' yield determinant is pollen sterility. There are eight stages in chickpea for pollen development, out of which two are most vulnerable to heat i.e. microspore mother cell (MMC) meiosis and mature microspores. Studies on pollen of chickpea are a bit behind others but it has been studied for cold tolerance only where meiosis stage is not affected. But in case of heat stress, meiosis is severely affected [136-138].

Reduced pollen viability is common to all legumes. At temperature from 7-25^{o}C it was observed that pollen germinations was 80-90%. As temperature is increased from this range pollen viability and germination is reduced. At higher temperatures pollen abnormalities are detected. Anther indehiscence in beans is common. Due to sterility of pollens flowers abortion occurs. But for this parameter there is huge variation present in chickpea germplasm [139, 140].

Heat stress is also detrimental to chickpea at post anthesis stage. This is characterized by loss of stigma receptivity, poor pollen germination and growth, failure of

fertilization and ovule formation. Heat also effects flowers in such a way that there is lack of synchronization in male and female organs. Stigma receptivity in chickpea is reported to be lowered at low temperature but not studied on high temperature. But it is hypothesized that due to low exudate on stigma at elevated temperature, receptivity will be lowered or completely lost [141-143].

Working on heat stress, day temperature is normally taken and studied but it is observed that night temperature also play a vital role in legumes and affect seed setting. At high night temperature anther indehiscence, reduced pod development, shrunken and small pollens are observed. Hence diurnal temperatures play a role in chickpea reproductive organ development [144, 145].

Seed setting depends upon time duration and rate of embryo growth, which is affected by higher temperature. It leads to small endosperm and embryo abortion. Endosperm filling is done by movement of carbohydrates to seed from other parts. When temperature goes up mobilization of photosynthates is stopped thus endosperm remains unfilled. This results in reduced seed size and lower weight of the seed [145-148].

1.8.3 Effect on Physiology

Photosynthesis is important physiological parameter to study effect of stress on plants. High temperature affects directly the rate of photosynthesis and it has a negative linear relationship with each other. Best temperature for photosynthesis is 22 ^{0}C. As temperature goes high, rate of photosynthesis is lowered. Photosynthesis rate and duration is determined by available assimilate and environment [149, 150].

Electrolyte leakage is a measure of membrane integrity, also said to be cell membrane thermostability. However there is no evidence regarding relationship of membrane integrity and seed setting or yield. But this is used as measure of heat tolerance [151, 152].

Leaf senescence and assimilate partitioning are other important parameters which can disturb seed and pods setting. At high temperature assimilates are unable to move to seeds, decreasing yield of chickpea. However limited study is available in chickpea regarding physiological parameters in heat stress [153, 154].

1.8.4 Effect on Nitrogen Fixation

Root nodulation is amazing feature of legumes which helps to fix the nitrogen in such a way that is available to plants. Heat stress causes impairs nodule function, lower nodule formation and deformed nodules. Slight increase in temperature delays nodule formation, decrease nitrogen fixation and reduces symbiosis [155, 156]. Optimal soil temperature defined for chickpea growth and development is 22 ^{0}C. If soil temperature goes up to 35 or higher then nodules are not formed even if some are already formed they do not work properly [156].

Mostly nitrogen is fixed during vegetative phase in chickpea. It starts decreasing after pod formation. From different studies it is observed that temperature above 30 ^{0}C, deterioration in nodules and nitrogen fixation starts [157].

1.9 Rainfall Stress in Plants

Downscaling climate change processes has intensified the necessity for correct evidences about distribution of climate variables on different time and space scales. In the case of rainfall a comprehensive study is required as intensity of rainfall is more affected than other factors of climate. Hence to assess and construct actual climate scenario it is needed to collect and analyze local and global climatic data [158]. Simulation of GCMs shows that there is increase in emission of greenhouse gasses which ultimately increase daily mean temperature and precipitation events on a global [159, 160] and local scale [161, 162]. However models still have restrictions that disturb the simulations of the extreme events in terms of spatial purpose, it is predictable that changes in daily rainfall systems are reliant on local factors [163].

On the other hand, there is indication in some areas of the increasing incidence of wet days and an increased rate of total rainfall occurring during the heaviest events [164, 165] but only few regions have been studied on a daily basis because high quality data are desirable, these data are not, in general available [166] and because there are complications in defining trends of very infrequent events [167]. In this situation, the relationship between annual precipitation and daily precipitation events is a priority research task in environments with limited rainfall events and where temporal rainfall variability is high, such as Mediterranean climatic regions. In these atmospheres, the annual maximum rainy day (or few) is able to produce enormous quantities a few events can severely change

monthly, seasonal or annual rainfall, and all of them are capable to disturb the water cycle and environmental processes [167].

Water organization (WO) is an organization which is handling water resources and their conservation regarding climatic changes and its impacts on field crops. When time of rainfall and its intensity is changed then water resources and its usage is changed [168]. It is evident from experiments that extreme conditions are more important to describe than mean climatic conditions because extreme conditions results in drastic conditions [169-172]. Generally, both the time and intensity of rain in a specific area are important events as an ecological process [173, 174].

1.10 Plant Responses to Rainfall Stress

Water present in soil is a determinant of species distribution all over the world. It can change into stress e.g. waterlogging, drought, excessive or shortage of water. Crop production is limited in tropics and subtropics due to excessive rainfall. Excessive rainfall causes floods. Flooding is the stress when plants are submerged in rain water and are unable to get oxygen from soil which harshly affects crop production. It is now a major problem of some areas in the world. Rain fed areas are more subjected to rainfall stress, especially those soils where drainage in very poor. Floods and excessive rainfall causes waterlogging, which disturbs 10% of land from all over the world and hence it is major constraint in agricultural production.

Yield loss due to waterlogging ranges from 15-80%. Intensity of loss depends upon time span of stress, crop species and type of soil. Flooding compared to other stresses, critically decreases yield as considered by FAO and International Institute for Applied Statistical Research in their experiments locally and worldwide [175].

Leaf is a good friend of plant in rainfall stress as it respires and photosynthesis is carried out. Actually chlorophyll fluorescence is affected due to heavy rainfall. In this condition major decrease occurs in quantum yield of photosystem [176]. This may be due to changed hormonal status, mineral contents and stomatal conductance. All these affect non-photochemical quenching, photochemical quenching and electron transport chain. After rainfall, reclamation of soil is necessary, if it is not done crop species cannot withstand. Ideal rainfall tolerant species is one which tolerate waterlogged condition and

recovers itself quickly. There is variation present in plants regarding ability to recover, some can recover quickly while others don't [177].

1.11 Physio-Chemical Changes during Rainfall Stress

1.11.1 Change Rhizosphere Chemistry

Soil redox potential is dependent on concentration of water available in soil. When excessive water is present soil redox potential is severely reduced which changes composition of soil profile. Depletion of oxygen in soil provokes microorganisms to use nitrates from soil as electron acceptor. Hence soil solution is filled with ions more than toxic levels. However toxicity of these cations depends upon pH of soil e.g. manganese is harmful in acidic soils. Flooding results in neutrality of soils due to which many nutrients becomes unavailable to plants. As the stress progresses redox potential is further reduced along with sulphate and hydrogen sulphide ions. Consequently methane formation from the reduction of carbon dioxide and definite organic acids, is started at redox potentials of 200 mV (at pH7). Repressed gas exchange during waterlogging leads to high partial pressure of CO_2 (pCO_2) in the roots. Hence plant metabolism and root growth is severely affected. During extended period of waterlogging many phenolic and short chain fatty acids are mixed with soil.

As nutritional composition is changed in soil, plant growth is affected negatively. In internal parts of plants endogenous levels of nutrients is reduced [178]. Root zone becomes deficient in oxygen so plant is unable to select sodium or potassium and hinders in transportation of sodium. The early senescence of leaves and the stunted growth of shoots in flooded plants are affected by the inhibition of nitrogen (N) uptake and the consequent redistribution of nitrogen inside the shoots. Moreover, the hampered effectiveness of photosystem (PS) II is recognized to the deficiencies of N, P, K, Ca and Mg occur.

1.11.2 Plant Metabolism and Energetic Alteration

Aerobic respiration may be hindered due to activity of microorganisms and roots respiration, which leads to conditions said to be hypoxia or anoxia. Here oxidative phosphorylation get hindered so plants tend to change their metabolism to anaerobic mode. As oxygen in not available so electron transport chain and intermediate compounds or electron carriers becomes completely unavailable or reduced. This disturbs the metabolic

activities requiring redox active ingredients. Cells needs to maintain their redox characteristics i.e. NADH should be unaffected under flooding [179].

Reactive oxygen species are produced in response to any stress in higher quantities while in normal cells these are present in moderate concentrations for signaling. When electron transport components are saturated and intracellular components are highly reduced due to excessive water in root zone, it produces ROS in elevated concentrations. ROS generation results in inactivation of enzymes, lipid peroxidation and damages DNA [180]. It is studied that in flooding situations how calcium concentration is elevated in the cell and membrane permeability is disturbed. ATP synthesis is reduced or completely stopped, photosynthesis is reduced and mitochondrial respiration is also disturbed. Tolerant genotypes are those which can maintain their energy level in flooding situation through fermentation [181].

1.11.3 Alterations in Physiological Mechanisms

One of the immediate response to soil flooding is a decrease of stomatal aperture and stomatal conductance. Low O_2 level may also decrease hydraulic conductivity (*Lp*) subsequently to reduced root permeability. The reduction in *Lp* may associated to aquaporin's gating by cytosolic pH [182] and also limit water uptake thus leading inner water shortage [183, 184]. O_2 scarcity usually brought a quick decrease in the rate of photosynthesis which is normally considered as consequences of reduced stomatal aperture, reduction in leaf chlorophyll contents, early leaf senescence and a decrease in leaf area may also contribute to inhibition of photosynthesis in later stage. Indeed flooded soils tends to decrease the translocation of photosynthesis product from 'source' leaf to 'sink' root [185] as a results the maintenance of photosynthetic activity and accumulation of soluble sugar to roots is obviously an significant adaptation to flooding. In vegetables flooding caused a noticeable decrease in stomata conductance of bitter melon. This reduction resulted in increased leaf water potential [186]. In contrast, it is observed that there is no significant correlation was found between water potential and stomatal conductanc of okra plants under flooded conditions [187].

1.11.4 Anaerobic Metabolism of Roots

In excessive water, oxygen is unavailable to plant roots so they have to respire anaerobically. Additionally proteins are formed by other mechanisms and anaerobic

polypeptides (ANPs) are formed [188]. Till now many ANPs have been categorized and studied by molecular approach or protein chemistry. All ANPs are glycolytic enzymes. Among these, ADH is most important and widely found in different studies. Its isozymes are found in different plant species during flooding, each with its unique properties [188-194].

1.11.5 Photosynthetic Capacity of Mesophyll Cells

There is fluctuations in activation of Rubisco, in excessive water it has been observed that its activation level is carbamylation [195], which is controlled by intensity and duration of light, and CO_2 assimilation [196]. Generally when CO_2 assimilation is increased, activity of Rubisco is decreased [196, 197]. Activation level of Rubisco was noticed to be 92% when CO_2 assimilation was 240 µbars in control plants [186]. Under elevated level of carbon dioxide assimilation the activity of Rubisco was found to decrease and the level of phosphoglycerate (PGA) to rise in different plant systems [198, 199].

Gaseous exchange in leaves was observed to reduce photosynthetic capacity of crop plants during excessive water. Flooding results in reduced capacity of leaves for gaseous exchange. Examples are *Lycopersicon esculentum* [200], *Pisum sativum* [201]. When pea plants were subjected to flooding abscisic acid level was arisen along with stomatal closure [202, 203]. Same results have been witnessed in tomato [204] and apples [205]. Water potential of leaf is also increased which fluctuates conductance of stomata [186]. Hence it can be concluded that increased water potential in leaf results in stomatal closure, which reduces transpiration rate which ultimately saves the leaves from dehydration [206].

1.11.6 Anatomical and Morphological Modifications

Excessive rainfall stress is also known to cause a number of anatomical and morphological changes in e.g. formation of arenchyma, hypertrophied lenticels, suberized epidermis and adventitious roots etc. Under rainfall stress the common anatomical change is the existence of hypertrophied lenticels detected in different woody species [207]. Hypertrophic growth results in nearby stem base due to radical cell division and cell expansion. Additionally, it is furthermore supposed to be related with synthesis of ethylene and auxin [208]. Function of lenticels is downward diffusion of O_2 and the compounds that are synthesized as byproducts of anaerobic metabolism (CO_2, CH_4 and ethanol). Though, the exact physiological function of lenticels is still unknown, sometimes they are considered to

creating tolerance in plants under rainfall stress [209]. Moreover, it has been studied that under water surface the number of hypertrophied lenticels are more that helps the argument stating their involvement in preservation of plant water homeostasis. These specialized cells play their role as significant helpers of plants for entry of oxygen to the root system. In plant water homeostasis the role of hypertrophied lenticels is clear as they actively participate in facilitating water uptake for the shoots by replacing crumbling roots [184]. In plants under rainfall stress potential morphological changes takes place as adventitious roots formed that replaced the basal roots [210]. When the basal root system fails the continuous supply of water and minerals under rainfall stress then the adventitious roots helps the plants to do so. Additionally, the failure of the basal root system is supposed as the expense of supplying energy for the formation of well adapted root system [211]. However, in plants under rainfall stress the formation of adventitious roots is directly associated with developing rainfall stress tolerance [212].

In plants one more vital morphological change occurs in the root cortex region is the formation of aerenchyma (lacunae gas spaces). So it is thought that development of lacunae gas spaces is reflected as an adaptive change of the plant in rainfall stress [213]. In formation of lacunae gas spaces two main processes takes place. The first change that takes place which is not related with abiotic stress is constitutive formation of lacunae gas spaces. During the process of tissue development the cells gets detached and formed this type of lacunae gas spaces. As a consequence of cell separation this type of cell death is known as shizogeny and it is not effected by abiotic stress conditions. The other type of lacunae gas spaces formation is termed as Isogeny and it is developed as a result of cortex partial breakdown that appears like programmed cell death and its formation usually takes place as a result of external stimulus like abiotic stress [214].

1.12 Response of Chickpea to Irrigation Stress

A study was conducted to check response of chickpea genotypes to irrigation stress. It was found that irrigation has no significant effect on plant height, number of branches, number of pods, biological yield and grain yield [215]. In another study it was found that doubling the irrigation reduced 30% of dry matter. Lowest number of seeds was obtained and percentage of decrease was 40%. Increased irrigation has no effect on canopy and radiation

interception. It was also suggested that yield of chickpea can be 100% reduced with water logged conditions [216].

It is estimated that heat stress and rainfall stress alters plant's morphological, physiological and biochemical behavior and accounts for about 40–50% of the yield losses in chickpea. Vegetative growth is increased and reproductive growth is delayed. Prolonged conditions of excessive water results in decline of chickpea's vigor and growth [217].

In the view of earlier reported literature, present study was designed with following objectives

- Development of protocol for screening of chickpea against irrigation, heat and combined stresses
- Assessment of effects of irrigation on morphology, physiology, and biochemistry of chickpea
- Screening of tolerant genotypes for irrigation (I), heat (H) and combined stress (I+H)

2 Materials and Methods

Two Experiments were designed for screening of Irrigation (I) stress tolerance, heat (H) stress tolerance and combine (I+H) stress tolerance in 9 Desi and 8 kabuli chickpea elite lines collected from different research centers of Pakistan. The screening experiments were conducted at Nuclear Institute for Agriculture and Biology (NIAB), protocols and conditions for these experiments were optimized. Experiments were layed out in completely randomized design (CRD) with 4 replications to investigate the effect of treatments on morphology, physiology and biochemistry of chickpea.

2.1 Seed Material

To screen the different chickpea lines against Irrigation, Heat and combine stress seeds of 17 genotypes (elite lines) of chickpea were used, 8 of Kabuli type and 9 of Desi type, from different institutes of Pakistan; AARI Faisalabad, NIAB Faisalabad, AZRI Bhakkar, RARI Bahawalpur, NARC Islamabad and NIFA Peshawar (Table 2.1).

Table 2.1: Seed material used in the study

Sr. No.	Desi Chickpea	Source	Sr. No.	Kabuli Chickpea	Source
1	D-0913	AARI Faisalabad	1	9 KCC-166	BARI,Chakwal
2	D-09027	AARI Faisalabad	2	K-09015	AARI,Faisalabad
3	CH 24/07	NIAB Faisalabad	3	CH888/06	NIAB,Faisalabad
4	CH104/06	NIAB Faisalabad	4	CM1235/08	NIAB,Faisalabad
5	TGX 225	AZRI, Bhakhar	5	FG0902	NIAB,Faisalabad
6	BRC -390	RARI,Bahawalpur	6	K0039-09	NIAB,Faisalabad
7	CMC 211S	NARC ,Islamabad	7	BKK 02174	NIAB,Faisalabad
8	NIFA -2	NIFA,Peshawar	8	NOOR-2013	Check
9	Bhakhar-2011	Check			

2.2 Experiment # I: (Screening of Kabuli Type against Heat, Irrigation and Combined Stress of Irrigation and Heat)

The experiment was performed under the net house conditions of (NIAB) where the average temperature was 33ºC and 13ºC of day and night respectively. There were four sets of pots one set for control second set for irrigation stress, third set for heat stress and fourth set for combined stress application. In each set 12 seeds of kabuli genotypes were sown in each pot. For this purpose pots were filled with 1kg of autoclaved sieved pure sand. Then the water holding capacity of the sand were measured which was 25% and according to measurements added 250ml distilled water in each pot to attain the field capacity conditions in the pots. Then small holes of 2-3 inches were made in the sand and seeds were sown in these holes.

2.2.1 Stress Treatments

After 5-6 days of sowing date average germination takes place. Then hog land solution of one eighth strength was applied on 10^{th} day of sowing to each set. The controlled and heat stressed pots were irrigated at half of their field capacity at 5^{th} day after germinating while that of irrigated and combined stressed pots were irrigated at full of their field capacity at the same day. Second and 3^{rd} irrigation according to above mention conditions were applied at 17^{th} day and 21^{th} day of sowing. After 2 weeks of germination the heat stressed and combine stressed set of plants were shifted in glasshouse for stress application where the average temperature was 43ºC and 20ºC of day and night respectively and experiment was harvested on 30^{th} day. Certain morphological parameters were measured and sample was collected for physiological and biochemical analysis (Figure 2.1).

2.3 Experiment # II: (Screening of Desi Type against Heat, Irrigation and Combined Stress of Irrigation and Heat)

The second experiment was performed under the net house conditions of (NIAB) where the average temperature was 32ºC and 10ºC of day and night respectively. There were four sets of pots one set for control second set for irrigation stress, third set for heat stress and fourth set for combined stress application. In each set 12 seeds of desi genotypes were sown in each pot. For this purpose pots were filled with 3 kg of autoclaved sieved pure sand. Then the water holding capacity of the sand were measured which was 25% and according

to measurements added 750ml distilled water in each pot to attain the field capacity conditions . Then small holes of 2-3 inches were made in the sand and seeds were sown in these holes.

2.3.1 Stress Treatments

After 5-6 days of sowing date germination takes place. Then hog land solution of one eighth strength was applied on 10th day of sowing to each set. The controlled and heat stressed pots were irrigated at half of their field capacity at 5th day after germinating while that of irrigated and combined stressed pots were irrigated at full of their field capacity at the same day. Second and 3rd irrigation according to above mention conditions were applied at 17th and 21th day of sowing. After 2 weeks of germination the heat stressed and combine stressed set of pots were shifted in glasshouse for stress application where the average temperature was 38^{o}C and 17^{o}C of day and night respectively and experiment was harvested on 30th day. Certain morphological parameters were measured and sample was collected for physiological and biochemical analysis (Figure 2.1).

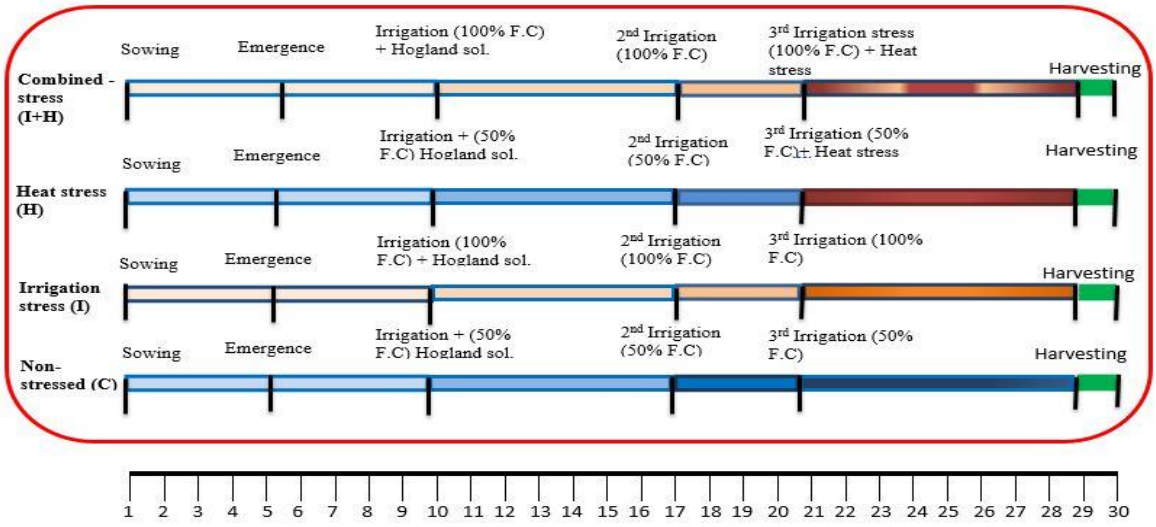

Figure 2.1: Protocol developed for screening against irrigation, heat and combined stress on Chickpea seedlings. Heat stress 43/20^{o}C for Kabuli type, 38/17^{o}C for Desi type, Hogland Solution of 1/8 strength.

2.4 Growth and Morphological Parameters

Samples of leaf for physio-biochemical analysis were collected at 30th day of both experiment and store at -20^{o}C. Seedlings fresh weights, roots fresh weights and shoots

fresh weights were recoded instantaneously after harvesting to avoid evaporation. Shoots and roots were separated and their weights recoded individually and plant biomass was also recoded at the same time. Root and shoot lengths were measured by spreading them on a graduated scale and calibrated in cm. For dry weights estimations, pre-weighted plants were kept in brown paper bags and placed them in oven at 70^{o}C for drying purpose. Then seedling, root and shoot dry weights were measured after complete drying when there was no further reduction in weights.

2.5 Physiological Parameters

Treatments may induced different physiological modifications in the plants that were analyzed. In this study various physiological parameters were assessed in control, heat stressed, irrigation stressed and for combine stressed. Data was collected for all treatments according to the following methods.

2.5.1 Water Relations

The second leaf from each plant was excised at 7.00 a.m. and the leaf water potential measurements were made with Scholander type pressure chamber (Arimad, UK). A proportion of the same leaf used for water potential measurements, was frozen into 2 cm 3polypropylene tubes at -20 ^{O}C in an ultra-low freezer for 2 weeks, after which plant material was thawed and the frozen sap was extracted by crushing the material with a glass rod. After centrifugation (8000×g) for 4 min, the sap was directly used for osmotic potential determination using a vapor pressure osmometer (Wescor 5500). Leaf turgor pressure was calculated as the difference between leaf water potential and leaf osmotic potential values [218].

2.5.2 Relative Water Content (R.W.C)

For measurement of relative water content the leaf samples were detached and their fresh weights were calculated by using a sensitive weighing balance. Then the same leaf samples were placed in small glass tubes filled with distilled water and placed them under dark conditions for 24 hours. After that again weighed them for measuring their saturated weights. Then the same leaf samples were placed in brown paper bags and these bags placed in oven at 70^{o}C for complete drying purpose. When the leaf samples were completely dried then again weighed them for collecting the data of dry weights. After that the relative water contents (RWC) were calculated with the following formula [219, 220].

RWC= (leaf fresh weight –leaf dry weight)/ (leaf saturated weight-leaf dry weight) × 100

2.5.3 Leaf Chlorophyll Florescence Parameters

Leaf chlorophyll florescence works on the principle that photosynthesis is one of the core functions in the physiology of plants. The functional state of photosynthesis has been considered an ideal physiological activity to monitor the vigor of plants. However, the data for chlorophyll florescence were recorded in this study following nomenclature and the literature available on the websites of chlorophyll florescence meters. Leaves that were to be analyzed adapted to darkness for 30 min by attaching light exclusion clips to the leaf surface. Chlorophyll florescence was measure by a portable florescence spectrometer "plant efficiency analyzer" (PEA, Handsatech instruments Ltd) following [221]. After a dark adaptation period of 30 min using specific leaf clips the minimal florescence level (Fo) with all photosystem II (PSII) reaction centers open was measured by a weak red light which was sufficiently low (<0.1 µmol $m^{-2}s^{-1}$) not to induce any significant variable florescence. Maximum fluorescence of dark –adapted leaves (Fm) PSII reaction cent reclosed was measured by a 0.8 s saturating pulse (8000µmol $m^{-2}s^{-1}$). All measurements of F^O were performed with measuring beam set to a frequency of 6000 Hz, whereas all measurements of F_m were performed with the measuring beam automatically switching to 20 kHz during the saturating flash. All the parameters were recorded according to [222]. Using the above fluorescence parameters, the following parameters were calculated:

2.5.4 Minimal Chlorophyll Fluorescence (F_o) of Dark Adapted Leaf

It is a measure of the stability of the light-harvesting complex. Increased values indicate damage of the photosynthetic apparatus while reduced values indicate impairment of the photosynthetic apparatus.

2.5.5 Maximal Chlorophyll Fluorescence (F_m) of Dark Adapted Leaf

Its values indicate the physiological state of acceptor side of photosynthetic apparatus.

2.5.6 Variable Chlorophyll Fluorescence (F_v) of Dark Adapted Leaf

Its values(comparison of non-stressed and stressed leaves) indicate the physiological state of donor side of photosynthetic apparatus (PSII), i.e., oxygen evolving center (OEC) or electron flow from OEC to acceptor side.

2.5.7 Time of Achieving Maximum Fluorescence Yield(T_m)

It gives the time taken by dark adapted leave to achieve maximum fluorescence yield.

2.5.8 Ratio of Variable Chlorophyll Fluorescence and Minimal Chlorophyll Fluorescence (F_v/F_m)

It estimate the maximum primary yield of photochemistry of PSII and provides an estimation of photosynthetic capacity.

2.6 Biochemical Analysis

Treatments induced biochemical modifications in the plants leaves were analyzed. In this experiment various biochemical parameters were assessed in control, heat stressed, irrigation stressed and for combine stressed. These analysis was performed on leaf samples for all treatments according to the following methods.

2.6.1 Extraction of Antioxidant Enzymes

Fresh leaves (0.5 g) of all chickpea genotypes were ground in 2 ml of 50 mM cold phosphate buffer (pH 7.8) and centrifuged at 15,000×g for 20 min at 4◦C. The supernatant was used for the determination of activities of enzymes.

2.6.2 Total Soluble Proteins

For protein estimation in chickpea leaves, 5 µL of supernatant and 95 µL 150 mM NaCl were mixed with 1.0 mL of dye reagent (100 mg). Coomassie Brilliant Blue G-250 dye was dissolved in 50 mL of 95% ethanol and 100 mL 58% (w/v) phosphoric acid and dilute to one litter and the mixture was left for 5 min to form a protein dye complex. Then the absorbance was measured at 595 nm [223].

2.6.3 Superoxide Dismutase (SOD)

For the estimation of SOD activity, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA and 1 mM dithiothreitol (DTT) as described by [224]. The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of [225]. One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT**.**

2.6.4 Peroxidase (POD)

For the estimation of POD, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA and 1 mM DTT. Activity of POD was measured using the method of [226] with some modification. For measurement of

POD activity, assay solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H2O2and 0.1 mL enzyme extract. The reaction was initiated by adding the enzyme extract. Increase in absorbance of the reaction solution at 470 nm was recorded after every 20 s. One unit POD activity was defined as an absorbance change of 0.01 units' min.

2.6.5 Catalase (CAT)

For the estimation of CAT, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer, pH 7.0 and 1 mM dithiothreitol (DTT). CAT was estimated by the following method described by [226]. For measurement of CAT activity, 5.9 mM H_2O_2 and 0.1 mL enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 s. An absorbance change of 0.01 unit's min $^{-1}$ was defined as one unit CAT activity. Enzyme activities were expressed on fresh weight basis.

2.6.6 Ascorbate Peroxidase (APX)

For the estimation of APX activity, 0.5 g plant samples were extracted in 2.5 mL homogenizing medium containing 100 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA, 0.1 mM ascorbate and 2% (v/v) b-mercaptoethanol. For assay of the enzyme activity, the rate of hydrogen peroxide-dependent oxidation of ascorbic acid was determined in a reaction mixture that contained 50 mM potassium phosphate buffer, pH 7.0, 0.6 mM ascorbic acid and enzyme extract. The reaction was initiated by addition of 10mlof 10% (v/v) H_2O_2 and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm for 3 min [227].

2.6.7 Protease Activity

For the estimation of protease activity, leaves were homogenized in a medium composed of 50 mM potassium phosphate buffer (pH 7.8). Protease activity was determined by the casein digestion assay described by [228]. By this method one unit is that amount of enzyme, which releases acid soluble fragments equivalent to 0.001 A280 per minute at 37 ^{O}C and pH 7.8. Enzyme activity was expressed on fresh weight basis.

2.6.8 Malondialdehyde (MDA) Content

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using method of [229] with minor modifications as described by [230] and

[231]. A 0.25 g leaf sample was homogenized in 5 mL 0.1% TCA. Homogenate was centrifuged at 10,000 ×g for 5 min. In 1 mL aliquot of the supernatant, 4 mL of 20% TCA containing 0.5% TBA were added. The mixture was heated at 95^{o}C for 30 min and then quickly cooled in an ice-bath. After centrifuging at 10,000×gfor 10 min, the absorbance of the supernatant at 532 nm was read and the value for the non-specific absorption at 600 nm was subtracted. The MDA content was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1}.

2.6.9 Total Oxidant Status (TOS)

Total Oxidant Status was determined by using a novel automated formulated method [232]. In this assay the ferrous ion-odianisidine complex was oxidize to ferric ion by oxidants reaction was increased by the glycerol molecules which were in abundant quantity in the reacting media. During reaction a colored complex was produced in an acidic media by the reaction of ferric ion with xylene orange solution. The reaction solution's color intensity which related to the oxidant molecules quantity was measured by spectrophotometer (HITACHI U-2800). The assay mixture contained reagent R1 (stock xylenol orange solution (0.38g in 500µL of 25Mm H_2SO_4) 0.4g NaCl, 500 µL glycerol and volume up to 50Ml with 25nM H_2SO, sample extract and reagent R2 (0.0317g o-dianisidine, 0.0196g ferrous ammonium sulphate (II). After 5 min the absorption was measured at 560nm by using spectrophotometer (HITACHI-2800).

2.6.10 Pigment Analysis

The concentration of chlorophyll (*a* and *b*) and carotenoids were determined following the method of [233]. Fresh leave (0.2) were grind well and extracted in 80%acetone at -4 ^{O}C. The extract was centrifuged at 10,000 g for 5 minutes at 4 ^{O}C. Absorbance of the supernatant was read at 645,663 and 480 nm using a spectrophotometer (HITACHI, U28000. The concentration of total chlorophyll contents, chlorophyll a, b and carotenoids were calculated by the following formulae:

Chl *a* (mg/g f.wt.) = [12.7 (OD 663) – 2.69 (OD 645) ×V/1000×W]

Chl *b* (mg/g f.wt.) = [22.9 (OD 645) – 2.69 (OD 663) ×V/1000×W]

Carotenoids (mg/g f.wt.) = [Acar/EM] ×1000 Acar = OD 480 +0.114(OD663)-.638(OD 645)

Where V is the volume of the sample, W is the weight of fresh tissue taken for extraction and Em is 2500.

2.6.11 Esterase Activity

Esterase activity was determined according to method adapted from [234] with some modification assumed. Leave sample of 500mg homogenized in 5 ml phosphate buffer (100mM, pH7.0), containing 1 mM of each EDTA, PMSF, PTU and 20% glycerol by using mini bead beater and then centrifuged at 10,000 g for 20 minutes at 4 ^{O}C. The supernatant was used for enzyme estimation. Different standards (0.1-0.9 µM interval) of esterase from working stock solution were prepared in 1000µl distilled water. Then, added 5 ml of phosphate buffer (40mM, pH 6.8) to all of standards solution including blank, finally added 1 ml of staining solution to each and placed them for 20 minutes in dark at 30^{o}C for incubating with gently shaking. Then absorbance at 590nm was measured .standard curves for a-naphthyl acetate esterase were prepared by using standards.

2.6.12 Estimation of Esterase Activity Using A-Naphthyl Acetate as Substrate

A substrate solution was made by adding 30Mm A- naphthyl acetate in 1ml acetone and added to 99 mi of phosphate buffer (40 mM, pH 6.8).Fast blue BB salt 1%(w/v) in phosphate buffer (40mM,Ph 6.8). and sodium dodecyl sulphate (SDS) 5% (w/v) in double distilled water was prepared separately to make staining solution .quantity of staining solution was taken by adding 2 parts of fast blue BB salt 1% solution into 5 parts of sodium dodecyl sulphate (SDS) 55 solution. enzyme stock was prepared by adding 10µl of enzyme solution to 990µl phosphate buffer (40 mM, pH 6.8).Assay mixture was prepared by adding 1ml of enzyme stock into 5ml of substrate solution and 1ml of phosphate buffer (40 mM, pH 6.8) . A total 5ml of substrate solution was mixed into 1ml of phosphate buffer (40mM, pH 6.8) for using blank as control sample. Finally, all the assay samples including blank were incubated in dark for 20 minutes at 30 ^{O}C with gently shaking .after 20 minutes, added 1ml of staining solution to all the samples including control and again incubate in dark for 20 minutes at 30 ^{O}C gently shaking. Then absorbance at 590 nm was measured using spectrophotometer (U-2800, 122-003 HITACHI, JAPAN).

2.6.13 Total Phenolic Contents (TPC)

A micro colorimetric method, as described by [235] was used for total phenolic assay, which utilizes Folin-Ciocalteu (F-C) reagent. A standard curve was prepared using

different concentrations of gallic acid and a linear regression equation was calculated. Phenolic content (gallic acid equivalents) of samples was determined by using the linear regression equation.

2.7 Stress Tolerance Index (STI)

Stress tolerance index for each parameter was calculated by using following formula.

Stress Tolerance Index (STI) = (value under stress/value under control) × 100

2.8 Statistical Analysis

Screening experiments were conducted in four repeats using completely randomized design (CRD). The significance was ascertained by analysis of variance and Tukey (HSD) test at $p<0.05$ by using XL-STAT software. Values presented in graphs are mean ± S.E. In graphs, bars with different alphabets differ significantly from each other. After stress tolerance index (STI) calculations from data under control and stressed conditions, Correlation and cluster analysis were performed for association and grouping by using XL-STAT 2012.1.02.

3 Results

3.1 Kabuli (White Seeded) Genotypes

3.2 Morphological Parameters

3.2.1 Shoot Length (S.L)

Under non-stress condition, significant differences were observed in tested genotypes for shoot length (S.L) (Figure 3.1). The highest (19.5 cm) shoot length was observed in K-09015 while the lowest (16.12 cm) in BKK 02174. Under the irrigation stress, shoot length was more (21.38 cm) in 9- KCC-166 while least (18.75 cm) in BKK 02174. Under heat stress K-09015 showed higher (21.88 cm) length while BKK 02174 showed least (17.98 cm) length. Under combined stress shoot length was higher (21.35 cm) in K-09015 while lowest (17.9 cm) in FG0902.

As compared to non-stress condition, shoot length was observed as not effected in tested genotypes under irrigation, heat and combined stresses (Figure 3.1).

3.2.2 Root Length (R.L)

Under non-stress condition, significant differences were observed in tested genotypes for root length (R.L) (Figure 3.2). The highest (13.5 cm) root length was observed in FG0902 while the lowest (12.00 cm) in CH888/06. Under the irrigation stress, root length was more (14.37 cm) in K0039-09 while least (11.95 cm) in NOOR-2013. Under heat stress K0039-09 showed higher (14.18 cm) length while K-09015 showed lowest (11.60 cm) length. Under combined stress NOOR-2013 root length was higher (18.10 cm) while CM1235/08 lowest (10.67 cm).

Root length was found to be same in all tested genotypes under all stress conditions except for NOOR-2013 in which it was significantly increased under combined stress. (Figure 3.2).

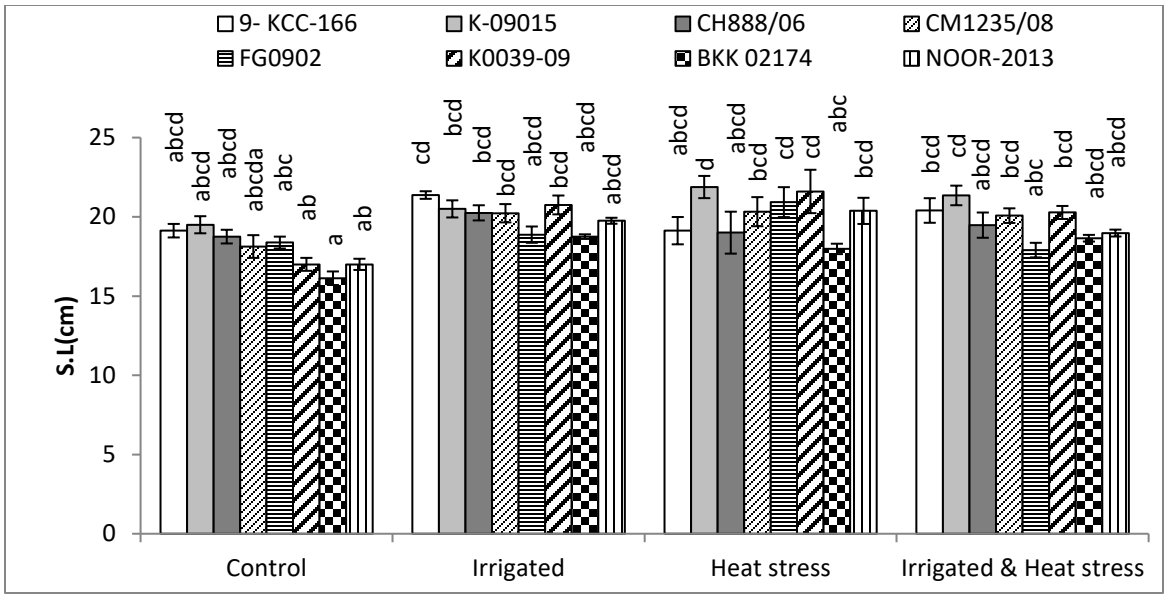

Figure 3.1: Shoot length under control, irrigation, heat and combined stress

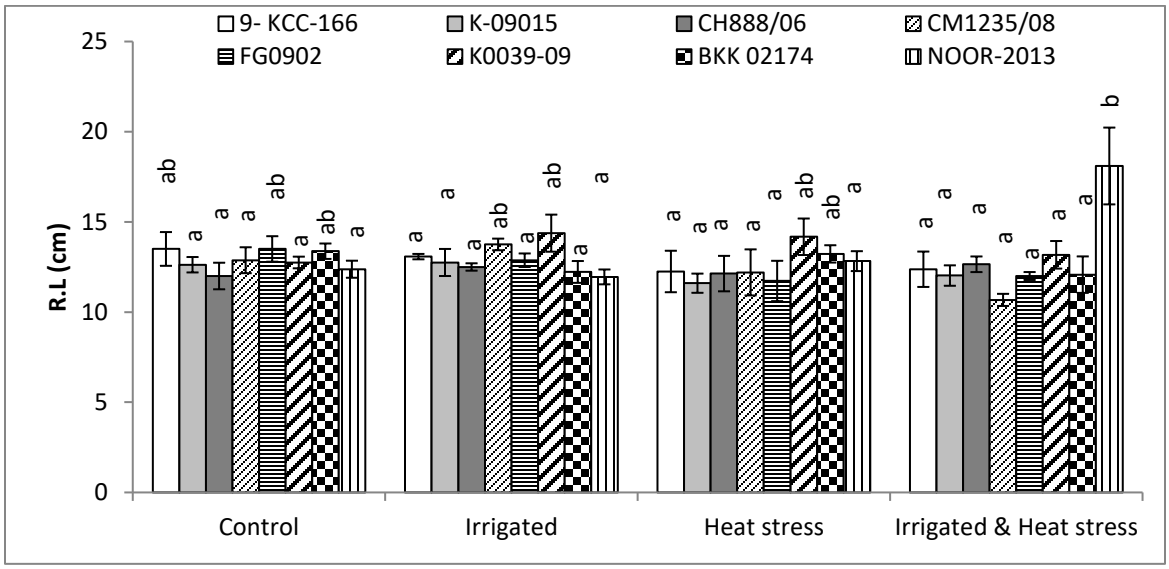

Figure 3.2: Root length under control, irrigation, heat and combined stress

3.2.3 Seedling Fresh Weight (S.F.W)

Under non-stress condition, significant differences were observed in tested genotypes for seedling fresh weight (S.F.W) (Figure 3.3). The highest (3.06 g) S.F.W was observed in CM1235/08 while the lowest (1.96 g) in BKK 02174. Under the irrigation stress, S.F.W was highest (3.36 g) in CM1235/08 and lowest (2.25 g) in BKK 02174. Under heat stress highest (2.28 g) S.F.W was present in CM1235/08 while lowest (1.42 g) were in NOOR-2013. In case of combined (heat and irrigation) stress, the highest (1.90 g) S.F.W was found in CH888/06 and the lowest (1.30 g) value was observed in K-09015.

As compared to non-stress condition, S.F.W was not effected in irrigation and heat stress while under combined stress S.F.W. was decreased in K-09015 and CM1235/08 while other genotypes were not affected (Figure 3.3).

3.2.4 Shoot Fresh Weight (SH.F.W)

Under non-stress condition, significant differences were observed in tested genotypes for shoot fresh weight (SH.F.W) (Figure 3.4). The highest (1.14 g) SH.F.W was observed in CM1235/08 while the lowest (0.85 g) in FG0902. Under the irrigation stress, SH.F.W was highest (1.39 g) in CM1235/08 and lowest (1.08 g) in K-09015. Under heat stress highest (1.11 g) SH.F.W was present in CH888/06 while lowest (0.78 g) was in NOOR-2013. In case of combined (heat and irrigation) stress, the highest (1.03 g) S.F.W was found in BKK 02174 and the lowest (0.69 g) value was observed in NOOR-2013.

As compared to non-stress condition, SH.F.W was found non-significant under irrigation heat and combined stress (Figure 3.4).

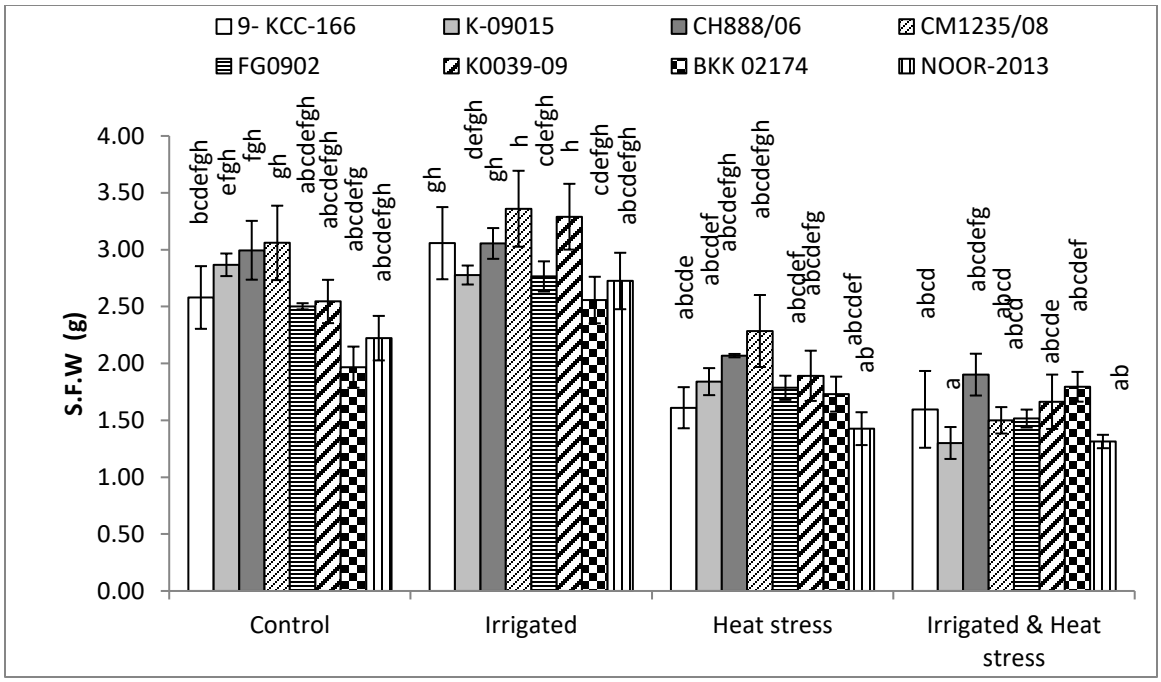

Figure 3.3: Seedling fresh weight under control, irrigation, heat and combined stress

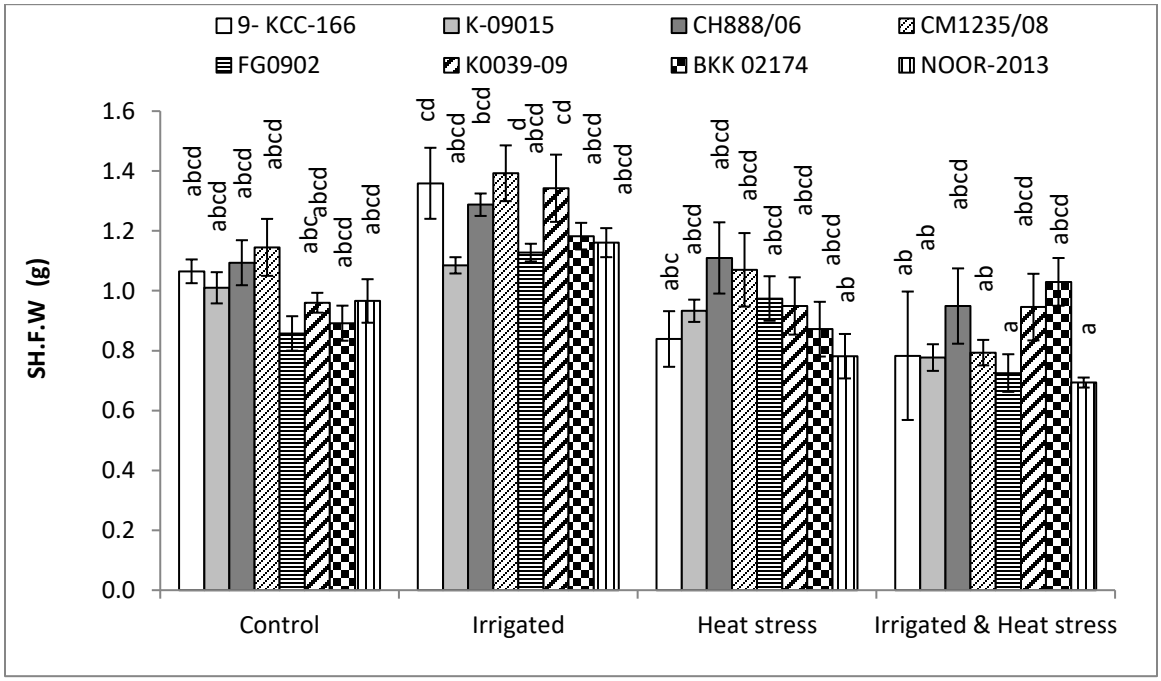

Figure 3.4: Shoot fresh weight under control, irrigation, heat and combined stress

3.2.5 Root Fresh Weight (R.F.W)

Under non-stress condition, significant differences were observed in tested genotypes for shoot fresh weight (R.F.W) (Figure 3.5). The highest (1.91 g) R.F.W was observed in CH888/06 while the lowest (1.07 g) in BKK 02174. Under the irrigation stress, R.F.W was highest (1.96 g) in CM1235/08 and lowest (1.37 g) in BKK 02174. Under heat stress highest (1.21 g) R.F.W was present in CM1235/08 while lowest (0.65 g) were in NOOR-2013. In case of combined (heat and irrigation) stress, the highest (0.95 g) R.F.W was found in CH888/06 and the lowest (0.52 g) value was observed in K-09015.

As compared to non-stress condition, R.F.W was found to be not effected under irrigation stress. Under heat stress R.F.W in K-09015 and CH888/06 was found significantly decreased while in all others remained unchanged. Under combined stress R.F.W K-09015, CM1235/08 and CH888/06 was found to be significantly decreased while others remained unchanged (Figure 3.5).

3.2.6 Root Dry Eight (R.D.W)

Under non-stress condition, significant differences were observed in tested genotypes for root dry weight (R.D.W) (Figure 3.6). The highest (0.13 g) R.D.W was observed in CM1235/08 while the lowest (0.09 g) in BKK 02174. Under the irrigation stress, R.D.W was highest (0.11 g) in K0039-09 and lowest (0.07 g) in BKK 02174. Under heat stress highest (0.11 g) R.D.W was present in CH888/06 while lowest (0.07 g) were in FG0902. In case of combined (heat and irrigation) stress, the highest (0.20 g) R.D.W was found in NOOR-2013 and the lowest (0.14 g) value was observed in 9- KCC-166.

As compared to non-stress condition, R.D.W was found non-significant under irrigation and heat stress. Under combined stress R.D.W was significantly increased in NOOR-2013 (Figure 3.6).

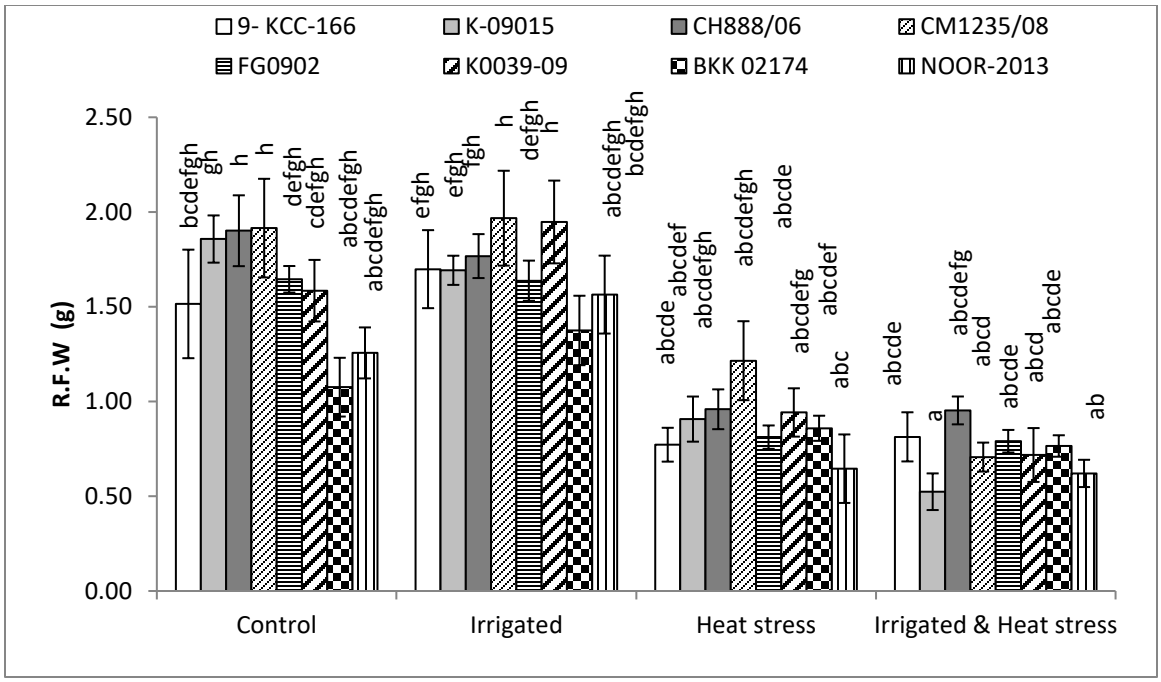

Figure 3.5: Root fresh weight under control, irrigation, heat and combined stress

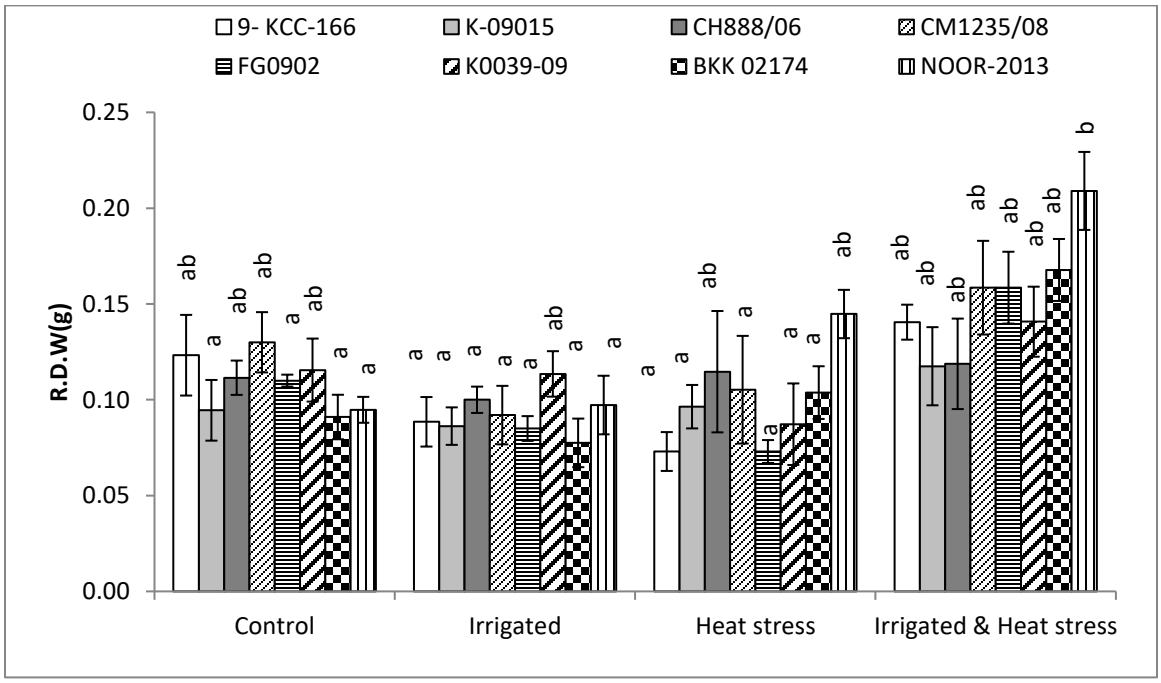

Figure 3.6: Root dry weight under control, irrigation, heat and combined stress

3.2.7 Seedling Dry Weight and Shoot Dry Weight

Under non-stressed, irrigation stress, heat stress and combined stress non-significant differences were observed among all genotypes for seedling dry weight and shoot dry weight (Figure 3.7,

Figure 3.8).

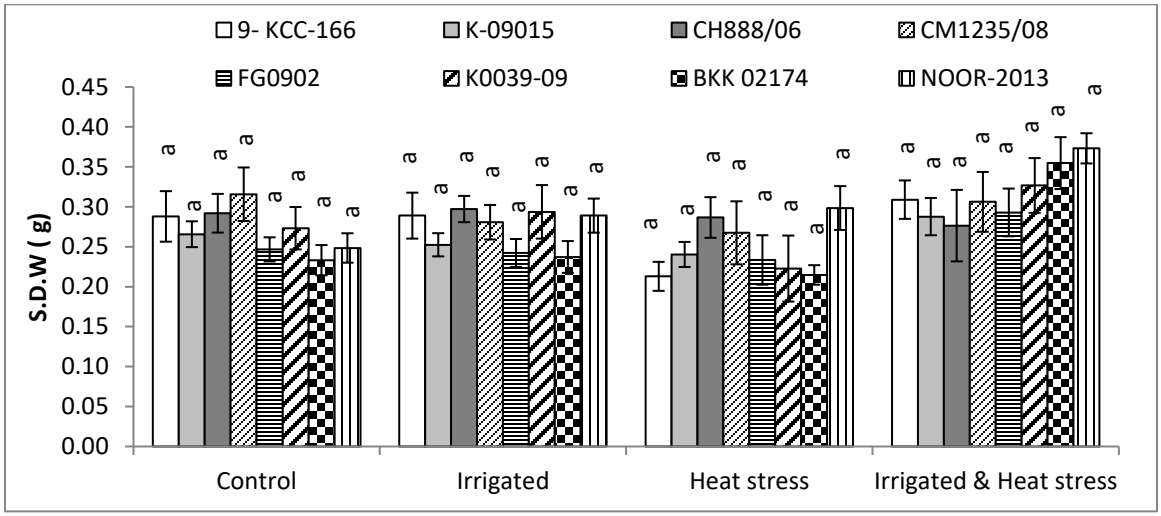

Figure 3.7: Seedling dry weight under control, irrigation, heat and combined stress

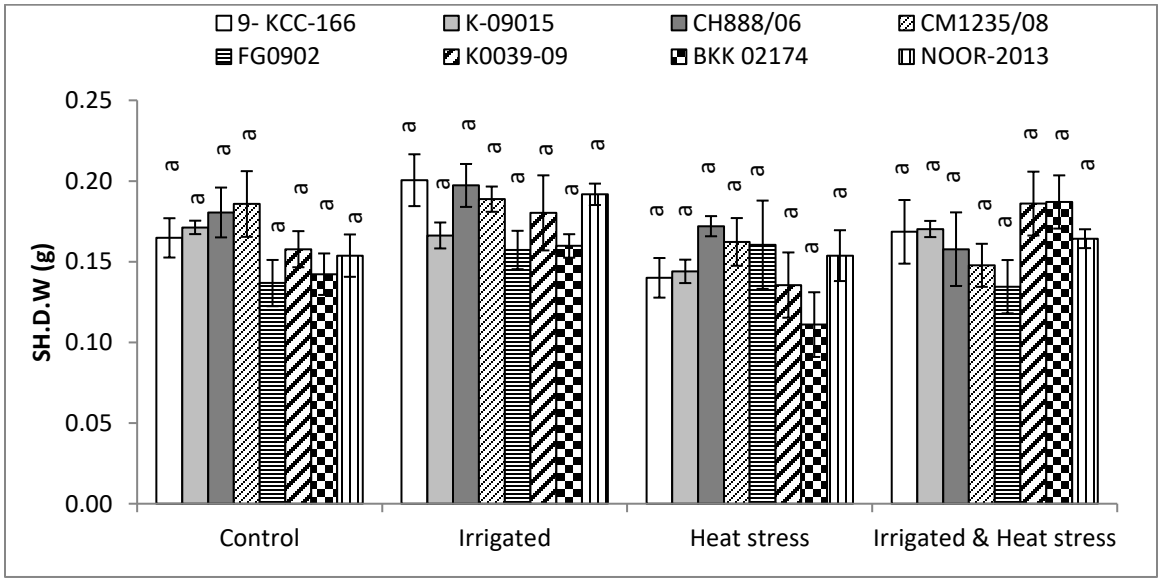

Figure 3.8: Shoot dry weight under control, irrigation, heat and combined stress

3.3 Morphological Trait

Screening of kabuli genotypes under irrigation, heat and combined stress on the basis of phenotypically appearance. Certain morphological symptoms were appeared under these stresses as yellowness of leaves, wilting and senescence under heat and combined stress, while a reasonable increased in vegetative growth was observed in some genotypes under irrigation stress (Figure 3.9, Figure 3.10, Figure 3.11, Figure 3.12).

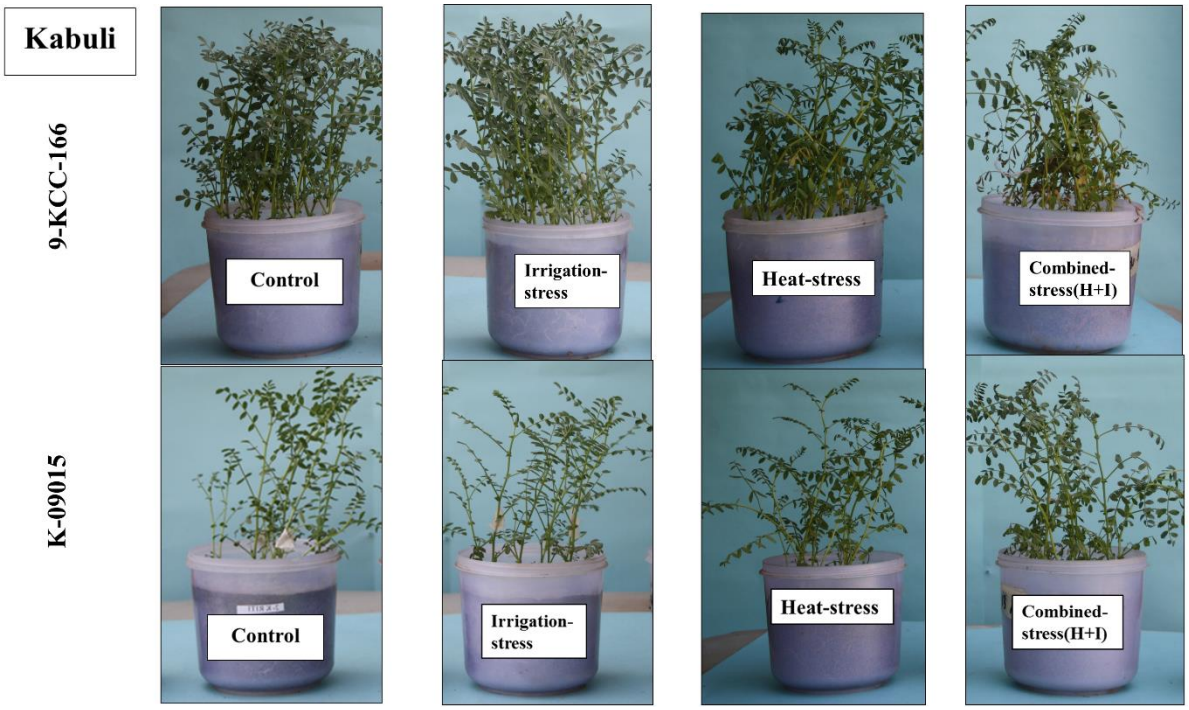

Figure 3.9: 9-KCC-166 and K-09015 under control, irrigation, heat and combined stress

Figure 3.10: CH888/06 and CM1235/08 under control, irrigation, heat and combined stress

Figure 3.11: FG0902 and K0039-09 under control, irrigation, heat and combined stress

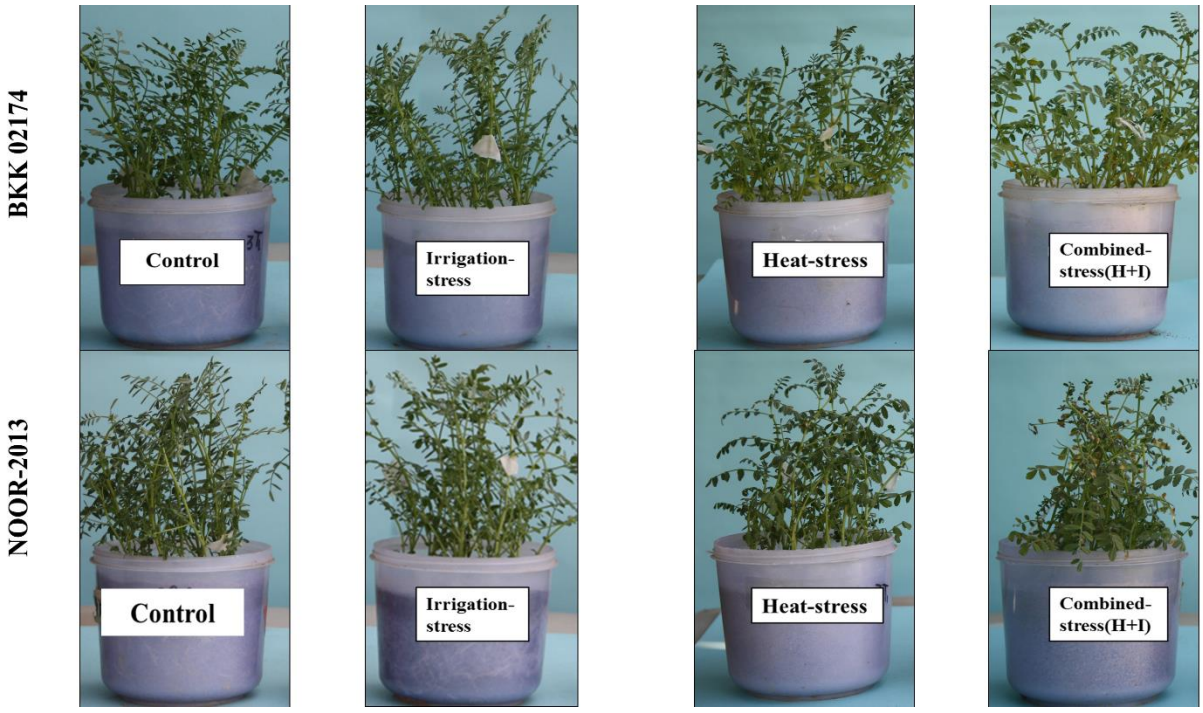

Figure 3.12: BKK02174 and NOOR-2013 under control, irrigation, heat and combined stress

In kabuli genotypes on the basis of physical appearance under irrigation stress it was observed that K-09015 (Figure 3.9) and FG0902 (Figure 3.11) showed tolerance because shoot length was maintained under stress. K0039-9 was proved to be sensitive genotype as there was maximum increase in shoot length (Figure 3.11). Under heat stress BKK02174 and CM1235/08 were observed tolerant genotypes because chlorosis, wilting and senescence was not detected in these (Figure 3.12,Figure 3.10). 9KCC166 was proved to be susceptible genotype because heat stress caused wilting, senescence and chlorosis in this genotype (Figure 3.9). Under combined stress K- 09015 and CM1235/08 were detected tolerant genotypes as there was no change in shoot length and no effect of chlorosis, wilting and senescence (Figure 3.9, Figure 3.10). FG0902 was susceptible genotype under combined stress as there was increase in shoot length along with senescence, wilting and chlorosis (Figure 3.11).

3.4 Physiological Parameters

3.4.1 Total Water Potential (TWP)

Under non-stress condition, significant differences were observed in tested genotypes for total water potential (TWP) (Figure 3.13). The highest (0.85 -Mpa) value of TWP was detected in FG0902 while the lowest (0.65 -Mpa) value of TWP was observed in CM1235/08. Under the irrigation stress, value of TWP was highest (0.80 -Mpa) in K-09015 and lowest (0.61 -Mpa) in CM1235/08. Under heat stress, highest (1.07 -Mpa) value of TWP was noticed in K0039-09 and lowest (0.92 -Mpa) for K-09015. In case of combined stress, the highest (1.03 -Mpa) value of TWP was found in 9- KCC-166 and the lowest (0.96 -Mpa) value was observed in K-09015.

As compared to non-stress condition, all the genotypes indicated non- significant change for TWP value under irrigation, heat and combined stress (Figure 3.13).

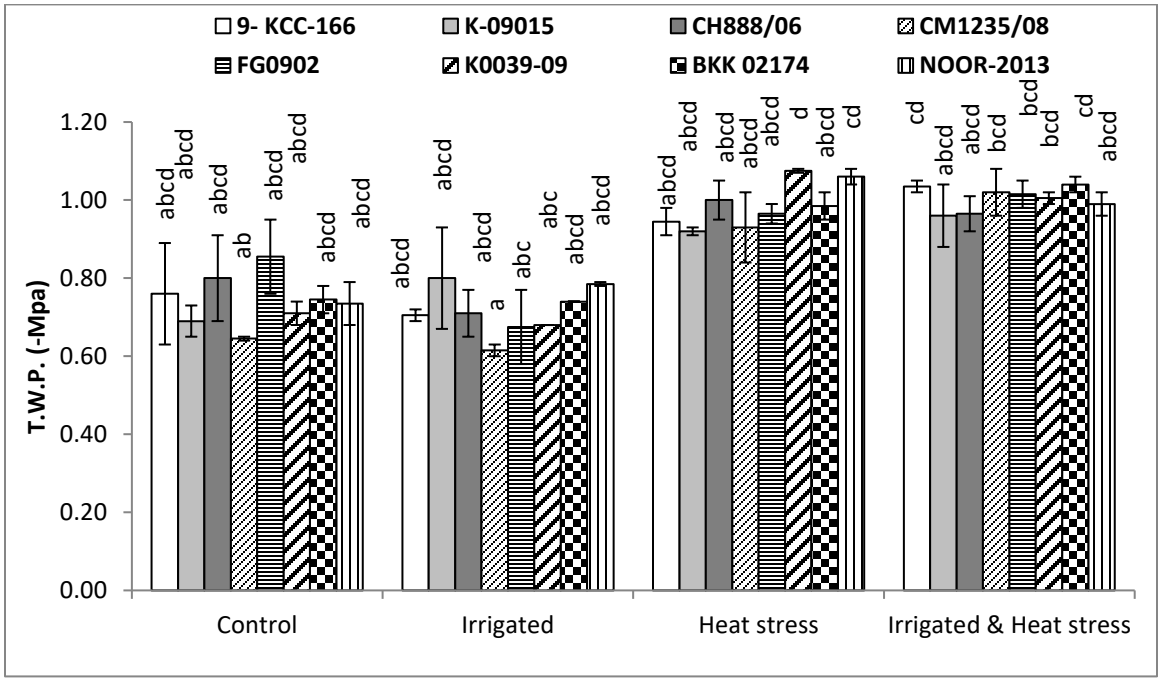

Figure 3.13: Total water potential under control, irrigation, heat and combined stress

3.4.2 Other Physiological Parameters

Under non-stress condition and all stresses non-significant differences were observed in tested genotypes for osmotic potential (O.P), turgor potential (Tr. P), relative water content

(R.W.C), Minimal chlorophyll fluorescence (Fo), Maximal chlorophyll fluorescence (Fm), variable chlorophyll fluorescence (Fv) and ratio of variable and maximal, chlorophyll fluorescence (Fv /Fm) and time of achieving maximum fluorescence yield (Tm) (Figure 3.14, Figure 3.15, Figure 3.16, Figure 3.17, Figure 3.18, Figure 3.19, Figure 3.20, Figure 3.21).

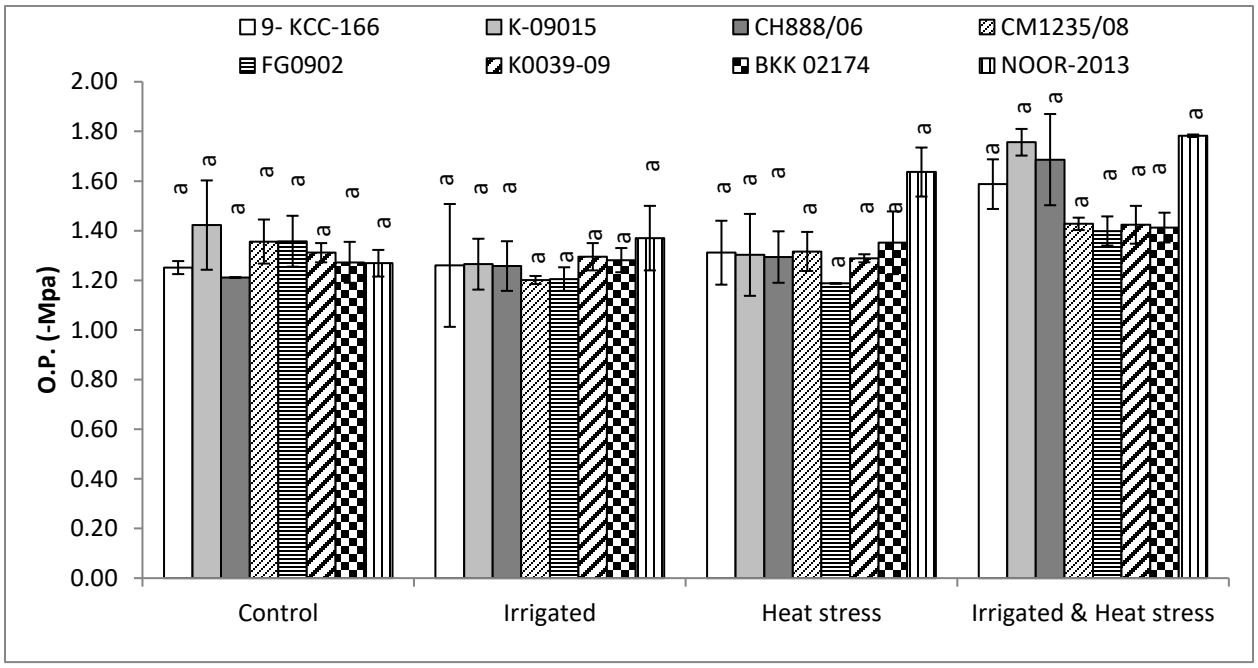

Figure 3.14: Osmotic potential under control, irrigation, heat and combined stress

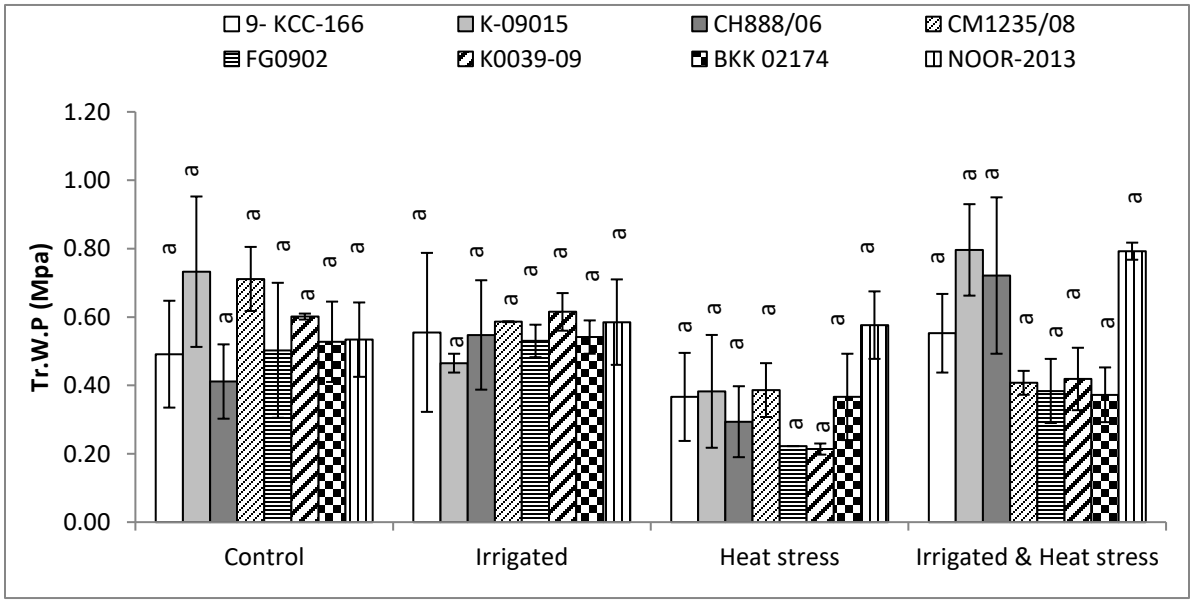

Figure 3.15: Turgor potential under control, irrigation, heat and combined stress

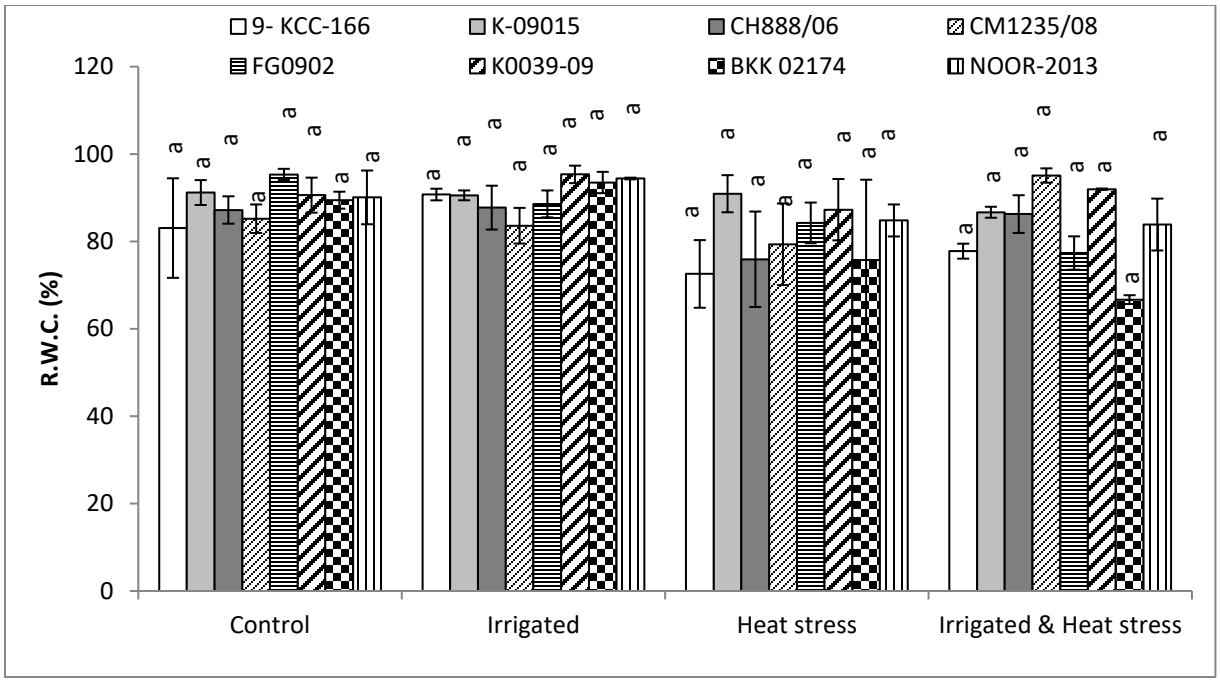

Figure 3.16: Relative water contents under control, irrigation, heat and combined stress

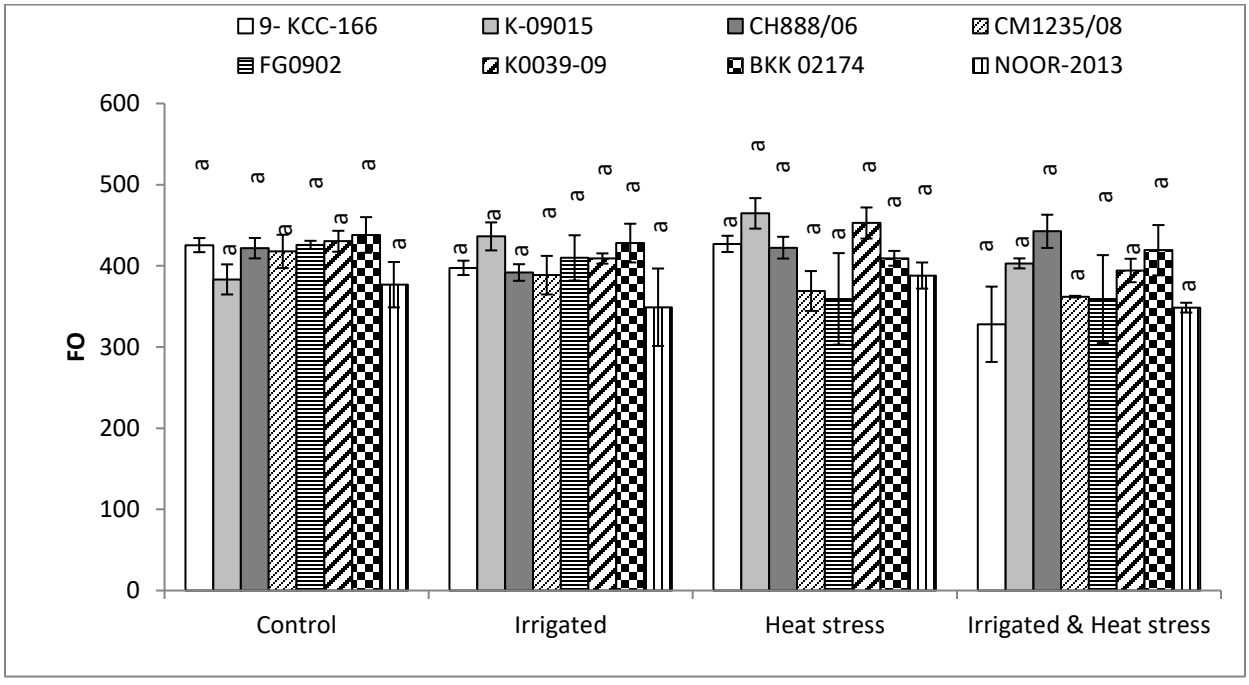

Figure 3.17 : F_O under control, irrigation, heat and combined stress

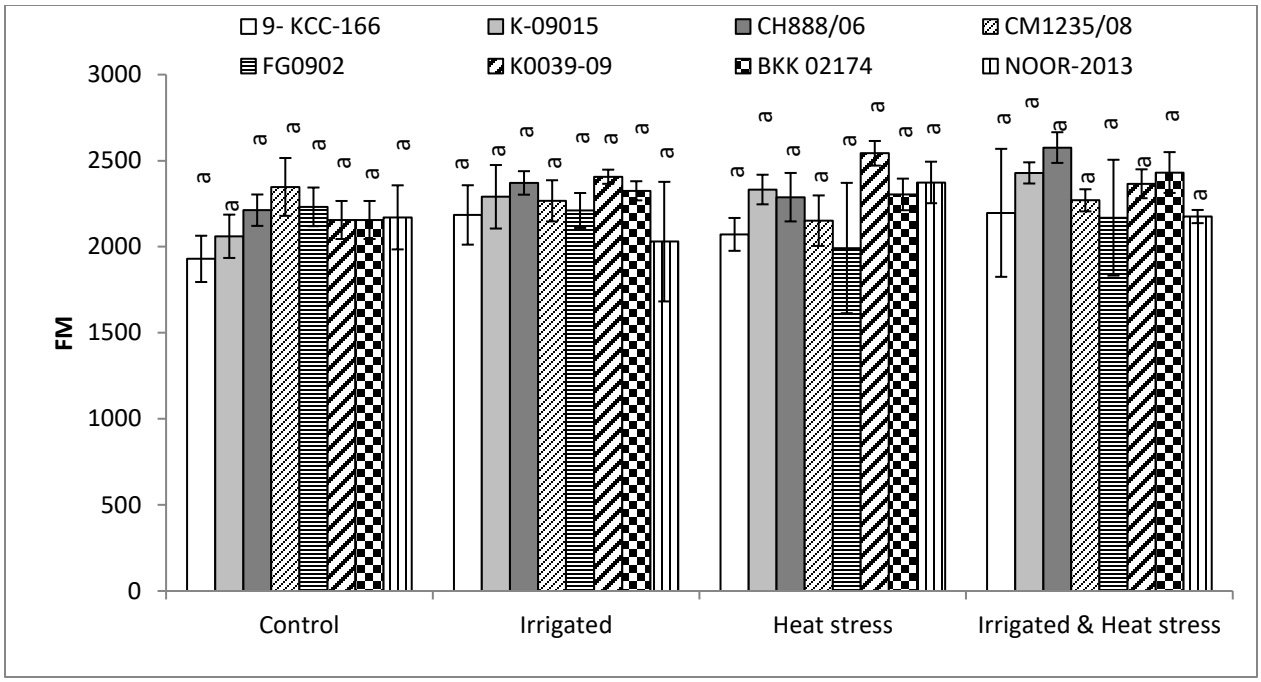

Figure 3.18: F_m under control, irrigation, heat and combined stress

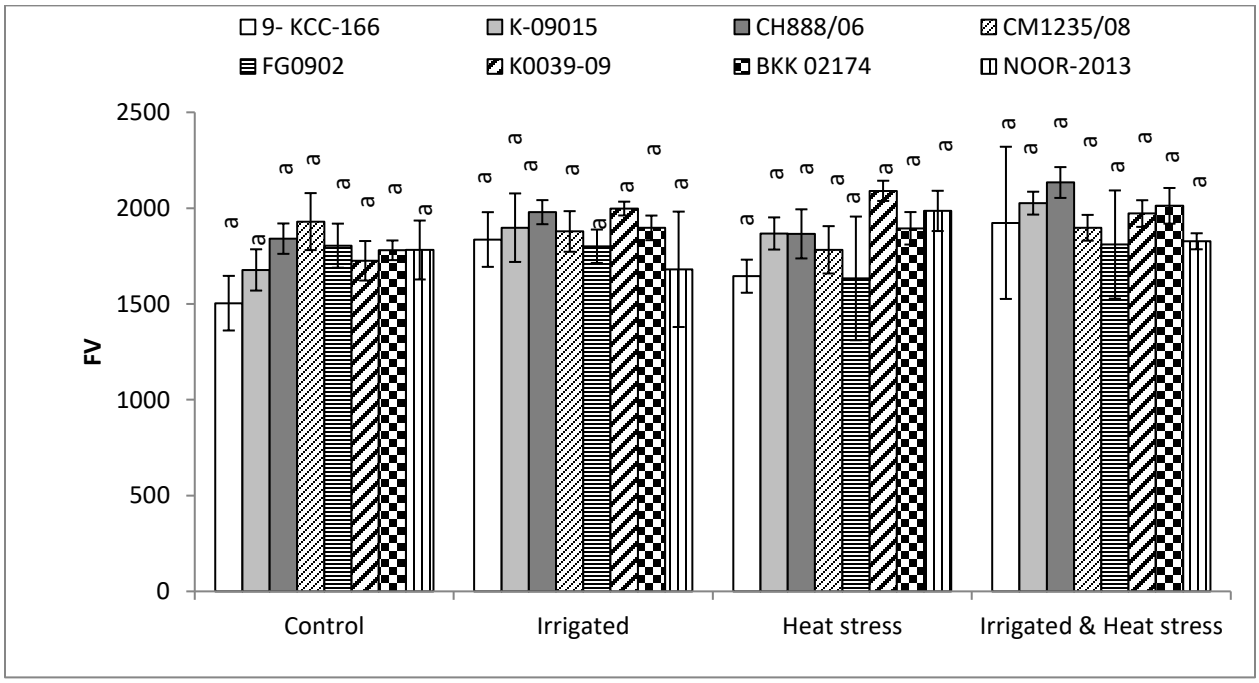

Figure 3.19: F_V under control, irrigation, heat and combined stress

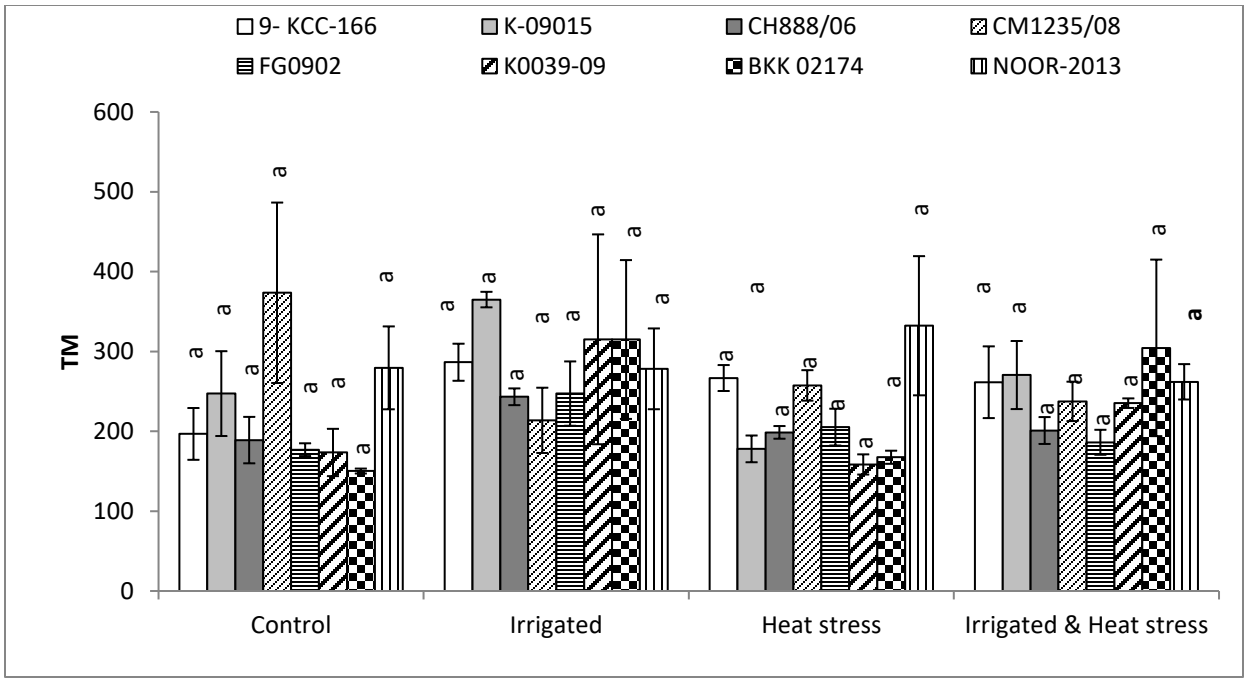

Figure 3.20: TM under control, irrigation, heat and combined stress

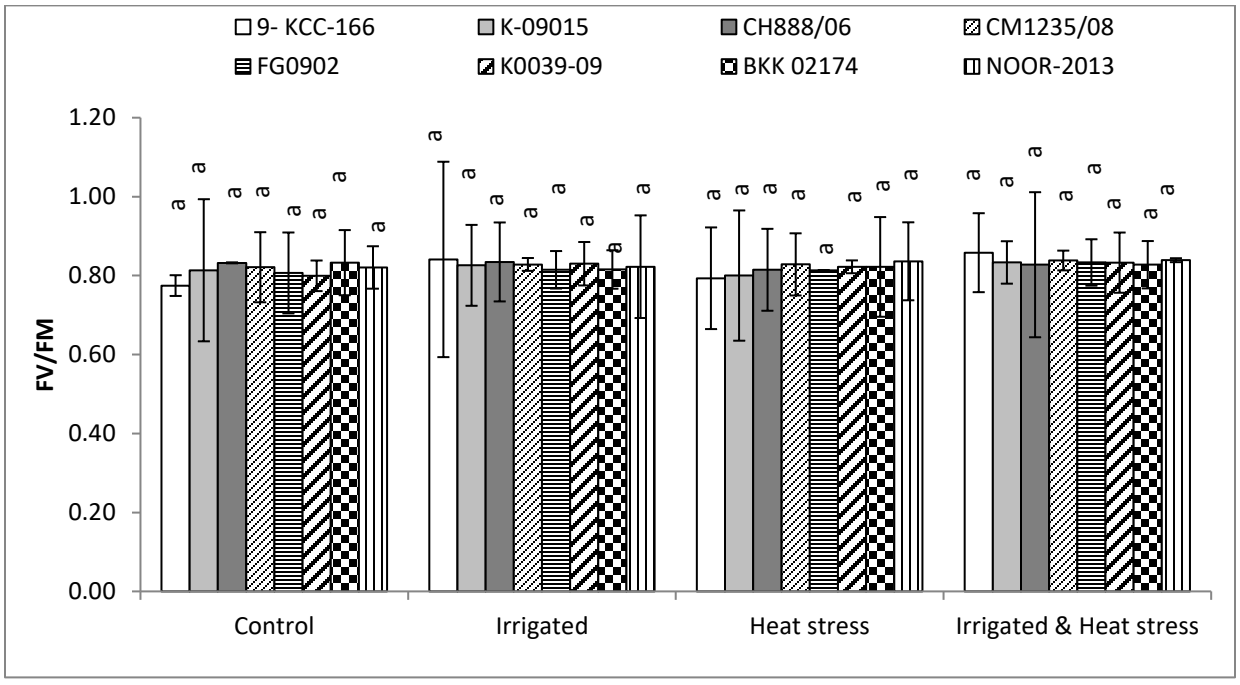

Figure 3.21: FV/FM under control, irrigation, heat and combined stress

3.5 Biochemical Parameters

3.5.1 Total Oxidant Status (TOS)

Under non-stress condition, significant differences were observed in tested genotypes for total oxidant status (TOS) (Figure 3.22). The highest (21825 uM/g. f.wt) value of TOS was detected in K0039-09 while the lowest (6600 uM/g. f.wt) value of TOS was observed in 9-KCC-166. Under the heat and irrigation stress, TOS was highest (19275 uM/g. f.wt, 18775 uM/g. f.wt) in K-09015 and lowest (10725 uM/g. f.wt, 7775 uM/g. f.wt) in CM1235/08. In case of combined stress, the highest (22150 uM/g. f.wt) value of TOS was found in CM1235/08 and the lowest (12000 uM/g. f.wt) value was observed in K-09015.

As compared to non-stress condition, TOS was significantly increased in 9-KCC-166 and K-09015 under irrigation stress while it remained unchanged in NOOR-2013. On other hand, TOS decreased significantly in all other genotypes under irrigation stress. Under heat stress, TOS value was increased in 9 KCC-166 and K-09015, remained unchanged in BKK 02174 while it decreased in all other genotypes as compare to non-stress condition. Under combined stress, a significant increase in TOS value was observed for 9-KCC-166, CH888/06 and CM1235/08 while a significant decrease was noticed in K-09015 as compared to that under non-stress condition. In all other genotypes the TOS value remained unchanged under combined stress (Figure 3.22).

3.5.2 Superoxide Dismutase (SOD)

Under non-stress condition, significant differences were observed in tested genotypes for superoxide dismutase (SOD) (Figure 3.23). The highest (265.43 Units/g f. wt) activity of SOD was detected in FG0902 while the lowest (169.72 Units/g f. wt) activity of SOD was observed in NOOR-2013. Under heat stress, SOD activity was highest (265.88 Units/g f. wt) in K-09015 and lowest (135.17 Units/g f. wt) in CH888/06. Under heat stress, SOD activity was highest (266.72 Units/g f. wt) in FG0902 and lowest (122.05 Units/g f. wt) in CH888/06. In case of combined stress, the highest (295.69 Units/g f. wt) activity of SOD was found in K-09015 and the lowest (116.58 Units/g f. wt) value was observed in NOOR-2013.

As compared to non-stress condition, SOD activity was significantly increased in BKK 02174 under irrigation stress while it remained unchanged for all other genotypes. Under heat stress, SOD activity was increased in BKK 02174 and remained unchanged in

all other genotypes as compare to non-stress condition. Under combined stress, a non-significant change was observed for all genotypes as compared to non-stress conditions (Figure 3.23).

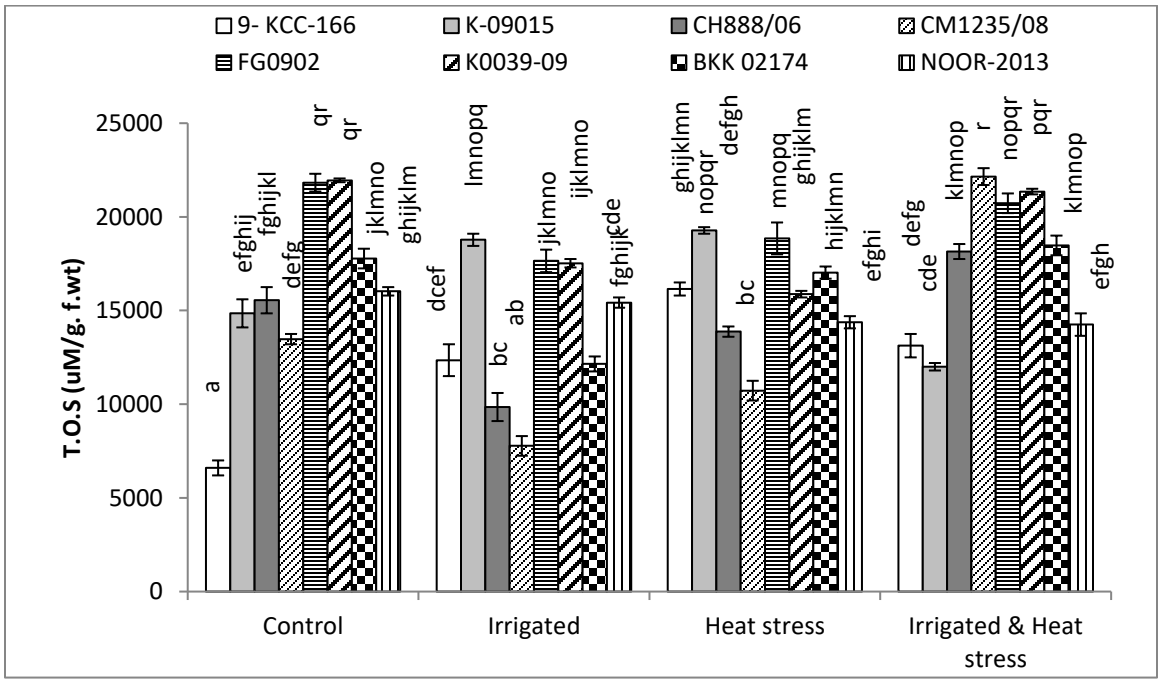

Figure 3.22: Total oxidant status under control, irrigation, heat and combined stress

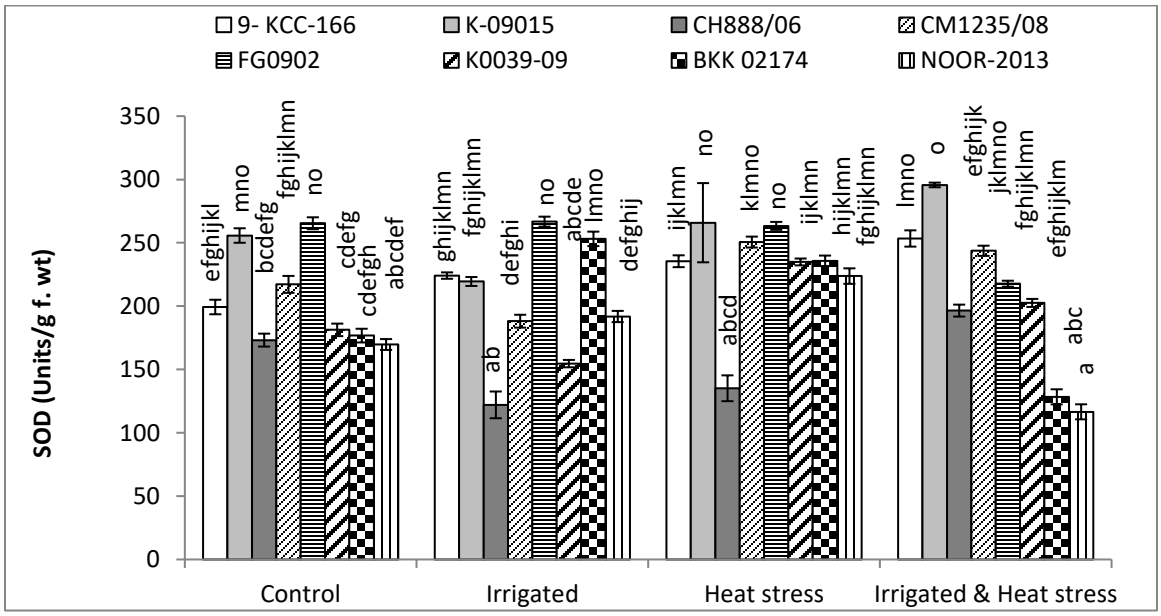

Figure 3.23: Superoxide dismutase activity under control, irrigation, heat and combined stress

3.5.3 Peroxidase (POD)

Under non-stress condition, significant differences were observed in tested genotypes for peroxidase (POD) activity (Figure 3.24). The highest (7126.20 Units/g f. wt) activity of POD was detected in 9- KCC-166 while the lowest (4961.70 Units/g f. wt) value of POD was observed in BKK 02174. Under the irrigation stress, POD activity was highest (19647.00 Units/g f. wt) in 9-KCC-166 and lowest (7642.35 Units/g f. wt) in K-09015. While under heat stress the highest (14335.65 Units/g f. wt) activity of POD was noticed in FG0902 and lowest (8225.10 Units/g f. wt) in 9-KCC-166. In case of combined (heat and irrigation) stress, the highest (21378.60 Units/g f. wt) value of POD was found in FG0902 and the lowest (9140.85 Units/g f. wt) value was observed in 9-KCC-166.

As compared to non-stress condition, POD was significantly increased in 9-KCC-166 and K-09015 under irrigation stress while it remained unchanged in NOOR-2013. On other hand, POD decreased significantly in all other genotypes under irrigation stress. Under heat stress, POD value was increased in 9 KCC-166 and K-09015, remained unchanged in BKK 02174 while it decreased in all other genotypes as compare to non-stress condition. Under combined stress, a significant increase in POD value was observed for 9-KCC-166, CH888/06 and CM1235/08 while a significant decrease was noticed in K-09015 as compared to that under non-stress condition. In all other genotypes the POD value remained unchanged under combined stress (Figure 3.24).

3.5.4 Melanodialdihyde (MDA)

Under non-stress condition, significant differences were observed in tested genotypes for melanodialdihyde (MDA) (Figure 3.25). The highest (189.48 uM/g. f.wt) value of MDA was detected in K-09015 while the lowest (112.25 uM/g. f.wt) value of MDA was observed in CH888/06. Under the irrigation stress, MDA was highest (161.03 uM/g. f.wt) in NOOR-2013 and lowest (126.19 uM/g. f.wt) in CM1235/08. Under heat stress highest (166.38 uM/g. f.wt) MDA contents were present in CH888/06 while lowest (120.77 uM/g. f.wt) were in K-09015. In case of combined stress, the highest (163.35 uM/g. f.wt) value of MDA was found in CM1235/08 and the lowest (100.64 uM/g. f.wt) value was observed in K-09015.

As compared to non-stress condition, MDA was found to be non-significant in irrigation and heat stress. In combined stress K-09015 showed decreased, CM1235/08 showed increased MDA contents while others remained unchanged (Figure 3.25).

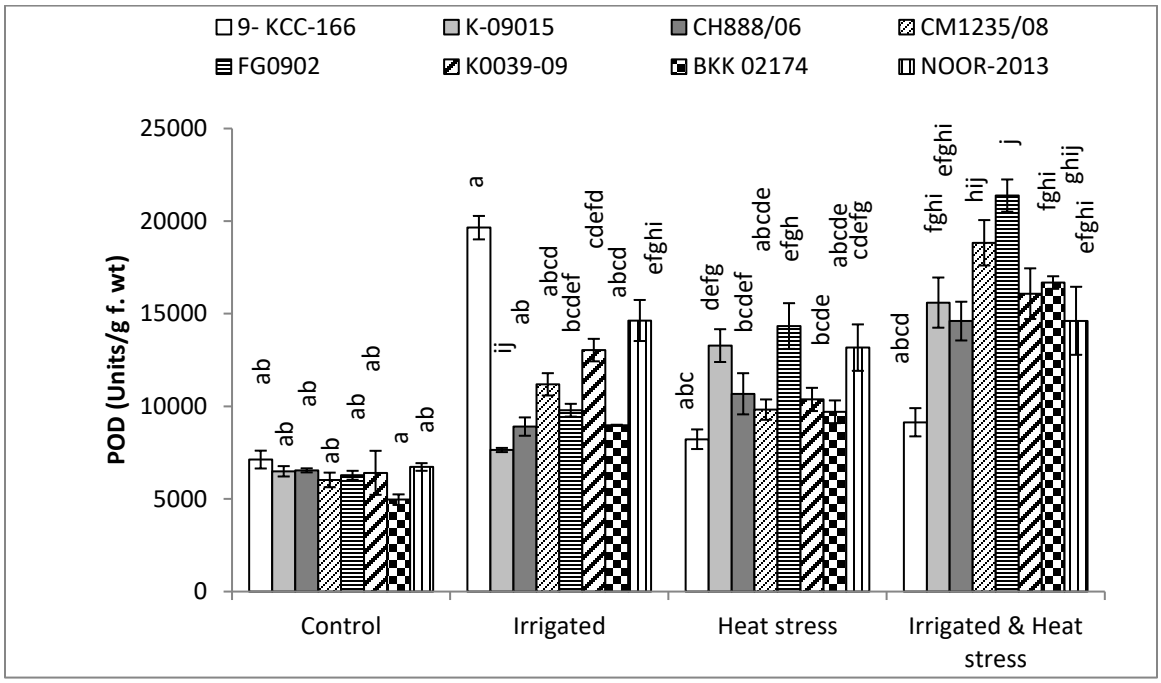

Figure 3.24: Peroxidase activity under control, irrigation, heat and combined stress

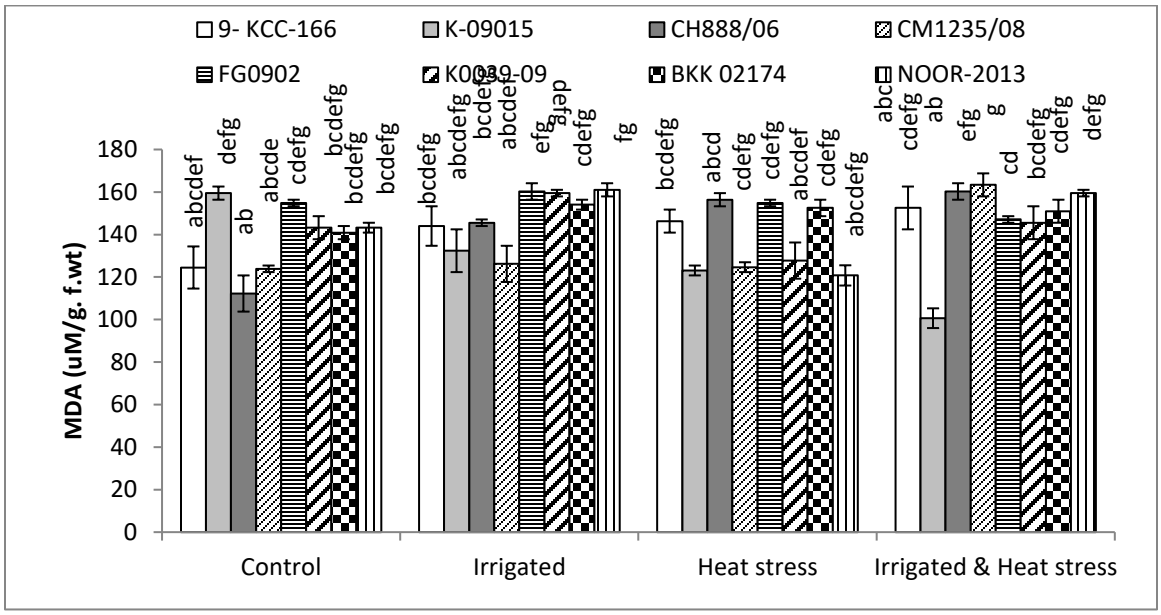

Figure 3.25: Melanodialdihyde activity under control, irrigation, heat and combined stress

3.5.5 Total Phenolic Contents (TPC)

Under non-stress condition, significant differences were observed in tested genotypes for total phenolic contents (TPC) (Figure 3.26). The highest (38925 uM/g. f.wt) value of TPC was detected in CH888/06 while the lowest (26725 uM/g. f.wt) value of TPC was observed in NOOR-2013. Under the irrigation stress, TPC was highest (45525 uM/g. f.wt) in 9-KCC-166 and lowest (28150 uM/g. f.wt) in FG0902. Under heat stress highest (37600 uM/g. f.wt) TPC contents were present in K0039-09 while lowest (25575 uM/g. f.wt) were in 9- KCC-166. In case of combined stress, the highest (39075 uM/g. f.wt) value of MDA was found in K0039-09 and the lowest (28525 uM/g. f.wt) value was observed in K-09015.

As compared to non-stress condition, TPC contents in irrigation stress 9- KCC-166, CH888/06 and NOOR-2013 increased significantly, CM1235/08 and K0039-09 decreased while others remained unchanged. Under heat stress, 9- KCC-166 and CH888/06 showed decreased TPC contents while all others remained unchanged. Under combined stress CH888/06 was observed with significantly lower contents while CM1235/08 with increased contents and others remained unchanged (Figure 3.26).

3.5.6 Total Soluble Protein

Under non-stress condition, significant differences were observed in tested genotypes for total soluble protein (TSP) (Figure 3.27). The highest (347.66 mg/g f.wt) TSP contents were observed in FG0902 while the lowest (204.00 mg/g f.wt) contents in BKK 02174. Under the irrigation stress, protein was highest (388.50 mg/g f.wt) in FG0902 and lowest (165.00 mg/g f.wt) in 9- KCC-166. Under heat stress highest (317.50 mg/g f.wt) protein contents were present in BKK 02174 while lowest (92.33 mg/g f.wt) were in FG0902. In case of combined (heat and irrigation) stress, the highest (246.33 mg/g f.wt) protein content was found in K-09015 and the lowest (105.33 mg/g f.wt) value was observed in NOOR-2013.

As compared to non-stress condition, TSP was significantly decreased under irrigation stress in 9- KCC-166, K-09015, CH888/06 and NOOR-2013 while increased in BKK 02174 and other genotypes remained unchanged. Under heat stress 9- KCC-166, K-09015, CH888/06, FG0902, K0039-09 and NOOR-2013 decreased significantly while CM1235/08 and BKK 02174 increased in protein contents. Under combined stress CM1235/08 remained unchanged while all others decreased significantly (Figure 3.27).

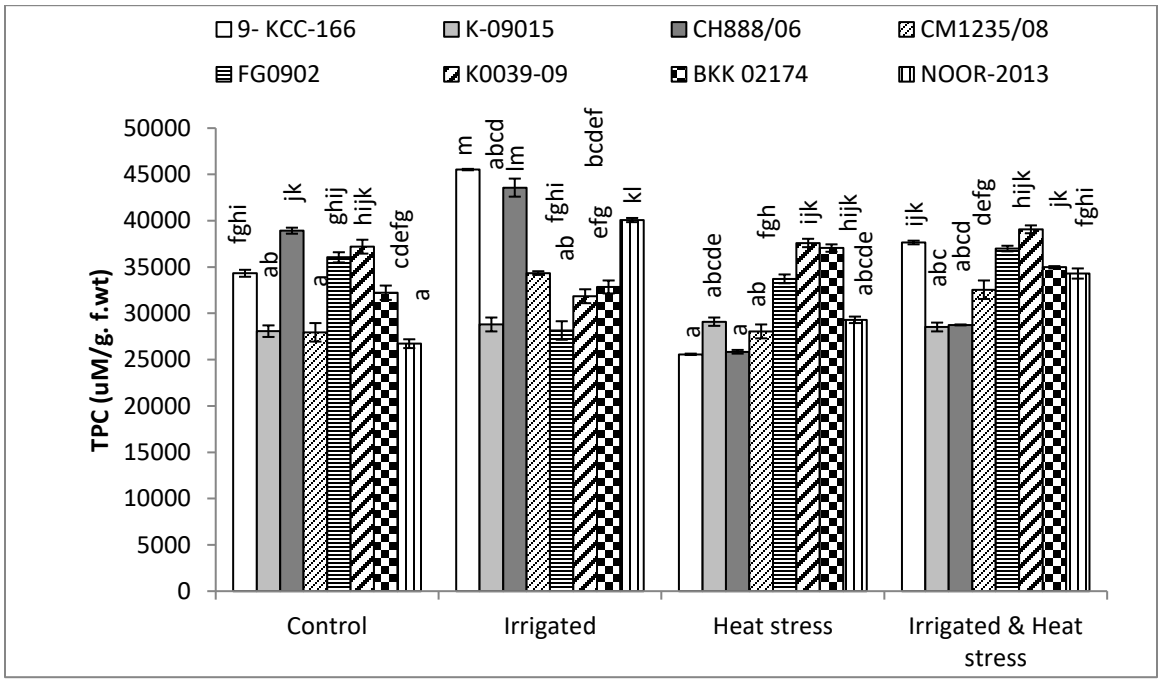

Figure 3.26: Total phenolic contents under control, irrigation, heat and combined stress

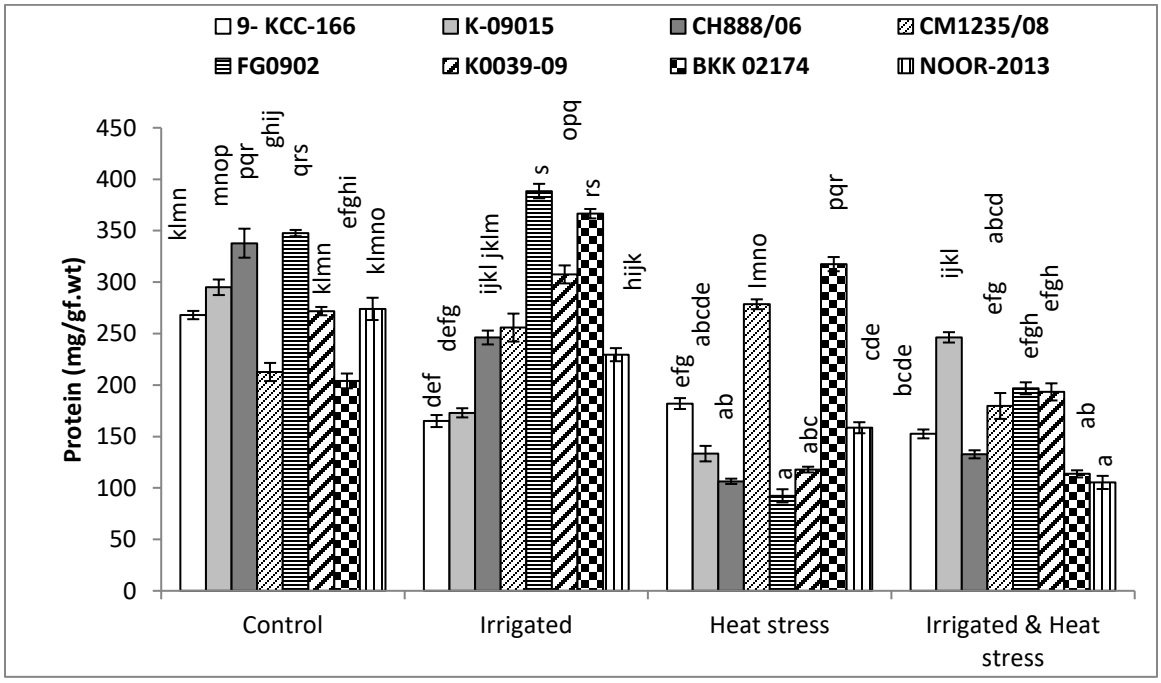

Figure 3.27: Protein contents under control, irrigation, heat and combined stress

3.5.7 Lycopene

Under non-stress condition, significant differences were observed in tested genotypes for lycopene contents (Figure 3.28). The highest (18.35 mg/g f.wt) lycopene contents were observed in CM1235/08 while the lowest (13.03 mg/g f.wt) in K0039-09. Under the irrigation stress, lycopene contents were highest (19.60 mg/g f.wt) in CM1235/08 and lowest (12.46 mg/g f.wt) in NOOR-2013. Under heat stress highest (20.98 mg/g f.wt) lycopene content was present in BKK 02174 while lowest (13.27 mg/g f.wt) were in CH888/06. In case of combined (heat and irrigation) stress, the highest (18.37 mg/g f.wt) lycopene contents were found in 9- KCC-166 and the lowest (8.22 mg/g f.wt) value was observed in K0039-09.

As compared to non-stress condition, lycopene contents were found significantly different under irrigation stress. CH888/06 and FG0902 increased significantly while BKK 02174 and NOOR-2013 decreased, others remained unchanged. Under heat stress K0039-09 and BKK 02174 were increased while K-09015 and CH888/06 showed decreased lycopene contents and others remained unchanged. Under combined stress CM1235/08 and K0039-09 showed decreased lycopene contents while in all others it remained unchanged (Figure 3.28).

3.5.8 Chlorophyll ‘a’

Under non-stress condition, significant differences were observed in tested genotypes for chlorophyll ‘a’ contents (Figure 3.29). The highest (238.80 µg/g f.wt) chlorophyll ‘a’ contents were observed in 9- KCC-166 while the lowest (77.56 µg/g f.wt) in K0039-09. Under the irrigation stress, chlorophyll ‘a’ were highest (212.01 µg/g f.wt) in CM1235/08 and lowest (63.19 µg/g f.wt) in CH888/06. Under heat stress, highest (286.46 µg/g f.wt) chlorophyll ‘a’ was detected in BKK 02174 while lowest (96.53 µg/g f.wt) was in K0039-09. In case of combined (heat and irrigation) stress, the highest (142.08 µg/g f.wt) chlorophyll ‘a’ were found in 9- KCC-166 and the lowest (20.24 µg/g f.wt) was observed in FG0902.

As compared to non-stress condition, chlorophyll ‘a’ contents were found non-significant under irrigation stress. BKK 02174 showed significantly high chlorophyll ‘a’ contents under heat stress while in other genotypes it remained unchanged. Under

combined stress all genotypes were found to have non-significant differences regarding chlorophyll 'a' contents (Figure 3.29).

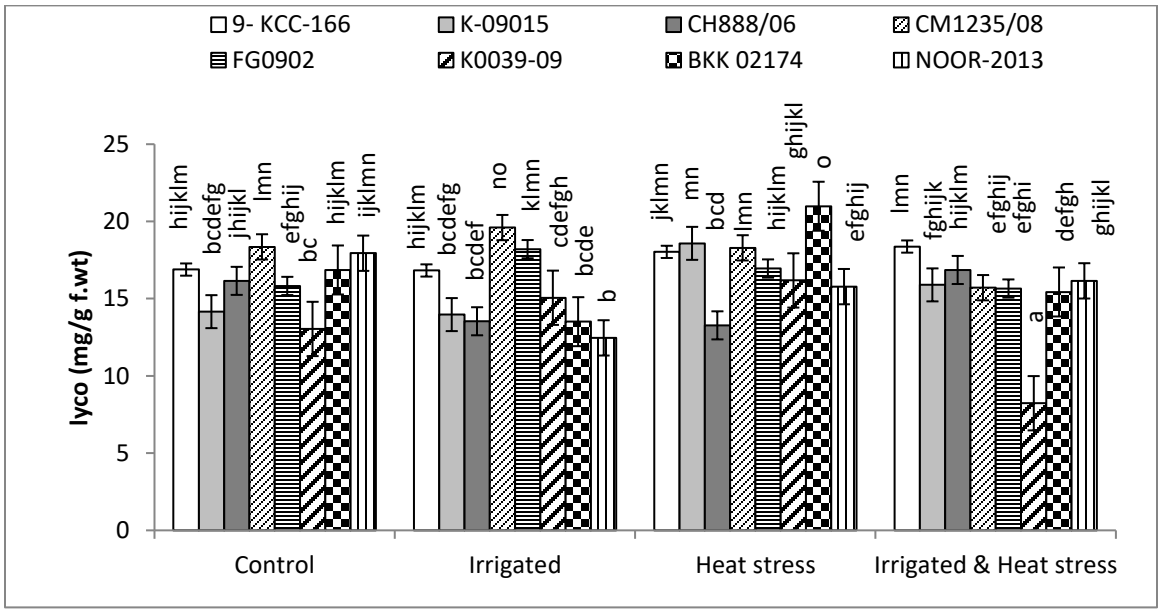

Figure 3.28: Lycopene contents under control, irrigation, heat and combined stress

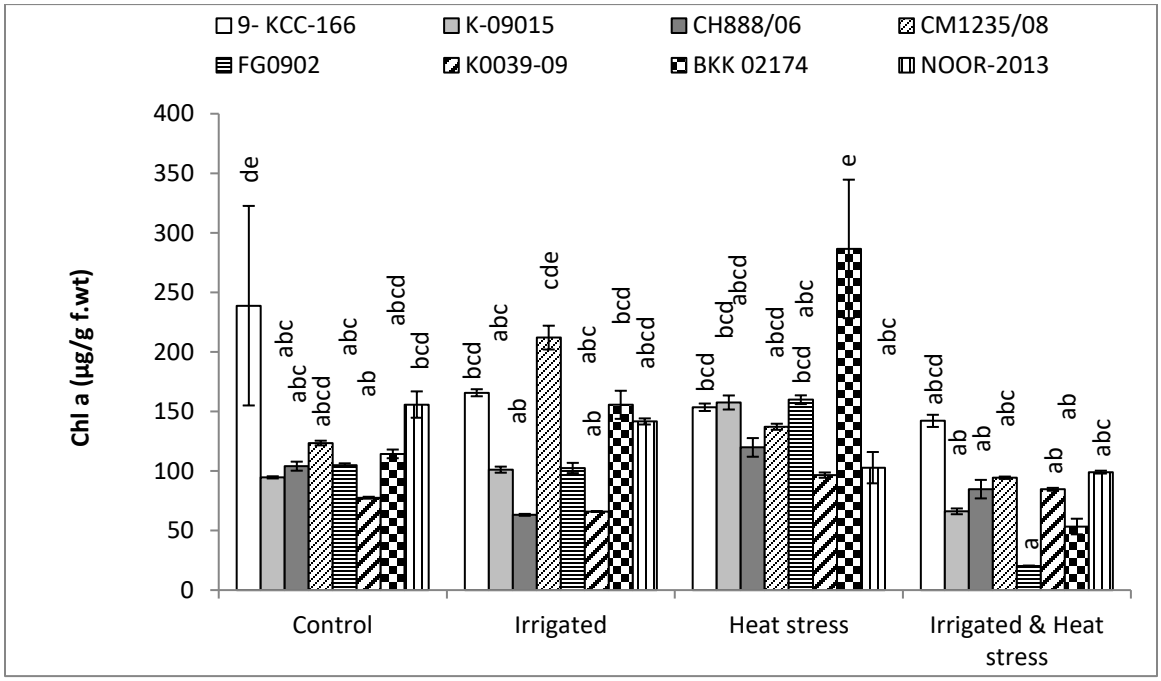

Figure 3.29: Chlorophyll 'a' contents under control, irrigation, heat and combined stress

3.5.9 Chlorophyll ‘b’

Under non-stress condition, significant differences were observed in tested genotypes for chlorophyll ‘b’ contents (Figure 3.30). The highest (612.40 µg/g f.wt) chlorophyll ‘b’ contents were observed in CM1235/08 while the lowest (403.08 µg/g f.wt) in K0039-09. Under the irrigation stress, chlorophyll ‘b’ were highest (633.18 µg/g f.wt) in FG0902 and lowest (332.68 µg/g f.wt) in NOOR-2013. Under heat stress highest (676.55 µg/g f.wt) chlorophyll ‘b’ was present in BKK 02174 while lowest (373.34 µg/g f.wt) were in CH888/06. In case of combined (heat and irrigation) stress, the highest (595.44 µg/g f.wt) chlorophyll ‘b’ were found in 9- KCC-166 and the lowest (206.65 µg/g f.wt) value was observed in K0039-09.

As compared to non-stress condition, chlorophyll ‘b’ contents were significantly affected. A reasonable increase in chlorophyll ‘b’ was observed in FG0902 while decreased in BKK 02174 and NOOR-2013 while others remained non-significant. Under heat stress 9- KCC-166 and K-09015 showed increased while CH888/06 decreased significantly regarding chlorophyll ‘b’ contents and others remained unchanged. Under combined stress of heat and irrigation 9- KCC-166 was increasing while CM1235/08 and K0039-09 showed decreasing chlorophyll ‘b’ contents and others remained unchanged (Figure 3.30).

3.5.10 Total Carotenoids

Under non-stress condition, significant differences were observed in tested genotypes for carotenoid contents (Figure 3.31). The highest (29.91 mg/g f.wt) carotenoid contents were observed in 9- KCC-166 while the lowest (19.30 mg/g f.wt) in K0039-09. Under the irrigation stress, carotenoid were highest (30.40 mg/g f.wt) in CM1235/08 and lowest (19.88 mg/g f.wt) in CH888/06. Under heat stress highest (32.77 mg/g f.wt) carotenoid was present in BKK 02174 while lowest (20.82 mg/g f.wt) were in CH888/06. In case of combined (heat and irrigation) stress, the highest (91.95 mg/g f.wt) carotenoids were found in K0039-09and the lowest (66.08 mg/g f.wt) value was observed in CH888/06.

As compared to non-stress condition, carotene contents were found non-significant under irrigation stress and combined stress of heat and irrigation. Under heat stress K-09015 showed significantly increased carotene contents while other remained unchanged (Figure 3.31).

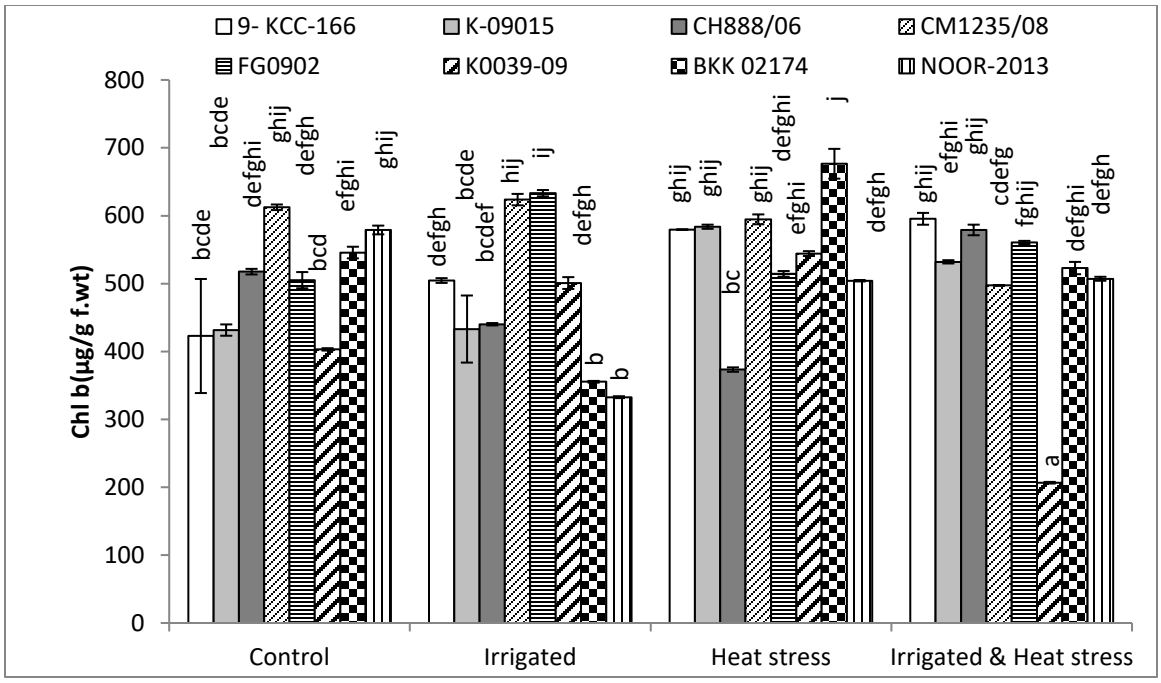

Figure 3.30: Chlorophyll 'b' contents under control, irrigation, heat and combined stress

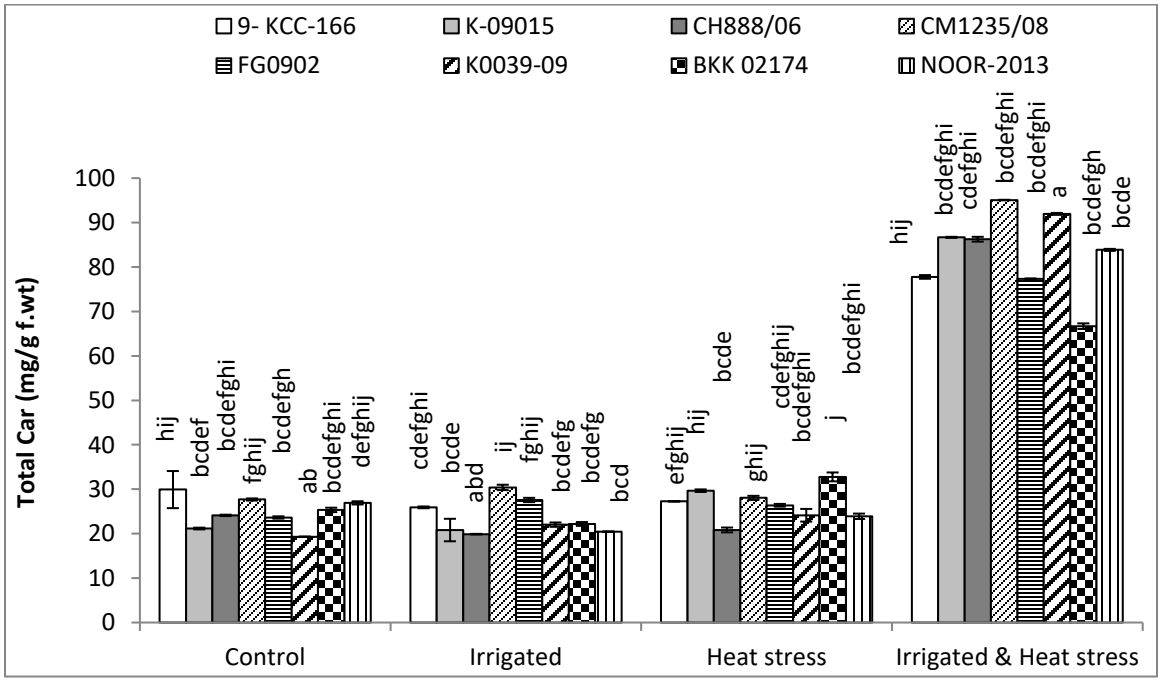

Figure 3.31: Total carotene contents under control, irrigation, heat and combined stress

3.5.11 Total Chlorophyll

Under non-stress condition, significant differences were observed in tested genotypes for total chlorophyll contents (Figure 3.32). The highest (735.89 µg/g f.wt) total chlorophyll contents were observed in CM1235/08 while the lowest (480.65 µg/g f.wt) in K0039-09. Under the irrigation stress, total chlorophyll contents were highest (835.76 µg/g f.wt) in CM1235/08 and lowest (474.28 µg/g f.wt) in NOOR-2013. Under heat stress highest (963.02 µg/g f.wt) total chlorophyll contents were present in BKK 02174 while lowest (493.15 µg/g f.wt) were in CH888/06. In case of combined (heat and irrigation) stress, the highest (737.53 µg/g f.wt) total chlorophyll contents were found in 9- KCC-166 and the lowest (291.27 µg/g f.wt) contents were observed in K0039-09.

As compared to non-stress condition, total chlorophyll contents were found significantly different under irrigation stress i.e. BKK 02174 and NOOR-2013 were observed to be decreasing for chlorophyll contents while others remained unchanged. Under heat stress K-09015 and K0039-09 were observed as increasing chlorophyll contents while others remained unchanged. K0039-09 and CM1235/08 showed significantly decreased chlorophyll contents under combined stress of heat and irrigation (

Figure **3.32**).

3.5.12 Protease

Under non-stress condition, significant differences were observed in tested genotypes for protease activity (Figure 3.33s). The highest (7415 Units/g. f.wt) activity of protease was detected in NOOR-2013 while the lowest (3665 Units/g. f.wt) activity of protease was observed in BKK 02174. Under the irrigation stress, protease activity was highest (6020 Units/g. f.wt) in K0039-09 and lowest (2410 Units/g. f.wt) in CM1235/08. While under heat stress the highest (8740 Units/g. f.wt) activity of protease was observed in 9- KCC-166 and lowest (3580 Units/g. f.wt) activity was found in K0039-09. In case of combined (heat and irrigation) stress, the highest (8575 Units/g. f.wt) activity of protease was found in CH888/06 and the lowest (4475 Units/g. f.wt) activity was observed in NOOR-2013.

As compared to non-stress condition, protease activity was significantly increased in FG0902 under irrigation stress. On other hand, protease activity was decreased significantly in CH888/06, CM1235/08 and for NOOR-2013 while the protease activity remained unchanged in all other genotypes under irrigation stress. Under heat stress,

protease activity was significantly increased in 9- KCC-166, CM1235/08 and in BKK 02174, remained unchanged in BKK 02174 while it decreased in all other genotypes as compare to non-stress condition. Under combined stress, a significant decrease in protease was observed for NOOR-2013, and remained unchanged K0039-09. While, a significant increase was noticed in all other genotypes as compared to that under non-stress condition (Figure 3.33).

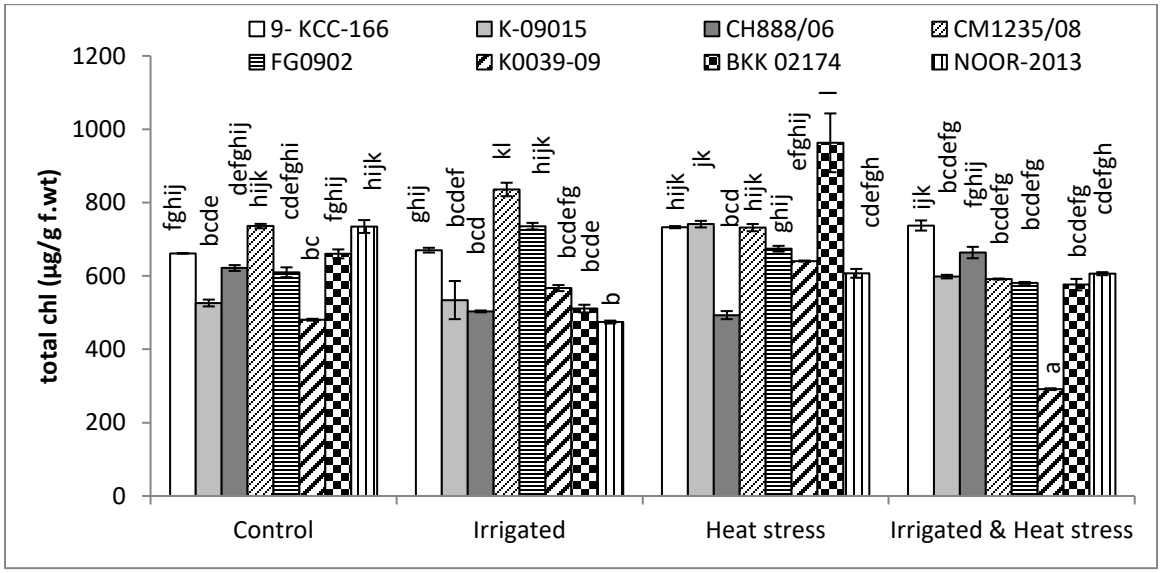

Figure 3.32: Total chlorophyll contents under control, irrigation, heat and combined stress

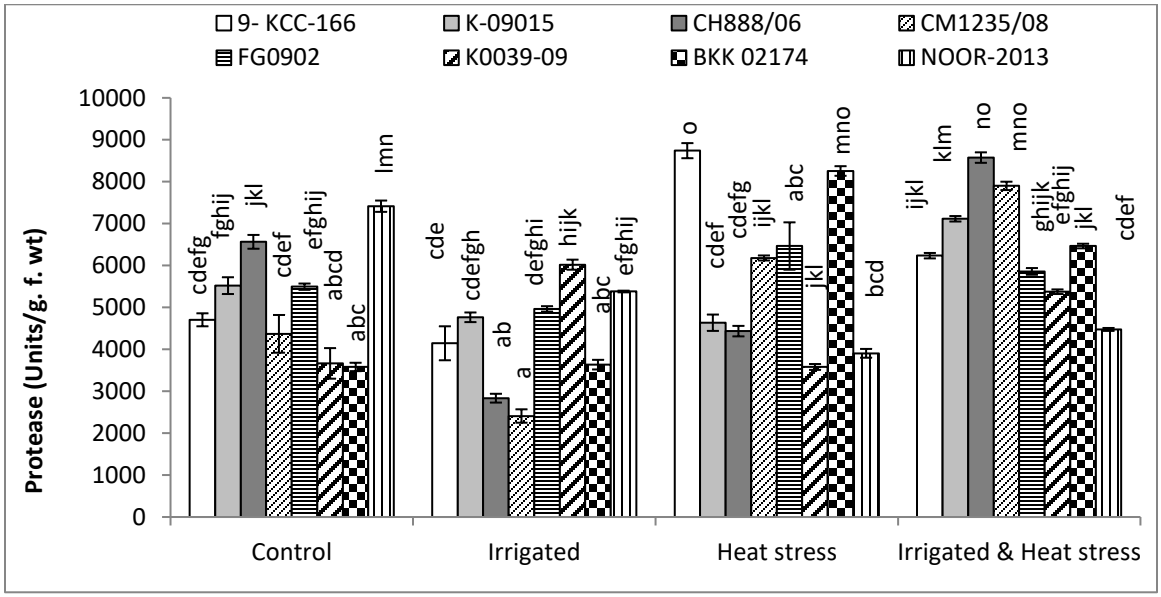

Figure 3.33: Protease activity under control, irrigation, heat and combined stress

3.5.13 Esterase

Under non-stress condition, significant differences were observed in tested genotypes for esterase activity (Figure 3.34). The highest (1612.95 µM/g f.wt) activity of esterase was detected in CM1235/08 while the lowest (1093.81 µM/g f.wt) activity of esterase was observed in K-09015. Under the irrigation stress, esterase activity was highest (1646.66 µM/g f.wt) in BKK 02174 and lowest (1409.56 µM/g f.wt) in K-09015. Under heat stress highest (1684.87 µM/g f.wt) activity of esterase was noticed in BKK 02174 and lowest91452.27 µM/g f.wt) for FG0902. In case of combined (heat and irrigation) stress, the highest (1183.70 µM/g f.wt) activity of esterase was found in 9- KCC-166 and the lowest (315.08 µM/g f.wt) activity was observed in FG0902.

As compared to non-stress condition, esterase activity was significantly increased in K-09015 under irrigation stress while it remained unchanged in all other genotypes. Under heat stress, esterase activity was increased in K-09015, remained unchanged in all other genotypes as compare to non-stress condition. Under combined stress, a significant decrease in esterase activity was observed for all genotypes as compared to non-stress condition (Figure 3.34).

3.5.14 Ascorbate Peroxidase (APX)

Under non-stress condition, significant differences were observed in tested genotypes for activity of ascorbate peroxidases (APX) (Figure 3.35). The highest (670 Units/g f.wt) activity of APX was detected in CM1235/08 while the lowest (440 Units/g f.wt) activity of APX was observed in FG0902. Under the irrigation stress, APX activity was highest (2120 Units/g f.wt) in FG0902 and lowest (440 Units/g f.wt) in K-09015. Under heat stress highest (1150 Units/g f.wt) activity of APX was noticed in NOOR-2013 and lowest (570 Units/g f.wt) for K0039-09. In case of combined (heat and irrigation) stress, the highest (1180 Units/g f.wt) activity of APX was found in K-09015 and the lowest (520 Units/g f.wt) activity was observed in BKK 02174.

As compared to non-stress condition, APX activity was significantly increased in FG0902 and K0039-09 under irrigation stress while it remained unchanged in all other genotypes. Under heat stress, APX activity was increased in 9- KCC-166, K-09015 and NOOR-2013 while it remained unchanged in all other genotypes as compare to non-stress condition. Under combined stress, a significant increase in activity of APX was noticed in

K-09015, FG0902 and K0039-09 while remained unchanged for all other genotypes as compared to non-stress condition (Figure 3.35).

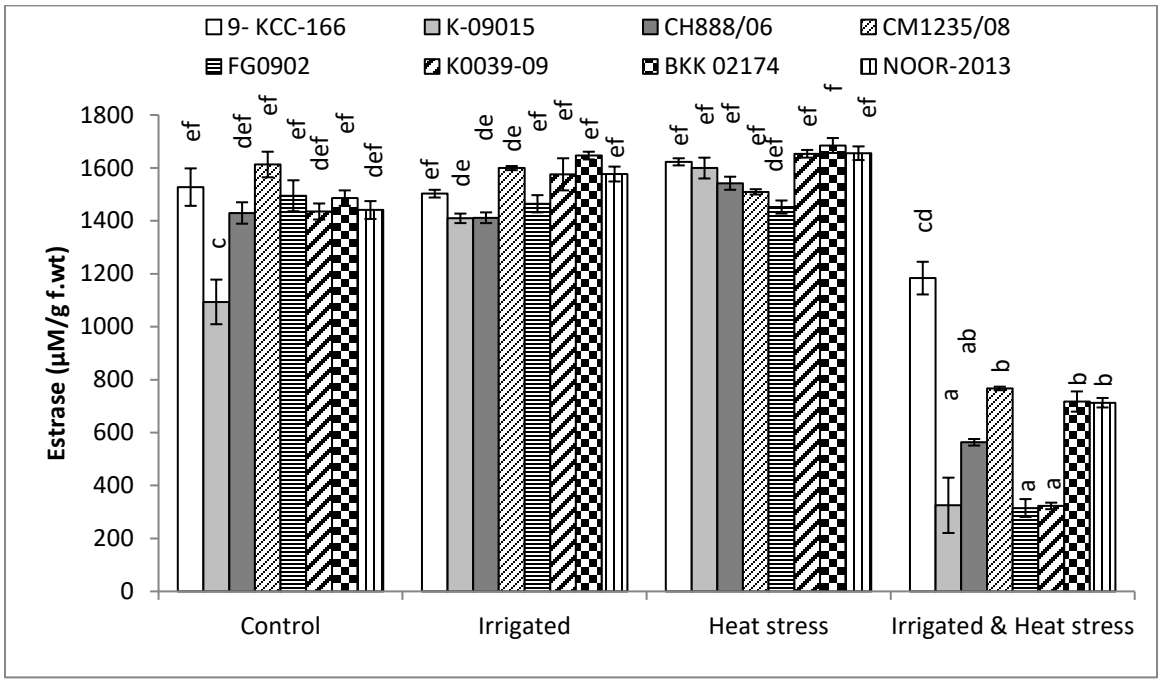

Figure 3.34: Esterase activity under control, irrigation, heat and combined stress

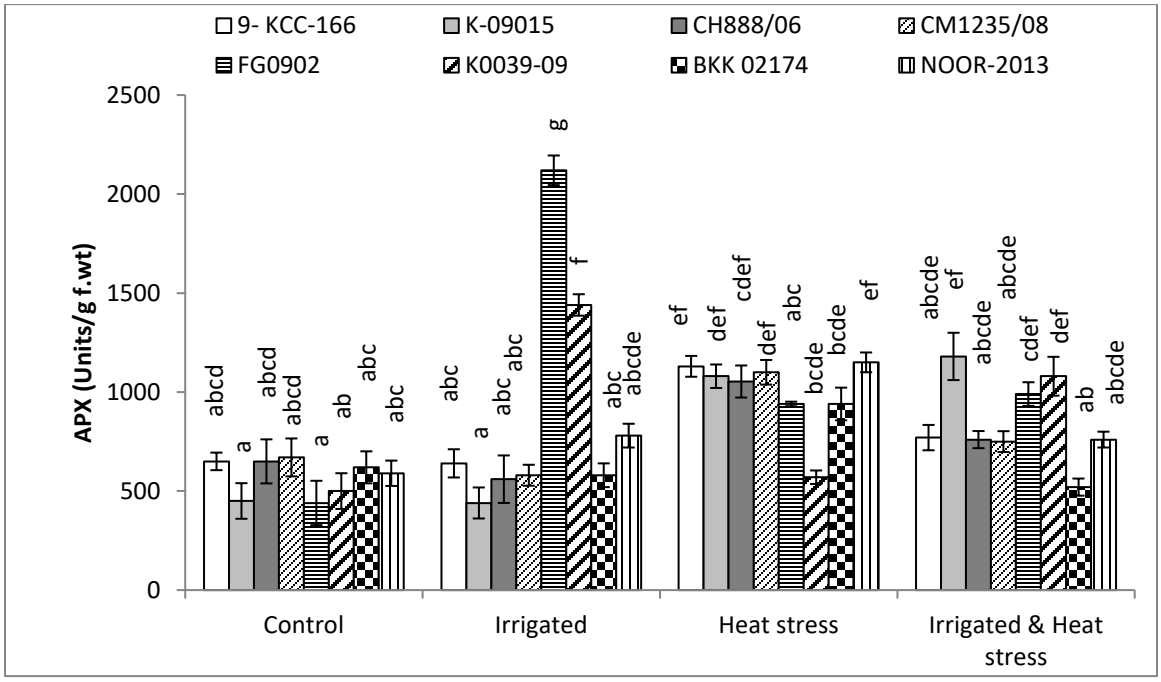

Figure 3.35: Ascorbate peroxidase activity under control, irrigation, heat and combined stress

3.5.15 Catalase (CAT)

Under non-stress condition, significant differences were observed in tested genotypes for activity of catalase (CAT) (Figure 3.36). The highest (32365 Units/g f.wt) activity of CAT was detected in BKK 02174 while the lowest (19580 Units/g f.wt) value of CAT was observed in K0039-09. Under the irrigation stress, CAT activity was highest (23825 Units/g f.wt) in NOOR-2013 and lowest (15085 Units/g f.wt) in 9- KCC-166. Under heat stress highest (34390 Units/g f.wt) activity of CAT was noticed in NOOR-2013 and lowest (17305 Units/g f.wt) for FG0902. In case of combined (heat and irrigation) stress, the highest (28310 Units/g f.wt) activity of CAT was found in 9- KCC-166 and the lowest (18055 Units/g f.wt) activity was observed in K-09015.

As compared to non-stress condition, CAT activity was significantly decreased in BKK 02174 under irrigation stress while it remained unchanged in all other genotypes. Under heat stress, CAT activity was increased in NOOR-2013, while it remained unchanged in all other genotypes. Under combined stress, a non-significant change was observed for catalase activity in all genotypes as compare to non-stress condition (Figure 3.36).

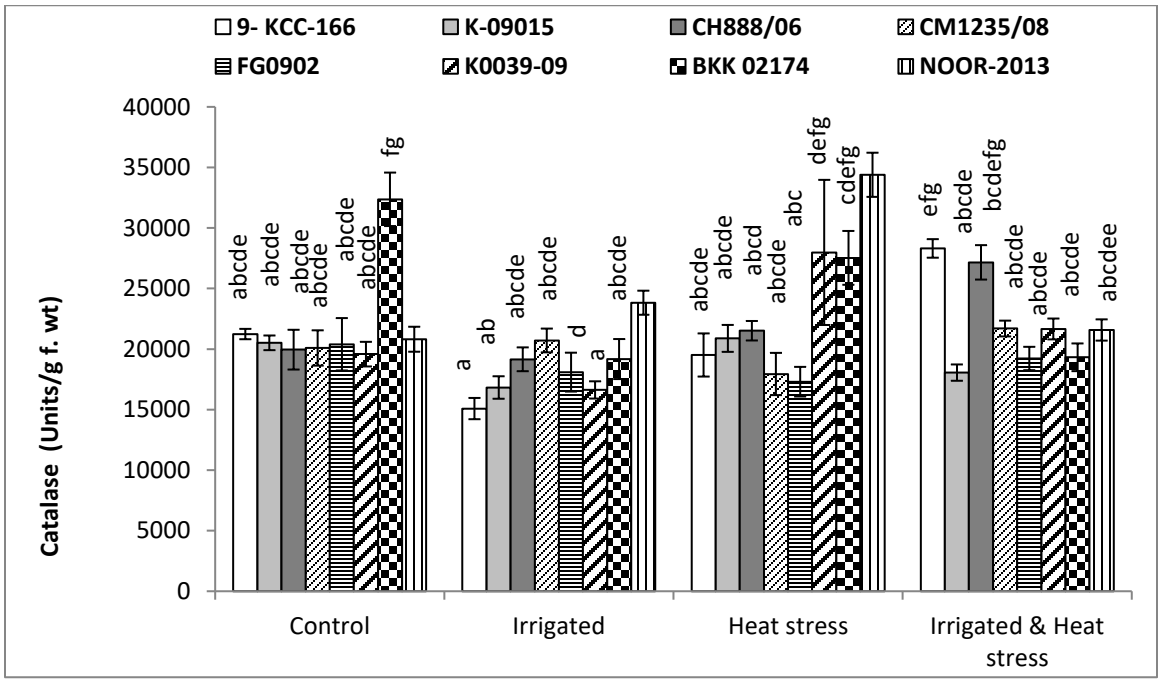

Figure 3.36: Catalase activity under control, irrigation, heat and combined stress

3.6 Correlation Analysis

Correlation (Pearson) for all morphological and physio biochemical parameters was carried out using XLSTAT 2012.1.02, using 95% confidence interval.

Under irrigation stress, shoot length was positively correlated with F_v/F_m/I, FV/FM/STI and S.D.W/I while negatively correlated with Protein/I. Root length was positively correlated with Lyco/STI, Chl b/STI, Carotene/STI, Total chlorophyll/STI, R.L/STI, S.F.W/I, R.F.W/I, R.D.W/C while negative correlation was not observed. Seedling fresh weight was positively correlated with R.L/I, R.L/STI, S.D.W/C, S.F.W/I, R.F.W/I, R.D.W/C while negatively correlated with T.W.P/I. Seedling dry weight was positively correlated with Fv/Fm/I, S.L/I, S.D.W/I and R.D.W/I while negatively correlated with SOD/I. Under irrigation stress shoot fresh weight was positively correlated with S.F.W/I, S.D.W/C and RDW/C while negatively correlated with TWP/I, R.W.C/C and MDA/C. Shoot dry weight was positively correlated with TPC/I, TPC/STI, F_v/F_m/I, S.D.W/I and SH.F.W/C while negatively correlated with R.W.C/C and MDA/C. Root fresh weight is positively correlated with R.L/I, R.L/STI, S.F.W/C, S.F.W/I, S.D.W/C and R.D.W/C while negatively correlated with SOD/STI and CAT/C. Root dry weight is positively correlated with R.L/STI and S.D.W/C while negatively correlated with SOD/C (Table A1).

Under heat stress shoot length was positively correlated with O.P/C, R.W.C/H and R.W.C/STI while negatively correlated with MDA/STI and APX/C. Root length was positively correlated with T.W.P/H, FM/H, FV/H and R.L/STI while negatively correlated with S.L/C, R.L/STI, APX/H and APX/STI. Seedling fresh weight was positively correlated with S.F.W/C, SH.F.W/H, R.F.W/C and R.F.W/H while negatively correlated with TM/STI. Seedling dry weight was positively correlated with Protease/C, S.D.W/STI, SH.D.W/H and R.D.W/C while negatively correlated with Lyco/STI, Chl b/STI, Tot CH/STI, Protease/STI and R.L/C. Shoot fresh weight was positively correlated with S.F.W/C, S.F.W/H, R.F.W/C and R.F.W/H while negatively correlated with SOD/STI. Shoot dry weight was positively correlated with S.F.W/C, S.D.W/H and R.F.W/C while negatively correlated with Lyco/H, Lyco/STI, Chl b/H, CH b/STI, Tot Chl/H, tot Chl/STI and Esterase/H. Root fresh weight was positively correlated with S.F.W/C and R.F.W/H while negatively correlated with T_m/STI. Root dry weight was positively correlated with

O.P/H, O.P/STI, Tr.P/H, Tr.P/STI, S.D.W/H, S.D.W/STI and R.D.W/STI while negatively correlated with R.L/C (Table A2).

Under combined stress shoot length was positively correlated with S.O.D/STI while negative correlation was not observed. Root length was positively correlated with R.L/STI and R.D.W/STI while negatively correlated with Protease/STI. Seedling fresh weight was positively correlated with T.P.C/C, FO/C, S.F.W/HI and R.F.W/HI while negative correlation was not found. Seedling dry weight was positively correlated with T.P.C/STI, S.D.W/STI, R.D.W/HI and R.D.W/STI while negatively correlated with Protease/HI, S.L/C, S.F.W/C, R.F.W/C and S.O.D/HI. Shoot fresh weight was positively correlated with FO/HI, FM/HI, FV/HI, S.F.W/HI, S.F.W/STI and SH.F.W/STI while no negative correlation was observed. Shoot dry weight was positively correlated with TM/HI, TM/STI, S.L/STI and SH.D.W/STI while negative correlation was not observed. Root fresh weight was positively correlated with MDA/STI, TPC/C, F_o/C and S.F.W/HI while negatively correlated with OP/C, Tr.P/C and MDA/C. Root dry weight was positively correlated with TPC/STI, SDW/H/I, SDW/STI and RDW/STI while negatively correlated with F_v/HI, SOD/HI and SOD/STI (Table A3).

3.7 Stress Tolerance Index (STI)

Stress tolerance index was used in crop plants for determining high yield and tolerance potential of genotypes under different stress conditions. It actually tells us the percent increase or decrease in a specific character by a specific genotype. On the basis of this increase or decrease we can select the tolerant and sensitive genotypes for specific traits.

In this study under irrigation stress STI was calculated for 32 parameters (Table 3.1). On the basis of these traits susceptible and tolerant genotypes were selected. According to desired parameters K-09015 was proved to be tolerant genotype because 14% and 17% decrease in protease and malanodialdehyde activity was observed respectively. Peroxidase is antioxidant which prevents from reactive oxygen species. There was 17% increase in activity of peroxidase. MDA and protease should be decreased and peroxidase should be increased to maintain the membrane integrity hence causing the tolerance. Among morphological parameters there was just 5%, 1% and 7% increase was found in shoot length, root length and shoot fresh weight respectively. K0039-09 was proved to be susceptible under irrigation stress as there was 64%, 11%, 22%, 12%, 40% increase in

protease, MDA, shoot length, root length and shoot fresh weight respectively. Reasonable increase in these parameters confers susceptibility. These parameters can be used as efficient markers for irrigation stress tolerance studies in chickpea.

Under heat stress 32 parameters were used for STI calculation. According to STI values tolerant and susceptible genotypes were selected. CM 1238/08 was detected as tolerant genotype for heat stress. As there was 7%, 7%, 26% and 7% decrease relative water contents, esterase, seedling fresh weight and shoot fresh weight respectively. MDA and total phenolic contents were maintained. Protein contents and POD activity was increased 30% and 62% respectively. 9KCC-166 was proved to be susceptible genotype under heat stress as there was 13%, 26%, 38%, 22% and 33% decrease in relative water contents, total phenolic contents, seedling fresh weight, shoot fresh weight and protein contents respectively. Esterase, MDA and POD were increased 6%, 18% and 15% respectively. It was concluded that these parameters can be used as efficient markers in heat tolerance studies in chickpea (Table 3.1).

STI was calculated for 32 parameters under combined stress of heat and irrigation. According to STI values tolerant and sensitive genotypes were selected. K-09015 exhibited tolerance as there was 5%, 37%, 5% and 17% decrease was found in relative water contents, MDA, root length and protein contents respectively. Protease, shoot length, root dry weight were increased 28%, 9% and 24% respectively. FG0902 was detected as susceptible genotype under combined stress as there was 19%, 5%, 3%, 12% and 44% decrease in relative water contents, MDA, shoot length, root length and protein contents respectively. It was concluded that these parameters can be used as efficient markers in heat and irrigation stress tolerance studies in chickpea (Table 3.1).

Table 3.1: Stress tolerance index (STI) of Kabuli chickpea

Genotypes	TWP	OP	TRP	RWC	TOS	Lyco	Chl a	Chl b	CAR	T-Chl
STI values under irrigation stress										
9 KCC-166	92.76	100.70	112.98	109.24	187.12	99.65	69.39	119.33	86.63	101.30
K-09015	115.94	88.93	63.48	99.30	126.43	98.65	106.83	100.35	98.38	101.52
CH888/06	88.75	103.82	133.13	100.62	63.34	83.80	60.73	85.03	82.45	80.97
CM1235/08	95.35	88.57	82.43	98.12	57.70	106.81	171.68	101.85	109.81	113.57
FG0902	78.95	88.77	105.47	92.94	80.87	115.05	97.65	125.37	116.67	120.60
K0039-09	95.77	98.76	102.29	105.24	79.84	115.48	85.08	124.25	114.11	117.93
BKK 02174	99.33	100.69	102.61	104.52	68.35	80.14	136.01	65.12	87.50	77.40

NOOR-2013	106.80	107.98	109.60	104.80	96.26	69.45	90.90	57.46	75.93	64.55
STI values under heat stress										
9 KCC-166	124.34	104.80	74.55	87.36	244.70	106.78	64.28	137.07	91.17	110.80
K-09015	133.33	91.56	52.22	99.71	129.79	131.22	166.42	135.28	140.25	140.88
CH888/06	125.00	106.81	71.43	87.08	89.23	82.16	115.15	72.13	86.37	79.33
CM1235/08	144.19	97.05	54.31	93.13	79.59	99.66	111.05	97.10	101.31	99.44
FG0902	112.87	87.48	44.28	88.38	86.37	107.13	152.25	101.84	111.62	110.51
K0039-09	151.41	98.28	35.55	96.33	72.32	124.08	124.45	135.01	125.04	133.31
BKK 02174	132.21	106.19	69.43	84.66	95.78	124.48	250.44	123.96	129.30	145.88
NOOR-2013	144.22	128.97	107.96	94.14	89.70	87.94	65.99	87.09	88.77	82.62
STI values under combined stress										
9 KCC-166	136.18	126.87	112.47	93.64	198.86	108.82	59.50	140.84	98.52	111.48
K-09015	139.13	123.46	108.70	95.06	80.81	112.24	69.85	123.30	117.67	113.69
CH888/06	120.63	139.22	175.38	98.93	116.72	104.36	81.47	111.87	107.61	106.78
CM1235/08	158.14	105.25	57.29	111.61	164.38	85.60	76.39	81.20	88.83	80.40
FG0902	118.71	103.04	76.37	81.14	94.96	98.94	19.28	111.01	100.90	95.22
K0039-09	141.55	108.58	69.65	101.51	97.27	63.11	109.09	51.27	71.13	60.60
BKK 02174	139.60	111.00	70.62	74.56	103.94	91.55	46.65	95.81	92.42	87.29
NOOR-2013	134.69	140.49	148.48	93.10	88.92	90.03	63.59	87.59	76.93	82.50

Genotypes	EST	Protease	MDA	TPC	FO	FM	FV	TM	FV/FM	SL
STI values under irrigation stress										
9 KCC-166	98.38	88.10	115.67	132.63	93.42	113.23	122.09	145.62	108.55	111.76
K-09015	128.87	86.32	83.01	102.58	113.83	111.14	113.14	147.62	101.53	105.13
CH888/06	98.74	43.18	129.66	111.95	92.89	107.15	107.51	128.70	100.28	108.00
CM1235/08	99.16	55.15	101.88	122.90	93.00	96.56	97.33	57.23	100.86	111.59
FG0902	97.97	90.35	103.50	78.09	96.24	99.06	99.83	139.69	100.99	102.72
K0039-09	109.79	164.26	111.35	85.62	95.06	111.66	115.79	181.70	103.82	122.06
BKK 02174	110.81	101.54	109.34	101.94	97.72	107.86	106.57	209.65	97.93	116.28
NOOR.2013	109.44	101.54	112.43	149.95	92.63	93.50	94.34	99.55	100.24	116.18
STI values under heat stress										
9 KCC-166	106.25	185.76	117.54	74.51	100.35	107.37	109.36	135.58	102.40	100.00
K-09015	146.23	83.97	77.18	103.65	121.23	113.18	111.34	71.99	98.34	112.21
CH888/06	107.86	67.56	139.31	66.41	100.14	103.41	101.35	105.11	97.89	101.33
CM1235/08	93.59	141.42	100.63	100.36	88.33	91.65	92.37	68.94	100.88	112.14
FG0902	97.14	117.65	100.00	93.55	84.38	89.28	90.53	115.96	100.77	113.89
K0039-09	115.19	97.68	89.19	101.08	105.23	117.98	121.16	91.35	102.84	127.06
BKK 02174	113.38	230.59	108.24	114.97	93.44	106.89	106.45	111.48	98.70	111.47
NOOR2013	114.89	52.66	84.32	109.64	102.99	109.34	111.45	118.87	101.91	119.85

						STI values under combined stress				
9 KCC-166	77.49	132.52	122.51	109.69	77.09	113.86	127.88	132.91	110.76	106.67
K-09015	29.73	128.89	63.11	101.60	105.15	117.87	120.77	109.40	102.43	109.49
CH888/06	39.41	130.62	142.76	73.86	104.92	116.44	115.92	106.35	99.43	103.87
CM1235/08	47.54	180.78	131.88	116.46	86.65	96.69	98.33	63.59	102.08	110.76
FG0902	21.08	106.64	95.00	102.64	84.32	97.19	100.33	105.23	103.29	97.41
K0039-09	22.50	146.66	101.62	105.04	91.63	109.77	114.29	135.59	104.17	119.26
BKK 02174	48.28	180.59	107.14	108.53	95.72	112.79	113.03	202.66	99.37	115.66
NOOR.2013	49.47	60.35	111.35	128.34	92.50	100.22	102.55	93.74	102.24	111.62
Genotypes	SDW	SH.FW	SH.DW	RFW	RDW	PROT	SOD	POD	APX	CAT
9 KCC-166	100.35	127.61	121.70	112.11	71.81	61.57	112.44	275.70	98.46	71.02
K-09015	95.01	107.43	97.08	91.12	91.27	58.64	85.81	117.69	97.78	82.01
CH888/06	101.80	117.71	109.28	92.97	89.69	72.87	70.48	136.13	86.15	96.02
CM1235/08	88.92	121.62	101.62	102.74	70.77	120.30	86.61	185.64	86.57	103.09
FG0902	98.18	131.49	114.99	99.54	77.27	111.74	100.49	155.97	481.82	88.72
K0039-09	107.50	139.84	114.26	122.87	98.27	113.12	85.30	203.38	288.00	84.91
BKK 02174	101.71	132.60	112.30	127.82	85.16	179.66	143.23	180.87	93.55	59.25
NOOR-2013	116.30	120.16	124.72	124.49	102.64	83.76	113.03	217.57	132.20	114.49
9 KCC-166	73.96	78.78	84.98	50.96	59.23	67.91	118.13	115.42	173.85	91.85
K-09015	90.46	92.40	84.09	48.84	102.01	45.20	103.96	204.51	240.00	101.78
CH888/06	98.17	101.46	95.29	50.44	102.84	31.51	78.05	163.19	162.05	107.84
CM1235/08	84.72	93.45	87.35	63.47	80.96	130.96	115.38	162.98	164.18	89.30
FG0902	94.63	113.62	117.37	49.36	66.36	26.56	99.22	228.38	213.64	84.85
K0039-09	81.52	98.88	85.90	59.45	75.54	43.35	129.57	161.82	114.00	142.90
BKK 02174	92.07	97.76	78.03	79.83	114.01	155.64	133.42	195.64	151.61	85.06
NOOR-2013	120.12	80.90	100.00	51.37	152.77	57.85	131.83	195.79	194.92	165.26
9 KCC-166	107.29	73.52	102.28	53.70	114.00	56.90	127.14	128.27	118.46	133.29
K-09015	108.28	76.93	99.42	28.21	124.34	83.50	115.61	240.26	262.22	88.01
CH888/06	94.69	86.77	87.40	50.12	106.50	39.27	113.46	223.16	116.92	136.14
CM1235/08	96.99	69.30	79.54	36.91	121.92	84.48	112.21	312.43	111.94	107.99
FG0902	118.74	84.58	98.35	48.05	144.09	56.62	82.02	340.58	225.00	94.24
K0039-09	119.58	98.49	117.91	45.28	121.86	71.12	111.73	250.91	216.00	110.62
BKK 02174	152.09	115.48	131.46	71.14	184.34	55.72	72.65	336.24	83.87	59.69
NOOR-2013	150.20	71.79	106.83	49.38	220.58	38.44	68.69	217.33	128.81	103.68

3.8 Cluster Analysis

Based on association among phenomic data (24 attributes) and physio biochemical markers (29 for irrigation, 41 for heat and 31 for combined stress) relative stress tolerance of

genotypes was assessed. Genotypes were classified as tolerant, moderately tolerant, moderately sensitive and sensitive. Following are dendrograms obtained by clustering under irrigation, heat and combined stress.

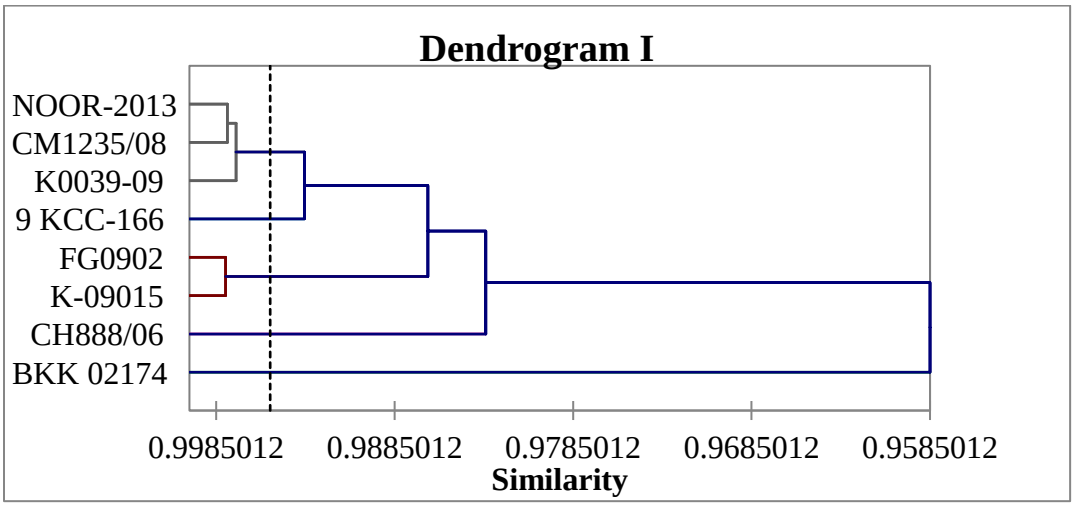

Figure 3.37: Dendrogram of kabuli genotypes under irrigation stress

K-09015 and FG0902 made one cluster and were categorized as tolerant genotypes because S.L, R.L, S.F.W and R.W.C were maintained in stress condition. Moderately tolerant was CH888/06 and BKK 02174 because these performed in between of tolerant and moderately sensitive. Moderately sensitive were NOOR-2013, CM 1235/08 and 9 KCC-166 because these performed better than sensitive. K0039-9 was sensitive genotype as it performed poorly in irrigation stress (Figure 3.37).

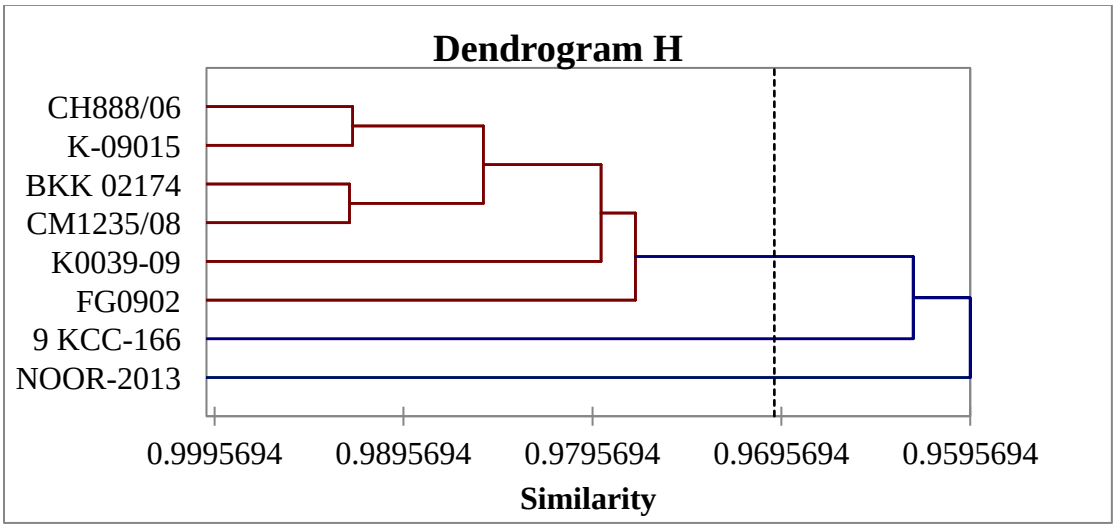

Figure 3.38: Dendrogram of kabuli genotypes under heat stress

BKK 02174 and CM 1235/08 were categorized as tolerant genotypes because S.L, R.L, S.F.W and R.W.C were maintained in stress condition. Moderately tolerant were CH888/06 and K-09015 because these performed in between of tolerant and moderately sensitive. Moderately sensitive were NOOR-2013, K0039-9 and FG0902 because these performed better than sensitive. 9 KCC-166 was sensitive genotype as it performed less in heat stress (Figure 3.38).

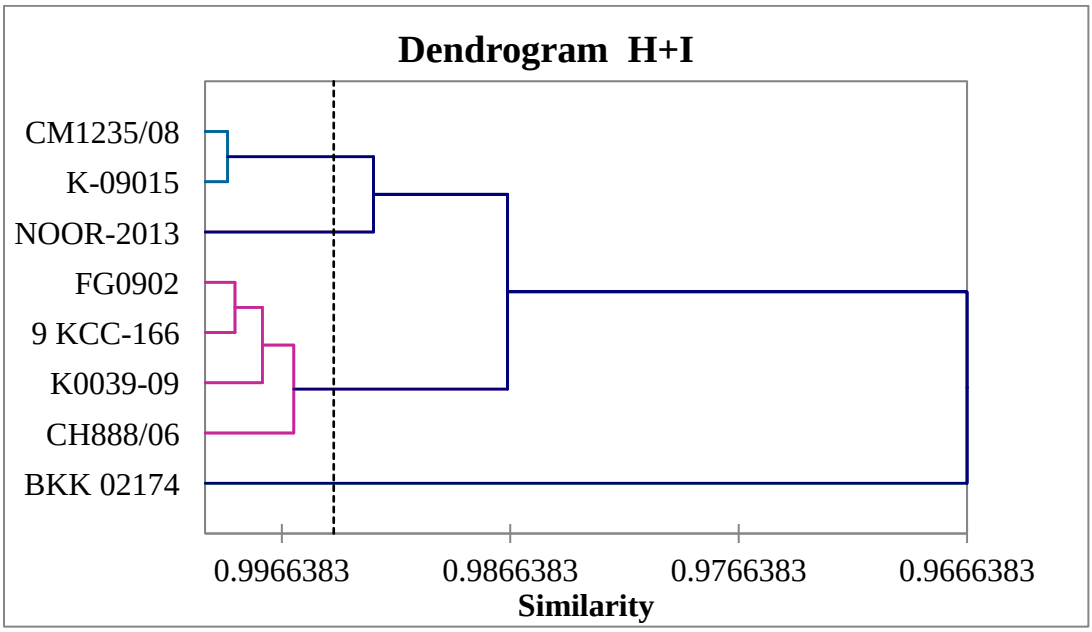

Figure 3.39: Dendrogram of kabuli genotypes under combined stress

K-09015 and CM 1235/08 were categorized as tolerant genotypes because S.L, R.L, S.F.W and R.W.C were maintained in stress condition. Moderately tolerant was BKK 02174 because it performed in between of tolerant and moderately sensitive. Moderately sensitive were 9 KCC-166 K0039-9 and NOOR-2013 and CH888/06 because these performed better than sensitive. FG0902 was sensitive genotype as it performed least in combined stress (Figure 3.39).

3.9 Desi Genotypes

3.10 Morphological Parameters

3.10.1 Shoot Length (S.L)

Under non-stress condition, significant differences were observed in tested genotypes for shoot length (S.L) (Figure 3.40). The highest (15.75 cm) shoot length was observed in TGX 225 while the lowest (12.22 cm) in D-09027. Under the irrigation stress, shoot length was more (17.12 cm) in TGX 225 while lowest (12.62) in D-09027. Under heat stress D-0913 showed higher (21.70 cm) length while CH 24/07 showed lowest (13.30 cm) length. Combined stress showed CH104/06 shoot length was increasing (21.12 cm) while D-09027 with decreasing (14.32 cm) length.

As compared to non-stress condition, shoot length was found non-significant under irrigation stress. In case of heat stress, shoot length of D-0913 was observed significantly high while others remained unchanged. When shoot length was observed under combined stress of heat and irrigation it was found that shoot length of CH104/06, BRC -390, CMC 211S and Bhakhar-2011 was significantly high while others were non-significant (Figure 3.40).

3.10.2 Root Length (R.L)

Under non-stress condition, significant differences were observed in tested genotypes for root length (R.L) (Figure 3.41). The highest (31.87 cm) root length was observed in CMC 211S while the lowest (16.80 cm) in D-0913. Under the irrigation stress, root length was more (33.87 cm) in CH 24/07 while lowest (19.55 cm) in D-0913. Under heat stress CMC 211S showed higher (29.90 cm) length while D-09027 showed smaller (22.40cm) length. Combined stress depicted increased (34.10 cm) root length in Bhakhar-2011 while decreased (19.85 cm) in D-0913.

As compared to non-stress condition, root length was found to be non-significant under irrigation, heat and combined stress (Figure 3.41).

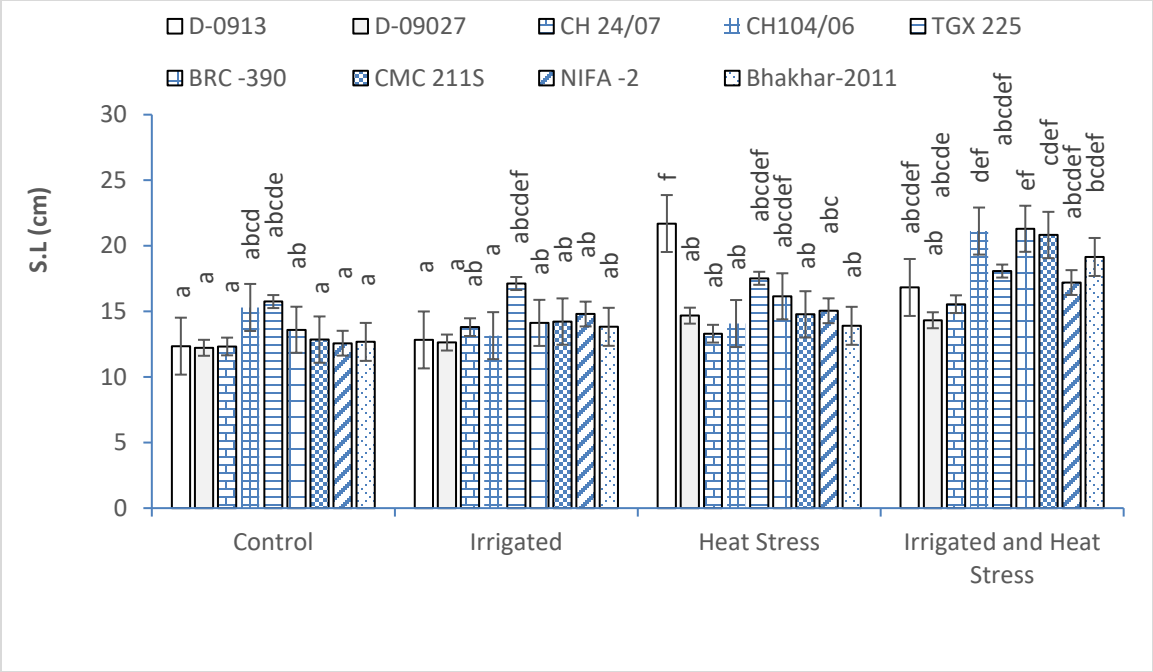

Figure 3.40: Shoot length under control, irrigation, heat and combined stress

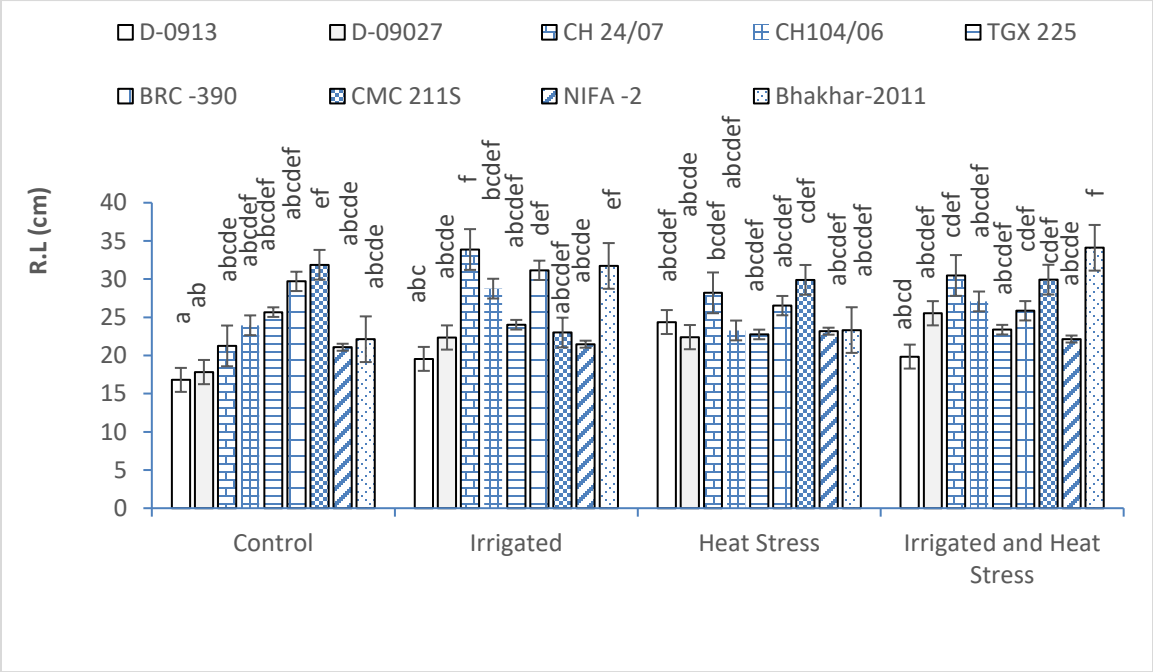

Figure 3.41: Root length under control, irrigation, heat and combined stress

3.10.3 Seedling Fresh Weight (S.F.W)

Under non-stress condition, significant differences were observed in tested genotypes for seedling fresh weight (S.F.W) (Figure 3.42). The highest (3.39 g) S.F.W was observed in BRC -390 while the lowest (2.61 g) in Bhakhar-2011. Under the irrigation stress, S.F.W was highest (5.32 g) in BRC -390 and lowest (2.083 g) in D-0913. Under heat stress highest (4.27 g) S.F.W was present in D-0913 while lowest (1.59 g) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (5.84 g) S.F.W was found in BRC -390 and the lowest (2.41 g) value was observed in TGX 225.

As compared to non-stress condition, S.F.W was non-significant in irrigation stress for all genotypes. Under heat and combined stress all genotypes showed significantly decreasing behavior (Figure 3.42).

3.10.4 Seedling Dry Weight (S.D.W)

Under non-stress condition, significant differences were observed in tested genotypes for seedling dry weight (S.D.W) (Figure 3.43). The highest (0.40 g) S.D.W was observed in BRC -390 while the lowest (0.19 g) in CH 24/07. Under the irrigation stress, S.D.W was highest (0.40 g) in BRC -390 and lowest (0.13 g) in CH 24/07. Under heat stress highest (0.37 g) S.D.W was present in TGX 225 while lowest (0.17 g) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (0.43 g) S.D.W was found in BRC -390 and the lowest (0.21 g) value was observed in CH 24/07.

As compared to non-stress condition, S.D.W was found non-significant in irrigation heat and combined stress as well (Figure 3.43).

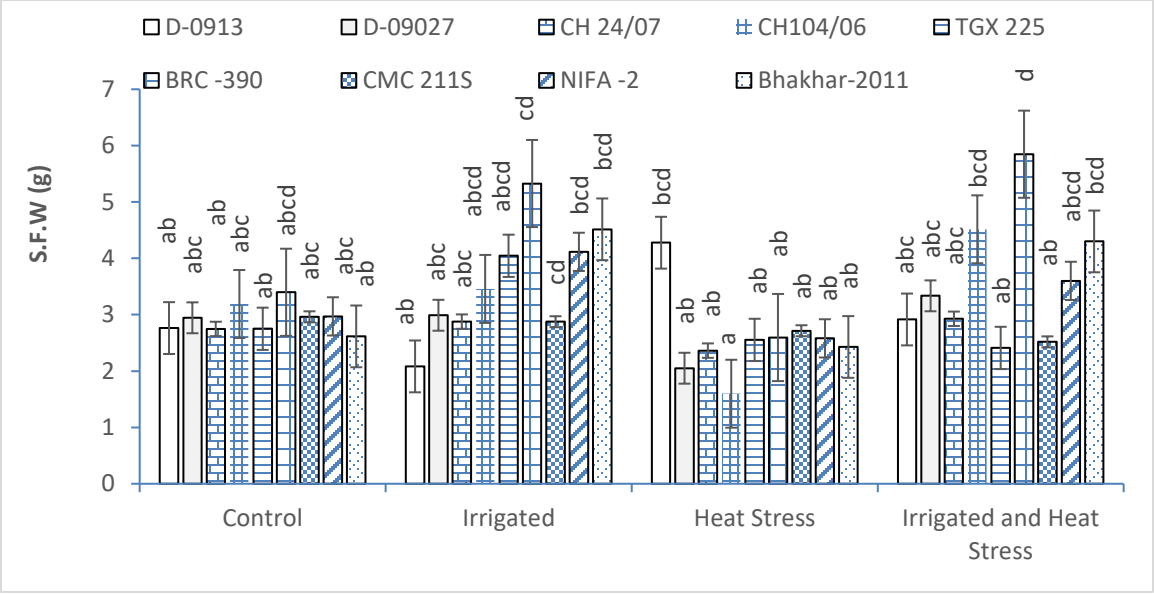

Figure 3.42: Seedling fresh weight under control, irrigation, heat and combined stress

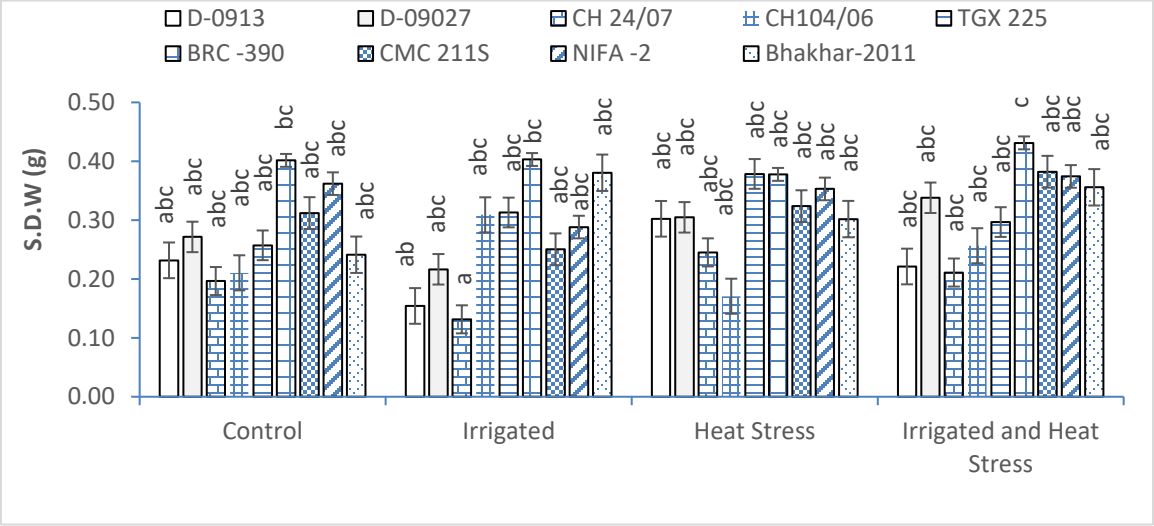

Figure 3.43: Seedling dry weight under control, irrigation, heat and combined stress

3.10.5 Shoot Fresh Weight (SH.F.W)

Under non-stress condition, significant differences were observed in tested genotypes for shoot fresh weight (SH.F.W) (Figure 3.44). The highest (1.25 g) SH.F.W was observed in BRC -390 while the lowest (0.91 g) in CMC 211S. Under the irrigation stress, SH.F.W was highest (1.58 g) in BRC -390 and lowest (0.76 g) in D-0913. Under heat stress highest (2.21 g) SH.F.W was present in D-0913 while lowest (0.79 g) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (3.13 g) SH.F.W was found in BRC -390 and the lowest (0.95 g) value was observed in CH 24/07.

As compared to non-stress condition, SH.F.W was found non-significant under irrigation stress. Under heat stress D-0913 showed significantly high SH.F.W while all others remained unchanged. Under combined stress, it was found that BRC -390 differed significantly and weight was high while all others remained unchanged (Figure 3.44).

3.10.6 Shoot Dry Weight (SH.D.W)

Under non-stress condition, significant differences were observed in tested genotypes for shoot dry weight (SH.D.W) (Figure 3.45). The highest (0.16 g) SH.D.W was observed in BRC -390 while the lowest (0.72 g) in CH 24/07. Under the irrigation stress, SH.D.W was highest (0.15 g) in Bhakhar-2011 and lowest (0.05 g) in CH 24/07. Under heat stress highest (0.21 g) SH.D.W was present in D-0913 while lowest (0.11 g) were in CH 24/07. In case of combined (heat and irrigation) stress, the highest (0.30 g) SH.D.W was found in BRC -390 and the lowest (0.09 g) value was observed in CH 24/07.

As compared to non-stress condition, SH.D.W was found non-significant under irrigation and heat stress. When heat and irrigation stress was observed combined, it was found that BRC -390 differed significantly and weight was high while all others remained unchanged (Figure 3.45).

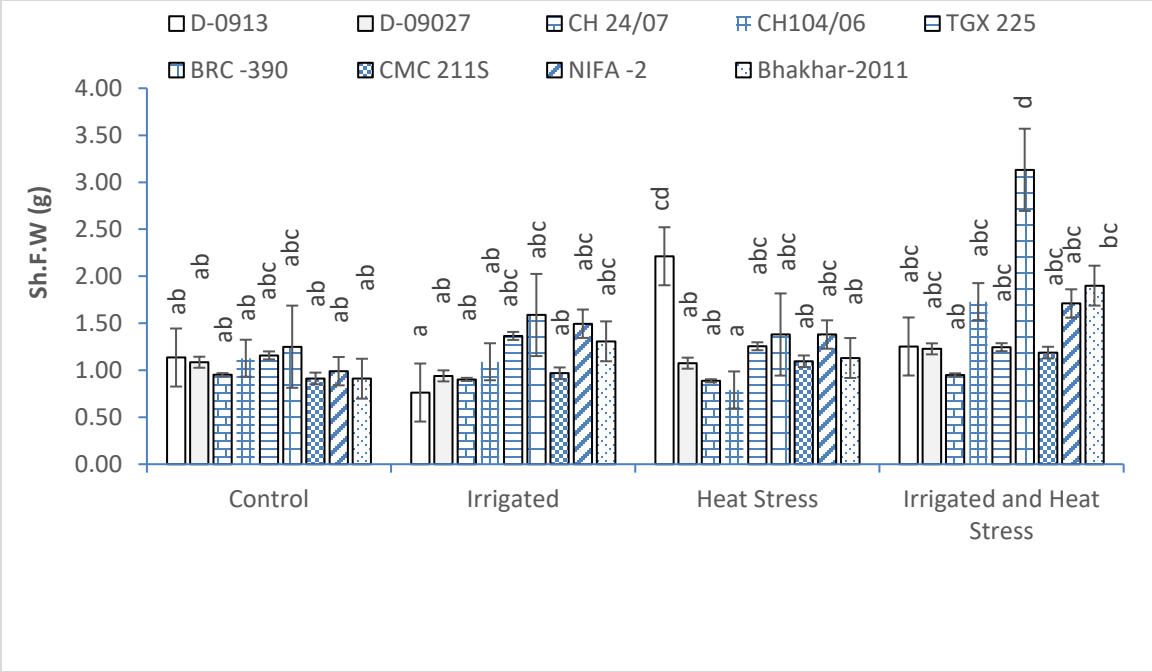

Figure 3.44: Shoot fresh weight under control, irrigation, heat and combined stress

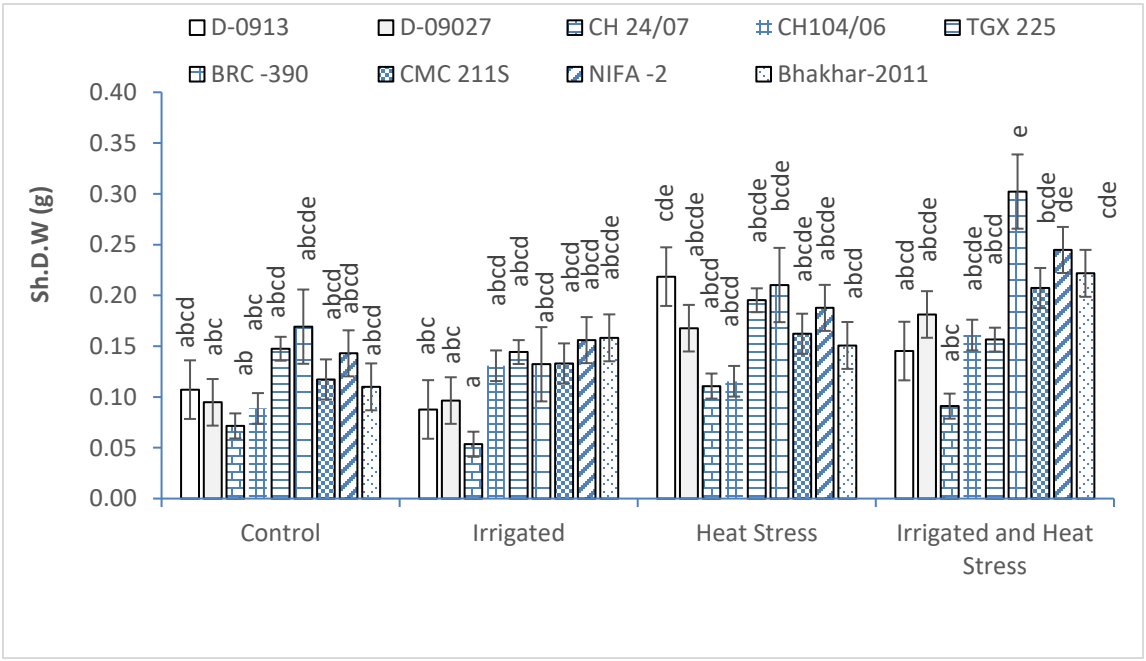

Figure 3.45: Shoot dry weight under control, irrigation, heat and combined stress

3.10.7 Root Fresh Weight (R.F.W)

Under non-stress condition, significant differences were observed in tested genotypes for shoot fresh weight (R.F.W) (Figure 3.46). The highest (2.14 g) R.F.W was observed in BRC -390 while the lowest (1.59 g) in TGX 225. Under the irrigation stress, R.F.W was highest (3.74 g) in BRC -390 and lowest (1.32 g) in D-0913. Under heat stress highest (2.06 g) R.F.W was present in D-0913 while lowest (0.80 g) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (2.78 g) R.F.W was found in CH104/06 and the lowest (1.16 g) value was observed in TGX 225.

As compared to non-stress condition, R.F.W was found non-significant under irrigation, heat and combined stresses (Figure 3.46).

3.10.8 Root Dry Weight (R.D.W)

Under non-stress condition, significant differences were observed in tested genotypes for root dry weight (R.D.W) (Figure 3.47). The highest (0.23 g) R.D.W was observed in BRC -390 while the lowest (0.11 g) in TGX 225. Under the irrigation stress, R.D.W was highest (0.27 g) in BRC -390 and lowest (0.06 g) in D-0913. Under heat stress highest (0.18 g) R.D.W was present in TGX 225 while, lowest (0.05 g) were observed in CH104/06. In case of combined (heat and irrigation) stress, the highest (0.17 g) R.D.W was found in CMC 211S and the lowest (0.07 g) value was observed in D-0913.

As compared to non-stress condition, R.D.W was found non-significant under irrigation and heat stress and under combination of both heat and irrigation as well (Figure 3.47).

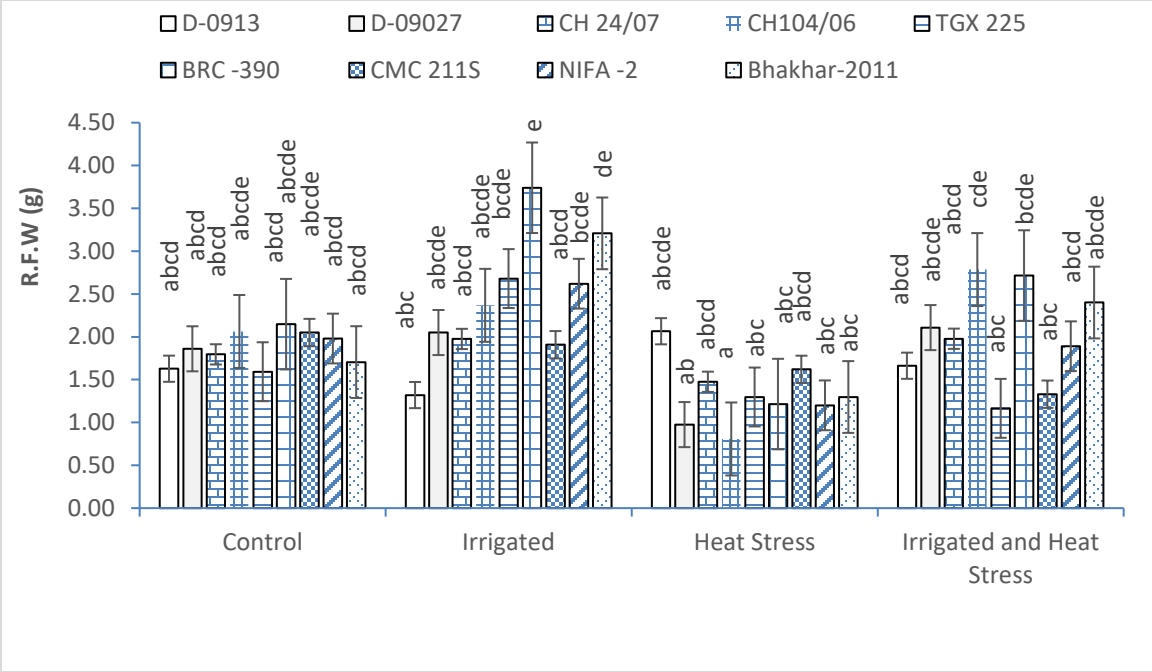

Figure 3.46: Root fresh weight under control, irrigation, heat and combined stress

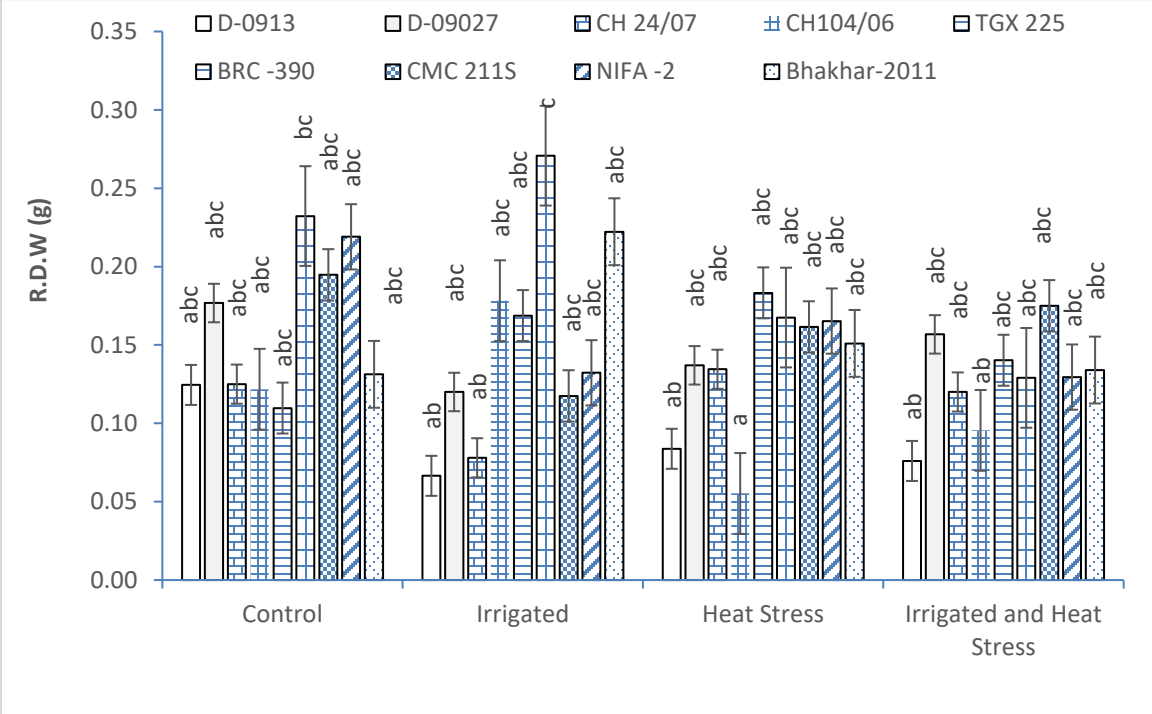

Figure 3.47: Root dry weight under control, irrigation, heat and combined stress

3.10.9 Morphological Trait

Screening of desi genotypes under irrigation, heat and combined stress on the basis of visual observation. Certain morphological symptoms were appeared under these stresses as yellowness of leaves, wilting and senescence under heat and combined stress, while a sreasonable increased in vegetative growth was observed in some genotypes (Figure 3.48, Figure 3.49, Figure 3.50).

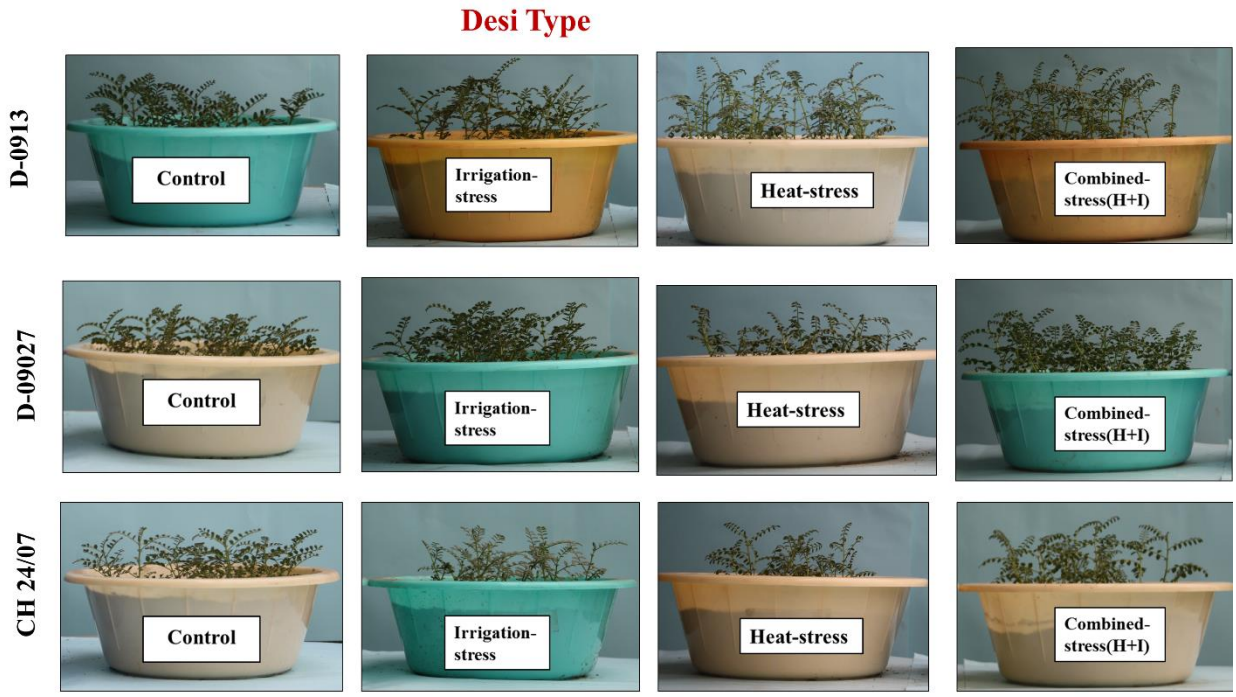

Figure 3.48: D-0913, D-09027 and CH 24/07 under control, irrigation, heat and combined stress

Figure 3.49: CH104/06 TGX 225 and BRC -390 under control, irrigation, heat and combined stress

Figure 3.50: CMC 211S, NIFA-2 and Bhakhar-2011 under control, irrigation, heat and combined stress

In Desi genotypes on the basis of physical appearance under irrigation stress it was observed that D-09027 (Figure 3.48) showed tolerance because shoot length was maintained under stress. NIFA-2 was proved to be sensitive genotype as there was maximum increase in shoot length (Figure 3.50). Under heat stress D-0913 were observed tolerant genotypes because chlorosis, wilting and senescence was not detected in these genotypes (Figure 3.48). NIFA-2 and TGX-225 was proved to be susceptible genotype because heat stress caused wilting, senescence and chlorosis in this genotype (Figure 3.50, Figure 3.49). Under combined stress D-0913 and CH 24/07 were detected tolerant genotypes as there was no change in shoot length and no effect of chlorosis, wilting and senescence (Figure 3.48). CMC211S was susceptible genotype under combined stress as there was increase in shoot length along with senescence, wilting and chlorosis (Figure 3.50).

3.11 Physiological Parameters

3.11.1 Total Water Potential (TWP)

Under non-stress condition, significant differences were observed in tested genotypes for total water potential (TWP) (Figure 3.51). The highest (0.88 -Mpa) TWP was observed in D-09027 while the lowest (0.51 -Mpa) in BRC -390. Under the irrigation stress, TWP was highest (0.42 -Mpa) in CH 24/07 and lowest (0.26 -Mpa) in Bhakhar-2011. Under heat stress highest (1.01 -Mpa) TWP was present in TGX 225 while lowest (0.74 -Mpa) were in D-0913. In case of combined (heat and irrigation) stress, the highest (0.97 -Mpa) TWP was found in NIFA -2 and the lowest (0.72 -Mpa) potential was observed in BRC -390.

As compared to non-stress condition, TWP was found non-significant under irrigation and heat stress and under combination of both heat and irrigation as well (Figure 3.51).

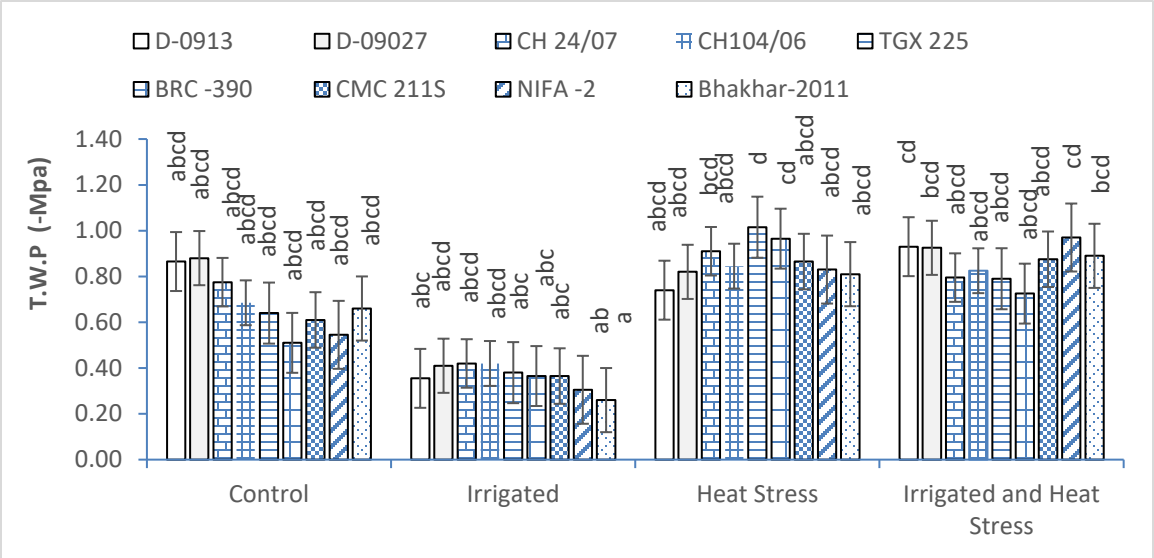

Figure 3.51: Total water potential under control, irrigation, heat and combined stress

3.11.2 Other Physiological Parameters

Under non-stress condition, irrigation, heat and combined stress non-significant differences were observed in tested genotypes for osmotic potential (O.P), turgor potential (Tr. P), relative water content (R.W.C), Minimal chlorophyll fluorescence (F_o), Maximal chlorophyll fluorescence (F_m), variable chlorophyll fluorescence (F_v) and time of achieving maximum fluorescence yield (Tm) (Figure 3.52 , Figure 3.53, Figure 3.54, Figure 3.55, Figure 3.56, Figure 3.57, Figure 3.58, Figure 3.59).

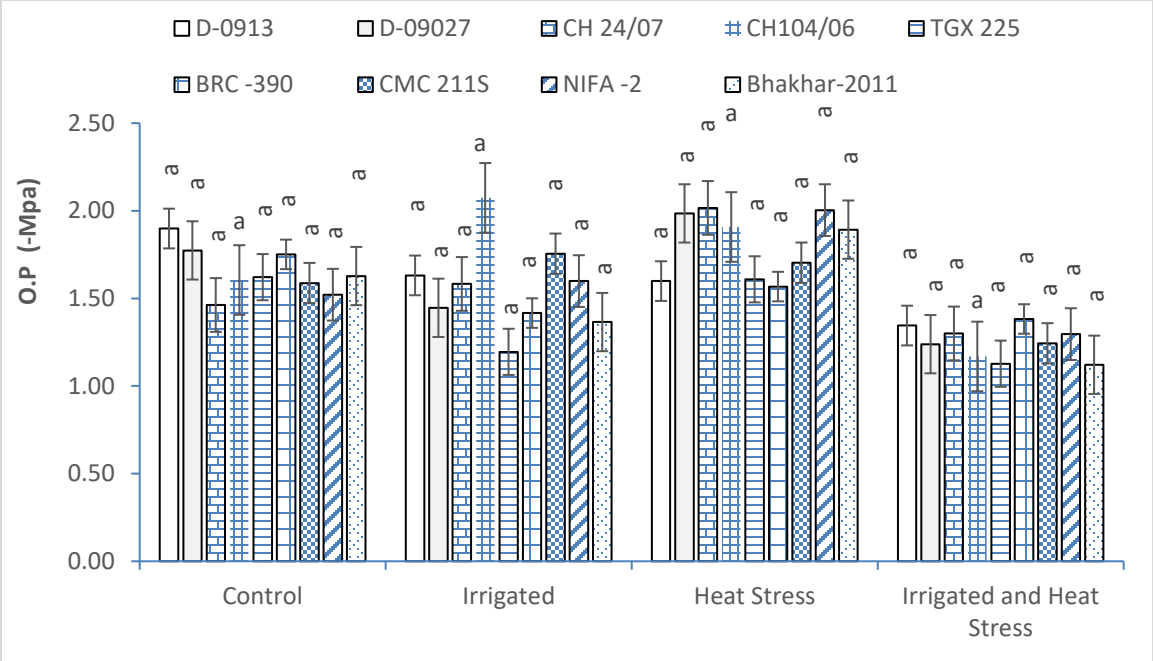

Figure 3.52: Osmotic potential under control, irrigation, heat and combined stress

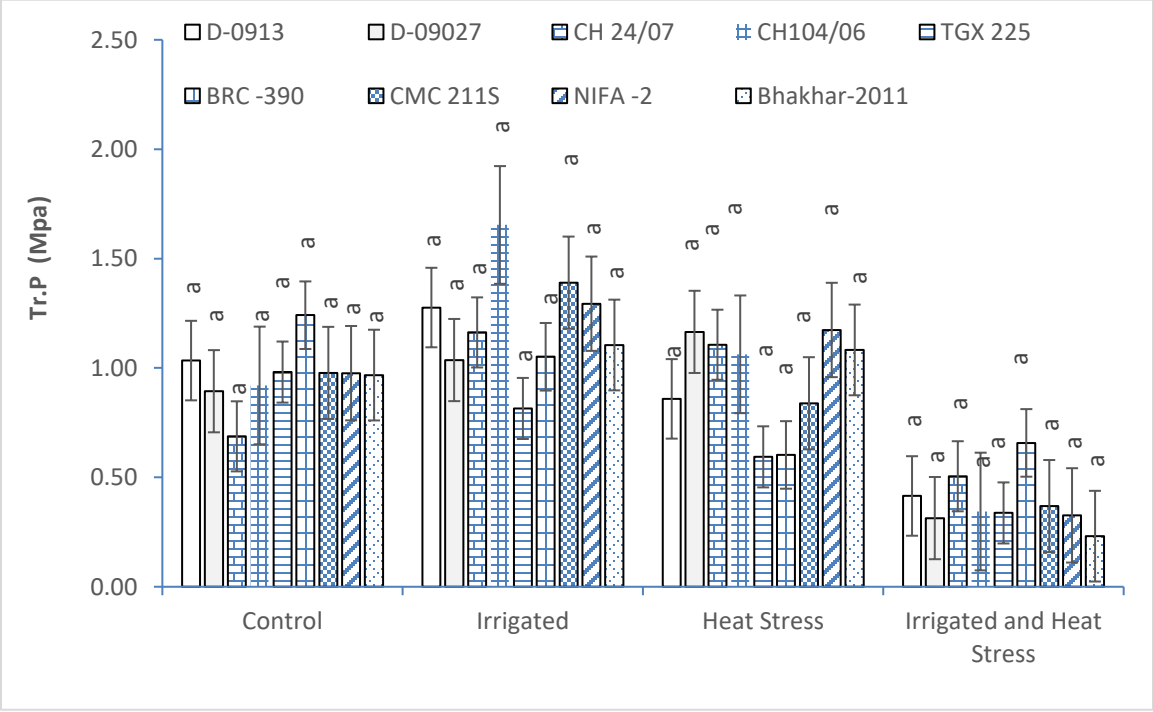

Figure 3.53: Turgor potential under control, irrigation, heat and combined stress

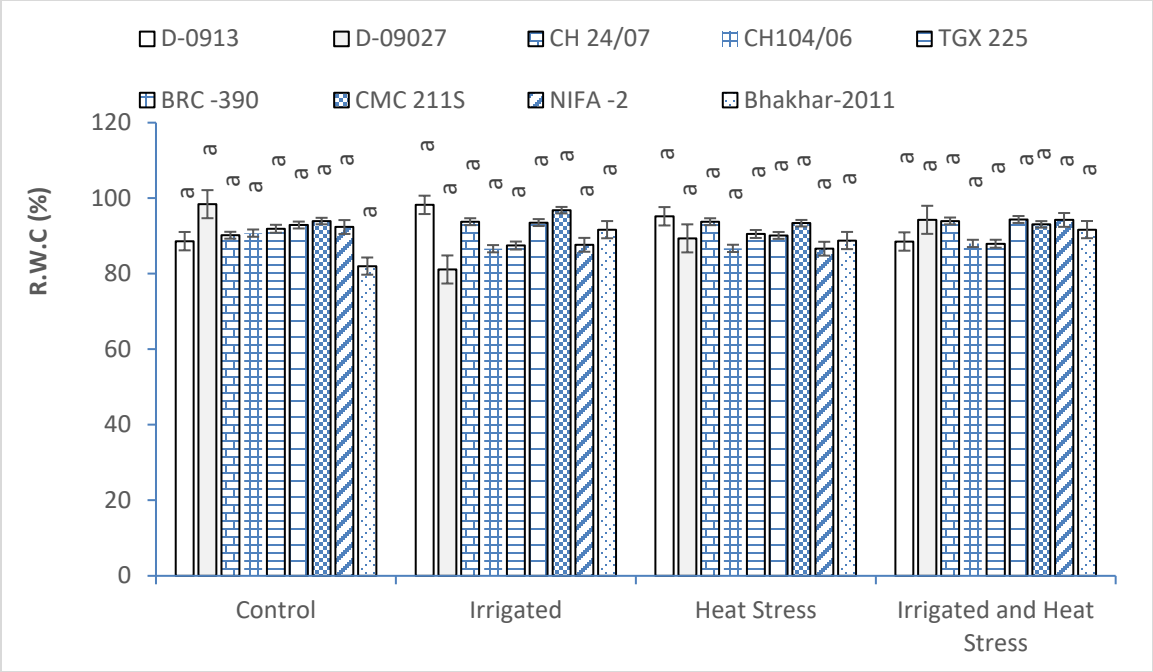

Figure 3.54: Relative water contents under control, irrigation, heat and combined stress

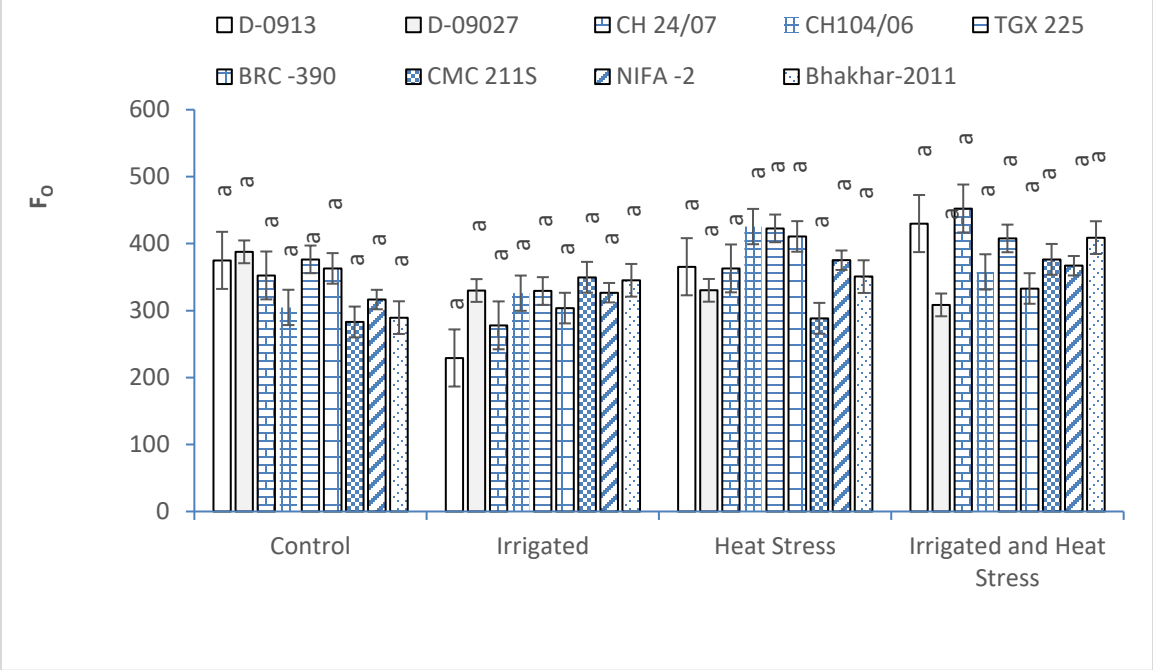

Figure 3.55: Minimal chlorophyll fluorescence under control, irrigation, heat and combined stress

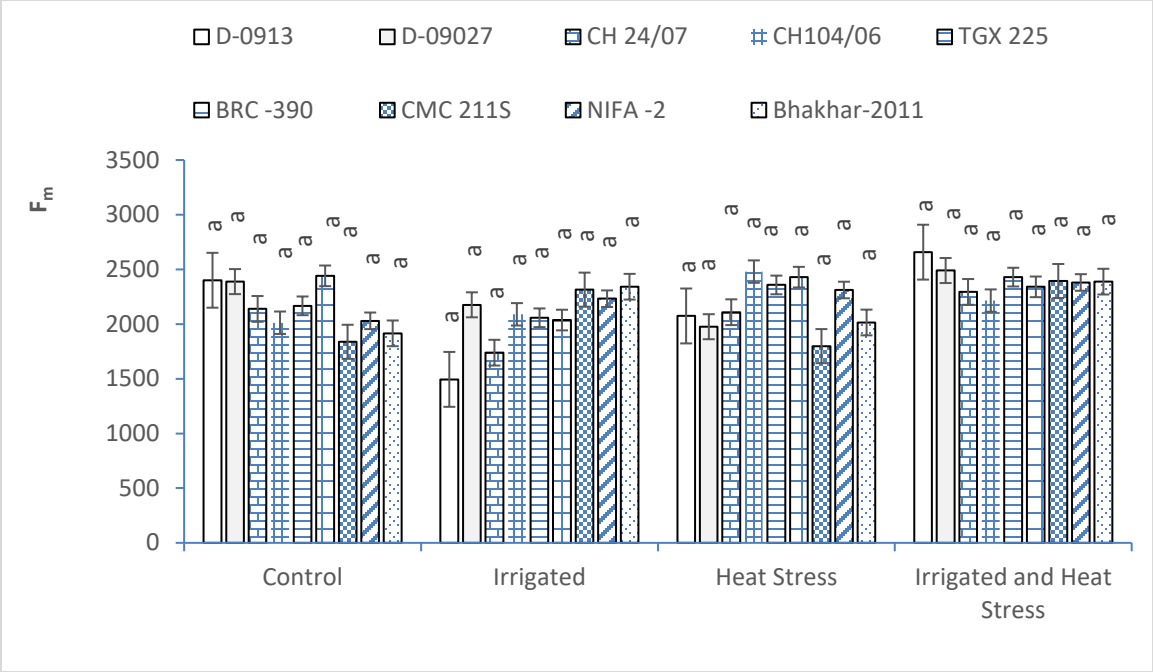

Figure 3.56: Maximum chlorophyll fluorescence under control, irrigation, heat and combined stress

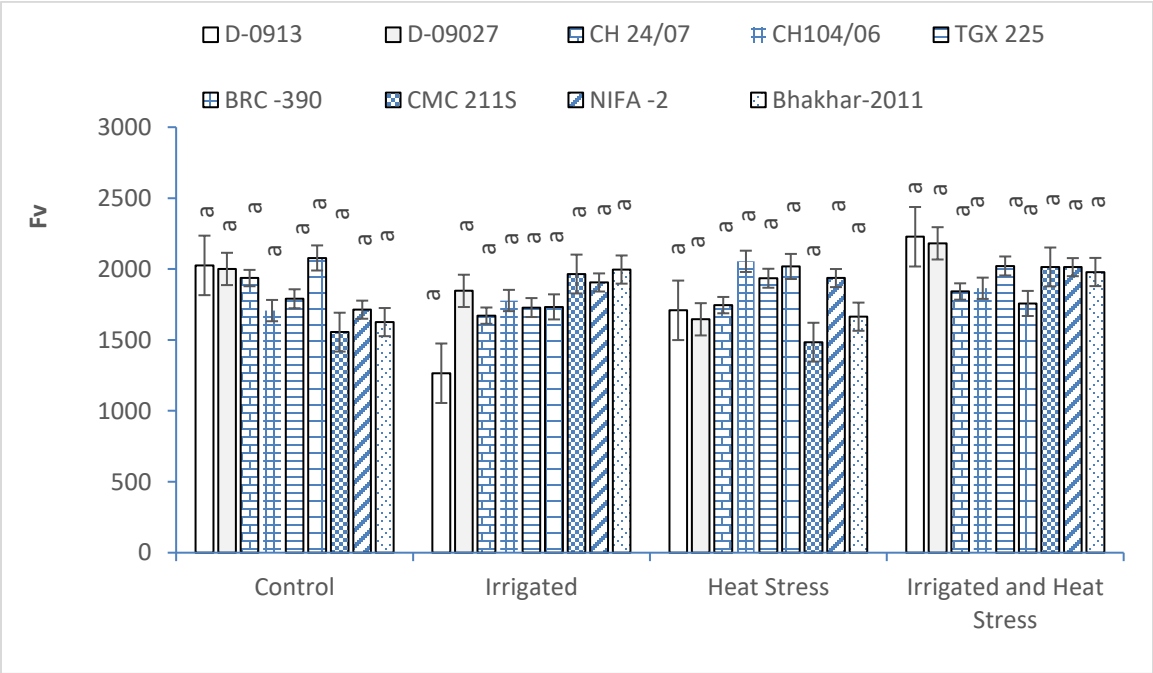

Figure 3.57: Variable chlorophyll fluorescence under control, irrigation, heat and combined stress

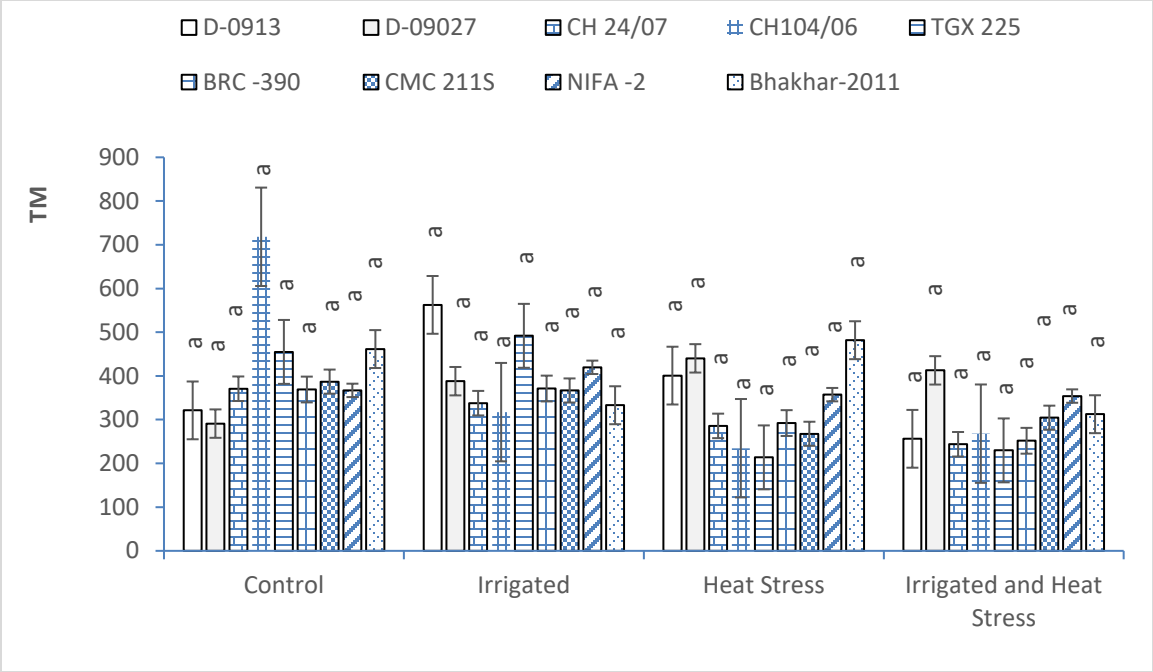

Figure 3.58: T_m under control, irrigation, heat and combined stress

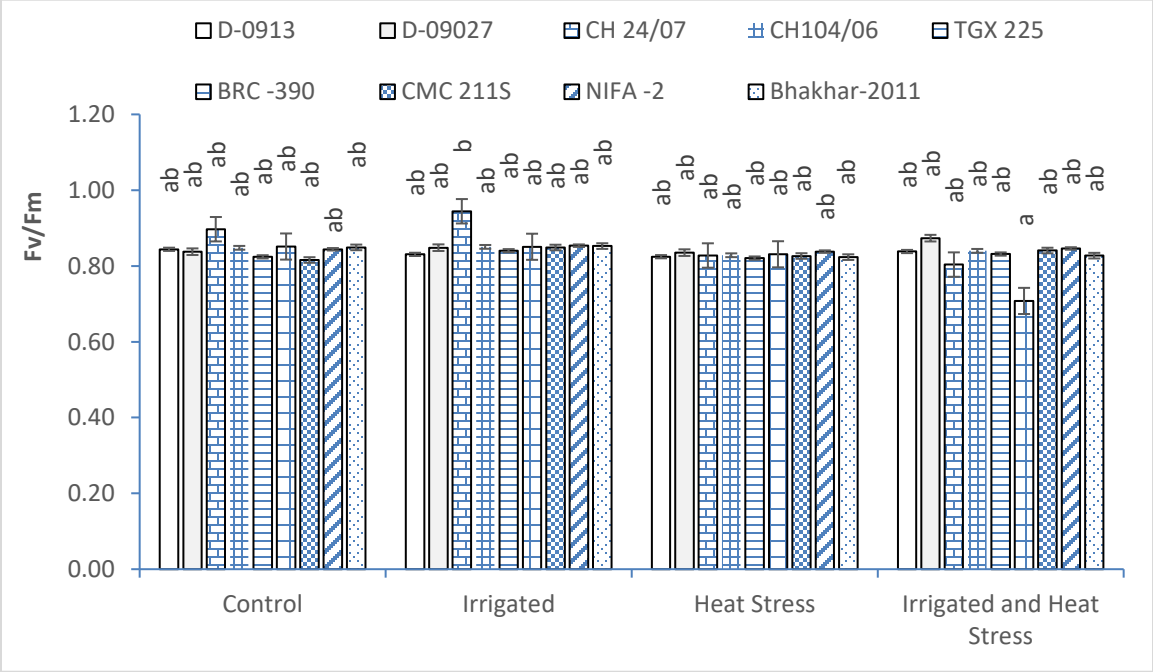

Figure 3.59: F_v/F_m under control, irrigation, heat and combined stress

3.12 Biochemical Parameters

3.12.1 Lycopene

Under non-stress condition, significant differences were observed in tested genotypes for lycopene contents (Figure 3.60). The highest (17.76 mg/g f.wt) lycopene contents were observed in CH 24/07 while the lowest (10.29 mg/g f.wt) in Bhakhar-2011. Under the irrigation stress, lycopene contents were highest (18.47 mg/g f.wt) in CH 24/07 and lowest (9.31 mg/g f.wt) in Bhakhar-2011. Under heat stress highest (17.35 mg/g f.wt) R.D.W was present in CMC 211S while lowest (10.86 mg/g f.wt) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (19.88 mg/g f.wt) lycopene contents were found in CH104/06 and the lowest (12.43 mg/g f.wt) value was observed in CH 24/07.

As compared to non-stress condition, lycopene contents were found significantly different under irrigation stress. D-0913, D-09027 showed decreased lycopene contents and CMC 211S, NIFA -2 and CH104/06 increased significantly while others remained unchanged. Under heat stress, D-0913, D-09027, CH 24/07 and TGX 225 were decreased while BRC -390, CMC 211S and Bhakhar-2011 showed increased lycopene contents and in others it remained unchanged. Under combined stress, D-0913 and CH 24/07 showed decreased lycopene contents, CH104/06, NIFA-2 and Bhakhar-2011 were increased in lycopene contents while all others remained unchanged (Figure 3.60).

3.12.2 Chlorophyll ‘a’

Under non-stress condition, significant differences were observed in tested genotypes for chlorophyll ‘a’ contents (Figure 3.61). The highest (159.88 ug/g f.wt) chlorophyll ‘a’ contents were observed in CH 24/07 while the lowest (59.29 ug/g f.wt) in Bhakhar-2011. Under the irrigation stress, chlorophyll ‘a’ were highest (160.47 ug/g f.wt) in CH 24/07 and lowest (44.54 ug/g f.wt) in Bhakhar-2011. Under heat stress highest (124.92 ug/g f.wt) chlorophyll ‘a’ was present in CMC 211S while lowest (27.10 ug/g f.wt) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (339.82 ug/g f.wt) chlorophyll ‘a’ were found in CH104/06 and the lowest (43.66 ug/g f.wt) value was observed in CH 24/07.

As compared to non-stress condition, chlorophyll ‘a’ contents were found non-significant under irrigation stress. D-0913, CH 24/07 and CH104/06 showed significantly decreased chlorophyll ‘a’ contents under heat stress while other genotypes remained

unchanged. Under combined stress CH104/06 was observed to have significantly highest contents while in others it remained unchanged (Figure 3.61).

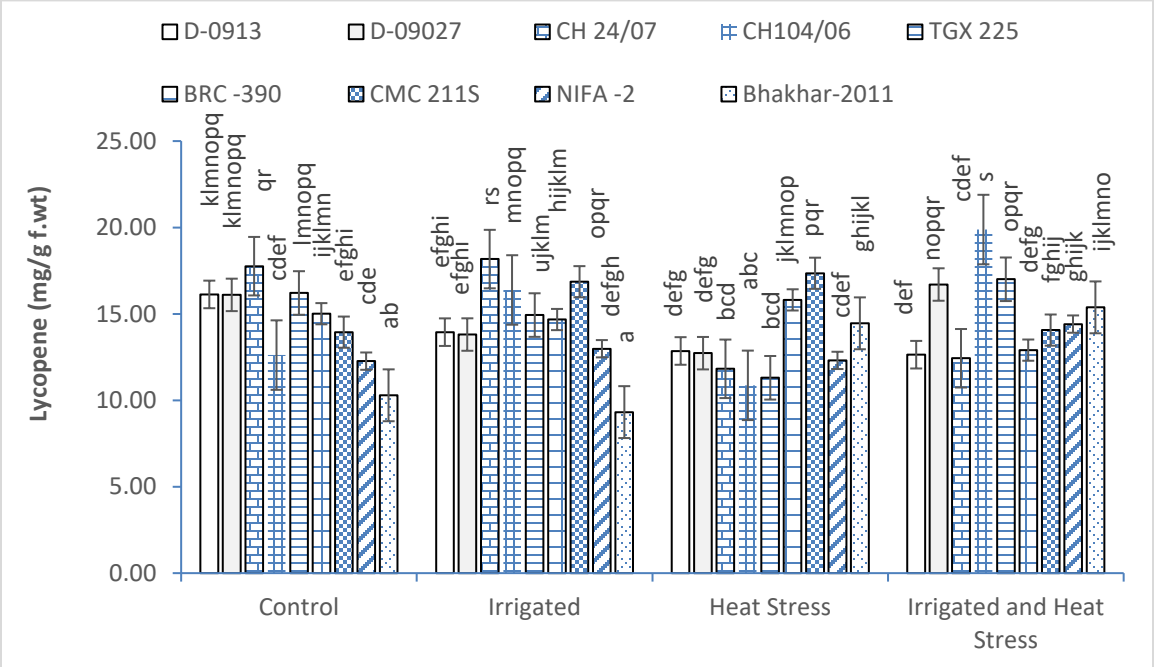

Figure 3.60: Lycopene contents under control, irrigation, heat and combined stress

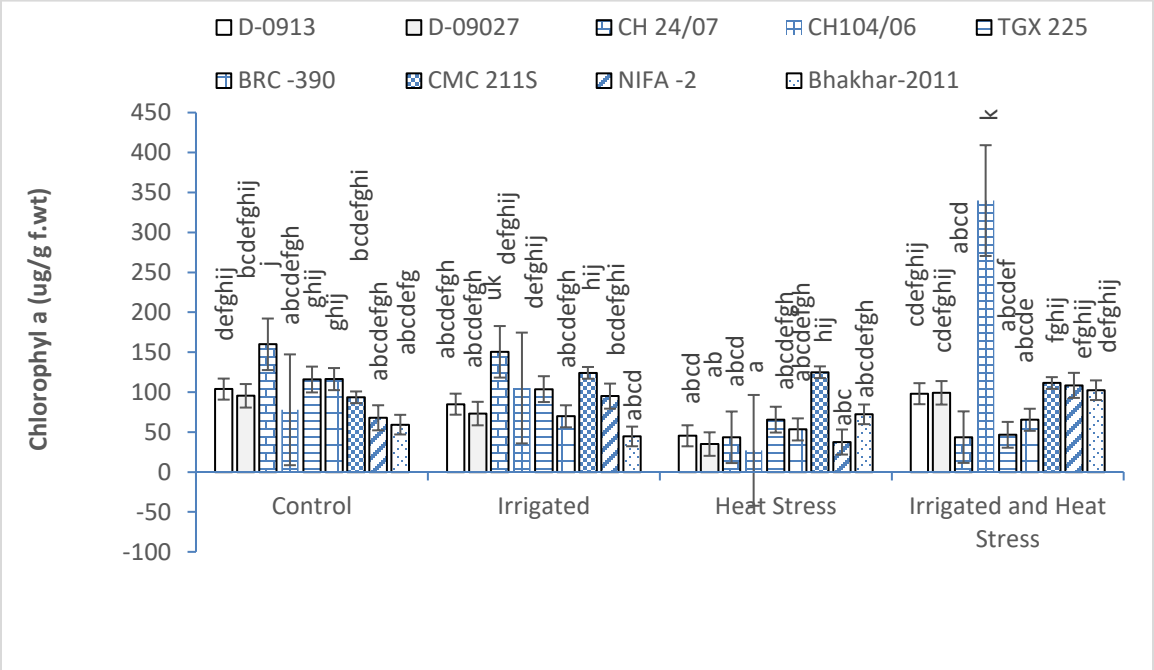

Figure 3.61: Chlorophyll 'a' under control, irrigation, heat and combined stress

3.12.3 Chlorophyll ‘b’

Under non-stress condition, significant differences were observed in tested genotypes for chlorophyll ‘b’ contents (Figure 3.62). The highest (573.50 ug/g f.wt) chlorophyll ‘b’ contents were observed in CH 24/07 while the lowest (301.51 ug/g f.wt) in Bhakhar-2011. Under the irrigation stress, chlorophyll ‘b’ were highest (584.02 ug/g f.wt) in CH 24/07 and lowest (273.63 ug/g f.wt) in Bhakhar-2011. Under heat stress highest (594.73 ug/g f.wt) chlorophyll ‘b’ was present in CMC 211S while lowest (351.49 ug/g f.wt) were in TGX 225. In case of combined (heat and irrigation) stress, the highest (640.66 ug/g f.wt) chlorophyll ‘b’ were found in TGX 225 and the lowest (374.35 ug/g f.wt) value was observed in D-0913.

As compared to non-stress condition, in irrigation stress chlorophyll ‘b’ contents were found significantly different i.e. D-0913 and D-09027 decreases and CH104/06 increased while others remained non-significant. Under heat stress D-0913, D-09027, CH 24/07 and TGX 225 showed decreased chlorophyll ‘b’ contents while BRC -390, CMC 211S and Bhakhar-2011 were observed with increased contents and others remained unchanged. Under combined stress D-0913 and CH 24/07 showed decreased contents while TGX 225 and Bhakhar-2011 were observed as high accumulators and others remained unchanged (Figure 3.62).

3.12.4 Total Carotenoids

Under non-stress condition, significant differences were observed in tested genotypes for carotenoid contents (Figure 3.63). The highest (25.64 ug/g f.wt) carotenoid contents were observed in CH 24/07 while the lowest (15.28 ug/g f.wt) in Bhakhar-2011. Under the irrigation stress, carotenoid were highest (27.24 ug/g f.wt) in CH 24/07 and lowest (13.31 ug/g f.wt) in Bhakhar-2011. Under heat stress highest (24.53 ug/g f.wt) carotenoid contents were present in CMC 211S while lowest (13.57 ug/g f.wt) were in CH104/06. In case of combined (heat and irrigation) stress, the highest (31.60 ug/g f.wt) carotenoids were found in CH104/06 and the lowest (16.50 ug/g f.wt) value was observed in CH 24/07.

As compared to non-stress condition, carotenoid contents were exhibited significantly affected i.e. CH104/06 and CMC 211S showed higher carotenoid contents and other remained unchanged. Under heat stress genotypes with lowered contents were D-0913, D-09027, CH 24/07, CH104/06 and TGX 225. Higher contents was found in CMC

211S and Bhakhar-2011 while others remained unchanged. D-0913 and CH 24/07 were found to have significantly least contents while CMC 211S and Bhakhar-2011 with higher contents and others remained unchanged under combined stress (Figure 3.63).

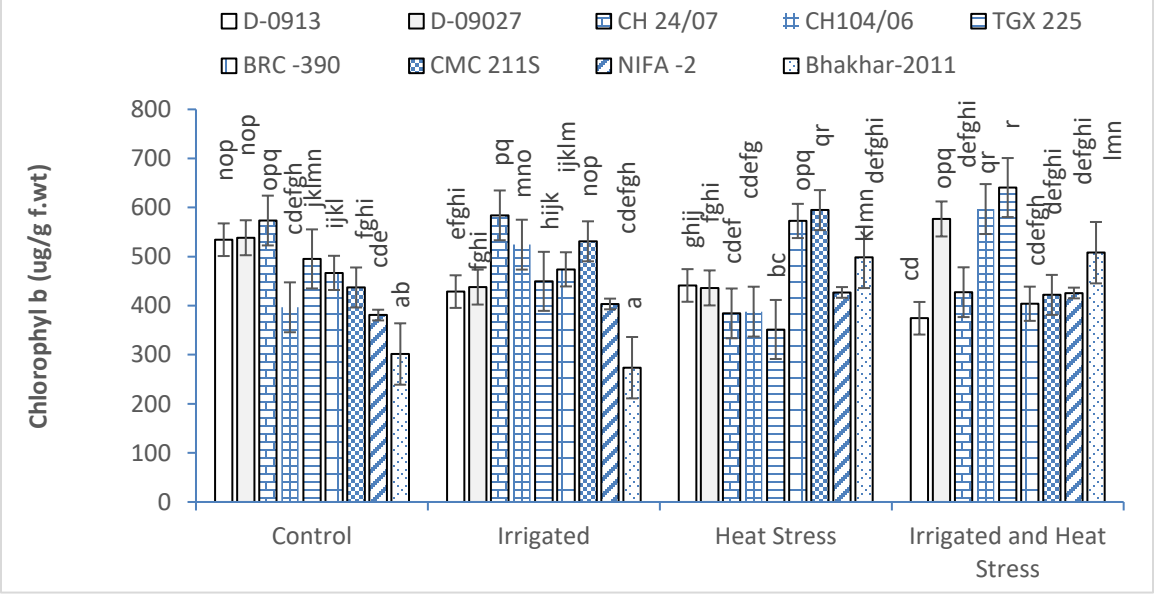

Figure 3.62: Chlorophyll 'b' under control, irrigation, heat and combined stress

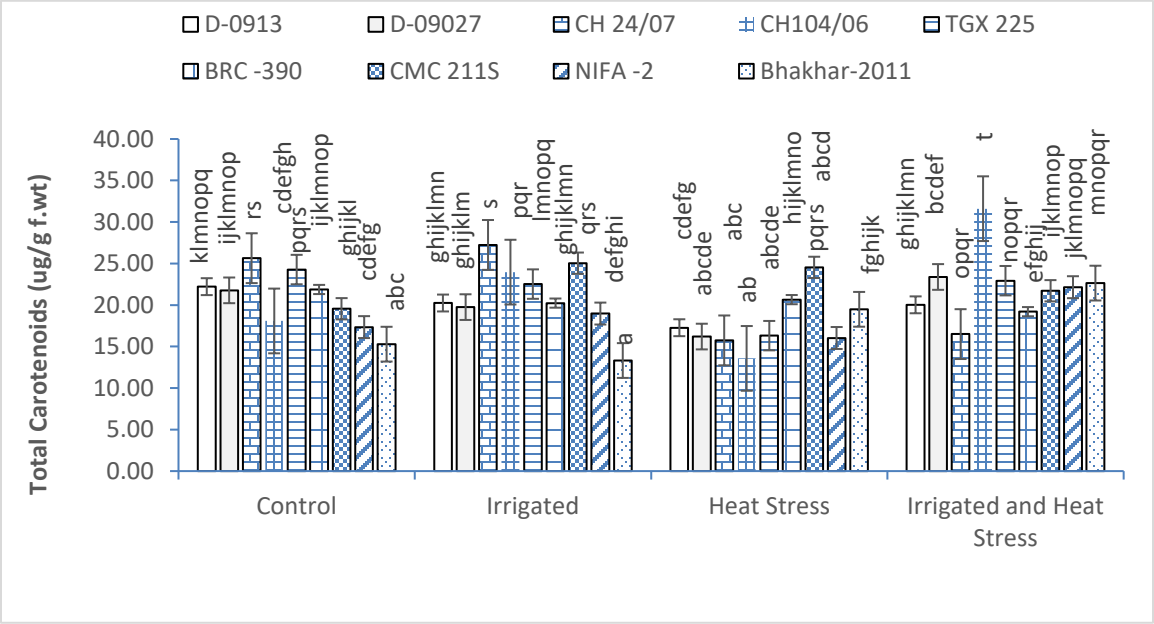

Figure 3.63: Total carotene contents under control, irrigation, heat and combined stress

3.12.5 Total Chlorophyll

Under non-stress condition, significant differences were observed in tested genotypes for total chlorophyll contents (Figure 3.64). The highest (733.38 ug/g f.wt**)** total chlorophyll contents were observed in CH 24/07 while the lowest (360.81 ug/g f.wt) in Bhakhar-2011. Under the irrigation stress, total chlorophyll contents were highest (734.39 ug/g f.wt) in CH 24/07 and lowest (318.17 ug/g f.wt) in Bhakhar-2011. Under heat stress highest (719.66 ug/g f.wt) total chlorophyll contents were present in CMC 211S while lowest (417.04 ug/g f.wt) were in TGX 225. In case of combined (heat and irrigation) stress, the highest (936.71 ug/g f.wt) total chlorophyll contents were found in CH104/06 and the lowest (471.15 ug/g f.wt) contents were observed in CH 24/07.

As compared to non-stress condition, total chlorophyll contents were detected significantly affected under irrigation stress i.e. D-0913 and D-09027 depicted decreased contents, CH 24/07 and CMC 211S showed elevated chlorophyll contents while in others it remained unchanged. Under heat stress D-0913, D-09027, TGX 225 and CH 24/07 were observed as decreasing chlorophyll contents, BRC -390, CMC 211S and Bhakhar-2011 were observed with significantly increased chlorophyll contents while others remained unchanged. Under combined stress of heat and irrigation it was found that D-0913 and CH 24/07 showed significantly lowered contents, CH104/06, TGX 225 and TGX 225 with significantly higher contents while others remained unchanged (Figure 3.64).

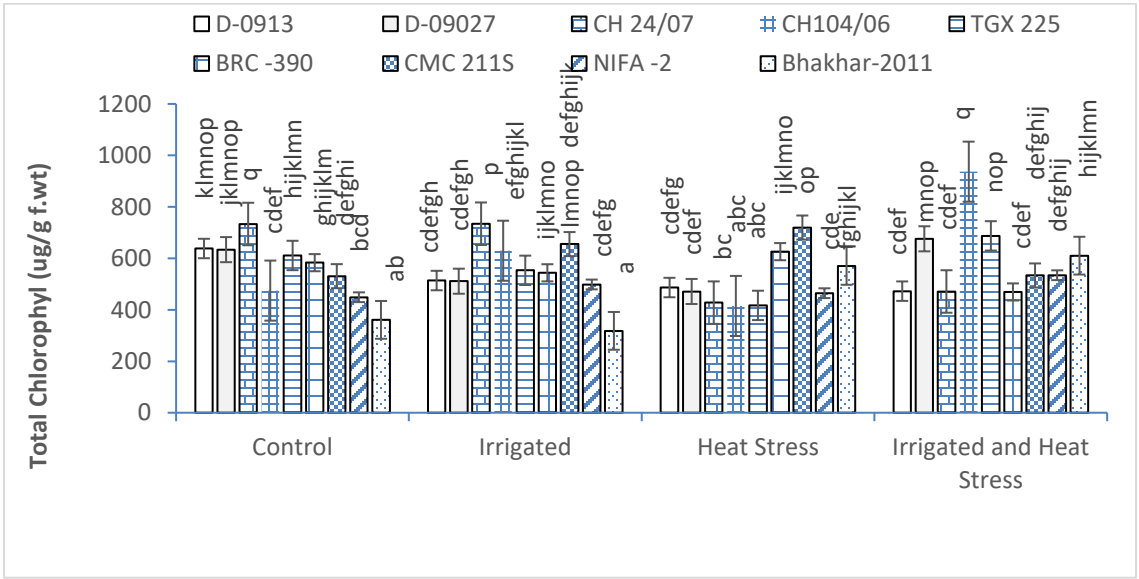

Figure 3.64: Total Chlorophyll contents under control, irrigation, heat and combined stress

3.12.6 Total Oxidant Status (TOS)

Under non-stress condition, significant differences were observed in tested genotypes for total oxidant status (TOS) (Figure 3.65). The highest (10650 uM/g f.wt) value of TOS was detected in Bhakhar-2011 while the lowest (950 uM/g f.wt) value of TOS was observed in D-0913. Under the irrigation stress, TOS was highest (10225 uM/g f.wt) in D-0913 and lowest (1875 uM/g f.wt) in CH 24/07. Under heat stress the highest (18125 uM/g f.wt) value of TOS was observed in Bhakhar-2011 and lowest (1400 uM/g f.wt) value was detected in CH 24/07. In case of combined (heat and irrigation) stress, the highest (13150 uM/g f.wt) value of TOS was found in Bhakhar-2011 and the lowest (3100 uM/g f.wt) value was observed in CH 24/07.

As compared to non-stress condition, TOS was significantly increased in D-0913 and CH104/06 under irrigation stress while it decreased significantly in Bhakhar-2011 and remained unchanged in all other genotypes. Under heat stress, TOS value was increased in CH104/06 and Bhakhar-2011, remained unchanged in all other genotypes. Under combined stress, TOS value remained unchanged CH 24/07, TGX 225 and in BRC -390. While a significant increase in TOS value was observed for all other genotypes as compared to that under non-stress condition (Figure 3.65).

3.12.7 Superoxide Dismutase (SOD)

Under non-stress condition, significant differences were observed in tested genotypes for superoxide dismutase (SOD) (Figure 3.66). The highest (298.11 Units/g f. wt) activity of SOD was detected in NIFA -2 while the lowest (207.48 Units/g f. wt) activity of SOD was observed in Bhakhar-2011. Under irrigation stress, SOD activity was highest (296.85 Units/g f. wt) in D-09027 and lowest (184.67 Units/g f. wt) in TGX 225. Under heat stress, SOD activity was highest (282.96 Units/g f. wt) in NIFA -2 and lowest (130.47 Units/g f. wt) in CH104/06. In case of combined (heat and irrigation) stress, the highest (227.82 Units/g f. wt) activity of SOD was found in NIFA -2 and the lowest (107.08 Units/g f. wt) value was observed in D-0913.

As compared to non-stress condition, SOD activity was significantly increased in D-09027 under irrigation stress however, a significant decrease in SOD activity was observed in TGX 225 while it remained unchanged for all other genotypes. Under heat stress, SOD activity was remained unchanged in D-09027 and in NIFA-2 while it decrease

significantly in all other genotypes as compare to non-stress condition. Under combined stress, a significant decrease was observed for all genotypes as compared to non-stress condition (Figure 3.66).

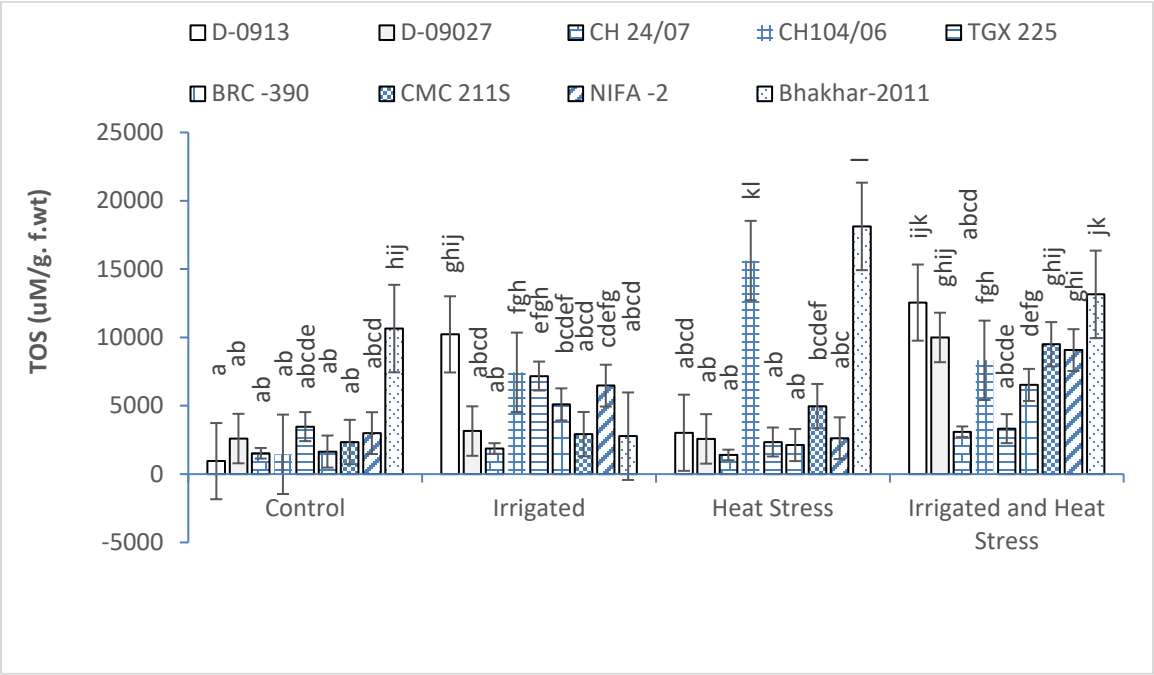

Figure 3.65: Total oxidant status under control, irrigation, heat and combined stress

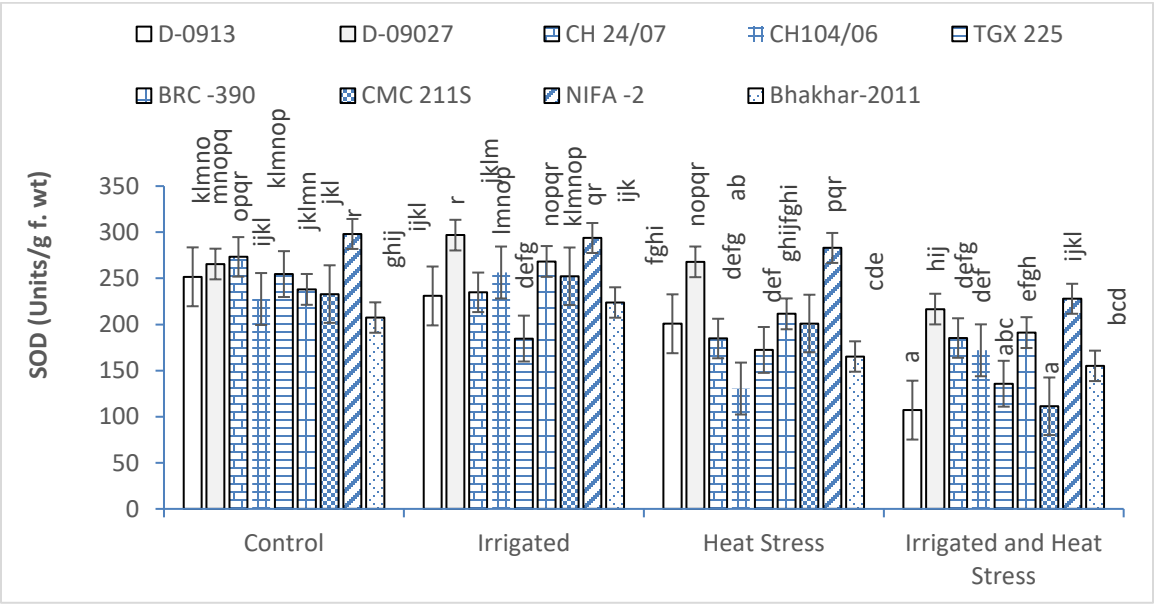

Figure 3.66: Superoxide dismutase activity under control, irrigation, heat and combined stress

3.12.8 Protease

Under non-stress condition, significant differences were observed in tested genotypes for protease activity (Figure 3.67). The highest (8690 Units/g f.wt) activity of protease was detected in TGX 225 while the lowest (2010 Units/g f.wt) activity of protease was observed in Bhakhar-2011. Under the irrigation stress, protease activity was highest (6925 Units/g f.wt) in D-09027 and lowest (2490 Units/g f.wt) in NIFA-2. While under heat stress the highest (7180 Units/g f.wt) activity of protease was observed in Bhakhar-2011 and lowest (2225 Units/g f.wt) activity was found in D-0913. In case of combined (heat and irrigation) stress, the highest (8780 Units/g f.wt) activity of protease was found in CH104/06 and the lowest (3055 Units/g f.wt) activity was observed in NIFA -2.

As compared to non-stress condition, protease activity was remained unchanged in CH104/06 under irrigation stress. On other hand, protease activity was decreased significantly in all other genotypes under irrigation stress. Under heat stress, protease activity was significantly increased in Bhakhar-2011. While it decreased significantly in all other genotypes as compare to non-stress condition. Under combined stress, a significant increase in protease was observed for CH104/06, and remained unchanged in TGX 225 while, a significant decrease was noticed in all other genotypes as compared to that under non-stress condition (Figure 3.67).

3.12.9 Esterase

Under non-stress condition, significant differences were observed in tested genotypes for esterase activity (Figure 3.68). The highest (680.28 uM/g f.wt) activity of esterase was detected in CH 24/07 while the lowest (225.18 uM/g f.wt) activity of esterase was observed in NIFA-2. Under the irrigation stress, esterase activity was highest (1966.19 uM/g f.wt) in NIFA-2 and lowest (160.01 uM/g f.wt) in CH 24/07. Under heat stress highest (828.61 uM/g f.wt) activity of esterase was noticed in BRC -390 and lowest (307.21 uM/g f.wt) for NIFA -2. In case of combined (heat and irrigation) stress, the highest (663.43 uM/g f.wt) activity of esterase was found in CH 24/07 and the lowest (219.57 uM/g f.wt) activity was observed in D-09027.

As compared to non-stress condition, esterase activity was significantly increased in NIFA -2 under irrigation stress while it remained unchanged in all other genotypes. On the other hand, a significant decrease in activity of esterase was observed in Bhakhar-2011.

Under heat stress, esterase activity was increased in BRC-390, remained unchanged in all other genotypes as compare to non-stress condition. Under combined stress, a non-significant change was observed for all genotypes as compared to non-stress condition (Figure 3.68).

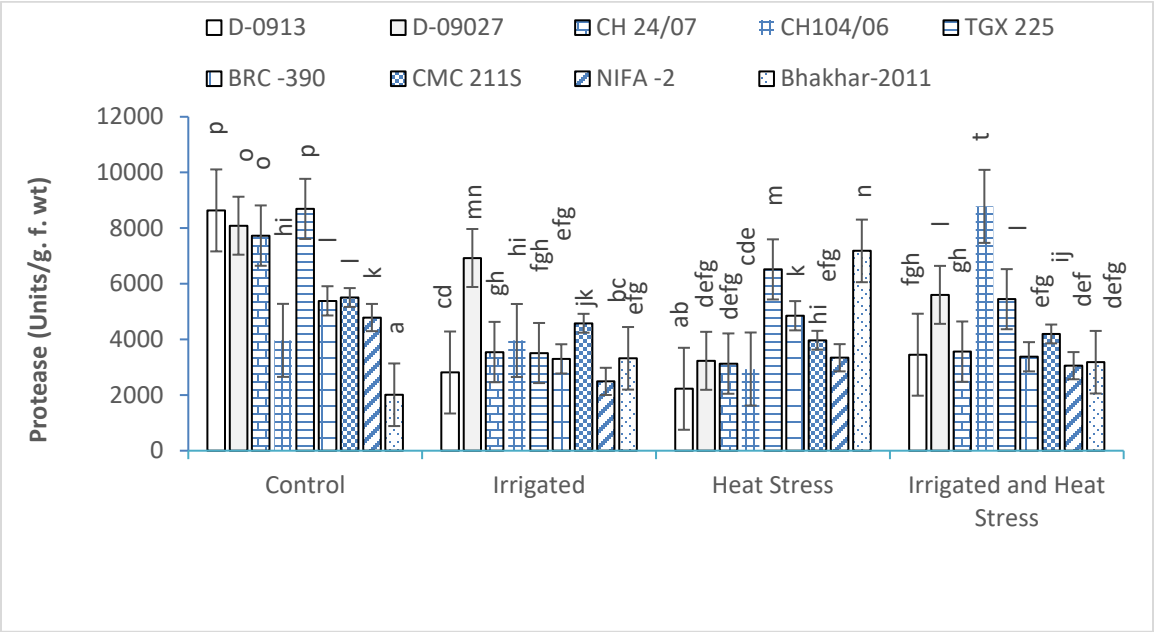

Figure 3.67: Protease activity under control, irrigation, heat and combined stress

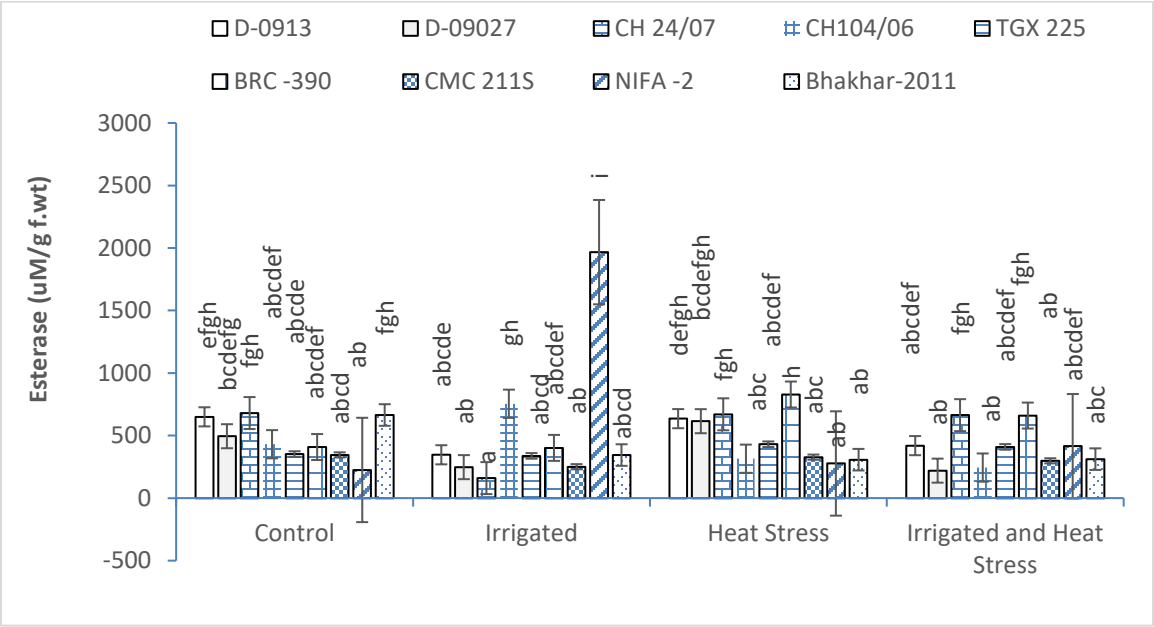

Figure 3.68: Esterase activity under control, irrigation, heat and combined stress

3.12.10 Catalase

Under non-stress condition, significant differences were observed in tested genotypes for activity of catalase (CAT) (Figure 3.69). The highest (25775 Units/g f. wt) activity of CAT was detected in CMC 211S while the lowest (17065 Units/g f. wt) value of CAT was observed in D-0913. Under the irrigation stress, CAT activity was highest (31070 Units/g f. wt) in D-0913 and lowest (19920 Units/g f. wt) in CH 24/07. Under heat stress highest (33940 Units/g f. wt) activity of CAT was noticed in CH 24/07 and lowest (13075 Units/g f. wt) for BRC -390. In case of combined (heat and irrigation) stress, the highest (34425 Units/g f. wt) activity of CAT was found in NIFA-2 and the lowest (16040 Units/g f. wt) activity was observed in CH 24/07.

As compared to non-stress condition, CAT activity was significantly increased in NIFA -2 under irrigation stress while it remained unchanged in all other genotypes. Under heat stress, CAT activity was increased in CH 24/07. On the other hand significant decrease in CAT activity was observed in BRC-390 while it remained unchanged in all other genotypes. Under combined stress, a significant increase in CAT activity was noticed in NIFA -2 and remained unchanged in all other genotypes as compare to non-stress condition.

3.12.11 Total Soluble Protein (TSP)

Under non-stress condition, significant differences were observed in tested genotypes for total soluble protein (TSP) (Figure 3.70). The highest (518.00 mg/g f.wt) TSP content was observed in Bhakhar-2011 while the least (140.33 mg/g f.wt) TSP were observed in D-0913. Under the irrigation stress, TSP were highest (400 mg/g f.wt) in D-09027 and lowest (134.33 mg/g f.wt) in TGX 225. Under heat stress highest (356.83 mg/g f.wt) TSP were noticed in D-0913 and lowest (84.33 mg/g f.wt) for CMC 211S. In case of combined (heat and irrigation) stress, the highest (490.50 mg/g f.wt) TSP were found in Bhakhar-2011 and lowest (81.83 mg/g f.wt) value was observed in BRC-390.

As compared to non-stress condition, TSP were significantly increased in D-0913 and CMC 211S under irrigation stress while it remained unchanged in D-09027, CH 24/07 and NIFA-2 while it decreased significantly in all other genotypes. Under heat stress, TSP were increased significantly in D-0913 while it remained unchanged in CH 24/07 and in NIFA-2 and in all other genotypes a significant decrease in protein content was observed.

Under combined stress, a significant increase in TSP were noticed in D-0913 and remained unchanged in Bhakhar-2011 while a significant decrease in TSP were observed in all other genotypes as compare to non-stress condition.

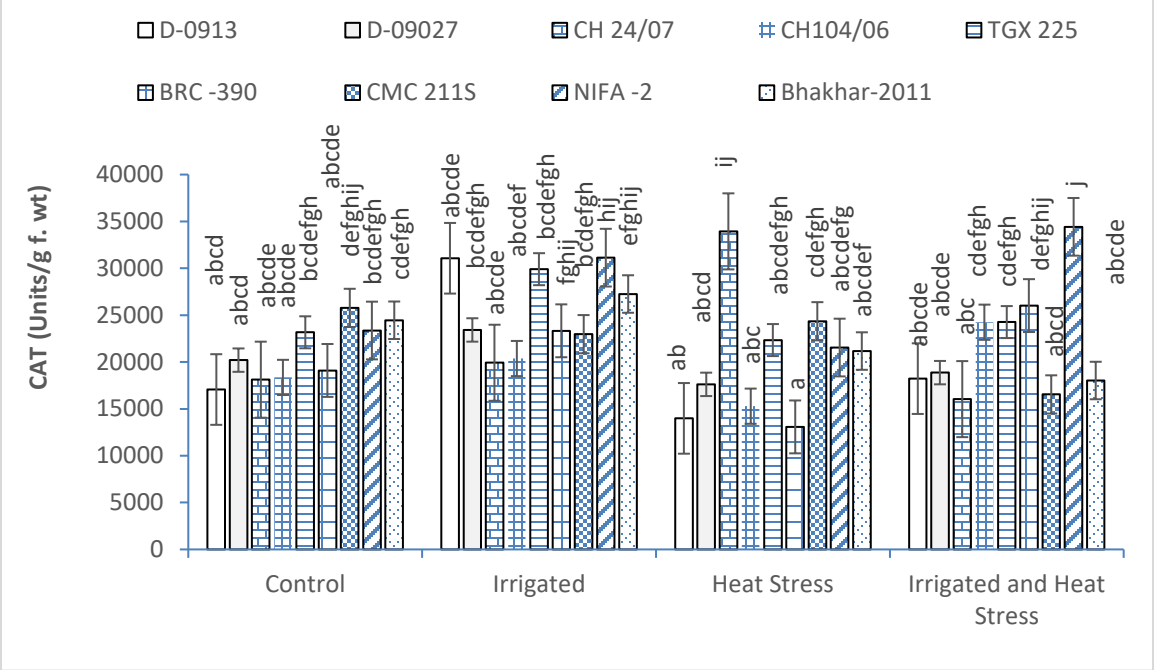

Figure 3.69: Catalase activity under control, irrigation, heat and combined stress

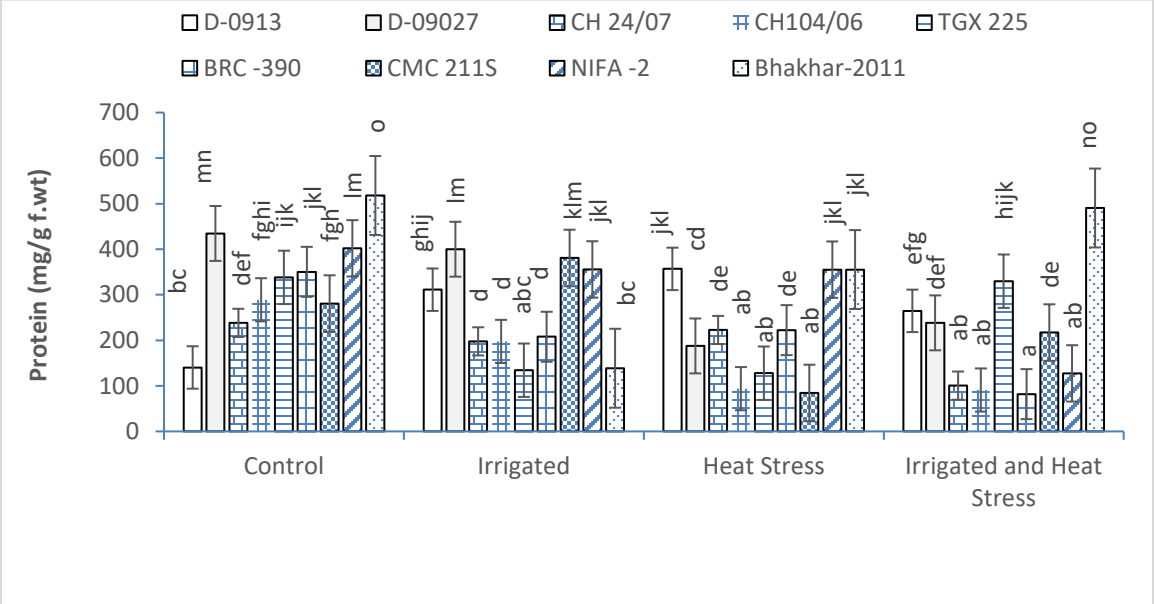

Figure 3.70: Protein contents under control, irrigation, heat and combined stress

3.12.12 Malondialdihyde (MDA)

Under non-stress condition, significant differences were observed in tested genotypes for Malondialdehyde (MDA) content (Figure 3.71). The maximum (171.74 uM/g f. wt) MDA contents were observed in D-0913 while the minimum (91.35 uM/g f. wt) MDA contents were observed in NIFA-2. Under the irrigation stress, maximum (149.41 uM/g f. wt) MDA content were detected in CH 24/07 and minimum (120.77 uM/g f. wt) were observed in TGX 225. Under heat stress maximum (159.48 uM/g f. wt) MDA contents were noticed in BRC-390 and minimum (117.67 uM/g f. wt) in TGX 225. In case of combined (heat and irrigation) stress, maximum (167.93 uM/g f. wt) MDA contents were found in CH104/06 and the minimum (120.77 uM/g f. wt) were observed in Bhakhar-2011.

As compared to non-stress condition, MDA contents were non-significantly affected in all genotypes under irrigation stress and heat stress condition. Under combined stress, a significant increase in MDA contents were noticed in NIFA-2 and remained unchanged in all other genotypes as compare to non-stress condition (Figure 3.71).

3.12.13 Total Phenolic Contents (TPC)

Under non-stress condition, significant differences were observed in tested genotypes for total phenolic contents (TPC) (Figure 3.72). The maximum (47850 uM/g. f.wt) TPC were observed in CH 24/07 while the minimum (39575 uM/g. f.wt) TPC were observed in CH104/06. Under the irrigation stress, maximum (46075 uM/g. f.wt) TPC were detected in TGX 225 and minimum (35725 uM/g. f.wt) were observed in Bhakhar-2011. Under heat stress maximum (28900 uM/g. f.wt) TPC were noticed in CH 24/07 and minimum (21250 uM/g. f.wt) were in D-09027. In case of combined (heat and irrigation) stress, maximum (41825 uM/g. f.wt) TPC were found in D-0913 and the minimum (30575 uM/g. f.wt) were observed in NIFA-2.

As compared to non-stress condition, TPC were significantly decreased in CH 24/07, NIFA -2 and in Bhakhar-2011 under irrigation stress while it remained unchanged in all other genotypes. Under heat stress, TPC were remained unchanged in all genotypes as compare to non-stress condition .Under combined stress, TPC were remained unchanged in D-0913, TGX 225, BRC -390 and in Bhakhar-2011. While a significant decrease in TPC was observed in all other genotypes as compare to non-stress condition (Figure 3.72).

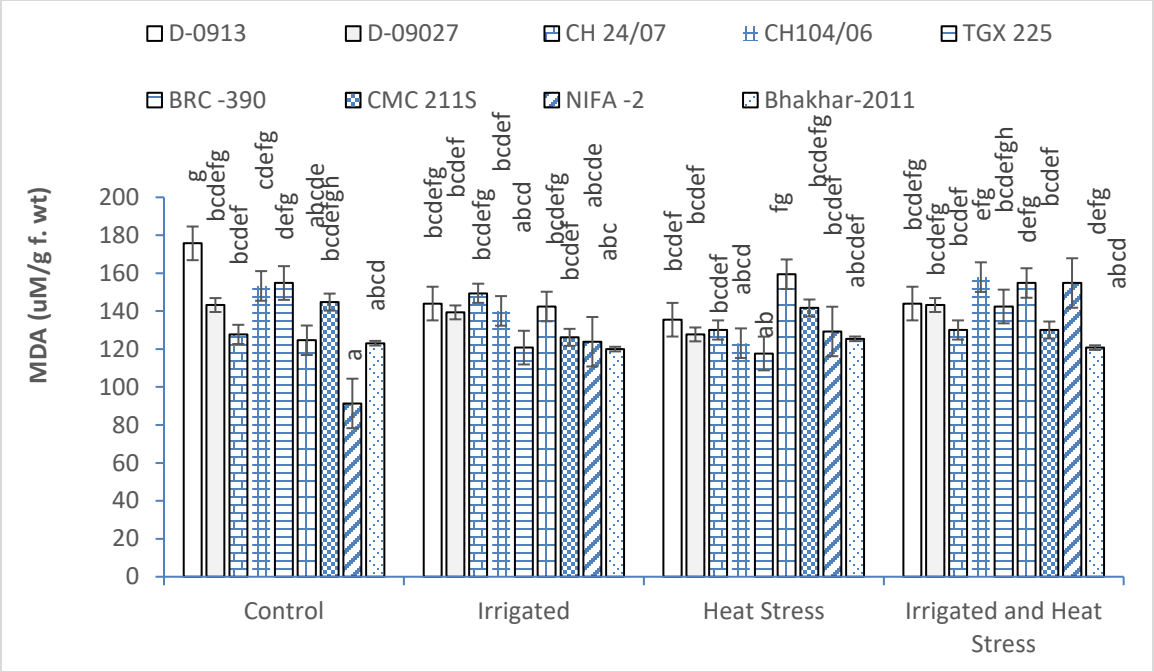

Figure 3.71: Malenodialdihyde under control, irrigation, heat and combined stress

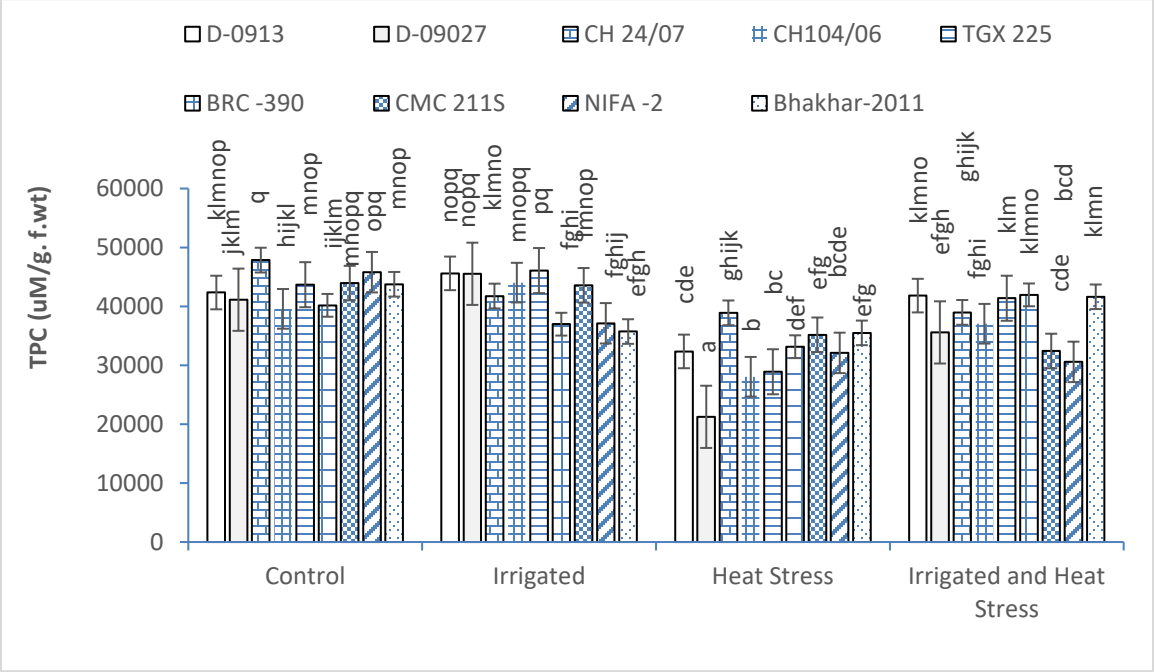

Figure 3.72: Total phenolic contents under control, irrigation, heat and combined stress

3.12.14 Peroxidase and Ascorbate Peroxidase

Under non-stress condition, irrigation, heat and combined stress non-significant differences were observed in tested genotypes for peroxidases (POD) and ascorbate peroxidases (APX) (Figure 3.73, Figure 3.73).

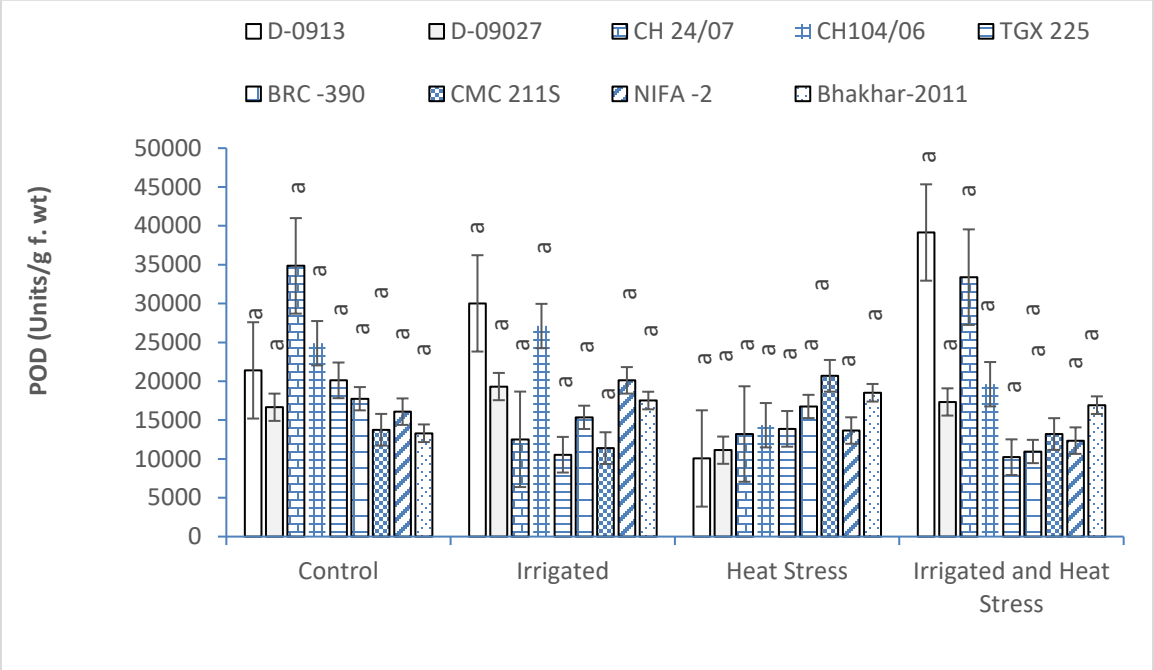

Figure 3.73: Peroxidase activity under control, irrigation, heat and combined stress

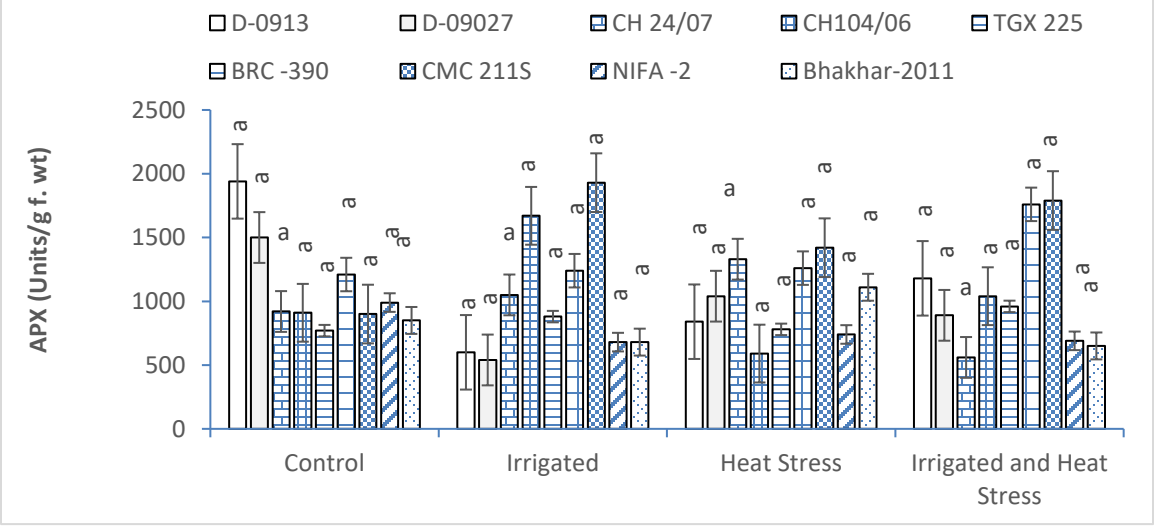

Figure 3.74: Ascorbate peroxidases under control, irrigation, heat and combined stress

3.13 Correlation Analysis

Correlation (Pearson) for all morphological and physio biochemical parameters was carried out using XL-STAT 2014.1.02, using 95% confidence interval. Correlation matrix of most important parameters is given below.

Under control condition shoot length was not correlated with any parameter. Root length was positively correlated with FV/FM/C while, negatively correlated with T_m/I, T_m/STI and Protein/I. Seedling fresh weight was positively correlated with S.F.W/STI, S.D.W/I, SH.F.W/I, SH.F.W/STI, SH.D.W/C, R.F.W/I, R.F.W/STI and R.D.W/I while negatively correlated with T.W.P/C, T.P.C/I and Protein/STI. Seedling dry weight was positively correlated with S.F.W/I, S.F.W/STI, S.D.W/STI, SH.F.W/I, SH.F.W/STI, SH.D.W/I, R.F.W/I, R.F.W/STI, R.D.W/I and R.D.W/STI while negatively correlated with T.W.P/C, Chl b/C, TOT Chl/C and Protein/STI. Shoot fresh weight was positively correlated with S.F.W/I, S.F.W/STI, S.D.W/C, S.D.W/I, SH.F.W/STI, SH.D.W/C, R.D.W/I, R.F.W/I and R.F.W/STI while negatively correlated with T.W.P/C, T.P.C/I and Protein/STI. Shoot dry weight was positively correlated with F_o/I, F_o/STI, F_m/I, F_m/STI, S.F.W/STI, S.D.W/I, SH.F.W/I, SH.F.W/STI and CAT/C while negative correlated with T.W.P/C, lyco/C, chl a/C, chl b/C, Tot CAR/C, TOS Chl/C, MDA/I and POD/C. Root fresh weight was positively correlated with S.F.W/I, S.F.W/STI, S.D.W/I, SH.F.W/I, SH.F.W/STI, R.F.W/STI and R.D.W/I while negatively correlated with T.W.P/C, TPC/I and Protein/STI. Root dry weight was positively correlated with S.F.W /me, S.F.W/STI, S.D.W/I, S.D.W/STI, SH.F.W/I, R.F.W/I, R.F.W/STI and R.D.W/STI while negatively correlated with Protein/STI (Table. A4).

Under heat stress shoot length was positively correlated with O.P/C, S.L/STI, S.F.W/H, S.F.W/STI, SH.F.W/H, SH.F.W/STI, SH.D.W/H, R.F.W/STI, Protein/STI and APX/C while negatively correlated with O.P/H and O.P/STI. Root length was positively correlated with TPC/H, APX/H and APX/STI while negative correlation was not found. Seedling fresh weight was positively correlated with R.W.C/H, S.L/H, S.L/STI, S.F.W/STI, SH.F.W/H, SH.F.W/STI, SH.D.W/H, R.F.W/H, R.F.W/STI and Protein/STI while negative correlation was not found. Seedling dry weight was positively correlated with S.D.W/C, SH.D.W/C, SH.D.W/H and R.D.W/H while negatively correlated with TOS/STI. Shoot fresh weight was positively correlated with O.P/C, S.L/H, S.L/STI,

S.F.W/H, S.F.W/STI, SH.F.W/STI, SH.D.W/H, R.F.W/H, R.F.W/STI, Protein/STI and APX/C while negative correlation was not found. Shoot dry weight was positively correlated with Tr.P/C, S.L/H, S.L/STI, S.F.W/H, S.D.W/H, SH.F.W/H, SH.F.W/STI and SH.D.W/C while negatively correlated with O.P/H, O.P/STI and Tr.P/STI. Root fresh weight was positively correlated with R.W.C/H, R.W.C/STI, S.L/STI, S.F.W/H, S.F.W/STI, SH.F.W/H, SH.F.W/STI, R.F.W/STI and Protein/STI while negative correlation was not observed. Root dry weight was positively correlated with S.D.W/H while negatively correlated with TOS/STI (Table .A5).

Under combined stress shoot length was positively correlated with S.L/STI, R.L/C and APX/STI while negatively correlated with T.W.P/C. Root length was positively correlated with S.D.W/STI while negatively correlated with MDA/H/I. Seedling fresh weight was positively correlated with S.F.W/C, S.F.W/STI, SH.F.W/HI, SH.F.W/STI, R.F.W/HI, R.F.W/STI and SOD/STI while negatively correlated with F_v/F_m/HI. Seedling dry weight was positively correlated with T.W.P/STI, S.D.W/C, SH.F.W/STI, SH.D.W/C, SH.D.W/H/I and R.D.W/C while negatively correlated with T.W.P/C, POD/C and POD/HI. Shoot fresh weight was positively correlated with Tr.P/C, S.F.W/C, S.F.C/HI, S.F.W/STI, S.D.W/C, SH.F.W/STI and SH.D.W/HI while negatively correlated with FV/FV/HI. Shoot dry weight was positively correlated with T.W.P/STI, Tr.P/C, S.D.W/C, S.D.W/H/I, SH.F.W/HI, SH.F.W/STI, SH.D.W/C and R.D.W/C while negatively correlated with T.W.P/C, POD/C and POD/HI. Root fresh weight was positively correlated with S.F.W/STI, S.F.W/C, R.F.W/STI, SOD/STI while negative correlation was not observed. Root dry weight was positively correlated with CAT/C while negatively correlated with POD/HI (Table. A6).

3.14 Stress Tolerance Index (STI)

Stress tolerance index was used in crop plants for determining high yield and tolerance potential of genotypes under different stress conditions. It actually tells us the percent increase or decrease in a specific character by a specific genotype. On the basis of this increase or decrease we can select our desirable genotypes for specific traits.

Under irrigation stress STI was calculated for 32 parameters. On the basis of these traits susceptible and tolerant genotypes were selected. According to desired parameters D-09027 was proved to be tolerant genotype because 14% and 3% decrease in shoot fresh

weight and malondialdehyde activity was observed respectively. Superoxide dismutase is antioxidant which prevents from reactive oxygen species (Table 3.2). There was 12%, 10%, 3% and 1% increase in activity of superoxide dismutase, TPC, shoot length and seedling fresh weight. NIFA-2 was proved to be susceptible under irrigation stress as there was 35%, 17%, 51% and 38% increase in MDA, shoot length, shoot fresh weight and seedling fresh weight respectively. Total phenolic contents and superoxide dismutase were decreased 19% and 3% respectively. These parameters can be used as efficient markers for irrigation stress tolerance studies in chickpea.

Under heat stress 32 parameters were used for STI estimation. According to STI values tolerant and susceptible genotypes were selected. D-0913 was detected as tolerant genotype for heat stress. There was 3% decrease in esterase activity. Relative water contents, seedling fresh weight and shoot fresh weight were increased 7%, 64% and 94% respectively. NIFA-2 was proved to be susceptible genotype under heat stress as there was 7%, 14% decrease in relative water contents and seedling fresh weight. Esterase and shoot fresh weight were increased 22% and 39% respectively. It was concluded that these parameters can be used as efficient markers in heat tolerance studies in chickpea.

STI was estimated for 32 parameters under combined stress of heat and irrigation. According to STI values tolerant and genotypes were selected. D-0913 exhibited tolerance as there was 61% and 36% decrease was found protease and esterase respectively. Total phenolic contents were maintained. Seedling fresh weight, shoot fresh weight, root fresh weight, protein, peroxidase and catalase were increased 5%, 10%, 2%, 88%, 82% and 6% respectively. CMC 211S was detected as susceptible genotype under combined stress as there was 16%, 36%, 23%, 4%, 14%, 24%, 27% and 36% decrease in seedling fresh weight, root fresh weight, protein, peroxidase, esterase, protease, total phenolic contents and catalase respectively. It was concluded that these parameters can be used as efficient markers in heat and irrigation stress tolerance studies in chickpea (Table 3.1Table 3.2).

Table 3.2: Stress tolerance index (STI) of Desi Chickpea

Genotypes	TWP	O.P	Tr.P	R.W.C	TOS	Lyco	chl a	chl b	T. car
STI values under irrigation stress									
D-0913	41.04	85.91	123.46	110.84	1076.32	86.46	81.84	80.22	91.13
D-09027	46.59	81.54	115.94	82.43	121.15	85.75	76.71	81.33	90.72
CH 24/07	54.19	108.21	169.09	103.97	122.95	102.31	94.12	101.83	106.22
CH104/06	61.31	129.21	179.76	95.45	513.79	129.87	135.04	132.17	132.53
TGX 225	59.38	73.71	83.06	95.17	206.47	92.12	89.61	90.77	92.79
BRC -390	71.57	80.87	84.69	100.70	309.09	97.76	59.95	101.55	92.50
CMC 211S	59.84	110.55	142.20	103.07	124.47	120.93	132.72	121.51	128.06
NIFA -2	55.96	105.09	132.52	94.89	215.83	105.85	139.93	105.93	109.40
Bhakhar-2011	39.39	83.87	114.21	111.77	26.06	90.55	75.12	90.75	87.12
STI values under heat stress									
D-0913	85.55	84.20	83.07	107.43	318.42	79.71	43.67	82.57	77.74
D-09027	93.18	111.91	130.35	90.80	99.04	79.05	36.66	81.01	74.44
CH 24/07	117.42	137.86	160.91	103.97	91.80	66.56	27.20	67.01	61.38
CH104/06	123.36	118.85	115.49	95.55	1077.59	86.13	34.81	97.76	75.06
TGX 225	158.59	99.23	60.51	98.50	67.63	69.73	56.61	70.97	67.21
BRC -390	189.22	89.51	48.54	97.04	128.79	105.33	45.85	122.67	94.41
CMC 211S	141.80	107.32	85.81	99.40	211.70	124.43	133.56	136.06	125.49
NIFA -2	152.29	131.72	120.23	93.78	87.50	100.34	55.27	112.10	92.44
Bhakhar-2011	122.73	116.28	111.89	108.26	170.19	140.47	121.76	165.31	127.46
STI values under combined stress									
D-0913	107.51	70.84	40.15	99.89	1321.05	78.37	94.47	70.05	90.15
D-09027	105.11	69.84	35.10	95.79	384.62	103.72	103.93	107.11	107.40
CH 24/07	102.58	88.89	73.45	104.19	203.28	70.01	27.31	74.54	64.35
CH104/06	120.44	72.82	37.36	97.00	574.14	157.58	436.31	150.47	174.77
TGX 225	123.44	69.55	34.39	95.68	95.68	104.90	40.24	129.35	94.48
BRC -390	142.16	78.94	52.97	101.58	395.45	85.96	56.20	86.53	87.77
CMC 211S	143.44	78.35	37.72	99.07	404.26	100.85	119.12	96.54	111.09
NIFA -2	177.98	85.21	33.42	102.03	302.50	117.47	159.74	111.80	127.72
Bhakhar-2011	134.85	68.89	23.90	111.78	123.47	149.45	172.69	168.49	148.11

Genotypes	T. chl	Esterase	Prote.	MDA	TPC	FO	FM	FV	TM	(Fv/Fm)
STI values under irrigation stress										
D-0913	80.48	53.32	32.54	81.94	107.67	61.13	62.26	62.47	175.10	98.42
D-09027	80.64	50.05	85.65	97.30	110.70	85.11	91.11	92.28	133.33	101.27
CH 24/07	100.15	23.52	45.86	116.97	87.25	78.87	81.29	86.31	91.10	105.28
CH104/06	132.65	175.12	99.87	91.41	111.24	106.97	103.81	104.16	44.17	100.29
TGX 225	90.55	95.85	40.39	78.00	105.50	87.45	94.96	96.54	108.08	101.95
BRC -390	93.25	98.35	61.28	114.29	92.03	83.68	83.44	83.40	100.61	99.92
CMC 211S	123.49	72.67	83.20	87.17	99.15	123.59	125.94	126.35	94.77	104.04
NIFA -2	111.08	873.45	52.04	135.59	81.06	103.24	109.96	111.21	114.37	101.13
Bhakhar-2011	88.18	51.81	165.17	97.48	81.66	119.26	122.26	122.79	72.16	100.41
STI values under heat stress										
D-0913	76.24	97.75	25.77	77.09	76.39	97.47	86.41	84.36	124.75	97.68
D-09027	74.34	124.30	39.95	89.19	51.67	85.17	82.73	82.25	151.29	99.71
CH 24/07	58.33	98.51	40.49	101.82	81.30	102.98	98.59	90.15	77.07	92.26
CH104/06	87.43	73.14	74.02	80.30	70.88	139.62	123.23	120.34	32.68	97.68
TGX 225	68.25	122.34	74.97	76.00	66.17	112.28	108.82	108.09	46.98	99.60
BRC -390	107.35	202.92	90.06	127.95	82.51	113.15	99.50	97.11	79.20	97.60
CMC 211S	135.62	94.80	72.12	97.86	80.03	101.94	97.85	95.45	69.19	101.29
NIFA -2	103.50	122.95	69.80	141.53	70.09	118.56	113.88	113.02	97.34	99.27
Bhakhar-2011	158.16	46.23	357.21	101.89	81.14	121.16	105.18	102.34	104.39	96.98
STI values under combined stress										
D-0913	74.02	64.56	39.95	81.94	98.76	114.67	110.71	109.97	79.77	99.31
D-09027	106.63	44.37	69.26	100.00	86.50	79.56	104.24	109.02	141.84	104.27
CH 24/07	64.24	97.52	46.05	101.82	81.45	128.37	107.26	95.11	65.75	89.60
CH104/06	197.38	56.70	221.44	103.03	93.62	117.39	110.05	109.21	37.31	99.06
TGX 225	112.47	116.27	62.66	92.00	94.73	108.30	112.11	112.92	50.49	100.91
BRC -390	80.48	161.64	62.67	124.22	104.42	91.74	95.87	84.56	68.22	83.14
CMC 211S	100.52	86.34	76.20	89.84	73.78	133.04	130.22	129.57	78.68	103.07
NIFA -2	119.05	184.33	63.85	169.49	66.76	115.96	117.27	117.51	96.46	100.26
Bhakhar-2011	169.18	46.91	158.21	98.11	95.14	141.28	124.68	121.73	67.71	97.43

Genotypes	S.L	R.L	S.F.W	S.D.W	SH.F.W	SH.D.W	R.F.W
STI values under irrigation stress							
D-0913	103.85	116.37	75.38	66.56	67.18	81.82	81.11
D-09027	103.27	125.39	101.53	79.74	86.64	101.85	110.22
CH 24/07	111.97	159.41	104.73	66.92	94.75	74.83	110.03
CH104/06	85.95	120.04	108.39	146.79	96.67	147.32	114.79
TGX 225	108.73	93.57	147.09	121.67	117.93	97.80	168.29
BRC -390	103.86	104.88	156.81	100.37	127.00	78.14	174.16
CMC 211S	110.70	72.16	97.05	80.29	106.03	113.43	93.05

NIFA -2	117.69	101.90	138.55	79.63	151.01	109.09	132.32
Bhakhar-2011	109.07	143.39	172.66	157.72	143.68	143.86	188.12
STI values under heat stress							
D-0913	175.71	145.09	154.84	130.42	194.93	203.73	126.88
D-09027	120.04	125.67	69.61	112.25	99.08	177.04	52.42
CH 24/07	107.91	132.71	85.99	124.81	93.18	154.90	82.17
CH104/06	91.99	97.18	50.08	81.12	70.07	130.14	39.15
TGX 225	111.27	88.61	92.82	147.13	108.42	132.37	81.48
BRC -390	118.75	89.31	76.38	94.08	110.40	124.22	56.58
CMC 211S	114.98	93.80	91.65	103.77	120.00	138.38	79.02
NIFA -2	119.68	109.96	86.87	97.51	139.39	131.29	60.61
Bhakhar-2011	109.66	105.42	92.83	125.08	124.18	137.05	76.10
STI values under combined stress							
D-0913	136.23	118.15	105.52	95.47	110.35	135.43	102.15
D-09027	117.18	143.20	113.24	124.49	113.13	191.29	113.31
CH 24/07	126.17	143.41	106.55	107.38	99.74	127.27	110.17
CH104/06	138.07	113.05	141.54	121.85	153.44	181.41	135.03
TGX 225	114.76	91.04	87.64	115.35	107.56	106.10	73.16
BRC -390	156.62	87.04	172.11	107.41	250.60	178.58	126.43
CMC 211S	162.06	93.88	84.98	122.52	130.14	176.76	64.88
NIFA -2	136.78	105.10	121.21	103.38	172.73	171.15	95.45
Bhakhar-2011	151.08	154.12	164.44	147.46	208.79	201.59	140.76

Genotypes	R.D.W	PROT	SOD	POD	APX	CAT
STI values under irrigation stress						
D-0913	53.41	221.73	91.75	140.31	30.93	182.07
D-09027	67.89	92.02	111.79	116.00	36.00	115.94
CH 24/07	62.40	82.94	85.93	35.93	114.13	109.96
CH104/06	146.41	68.34	112.63	108.90	183.52	110.98
TGX 225	153.76	39.72	72.53	52.40	114.29	129.07
BRC -390	116.58	59.32	112.73	86.49	102.48	122.15
CMC 211S	60.33	135.69	108.29	82.81	214.44	89.12
NIFA -2	60.39	88.47	98.54	125.05	68.69	133.28
Bhakhar-2011	169.33	26.77	107.86	131.66	80.00	111.45
STI values under heat stress						
D-0913	67.27	254.28	79.77	47.00	43.30	81.95
D-09027	77.51	43.17	100.87	66.80	69.33	87.20
CH 24/07	107.60	93.43	67.60	37.89	144.57	187.36
CH104/06	45.38	32.47	57.33	57.59	64.84	83.27
TGX 225	166.97	37.80	67.69	68.96	101.30	96.42
BRC -390	72.12	63.46	88.85	94.37	104.13	68.46
CMC 211S	82.93	30.05	86.30	150.48	157.78	94.47
NIFA -2	75.46	88.35	94.92	84.89	74.75	92.23

Bhakhar-2011	115.05	68.60	79.63	139.17	130.59	86.57
	STI values under combined stress					
D-0913	61.04	188.60	42.55	182.96	60.82	106.80
D-09027	88.68	54.83	81.59	104.10	59.33	93.39
CH 24/07	96.00	42.17	67.79	95.84	60.87	88.55
CH104/06	78.44	31.43	75.56	78.80	114.29	132.09
TGX 225	127.79	97.54	53.30	50.83	124.68	104.70
BRC -390	55.54	23.36	80.32	61.73	145.45	136.23
CMC 211S	89.86	77.32	47.77	96.00	198.89	64.17
NIFA -2	59.13	31.67	76.42	76.81	69.70	147.37
Bhakhar-2011	102.10	94.69	74.74	127.16	76.47	73.71

3.15 Cluster Analysis

Based on association among phenomic data (24 attributes) and physio biochemical markers (37 for irrigation, 36 for heat and 33 for combined stress) relative stress tolerance of genotypes was assessed.

Following dendrograms were made for desi chickpea genotypes under irrigation, heat and combined stress by using STI values.

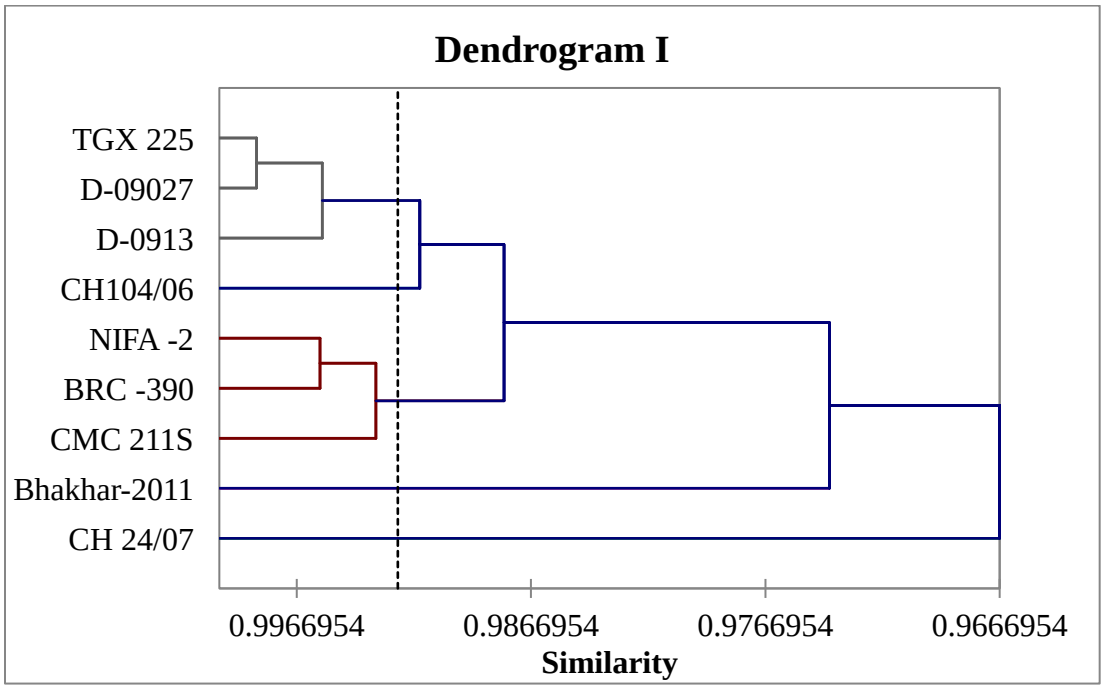

Figure 3.75: Dendrogram of desi genotypes under irrigation stress

D-09027 was categorized as tolerant genotypes because S.L, R.L, S.F.W and R.W.C were maintained in stress condition. Moderately tolerant was TGX 225, D-0913 and CH104/06 because it performed in between of tolerant and moderately sensitive. Moderately sensitive genotype were BRC -390, CMC 211S, Bhakhar-2011 and CH 24/07 because these performed better than sensitive. NIFA -2 was sensitive genotype as it performed less in irrigation stress (Figure 3.75).

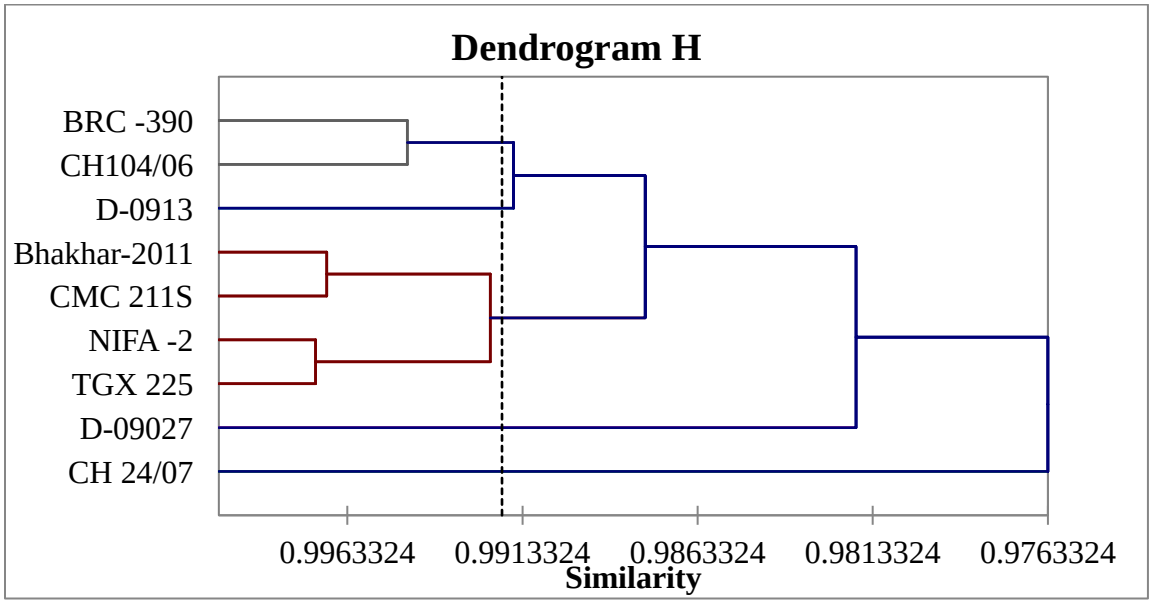

Figure 3.76: Dendrogram of desi genotypes under heat stress

D-0913 was categorized as tolerant genotypes because S.L, R.L, S.F.W and R.W.C were maintained in stress condition. Moderately tolerant was D-09027, CH 24/07 and CH104/06 because it performed in between of tolerant and moderately sensitive. Moderately sensitive were BRC -390, CMC 211S and Bhakhar-2011because these performed better than sensitive. NIFA -2 and TGX 225 were sensitive genotype as it performed less in heat stress (Figure 3.76).

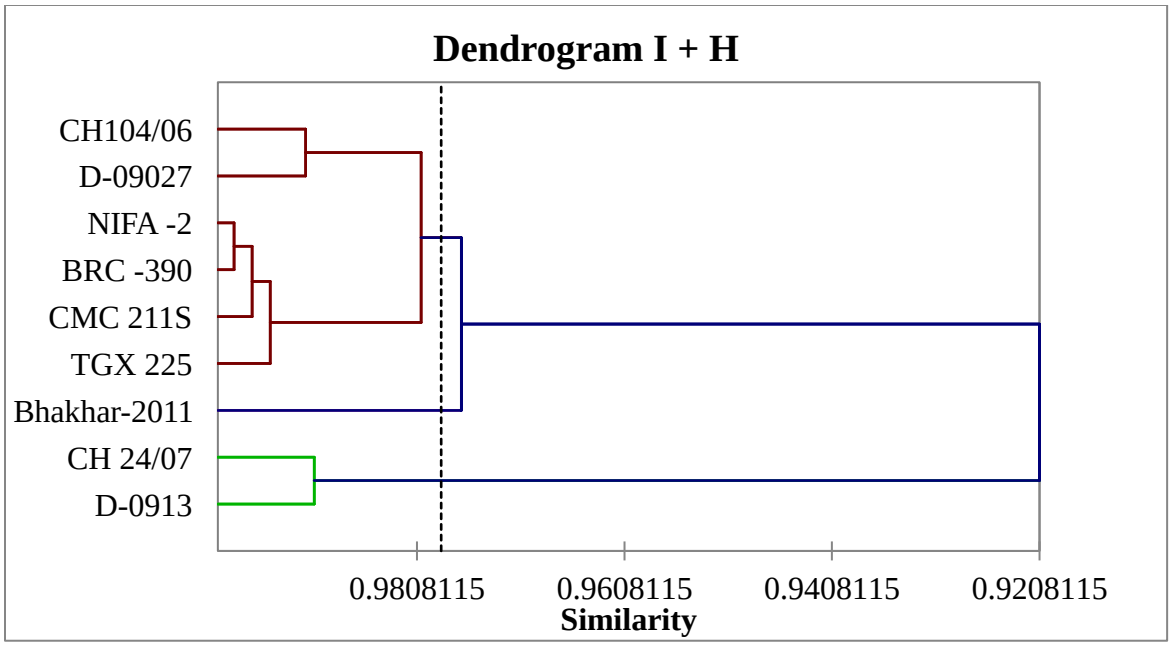

Figure 3.77: Dendrogram of desi genotypes under combined stress

D-0913 and CH 24/07 were categorized as tolerant genotypes because S.L, R.L, S.F.W and R.W.C and certain biochemical parameters like TSP, MDA etc. were maintained in stress condition. Moderately tolerant was NIFA-2 and D-09027 because it performed in between of tolerant and moderately sensitive. Moderately sensitive were CH104/06, BRC-390, TGX 225 and Bhakhar-2011 because these performed better than sensitive. CMC 211S was sensitive genotype as it performed less in heat stress (Figure 3.77).

4 Discussion

4.1 Heat Stress

Different crops including chickpea are observed to show differential response to heat stress. Differential response is regarding morphological, physiological and biochemical attribute of plants and regarding genotypes. It was reported that heat stress significantly alters all these attributes of chickpea. Heat stress results in drought stress and affects plants' initial growth phase. Expansion and elongation growth is badly affected. It is reported that early phenological stages in chickpea are most important for heat stress tolerance. A holistic approach is needed to investigate tolerance mechanism and heat tolerant genotypes which includes not only morphological but physiological and biochemical parameters. Main objective of this experiment was to find out heat tolerant genotypes using integrated approaches of morpho-physiological and biochemical parameters.

Heat stress significantly effects morphological, physiological and biochemical parameters of a plant [236]. Heat stress significantly alters water relations, hormone levels, photosynthetic rate, respiration and metabolic rate. Seedling fresh weight and shoot fresh weight was reduced under high temperature stress in all kabuli genotypes and was maintained in desi genotypes. Root fresh weight was found to decrease in desi and kabuli genotypes except D-0913 which indicated increased root fresh weight under heat stress. Similar results were found in wheat, as root fresh weight was reduced in wheat and significant variation was found in genotype [237].

Leaf water relations are altered upon exposure to heat stress. It was found in our study that total water potential was increased in both desi and kabuli genotypes. Similar results were found in sugarcane and tomato [74]. Transpiration is increased due to high temperature which ultimately effects water potential. Photosynthetic attributes were found to be non-significant contrary to other studies.

Biochemical response of plants are altered due to heat stress. An increase in activity of antioxidant enzymes was observed in heat tolerant varieties. Several heat shock proteins are activated in response to upregulation of heat responsive genes under high temperature stress. Heat resilience in plants is improved as cell's structural and functional proteins are

protected by heat shock proteins. Total soluble proteins (TSP) were found to decrease in both desi and kabuli genotypes except CM1235/08 and BKK 02174 in kabuli and D-0913 in desi. Our results were agreed to the previous ones that were observed in case of wheat. As TSP were observed to decrease in wheat under high temperature stress in some genotypes while increased in some others [237].

Different phenolics e.g. flavonoids, lignins, anthocyanins are important class of secondary metabolites which play a vital role in eluviation of stress and induction of abiotic stress tolerance. It is clear from studies that accumulation of phenolic contents induces heat tolerance and is an important parameter for heat tolerance studies [91, 108]. Total phenolic contents were decreased in all desi and some kabuli genotypes except BKK 02174 and CM1235/08, which were proved as tolerant genotypes. Same results were found in wheat and rice. Hence increased phenolic contents is linked with heat tolerance and can be used as important biochemical marker. Phenolics were found to decrease under high temperature in apples, chrysanthemums and casters [112, 238]. Phenolics are accumulated in plants under heat stress by increasing their biosynthesis and reducing its breakdown. This was found to be an acclimation process in plants for thermotolerance [108].

Total oxidant status (TOS) is increased whenever plant faces elevated temperature. But if antioxidants increase, TOS level is lowered. In desi genotypes D-0913 was tolerant which showed non-significant change in TOS, while TGX 225 and NIFA-2 were sensitive in which TOS level was significantly increased. In kabuli, genotypes TGX 225 and CMC 211S were proved to be tolerant because TOS level was significantly decreased. A decrease in TOS level was required for thermotolerance as in tolerant genotypes antioxidants were increased [239].

Lycopene is an antioxidant which was observed to decrease under high temperature except in tolerant genotypes. This is important for heat stress alleviation as it scavenges reactive oxygen species and helps to protect the cell structure and function. It was found in our study that lycopene contents showed decreasing behavior in desi genotypes except BRC-390, CMC 211S and NIFA-2. In kabuli genotypes it increased in most genotypes except CH888/06 and 8k. It was found that lycopene contents are decreased in tomato under heat stress [240].

Changes in different photosynthetic attributes of plants e.g. chlorophyll a, chlorophyll b, chlorophyll fluorescence and ratio of chlorophyll contents to carotenoids confers heat tolerance. Under high temperature it was observed that due to production of active oxygen species, photosynthetic apparatus is disturbed leading to lowered photosynthetic rate. Genotypes which can withstand this situation and maintain their pigments' levels show thermotolerance. In our study it was observed that in desi genotypes chlorophyll a contents were lowered in all genotypes except CMC 211S. In kabuli genotypes it was increased in BKK 02174 which was tolerant genotype. Chlorophyll b contents were lowered in all desi genotypes except BRC-390, CMC 211S and NIFA-2. In kabuli genotypes it was increased mostly but maximum increase was in BKK 02174 which was tolerant genotype. Exactly same results were found in total chlorophyll and total carotene contents as in chlorophyll b. Here it was found that chlorophyll b contents are more responsible for thermotolerance, which can be used as biochemical marker for heat tolerance studies in chickpea. Similar results were found in tomato chlorophyll contents under heat stress. The attributes which were found non-significant in our study were same as found in chickpea earlier in India [241].

Activity of antioxidant enzymes is increased under high temperature stress. Different oxygen reactive species which are produced in oxidative stress are singlet oxygen, superoxide radical, hydrogen peroxide and hydroxyl radical. Plants have defense mechanism against these species i.e. antioxidants defence system. Which are utilized to detoxify the cytotoxic effects and injuries caused by oxidative species at cellular level. Plants have several enzymatic (tocopherol, phenols) and non-enzymatic antioxidants (superoxide dismutase, ascorbate peroxidase, peroxidase, catalase, esterase and protease). Each has its special characteristics and pathway to scavenge active oxygen species. Whenever plant faces any stress, oxidative stress also becomes a part of that. To alleviate active oxygen species antioxidants are produced in higher quantity. Hence tolerant genotypes should have higher antioxidant status than normal growing plants [242].

Superoxide dismutase (SOD) is an enzyme which dismutates superoxide into either oxygen or hydrogen peroxide. Hydrogen peroxide is less toxic than superoxide and is detoxified by catalase. It was found in desi genotypes that SOD was increased in some genotypes while decrease in others. In TGX 225 and NIFA-2 it was severely reduced which

showed susceptibility towards heat stress. In kabuli genotypes SOD was mostly increased except CH888/06. CM1235/08 and BKK 02174 were found to be significantly increased SOD which were proved to be resilient genotypes. Same results were found in chickpea earlier [243, 244].

Peroxidase is another antioxidant enzyme which detoxifies hydrogen peroxide into water, helps in biosynthesis of cell wall and oxidize the toxic compounds. It was found that in all kabuli genotypes its amount was increased, lest increase was found in sensitive genotype i.e. 9-KCC-166. While in desi genotypes no significant change was observed. POD were found to behave same as studied earlier in wheat varieties [242]. Same results were found in chickpea [243].

Melanodialdehyde (MDA) is associated with deterioration of membrane lipids. Under heat stress it was found to decrease in tolerant genotypes. In our study same results were found that in desi genotypes, tolerant genotype D-0913 exhibited lower MDA contents while increase in susceptible genotypes. In kabuli genotypes MDA contents were maintained in tolerant genotypes while increased in susceptible i.e. 9-KCC-166. As malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress. MDA was reported to increase in susceptible wheat varieties under high temperature stress which deteriorates membranes [245].

Protease is involved in breakdown of proteins. Proteolysis is the breakdown of peptide bonds present among proteins. It should be decreased in heat tolerant genotypes. In desi genotypes D-0913 showed lowered protease contents which was tolerant genotype, while susceptible genotype NIFA-2 showed highest contents. In kabuli genotypes susceptible genotype 9-KCC-166 showed highest protease contents while in CM1235/08 it was maintained which was tolerant. Protease was found to increase in wheat varieties under heat stress [237].

In kabuli chickpea seedling dry weight, shoot dry weight, osmotic potential, turgor water potential, relative water contents, chlorophyll fluorescence, ascorbate peroxidase, peroxidase and esterase were found non-significant. Which shows that these parameters are not reliable for thermotolerance studies at seedling stage in chickpea. In desi genotypes

root length, seedling dry weight, shoot dry weight, root fresh weight, root dry weight, total water potential, chlorophyll fluorescence, relative water content, osmotic potential, turgor potential, ascorbate per oxidase, peroxidase and esterase were found non-significant. Which means that these parameters are not reliable for thermotolerance studies at seedling stage in desi chickpea.

4.2 Irrigation Stress

Water present in soil is a determinant of species distribution all over the world. It can change into stress e.g. waterlogging, drought, excessive or shortage of water. Crop production is limited in tropics and subtropics due to excessive rainfall. Excessive rainfall causes floods. Flooding is the stress when plants are submerged in rain water and are unable to get oxygen from soil which harshly affects crop production. It is now a major problem of some areas in the world. Rainfed areas are more subjected to rainfall stress, especially those soils where drainage in very poor. Floods and excessive rainfall causes waterlogging, which disturbs 10% of land from all over the world and hence it is major constraint in agricultural production. Chickpea is major crop of rainfed areas in Pakistan. As these areas are subjected to excessive rainfall at specific times, crop is affected badly. This experiment was conducted to check response of chickpea elite lines towards excessive water stress and screen out resilient genotypes.

Excessive water significantly alters morphology of plants. More water conductance through roots and more transpiration are major observations. As more water is available some morphological attributes gain positive effect. In our study it was found that all desi genotypes showed non-significant change regarding shoot length. In kabuli, resilient genotypes K-09015 and FG0902 showed less increase in shoot length than susceptible genotype K0039-09. An overall increase was found in shoot length of kabuli genotypes. Same results were found in *Lotus tenuis* [246].

Root length revealed as non-significant increase in desi genotypes. In kabuli genotypes K-09015 and FG0902 maintained their root length while susceptible genotype K0039-09 showed increased length. In *Lotus tanuis* Non-significant increase in root length was observed under irrigation stress[246].

Seedling fresh weight showed an overall increase in desi and kabuli genotypes. In desi type it was found that susceptible genotype showed increase in fresh weight while D-

09027 maintained its weight which was proved to be resistant. In kabuli genotypes K-09015 and FG0902 maintained their S.F.W while K0039-09 showed increase in weight which was susceptible. Same results were found for shoot fresh weight and root fresh weight [246].

Total oxidant status (TOS) is increased whenever plant faces elevated temperature. But if antioxidants increase, TOS level is lowered. In desi genotypes D-09027 showed maintained oxidant status which was tolerant while susceptible Bhakhar-2011 showed increased oxidants.

Lycopene have antioxidant properties of quenching singlet oxygen. Lycopene was significantly lowered in desi genotypes under excessive water in resistant genotype while in susceptible genotype Bhakhar-2011 it was increased. In kabuli genotype K-09015 showed same contents as control while susceptible K0039-09 showed increased contents. In tomato it was observed that high lycopene contents does not tolerate waterlogging. Hence moderate lycopene contents may withstand with waterlogging.

Waterlogging causes lowering of chlorophyll 'a' contents and ultimately photosynthesis. In our experiment it was found that in case of desi resistant genotype D-09027 showed no change in chlorophyll 'a' contents while susceptible genotype Bhakhar-2011 showed increased chlorophyll 'a' contents. Chlorophyll 'b' indicated lower value in desi resistant genotype while no change in susceptible genotype. In kabuli genotypes K-09015 maintained its chlorophyll 'b' contents while K0039-09 showed increased behavior which was susceptible. Same results were found for total chlorophyll and total carotene contents for desi and kabuli as for chlorophyll 'b' contents. *Memordica charantia* showed the same results [247, 248].

Under excess of water reactive oxygen species are increased due to low supply of energy and changed redox condition of the cell. Higher level of ROS damages cell by lipid peroxidation and damage to DNA. Due to lipid peroxidation, membrane stability is decreased. This was found in maize, winter rape and barley [249, 250].

Presence of ROS was evident from injury to membranes. At the sites where membranes were damaged, melanodialdehyde (MDA) was found, hence it was cause of lowered membrane integrity. Electrolyte leakage was found in corn and barley [251]. In desi genotypes it was found that D-09027 showed maintained MDA contents while

increased contents in susceptible genotype i.e. Bhakhar-2011. In kabuli genotypes K-09015 and FG0902 showed maintained MDA while K0039-09 showed increasing behavior. It was concluded that presence of lower MDA level confers resistance by the membrane integrity.

Under water stress conditions, lowered nitrogen causes decrease in protein level. So in tolerant genotypes protease activity will also be lowered. In desi type D-09027 indicated lower protease activity while resistant Bhakhar-2011 indicated higher activity. Likewise in kabuli resistant genotypes K-09015 and FG0902 exhibited lower protease activity while susceptible K0039-09 revealed higher activity. These results were also found in mungbean [252].

Under high water stress it was reported that activity of superoxide dismutase was enhanced. In our results desi type D-09027 showed increased activity of SOD while Bhakhar-2011 showed no change in its activity which was susceptible genotype. In kabuli type resistant genotypes showed maintained SOD activity while susceptible K0039-09 showed decreased activity. Increased SOD activity was found when rhizomes of *Iris pseudocorus* were submerged in water [253].

Ascorbate peroxidase and peroxidase shown same behavior. Both were non-significant in desi type chickpea. In kabuli type activity of peroxidase was enhanced overall. But susceptible genotype (K0039-09) showed more activity than resistant ones (K-09015 and FG0902). Same was the result for ascorbate peroxidase. In maize activity of APX and POD were given which were slightly enhanced [254].

Enhanced catalase activity was found in mungbean [255]. Any stress immediately results in production of oxidative species which needs to be removed as early as possible to be save the cell from injury. In desi type it was found that tolerant genotype D-09027 showed enhanced catalase activity but susceptible genotype Bhakhar-2011 showed much higher level of catalase. In kabuli genotypes K-09015 and FG0902 showed higher level of catalase activity while susceptible (K0039-09) showed lower level of it.

When plants are submerged in the water, nitrogen becomes unavailable which results in lower level of protein formation. Genotypes which maintain their protein level even in high water can be categorized as tolerant. In desi type D-09027 maintained its protein level while in case of kabuli K-09015 maintained its level and FG0902 showed

much increase in protein level. Protein levels were studied in citrus plant and same results were found [256].

Esterase is involved in breakdown of ester bonds. It should be decreased for tolerant genotypes. In desi type it was found that tolerant genotype D-09027 showed lower level of esterase while susceptible Bhakhar-2011 showed highest level of esterase. In case of kabuli type K-09015 showed significant increase and FG0902 maintained its level of esterase activity. While in susceptible K0039-09 the activity of esterase was increased. Barley and wheat showed the same results [257].

In desi genotypes, osmotic potential, turgor potential, relative water contents, FO, FM, FV, TM, FV/FM, shoot dry weight, seedling dry weight, root dry weight, peroxidase, and ascorbate peroxidase were found non-significant. Which means these parameters are not reliable for irrigation stress studies in desi chickpea. In kabuli genotypes, seedling dry weight, shoot dry weight, root dry weight, total water potential, osmotic potential, turgor potential, relative water contents, FO, FM, FV, TM and FV/FM were found non-significant which means that these are not reliable for irrigation stress studies in kabuli chickpea.

4.3 Combined (Heat and Irrigation) Stress

Climate of earth is well described by changes in these factors. It is evident that from very beginning up to now earth has undergone many changes regarding climate but changes occurring in last century are so drastic that it has raised the overall temperature of earth which is said to be warming. It was suggested in IPCC that all this is result of human activities which causes rapid global warming [24]. Now the weather conditions are changing continuously. Average temperature is increasing each year which has increased average global temperature of earth. This increase is due to many factors e.g. glaciers and sea ice is melting, waves of heat are increased, this elevated temperature cause's rise in the sea level, precipitation is changes which causes drought in one area and heavy rainfall in other area [25].

In desi type root length was affected such as tolerant genotypes D-0913 and CH24/07 showed increased root length while susceptible 5 showed decreased root length. In kabuli type it was found non-significant. The cause for increased root length may be due to more temperature which made the water unavailable.

Seedling fresh weight was maintained in desi genotypes while decreased significantly in kabuli type. Same result was found for shoot and root fresh weight. Seedling and shoot dry weight was found non-significant for kabuli genotypes while it was maintained in desi type. Root dry weight was maintained in desi type while it was increased in kabuli type, where susceptible genotype FG0902 showed higher root dry weight.

Lycopene is an antioxidant which is used by plants for oxidative stress alleviation. In desi type it was found that tolerant genotypes D-0913 and CH24/07 showed lowered lycopene while susceptible NIFA-2 showed higher contents. In case of kabuli type tolerant (K-09015 and CM1235/08) and susceptible (FG0902) showed maintained lycopene contents.

Among photosynthates, chlorophyll 'a' contents were significantly decreased in tolerant desi genotypes (D-0913 and CH24/07) while maintained in susceptible NIFA-2. In case of kabuli K-09015 and CM1235/08 showed decreased chlorophyll 'a' contents while FG0902 showed significantly decreased contents which was susceptible genotype. For chlorophyll 'b' contents desi tolerant and susceptible genotypes showed decreasing behavior while kabuli genotypes showed overall increasing behavior. Same results were found for total carotene contents. Total chlorophyll contents showed decreased behavior in desi genotypes while maintained in kabuli genotypes [187, 258].

When roots of plants are submerged in the water, nitrogen becomes unavailable for transportation. This unavailability of nitrogen results in decrease of protein level. When there are two stresses combined i.e. heat and excessive water then protein level will be decreased drastically. In desi genotypes D-0913 showed increased level of protein which shows its resilience while NIFA-2 shows drastic decrease which means it is susceptible one. In case of kabuli type resilient genotypes showed maintained protein level.

Heat induces production of high level of reactive oxygen species and so do excessive water. So whenever both stresses are prevailing then it is inferable that more ROS will be present. In desi genotypes it was found that superoxide dismutase was lowered in all desi genotypes either resistant or susceptible. In case of kabuli tolerant genotypes K-09015 and CM1235/08 showed increased level of SOD activity while susceptible genotype FG0902 showed decreased level of SOD activity.

Ascorbate peroxidase and peroxidase were found non-significant in desi type. While in case of kabuli both were significantly increased in all genotypes. Which means that their activity was significantly enhanced to keep the cells safe from cellular injury. Catalase activity was maintained in both desi and kabuli types in tolerant genotypes.

Protease is involved in breakdown of lipids and proteins. Hence tolerant genotypes should have lower quantity. In desi genotypes protease activity was lowered in tolerant genotypes i.e. D-0913 and CH24/07. In case of kabuli type all the genotypes showed increasing behavior.

Esterase break downs ester bonds and should be decreased in tolerant genotypes. It was found in all desi and kabuli genotypes that its activity was lowered. Which means the genotypes showed resistance in this regard. Melanodialdehyde contents were also lowered in desi and kabuli type showing positive response towards tolerance.

In desi genotypes total water potential, osmotic potential, turgor potential, relative water contents, FO, FM, TM, FV, FV/FM, peroxidase and ascorbate peroxidase were found non-significant so these are not reliable for combined stress tolerance studies. In kabuli genotypes total water potential, osmotic potential, turgor potential, relative water contents, FO, FM, TM, FV and FV/FM were found non-significant so these are not reliable for combined stress tolerance studies.

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