The effect of high temperature on physiological and metabolic parameters and reproductive tissues of okra (*Abelmoschus esculentus* (L.) Moench)

By

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Certificate of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work and it contains no material previously published or written by another person for the award of any other degree in any university or institute. I certify that all the assistance received in preparing this thesis and sources have been acknowledged.

Shahnoosh Hayamanesh

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Conferences

Oral Presentations

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Dedication

I dedicate my PhD to my amazing father, BABA SHAFIEI, who is not in this world that I can celebrate my victory with.

My dear father, you always wanted the best for me. You wanted me to be educated and successful and you always wanted me to be a doctor. It was hard to start without your encouragement and it is even hard to finish without seeing your happiness and satisfaction. I am proud with who I am today, as I was raised by your love and kindness.

Summary

Okra is an important summer vegetable crop grown in tropical and subtropical areas of Asia, Africa, the Middle East, the southern United States and northern Australia. Demand for this crop has increased worldwide due to an increasing population and increasing demand for this nutritious vegetable. Edible pods (fruits) of okra are a good source of fibre, vitamins, proteins, carbohydrates and amino acids. Although okra is native to warm regions, high temperature has been shown to lower its germination, growth and yield.

High temperature damages the plant photosystem, impairs cellular function by damaging membranes, alters sugar content and affects reproductive tissues; all potentially lowering yield. There is, however, limited information on where heat damage occurs in okra and how heat stress affects okra at different growth stages. The current study aimed to characterise the effect of heat stress on the physiological, biochemical, morphological and histological level and identify the developmental stages that are more vulnerable in okra genotypes which have distant genetic background.

Physiological parameters and molecular techniques (e.g. SSR markers) are useful tools to screen for specific environmental stress (e.g. heat) and genetic diversity, respectively. In this study one hundred and nineteen genotypes from the Vegetable Research Institute (VRI) and the World Vegetable Centre, previously known as the Asian Vegetable Research and Development Center (AVRDC), were screened for their response to a short period of heat shock (45°C for 4 hours) in a controlled environment facility (CEF) by assessing chlorophyll fluorescence (F_v/F_m and F_v'/F_m') and stomatal conductance (g_s) in both control and heat. DNA of fresh green leaf of all genotypes was isolated by a modified CTAB method with additional PVP and RNase and amplified by 8 polymorphic SSR markers to generate a dendrogram. This preliminary screening identified thirty three polymorphic genotypes with less than 50% genetic similarity and contrasting response in F_v'/F_m' and g_s.

More detailed physiological measurements (i.e. F_v/F_m, F_v'/F_m', g_s, photosynthesis (A) and electrolyte leakage (EL)) were conducted on leaves after these genotypes (with three replicates) were exposed to 45°C for 6 hours in the CEF in both control and heat. No significant difference was observed in EL (in most genotypes except for 5 genotypes) between control (30°C) and heat, therefore, a short period of heat stress did not affect cell membranes in okra. In contrast, F_v'/F_m' and F_v/F_m reduced in the heat with varying response in g_s and A. Additionally, two genotypes of okra showed an acclimation response when grown at day temperatures of 45°C for two weeks in a growth cabinet. Heat-adapted plants had significantly higher F_v'/F_m', g_s, A and efficiency of the open reaction centre (Φ_{PSII}) than non-adapted plants, so that F_v'/F_m', A and Φ_{PSII} were constant and g_s increased when temperatures were raised from 33°C to 45°C.

Eight genotypes out of the 33 were selected based on their contrasting physiological responses (F_v'/F_m', g_s and EL) in the heat to evaluate their tolerance and acclimation under long periods of high temperature in an outdoor production setting. Physiological, agronomic and reproductive traits were assessed for two consecutive years (eight genotypes in the first year and four genotypes in the second year). Two adjacent plastic tunnel houses (heat and control) were used for this more detailed study, where the temperature was on average 10°C warmer in the heat tunnel house (maximum of 45°C) during the day compared to the control, and night temperatures were similar (c. 18°C). In Year 1 (summer 2015), photosynthetic parameters (i.e. F_v'/F_m', g_s and A), EL, agronomy traits (i.e. plant height, dry matter and yield) and *in vitro* pollen germination were assessed 15 weeks after transplanting into the tunnel houses. No significant effect on F_v'/F_m', A and dry matter was found after exposing okra to this long-term heat, whereas g_s and EL increased significantly. Plants in the heat were shorter and biomass was reduced (not significantly), they also showed significant decrease in pollen germination, accompanied by a severe reduction in yield.

In Year 2 (2016), the selection was further reduced to four genotypes based on their yield and physiological responses to heat stress. Physiological, agronomic and reproductive traits, as well as sugar content were assessed at two developmental stages of flowering and bud initiation. The selected genotypes were transplanted into control and

heat tunnel houses with three replicates per genotype and kept for 6 weeks under the same conditions explained earlier. Physiological (i.e. F_v'/F_m', g_s, A and EL) responses were assessed every two weeks, sugar content was measured at the beginning and at the end of the experiment, and reproductive tissues (e.g. morphology, *in vitro* pollen germination, anther numbers and stigma receptivity) as well as agronomy traits (e.g. plant height, biomass and yield) were assessed at the end of the experiment. EL declined over time in both heat and control. In the heat, g_s was similar to the control for two weeks and then increased over time, while A decreased significantly within the first two weeks and then increased. This physiological adjustment resulted in no significant difference of A between heat and control at the end of the experiment. Dry matter decreased under high temperature, but since plants were kept for shorter period compared to Year 1, biomass in the heat may have caught up to the control if plants had been kept longer.

Despite physiological adaptation to heat, yield decreased severely due to heat damage and alteration to reproductive tissues. Heat reduced anther numbers and pollen germination. The shape and structure of male tissues (anthers and pollen) was altered (i.e. deformed and flattened) in the early stage of bud development. In contrast, heat did not alter stigma receptivity and no damage was observed in female tissue under the microscope. The adverse effect of high temperature on male tissues may have resulted from alteration in carbohydrate content, for example lower concentrations of sucrose in reproductive tissues. This may have arisen from inhibition of sucrose movement through the phloem, as sugar concentrations were severely decreased in shoots, whereas in leaves (except in sucrose concentration at bud initiation) and roots the concentrations of sugars increased.

Okra was more affected by heat in its earlier stages of development in some of the assessed parameters. In the heat, there was higher F_v'/F_m' and A at flowering compared to bud initiation, whereas g_s and EL remained similar between the two stages. Although sucrose content was significantly lower at bud initiation in the heat but anthers numbers remained similar between two stages (with slightly higher number at bud initiation).

The results of this study suggest that okra shows a physiological acclimation response, but is more vulnerable in the early stages of growth and development. Despite

coping physiologically, heat severely damages the male reproductive tissue, resulting in strong yield reduction. Of all genotypes studied, one genotype (L4-48, Cajun jewel, VI056456) showed potential for a heat-tolerance breeding variety.

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List of Abbreviations

A	Photosynthesis rate (µmol m^{-2} s^{-1})
ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Center
bp	Base pair
CEF	Controlled Environment Facility
CO_2	Carbon dioxide
CTAB	Cetyl trimethylammonium bromide
DM	Dry matter
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
EDTA	Ethylenediaminetetraacetic acid
E	Transpiration rate (mmol $m^{-2}s^{-1}$)
EL	Electrolyte leakage (%)
F_m	Maximum fluorescence
F_o	Initial fluorescence
F_s	Steady state of fluorescence
F_v	Variable fluorescence
F_v/F_m	Chlorophyll fluorescence, dark adapted
F_v'/F_m'	Chlorophyll fluorescence, light adapted
GC-MS	Gas chromatography–mass spectrometry
g_s	Stomatal conductance (mol m^{-2} s^{-1})
k_{leaf}	Leaf hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1})
MCW	Methanol/chloroform/water
MEGA	Molecular Evolutionary Genetics Analysis
mM	Millimolar
n	Replicate
NTSYS	Numerical taxonomy and multivariate analysis system
O_2	Oxygen
qP	Photochemical quenching

p	Probability
PAR	Photosynthetically active radiation
PCR	Polymorphic chain reaction
PBI	Plant Breeding Institute
PIC	Polymorphic information content
PSI	Photosystem one
PSII	Photosystem two
PVP	Polyvinyl pyrrolidone
RH	Relative humidity
RNase	Ribonuclease
SE	Standard Error
SPS	Sucrose phosphate synthase
SS	Sucrose synthase
SSR	Simple sequence repeats
TBE	Tris/Borate/EDTA buffer
T_{leaf}	Leaf temperature
Tm	Annealing temperature
UPGMA	Unweighted pair group method with arithmetic mean
USA	United States of America
UV	Ultra violet
VRI	Vegetable Research Institute
Φ_{PSII}	Efficiency of the open reaction centre
Ψ	Water potential (MPa)

1 General Introduction

1.1 Background

Okra is an important, nutritious vegetable crop growing in tropical and subtropical areas mostly in Africa and Asia (Dhankhar and Singh, 2009, Kumar et al., 2010, Oppong-Sekyere et al., 2011, Tripathi et al., 2011). Recently, its commercial growing has been expanded to southern United States and Northern Australia, and it has been introduced into the European diet (Dhankhar and Singh, 2009). Although okra is a summer crop and it is well adapted to hot conditions, yield reduction has been observed under high temperature (Arulrajah and Ormrod, 1973, Dhankhar and Singh, 2013). Emission of greenhouse gases resulted in a 0.7°C increase in temperature during the twentieth century and it has been predicted to further increase between 1°C and 4°C during the twenty first century (IPCC, 2007), which will severely affect food production worldwide. In India – a leading okra producer – the hottest recorded temperature was 51°C in 2016 (FAO, 2015, Van Oldenborgh et al., 2017). The adverse effects of warmer temperatures on growth, development and productivity of crops in tropical and subtropical areas have already been documented (Wahid et al., 2007, Hatfield et al., 2011). Yet, there is limited information on heat damage to okra's reproductive tissues and its physiological and metabolic responses to the heat, as most studies to date focused on the effect of high temperature on its morphology and productivity (Arulrajah and Ormrod, 1973, Gunawardhana and De Silva, 2012, Dhankhar and Singh, 2013). In addition, vulnerability of different stages of development to the heat has not been identified. Although there are some studies in this regard, for example in wheat (Porter and Gawith, 1999), information about okra is limited. Demir (2001) reported okra seed incubation for two days at 50°C prior to sowing increased germination, while high temperature of 45ºC immediately after sowing caused rapid emergence, but delayed flowering (Dhankhar and Singh, 2013) or resulted in no bud development (Arulrajah and Ormrod, 1973). As global food security is under threat by increasing population and temperature (Lobell and Gourdji, 2012), and okra is a valuable food source in Eastern countries, evaluating okra heat sensitivity and identification of heat tolerant varieties are essential. In this regard, usefulness of different

techniques for screening and variation in okra germplasm needs to be evaluated to maximize the efficiency of the selection.

1.2 Aim

This study aimed to fill the knowledge gap of the effect of heat on physiological parameters of okra, such as photosynthesis, stomatal conductance, transpiration, fluorescence, carbohydrate concentration and electrolyte leakage in order to determine vulnerable processes affected by high temperature. It evaluated these physiological parameters under short- and long-term exposure to heat stress in a controlled environment and in an outdoor production setting (tunnel houses) at different stages of plant development. In addition, this study investigated heat damage to reproductive tissues and changes to morphology and yield.

1.3 Thesis outline

The thesis is composed of four research chapters, preceded by a general introduction (chapter 1) and literature review (chapter 2), and finally a synthesis that includes a general discussion (chapter 7) which identifies some of the research gaps and future investigation. In detail, the research chapters will focus on:

Chapter 3. Physiological screening tools to identify high temperature tolerance in okra (*Abelmoschus esculentus* (L.) Moench)

- Investigates and evaluates the usefulness of physiological parameters (e.g. chlorophyll fluorescence, stomatal conductance, photosynthesis and electrolyte leakage) under short-term high temperature stress in a controlled environment.
- Uses these tools (in combination with molecular techniques, chapter 4) to narrow down a big selection of genotypes for further testing in a production environment (tunnel houses).

- Assesses acclimation in a growth cabinet by evaluating okra physiological responses after a 2 week exposure to high temperature (45°C).

Chapter 4. Optimising molecular techniques for okra (*Abelmoschus esculentus* (L.) Moench) breeding and improvement

- Optimizes DNA extraction.
- Uses Simple Sequence Repeats markers, to amplify okra genomic DNA and identify genetic variation among the genotypes.

Chapter 5. Effect of high temperature on physiological parameters and productivity in okra (*Abelmoschus esculentus* (L.) Moench)

- Evaluates the effects of high temperature on the physiology of okra under long-term heat exposure in a production environment (tunnel houses), including photosynthetic parameters, electrolyte leakage and soluble carbohydrate concentrations.
- Determines the physiological sensitivity of okra at different stages of development.
- Assesses okra productivity, i.e. dry matter and yield, under heat and control conditions.

Chapter 6. The effect of high temperature on the reproductive development of okra (*Abelmoschus esculentus* (L.) Moench)

- Evaluates the effects of high temperature on vegetative growth (i.e. plant height and base diameter).
- Evaluates the effects of high temperature on reproductive tissues, including numbers of anthers, *in vitro* pollen germination, stigma receptivity and histology of male and female organs at different stages of development.
- Evaluates the effects of high temperature on fruit production.

2 Literature review

2.1 Background

2.1.1 Origin and distribution

Okra (*Abelmoschus esculentus* (L.) Moench) is a dicotyledon, warm-season annual vegetable crop with a growing season of 90 to 100 days which grows in tropical and subtropical regions of the world on sandy to clayey soil (Nwangburuka et al., 2011, Oppong-Sekyere et al., 2011, Tripathi et al., 2011). Okra belongs to the family *Malvacae* (Table 2.1) and taxonomists have described about 50 species in the genus *Abelmoschus*, eight of which are accepted as cultivated and wild species (Table 2.2) (Kumar et al., 2010, Tripathi et al., 2011, Kalloo and Bergh, 2012). The origin of okra is disputed; most of these eight species have been documented in Southeast Asia, mostly in India and neighbouring countries, but some studies have suggested that domestication of this crop took place in Africa due to its adaptation in the region (Bisht and Bhat, 2006, Kumar et al., 2010, Oppong-Sekyere et al., 2011, Tripathi et al., 2011). The word okra is assumed to be derived from the African language (Dhankhar and Singh, 2009), whereas the genus name *Abelmoschus* is derived from the Arabic language and refers to the musk-scented seeds.The species name *esculentus* comes from Latin and means edible (Dhankhar and Singh, 2009). This seed-propagated and self-pollinated crop can be cultivated as a garden crop or on large commercial farms, which are found in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopia, Cyprus, the Southern United States and northern Australia (Dhankhar and Singh, 2009, Kumar et al., 2010, Tripathi et al., 2011). In most parts of the world, *A. esculentus* is known as okra, ochro, okoro, quingumbo, ladies finger, gombo, kopi arab, kacang, bendi, bhindi (South Asia), bamia, bamya or bamieh (Middle East) or gumbo (Southern USA). In Portugal and Angola, okra is known as quiabo, and in Cuba as quimbombo. In Japan, it is known as okura (Kaur et al., 2013).

Table 2.1 Taxonomy of okra (USDA, 2017).

Rank	Scientific Name and Common Name
Kingdom	Plantae - Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – flowering plants
Class	Magnoliopsida - Dicotyledons
Subclass	Dilleniidae
Order	Malvales
Family	Malvaceae – Mallow family
Genus	*Abelmoschus*Medik - Okra
Species	*Abelmoschus esculentus*(L.) Moench – okra

Table 2.2 Classification in the genus *Abelmoschus* adopted by (IBPGR, 1990).

No	Species
1	*A. moschatus* Medikus- subsp. *moschatus* var. *moschatus*- subsp. moschatus var. *betulifolius* (Mast) Hochr- subsp. *biakensis* (Hochr.) Borss. Subsp. *tuberosus* (Span) Borss.
2	*A. manihot* (L.) Medikus- subsp. *tetraphyllus* (Roxb. ex Hornem.) Borss. var. *tetraphyllus* - var. *pungens*
3	*A. esculentus* (L.) Moench
4	*A. tuberculatus* Pal & Singh
5	*A. ficulneus* (L.) W &A.ex. Wight
6	*A. crinitus* Wall.
7	*A. angulosus* Wall. ex. W, & A.
8	*A. caillei* (A. Chev.) Stevels

2.1.2 Morphology

Okra's morphological characteristics show a large variation, but in general it is a robust, erect, fibrous plant with an indeterminate growth habit (Lamont, 1999). Okra has a deep tap root system, semi-woody green stem (Tripathi et al., 2011), with alternating and palmately veined leaves and solitary flowers, which have superior ovaries and numerous stamens (Nwangburuka et al., 2011). Its edible fruit is called a pod, which contains a large number of seeds (Oppong-Sekyere et al., 2011).

2.1.2.1 Root system, stem and leaves

Okra has a vertical tap root, which can penetrate up to a 1.3 m depth. Its 24 to 35 lateral roots spread horizontally and obliquely downward, and can extend up to 2 m (Figure 2.1) (Weaver and Bruner, 1927).

The erect, semi-woody stem of okra is always green (Tripathi et al., 2011). The height of okra differs between varieties and growth conditions, ranging between 0.9 and 2.4 m (Lamont, 1999, Nwangburuka et al., 2011, Tripathi et al., 2011), and even up to 4 m in central Nepal (Velayudhan and Upadhyay, 1994). The main stem may have short side-branches, which is variable among the different varieties of okra.

The palmate, green leaves of okra normally are hairy and alternate with three to five lobes and a 15 to 35 cm long petiole (Dhankhar and Singh, 2009, Tripathi et al., 2011). Some varieties are characterized by red pigmentation on the petiole and leaf veins (Dhankhar and Singh, 2009). The size and shape of the leaves may vary among different varieties (Figure 2.1); the length could reach up to 30.5 cm (Tripathi et al., 2011).

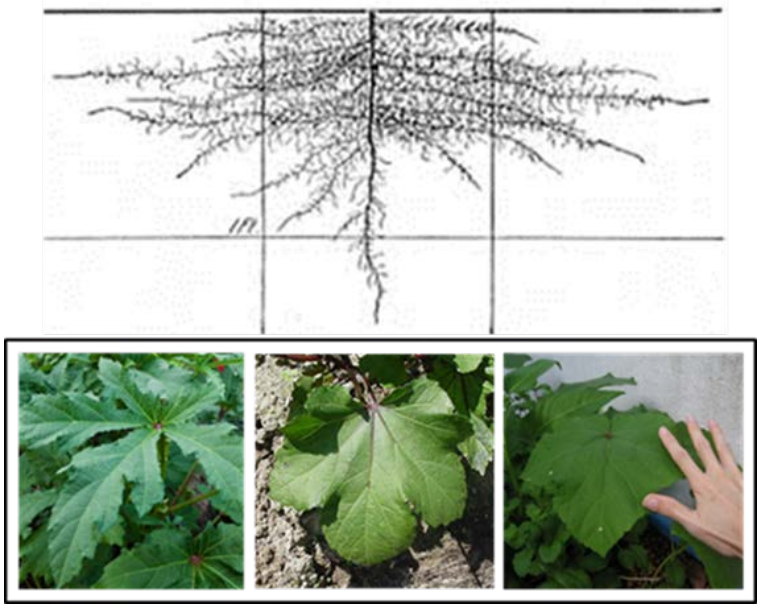

Figure 2.1 Okra root system about 3 weeks after planting (Weaver and Bruner, 1927) and different shapes of okra leaves.

2.1.2.2 Flower

The flower of okra has a superior ovary and five pale yellow petals with a red or purple spot at the base of each of them (Tripathi et al., 2011). The size of the flower varies from 5 to 8 cm in diameter (Dhankhar and Singh, 2009). The style is surrounded by many stamens (Figure 2.2) with kidney-shaped anthers that can open by a slit from the top. Each anther can produce c. 100 sticky pollen grains which contain many pores and are able to develop many pollen tubes (Figure 2.3) (Thakur and Arora, 1986, Dhankhar and Singh, 2009). As the staminal column grows, pollens come into contact with five hairy deep red or dark purple color stigmas, which can accommodate around 600 pollen grains (Dhankhar and Singh, 2009). Flower buds emerge in the axil of each leaf where the first one is normally above the 3rd to 8th leaf (Dhankhar and Singh, 2009); they always appear singly and as the plant elongates, a flower opens every 2 or 3 days (Tripathi et al., 2011).

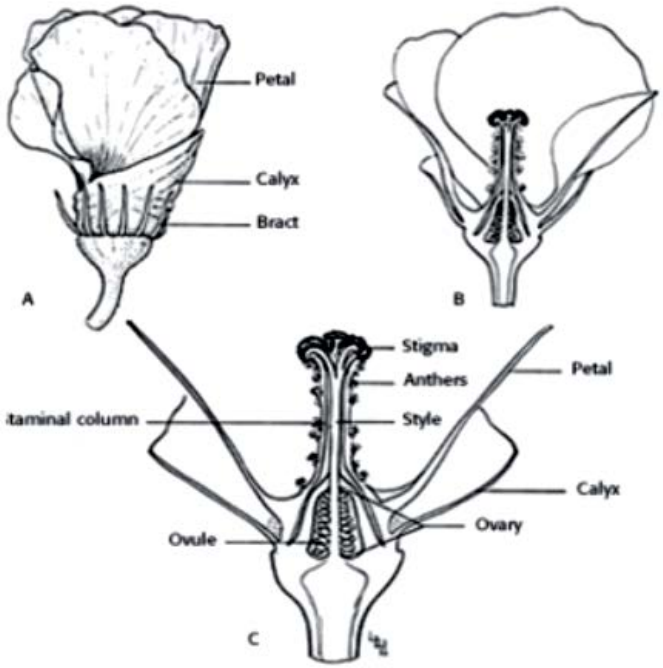

Figure 2.2 Okra flower. Side view (A); longitudinal section (B); longitudinal section of staminal column (C) (Tripathi et al., 2011).

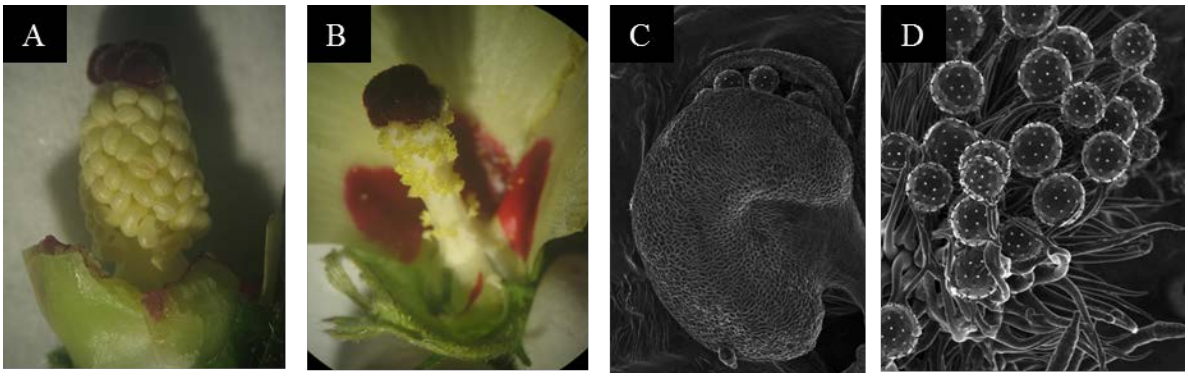

Figure 2.3 (A) closed pollen sacs and (B) opened sacs and pollen grain under light microscope (photo credit: S. Hayamanesh), (C) dehiscence of anther and (D) pollen deposition on the stigma under scanning electron microscope (Patil et al., 2013)

2.1.2.3 Fruit and seed

The fruit growth (the length, height and diameter) occurs in the first week after pollination (Tripathi et al., 2011). The capsule formed of okra fruit (called pod), which contains 30-80 seeds, is pale green to dark green or purple in colour. The pod is covered with hair and has a variety of shapes and sizes (c. 10-30 cm long) (Figure 2.4) (Dhankhar and Singh, 2009). The highest and lowest number of fruit per plant were reported between 144 and 16.8, respectively (Oppong-Sekyere, 2012).

Mature seed of Okra is round, dark brown, black, greyish, greenish, or khaki in colour with a hard coat (Figure 2.4) and has an average weight of 1.6 to 8.1 g per 100 seeds (Martin and Rhodes, 1983). Seeds reach maturity c. 40 days after fertilization (Dhankhar and Singh, 2009).

Figure 2.4 Okra pods of different shapes and sizes. Mature okra seeds (photo credit: agribuz.com).

2.1.3 Agronomy

2.1.3.1 Seed germination, growth and development

Okra seeds reach maturity 40 days after fertilization (Dhankhar and Singh, 2009). The highest germination rate was observed in harvested seed at 30 (Chauhan and Bhandari, 1971) or 34 to 41 days after anthesis (Castro et al., 2008). Mature seeds germinate within 5-14 days after sowing (Hamon and Noirot, 1991). The indeterminate growth habit with erect appearance provides uniform and maximum capture of sunlight for all leaves and other vegetative parts of the okra plant. This habit increases the biomass and yield, as well as reduces the chance of fruit rotting and allows a longer period of fruit harvest (Oppong-Sekyere et al., 2011).

Growth rate and development of okra is significantly influenced by genotypic variation, as well as water availability, relative humidity, day length, and most importantly, temperature (Dhankhar and Singh, 2009). Long growing period, high light intensity and optimal temperatures are required to maximize plant height and canopy development, which leads to increased plant productivity (Iremiren and Okiy, 1986). Optimum soil moisture is required during the first month after sowing for good crop establishment (Kumar et al., 2010). Drip irrigation of 25 to 38 mm of water per week supplied enough moisture to prevent loss of leaves and pods (Lamont, 1999), but in a tropical environment, this can be reduced to c. 8 mm per day due to high humidity (Benchasri, 2012).

2.1.3.2 Flower development

Flower initiation takes place 22-26 days after sowing (Sulikiri and Swamy Rao, 1972, Tripathi et al., 2011) and the first flower normally appears one or two months after sowing (Tripathi et al., 2011, Dhankhar and Singh, 2013), more specifically 41-48 days after sowing (Sulikiri and Swamy Rao, 1972). Generally, anthesis takes place at the end of the night (Hamon and Koechlin, 1991a) and the flower opens between 6 am to 10 am (Dhankhar and Singh, 2009). In the afternoon, petals close, wilt and fall off the following day (Tripathi et al., 2011). Anthers dehisce about 15-20 minutes after the flower opens

(Venkataramani, 1953), but may also occur 4 to 6 hours before opening of the flower (Dhankhar and Singh, 2009). The style is receptive before flowers open and self-pollination can occur before anthesis (Hamon and Koechlin, 1991b). Pollen is maximally fertile between an hour before and an hour after the flower opens, but pollen grains can be viable for 55 days at 55% relative humidity (Dhankhar and Singh, 2009). Fertilisation process increases during morning which can give a chance to all pollen to fertile the ovules by midday (Hamon and Koechlin, 1991a).

2.1.3.3 Fruit development

For human consumption, the fruits are be harvested immaturely when they contain a high level of mucilage (Figure 2.5) (Tripathi et al., 2011). After 7 days of fruit setting, fibre content will increase while moisture, protein and starch content decrease. Therefore, the fruits become tough and lose their quality (Dhankhar and Singh, 2009). More specifically, the production of fibre starts around the 6th day of fruit formation and from the 9th day, the fibre content increases dramatically (Nath, 1976). Therefore, the picking should be done 4 to 6 days after pollination and fruit setting (Iremiren et al., 1991, Dhankhar and Singh, 2013), normally early in the morning, every second day (Moekchantuk and Kumar, 2004).

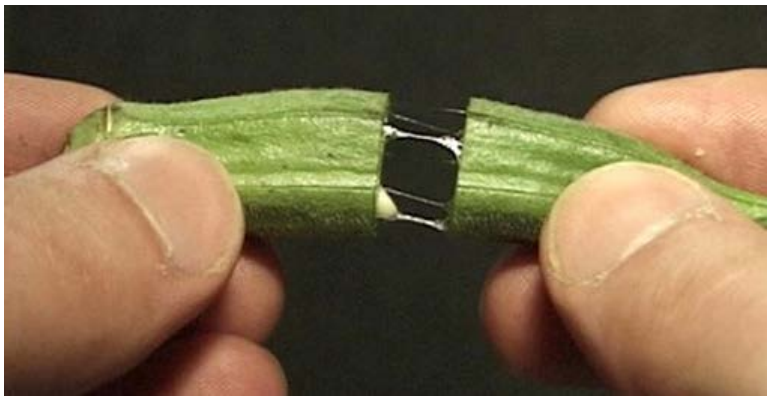

Figure 2.5 Okra pod mucilage (photo credit: diabetesadvocates.info).

2.1.4 Economic and nutritional values of okra

2.1.4.1 Nutrition

Okra is one of the most important vegetable crops in many countries in Africa, Asia, the Middle East, the southern United States and northern Australia due to considerable quantities of vitamins, proteins and carbohydrates, as well as essential and non-essential amino acids (Adeboye and Oputa, 1996). Pods, leaves, shoot, flower buds and calyxes can be eaten; pods can be boiled or fried and added to soups or different dishes to produce a unique flavor and mucilaginous texture (Lamont, 1999). Nutritional composition of 100g of fresh okra fruit and leaves are shown in Table 2.3 and Table 2.4, respectively (FAO, 1968, Haytowitz and Matthews, 1984). Okra seeds contain unsaturated fatty acids that can be used as margarine (Lamont, 1999). According to Berry et al. (1988) and Siemonsma and Kouame (2004), 100g of mature seed contains 20% and 20.23% edible oil and crude protein, respectively. Okra seed flour is used to fortify cereal flour (Adekalu et al., 2008), as well as to supplement corn flour in order to make better quality dough (Taha El-Katib, 1947). okra seeds also can be roasted and used as coffee substitute (Burkill et al., 1936, Moekchantuk and Kumar, 2004).

Table 2.3 The nutritional composition of 100 g edible portion of fresh okra fruit (Haytowitz and Matthews, 1984).

Nutrient	Value
Water (%)	90.00
Energy (kcal)	38.00
Protein	2.00
Fat (g)	0.10
Carbohydrate (g)	7.60
Fiber (g)	0.90
Ca (mg)	81.00
P (mg)	63.00
Fe (mg)	0.80
Na (mg)	8.00
K (mg)	303.00
Vitamin A (IU)	660.00
Thiamine (mg)	0.20
Riboflavin (mg)	0.06
Niacin (mg)	1.00
Ascorbic acid (mg)	21.10
Vitamin B_6 (mg)	0.22

Table 2.4 The nutritional composition of 100 g edible portion of the okra leaf (FAO, 1968).

Nutrient	Value
Water (mL)	1.00
Protein (g)	4.40
Fat (g)	0.60
Carbohydrate (g)	11.00
Calories	56.00
Fiber (g)	2.10
Minerals (mg)	602.70
Vitamins (mg)	447.25

2.1.4.2 Other uses

Okra stems contain long fibres which make them a suitable material for glace paper production (Lamont, 1999, Benchasri, 2012), and brightening agents in the electro-deposition of metals (Kumar et al., 2010). Stems are also a good source of fuel due to their considerable heat generating property without producing sparks, excessive smoke or bad odours (Ahiakpa et al., 2013). Okra mucilage is used as a medicine for irritating and inflammatory diseases (Lengsfeld et al., 2004), a plasma replacement or blood volume expander (Savello et al., 1980, Lengsfeld et al., 2004, Kumar et al., 2010).

2.1.5 World productivity

Okra is becoming a more popular vegetable worldwide, and is being introduced into the European diet (Dhankhar and Singh, 2009). In 2012, the total area under okra cultivation was around 1.1 million ha with a total production of around 8 million t worldwide. As demand for this crop is increasing, world production and cultivation area for okra has become larger (FAO, 2015) (Table 2.5). India is the largest okra producer, followed by Nigeria with a 71.77% and 13.16% share in world production in 2012. Nearly half of the world's okra cultivated area is in India, but Egypt is the most productive country with a yield of 13.96 tonnes ha^{-1} (Table 2.6).

Table 2.5 Area of okra cultivation, total production and yield per hectare from 2002-2012 (FAO, 2015).

Year	Area harvested (ha)	Production (t)	Yield (t ha^{-1})
2002	874489	5314001	6.08
2003	905373	5620173	6.21
2004	930693	5807445	6.24
2005	906908	5613188	6.19
2006	975952	6206495	6.36
2007	1025598	6548659	6.39
2008	994247	6437008	6.47
2009	1013049	6809175	6.72
2010	1038526	7066641	6.80
2011	1067591	8058753	7.55
2012	1085146	8359944	7.70

Table 2.6 Area of okra cultivation, total production and yield of leading okra producers in the world in 2002-2012, (FAO, 2015).

Area	Area harvested (ha)	Production (t)	Yield (t ha^{-1})	% Share in World Production
World	1085146	8359944	7.70	100.00
India	500000	6000000	12.00	71.77
Nigeria	385000	1100000	2.86	13.16
Iraq	20000	160000	8.00	1.91
Ivory Coast	50000	134260	2.69	1.61
Pakistan	14000	108000	7.71	1.29
Egypt	6957	97108	13.96	1.16
Cameroon	26500	64000	2.42	0.77
Ghana	3000	60000	20.00	0.72
Saudi Arabia	4000	51500	12.88	0.62
Others	75689	585076	7.73	7.00

2.2 Environmental stress

External factors which negatively influence plant growth and development can be summarised as biotic and abiotic stresses. Measurements such as plant growth, yield, carbon dioxide (CO_2) and water and mineral uptake have been proven useful to assess the effect of stress and its severity (Taiz and Zeiger, 2002). Of the abiotic stresses, water stress (drought and water logging), acidity, salinity and temperature (low and high) have been reported to affect okra production (Diizyaman, 1997, Lamont, 1999, Kumar et al., 2010).

2.2.1 Water stress

Water stress has a profound influence on plant development, growth and yield and it usually takes days to weeks to have a negative effect (Kirda, 2002). Water deficit is one of the major abiotic stresses in most countries and its impact on plant growth depends on the duration of stress, plant species, genotype and stage of development. In drought, water potential (Ψ), osmotic potential and relative water content decrease in corn (Atteya, 2003), whereas root sugar content, proline content and ion leakage increase (Baghizadeh. A. et al., 2009). The most sensitive plant growth phases to drought in okra are germination and seedling growth (Baghizadeh and Hajmohammadrezaei, 2011). Drought can also reduce yield by 45 to 70% if it occurs during flowering or pod filling period in okra (Mbagwu and Adesipe, 1987, Diizyaman, 1997, Naveed et al., 2010, Naveed et al., 2012). During drought, plant height is reduced, leaves senesce and less leaves per plant are produced. Moreover, decreasing cell enlargement causes leaf size to decrease, which leads to a reduction in photosynthesis and subsequently, lower yield (Premachandra et al., 1995, Shao et al., 2008) and lower dry matter (Naveed et al., 2010). Importantly, yield reduction is even more severe when water stress is accompanied by heat stress (Gunawardhana and De Silva, 2012).

2.2.2 Temperature stress

Temperature is one of the other major environmental stress factors that limits plant growth, metabolism and productivity, and can be harmful to plants even when stress exposure is only for a few minutes (Krasensky and Jonak, 2012, Hasanuzzaman et al., 2013, Zhao et al., 2017). Optimum temperature for plant growth and development is different from one species to the other; in some species, for example cotton, significant differences between cultivars have even been observed (Kakani et al., 2005a). Low temperature is particularly harmful to tropical and subtropical species which are sensitive to low temperature where it affects plant growth by inhibiting photosynthesis, protein synthesis and carbohydrate translocation, as well as decreasing respiration and enzyme activity and causing loss of membrane fluidity (Mangrich and Saltveit, 2000, Taiz and

Zeiger, 2002, Krasensky and Jonak, 2012). In low temperature, internal and external discoloration, low growth rate and abnormal fruit ripening can be observed (Mangrich and Saltveit, 2000). Minimum temperature for okra germination is 25°C (Grubben, 1977), but seeds can still germinate at 18°C (Jamala et al., 2011) and 15°C (Diizyaman, 1997). After 1 day at 2.5°C, radicle elongation is inhibited by 47% and an additional 30% on the second day (Mangrich and Saltveit, 2000).

2.2.2.1 High temperature stress

High temperature has a negative effect on crop yield and reduction of 1.5% per decade is predicted for global yield (Fedoroff et al., 2010, Lobell and Gourdji, 2012). During extreme climate events, crop yield can decrease very dramatically. For example, in Europe during the summer of 2003, higher average temperature of 3.5°C reduced grains and fruit yield by 20-36% (Battisti and Naylor, 2009). In tropical and subtropical areas, high temperature is a major limiting factor which causes yield reduction (Wahid et al., 2007). More than 3 billion people live in these areas, who not only need food, but also use agriculture as a source of income (Battisti and Naylor, 2009). In these climates, soil temperature exceeds 50°C for several hours which reduces seed emergence, as it slows or inhibits seed germination (Onwueme, 1975, Wahid et al., 2007). Morphological symptoms of heat damage on tropical plants that are exposed to high temperature and excess radiation include, sun burnt and scorched leaves and branches and increased leaf senescence and abscission. (Guilioni et al., 1997, Ismail and Hall, 1999, Vollenweider and Günthardt-Goerg, 2005). There are only a few plant species that can survive a steady temperature above 45°C (Taiz and Zeiger, 2002).

Physiologically, high temperature inhibits carbon assimilation and consequently alters carbohydrate content, which leads to a reduction in plant productivity (Gawronska et al., 1992, Lafta and Lorenzen, 1995, Maxwell and Johnson, 2000, Taiz and Zeiger, 2002, Zhang, 2012). For example, Hatfield et al. (2011) reported optimum temperature for vegetative growth in cotton to be 37ºC, whereas maximum photosynthesis was observed at temperatures between 30°C and 33°C (Wise et al., 2004, Bibi et al., 2008). However, high temperature of 45°C reduced photosynthetic rate by 22% (Wise et al., 2004) and efficiency of the open reaction centre in photosystem II (PSII) decreased at

36°C and above (Bibi et al., 2008). Heat alters membrane structure and increases membrane permeability (occurring mostly in leaves) which leads to leakage of ions, termed electrolyte leakage (EL), thereby, disrupting cellular activities (Leveitt, 1980). For example, thylakoid membranes, which is where the photosynthetic apparatus is located, become unstable due to instability of lipid-protein interaction, which leads to changes in photosynthetic activity (Mc Daniel, 1982, Pilon et al., 2016). In cotton, high temperature increased EL, whereas cotton adapted to high temperature could maintain membrane stability (Schrader et al., 2004). In addition, alteration of stomatal conductance (g_s) plays an important role in exchange of CO_2 and water between the leaf and atmosphere. In heat-sensitive plants, closure of stomata prevents water loss, but results in reduction of *A* and higher leaf temperatures due to less evaporative cooling under high temperature (Cui et al., 2006, Reece et al., 2011). Heat-tolerant plants may have higher g_s, lower leaf temperature and higher internal leaf CO_2 concentration and transpiration (*E*) (Camejo et al., 2005, Martinazzo et al., 2012, Simonin et al., 2014). Although, CO_2 supply is not limiting in these plants, *A* might be reduced due to modifications in PSII (e.g. changes to electron transport rate), which can be measured by fluorescence techniques (Camejo et al., 2005, Murchie and Lawson, 2013).

Further downstream, the reduction of *A* may lead to a decrease in carbohydrate reserves which alters plants growth, development and productivity (Zinn et al., 2010), so that plant biomass and yield reduce. Reduction in carbohydrate content can also be caused by modification of enzyme activities under high temperature. Sucrose synthesis enzymes (i.e. sucrose phosphate synthase, sucrose synthase) and sucrose hydrolysis enzymes (i.e. invertase), which are important enzymes in sugar production, are inhibited or activated under high temperature. For example, sucrose phosphate synthase activity increased in rose and potato (Rufty et al., 1985, Khayat and Zieslin, 1987, Lafta and Lorenzen, 1995) whereas it decreased in rice, chickpea, spinach and lentil (Holaday et al., 1992, Li et al., 2006, Kaushal et al., 2013, Bhandari et al., 2016) under high temperature. Additionally, high temperature alters movement of sugars to sink tissues, which include vegetative (e.g. roots) and reproductive (e.g. flowers and fruit) tissues, and fruit setting decreases while fruits which have been formed lose their sweetness and quality (Taiz and Zeiger, 2002, Wahid et al., 2007).

Despite physiological changes due to heat, alteration in reproductive tissues is the main reason in yield reduction. Infertility can be caused by insufficient nutrition in male and female tissues from early stages of bud development (Datta et al., 2001, Karni and Aloni, 2002, Pressman et al., 2002). If heat stress is applied before or during anthesis, sterility and pod or grain abortion follow as plant reproductive processes such as anther development, pollen production, pollen germination and pollen tube growth, ovule viability, stigma receptivity and fertilisation processes are affected (Charles and Harris, 1972, Herrero and Johnson, 1980, Burke et al., 2004, Singh et al., 2007, Wahid et al., 2007), which results in reduced fruit numbers. In cotton, a close relative to okra, *in vitro* pollen germination was highest between 28°C to 31°C, but it decreased when temperature reached 37°C. Pollen tube elongation was also reduced at temperatures above 32°C, resulting in yield reduction. Additionally, high temperature during flowering reduces boll retention and consequently decreases yield (Burke et al., 2004).

2.2.2.2 Effect of heat on okra

Okra is classified as slightly heat tolerant as its EL is moderate compared to other tropical summer crops (Leveitt, 1980). Optimum temperature for okra germination is 35°C (Diizyaman, 1997, Jamala et al., 2011), although heat treatment can increase the germination rate either by incubating seeds at 50°C for 2 days (Demir, 2001) or soaking the seeds in 45°C water for 1-5 h before sowing for rapid field emergence (Onwueme, 1975). Exposure to 45°C immediately after sowing causes more rapid emergence and hastens plant growth but delays flowering (Dhankhar and Singh, 2013). More specifically, on day 0 and 1 of sowing, temperatures of 45°C for 10 hours promotes elongation of hypocotyl but the same treatment on the second day retards it (Onwueme, 1975). The same study showed that if the temperature is 50°C on the sowing day, there is no emergence and this temperature probably kills the seed (Onwueme, 1975). The highest numbers of flower, highest pod weight and rapid growth in pods and diameter have been observed at 34°C in Sri Lanka while plants received adequate water (Gunawardhana and De Silva, 2012). High temperature at night helps increase plant height, whereas high temperature during the day causes delay in floral development (Dhankhar and Singh, 2009) but increase in carbohydrate (Arulrajah and Ormrod, 1973) and fibre content

(Gunawardhana and De Silva, 2012). Increasing plant height is due to elongation of internodes if there is no water stress (Gunawardhana and De Silva, 2012). Additionally, the position of the first flowering node is greater when the plant is exposed to high temperature. Some cultivars show slower bud development and delay in flowering (flower opening) whereas others show no bud development and growth (Arulrajah and Ormrod, 1973). Okra raised under short days are sensitive to a long photoperiod and lose their ability to produce buds in high temperature; if they do produce buds, there is a delay in bud initiation and the buds cannot develop fully, and therefore, they dry out and are dropped, resulting in yield reduction (Figure 2.6) (Arulrajah and Ormrod, 1973).

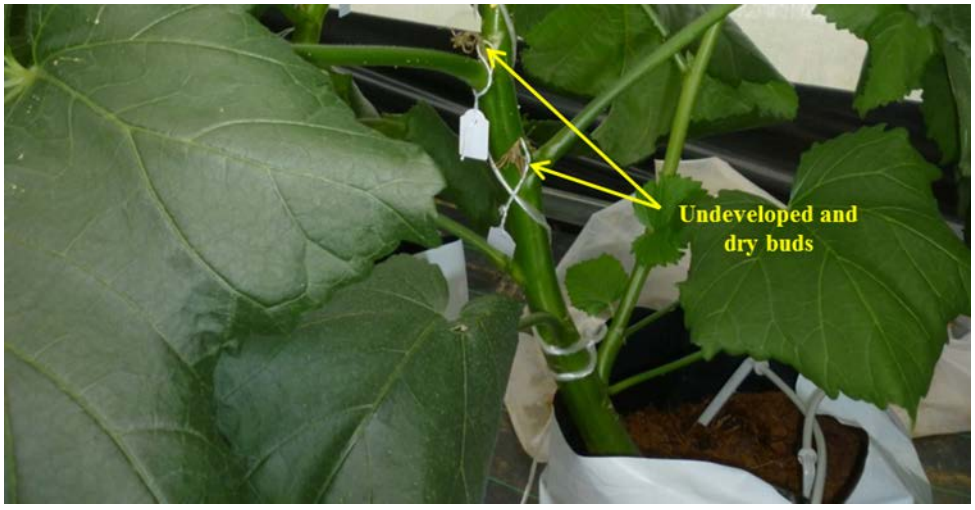

Figure 2.6 Okra dried immature buds which were damaged by high temperature (photo credit: S. Hayamanesh)

Many studies have been carried out to assess the effect of high temperature on the morphology of okra. However, adverse effect of heat on its physiology and metabolism are not fully understood. As global warming becomes a serious issue, development of high temperature tolerant plants with maximum yield for heat prone environments is important. To achieve this, identifying tolerant cultivars and breeding for heat tolerant varieties are two strategies for reducing the associated loss in yield. More detailed physiology and metabolic studies are needed to examine the degree of damage in different stages of plant growth and development and understanding the adaptation mechanisms under heat stress in okra.

3 Physiological screening tools to identify high temperature tolerance in okra (*Abelmoschus esculentus* (L.) Moench)

3.1 Introduction

Plants are immobile organisms and this increases their susceptibility to environmental stresses. However, they have different responses and mechanisms to either avoid or tolerate stress. Avoidance is generally related to morphology and phenology; for example changing leaf orientation to avoid high incident radiation, open stomata to increase water loss and evaporative cooling that reduce leaf temperature, or alteration of membrane lipid composition. In contrast, tolerance allows the plant to not only survive but be economically productive under stress. Mechanisms of tolerance include osmo-protectants, antioxidant defence compounds and regulation of transcription (Kuo et al., 1992, Vinocur and Altman, 2005, Cui et al., 2006, Ribeiro et al., 2012, Hasanuzzaman et al., 2013, Murchie and Lawson, 2013).

High temperature exposure (either short- or long-term) has a negative impact on plant growth and development. Different plant species and cultivars within species need different temperatures to optimize their productivity (Kakani et al., 2005b); however even just a few minutes exposure to temperatures above these optimums can disrupt plant function (Taiz and Zeiger, 2002, Hasanuzzaman et al., 2013). Such short-term exposure to heat is termed heat-shock and is defined as a sudden increase in temperature over a short period of time (Faralli et al., 2015). Plant physiological studies help understand plant function and plant adaptation to environmental changes. Heat shock between 2 minutes and 2 hours was shown to reduce photosynthesis, chlorophyll fluorescence and stomatal conductance in Arabidopsis, peach, maize and cucumber (Sinsawat et al., 2004, Zhang and Sharkey, 2009, Martinazzo et al., 2012, Ding et al., 2016).

Photosynthesis is conversion of light energy to chemical energy for plant growth, productivity and survival. Under high temperature, photosynthesis is inhibited before other functions (Camejo et al., 2005, Chen and Cheng, 2009) and this inhibition results mostly from alteration in photosystem II (PSII) which is responsible for light energy

capture, oxygen (O_2) generation and the provision of electrons to the electron transport chain to maintain photosynthesis (Lambers et al., 1998). Plants capture light through chlorophyll molecules, which are located in the light harvesting complex of PSII in the thylakoid membrane. Light energy is passed to a special chlorophyll molecule in the reaction centre (P680 chlorophyll a molecule), which excites electrons to a higher energy state. These electrons are passed to the electron transport chain and P680 returns to a non-excited state. In this process, light energy can be used for photochemistry, emitted as heat or emitted as lower-energy light known as chlorophyll fluorescence.

Chlorophyll fluorescence measurement is a common technique that allows the evaluation of photosynthesis in plants. This technique is especially useful for assessing the activity of PSII (where fluorescence mostly occurs) and the electron transport chain in intact leaves in response to stresses (Yamane et al., 2000, Strasser et al., 2004, Chen and Cheng, 2009, Misra et al., 2012, Murchie and Lawson, 2013). Chlorophyll fluorescence is measured as the ratio of variable to maximum fluorescence in the dark (F_v/F_m) or the light (F_v'/F_m') where F_v' is the difference between the maximum (F_m') and the initial (F_o') fluorescence (Murchie and Lawson, 2013). Under high temperature, reduction of this ratio results from an increase in F_o and decrease in F_m (Yamane et al., 1997, Cui et al., 2006, Martinazzo et al., 2012). The other fluorescence parameter that is used under light conditions and suitable for field investigation is the efficiency of the open reaction centre (Φ_{PSII}). Φ_{PSII} shows the proportion of PSII reaction centres that are active for photochemical processes and it is measured as $(F_m'- F_s)/F_m'$ where F_s is the steady state of fluorescence under actinic light (Maxwell and Johnson, 2000, Roháček et al., 2008). Under high temperature, Φ_{PSII} reduces due to decrease in F_m'. The reduction of these two fluorescence parameters under high temperature indicates structural and functional damage to PSII; more specifically, damage to an important portion of the reaction centre (Camejo et al., 2005, Cui et al., 2006). These structural and functional alterations lead to reduction in photosynthesis (A) and subsequent reduction in plant growth and development.

Another process that lowers photosynthetic rates under heat is the regulation of stomatal conductance (g_s). H_2O and CO_2 diffuse in and out of the leaf through stomata

and the rate of water exchange is estimated by g_s. Under high temperature, g_s decreases as plants close their stomata to prevent water loss, resulting in limited CO_2 availability to the Calvin cycle (Lu et al., 1994, Cui et al., 2006, Reece et al., 2011, Martinazzo et al., 2012). As leaf temperature increases with closed stomata, well-watered plants may increase their g_s to increase evaporative cooling and lower the leaf temperature, resulting in elevated internal CO_2 concentration (Camejo et al., 2005, Martinazzo et al., 2012).

In addition to the damage to PSII and changes in g_s, heat can have a detrimental effect on cell membranes, specifically the thylakoid membranes where the photosynthetic apparatus is located. Cell membranes are made of a phospholipid bilayer, the structure of which becomes unstable and causes instability of the lipid-protein interaction at high temperature, leading to an increase in membrane permeability, loss of electrolytes and disturbance to PSII activity (Mc Daniel, 1982, Pilon et al., 2016).

Sustained high growing temperature has been found to increase the heat tolerance of maize and potato (Havaux, 1993, Sinsawat et al., 2004). Havaux and Gruszecki (1993) and Havaux and Tardy (1996) reported that acclimation in potato was a result of accumulation of zeaxanthin in the leaves which increased membrane stability. Growth at high temperature and induced resistance to heat stress can also be a result of PSII protection from photo-inhibition by chloroplast small heat-shock proteins (Downs et al., 1999).

Okra is a summer vegetable crop which is well adapted to elevated temperatures and has been classified as generally heat tolerant (Leveitt, 1980) with optimal growth and productivity at 34°C (Gunawardhana and De Silva, 2012). Nevertheless, high temperature can have a negative effect on okra development and productivity, including delayed floral development and flowering, elongation of internodes and yield loss (Arulrajah and Ormrod, 1973, Diizyaman, 1997, Dhankhar and Singh, 2009, Jamala et al., 2011, Gunawardhana and De Silva, 2012, Dhankhar and Singh, 2013). Cotton, a close relative of okra, also showed a decline in photosynthesis and increase in membrane permeability under heat stress (UR Rahman et al., 2004, Bibi et al., 2008, Azhar et al., 2009, Abro et al., 2015).

F_v/F_m, F_v'/F_m', g_s, A and EL have been recognized as useful traits for the assessment of physiological heat damage to plants and they have frequently been used for screening (Camejo et al., 2005, Cottee et al., 2012, Abro et al., 2015, Ding et al., 2016). These physiological traits also have an advantage as screening tools over phenotypic parameters such as yield, as the latter are laborious and expensive to use on a large number of lines. Hence, the aim of the current study was to:

- Screen a large set of okra germplasm (119 genotypes) for variation in heat tolerance.
- Identify a subset of contrasting lines for subsequent more in depth examinations to identify the mechanistic basis of heat tolerance.
- Use F_v/F_m, F_v'/F_m', g_s, A and EL parameters to evaluate the effect of a short period of heat shock in a controlled environment
- Assess possible acclimation in okra by evaluating the physiological effect of short-term increases in temperatures on heat-adapted and non-adapted okra plants grown in growth cabinets.

3.2 Material and Methods

3.2.1 Plant material

Okra seed from the Vegetable Research Institute (VRI) and The World Vegetable Center, previously known as the Asian Vegetable Research and Development Center (AVRDC) were provided (Appendix 1). Seeds were sown in 90 mm-diameter pots filled with 8 mm composted pine bark, 3 mm composted pine bark and sand mixture (8:1:1) and supplemented with trace elements at 0.4 kg m^{-3} including gypsum (1 kg m^{-3}), superphosphate (1 kg m^{-3}), KNO_3 (13% N) (0.25 kg m^{-3}), nitroform (38% N)(0.25 kg m^{-3}) and magrilime (1.5 kg m^{-3}). Plants were kept in a glasshouse with natural light for 4 weeks at the Plant Breeding Institute (PBI), University of Sydney, NSW, Australia, with day/night temperatures of 30°C/25°C. Plants were transplanted into 4 L pots in the third week after sowing.

Genotypes were labelled by their list number and genotype number (e.g. the first genotype in Appendix 1 was labelled L2-1) as not all genotypes had a name or VI number (i.e. the number provided by AVRDC).

3.2.2 Physiological assessments

3.2.2.1 Photosynthetic parameters

$F_v/$, F_v'/F_m', F_m, F_o,qP, Φ_{PSII}, A and g_s were measured on the most recent, top mature leaf in both the control and heat treatments using a Licor 6400xt fitted with a fluorescence light source (LI-COR, Lincoln, NE, USA). Reference CO_2 was set to 400 µmol mol^{-1}, photosynthetically active radiation (PAR) to 1300 µmol m^{-2} s^{-1} and flow rate to 300 mL min^{-1}. Fluorescence parameters were assessed in the light (F_v'/F_m') and again in the dark (F_v/F_m) after dark adaptation of all plants for 1h at 30°C. Due to the large number of genotypes (119), stomatal conductance was initially (4h heat shock) measured using a porometer (SC-1, Decagon Devices, Pullman, WA, USA) which was calibrated in Auto Mode, following the manufacturer's calibration procedure.

3.2.2.2 Electrolyte leakage

Electrolyte leakage was measured on the same leaf from which photosynthetic measurements were taken using the conductivity method described by Sullivan (1972), Lafuente et al. (1991) and Camejo et al. (2005). Eight 7 mm diameter leaf disks were cut using a cork borer and were placed in 50 mL Greiner centrifuge tubes (Sigma Aldrich, Australia) with 20 mL of double deionised water and maintained on a shaker at 80 rpm for 20 hours at room temperature. The conductivity of the solution was read with a conductivity meter (Edge, Hanna Instruments Inc. HI11310 single ceramic, double junction, and refillable pH electrode with temperature sensor, UK) before the samples were autoclaved for 15 min at 121°C to burst the cells. Conductivity was remeasured after autoclaving. Electrolyte leakage was expressed as the ratio of the conductivities in percentage as follows:

% electrolyte = (T1/T2)*100, where T1 and T2 are the conductivities before and after autoclaving the tissues, respectively.

3.2.3 Four-hour heat shock (45˚C): initial screening of 119 genotypes (1 replicate)

Due to the large number of genotypes (119), one plant per genotype was considered for the initial screen. After 4 weeks (in the glass house), plants were moved into a controlled environment facility (CEF) with a 12h day-night cycle set at 30°C during the day and 22°C at night, and 70% RH for a week. Light and heat were provided by 6 high-pressure sodium lights in the chamber and plants were watered regularly to avoid moisture stress. For the high temperature treatment, plants were subjected to a short increase in temperature for a few hours (heat shock) where temperature was increased to 45˚C for 4 hours before measuring the physiological responses at 30°C. After heat shock, temperature was reduced to 30°C for an hour before measurement. The plants were measured for F_v/F_m, F_v'/F_m' (using a LI-COR 6400) and g_s (using a porometer) in the control prior to the heat shock being applied.

3.2.4 Six-hour heat shock (45˚C): advanced screening of 33 genotypes (3 replicates)

Thirty three okra genotypes selected from the initial screening were moved from the glass house to the CEF (4 weeks after sowing) and arranged in 3 replications (completely randomized design) under the same conditions as the initial screen. Since the fluorescence measurement did not show clear differences between control and heat treatments in the initial screening, the duration of the heat shock was extended to 6 hours at 45˚C before taking the measurements at 30°C. After heat shock, temperature was reduced to 30°C for an hour before measurement. The same plants were measured for F_v/F_m, F_v'/F_m', A, Φ_{PSII} and g_s using a LI-COR 6400 and EL using conductivity meter in the control prior to the application of heat shock.

3.2.5 Acclimation of okra genotypes under gradual increase in temperature

The okra genotypes Rama Krishna (L2-30) and Choitali (L3-1) were transferred (6 weeks after sowing) from the glass house into a growth cabinet (Premium Plant Growth Chambers, Climatron, Model: TPG-1260-TH-Co2, Thermoline Scientific, Australia) and kept at 30°C during the day and 25°C during the night with a 12h day-night cycle for two weeks. Three replicates of each genotype were assessed in the growth chamber. F_v'/F_m', A, Φ_{PSII} and g_s (using a LI-COR 6400) were measured at 33, 36, 39, 42 and 45°C. Both the cabinet and the block temperature of the Licor were set to the different temperatures. Plants were left to adjust to changes in temperature between 30 to 60 min prior to measurement. F_v/F_m (overnight) was measured prior to the temperature increase at 30 °C and again at 45°C after a dark-adaptation period of 30 min and finally after reducing the temperature to 30°C. The plants were then exposed to day/night temperatures of 42°C/25°C for two weeks and all parameters reassessed.

3.2.6 Statistical analysis

Data were subjected to a one- and two-sample t-test with the level of significance at $P<0.05$ and a general analysis of variance (ANOVA) with the level of significance at $P<0.05$ and a comparison of means was performed using Fisher's unprotected LSD using GenStat 17th Edition software (VSN International Ltd, London, UK).

3.3 Results

3.3.1 Four-hour heat shock (45˚C): initial screening of 119 genotypes (1 replicate)

The difference between F_v'/F_m' in control and heat was significant ($p<0.001$). Under high temperature, F_v'/F_m' increased relative to the control in 15% of the genotypes, decreased in 45% of the genotypes and remained similar in 40% of genotypes (Appendix 2). In both control and heat treatments, chlorophyll fluorescence was close to 0.8 in the dark and between 0.7 and 0.75 in the light. The difference between g_s in control

and heat was significant ($p<0.001$). g_s varied strongly among genotypes in each treatment and 63% of genotypes had lower g_s in the heat treatment compared to the control, 23% had higher g_s and 14% were similar to the control.

Thirty-three genotypes were then selected based on contrasting responses in chlorophyll fluorescence and stomatal conductance. These genotypes fell within seven groups with the different responses to heat stress listed below:

1. 42.4% with low F_v'/F_m', low g_s
2. 9.1% with low F_v'/F_m', high g_s
3. 6.1% with low F_v'/F_m', same g_s
4. 6.1% with high F_v'/F_m', high g_s
5. 21.2% with same F_v'/F_m', low g_s
6. 12.1% with same F_v'/F_m', high g_s
7. 3% with same F_v'/F_m', same g_s

The genotypes chosen with the physiological screening were tested with molecular markers (chapter 4) to ensure that broad genetic variation was maintained among the selected genotypes.

3.3.2 Six-hour heat shock (45°C): advanced screening of 33 genotypes (3 replicates)

F_v/F_m, F_v'/F_m', A, Φ_{PSII}, g_s and EL of 33 genotypes were measured in the control and heat treatments. No photosynthetic data was recorded for L2-32, L3-1, L3-58 and L3-59; however, EL was assessed on all 33 genotypes. Significant Genotype*Treatment interaction indicated that genotypes reacted differently to temperature (Table 3.1).

Genotypes varied significantly for all measured parameters (Table 3.1). The 6-hour heat treatment also had a significant effect on all traits except electrolyte leakage ($p=0.585$); however, the Genotype*Treatment interaction was significant for all traits including electrolyte leakage.

Table 3.1 ANOVA for chlorophyll fluorescence (in the dark F_v/F_m and in the light F_v'/F_m'), photosynthesis rate (A), efficiency of the open reaction center (Φ_{PSII}), stomatal conductance (g_s) and electrolyte leakage (EL) of 29 genotypes (except for EL which were 33 genotypes) in the control and heat (6h, 45°C).

		F_v/F_m	F_v'/F_m'	A	Φ_{PSII}	g_s	EL
Genotype	F	2.14	6.23	6.41	3.09	9.94	4.34
	P	0.003	<.001	<.001	<.001	<.001	<.001
Treatment	F	61.27	27.68	11.28	16.4	29.61	0.3
	P	<.001	<.001	0.001	<.001	<.001	0.585
Genotype* Treatment	F	2.27	1.8	4.88	2.99	5.29	1.62
	P	0.001	0.017	<.001	<.001	<.001	0.031

The change in F_v/F_m and F_v'/F_m' due to the heat treatment depended on the genotype, with values decreasing, increasing or remaining the same. However, most genotypes showed lower F_v/F_m and F_v'/F_m' under high temperature, especially for F_v'/F_m' (Table 3.2). Genotype L4-13 showed similar F_v/F_m and F_v'/F_m' in the control and heat treatments whereas L3-47, L4-6, L4-14, L4-24 and L4-26 had higher F_v'/F_m' under heat.

Most genotypes had lower g_s in response to the heat (Table 3.3). Eight genotypes (L2-11, L2-12, L3-36, L4-6, L4-13, L4-18, L4-24 and L4-26) increased g_s in the heat and six genotypes (L2-14, L2-27, L2-28, L3-31, L4-14 and L4-48) maintained similar g_s in both treatments. As a result of higher g_s, A was significantly higher in those genotypes exposed to heat, with the exception of L2-11 and L4-6 which showed similar A in both treatments and L3-36 which had lower A in the heat treatment. Genotypes with similar g_s in both treatments showed lower A except for the genotypes L2-28 and L4-14 which had slightly higher A in the heat treatment. Genotypes with lower g_s generally showed lower A except for genotypes L2-22, L2-30 and L4-42 which showed slightly higher A in the heat treatment (Table 3.3). Overall A and g_s positively correlated under heat shock ($R = 0.624$, $p<0.001$). Φ_{PSII} followed the same pattern as photosynthesis.

The six-hour heat shock had a significant effect on EL Genotype*Treatment interaction (Table 3.1), however, there were only 5 genotypes with significant difference between treatments, indicating that the cell membranes of the other 28 genotypes were undamaged by high temperature.

Table 3.2 Average chlorophyll fluorescence (in the dark, F_v/F_m and in the light F_v'/F_m') and efficiency of the open reaction centre (Φ_{PSII}) of 33 okra genotypes (n=3) in the control and heat (6h, 45°C), measured by LI-COR. Values are presented with ±SE and least significant differences (LSD) of means for Genotype*Treatment interaction, when the interaction was significant at p<0.05. Missing data left blank.

	Chlorophyll Fluorescence				**Φ_{PSII}**	
Genotype	**Control Dark**	**Heat Dark**	**Control Light**	**Heat Light**	**Control**	**Heat**
	F_v/F_m	**F_v/F_m**	**F_v'/F_m'**	**F_v'/F_m'**		
L2-11	0.81 ± 0.003	0.80 ± 0.002	0.51 ± 0.007	0.47 ± 0.003	0.11 ± 0.003	0.11 ± 0.013
L2-12	0.82 ± 0.002	0.81 ± 0.003	0.52 ± 0.009	0.51 ± 0.019	0.14 ± 0.012	0.17 ± 0.014
L2-14	0.81 ± 0.003	0.79 ± 0.009	0.53 ± 0.003	0.53 ± 0.002	0.12 ± 0.011	0.13 ± 0.019
L2-15	0.81 ± 0.001	0.77 ± 0.007	0.52 ± 0.006	0.46 ± 0.008	0.22 ± 0.009	0.08 ± 0.003
L2-22	0.81 ± 0.005	0.79 ± 0.008	0.51 ± 0.011	0.49 ± 0.025	0.14 ± 0.031	0.13 ± 0.028
L2-23	0.82 ± 0.002	0.81 ± 0.001	0.53 ± 0.021	0.51 ± 0.009	0.19 ± 0.029	0.16 ± 0.005
L2-24	0.81 ± 0.002	0.80 ± 0.006	0.54 ± 0.004	0.51 ± 0.003	0.18 ± 0.018	0.14 ± 0.032
L2-27	0.81 ± 0.002	0.80 ± 0.001	0.52 ± 0.014	0.50 ± 0.009	0.11 ± 0.016	0.11 ± 0.004
L2-28	0.82 ± 0.003	0.79 ± 0.004	0.46 ± 0.008	0.44 ± 0.013	0.09 ± 0.001	0.09 ± 0.006
L2-30	0.81 ± 0.002	0.78 ± 0.012	0.51 ± 0.004	0.49 ± 0.030	0.18 ± 0.006	0.17 ± 0.020
L2-32						
L3-1						
L3-31	0.81 ± 0.003	0.80 ± 0.002	0.52 ± 0.007	0.52 ± 0.013	0.17 ± 0.031	0.15 ± 0.019
L3-36	0.80 ± 0.003	0.77 ± 0.009	0.49 ± 0.015	0.48 ± 0.028	0.12 ± 0.020	0.10 ± 0.023
L3-47	0.80 ± 0.007	0.79 ± 0.002	0.49 ± 0.017	0.50 ± 0.009	0.18 ± 0.003	0.08 ± 0.000
L3-51	0.80 ± 0.004	0.79 ± 0.006	0.47 ± 0.011	0.44 ± 0.003	0.13 ± 0.010	0.10 ± 0.007
L3-54	0.81 ± 0.004	0.79 ± 0.000	0.49 ± 0.001	0.46 ± 0.008	0.15 ± 0.011	0.11 ± 0.002
L3-58						
L3-59						
L4-6	0.81 ± 0.003	0.81 ± 0.002	0.50 ± 0.011	0.52 ± 0.009	0.13 ± 0.017	0.14 ± 0.009
L4-13	0.81 ± 0.004	0.81 ± 0.002	0.52 ± 0.001	0.52 ± 0.006	0.10 ± 0.001	0.14 ± 0.009
L4-14	0.80 ± 0.003	0.80 ± 0.008	0.50 ± 0.012	0.51 ± 0.004	0.13 ± 0.016	0.11 ± 0.014
L4-18	0.81 ± 0.001	0.80 ± 0.003	0.52 ± 0.007	0.50 ± 0.003	0.13 ± 0.011	0.13 ± 0.005
L4-19	0.80 ± 0.010	0.79 ± 0.009	0.52 ± 0.004	0.46 ± 0.019	0.13 ± 0.013	0.10 ± 0.015
L4-22	0.80 ± 0.003	0.79 ± 0.002	0.50 ± 0.020	0.47 ± 0.007	0.12 ± 0.017	0.11 ± 0.004
L4-23	0.80 ± 0.002	0.80 ± 0.001	0.51 ± 0.019	0.49 ± 0.008	0.11 ± 0.008	0.10 ± 0.004
L4-24	0.81 ± 0.003	0.80 ± 0.000	0.50 ± 0.002	0.52 ± 0.008	0.10 ± 0.012	0.10 ± 0.013
L4-26	0.81 ± 0.004	0.80 ± 0.002	0.51 ± 0.002	0.53 ± 0.008	0.09 ± 0.005	0.12 ± 0.024
L4-29	0.81 ± 0.003	0.76 ± 0.008	0.50 ± 0.019	0.49 ± 0.014	0.13 ± 0.014	0.10 ± 0.013
L4-31	0.82 ± 0.002	0.81 ± 0.004	0.48 ± 0.009	0.47 ± 0.018	0.16 ± 0.014	0.15 ± 0.010
L4-37	0.80 ± 0.012	0.79 ± 0.009	0.51 ± 0.008	0.49 ± 0.001	0.18 ± 0.009	0.080 ± 0.02
L4-42	0.81 ± 0.004	0.79 ± 0.009	0.52 ± 0.000	0.48 ± 0.009	0.10 ± 0.019	0.08 ± 0.025
L4-48	0.81 ± 0.004	0.78 ± 0.018	0.47 ± 0.011	0.46 ± 0.020	0.15 ± 0.006	0.14 ± 0.008
LSD	0.021		0.034		0.049	

Table 3.3 Average photosynthesis (*A*), stomatal conductance (g_s) and electrolyte leakage (EL) of 33 genotypes (n=3) in the control and heat (6h, 45°C) measured by LI-COR and conductivity meter. Values are presented with ±SE and least significant differences (LSD) of means for Genotype*Treatment interaction, when the interaction was significant at $p < 0.05$. Missing data left blank.

Genotype	*A* (µmol m^{-2} s^{-1})		g_s (mol m^{-2} s^{-1})		EL (%)	
	Control	Heat	Control	Heat	Control	Heat
L2-11	6.84 ± 0.53	6.17 ± 1.60	0.03 ± 0.001	0.06 ± 0.012	52 ± 5.3	36 ± 1.7
L2-12	13.23 ± 0.4	15.39 ± 2.20	0.06 ± 0.019	0.19 ± 0.054	34 ± 1.2	34 ± 0.9
L2-14	13.50 ± 0.23	8.26 ± 1.36	0.10 ± 0.018	0.11 ± 0.022	37 ± 2.3	33 ± 0.9
L2-15	20.50 ± 1.70		0.12 ± 0.024	0.01 ± 0.001	35 ± 0.8	31 ± 0.6
L2-22	12.91 ± 0.84	14.02 ± 0.40	0.19 ± 0.055	0.06 ± 0.030	36 ± 1.3	36 ± 0.6
L2-23	20.12 ± 1.23	11.39 ± 0.94	0.12 ± 0.037	0.05 ± 0.006	35 ± 3.4	24 ± 1.0
L2-24	21.16 ± 0.26	13.85 ± 0.57	0.17 ± 0.047	0.07 ± 0.027	35 ± 0.6	29 ± 4.6
L2-27	10.50 ± 1.41	6.85 ± 0.78	0.04 ± 0.015	0.03 ± 0.007	36 ± 2.1	37 ± 1.0
L2-28	1.01 ± 0.16	6.29 ± 0.72	0.02 ± 0.002	0.03 ± 0.005	33 ± 1.8	32 ± 1.5
L2-30	17.64 ± 0.52	17.87 ± 2.39	0.21 ± 0.035	0.13 ± 0.024	37 ± 2.0	39 ± 3.6
L2-32					42 ± 1.9	36 ± 0.0
L3-1					45 ± 3.0	41 ± 1.4
L3-31	20.22 ± 0.90	13.25 ± 0.76	0.10 ± 0.029	0.09 ± 0.027	36 ± 2.0	34 ± 2.3
L3-36	13.44 ± 3.70	10.09 ± 0.29	0.09 ± 0.030	0.14 ± 0.006	38 ± 0.7	40 ± 2.7
L3-47	18.04 ± 0.77	14.88 ± 1.24	0.19 ± 0.040	0.04 ± 0.011	30 ± 0.9	32 ± 1.8
L3-51	13.91 ± 0.28	10.64 ± 0.38	0.22 ± 0.044	0.14 ± 0.011	36 ± 1.6	35 ± 2.0
L3-54	16.26 ± 0.97	11.91 ± 0.24	0.24 ± 0.013	0.20 ± 0.013	35 ± 3.8	32 ± 2.6
L3-58					44 ± 1.5	39 ± 1.0
L3-59					41 ± 1.3	34 ± 3.9
L4-6	12.36 ± 3.01	13.32 ± 2.33	0.06 ± 0.016	0.11 ± 0.022	37 ± 0.7	36 ± 1.9
L4-13	5.25 ± 0.33	13.17 ± 1.08	0.03 ± 0.002	0.12 ± 0.023	44 ± 1.3	38 ± 1.2
L4-14	11.16 ± 0.48	14.19 ± 0.66	0.05 ± 0.006	0.06 ± 0.016	36 ± 0.5	36 ± 1.3
L4-18	9.78 ± 0.40	15.16 ± 0.20	0.05 ± 0.006	0.07 ± 0.002	43 ± 1.2	42 ± 2.9
L4-19	12.40 ± 0.02	3.11 ± 1.97	0.09 ± 0.028	0.02 ± 0.009	39 ± 1.6	44 ± 2.0
L4-22	12.99 ± 2.02	5.96 ± 0.48	0.16 ± 0.032	0.04 ± 0.008	36 ± 1.7	39 ± 1.9
L4-23	10.13 ± 0.57	5.43 ± 2.50	0.10 ± 0.016	0.04 ± 0.014	36 ± 2.1	39 ± 1.9
L4-24	5.11 ± 0.63	13.52 ± 2.49	0.05 ± 0.002	0.07 ± 0.028	50 ± 6.9	44 ± 1.5
L4-26	4.06 ± 0.15	13.83 ± 0.17	0.04 ± 0.007	0.07 ± 0.019	49 ± 5.2	49 ± 1.1
L4-29	13.31 ± 1.37	4.32 ± 0.50	0.12 ± 0.028	0.05 ± 0.015	37 ± 0.9	41 ± 2.4
L4-31	16.85 ± 0.82	15.64 ± 1.00	0.26 ± 0.012	0.18 ± 0.051	39 ± 0.6	41 ± 1.1
L4-37	18.19 ± 0.84	9.14 ± 0.65	0.25 ± 0.008	0.04 ± 0.002	33 ± 2.3	32 ± 2.0
L4-42	5.80 ± 2.27	7.69 ± 0.00	0.05 ± 0.017	0.02 ± 0.013	50 ± 8.1	43 ± 0.3
L4-48	15.59 ± 0.75	14.93 ± 0.86	0.12 ± 0.010	0.12 ± 0.010	44 ± 0.7	38 ± 1.0
LSD	5.73		0.066		13.95	

3.3.3 Acclimation of okra genotypes under gradual increase in temperature

In general, heat-adapted plants of both genotypes showed significantly higher F_v'/F_m', g_s, A and Φ_{PSII} than non-adapted plants at all temperatures (Figure 3.1). However, genotype responses did vary. The F_v'/F_m' of heat adapted L2-30 stayed constant with increasing temperature while L3-1 stayed constant, but declined at 45 degrees (p=0.003). In non-adapted plants, F_v'/F_m' tended to decline more steeply. L2-30 also showed significantly lower F_v'/F_m' at 45°C (p=0.018) compared to lower temperatures, whereas the changes in L3-1 were not significant, mainly due to large variation among replicates. The g_s of adapted plants of both genotypes increased from 39°C whereas it remained the same in non-adapted plants. However, in adapted plants this increase was not significant in L2-30 but, it was significant in L3-1 at 39°C (p=0.043). Φ_{PSII} was constant in both adapted and non-adapted plants of both genotypes (with lower values for L3-1). Despite similar Φ_{PSII} and a constant or increased g_s observed with increasing temperature, A decreased in both adapted (not significant) and non-adapted (significant) plants. This decrease was less noticeable in heat-adapted plants; A decreased by 51.6% (L2-30) and 56.8% (L3-1) in non-adapted, and by 22.3% (L2-30) and 22.4% (L3-1) in heat-adapted plants between the lowest and highest temperatures.

Adapted and non-adapted plants of both genotypes showed lower F_v/F_m (after 30 min in the dark) at 45°C (not significant in adapted L3-1 and significant in the others) compared to the initial measurement at 30°C (prior to switching the light on), and did not recover after returning the temperature to 30°C and exposure to dark-adaptation for 1-2 hours (Figure 3.2). The reduction of F_v/F_m resulted from an increase in F_o and decrease in F_m. The F_o of non-adapted plants was similar in both genotypes (slightly lower for L2-30) at each temperatures whereas in adapted plants, L3-1 showed higher F_o (on average 19%) compare to L2-30 at each temperature. The F_m of non-adapted plants was similar in both genotypes (slightly lower for L3-1) at each temperature whereas L3-1 showed higher F_m (in average 13%) compared to L2-30 at each temperature following adaptation. Therefore, the higher F_o and F_m observed in the adapted L3-1 compared to the adapted L2-30 produced a similar F_v/F_m in both genotypes at each temperature.

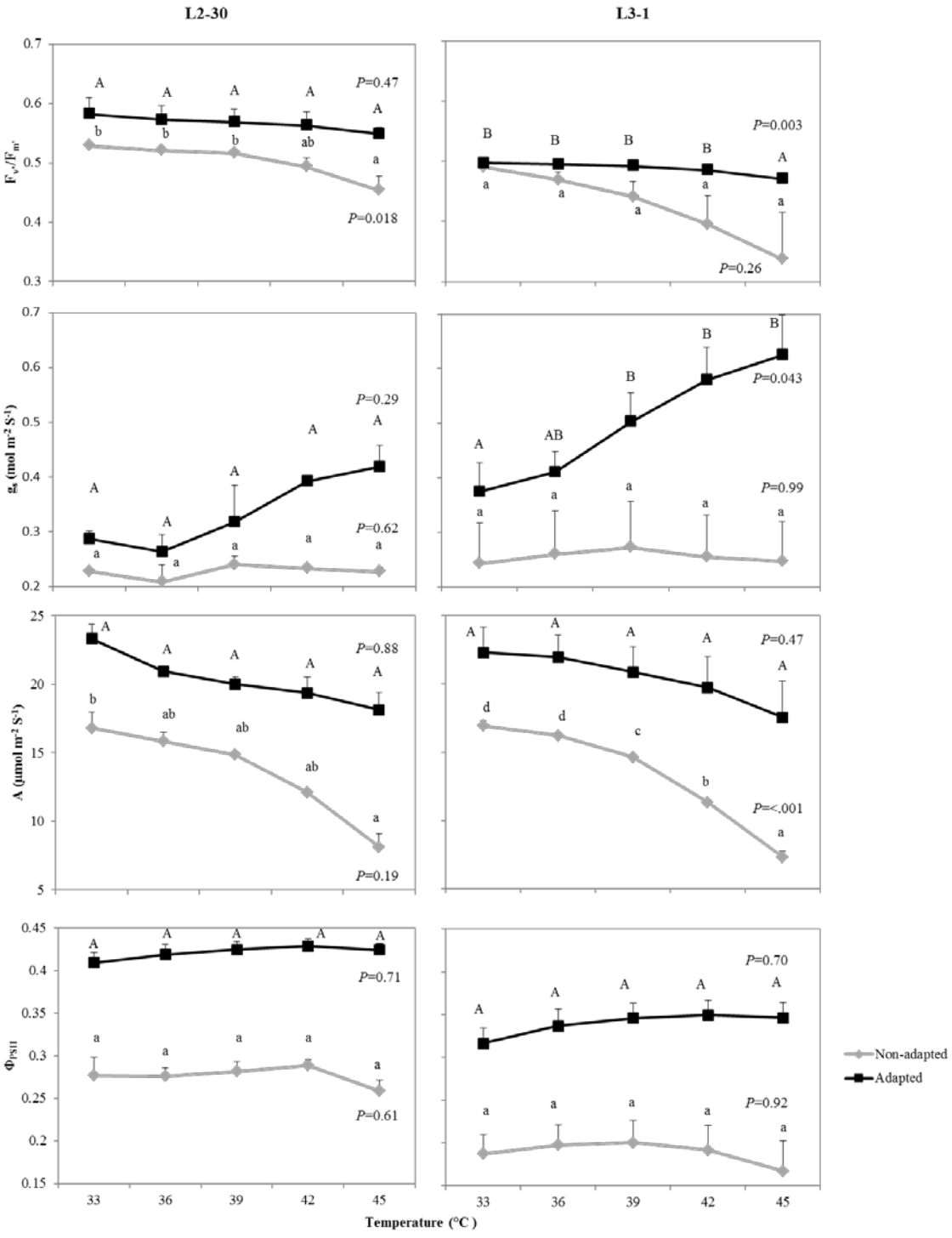

Figure 3.1 Average chlorophyll fluorescence (F_v'/F_m'), stomatal conductance (g_s, mol m^{-2} s^{-1}), photosynthesis (A, µmol m^{-2} s^{-1}) and efficiency of the open reaction center (Φ_{PSII}) in heat adapted and non-adapted genotypes L2-30 and L3-1 (n=3) at different temperatures. Bars represent standard error (+SE).

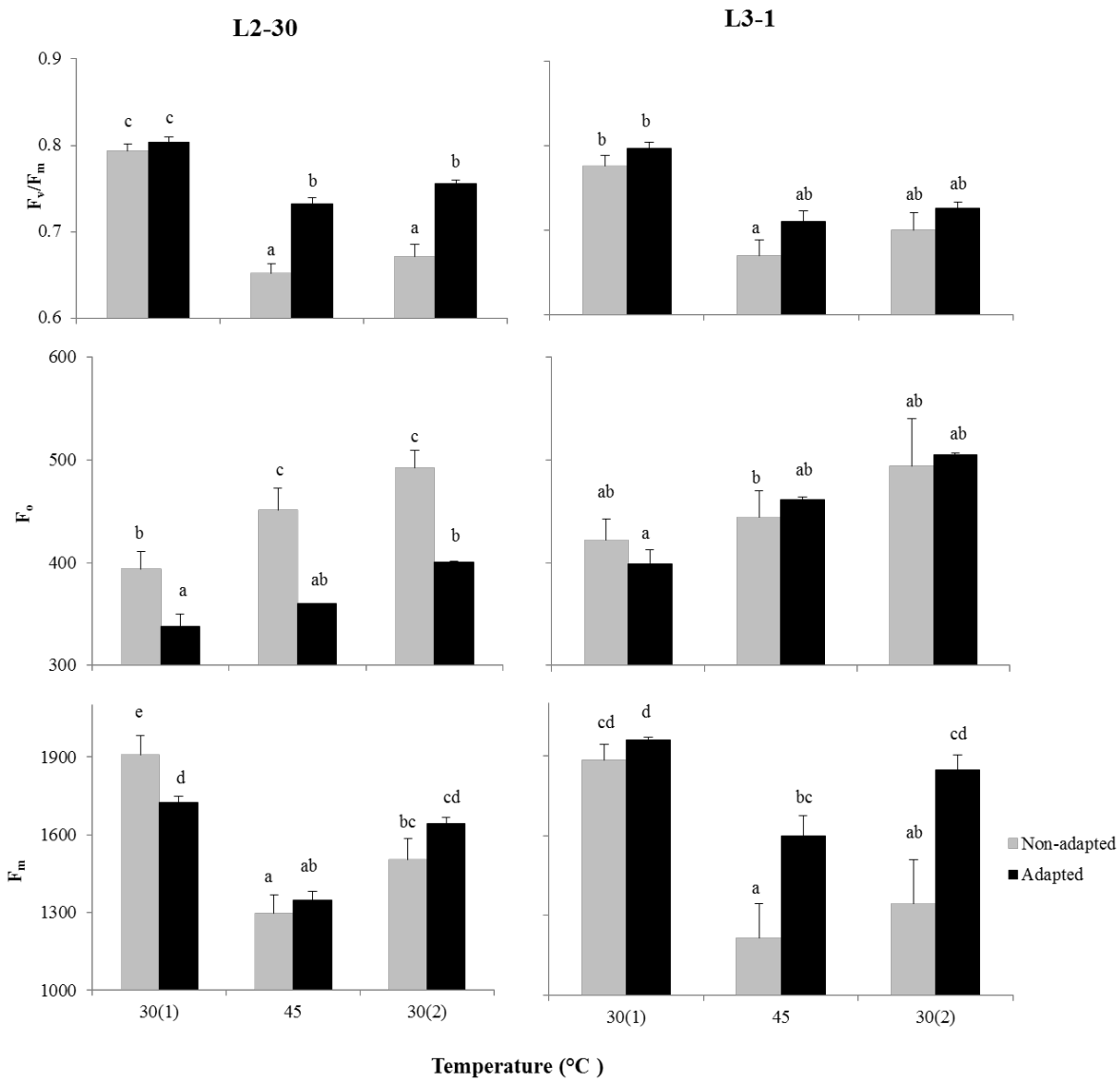

Figure 3.2 Average chlorophyll fluorescence (in the dark F_v'/F_m'), initial fluorescence (F_o) and maximum fluorescence (F_m) in the heat adapted and non-adapted genotypes L2-30 and L3-1 (n=3) at 30°C before light measurement (30(1)), at 45°C after light measurement (45) and at 30°C after measurement at 45°C (30(2)).Bars represent standard error (+SE).

3.4 Discussion

High temperature stress is a major limitation to agricultural production and food security and it is expected to become more frequent with climate change (IPCC, 2007, Fedoroff et al., 2010, Lobell and Gourdji, 2012). The photosynthetic pathway is highly sensitive to high temperature and can be affected by alteration of the PSII function, closure of stomata resulting in CO_2 limitation and disruption of cellular function from damaged thylakoid membranes (Mc Daniel, 1982, Cui et al., 2006, Pilon et al., 2016).

In tomato and cucumber, a 2 hour-heat shock at 45°C was observed to damage PSII, decrease in F_v'/F_m' and increase g_s (Camejo et al., 2005, Ding et al., 2016). In contrast, a 4 hour-heat shock at 45°C did not significantly damage PSII in okra, as F_v'/F_m' did not show a clear reduction in most genotypes and in fact , F_v'/F_m' increased in some (in both light and dark adapted plants) compared to the control. Okra are clearly more high temperature tolerant than either tomato or cucumber. However, a 6-hour heat shock applied to the 33 selected okra genotypes did reduce F_v'/F_m' more effectively indicating a reduction in functionality of the PSII reaction centre. If a photon is absorbed in a non-functional PSII reaction centre, its energy is dissipated as heat and cannot be used for photochemistry (Osmond and Grace, 1995). The conversion of light energy to photochemistry and the energy dissipation processes (i.e. dissipation as heat or reemission as fluorescence) operate in competition with one another, and if one increases there will be reduction in the other two (Maxwell and Johnson, 2000). Therefore, under high temperature, the damaged PSII reaction centre releases more heat than fluorescence and energy for photochemistry. In higher plants, Yamane et al. (1997) observed a decrease in F_m that was in parallel with denaturation of chlorophyll-protein complexes in PSII by high temperature, while an increase in F_o was the result of separation of PSII light harvesting complexes (Yamane et al., 1995). In the current study, the adapted and non-adapted plants showed that F_v/F_m reduced significantly at 45°C compared to 30°C, resulting from an increase in F_o and a decrease in F_m. However, F_o continued to increase even when temperature was reduced from 45°C to 30°C in both adapted and non-adapted plants, possibly pointing to the irreversible detachment of light harvesting complexes as observed earlier by Yamane et al. (1997). In contrast, in adapted plants, F_m recovered to

pre-heat levels when the temperature was reduced from 45°C to 30°C. Consequently, F_v/F_m reduced significantly at 45°C whereas Φ_{PSII} in both adapted and non-adapted plants stayed constant from 33 to 45°C, with a slight reduction in non-adapted plants at 45°C.

Other warm season crops such as cotton and tomato, in general showed lower F_v'/F_m' when exposed to high temperature (Camejo et al., 2005, Bibi et al., 2008, Cottee et al., 2012), whereas some of the okra genotypes showed higher fluorescence, which coincided with higher stomatal conductance and photosynthesis under stress. Nevertheless, these results were mostly due to the poor performance of the control (not the sensitivity of these genotypes to heat) as their F_v'/F_m' was lower than the average of all genotypes (0.51) and their g_s and A were nearly half value of the average (0.11 mol m^{-2} s^{-1} and 12.84 µmol m^{-2} s^{-1}). Although plants were watered regularly, the closure of stomata in the control and consequent reduction in A was accompanied by a decrease in F_v'/F_m', this may have been a result of transient water stress which has also been reported in bean (Zlatev and Yordanov, 2004).

Three different g_s responses to heat were observed among the okra genotypes (higher, lower and similar to the control). Genotypes with higher g_s opened their stomata, which potentially reduced leaf temperature when exposed to high temperature, as seen in Arabidopsis (Rizhsky et al., 2004, Mittler, 2006, Zhang and Sharkey, 2009). Most of these genotypes had higher A in the heat treatment (except for L2-11 and L4-6 which showed similar A in both treatments and L3-36 which was lower) indicating that photosynthesis was not strongly inhibited despite lower F_v'/F_m' under heat treatment. Although electron transport through PSII is interrupted by high temperature, the electrons can be contributed by cyclic electron transport to photosystem I (PSI) so that a reduction in A is prevented (Bukhov et al., 1999). Despite high g_s in some of the genotypes, A reduced under high temperature. The reduction of A under heat could be a consequence of reduction in Rubisco activase activity which was reported in wheat and cotton (Feller et al., 1998). Lower g_s under high temperature indicates partial stomatal closure and hence, less CO_2 assimilation resulting in lower A (Cui et al., 2006, Pilon et al., 2016). Those genotypes which exhibited similar g_s in the control and heat treatments may possess heat tolerance and similar responses were reported in heat tolerant genotypes of

tomato (Camejo et al., 2005). Despite heat effects on the photosynthetic apparatus, a six-hour heat shock only had a significant effect on electrolyte leakage of five okra genotypes, confirming results by Kuo et al. (1992) who ranked okra as a slightly heat tolerant vegetable. Although EL is a practical parameter to evaluate heat damage to cell membranes and was successfully applied to cowpeas, wheat, holly, turf grass and cotton (Bibi et al., 2008), only a small number of okra were affected in this study, and EL may not be an efficient screening technique for short-term heat damage in okra.

A short period of heat shock altered the physiological responses of okra; however, after exposure to high temperature for two weeks okra demonstrated an acclimation response with similar F_v'/F_m' and A under increased temperature (from 33°C to 45°C) and increased g_s. Okra plants which grew in high temperature for two weeks showed adaptation to the stressed environment and performed better relative to non-adapted plants when exposed to high temperature, this result was in a parallel with study in maize (Sinsawat et al., 2004) and indicating high heat tolerance in okra.

3.5 Conclusion

The current study showed photosynthetic traits were good indicators of the physiological response of okra genotypes to heat and that these traits can be used to assess genotype sensitivity following short periods of heat shock (6 hours or more at 45°C). In contrast, electrolyte leakage may be of limited use to detect damage from short-term heat exposure. Okra showed physiological adaptation when acclimatized to high temperature. However, this response must be assessed under field conditions before predictions can be made for okra breeding and productivity under high temperature.

4 Optimising molecular techniques for okra (*Abelmoschus esculentus* (L.) Moench) breeding and improvement

4.1 Introduction

Okra (*Abelmoschus esculentus*) belongs to the Malvaceae family. It is an allopolyploid and varies in chromosome number (2n) from 72 to 144 (Datta and Naug, 1968); however, most observations classify okra as 2n=130 as reviewed by Dhankhar and Singh (2009). Although the origin of okra is disputed, it was domesticated in Africa (Kumar et al., 2010, Oppong-Sekyere et al., 2011). Okra is now one of the most important vegetable crops in Africa and Asia due to its nutritional value (Adeboye and Oputa, 1996).

Okra has large variation in morphology (Lamont, 1999), but little is known about the extent of its genetic diversity. Diversity has been assessed using morphological markers (Ariyo, 1993, Aladele, 2009, Osawaru et al., 2013), but these markers are unstable in different environmental conditions. For this reason, molecular markers (DNA markers) provide better estimates of genetic diversity as they are not influenced by the environment (Leal et al., 2000, Gulsen et al., 2007).

Genetic diversity can be assessed using various molecular techniques including Sequence Related Amplified Polymorphism (SRAP), Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR). Of these, SSR markers are the most polymorphic because they distinguish between homozygotic and heterozygotic alleles (Gutierrez et al., 2005, Gulsen et al., 2007, Sawadogo et al., 2009, Shehata et al., 2009). Codominant SSR markers were discovered in the 1980s and use forward and reverse primers (Vieira et al., 2016). These markers are highly informative because different alleles at a given locus can be identified, and they are widely used for plant genotyping and the estimation of genetic distance (Shehata et al., 2009). The degree of polymorphism can then be estimated using the polymorphic information content (PIC) (Nagy et al., 2012).

Although studies of the genetic diversity in okra is limited, some evidence has been published using SSR markers (Sawadogo et al., 2009, Schafleitner et al., 2013, Kumar et al., 2017), RAPDs (Martinello et al., 2000, Aladele et al., 2008, Khan and Azmat, 2013) and AFLPs (Akash et al., 2013). In the current study, thirty five SSR markers were used to amplify okra genomic DNA to identify genetic variation among the genotypes.

Because okra has high polysaccharide content in its tissue, it is challenging to isolate its DNA which makes an effective molecular genetic analysis difficult. Hence, the aims of this chapter were:

- Optimise a cetyl trimethylammonium bromide (CTAB) isolation method (Brake et al., 2001) in order to obtain sufficient DNA of high quality
- Isolate DNA from all 107 genotypes (due to limitation in seed availability and failure in germination of 12 genotypes, the number of tested genotype reduced from 119 to 107 in this experiment) and use 35 SSR markers to amplify genomic DNA
- Test a variety of SSR markers (from okra and *Medicago truncatula* which are successfully used before) to establish polymorphism in the different genotypes
- Determine the most diverse okra genotypes to avoid accessions with similar genetic background in the selection of genotypes for the detailed physiological measurements to high temperature (Chapter 3 and 5).

4.2 Materials and methods

4.2.1 Source of genetic materials

One hundred and seven okra genotypes were used in this study (Appendix 3) from Vegetable Research Institute (VRI) (genotypes 1-19) and The World Vegetable Center, previously known as the Asian Vegetable Research and Development Center (AVRDC) (genotypes 20-107). Seeds were sown in 9 cm pots filled with a mixture of pine bark and

river sand (2:1) and kept in a glass house for 4 weeks at the Plant Breeding Institute (PBI), The University of Sydney, with day/night temperatures of 30°C/25°C. Young, expanding and tender fresh leaves (around 100 mg) were collected for DNA isolation.

4.2.2 Plant samples and preparation for DNA isolation

Two different methods were used to evaluate the most efficient procedure for sample preparation prior to DNA extraction from okra leaves:

1. Samples were collected in 2 mL labelled Eppendorf tubes, frozen immediately in liquid nitrogen and stored at −80°C. The samples were then freeze-dried using a KINETICS THERMAL SYSTEM (Model: FD-1-54D, England) for 24 hours then ground immediately.
2. Samples were collected in 2 mL labelled Eppendorf tubes dried on silica gel at room temperature for 3 days and then ground subsequently.

4.2.3 Molecular analysis

Two different methods were used to evaluate the effectiveness and quality of DNA extraction from mucilaginous okra leaves:

1. DNA was extracted using an ISOLATE Plant DNA mini kit (Bioline, Cat No. BIO-52069) following manufacturer's protocol (Appendix 4).
2. A CTAB extraction method (Brake et al., 2001) was modified and used to extract okra DNA. The ground leaf samples were transferred to Eppendorf tubes and 1 mL of 2xCTAB extraction buffer (Table 4.1) containing 2-mercaptoethanol (2%) was added. The solution was mixed well and incubated in a 65°C water bath for 30 minutes. Mixing by inversion was applied 3 times every 10 minutes. The preparation was divided into two 2 mL Eppendorf tubes (500 µl in each tube) to create more space. Then 250 µL of cold phenol (to separate protein and other cell material from DNA) and 250 µL of cold chloroform:isoamyl alcohol (chloroform contributes to the denaturation of

proteins and removes excess phenol whereas isoamyl alcohol works as anti-foaming agent) were added to each tube. Tubes were mixed by inversion until a thick emulsion formed; they were then centrifuged at 13,000 rpm for 30 minutes. Supernatants were transferred to a new sterile 2 mL Eppendorf tube. One volume (equivalent to supernatants volume) of cold chloroform:isoamyl alcohol was added to each tube, mixed by inversion, centrifuged at 13000 rpm for 15 minutes and the top phase subsequently transferred to new sterile 2 mL Eppendorf tubes. To precipitate DNA, 0.1 volume (equivalent to top phase volume) of 3M NaOAc (pH 5.2) and 1 volume (equivalent to top phase volume) of cold isopropanol were added and the tubes were stored at -20°C overnight. The next day, tubes were centrifuged at 13,000 rpm for 30 minutes, pellets were drained and 1 mL of cold ethanol (70%) was added, mixed and centrifuged at 13000 rpm for 20 minutes. Again pellets were drained then air dried and re-suspended in 200 µl of double deionised water and stored at 4°C overnight. The next day, 1 µl of RNaseA (100 mg mL^{-1}) was added to each tube before incubation at 37°C overnight.

Table 4.1 Components of the 2xCTAB extraction buffer.

Components	Quantity for 500 mL
2% w/v CTAB	10 g
20 mM EDTA (pH8)	20 mL
1.4 M NaCl	140 mL
2% w/v Polyvinyl pyrrolidone (PVP)	10 g
100 mm Tris-HCl (pH8)	50 mL
ddH_2O	290 mL

4.2.4 DNA quantification and qualification

DNA quantification was performed using 1 µL of material on a Nanodrop, ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA). DNA quality was assessed using a 2% agarose gel (3g agarose in 150 mL of Tris/Borate/EDTA (1xTBE) buffer (Table 4.2), stained with 3 µL of gel red and run in an electrophoresis system at 120 volts for 30 minutes. The DNA bands were analysed against the standard lambda

DNA and bands were visualized under UV light (ChemiDoc™ MP Imaging System, Image Lab™ Software, Version 5.1).

The stock-DNA was then diluted with double deionised water to a final working concentration of 25 ng μl^{-1} in 200 µL.

Table 4.2 Concentrations of the 1xTBE buffer components

Components	Quantity for 20 L
Tris	216 g
Boric acid	110 g
0.5M EDTA (pH8)	80 mL
ddH_2O	20 L

4.2.5 SSR primers

Nineteen SSR primers from *A. esculentus* (Schafleitner et al., 2013) (Appendix 5, No. 1-19) and sixteen SSR primers from *Medicago truncatula* (Sawadogo et al., 2009) (Appendix 5, No. 20-35) were ordered from Sigma-Aldrich and used to amplify okra genomic DNA (total of 35 primers). The stock-primers were diluted to a final working solution of 10 ng μL^{-1} in 200 µL ddH_2O.

4.2.6 Polymerase chain reaction (PCR)

PCR amplifications were conducted in a final volume of 15 µl containing 5 µl of 25 ng μl^{-1} DNA, 4.05 µl of ddH_2O and 5.95 µl of master mix (Table 4.3). PCR was performed using two different programs for either Medicago or okra SSR markers.

Table 4.3 PCR master mix for DNA amplification (10X Buffer, $MgCl_2$ and *Taq* polymerase were from BIOLINE, Cat No. BIO-21040)

Components	Concentration (µl)
10X Buffer	1.50
2 mM deoxyribonucleotide triphosphates (dNTPs)	1.50
50 mM $MgCl_2$	0.90
Forward primer (P_F)	0.90
Reverse primer (P_R)	0.90
Taq polymerase (5 units per µl)	0.25
Total	5.95

4.2.6.1 Program 1: Medicago SSR markers

An initial incubation of 1 min at 94°C was followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and elongation at 72°C for 2 min. A final extension of 72°C for 10 min was followed by an incubation at 4°C (Sawadogo et al., 2009).

4.2.6.2 Program 2: Okra SSR markers

The program described by Schafleitner et al. (2013) was modified and used for okra SSR markers. An initial incubation at 95°C for 10 min was followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 45 s and elongation at 72°C for 45 s. A final extension of 7 min at 72°C was followed by incubation at 4°C.

PCR products were separated using 3.5% agarose gel (7 g of agarose, 200 mL of 1xTBE buffer and 4 µl of gel red) electrophoresis while 1.5 µl of loading buffer was added to 4 µl of PCR products and loaded on the gel against 2 µl of Hyper ladder IV (BIOLINE) and the electrophoresis was run at 100 volts for 3 hours. Bands were visualized under UV light (ChemiDoc™ MP Imaging System, Image Lab™ Software, v5.1).

4.2.7 Statistical data analysis

Allele frequencies for each microsatellite locus were used to calculate the polymorphic information content (PIC) manually, using the following equation where *Pi* is the frequency for the *i*th allele and *l* is the total number of alleles (Nagy et al., 2012). Markers with PIC value greater than 0.5 are highly informative (Botstein et al., 1980).

$$\text{PIC} = 1 - \sum_{i=1}^{l} (P_i^2)$$

The images obtained from agarose gels were scored manually by indicating presence (1) or absence (0) of a specific allele. The “1” or “0” data was used to generate a dendrogram using unweighted pair-group method with arithmetic means (UPGMA) (Sneath and Sokal, 1973) clustering in NTSYS-pc v2 (Rohlf, 1998). Then “1” and “0”

data were converted to “A” and “T”, respectively, to generate a dendrogram in MEGA v6 (Molecular Evolutionary Genetics Analysis) to compare with the results from NTSYS-pc v2 (Kankwatsa, 2016). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 rep) are shown above the branches on generated dendrogram (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood (Tamura et al., 2004).

4.3 Results

4.3.1 Molecular analysis

Two different methods were used to optimize sample preparation for DNA extraction and they were tested against two methods of extraction. No significant difference in the quality of the samples was observed between these two methods. For example, the nanodrop values for DNA isolates of 71, 79 and 83 were 11, 16 and 27 ng μl^{-1}, respectively (the unmodified CTAB method was used at this stage). However, the first method (the freeze-dried procedure) was adopted for this study as it was more practical and convenient.

The high mucilage content of okra tissue limited the value of the DNA extraction kit because the provided solutions were not strong enough to separate the DNA from other components. Thus the DNA isolation was not pure, resulting in a low nanodrop reading (less than 100 ng μl^{-1}). However, DNA extracted using the CTAB method had higher quality and quantity. The purity of DNA was presented by A260/A280 and A260/A230 ratios for protein and organic contaminants, respectively (Table 4.4), where nucleic acids absorb at 260 nm, proteins at 280 nm (strong absorbance around this point) and organic compounds at 230 nm (strong absorbance at around 225 nm). Ideally, purified DNA should have a A260/A280 ratio between 1.65 and 1.8 and a A260/A230 ratio between 1.5 and 1.8 (Maréchal-Drouard and Guillemaut, 1995). The average observed protein purity (A260/A280) was 1.75 among the 107 genotypes where 64% of the isolates produced values between 1.8 and 1.9, 22% between 1.6 and 1.7 and 14% between 1.2 and 1.5.

The DNA bands in 2% agarose gel against lambda DNA ($\lambda_{1=}$ 1µl and $\lambda_{2=}$ 2µl) for 28 genotypes (Table 4.4) is shown in Figure 4.1. Genotypes with lighter colour bands had lower amounts of DNA (e.g. genotypes with sample ID 10, 21, 25 and 27). For genotypes with low DNA concentration (based on both nanodrop and gel results), a second extraction was conducted using freshly picked leaves.

4.3.2 Polymerase chain reaction (PCR)

Thirty five SSR markers were used to amplify the genomic DNA of 107 okra genotypes. Eight of these markers (Table 4.5) amplified all genomic DNA under PCR at the annealing temperature of 53ºC, except for genotypes 39, 57 and 70 which did not amplify with any markers.

DNA amplifications were scored to indicate the presence or absence of specific alleles. The 8 primers amplified a total of 29 alleles among 104 isolates with an overall average of 3.6 alleles per primer, ranging from the highest amplification by primer 13 (7 alleles) and the lowest by primers 9 and 12 (2 alleles each). The scored band size ranged from 109 to 308 base pairs. An example of the gel images of 19 isolates (genotypes 1-19), amplified by eight primers is given in Figure 4.2. The data obtained was used to generate a UPGMA-based phylogenetic dendrogram for clustering and similarity analysis across the isolates. Data was analysed in both MEGA v6 and NTSYS-pc v2 software and similar results were obtained. However, the dendrogram generated from the bootstrap analysis using MEGA v6 software was retained (Figure 4.3). This dendrogram was used to screen germplasm in conjunction with physiological responses to high temperature in chapter 3. The yellow highlighted genotypes (33 in total) indicate the first selection of material (chapter 3) and those in red colour the second selection (chapter 5).

Table 4.4 The DNA quantity (ng μl^{-1}) of 28 genotypes (as an example) and A260/A280 and A260/A230 ratios assessed using a nanodrop.

Sample ID	Genotype	ng μl^{-1}	A260/A280	A260/A230
1	L2-1	64.20	1.53	0.56
2	L2-6	148.35	1.60	0.67
3	L2-8	496.96	1.90	2.04
4	L2-11	707.82	1.91	1.48
5	L2-12	793.67	1.85	1.28
6	L2-14	132.72	1.52	0.55
7	L2-15	302.42	1.62	0.69
8	L2-19	995.77	1.75	1.17
9	L2-21	278.39	1.67	0.89
10	L2-22	118.87	1.50	0.50
11	L2-23	352.98	1.66	0.82
12	L2-24	540.88	1.31	1.65
13	L2-25	831.74	1.69	1.13
14	L2-27	93.31	1.32	0.35
15	L2-28	455.19	1.89	1.46
16	L2-30	347.25	1.68	0.76
17	L2-31	159.32	1.67	0.85
18	L2-32	86.47	1.53	0.51
19	L2-33	114.33	1.68	0.74
20	L3-1	390.41	1.59	0.74
21	L3-2	169.59	1.67	0.90
22	L3-3	264.79	1.86	1.53
23	L3-4	86.88	1.84	1.26
24	L3-5	423.99	1.90	1.93
25	L3-7	100.57	1.88	1.60
26	L3-8	385.07	1.90	1.93
27	L3-10	306.35	1.46	0.65
28	L3-11	162.50	1.85	1.52

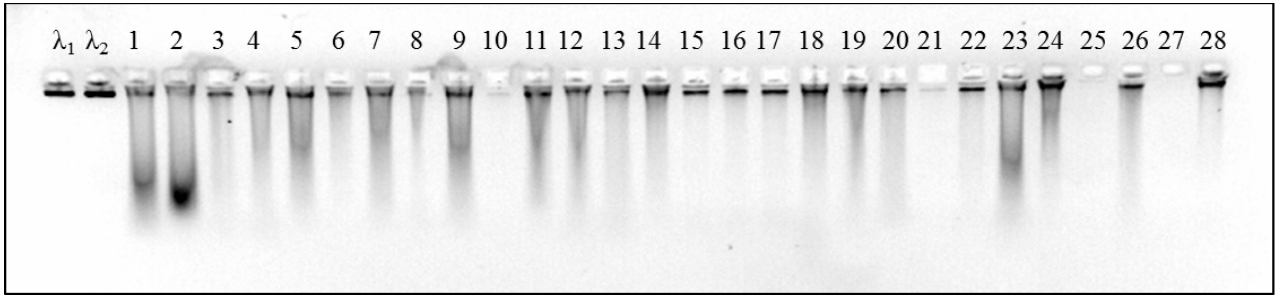

Figure 4.1 DNA bands of 28 genotypes and two lambda DNA bands (λ1= 1 µl and λ2= 2 µl) in 2% agarose gel using the modified CTAB method.

Table 4.5 List of 8 polymorphic SSR markers used to amplify 104 genomic DNA. The repeated motifs including, forward (F) and reverse (R) primer sequences, annealing temperature (°C), allele sizes (bp), number of alleles per locus and polymorphic information content (PIC) are defined.

Primer No.	Name	Repeat motif	Primer sequence (5' -3')	Annealing Tm (°C)	Estimated allele size range (bp)	No. of alleles	PIC
3	AVRDC-Okra9	(AAT)12	**F:**ACCTTGAACACCAGGTACAG **R:**TTGCTCTTATGAAGCAGTGA	53	150-250	4	0.56
6	AVRDC-Okra28	(ATT)8	**F:** CCTCTTCATCCATCTTTTCA **R:**GGAAGATGCTGTGAAGGTAG	53	200-300	3	0.53
9	AVRDC-Okra54	(GAA)10	**F:**CGAAAAGGAAACTCAACAAC **R:**TGAACCTTATTTTCCTCGTG	53	100-170	2	0.49
11	AVRDC-Okra57	(GAA)9-(GAG)7	**F:**CGAGGAGACCATGGAAGAAG **R:**ATGAGGAGGACGAGCAAGAA	53	170-310	4	0.43
12	AVRDC-Okra63	(TCT)12	**F:**GTGTTTGAAAGGGACTGTGT **R:**CTTCATCAAAACCATGCAG	53	200-300	2	0.27
13	AVRDC-Okra64	(TCT)22	**F:**AAGGAGGAGAAAGAGAAGGA **R:**ATTTACTTGAGCAGCAGCAG	53	100-300	7	0.71
18	AVRDC-Okra86	(AGC)8	**F:**ATGCAAACAAGCTAGTGGAT **R:**ATTCTCTTCAGGGTTTCCTC	53	250-400	4	0.65
19	AVRDC-Okra89	(AGC)8	**F:**TTTGAGTTCTTTCGTCCACT **R:**GTATTTGGACATGGCGTTAT	53	140-200	3	0.59

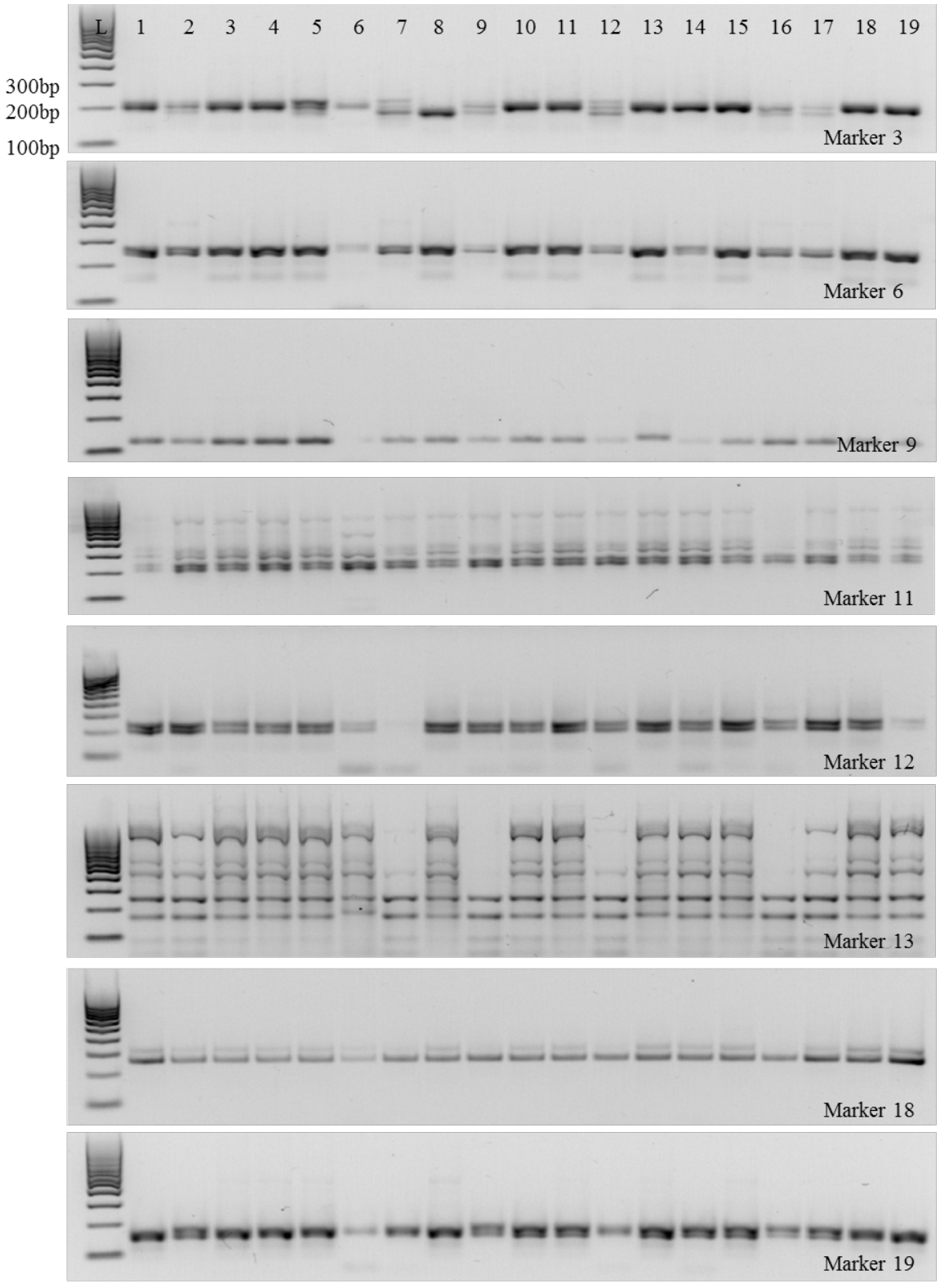

Figure 4.2 Amplification profiles of 19 genotypes with markers No. 3, 6, 9, 11,12,13,18 and 19. "L" represents Hyper ladder IV, numbers above each lane represent the isolates listed in Appendix 3.

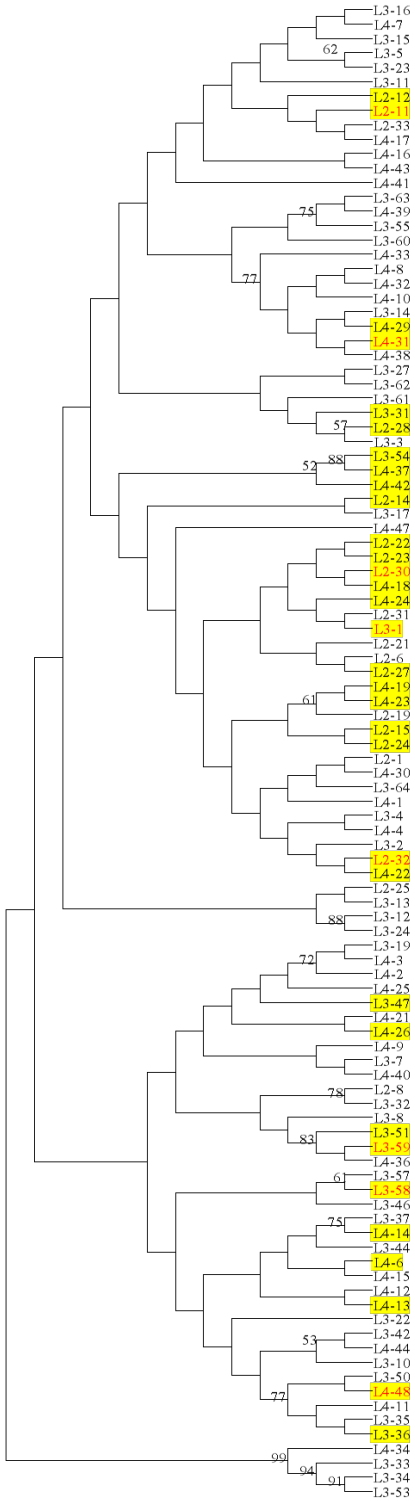

Figure 4.3 A phylogenetic analysis of 104 okra isolates based on 29 alleles produced by 8 SSR markers. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 iterations) are shown above the branches, values below 50 were omitted. 33 genotypes selected in chapter 3 are highlighted in yellow, 8 genotypes selected for chapter 5 are hightlighted in yellow with red font, demonstrating a large genotypic diversity of the selection.

4.4 Discussion

High quality genomic DNA of sufficient quantity is critical for effective molecular genetic analyses using PCR-based markers. Okra leaves contain polysaccharides and secondary metabolites such as polyphenols that interfere with the DNA isolation process, while the glue-like texture of polysaccharides makes extraction unmanageable during pipetting, resulting in poor DNA quality. Polysaccharide contamination also has a negative effect on PCR amplification and inhibits *Taq* polymerase activity (Fang et al., 1992). The current study optimized DNA isolation from young, fresh and green leaf tissue using different techniques and subsequently amplified genomic DNA using SSR markers to map genetic distance among lines.

To prevent polysaccharide contamination during DNA isolation, some studies used yellow and etiolated fresh leaves of 10-14 day old okra plants grown under dark conditions (Fougat et al., 2015, Kumar et al., 2017). However, our results show that DNA can be extracted from young, fresh, green leaf tissue of okra regardless of the drying method.

PVP was used to isolate DNA from polysaccharides and mucilage in young okra leaves by Manoj-Kumar et al. (2012) and mature/old leaves of strawberry by Porebski et al. (1997). Once the cell walls were lysed, the liberated polyphenols bound to nucleic acid by covalent bonding (Maréchal-Drouard and Guillemaut, 1995). PVP is a water soluble, solid polymer of high molecular weight that can bind polyphenols through hydrogen bonding, thus removing this contaminant from the DNA molecules (John, 1992). Our study showed that the commercial ISOLATE Plant DNA mini kit was not suitable for DNA isolation (the viscous substrate interfered with pipetting) whereas the modified CTAB method with addition of PVP and RNaseA produced good quality DNA, which was confirmed by the absorbance ratio at 260 nm/280 nm. Purity between 1.6 and 1.8 is ideal in DNA isolation (Maréchal-Drouard and Guillemaut, 1995). The average purity ratios observed of the 107 genotypes confirmed that the modified CTAB method used in this study can be utilized to extract high quality DNA with limited polysaccharide and secondary metabolite contamination. Therefore, the CTAB method is suitable for molecular analysis using SSR markers.

SSR markers can be used to assess okra genetic variability among accessions commonly used in breeding programs. The 16 *Medicago* SSR markers successfully used by Sawadogo et al. (2009) to study okra did not amplify the genomic DNA in the current study, whereas the 19 SSR markers specific to okra provided by Schafleitner et al. (2013) amplified 104 of 107 genotypes. Of the 19 markers studied, only eight showed polymorphism. In a previous study on Turkish okra (Yildiz et al., 2015), nine of the Schafleitner et al. (2013) SSR markers were used and five of these were common to the current study. The highest PIC value in both the current study and the Yildiz et al. (2015) study was observed for marker 13 (AVRDC-Okra64). This marker also had the third highest PIC value in Schafleitner et al. (2013) experiment and can be, therefore, considered as an informative SSR marker suitable for okra genotyping. However, marker 12 (AVRDC-Okra63) showed the lowest PIC value in the current study (0.27) but was higher than 0.6 in previous work. This suggests that this marker is probably polymorphic in only a few genotypes. The variation at the molecular level observed in the SSR-based dendrogram of 104 okra genotypes indicates that considerable genetic diversity exists among okra accessions. However, the genotypes did not group in relation to their geographical origin, indicating that materials have been introduced to different regions overtime, thus blurring geographical similarities.

The modified CTAB with additional PVP extraction method and molecular analysis using SSR markers could be useful in okra breeding programs. The results of this study were used in conjunction with germplasm screening for physiological responses to high temperature (chapter 3) to reduce the materials from 119 genotypes to 33 and subsequently, eight genotypes for more intensive phenotypic evaluation. Genotypes with low genetic similarity, generally less than 50%, were selected. Genotypes 55 (L3-54) and 98 (L4-37) from Cambodia showed 88% similarity (Figure 4.3); however, their responses to high temperature differed. In experiment 1 (4h heat shock), chapter 3, heat reduced stomatal conductance (g_s) and chlorophyll fluorescence (F_v'/F_m') in genotype 55 compared to genotype 98. These two genotypes also had 52% (Figure 4.3) similarity with genotype 103 (L4-42) (from the USA). However, under high temperature genotype 103 showed low g_s (similar to genotype 55) and the F_v/F_m response was similar to genotype 98 (experiment 1, 4h heat shock in chapter 3). Surprisingly, when the

experiment was repeated all three genotypes showed low g_s under heat treatment, whereas the rate of photosynthesis reduced in genotypes 55 and 98 (with higher reduction of these two parameters in 98) and increased in genotype 103 (experiment 2, 6h heat shock in chapter 3). The genotypes 60 (L3-59 from Australia) and 53 (L3-51 from the Philippines) were 83% similar (Figure 4.3) and had lower g_s under high temperature compare to the control, but F_v/F_m responses were different. These varied responses highlight the necessity to combine phenotyping with molecular methods in order to select genetically diverse material for breeding programs and productivity improvement. Hence, the current molecular chapter in conjunction with the chapter 3 have provided valuable information on genetic and phenotypic variation in okra that can be used to support the genetic improvement of this crop.

4.5 Conclusion

DNA isolated from young, fresh and green okra leaf tissue using the modified CTAB method with additional PVP and RNase was effective. This procedure enabled the extraction of high quality and sufficient quantity of genomic DNA for molecular analysis. The use of SSR markers showed that significant genetic variation exists among okra genotypes. These markers could also be used to screen germplasm in conjunction with phenotypic information to identify suitable okra genotypes for a study in a production environment. Finally, one previously published SSR marker was particularly effective in the current study and can be recommended for okra screening programs.

5 Effect of high temperature on physiological parameters and productivity in okra (*Abelmoschus esculentus* (L.) Moench)

5.1 Introduction

Temperature is a significant environmental stress factor which limits plant growth and productivity (Krasensky and Jonak, 2012, Hasanuzzaman et al., 2013). It has the potential to severely reduce yield, particularly in tropical and subtropical climates where temperature increases are expected to be the worst (Wahid et al., 2007, Lobell and Gourdji, 2012). High temperature stress has a negative impact on a plant's physiology (chapter 3) and can lead to a reduction in assimilation rate, an alteration in carbohydrate reserves and a reduction in plant productivity (i.e. dry matter and yield), which has been reported repeatedly under high temperature (Gawronska et al., 1992, Lewis, 2000, Siebert et al., 2014). In cotton for example, more than 50% reduction in boll development occurred when the temperature was raised above the optimum growth temperature (between 20ºC and 30ºC) (Oosterhuis, 1999).

Photosynthesis – the process of capturing light energy with chlorophyll molecules and converting it to chemical energy – is inhibited before other plant functions under high temperatures, as it is highly sensitive to heat stress (Camejo et al., 2005, Chen and Cheng, 2009). Chlorophyll fluorescence parameters (e.g. F_v'/F_m', Φ_{PSII} and qP) are commonly assessed to evaluate photosynthetic mechanisms in response to environmental stresses. This technique measures the excess energy which is not used in photochemistry, but emitted as heat and fluorescence (chapter 3) (Misra et al., 2012). F_v'/F_m' (the ratio of variable to maximum fluorescence in the light) and Φ_{PSII} (the efficiency of the open reaction centre) indicate efficiency of PSII (chapter 3), whereas qP (photochemical quenching) estimates the redox state of Q_A in the light and is measured as $(F_m'-F_s)/(F_m'-F_o')$(Baker, 2008). Often, these measurements are accompanied by assessing photosynthesis (A), stomatal conductance (g_s) and electrolyte leakage (EL) (Misra et al., 2012, Murchie and Lawson, 2013). In the heat, fluorescence parameters and A reduce and

EL increases due to a rise in membrane permeability (Cui et al., 2006, Murchie and Lawson, 2013, Pilon et al., 2016).

If adequate water is available, g_s (a measure of the rate of CO_2 and water movement in and out of the leaf) may increase in order to cool the leaf. Plants access water via the roots which is transported through the xylem governed by negative pressure due to leaf transpiration. Water movement upwards in the xylem is facilitated by a large difference in chemical potential (or water potential, Ψ) between the atmosphere and the leaves, and by attraction of water molecules to each other and the xylem walls, called cohesion and adhesion, respectively (Tyree, 1997). The efficiency of this water flow can be quantified as leaf hydraulic conductance (k_{leaf}), taking into account plant transpiration and the difference in water potential between the stem and the leaves (Sack and Holbrook, 2006). Well hydrated plants have high Ψ resulting in high g_s due to open stomata, high transpiration (E) and consequently, high k_{leaf} (Sack and Holbrook, 2006, Ribeiro et al., 2012, Locke et al., 2013, Simonin et al., 2014). If water is limiting, or water flow through the plant cannot be sustained, plants may close their stomata to conserve water in the heat (Hasanuzzaman et al., 2013).

Under heat stress, stomatal closure and lower A can lead to a reduction in carbohydrate production. Carbohydrates in leaves exist either as simple sugars (mono- and oligosaccharides) or starch, a polysaccharide. The most abundant sugar is glucose (a monosaccharide), which is the building block of transitory starch. Transitory starch builds up during the day and is hydrolysed at night to release glucose for cellular respiration and generating energy (in the form of adenosine triphosphate (ATP)). Another important monosaccharide is fructose, which is covalently bound to glucose to form sucrose, a disaccharide. Sucrose is the major product of photosynthesis which is made in the cytosol and used in leaf tissues, or exported via phloem to sink organs such as roots and fruit (Rolland et al., 2006). Sucrose phosphate synthase (SPS) and sucrose synthase (SS) are two major enzymes involved in sucrose synthesis (Nguyen-Quoc and Foyer, 2001). Sucrose is hydrolysed by invertase to produce glucose and fructose and it is located in the cell wall (CWIN), vacuole (VIN), and cytoplasm (CIN), and CWIN and VIN are major enzymes in response to stresses (Roitsch et al., 2003, Mc Laughlin and

Boyer, 2004, Essmann et al., 2008). Sucrose is also hydrolysed by sucrose synthase (SS), which is located in the cytoplasm, to produce UDP-glucose and fructose. Li et al. (2006) and Roitsch and González (2004) reported higher fructose content compared to glucose under optimum conditions, resulting from involvement of two enzymes in fructose production.

In the heat, these enzyme activities can be modified, which has been observed in many crops, affecting carbohydrate concentrations in leaves, shoots and grain. For instance, rose shoots and potato leaves had higher SPS activity in high temperature (Rufty et al., 1985, Khayat and Zieslin, 1987, Lafta and Lorenzen, 1995) increasing sucrose production, whereas in rice grain, chickpea, spinach and lentil leaves, SS and SPS activities were reduced, resulting in lower sucrose concentration (Holaday et al., 1992, Li et al., 2006, Kaushal et al., 2013, Bhandari et al., 2016). Heat reduced sucrose concentration by 9% and increased reducing sugar (glucose and fructose) content (due to an increase in invertase activity) by 47% in soybean leaves (Djanaguiraman et al., 2011, Camejo, 2013, Bhandari et al., 2016). Therefore, increasing reducing sugar concentrations can be the result of sucrose cleaving (Hawker and Jenner, 1993) or degradation of starch (Geigenberger et al., 1998). In rice grain, invertase activity decreased under high temperature resulting in the decrease of glucose and fructose concentrations (Li et al., 2006). Additionally, under high temperature plants produce ethylene (Djanaguiraman et al., 2011) as a stress hormone, which has been reported to possibly inhibit sugar and starch synthesis enzymes (Hasanuzzaman et al., 2013).

Long-term heat stress in field conditions has been studied in many crops and plant acclimation responses to high temperature were shown to avoid stress and allow survival, despite lowered productivity, e.g. in cotton (Hasanuzzaman et al., 2013, Abro et al., 2015). Okra is well adapted to warm regions; however, high temperature reduced the productivity of tropical and subtropical crops under field conditions (Wahid et al., 2007). Notably, in the short-term (2-week) heat-exposure study of chapter 3, okra displayed an acclimation response, and heat did not affect its photosynthetic performance in a growth cabinet. Nevertheless, the acclimation response of okra might be different in the field, as the duration of temperature changes may be longer and more frequent than the 30 to 60

min chosen for the cabinet. In addition, other factors such as light intensity, water availability, humidity and wind can play important roles in plant responses to high temperature. Hence, the objective of this chapter was to evaluate the effects of high temperature on the photosynthetic performance, carbohydrate metabolism and productivity (biomass and yield) of okra in an outdoor production setting with a condition between controlled environment and field. For this purpose, plants were placed in two tunnel houses, with and without raising the temperature above ambient. Measurements were taken in two consecutive years in order to confirm reliability of the data. More specifically, the study aimed to:

- Assess the physiological responses of okra under long-term elevated temperature compared to ambient, particularly photosynthesis, stomatal conductance, transpiration, fluorescence parameters and electrolyte leakage.
- Assess concentrations of leaf, shoot and root carbohydrates in okra under high temperature compared to ambient.
- Determine if different plant developmental stages (flowering and bud initiation stages) are similar in their physiological responses to heat.
- Compare dry matter and yield in elevated compared to ambient temperatures and relate them to the physiological parameters and sugar content.

5.2 Materials and methods

5.2.1 Plant material

Okra seed from the Vegetable Research Institute (VRI) and The World Vegetable Center, previously known as the Asian Vegetable Research and Development Center (AVRDC) were sown in 90 mm diamiter pots filled with 8 mm composted pine bark, 3 mm composted pine bark and sand mixture (8:1:1) and supplemented at 0.4 kg m^{-3} with all trace elements, gypsum (1 kg m^{-3}), superphosphate (1 kg m^{-3}), KNO_3 (13% N) (0.25 kg m^{-3}), nitroform (38% N) (0.25 kg m^{-3}) and magrilime (1.5 kg m^{-3}). Plants were kept in

a glass house with natural light at the Plant Breeding Institute (PBI), The University of Sydney, NSW, Australia, with day/night temperatures of 30°C/25°C before being transplanted to the tunnel houses.

5.2.2 Environmental parameters in the tunnel house

There were two, 450 m^2, adjacent plastic (180 micro thickness PEP film, anti-UV, anti-dripping) tunnel houses with 5 rows of platforms to accommodate potted plants in each tunnel house. The tunnel house used for the heat treatment was fully covered while the other tunnel house (control) had two opened sides and/or a ventilation system. In late January 2015, a data logger (CR200X, Campbell Scientific Australia, Townsville, QLD, Australia) was installed inside each tunnel house (heat and control) and one outside the tunnel houses (ambient) to record temperature, relative humidity (RH) and photosynthetically active radiation (PAR) every 5 minutes. During the day, temperatures were aimed at a maximum of 45°C in the heat treated tunnel house, and on hot days, the sides were rolled up to avoid excess heat in the heat treatment. Sunny days were chosen for measurements.

5.2.3 Physiological assessment

5.2.3.1 Photosynthetic parameters

F_o', F_m', F_v'/F_m', Φ_{PSII}, qP, E, A and g_s were measured on the most recent, top mature leaf in both control and heat between 10 am and 3 pm, using a Licor 6400xt fitted with a fluorescence light source (LI-COR, Lincoln, NE, USA). Reference CO_2 was set to 400 µmol mol^{-1}, photosynthetically active radiation (PAR) to 1300 µmol m^{-2} s^{-1} and flow rate to 300 mL min. As the effect of heat stress is more damaging and severe in the light (Zhang and Sharkey, 2009), and the result from chapter 3 showed a stronger response of chlorophyll fluorescence in the light, as well as logistical constraints on measurement days, only chlorophyll fluorescence of light adapted leaves was measured.

5.2.3.2 Electrolyte leakage

Electrolyte leakage was measured on the same leaf as photosynthetic parameters, using the conductivity method described by Sullivan (1972), Lafuente et al. (1991) and Camejo et al. (2005). Eight 7 mm diameter leaf disks were cut using a cork borer and were placed in 50 mL Greiner centrifuge tubes (Sigma Aldrich, Australia) with 20 mL of double deionised water and maintained on a shaker at 80 rpm for 20 hours at room temperature. The conductivity of the solution was read with a conductivity meter (Edge, Hanna Instruments Inc. HI11310 single ceramic, double junction, and refillable pH electrode with temperature sensor, UK), before and after autoclaving the samples for 15 min at 121°C. Electrolyte leakage was expressed as the ratio of the conductivities in percentage as follows:

% electrolyte = (T1/T2)*100 where T1 and T2 are the conductivities before and after autoclaving the tissues respectively.

5.2.3.3 Leaf hydraulic conductance

Water potential was measured according to Simonin et al. (2014) at mid-day using a Scholander-style pressure chamber (Soil Moisture Equipment Co., Santa Barbara CA, USA) on three positions per plant (low, mid and top canopy). Low canopy leaves were covered with plastic film and aluminium foil the night before as a proxy for stem water potential (Ψ_{stem}), top and mid canopy were wrapped in plastic film prior to leaf excision to avoid excessive water loss through the leaf surface before measurement. Leaf hydraulic conductance was calculated using $k_{leaf} = E/ \Delta\Psi_{stem\text{-}leaf}$ (Brodribb and Holbrook, 2003), where Ψ_{leaf} was the average water potential of top and mid canopy.

5.2.4 Metabolic assessment

Sugar concentrations were assessed for the same leaf after photosynthetic parameters measurements and EL samples were taken, i.e. the other half of the leaf was sampled for sugars, avoiding the mid-rib. Additionally, 7 to 8 cm shoot-sections from the middle of the shoot and root samples were taken per plant and frozen immediately in

liquid nitrogen. Roots were washed with double deionised water, and excess water shaken off before freezing. Samples were subsequently freeze dried and extracted in the laboratory for identification and quantification of the most abundant carbohydrates.

5.2.4.1 Carbohydrate extraction and derivatisation

One mL of a methanol/chloroform/water mixture (MCW, 12:5:3) was used to extract soluble organics from 40 mg of freeze-dried material (Merchant et al., 2006) and 10 µl penta-erythritol was added as an internal standard. Six µl of the extract was dried in a 2 mL vial with a 300 µl insert using a vacuum concentrator (Speed Vacuum Concentrator, Model: ScanSpeed 40, LaboGene, Denmark). A modified derivatisation method was used (Roessner et al., 2000) where the dried samples were methoximated in a solution (20 µL, 20 mg mL^{-1}) of methoxyamine hydrochloride (Sigma-Aldrich) in pyridine (Fluka) by heating at 60°C for 30 min. The samples were then silylated following the addition of BSTFA containing 1%TMCS (25 µL, Supelco) and heating at 60°C for 15 min.

5.2.4.2 Gas chromatography–mass spectrometry (GC-MS)

Samples were diluted with 150 µl of dichloromethane and 0.2 µL were injected onto a fused-silica capillary column (30 m x 0.25 mm i.d.) coated with a 5% diphenyl-95% dimethylpolysiloxane bonded phase (Restek; Rtx-5MS, film thickness 0.25 µm), which was eluted with He (inlet pressure 14.5 psi) directly into the ion source of a Thermo Polaris Q GC/MS (injection port 220°C; interface 250°C; source 250°C). The column was programmed from 60°C (hold 2 min) to 120°C at 5°C min^{-1} and then to 250°C (hold 2 min) at 10°C min^{-1}. The mass spectrometer was operated in the electron ionisation (EI) mode with ionisation energy of 70 eV, scanning the mass range of m/z 50-650. The GC/MS software was used to control the instrument and quantification of sugars was performed with Xcalibur v1.4 (Thermo Fisher Scientific, 2004).

5.2.5 Agronomy assessments

5.2.5.1 Dry matter

Biomass was measured at the end of experiment where plants were cut off at the base of the stem. Above ground biomass was separated into leaves and stems and fresh and dry weight assessed. Below ground biomass was assessed by weighing fresh roots after thorough washing, shaking off excess water and towel-drying and dry roots after oven drying.

5.2.5.2 Yield

Fruits were collected during the experiment, 6 to 9 days after flower-opening. In this period, fibre content is suitable for human consumption and okra fruit has high level of moisture, mucilage, proteins and starch (Dhankhar and Singh, 2009, Tripathi et al., 2011). The average number of fruits per genotype (n =3) was recorded as yield.

5.2.6 Year 1

In the first year of the field experiment, eight okra genotypes (e.g. L2-11, L2-30, L2-32, L3-1, L3-58, L3-59, L4-31 and L4-48) were selected (Chapter 3) and were subjected to a 15 week period of heat, and physiological and agronomic measurements taken at conclusion of the treatment. After raising okra seedlings for 4 weeks in the glass house, three replicates of each genotype were moved into tunnel houses in mid-December 2014 and transplanted into 10 L bags containing coco peat (Galuku Coir, Galuku Pty Ltd, Australia). The plants were fertilized using a recipe for growing commercial tomato and cucumber (Appendix 6). Fertigation of 200 mL h^{-1} was applied daily from 6am to 6pm (2.4 L of water per day) through dripped irrigation. Soil water moisture was checked periodically (multiple times during a single day) using water moisture meter. Plants in the control tunnel house were in the second row of the west-side but plants in heat treated tunnel house were in the first row of the east-side. On a very hot day in mid-February (8 weeks after transplantation) the top of the canopy was burnt in the heat tunnel house (Figure 5.1) as temperatures reached above 50°C and subsequently all plants in both

tunnel houses were cut from 50 cm above the soil. After 15 weeks, the plants were measured for F_v'/F_m', g_s, A, Φ_{PSII}, qP, E, aboveground biomass and yield.

Figure 5.1 Photo of burnt leaves in the heat treatment tunnel house, the day after a hot day in mid-February, when plants were 12 weeks old.

5.2.7 Year 2

In the second year of the outdoor study, four okra genotypes, (e.g. L2-11, L2-30, L3-1 and L4-48) were selected based on their performances and high yield in the control from year 1. The experiment was conducted in a 6 week period of heat (Table 5.1).

Table 5.1 Year 2, experimental plan.

Stage	Time of sowing (glass house)	Time of relocation (Tunnel house)	Time of measurement	Number of measurement
Flowering	Late-Dec 2015	4 weeks after sowing	every second week after relocation	3
Bud initiation	Mid-Jan 2016			

There were two sowing dates, three weeks apart. Plants were sown in late-December and early-January 2015-2016. In mid-February, three replicates of each genotype were moved into tunnel houses (control and heat) when the first set was at flowering stage (6 weeks old) and second set was at bud initiation stage (3 weeks old). The plants were kept inside the tunnel houses for 6 weeks. Plants were placed in 7 L pots filled with 8 mm composted pine bark, 3 mm composted Pine Bark and prop sand mixture (8:1:1) and supplemented at 0.4 kg m^{-3} with all trace elements, gypsum (1 kg m^{-3}), superphosphate (1 kg m^{-3}), KNO_3 (13% N) (0.25 kg m^{-3}), nitroform (38% N) (0.25 kg m^{-3}) and magrilime (1.5 kg m^{-3}). Plants were irrigated daily from 6am to 6pm at a rate of 400 mL h^{-1}; additionally, twice a week, a fertilizer solution formulated for growing commercial tomato and cucumber (Appendix 6) was applied through irrigation. Irrigation was always supplied in excess, so that there was runoff at the bottom of the pot, and on measurement days, water was supplied at 600 mL h^{-1} to prevent water deficiency. Excess water was collected using custom-built trays for collection of run-off, which were placed in sealed saucers. A tube sealed to the saucer collected run-off into a plastic container below the plant (Figure 5.2) and volume was measured in the morning, mid-day and late afternoon on measurement day. Additionally, soil water moisture was checked periodically (multiple times during a single day) using water moisture meter. Plants in the control tunnel house were placed in the first row of the west-side (opened sides) while in the heat, plants were placed in the middle row Figure 5.2 of the closed tunnel house for more protection against cooler outside temperatures. Side branches were tied to grow upwards instead of sideways to ensure uniform heat treatment of all plant branches, as in the previous experiment (Year 1), lower side branches had fruit production, since they grew horizontally in the cooler part of the tunnel house.

Physiological parameters (e.g. F_v'/F_m', g_s, A, Φ_{PSII}, qP and E) were assessed every two weeks, i.e. three times. Physiological assessments were conducted in weeks 2, 4 and 6. Agronomy parameters (e.g. above- and belowground biomass and yield) were assessed at week 6. Metabolic analyses were carried out using half a leaf per plant at weeks 2, 4 and 6, and using a 7-8 cm piece of shoot and some root material (washed with double deionised water) per plant at week 6. Samples were frozen immediately in liquid nitrogen, and subsequently freeze dried and extracted in the laboratory for identification and quantification of the most abundant carbohydrates. Additionally, k_{leaf} was measured at week 6.

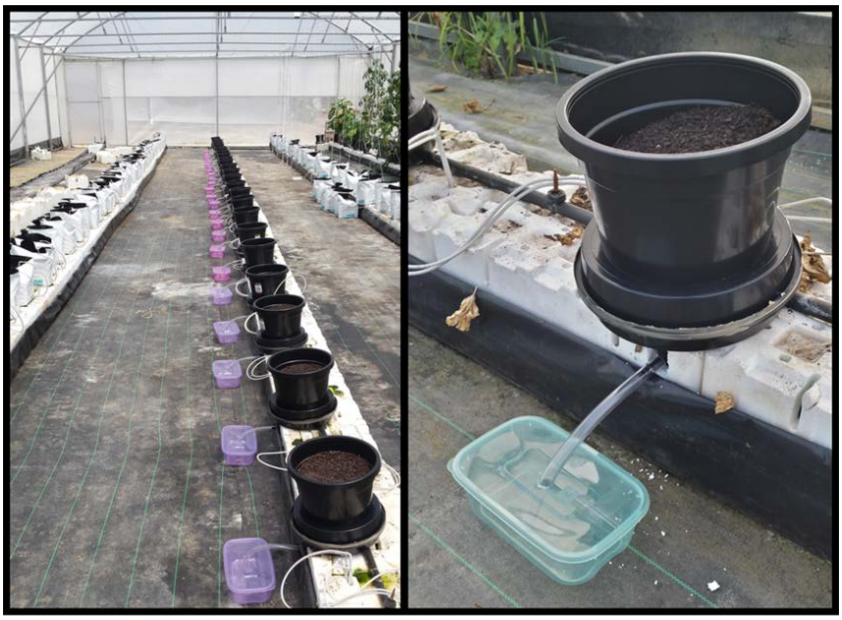

Figure 5.2 Tunnel house set-up with designed trays for run-off collection. Photo on the left shows the location of the experiment in middle of the heat tunnel house.

5.2.8 Statistical analysis

Data were subjected to a general analysis of variance (ANOVA) with level of significance measured at $P<0.05$ and a comparison of means were performed via the Fisher's unprotected LSD using GenStat 17th Edition software (VSN International Ltd, London, UK).

5.3 Results

5.3.1 Environmental parameters

On average, daytime temperature was 7-10°C warmer in the heat tunnel house with 10% higher RH compared to the control tunnel house, whereas night temperatures and RH were similar in both tunnel houses in year 1 and 2. During the experiment, average maximum/minimum temperature (ºC) in heat and control tunnel houses was 44/17 ºC and 34/16 ºC in Year 1 (2015) and 49/19°C and 42/18°C in Year 2 (2016). Maximum and minimum temperature in heat and control tunnel houses from 28th January 2015 (installation of logger) to 2nd April 2015 (end of Year 1) and from 1st January to 25th March 2016 is shown in Figure 5.3. On cloudy days, the temperature difference between the tunnel houses was strongly reduced (e.g. 2nd and 13th March 2015) and similar to outside temperatures. Inside the tunnel houses, photosynthetically active radiation was between 1500 and 2000 µmol m^{-2} s^{-1} on sunny days. There are some missing data for December 2015 and 6th to 14th February 2016 due to a logger error.

Figure 5.3 Maximum and minimum temperature (°C) in heat and control tunnel houses from 28th January to 2nd April 2015 and from 1st January to 25th March 2016.

5.3.2 Year 1

Average F_v'/F_m', Φ_{PSII}, qP, A, g_s, EL, dry matter and yield were assessed after 15 weeks of heat treatment (Appendix 7, Appendix 8 and Appendix 9) and statistical analyses (Table 5.2) applied for eight okra genotypes. In general, there was a genetic variation among the selection however this variation was not significant in all the parameters, only EL, DM and yield showed significant variation. The results of year 1 are presented in four sections, photosynthetic parameters, electrolyte leakage, dry matter and yield.

Table 5.2 ANOVA results for chlorophyll fluorescence in the light (F_v'/F_m'), efficiency of the open reaction centre (Φ_{PSII}), photochemical quenching (qP), photosynthesis rate (A), stomatal conductance (g_s), electrolyte leakage (EL), above ground dry matter (DM) and yield for eight okra genotypes grown in control and heat tunnel houses for 15 weeks.

		F_v'/F_m'	Φ_{PSII}	qP	A	g_s	EL	DM	Yield
Genotype	F	1.91	1.17	0.87	2	1.12	8.8	2.42	8.16
	P	0.101	0.348	0.538	0.086	0.377	<.001	0.041	<.001
Treatment	F	0.73	3.92	8.13	1.24	72.67	72.5	2.91	5.01
	P	0.4	0.056	0.008	0.273	<.001	<.001	0.098	0.034
Genotype* Treatment	F	1.54	2.15	1.85	1.75	1.61	5.53	0.77	2.02
	P	0.188	0.067	0.111	0.133	0.168	<.001	0.618	0.089

5.3.2.1 Photosynthetic parameters

In general, high temperature did not affect F_o' and F_m', which resulted in similar F_v'/F_m' in the control and heat, however there was a reduction in Φ_{PSII} (not significant) and qP (significant, p=0.008) under high temperature due to significant increase in F_s (p=0.032) (Table 5.3) and slight but not significant increase in F_m' (Figure 5.4). On average, there was a 12% qP reduction (significant, p=0.01) in all genotypes (except L4-48) under high temperature. Genotype L4-48 showed higher F_m' in the heat compared to the control (Appendix 7).

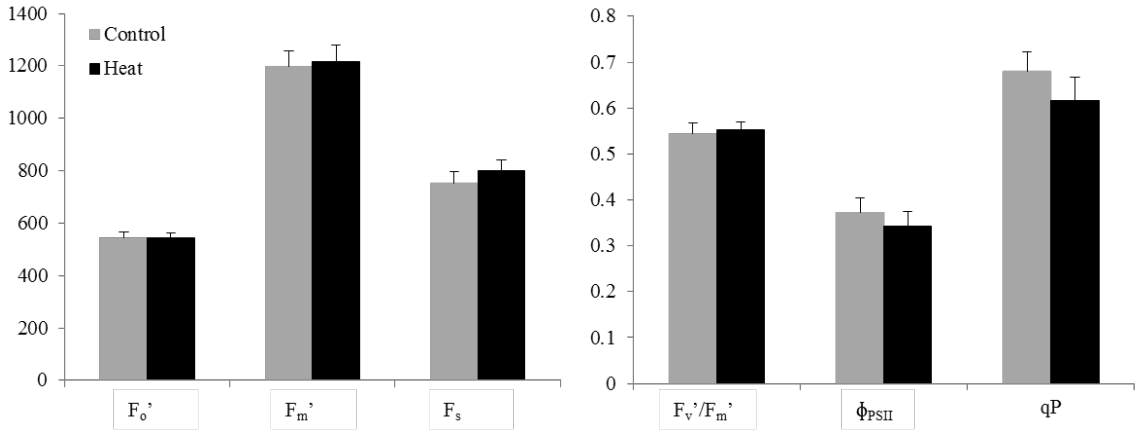

Figure 5.4 Average F_o', F_m', F_s, F_v'/F_m', Φ_{PSII} and qP of all eight okra genotypes (n = 24) grown in control and heat tunnel houses for 15 weeks in Year 1. Bars represent standard error (+SE).

Table 5.3 ANOVA results for initial fluorescence (F_o'), maximum fluorescence (F_m'), steady state of fluorescence (F_s), chlorophyll fluorescence in the light (F_v'/F_m'), efficiency of the open reaction center (Φ_{PSII}) and photochemical quenching (qP) for all 24 plants (8 genotypes, 3 replicates per genotype) in both tunnel houses (control and heat) for 15 weeks.

		F_o'	F_m'	F_s	F_v'/F_m'	Φ_{PSII}	qP
Treatment	F	0.00	0.42	4.88	0.60	3.27	7.32
	P	0.976	0.521	0.032	0.444	0.077	0.01

Although stomatal conductance increased significantly ($p<0.001$) in the heat and *A* was similar in both treatments (Figure 5.5), there was no significant genotypic variation (Table 5.2). Nevertheless, in the control, genotype L2-11, L2-30, L2-32, L3-1 and L3-58 showed higher g_s and *A* compared to the other three genotypes. In the heat, L4-31 showed the largest g_s where there was a 61% increase compared to the control. The largest difference between treatment was observed in L4-48 where g_s increased by 67% in the heat. These two genotypes (L4-31 and L4-48) also showed an increase in *A* under high temperature (Figure 5.5).

In the control, *A* was linearly related to g_s and fluorescence parameters (e.g. F_v'/F_m' and Φ_{PSII}) whereas under high temperature, *A* had a stronger relationship with fluorescence parameters (i.e. Φ_{PSII}, $R^2=0.90$, $p<0.001$) than g_s ($R^2=0.13$) (Figure 5.6).

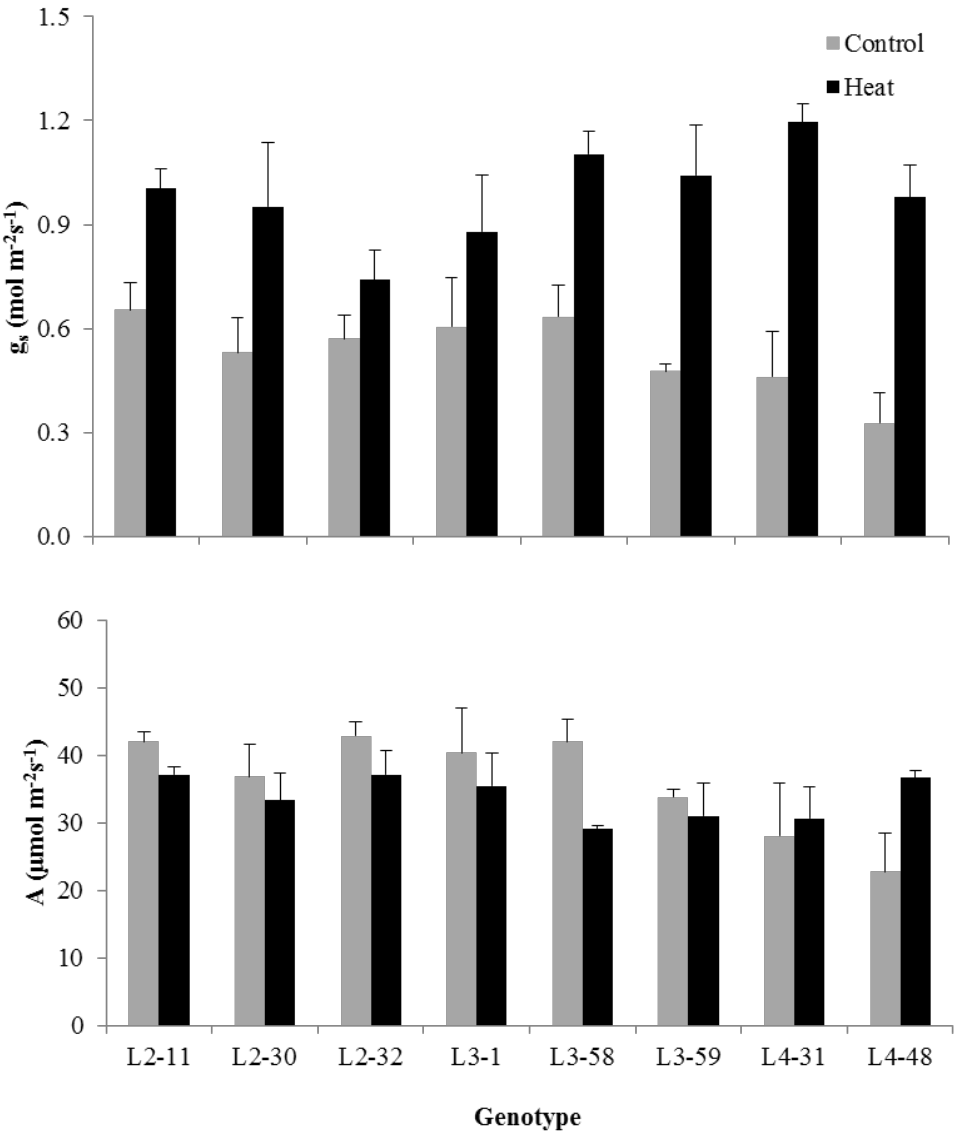

Figure 5.5 Average g_s and A for eight okra genotypes (n = 3) grown in control and heat tunnel houses for 15 weeks in Year 1. Bars represent standard error (+SE).

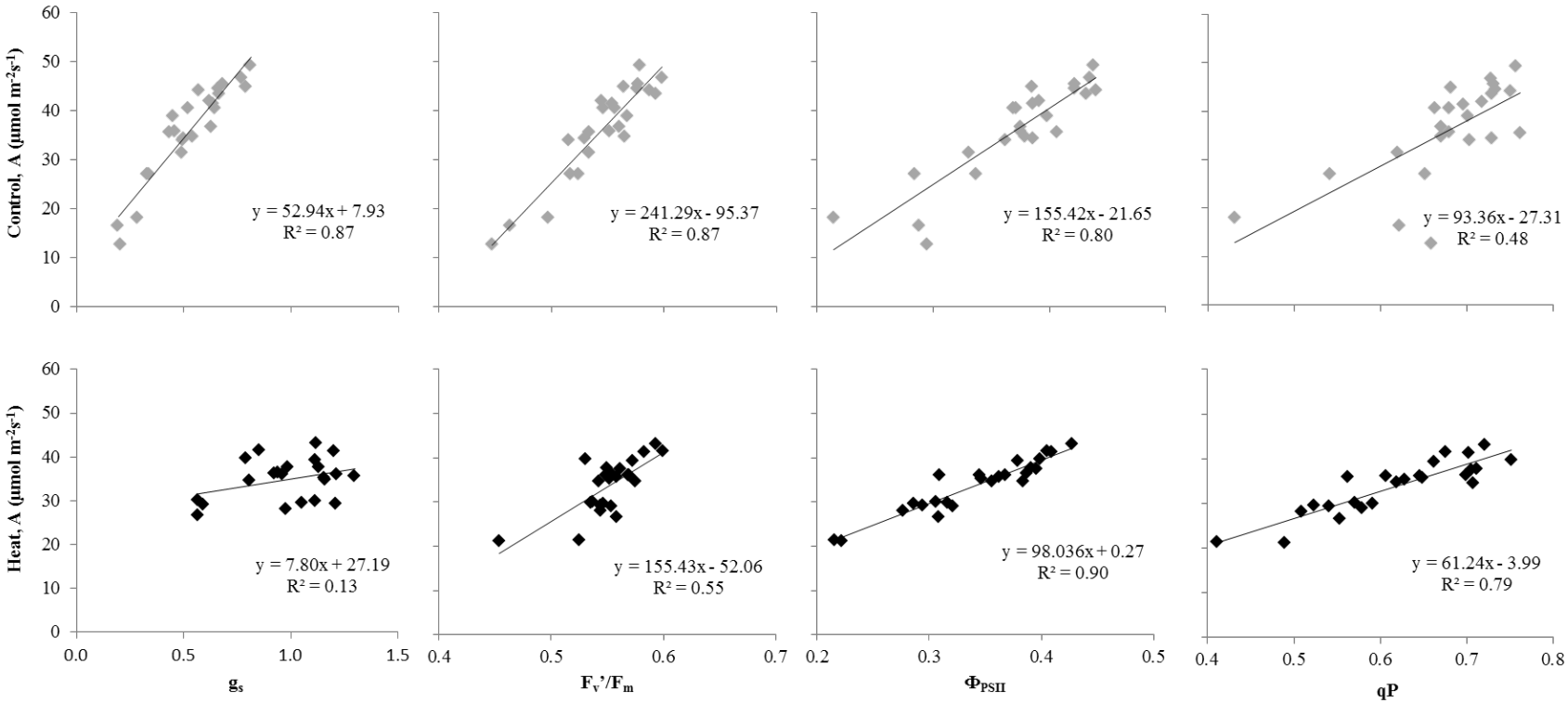

Figure 5.6 Linear regression between *A* and g_s, F_v'/F_m' and Φ_{PSII} in control (grey) and heat (black) treatments in year 1. Each point represents an individual plant.

5.3.2.2 Electrolyte leakage

In the heat, EL increased significantly ($p<0.001$) and was significantly different among genotypes under high temperature ($p<0.001$, Figure 5.7). In the heat, L4-31 showed 60% increase in EL followed by L2-11 and L3-59 (30%), whereas L2-30 had the lowest EL increase (1%) followed by L4-48 (5%).

5.3.2.3 Dry matter

There was no significant difference in dry matter between treatments. However, genotype variation was significant ($p=0.041$) where some genotypes (e.g. L2-30, L2-32 and L4-48) showing slightly reduced biomass and some genotypes (e.g. L2-11, L3-58 and L3-59) slightly increased biomass in the heat, except for genotype L3-1 and L4-31 which had 55% and 24% dry matter increase in the heat compared to the control respectively (Figure 5.8).

5.3.2.4 Yield (number of fruit)

There was a significant main effect of genotype and treatment, but no Genotype*Treatment interaction. This means that heat generally reduced fruit numbers significantly ($p=0.034$), and genotypes differed in overall yield. This reduction was observed in most genotypes, except for L2-30 and L2-32. As side branches were not tied up or pruned, most of the fruit in the heat grew on side branches which were close to the ground, where temperature was cooler and similar to the control. Percentages of fruit from side branches are shown in Figure 5.9. Genotype variation was observed in the control where L2-11 had the highest yield with an average of 46 fruits followed by L4-48 (34 fruits) and L3-1 (21 fruits).

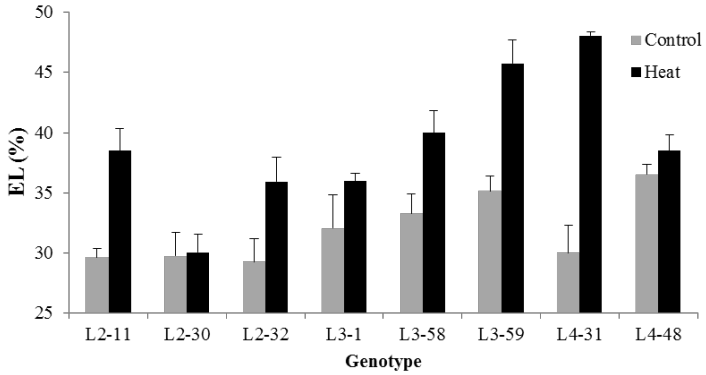

Figure 5.7 Average electrolyte leakage (EL) for eight okra genotypes (n = 3) grown in control and heat tunnel houses for 15 weeks in Year 1. Bars represent standard error (+SE).

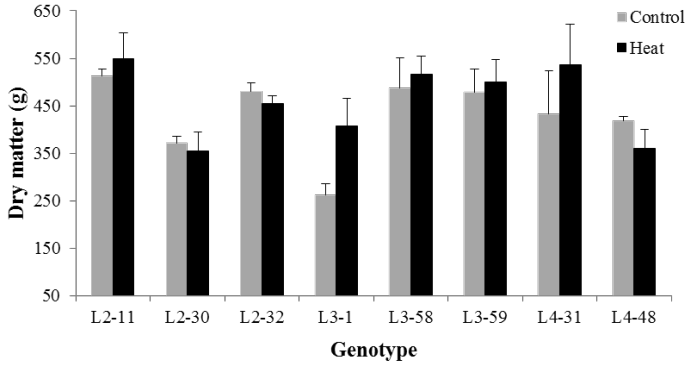

Figure 5.8 Average above ground dry matter (g) for eight okra genotypes (n = 3) grown in control and heat tunnel houses for 15 weeks in Year 1. Bars represent standard error (+SE).

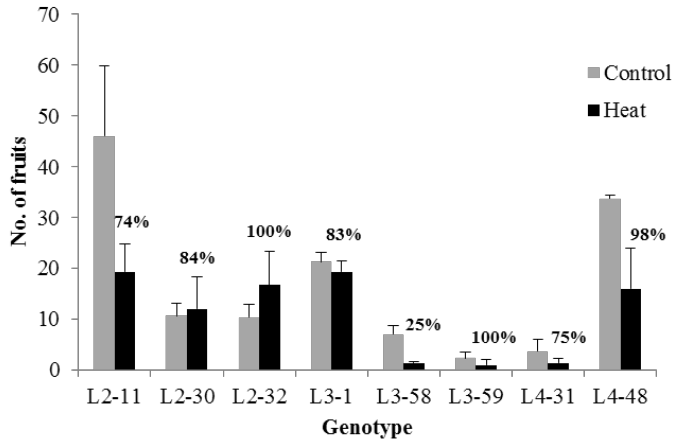

Figure 5.9 Average number of fruits (yield) for eight okra genotypes (n = 3) grown in control and heat tunnel houses for 15 weeks in Year 1. Most fruits in the heat were harvested from side branches close to the ground, and percentage of those fruit is shown on the graph. Bars represent standard error (+SE).

5.3.3 Year 2

Average F_v'/F_m', Φ_{PSII}, qP, A, g_s, E, leaf temperature (T_{leaf}), EL, k_{leaf}, dry matter and yield were assessed over a 6 week period. Statistical analyses are presented here (Table 5.4 Table 5.5 and Table 5.7) and the mean values can be found in Appendix 10, Appendix 11, Appendix 12 and Appendix 13. The results of year 2 are presented in the following five sections photosynthetic parameters (5.3.3.1), electrolyte leakage (5.3.3.2), soluble carbohydrates (5.3.3.3), dry matter (5.3.3.4) and yield (5.3.3.5).

Table 5.4 ANOVA for chlorophyll fluorescence (F_v'/F_m'), photosynthesis (A), efficiency of the open reaction center (Φ_{PSII}), photochemical quenching (qP), stomatal conductance (g_s), transpiration (E), leaf temperature (T_{leaf}) and electrolyte leakage (EL) for four okra genotypes (n=3) at flowering and bud initiation in the control and heat at week (W) 2, 4 and 6.

		F_v'/F_m'	A	Φ_{PSII}	qP	g_s	E	T_{leaf}	EL
Genotype (G)	F	0.3	0.52	1.01	0.58	1.33	1.94	0.35	8.13
	P	0.824	0.671	0.391	0.63	0.27	0.129	0.789	<.001
Treatment (T)	F	0.48	20.93	3.39	8.74	64.85	830.03	721.87	1.52
	P	0.49	0.1	0.069	0.4	<.001	<.001	<.001	0.221
Developmental stages (DS)	F	8.57	19.33	65.57	11.59	3.55	14.83	0.05	0.21
	P	0.004	<.001	<.001	<.001	0.063	<.001	0.822	0.649
Week (W)	F	2.36	0.31	1.75	1.14	2.58	35.81	60.74	18.29
	P	0.1	0.734	0.179	0.323	0.081	<.001	<.001	<.001
G*T	F	0.53	0.29	1	0.87	0.6	0.67	0.59	0.13
	P	0.663	0.832	0.395	0.459	0.617	0.574	0.624	0.943
G*DS	F	0.46	0.43	0.43	0.08	2.67	0.32	0.8	0.91
	P	0.713	0.733	0.732	0.971	0.052	0.809	0.495	0.44
G*W	F	0.85	1.19	0.53	0.66	1.5	0.75	0.75	2.31
	P	0.537	0.317	0.784	0.682	0.187	0.611	0.612	0.04
T*DS	F	1.52	0.43	0.03	0.33	0.36	0.4	1.5	6.95
	P	0.22	0.733	0.858	0.568	0.551	0.53	0.224	0.01
T*W	F	1.12	2.91	0.23	0.34	11.99	26.66	11.38	6.48
	P	0.33	0.06	0.798	0.713	<.001	<.001	<.001	0.002
DS*W	F	1.77	0.8	2.19	4.05	1.16	1.77	2.2	2.84
	P	0.176	0.451	0.118	0.021	0.318	0.177	0.116	0.064

Table 5.5 ANOVA for leaf hydraulic conductance (k_{leaf}) (average top and mid canopy) for four okra genotypes (n=3) at flowering and bud initiation in the control and heat at week 6.

		k_{leaf}
Genotype (G)	F	0.57
	P	0.636
Treatment (T)	F	99.17
	P	<.001
Developmental stages (DS)	F	2.4
	P	0.133
G*T	F	0.88
	P	0.463
G*DS	F	3.77
	P	0.022
T*DS	F	0.35
	P	0.562

5.3.3.1 Photosynthetic parameters

F_v'/F_m' was significantly lower at bud initiation in both control and heat (p=0.004), but there was no significant treatment effect (Table 5.4, Figure 5.10). Genotype L2-30 had relatively low average value, in the control and relatively high value in high temperature at both stages (flowering and bud initiation) in week 6, while all other genotypes at both stages had similar responses in both control and heat (Appendix 10).

In the heat, plants showed lower Φ_{PSII} only at week 2, but it was similar in the control and heat at week 4 and 6 (Figure 5.10), however, statistically there was no treatment effect (Table 5.4). At bud initiation, Φ_{PSII} was significantly (p<0.001) lower than flowering in both control and heat.

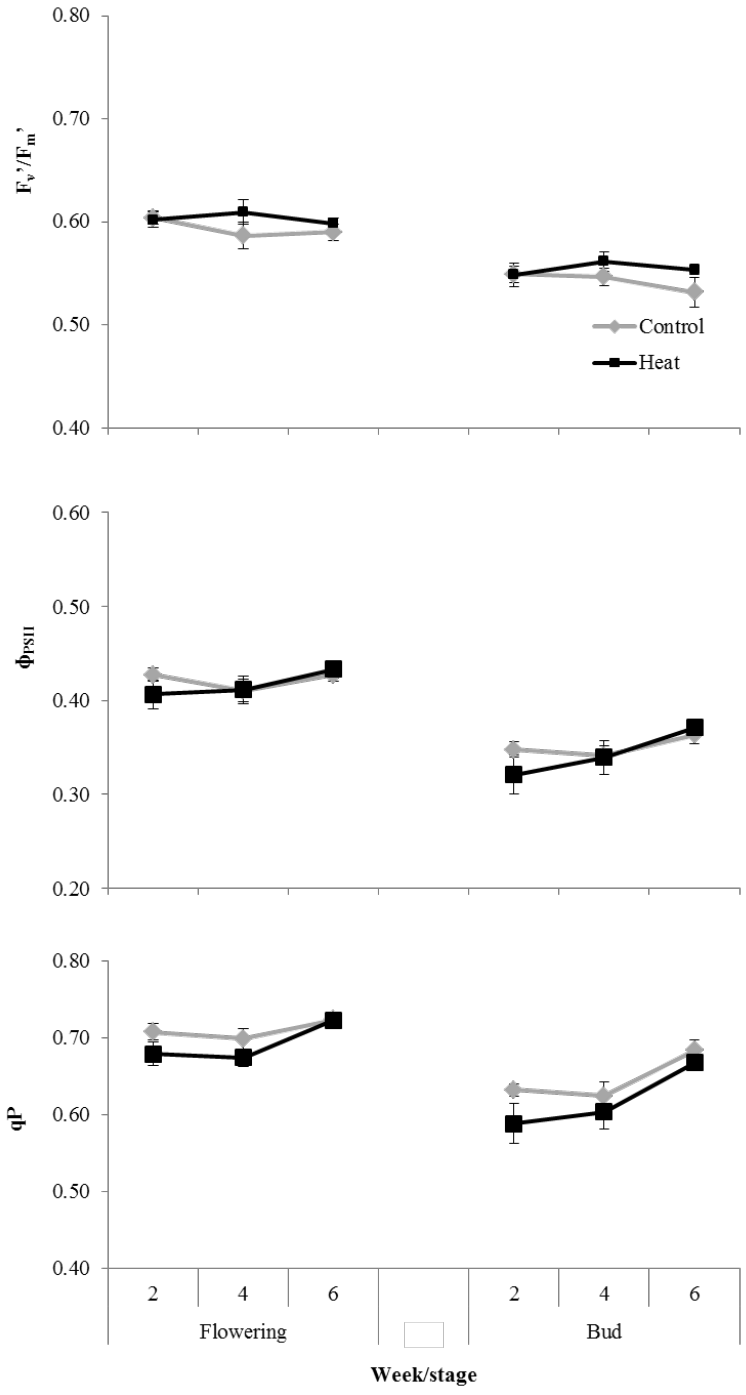

Figure 5.10 Average chlorophyll fluorescence (F_v'/F_m'), efficiency of the open reaction center (Φ_{PSII}) and photochemical quenching (qP) of all four okra genotypes (n = 12) at flowering and bud initiation in the control and heat at week 2, 4 and 6 in Year 2. Bars represent standard error (±SE).

*q*P significantly (p=0.021) increased over time in the heat at both stages of development (Figure 5.10). Correspondingly to $\Phi_{PSII,}$ *q*P was significantly (p<0.001) lower at bud initiation than flowering with no significant variation between genotypes.

Under high temperature, photosynthesis was significantly lower at the beginning and then increased (Figure 5.11), resulting in no significant difference between treatments at the end of the experiment. *A* was significantly higher at flowering compared to bud initiation in both control and heat (Figure 5.11) with no significant variation between genotypes (Table 5.4).

In the heat, g_s was significantly (p<0.001) higher than in the control and also significantly (p<0.001) increased over time resulting in a drop in leaf temperature (T_{leaf}) between weeks 2 and 4, and constant leaf temperature thereafter (Figure 5.11). Average leaf temperature was around 31°C in the control (similar to air temperature) whereas in the heat, T_{leaf} was 36°C where air temperature was c. 10°C higher. Due to open stomata, E was almost double in the heat compared to the control (p<0.001) and significantly (p<0.001) increased over time (Table 5.4 and Figure 5.11). Although there was no significant difference between developmental stages in g_s, *E* was significantly lower at bud initiation stage (p<0.001). There was a reduction in g_s and *E* at bud initiation at week 6 in the control (Figure 5.11). At week 6, *E* doubled under high temperature (Figure 5.12) resulting in higher k_{leaf} (Figure 5.13).

In the control, *A* was linearly related particularly to g_s (R^2 = 0.90) and F_v'/F_m' (R^2 = 0.81), as well as Φ_{PSII} (R^2 = 0.61) (p<0.001), whereas under high temperature *A* had a stronger relationship with Φ_{PSII} and *q*P (R^2 of 0.76 and 0.78) than g_s (R^2 = 0.48, Figure 5.14).

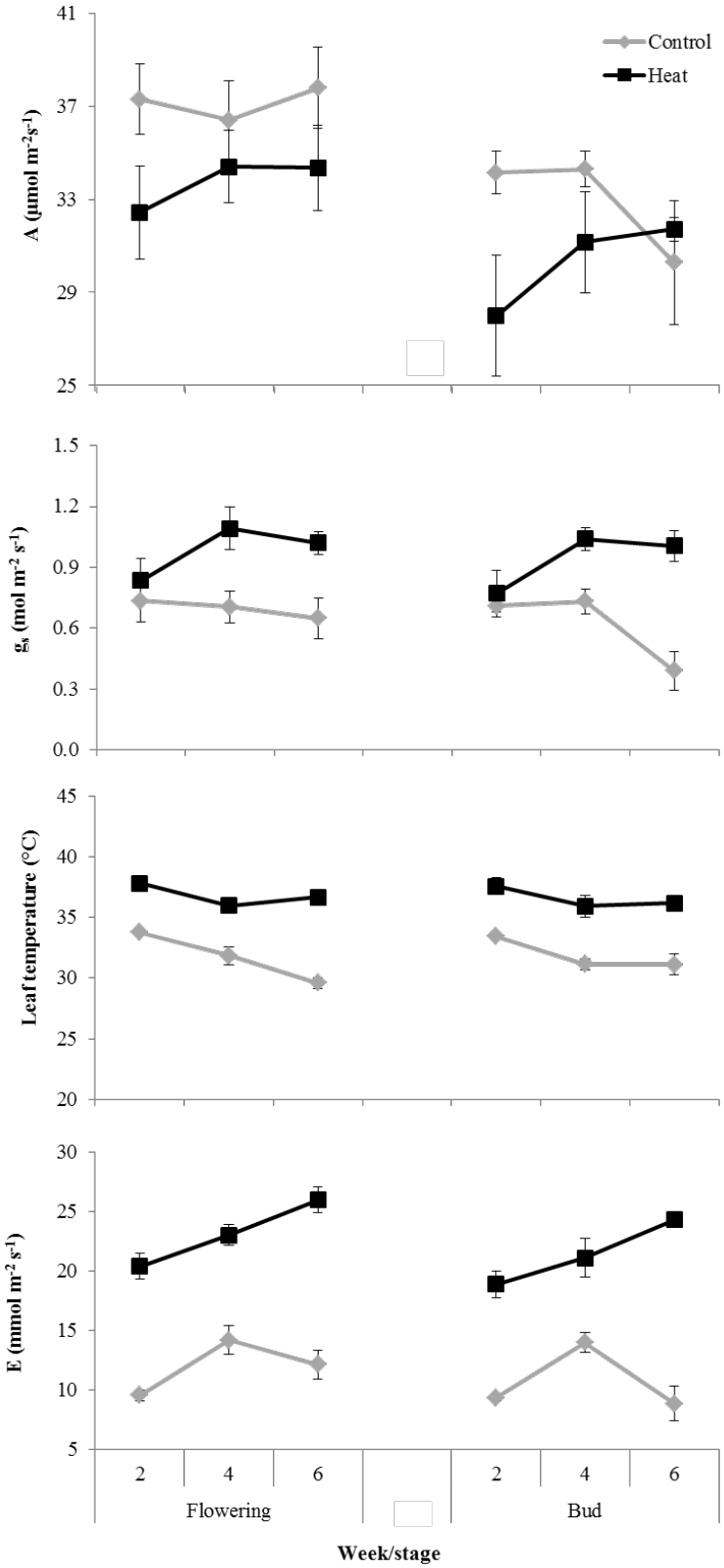

Figure 5.11 Average photosynthesis (*A*), stomatal conductance (g_s), leaf temperature and transpiration (*E*) of all four okra genotypes (n = 12) at flowering and bud initiation in the control and heat at week 2, 4 and 6 in Year 2. Bars represent standard error (±SE).

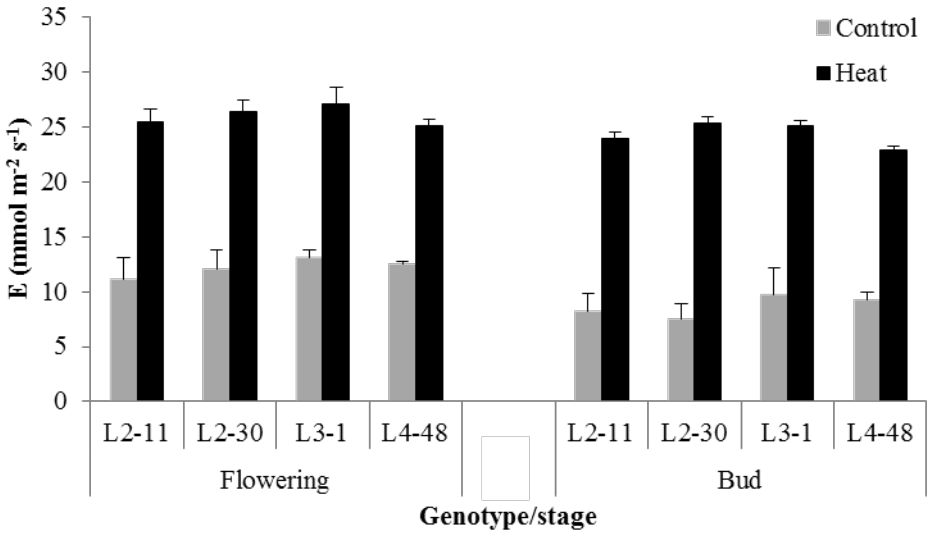

Figure 5.12 Average transpiration rate (*E*) for four okra genotypes (n = 3) at flowering and bud initiation in the control and heat at week 6 in Year 2. Bars represent standard error (+SE).

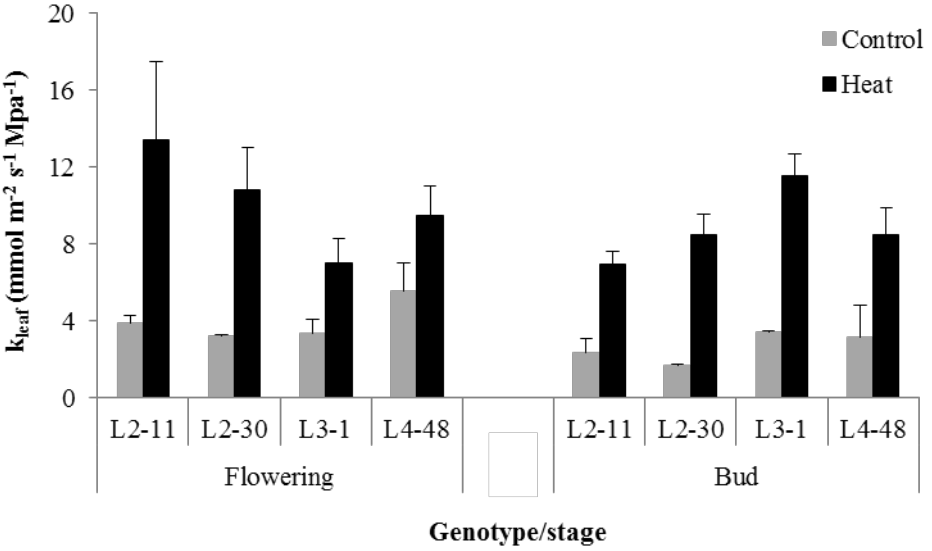

Figure 5.13 Average leaf hydraulic conductance (k_{leaf}) of top and mid canopy leaves for four okra genotypes (n = 6) at flowering and bud initiation in the control and heat tunnel houses at week 6 in Year 2. Bars represent standard error (+SE).

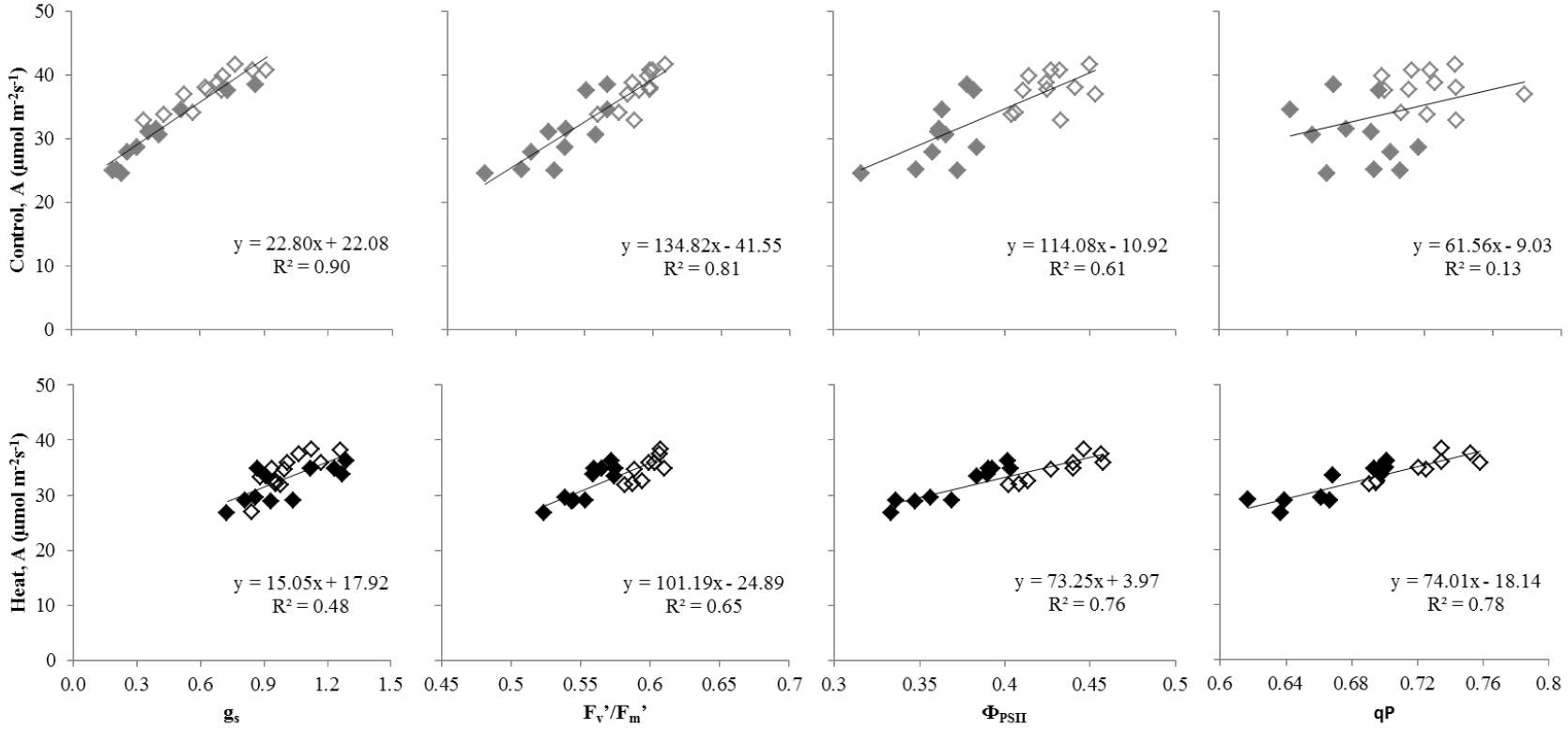

Figure 5.14 Linear regression between *A* and g_s, F_v'/F_m', Φ_{PSII} and *q*P in the control (grey) and heat (black) treatments at week 6 in year 2. Filled and open symbols are representative values at bud initiation and flowering stages respectively. Each point represents an individual plant.

5.3.3.2 Electrolyte leakage

EL reduced over time with significant Treatment*Week interaction (p=0.002) at both flowering and bud initiation. In the heat, EL was significantly higher at bud initiation (p=0.01). There was a genetic variation in EL regardless of treatment where L4-48 showed the highest leakage at both stages in both control and heat compared with the other genotypes (Figure 5.16).

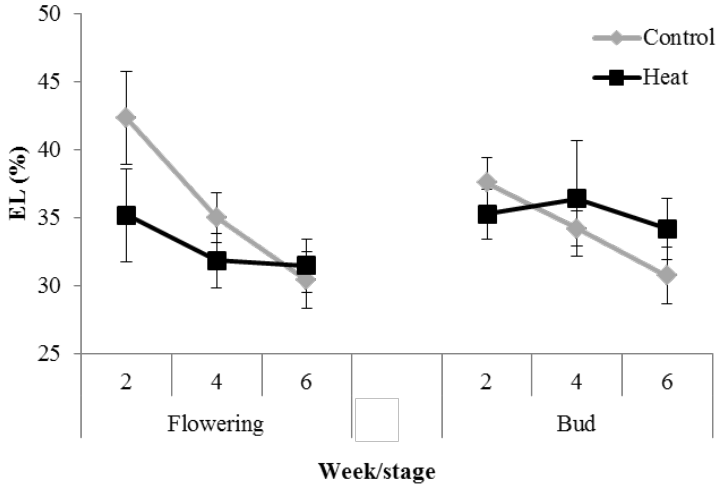

Figure 5.15 Average electrolyte leakage (EL) of all four okra genotypes (n = 12) at flowering and bud initiation grown in the control and heat tunnel houses at week 2, 4 and 6, in Year 2. Bars represent standard error (±SE).

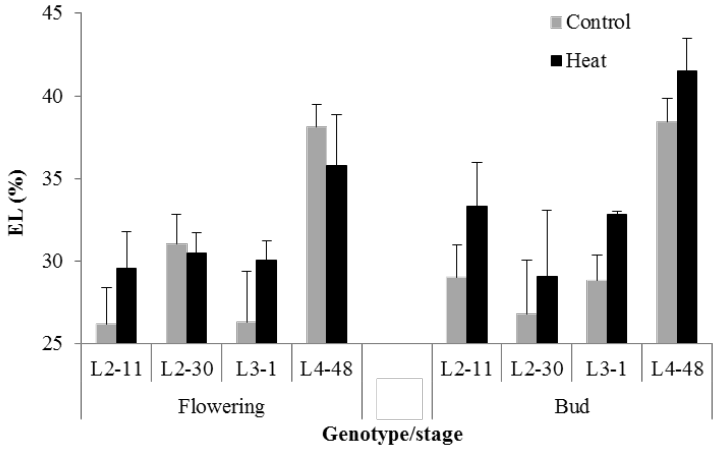

Figure 5.16 Average electrolyte leakage (EL) for four okra genotypes (n = 3) at flowering and bud initiation in the control and heat at week 6 in Year 2. Bars represent standard error (+SE).

5.3.3.3 Soluble carbohydrates (fructose, glucose and sucrose)

5.3.3.3.1 Whole plant sugar concentration

Average concentration of fructose, glucose and sucrose in the whole plant decreased at both stages for all 4 genotypes in the heat (Figure 5.17). Concentration of all sugars decreased in all the genotypes at both stages, however the highest sugar concentration (fructose, glucose and sucrose) at both stages and in both treatments was found in L2-30 (Figure 5.17). At flowering, this reduction was 49.8%, 48.6% and 46.4% for fructose, glucose and sucrose respectively. The reduction was slightly higher at bud initiation, where fructose, glucose and sucrose were reduced by 64.2%, 64% and 52.9% respectively (Figure 5.18). At bud initiation, plants had significantly ($p<0.001$) lower sucrose concentration (Table 5.6), whereas the concentration of fructose and glucose were similar between the stages in both control and heat (Figure 5.18).

Table 5.6 ANOVA for sugar concentrations (Fru: fructose, Glu: glucose and Suc: sucrose) in the whole plant as well as in leaf, shoot and root for four okra genotypes at flowering and bud initiation in the control and heat at week 6 in year 2.

		Whole plant			Leaf			Shoot			Root		
		Fru	**Glu**	**Suc**	**Fru**	**Glu**	**Suc**	**Fru**	**Glu**	**Suc**	**Fru**	**Glu**	**Suc**
Genotype (G)	F				5.79	3.23	0.48	7.7	7.91	0.99	4.96	5.29	1.85
	p				0.003	0.037	0.702	<.001	<.001	0.412	0.007	0.005	0.162
Treatment (T)	F	37.25	34.51	101.15	30.51	20.45	0.05	853.44	717.23	410.02	13.5	14.23	9.82
	p	<.001	<.001	<.001	<.001	<.001	0.833	<.001	<.001	<.001	0.001	<.001	0.004
Developmental stage (DS)	F	0.43	0	22.93	0.01	0.24	1.14	4.59	2.43	9.45	22.38	13.87	32.58
	p	0.525	0.998	<.001	0.943	0.627	0.294	0.041	0.131	0.005	<.001	<.001	<.001
G*T	F				3.99	3.09	0.23	6.26	7.41	0.75	3.76	4.27	1.45
	p				0.017	0.042	0.874	0.002	<.001	0.533	0.022	0.014	0.251
G*DS	F				0.43	0.38	0.25	0.04	0.24	0.13	1.17	0.83	0.35
	p				0.734	0.765	0.861	0.99	0.869	0.94	0.339	0.487	0.792
T*DS	F	0.67	1.24	0.47	0.01	0.01	3.96	19.34	20	1.77	0.6	0.02	0.74
	p	0.429	0.288	0.506	0.922	0.924	0.056	<.001	<.001	0.194	0.445	0.893	0.399

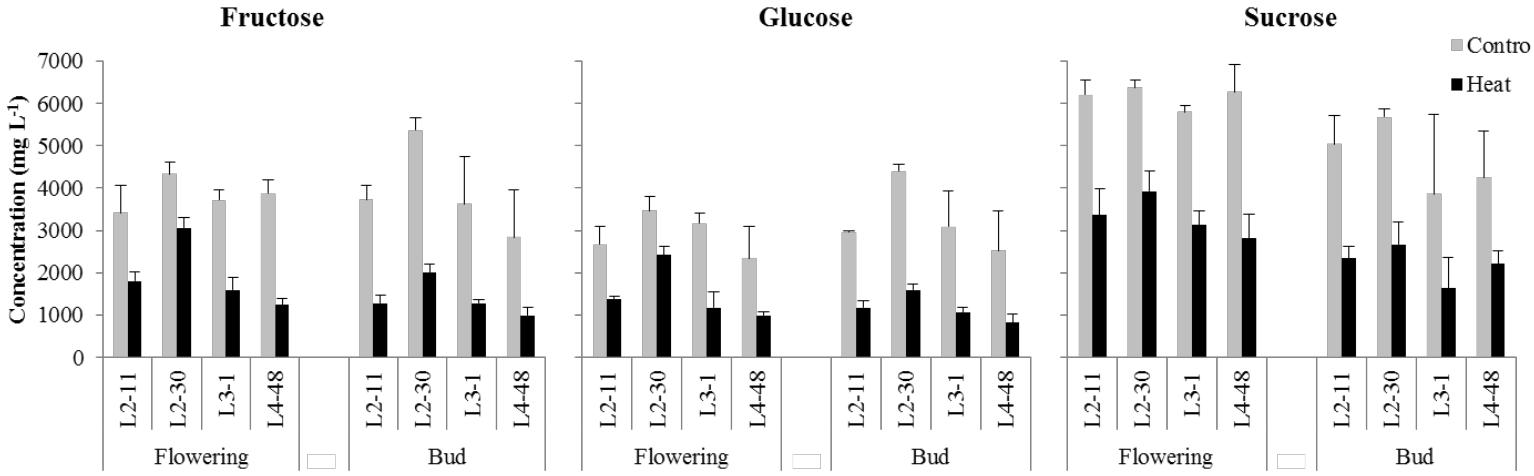

Figure 5.17 Average plant (leaf, shoot and roots) sugar concentrations (fructose, glucose and sucrose) (mg L^{-1}) for four genotypes (n = 3) at flowering and bud initiation in the control and heat, in Year 2. Bars represent standard error (+SE).

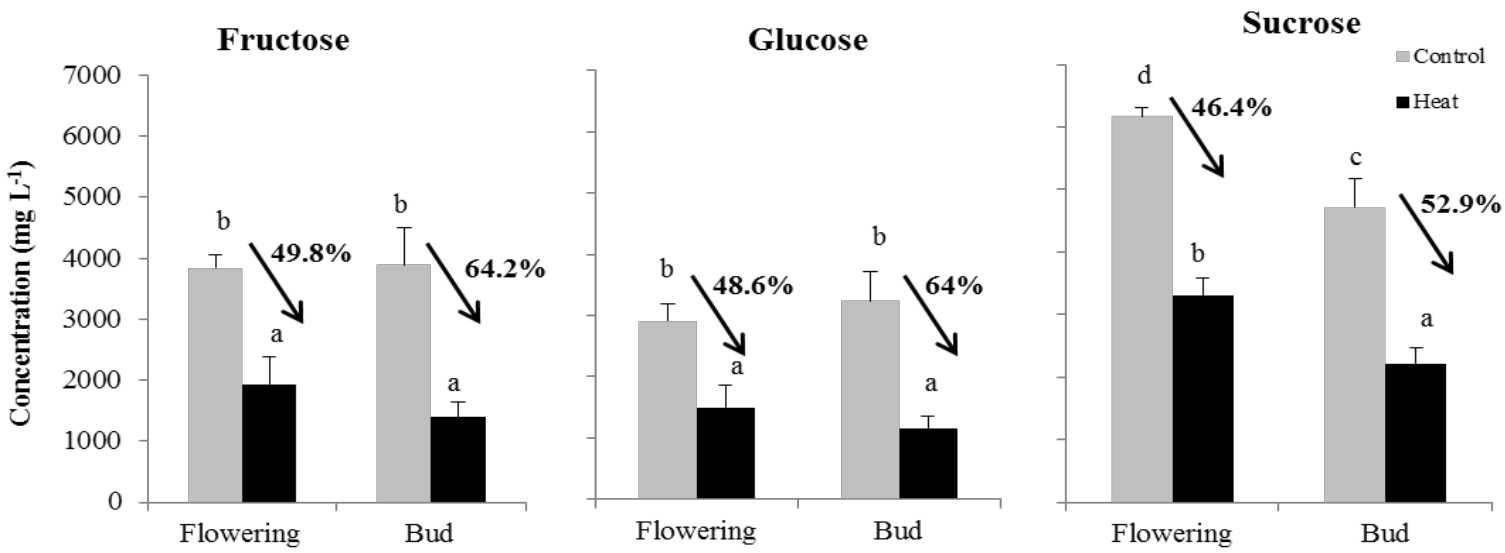

Figure 5.18 Average plant (leaf, shoot and roots) sugar concentrations (fructose, glucose and sucrose) (mg L^{-1}) at flowering and bud initiation in the control and heat, in Year 2. Histograms represent the average of sugar in all four genotypes (n = 12) and bars represent standard error (+SE).

5.3.3.3.2 Leaf, shoot and root soluble carbohydrate concentration

Average concentrations of the soluble carbohydrates fructose, glucose and sucrose were assessed, specifically at week 2 (Appendix 14) and at week 6 for leaves, and at week 6 for shoots and roots at completion of the experiment (Appendix 15, Appendix 16, Appendix 17).

In the control, sugar concentrations were higher in shoots compared to leaves and roots, whereas under high temperature, sugar concentrations decreased severely in the shoots. In the heat, carbohydrate concentrations were generally higher in leaves and roots at both stages, except for sucrose concentration at bud initiation (Figure 5.19).

In the leaves, concentration of fructose and glucose were significantly ($p<0.001$) higher in the heat, but no difference between stages was observed either in the control or in the heat. In contrast, sucrose concentration, which was 5 times higher than the other sugars in the control, did not show significant changes in the heat; however sucrose concentration was higher at flowering stage compared to bud initiation under high temperature (Figure 5.19).

In the shoots, higher concentrations of fructose and glucose were observed at bud initiation in the control whereas sucrose concentration was lower at bud initiation compare to flowering. However under high temperature there was no significant difference between stages (Figure 5.19).

In the roots, sugar concentration increased in the heat at both stages with higher concentration at the flowering stage. Sucrose concentration was higher at both stages and in both control and heat compare to fructose and glucose (Figure 5.19).

Comparison between leaf sugar concentration at week 2 and 6 showed there was a significant increase in both control (Figure 5.20) and heat (Figure 5.21) at week 6. However, there was no difference in sugar concentrations between stages in both treatments except for glucose concentration in the control at week 2 (higher concentration at flowering) (Figure 5.20) and sucrose concentration which was lower at bud initiation at both week 2 and 6 in the heat (Figure 5.21).

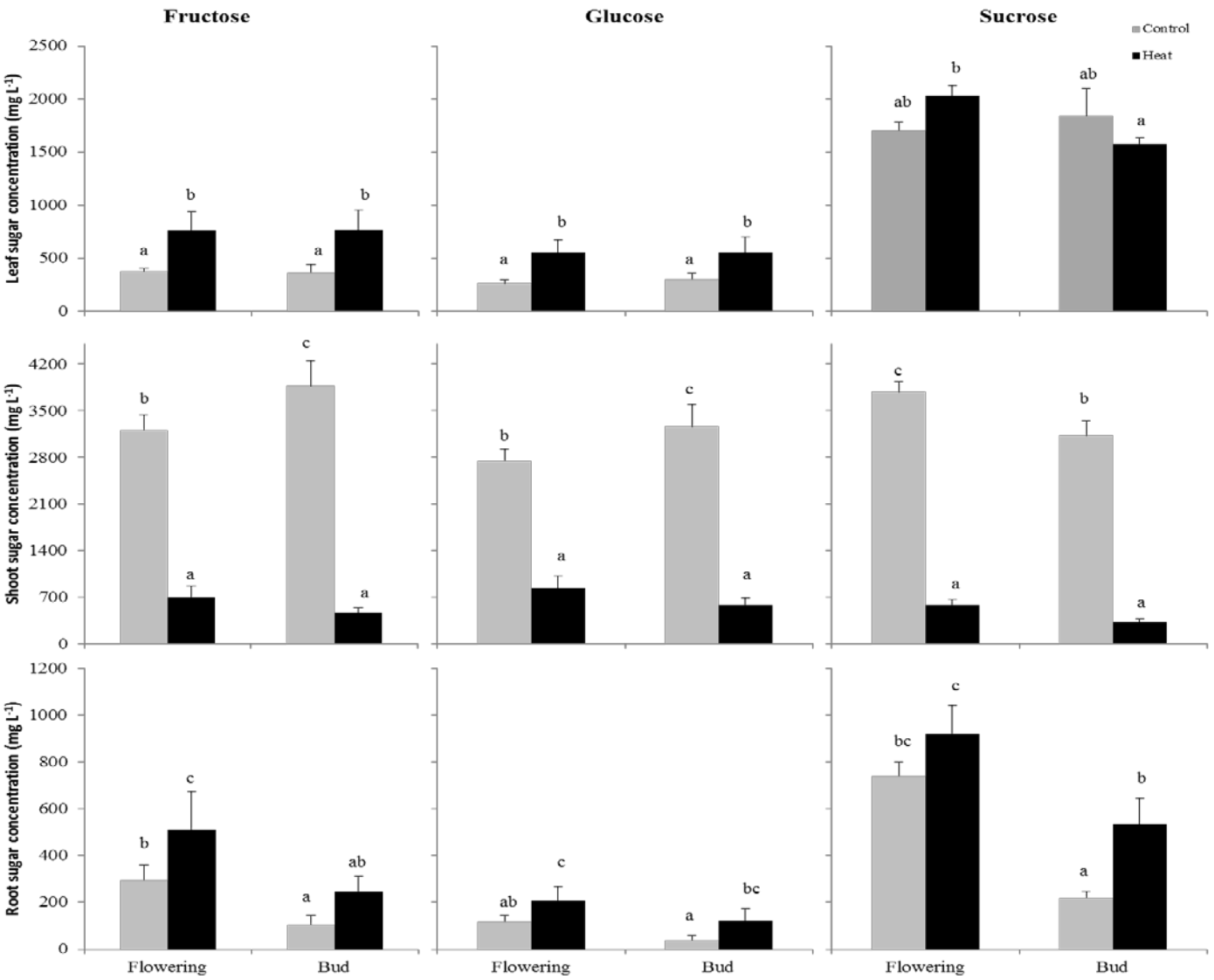

Figure 5.19 Concentrations of fructose, glucose and sucrose (mg L^{-1}) in leaves, shoots and roots at the end of experiment (week 6), in Year 2. Each histogram represents the average value of all four genotypes (n= 12) and bars represent standard error (+SE).

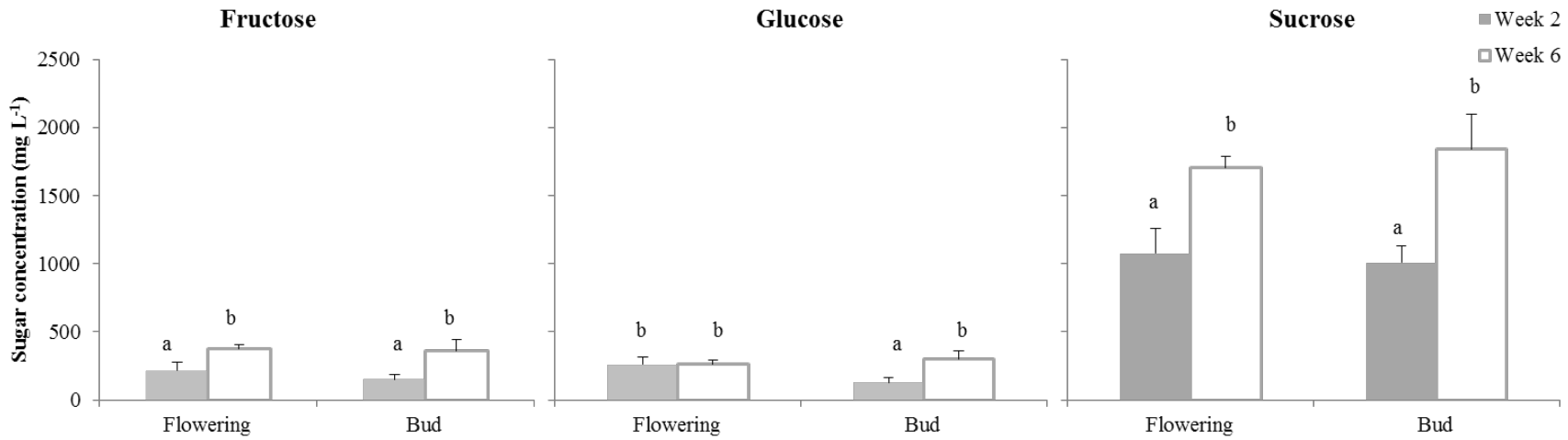

Figure 5.20 Sugar concentrations (fructose, glucose and sucrose (mg L^{-1}) in leaves at week 2 and 6 in the control. Each histogram represents the average value of four genotypes (n= 12) and bars represent standard error (+SE).

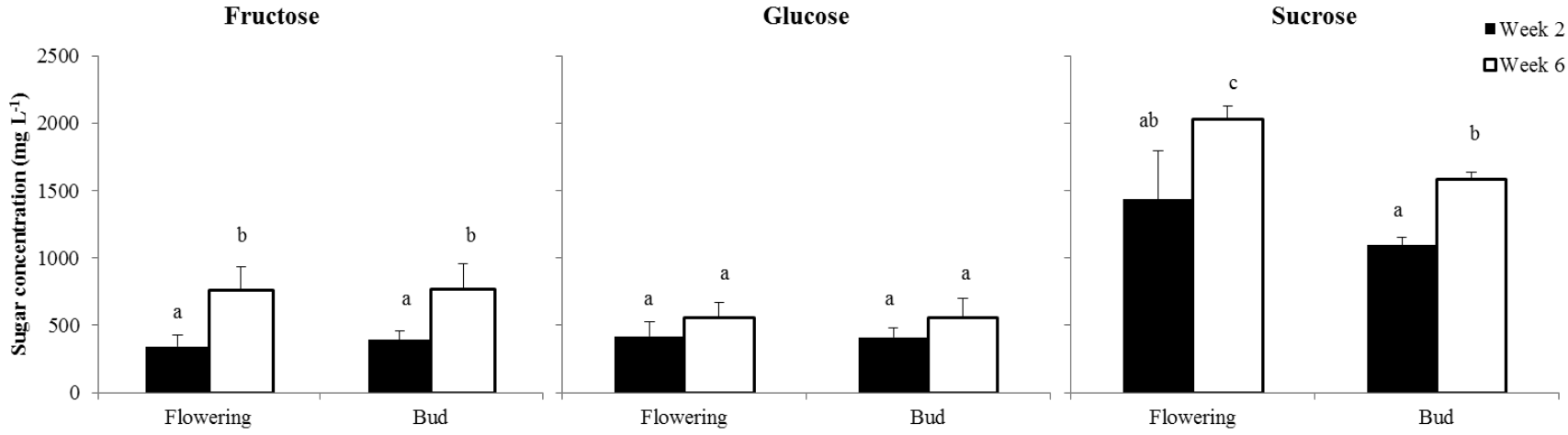

Figure 5.21 Sugar concentrations (fructose, glucose and sucrose (mg L^{-1}) in leaves at week 2 and 6 in the heat. Each histogram represents the average value of four genotypes (n= 12) and bars represent standard error (+SE).

5.3.3.4 Dry weight

Both above and below ground dry matter significantly ($p<0.001$) reduced under high temperature, and there was significantly ($p<0.001$) lower dry matter (above and below ground) at bud initiation as they had less time to grow (Table 5.7). Genotype variation was observed in below ground dry matter, where L4-48 had more root biomass compared to the other genotypes (Figure 5.22).

Table 5.7 ANOVA for above and below ground dry matter and yield for four okra genotypes at flowering and bud initiation in the control and heat after 6 weeks in Year 2.

		Above ground	**Below ground**	**Yield**
Genotype (G)	F	0.29	9.41	21.92
	p	0.835	<.001	<.001
Treatment (T)	F	17.93	42.21	163.15
	p	<.001	<.001	<.001
Developmental stages (DS)	F	20.45	13.46	29.66
	p	<.001	<.001	<.001
G*T	F	0.46	1.13	21.03
	p	0.711	0.351	<.001
G*DS	F	0.2	1.28	5.19
	p	0.897	0.299	0.005
T*DS	F	0.14	0.19	38.91
	p	0.713	0.668	<.001

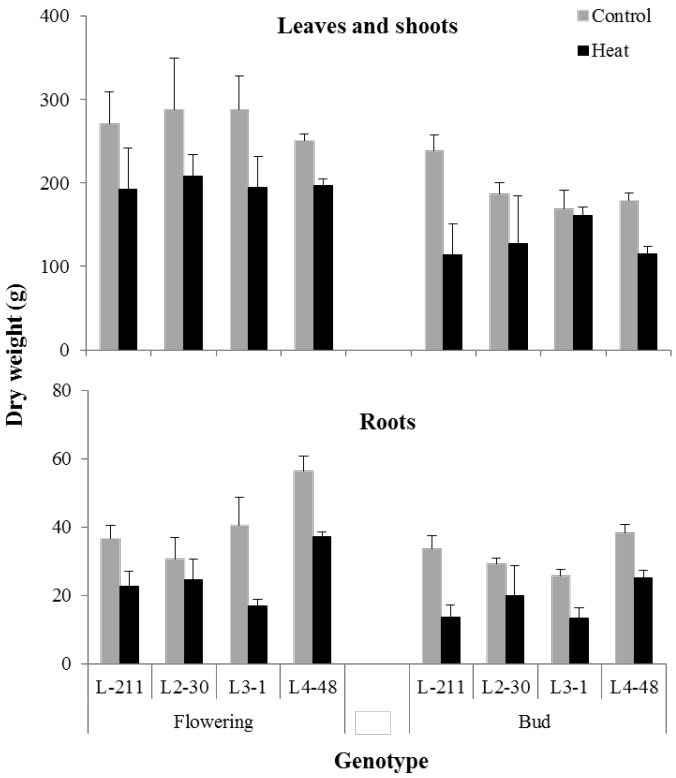

Figure 5.22 Average above (leaves and shoots) and below (roots) ground dry matter (g) for four okra genotypes (n = 3) at flowering and bud initiation grown in the control and heat tunnel houses at week 6 in Year 2. Bars represent standard error (+SE).

5.3.3.5 Yield (total number of fruit)

Under high temperature, the total number of fruit decreased dramatically (Figure 5.23). Yield was significantly ($p<0.001$) lower at bud initiation in both control and heat due to the 3 weeks age difference (Table 5.7). Among the genotypes, L3-1 had the highest yield at the flowering stage in the heat, and the highest at both stages in the control (Figure 5.23).

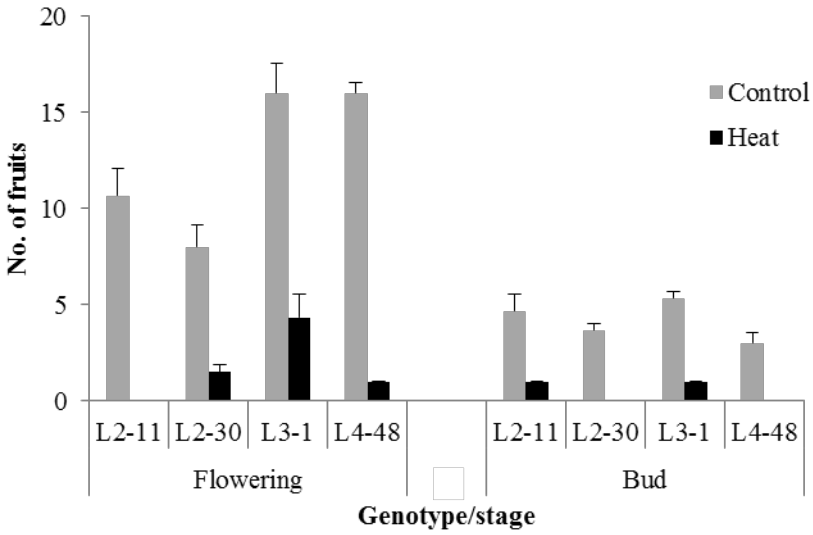

Figure 5.23 Average number of fruits for four okra genotypes at flowering and bud initiation grown in the control and heat tunnel houses after 6 weeks in Year 2. Bars represent standard error (+SE).

5.4 Discussion

Increased temperatures due to global warming have become more frequent and have negatively affected growth and development of tropical and subtropical crops, including okra, where it is a major factor in yield reduction (Wahid et al., 2007, Hatfield et al., 2011). Depending on the growth or developmental stage, crop species are characterized by different cardinal temperatures; for example, optimum temperatures for vegetative and reproductive growth in cotton are 37ºC and 28-30ºC, respectively (Hatfield et al., 2011). Plant growth is driven by photosynthesis which is highly sensitive to elevated temperatures, and a few hours of high temperature is enough to affect the photosynthetic apparatus (Camejo et al., 2005, Ding et al., 2016). Yet, plants use different mechanisms to acclimate or avoid stresses to survive when they are exposed to long-term high temperature (Hasanuzzaman et al., 2013). Okra demonstrated a tendency to acclimate after 2 weeks of growing at 45ºC (chapter 3). A 6-hours heat shock (45ºC) had a negative impact on the photosynthetic apparatus of non-acclimated plants (e.g. F_v'/F_m' reduced in the heat), whereas a 2-week heat treatment triggered a physiological acclimation response where heat-adapted plants showed improved physiological responses (F_v'/F_m', g_s, A and Φ_{PSII}).

When assessing okra in the production setting (tunnel houses), okra was able to adjust photosynthetic functionality even for a long-term heat exposure of an average maximum temperature of 44ºC in year 1 and 49°C in year 2. F_v'/F_m' became similar to the control, within two weeks of translocation to the heat tunnel house, so that there was no discernible treatment effect at the end of the experiment. Yamane et al. (1997) assumed irreversible detachment of light harvesting complexes by assessing changes in F_o' and F_m', which could not be confirmed in okra after 15 weeks of heat treatment. This suggest that okra shows changes in chlorophyll fluorescence to a short-term heat exposure, but can adapt to longer periods of heat stress.

After a 15-week of heat treatment (year 1) there was a significant reduction in Φ_{PSII} and qP, despite the increase over time observed in year 2. This reduction was due to a significant increase in F_s in the heat. Although F_s can be influenced by many factors (Baker 2008), increased F_s under high temperature was the main reason (accompanied by a slight increase in F_m) for Φ_{PSII} and qP reduction which lowered photochemical capacity of PSII, which may in turn have reduced A, albeit insignificantly. Bibi et al. (2008) reported a reduction in Φ_{PSII} under high temperature and Maxwell and Johnson (2000) observed a linear relationship between Φ_{PSII}, measured by fluorescence techniques, and carbon fixation, measured by gas exchange. Nevertheless, PSI fluorescence at wavelengths above 700 nm and reduction in plastoquinone quenching due to saturating light pulses can have an influence on Φ_{PSII} which leads to an underestimation of PSII functionality (Baker, 2008). In the control, okra photosynthesis was linearly related to g_s, F_v'/F_m' and Φ_{PSII} whereas under high temperature, A was decoupled from g_s and the linear relationship between A and Φ_{PSII} was more significant. This suggests that increases in g_s was a mechanism to avoid heat stress by lowering leaf temperature (Lu et al., 1994) and A was therefore more strongly determined by the efficiency of the photosynthetic apparatus.

There was no significant difference between F_v'/F_m' and A in the control and heat, but E increased over time and was more than double in the heat, which successfully reduced leaf temperature by 10°C compared to ambient. During the conversion of light energy to photochemistry, dissipation of excess energy as heat or fluorescence in the leaf

are in competition (Maxwell and Johnson, 2000), and the cooling provided by higher transpiration helps dissipate this energy. As water availability was not limited, high g_s and E under high temperature could be maintained, and k_{leaf} increased. This result was in parallel to a finding in linden and soybean, where k_{leaf} increased in high temperature when water supplied was sufficient (Sellin and Kupper, 2007, Locke et al., 2013). Higher k_{leaf} in acclimated leaves is influenced by vein traits where there is an increase in number of xylem conduits and vein length (Sack and Scoffoni, 2013). Leaf morphology has not been considered in this study but higher k_{leaf} suggests an adaptation in the water-conduit system and explains the sustained higher stomatal conductance and transpiration rate in the heat.

Relatively stable photosynthesis under heat stress can be the result of alternative electron flow which maintains electron-transfer through PSII (Havaux, 1996) and protects reaction centres in PSI and PSII (Tóth et al., 2007). Although PSII can be protected from photoinhibition by small heat-shock proteins (Downs et al., 1999), CO_2 was not fully utilized for sugar production and A did not increase at the same rate as g_s due to possible inhibition of Rubisco activation (Feller et al., 1998), which is more heat-sensitive than damage to PSII (Law and Crafts-Brandner, 1999, Schrader et al., 2004). Under high temperature, the affinity of Rubisco for CO_2 decreases resulting in an increase in photorespiration and a decrease in A (Laing et al., 1974). Photorespiration does not generate ATP; however, Schrader et al. (2004) reported that in heat-stressed cotton, ATP level did not change, therefore photorespiration was not considered to be the reason for A reduction. This author concluded that oxidation of PSI at 42°C was the reason for A reduction under high temperature.

Membrane damage was apparent, which is generally one of the primary effects of heat injury and influenced by genetic variation (Blum and Ebercon, 1981, Kuo et al., 1992). EL was higher in the control at week 2 and 4, which may have resulted from high (c. 40°C) temperatures inside the control tunnel house on the day measurements were taken. EL declined over time, and Camejo et al. (2005) suggested that this reduction in ion leakage might be due to plant adaptation to environment and maintenance of membrane function. Cotton and wheat adapted to high leaf temperature (Law and Crafts-

Brandner, 1999) by maintaining membrane stability (Schrader et al., 2004). Increased membrane stability can result from accumulation of zeaxanthin in the leaves as was seen in heat-acclimated potato (Havaux and Gruszecki, 1993, Havaux and Tardy, 1996).

Negative impact of high temperature on plant productivity (biomass and yield) is one of the main concerns which has been reported repeatedly (Arulrajah and Ormrod, 1973, Burke et al., 2004, Wahid et al., 2007, Hasanuzzaman et al., 2013). Plant biomass was lower in the heat after 6 weeks in Year 2; however as dry matter production was similar between control and heat after 15 weeks of treatment in Year 1, physiological acclimation in okra may have reduced dry matter loss under long exposure to high temperature. Despite similar *A* and dry matter in both treatments, yield decreased dramatically. Hence, this decrease was unrelated to photosynthetic capacity and growth.

Actively growing reproductive (sink) tissues demand a high level of energy, higher than vegetative tissues, which is obtained from carbohydrates (Herrero and Arbeloa, 1989). Carbohydrate supply to sink tissues can be provided by recently fixed photosynthates which are transported in the phloem (Turgeon and Wolf, 2009). Under high temperature, stored carbohydrates in roots and shoots are generally used for shoot growth rather than reproductive growth (but this is dependent on plant species) which results in taller plants; however, heat inhibits sucrose movement (through phloem) from leaves to the other organs (Ribeiro et al., 2012, Bhandari et al., 2016) and sucrose supplied to reproductive tissue is limited. Leaf sugar content increased in the heat, but *A* was lower, which indicates that photosynthetic rate was not the primary cause for varying leaf sugar concentration. As shoot carbohydrate concentrations decreased strongly, lower phloem loading rate and stronger competition by vegetative tissues for carbohydrates (Li et al., 2011) may have occurred in okra, as also found in creeping bentgrass (Liu and Huang, 2000). Fructose and glucose increased along with sucrose in leaves and roots, and suggests that the activity of sucrose hydrolyzing enzymes were not inhibited by high temperature in these two tissues.

Acclimation in okra was observed in both flowering and bud initiation. However at bud initiation plants were more vulnerable to high temperature, as fluorescence parameters, *A* and sugar content were significantly lower compared to flowering.

Although there were contrasting g_s and A responses among genotypes, statistical analysis did not show a significant genetic variation in response to effect of high temperature on photosynthetic apparatus. Nevertheless, genotype L4-48 (which was the shortest genotype) showed an increase in A and fluorescence parameters after 15 weeks of heat treatment and an increase in fluorescence parameters after 6 weeks of heat treatment. This genotype had the second highest yield in both year 1 and 2 in the control. Therefore L4-48 might have a potential to be considered as a heat tolerant genotype despite its dry matter reduction under high temperature. Since plants were grown in pots, root production was limited and likely influenced their vegetative growth; hence, dry matter production may differ when planted in the ground.

5.5 Conclusion

Physiological acclimation occurred in okra under heat stress, where stomatal conductance increased strongly to ensure evaporative cooling. Okra's physiological acclimation under high temperature was a mechanism to survive but not be productive. The photosynthetic apparatus recovered after a period of time in the heat which resulted in dry matter production similar to the control; nevertheless sugar content was altered in different vegetative tissues. Okra is vulnerable at early stages of growth and development (lower fluorescence parameters, A and sugar content at bud development compared to flowering), but can still tolerate heat stress. In the heat, yield reduced severely which might be due to changes to the reproductive tissue (potentially due to sugar imbalances) and the effect of heat on the reproductive development will be discussed in the next chapter.

6 The effect of high temperature on the reproductive development of okra *(Abelmoschus esculentus* (L.) Moench)

6.1 Introduction

High temperature limits plant growth, development and productivity by damaging tissues or physiological and reproductive functions leading to reduced dry matter production and lower fruit yield. There are many studies investigating the effect of heat stress on leaves and roots, and most focus on photosynthetic alteration (Zinn et al., 2010). Nevertheless, the sensitivity of reproductive tissues to high temperature has long been recognized as one of the reasons for yield reduction in crops (Charles and Harris, 1972, Herrero and Johnson, 1980) and reproductive tissues have proved more useful than vegetative organs for determining heat stress tolerance of plants (Salem et al., 2007). Still, the complexity of the fertilization process and gamete development, which occurs during a short period of time, makes it difficult to study reproductive tissues (Zinn et al., 2010). Chapter 5 demonstrated that heat stress did not severely affect the vegetative tissue as assimilation or biomass was similar between treatments, but a strong yield reduction was observed. Therefore, it is likely that yield loss was due to the impacts of high temperature on the reproductive tissues.

Okra has solitary flowers 5 to 8 cm in diameter, superior ovaries, five pale yellow petals with a red or purple colouration at their bases (Figure 6.1). There are numerous stamens with kidney shaped anthers. Okra anthers are monothecous (characterized by one lobe) containing two pollen sacs (bisporangiate) which open by a transverse slit from the middle of each anther producing approximately 100 spherical and porous pollen grains. The hairy deep red stigmas can accommodate around 600 pollen grains. After fertilisation, the capsule shaped fruit forms and each contains 30-80 seeds (Dhankhar and Singh, 2009). Anthesis takes place in the morning (c. 6-10 am) while the stigma becomes receptive before anthesis. Anther dehiscence occurs 4 to 6 hours before or 15-20 minutes after anthesis (Venkataramani, 1953, Hamon and Koechlin, 1991b, Dhankhar and Singh, 2009). Fertilization increases during the morning with pollen shedding, thus ensuring

most ovules are fertilized by midday (Hamon and Koechlin, 1991b). Cotton, takes 12 hours or more for fertilization to occur post pollination (Stewart, 1986), although this is not clear in okra.

Under high temperature, bud initiation can be delayed or inhibited in okra and this can cause the first flowering node to be produced on a higher position along the stem. Consequently, there is a high probability that the buds will not develop fully (Arulrajah and Ormrod, 1973, Dhankhar and Singh, 2009). In tomato and cotton, heat stress causes abnormalities in the male and female reproductive structures including impairment of pollen and anther development, reduced pollen production, deformed pollen morphology, anther indehiscence, reduced pollen and ovule viability and reduced stigma receptivity (Burke et al., 2004, Singh et al., 2007, Wahid et al., 2007, Oosterhuis, 2011). In wheat, temperature above 30°C damages male tissue from early meiosis to pollen maturity resulting in low fertilisation and seed setting (Saini et al., 1983). In rice, high temperature reduced pollen production, anther dehiscence and pollen germination (Matsui et al., 2001, Matsui and Omasa, 2002, Prasad et al., 2006a). In cotton, temperatures higher than 32°C reduce pollen tube elongation, and temperatures of 37°C significantly decrease pollen germination (Burke et al., 2004). Brown (2008) reported that most developed cotton buds did not fully open under heat stress and of those that did, small flowers were produced with extended stigmas.

Although a large body of information is available for other crops, such as tomato, cotton and cereals, there is limited information on the effects of heat damage on the reproductive tissues of okra. This chapter evaluates the changes to male and female organs as well as vegetative growth under high temperature in two consecutive years.

Figure 6.1 (A) Okra bud, flower and fruit in the axils of leaves. (B) Longitudinal section of the flower at anthesis showing ovary, ovules, style, staminal column, stigmas and stamens.

6.2 Materials and methods

6.2.1 Source of plants and tissues

The genotypes examined are described in chapter 5 (e.g. L2-11, L2-30, L2-32, L3-1, L3-58, L3-59, L4-31 and L4-48 in 2015 and L2-11, L2-30, L3-1 and L4-48 in 2016) and the same plants were used for vegetative (chapter 5) and reproductive assessments (chapter 6). These genotypes were evaluated for the impacts of stress on vegetative growth and reproductive tissues.

6.2.2 Morphological assessments

6.2.2.1 Vegetative growth

Plant height and main stem base diameter were recorded at the end of the experiment in 2015. Plant height, main stem base diameter, number of nodes and number of branches were recorded at the end of the experiments in 2016. All assessments were made using a measuring tape and digital callipers.

6.2.2.2 Flower size

One flower was collected from each plant under the control and high-temperature treatments (three replicates per genotype per treatment) before 10 am in 2015.

Flowers were photographed using a digital camera to screen flower size in each treatment. Flowers were scored as large when the diameter (in the fully open flower) was 6 cm or above and scored as small when the diameter was 6 cm or below.

6.2.2.3 Fruit size and shape

Fruits were picked 6 to 9 days after flowering. Their length, width and fresh weight were measured using a digital calliper and a digital scale. Photos were taken to provide comparisons of fruit shape.

6.2.3 Reproductive tissue assessment

Stereo and scanning electron microscopy were used to observe morphological changes in the reproductive tissues (stigmas, anthers and pollen) under both control and heat treatments, and photographs were recorded.

In 2016, floral materials (buds and flowers) at various developmental stages were collected. Genotypes L2-30 and L3-1 were selected for the morphological analysis. One opened flower and two buds (2^{nd} and 4^{th} level buds above the opened flower) were collected from each plant in both treatments. Collected materials were fixed in formalin acetic alcohol (FAA) (5:5:90 formalin, glacial acetic acid and 50% ethanol (v/v/v)) for 24 hours and stored in 70% ethanol. Fixed tissues were dehydrated through an ethanol series (50, 70, 95, and 100%) and then embedded in paraffin (melting point 58–60ºC) before sectioning (Ruzin, 1999). Serial transverse sections were cut using a rotary microtome (Leica RM2255, Germany) at 8 µm thickness. Safranin-Fast Green staining, dehydration with ethanol and mounting were applied according to (Sass, 1958, Ahmad et al., 2009). Samples were digitised by a slide-scanning microscope (ZEISS AxioScan Z.1, Germany).

6.2.4 Fertility assessment

6.2.4.1 Female organs (stigma receptivity)

Stigma enzymatic-activity was evaluated using Peroxtesmo KO (Machery-Nagel, Duren, Germany) (Dafni and Maués, 1998), which is a measure of stigma receptivity. One test paper (15*15 mm) was soaked in 1 mL of double deionised water. Twenty-four well culture plates were used to accommodate droplets of the solution and undamaged stigmas were held upside-down in the solution. After soaking for a few minutes, stigmas were gently pressed on a filter paper to examine the stain colour. In the presence of peroxidase, a blue-purple colour stain can be observed (evidence for stigma receptivity) otherwise the colour would be red.

6.2.4.2 Male organs

6.2.4.2.1 Anther count

The numbers of anthers on each staminal column were counted. Due to the large anther size, counting was conducted by the naked eye and anthers were pinched out from the staminal column to avoid miscounting.

6.2.4.2.2 Optimization of the pollen germination medium

Basic BK *in vitro* germination medium (Brewbaker and Kwack, 1963) with different concentrations of sucrose and polyethylene glycol were evaluated for optimal pollen germination. The basic BK medium contains 100 mg L^{-1} H_3BO_3, 300 mg L^{-1} $Ca(NO_3)_2.4H_2O$, 200 mg L^{-1}, $MgSO_4.7H_2O$, 100 mg L^{-1} KNO_3 and 10% sucrose (all w/v). Polyethylene glycol was used at three concentrations (0%, 2.5% and 5% w/v) and sucrose at six concentrations (0%, 5%, 10%, 15%, 20 and 25%, w/v) with the pH adjusted to 6.2.

For *in vitro* germination, a mix of pollen grains from all genotypes (in the year 2015) was collected in Petri dishes before 10 am in each tunnel house, and rehydrated by placing Petri dishes in a closed container lined with a wet tissue paper for an hour. Then, pollen grains were cultured on the medium and kept in an incubator at 26°C for an hour.

There were eighteen medium combinations to screen. A twenty-four well culture plate was used to accommodate eighteen different media at the same time. The experiment was repeated three times on three different days. Photos were taken from six random fields on each cell using a Nikon camera attached to a light microscope (Leica DMIL, Leica Microsystems CMS GmbH, Wetzlar, Germany). The saved photos were used to count the total number of pollen grains and those which germinated. A pollen grain was considered germinated when the length of its tube was twice the diameter of the pollen grain (Patil et al., 2013). Pollen germination percentage was calculated using the formula (Number of germinated pollens/total number of pollen grains)*100.

6.2.4.2.3 Pollen viability (pollen germination)

In vitro pollen germination was tested in 2015 on seven genotypes (L2-11, L2-30, L2-32, L3-1, L3-59, L4-31 and L4-48) grown under both temperature treatments. Pollen grains from all plants (3 plants per genotype per treatment) were collected before 10 am (at anthesis) in each tunnel house, placed in Petri dishes (one Petri dish per plant) and kept in a closed container lined with a wet tissue paper for rehydration. After one hour of rehydration, the pollen grains were cultured on a modified BK medium (Brewbaker and Kwack, 1963) supplemented with 10% sucrose and 2.5% polyethylene glycol and the pH adjusted to 6.2. The cultures were kept in an incubator at 26°C for one hour. Photos were taken from three random fields of each Petri dish using a Nikon camera attached to a light microscope (Leica DMIL, Leica Microsystems CMS GmbH, Wetzlar, Germany). The saved photos were used to count the total number of pollen grains and those which germinated. Pollen germination percentage was calculated using the formula: (Number of germinated pollens/total number of pollen grains)*100.

6.2.4.2.4 Pollen germination sensitivity to different temperatures

In vitro pollen germination was evaluated on two genotypes (L2-11 and L2-30) in different temperatures (28, 32, 37, 42 and 45°C) to (i) determine the optimum temperature for okra pollen germination and (ii) identify the most adverse temperature threshold for pollen germination. Pollen was collected from plants in control tunnel house and the same protocol (see above) applied to germinate pollen. Five incubators with

different temperatures were used at the same time. Photos were taken after one hour incubation for analysis (as described above).

6.2.5 Statistical analysis

The obtained data were subjected to a general analysis of variance (ANOVA) when the level of significance measured at $P<0.05$ and a comparison of means were performed using Fisher's unprotected LSD test using GenStat 17th Edition software (VSN International Ltd, London, UK).

6.3 Results

6.3.1 Morphological assessments

6.3.1.1 Vegetative growth

Means for plant height (cm), main stem base diameter (mm), number of nodes and number of lateral shoots under heat stress and non-stressed conditions in 2015 and 2016 are shown in Table 6.1,Table 6.2 and Table 6.3. The statistical summary of plant height, main stem base diameter, number of nodes and number of branches is shown in Table 6.4.

Heat treated plants were significantly shorter than the control in both years ($p<0.001$). Height variation among genotypes followed the same pattern in both treatments, but Genotype*Treatment interaction was not significant. L4-48 was the shortest with an average height of 38/17 cm in the control/heat treatments in 2015 and in 2016, the equivalent mean heights were 101/91cm and 73/65 cm for plants assessed at flowering and bud initiation, respectively. L2-32 was the tallest in 2015 with an average height of 169/125 cm in both treatments. In 2016, L3-1 was the tallest genotype with an average height of 264/159 cm at flowering and 212/183 cm at bud initiation. Under high temperature, height reduction between 26-55% and 10-40% were observed in 2015 and 2016, respectively.

There was no significant effect of high temperature on main stem diameter. Plants at bud initiation had significantly lower base diameter compared to plants at flowering in 2016.

The number of nodes was significantly reduced under high temperature and there was significant variation observed among genotypes. Plants were shorter at bud initiation compared to flowering and consequently, significantly fewer nodes were observed at this stage.

In 2016, L2-11 had the highest number of lateral shoots and L3-1 had the lowest in the control. However, high temperature did not have a significant effect on the production of the lateral shoots and no significant difference was observed between stages of development. However, there was a tendency to higher numbers of lateral shoots under high temperature at flowering.

Table 6.1 Average plant height (cm) and base diameter of the main stem (mm) for eight okra genotypes (n=3) in control and heat treatments in 2015. Values are presented with ±SE and least significant differences (LSD) of means for Genotype*Treatment interaction, when the interaction was significant at $p<0.05$. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test.

Genotype	Height (cm)				Base diameter (mm)	
	Control		Heat		Control	Heat
L2-11	150 ± 4.19	hi	104 ± 10.75	d	40.41 ± 1.27	48.53 ± 3.15
L2-30	156 ± 7.26	hi	110 ± 7.75	de	46.55 ± 4.11	40.48 ± 2.23
L2-32	169 ± 3.41	i	125 ± 4.99	efg	42.16 ± 2.76	42.72 ± 1.26
L3-1	151 ± 0.29	hi	101 ± 31.3	gh	34.97 ± 2.06	40.46 ± 2.37
L3-58	130 ± 4.72	fg	82 ± 0.82	c	52.48 ± 1.98	47.08 ± 3.02
L3-59	128 ± 6.34	efg	85 ± 2.60	c	49.92 ± 0.98	50.12 ± 1.72
L4-31	118 ± 8.18	def	76 ± 3.40	c	53.08 ± 3.07	46.69 ± 2.61
L4-48	38 ± 3.14	b	17 ± 1.91	a	44.20 ± 2.74	41.34 ± 2.59
LSD					7.72	

Table 6.2 Average plant height (cm) and number of nodes for four genotypes (n=3) in the control and heat treatments at flowering (F) and bud initiation (B) stages (DS: developmental stage) at week 6 in 2016. Values are presented with ±SE. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test.

DS	Genotype	Height (cm)		No. nodes	
		Control	Heat	Control	Heat
F	L2-11	229 ± 5 efg	180 ± 18 cde	46 ± 2 g	33 ± 2 cde
F	L2-30	238 ± 9 fg	176 ± 14 cde	44 ± 5 fg	33 ± 3 de
F	L3-1	264 ± 4 g	159 ± 55 cd	39 ± 1 ef	27 ± 0 abc
F	L4-48	101 ± 1 ab	91± 1 ab	31 ± 1 cd	29 ± 1 bcd
B	L2-11	186 ± 10 cdef	142 ± 16 bc	34 ± 1 de	28 ± 4 abcd
B	L2-30	180 ± 1 cde	133 ± 33 bc	31 ± 0 cd	33 ± 2 cde
B	L3-1	212 ± 18 defg	183 ± 9 cde	32 ± 3 cd	29 ± 1 bcd
B	L4-48	73 ± 2 a	65 ± 1 a	24 ± 0 ab	23 ± 1 a

Table 6.3 Base diameter of the main stem (mm) and number of lateral shoots for four genotypes (n=3) in the control and heat treatments at flowering (F) and bud initiation stages (B) (DS: developmental stage) at week 6 in 2016. Values are presented with ±SE. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test.

DS	Genotype	Base diameter (mm)		No. lateral shoots	
		Control	Heat	Control	Heat
F	L2-11	36 ± 3 bcd	34± 2 abcd	6 ± 1.2 de	7 ± 1.5 a
F	L2-30	32 ± 6 abcd	38± 5 cd	3 ± 0.6 abc	4 ± 0.3 abcd
F	L3-1	38 ± 2 cd	32± 5 abcd	2 ± 0.0 a	4 ± 0.4 abc
F	L4-48	37 ± 1 cd	38± 3 d	5 ± 0.3 bcd	4 ± 0.9 abcd
B	L2-11	33 ± 1 abcd	28± 3 a	5 ± 0.3 cde	3 ± 1.0 abc
B	L2-30	29 ± 1 ab	27± 5 a	2 ± 0.9 ab	4 ± 1.3 abcd
B	L3-1	30 ± 1 abc	33± 1 abcd	2 ± 0.6 a	2 ± 0.0 a
B	L4-48	33 ± 2 abcd	33± 1 abcd	5 ± 0.0 cde	5 ± 1.5 cde

Table 6.4 ANOVA for plant height, base diameter, number of nodes and number of lateral shoots for 8 okra genotypes in 2015 and for 4 okra genotypes at flowering and bud initiation in control and heat treatments at week 6 in 2016.

		2015		2016			
		Height	**Base diameter**	**Height**	**Base diameter**	**No. nodes**	**No. lateral shoots**
Genotype (G)	F	64.53	5.76	35.05	1.29	13.61	11.00
	P	<0.001	<0.001	<0.001	0.30	<0.001	<0.001
Treatment (T)	F	124.02	0.05	22.79	0.27	26.99	0.63
	P	<0.001	0.83	<0.001	0.61	<0.001	0.43
Developmental Stage (DS)	F	NA	NA	12.73	14.16	31.20	2.53
	P	NA	NA	0.001	<0.001	<0.001	0.12
G*T	F	2.02	2.90	1.80	0.56	2.80	0.98
	P	0.09	0.02	0.17	0.65	0.06	0.42
G*DS	F	NA	NA	0.72	0.47	1.66	2.46
	P	NA	NA	0.55	0.70	0.20	0.08
T*DS	F	NA	NA	1.74	0.01	11.55	0.99
	P	NA	NA	0.20	0.93	0.002	0.33

NA: not applicable.

6.3.1.2 Flower size

Under high temperature stress, plants produced smaller flowers. Flowers in the heat treatment were approximately two-thirds the size of flowers in the control. An example is shown in Figure 6.2 comparing flowers of the same genotype in the control (A) and heat treatment (B).

Figure 6.2 Flowers from plants of the same genotype in the control (A) and heat (B) treatments

6.3.1.3 Fruit size and shape

Fruit produced from plants in the heat treatment failed to fully develop (Figure 6.3B) and produced no seeds. Figure 6.3 shows an example of fruit from genotype L3-1 at flowering in the control tunnel house (A), collected 7 days after flowering (fruit of this genotype had an average of 6 ridges with 11 seeds per ridge) and fruit from the heat treated tunnel house (B), collected 11 days after flowering.

The average length and width of collected fruit for flowering and bud initiation stages in 2016 is summarized in Figure 6.4. Fruit produced under high temperature were seedless and shorter compared to those in the control. At flowering, this reduction in length was c. 50% while heat treated plants produced longer fruits at the bud stage compared to the flowering stage. There was a reduction in fruit width under high temperature in both stages of development and the fruit also appeared deformed and lighter in colour.

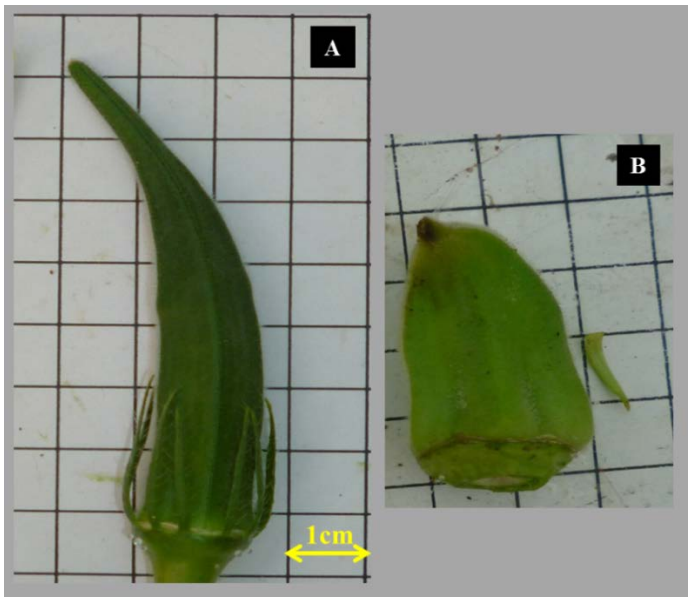

Figure 6.3 Fruit from genotype L3-1. A) Control: fruit picked 7 days after anthesis. B) Heat: fruit picked 11 days after anthesis.

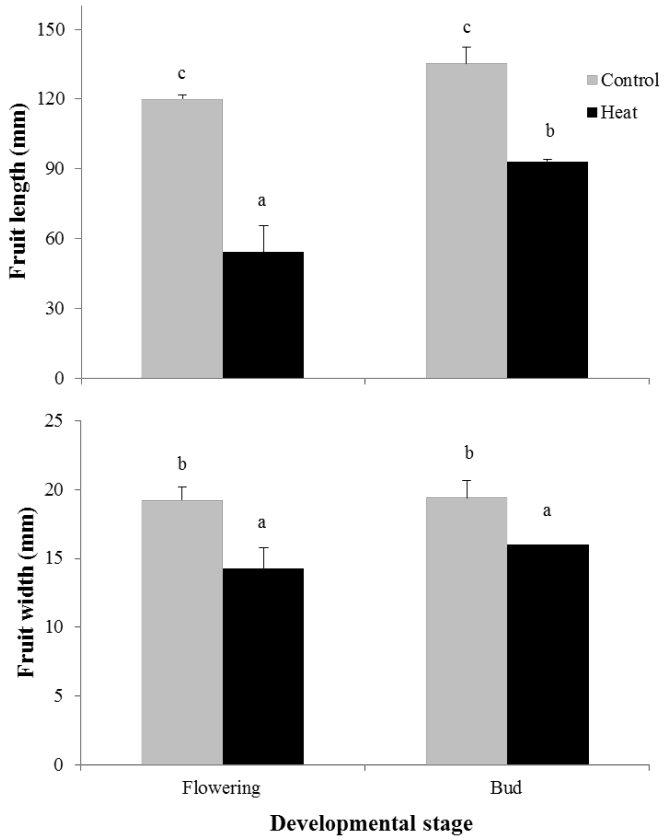

Figure 6.4 Average length and width (mm) of collected fruit (all four genotypes, n=12) in control and heat treatments at flowering and bud initiation in year 2 (2016). Bars represent standard error (+SE).

6.3.2 Reproductive tissue assessment

In the control, anthers were fully open and spherical pollen grains were dispersed whereas in the heat treatment, anthers were shrunken and did not fully open and pollen structure was severely affected (Figure 6.5, Figure 6.6, Figure 6.7). No pollen was shed on the surface of the stigmas whereas all the anthers fully opened and pollen was dispersed in the control. However, no sign of temperature damage was observed on the stigmas (Figure 6.6). Changes in anther and pollen structure under high temperature included incomplete dehiscence (Figure 6.5 and Figure 6.6) and flattened and shrunken pollen (Figure 6.7).

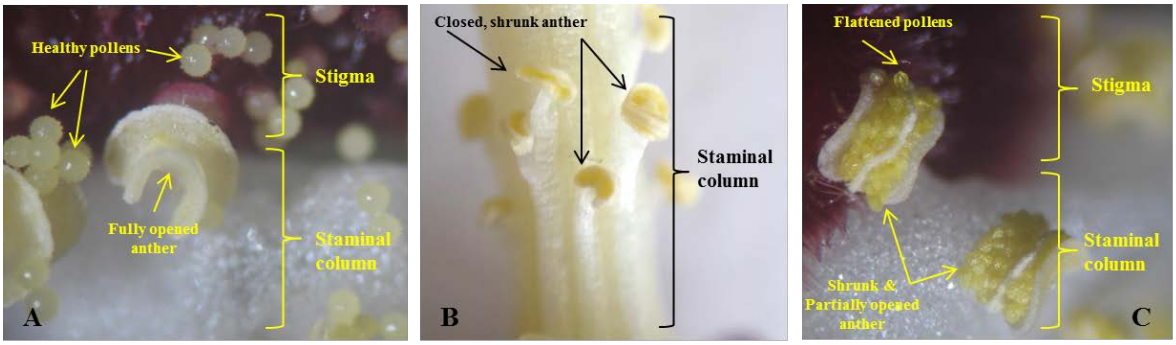

Figure 6.5 Stereo microscopic images of pollen grains and anthers in the control (A) and heat (B and C) treatments. Images A and C were taken with 40x magnification and B with 20x magnification.

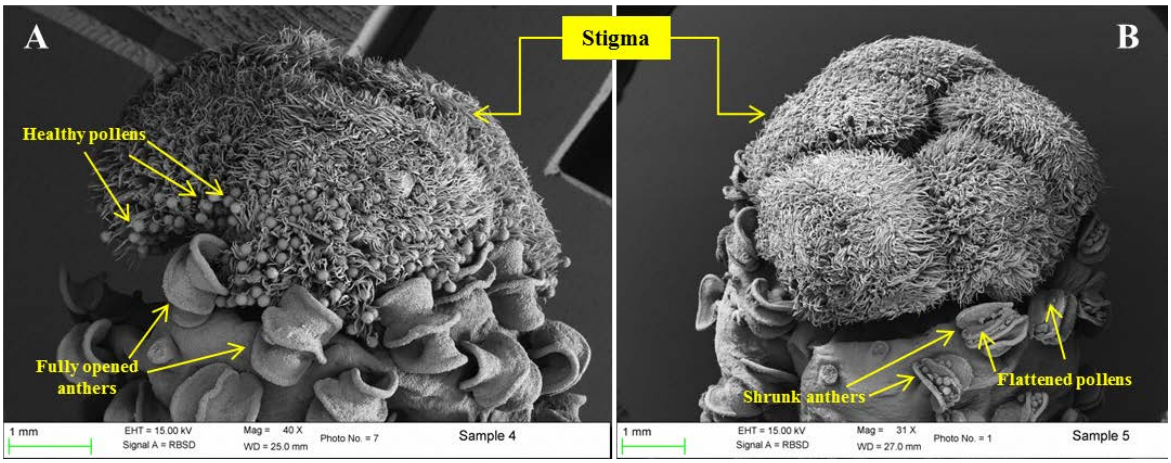

Figure 6.6 Scanning electron microscopy images of stigmas and fully opened anthers and healthy pollen grains in the control (A), and stigmas and affected stamens in the heat treatments (B).

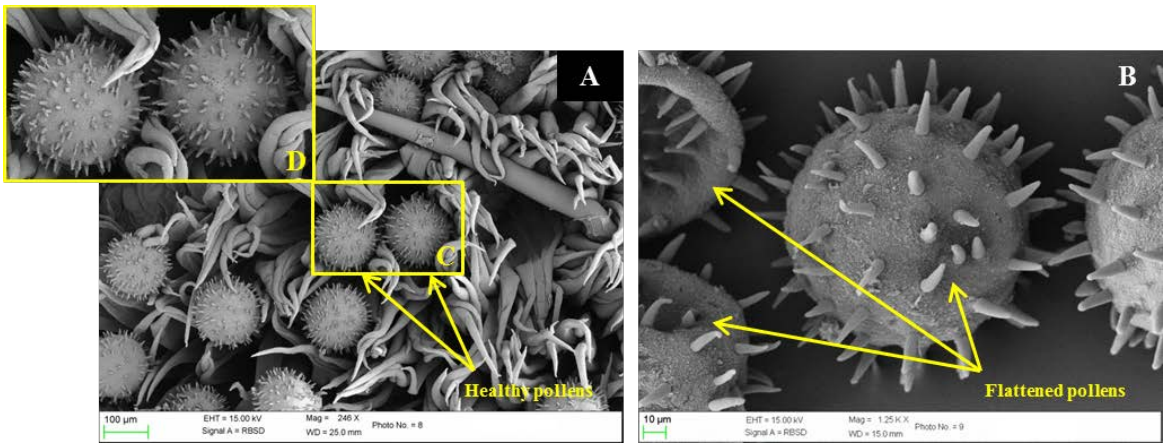

Figure 6.7 Scanning electron microscopy images of healthy (A) and heat affected pollen (B). Section (C) has been enlarged to improve the view (D).

High temperature had a negative effect on male reproductive structures. In the control, anthers were oriented evenly around the staminal column with larger pollen sacs and numerous pollen (Figure 6.8A and B), whereas under heat stress, anther sacs were smaller, pollen numbers reduced and the pollen was misshapen (Figure 6.8C and D). Cross sections showed clearly that under high temperature, anthers were affected in the early stages of bud development (Figure 6.8D). Despite the obvious alterations in male tissue, even in the early stages of bud development under high temperature, female tissue appeared to be intact. The flower ovaries and ovules were similar in the control (Figure 6.9A) and heat (Figure 6.9D) treatments and there was no evidence that heat affected early stage female tissue development (Figure 6.9 E and F).

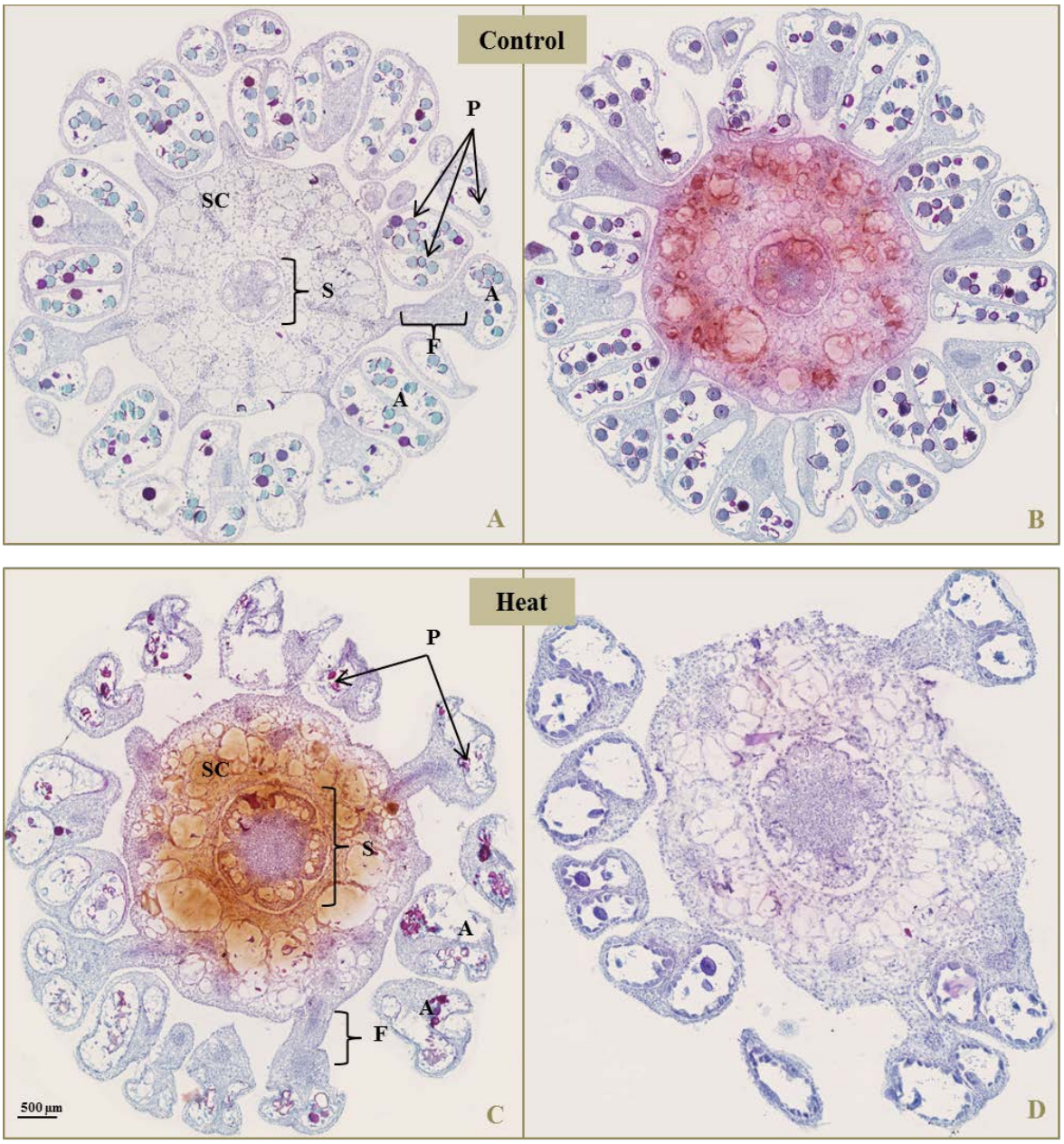

Figure 6.8 Histological observations showing cross sections of flower buds before anthesis in the genotype L2-30 in the control (A and B) and heat treatment (C and D) where A and C are cross section of 2nd bud above opened flower and B and D are cross section of 4th bud above opened flower. Abbreviations: style (S), stamina column (SC), stamens including anthers (A) and filaments (F) and pollens (P).

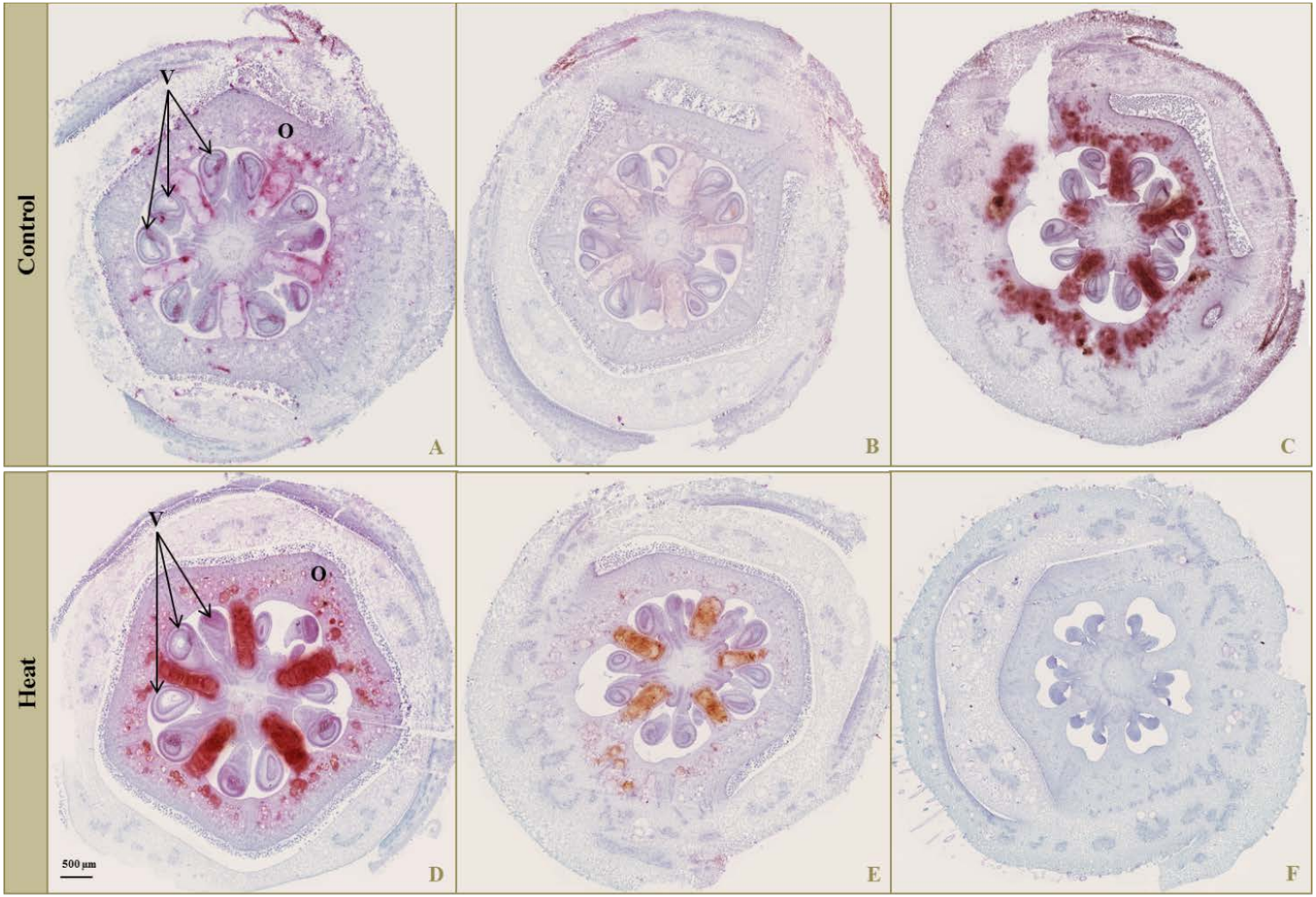

Figure 6.9 Histological observations showing cross sections of flower buds before and after anthesis in the genotype L2-30 in the control (A,B and C) and heat treatment (D, E and F) where A and D are ovary of opened flower, B and E are ovary of 2nd bud above opened flower and C and F are ovary of 4th bud above opened flower. Abbreviations: pentalocular ovary (O) containing ovules (V).

6.3.3 Fertility assessment

6.3.3.1 Female organs (stigma receptivity)

The stigma receptivity test showed that the stigmas reacted positively with Peroxtesmo KO. The presence of the peroxidase enzyme on the stigmas was indicated by the appearance of a blue-purple coloured stain. Figure 6.10 shows there was no colour difference between the stigmas collected from plants grown under both control and heat conditions.

Figure 6.10 Results of the stigma receptivity test. Blue-purple colour was produced in both the control (A) and heat (B) treatments.

6.3.3.2 Male organs

6.3.3.2.1 Anther count

Under high temperature, anther numbers decreased significantly ($p<0.05$) by 34% and 26% at flowering and bud initiation, respectively (Figure 6.11). The mean anther number at flowering and bud initiation were similar in the control (114.2 and 114.5), whereas there was a tendency to produce higher anther numbers at bud initiation under high temperature (84.4 versus 75.2 at flowering), although this was not statistically significant ($p=0.074$).

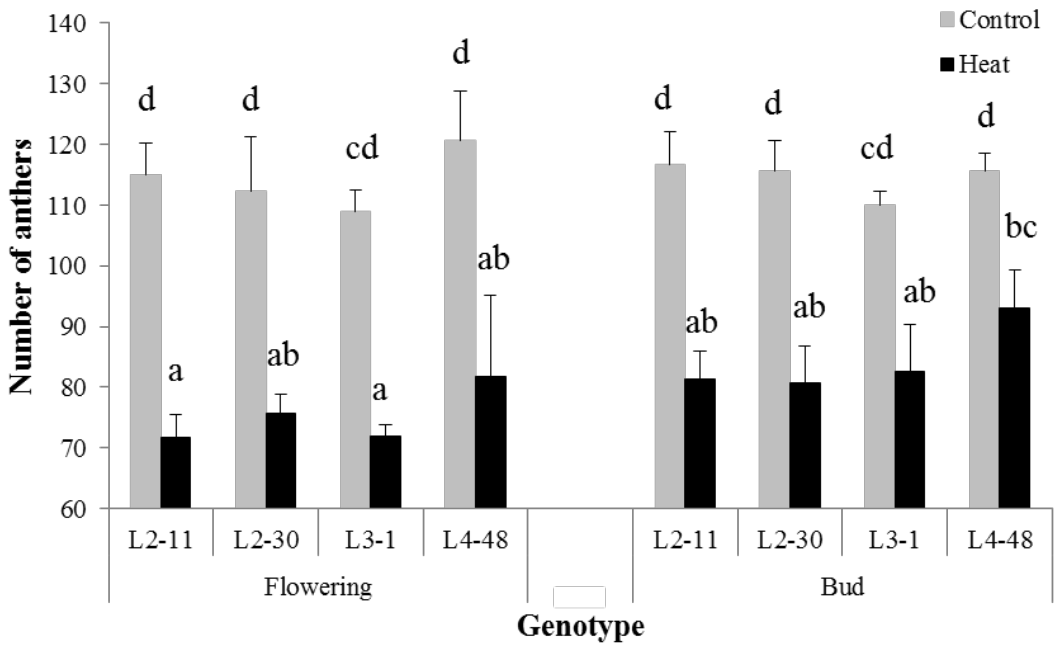

Figure 6.11 Average anther numbers for four okra genotypes (n=3) in control and heat treatments at flowering and bud initiation in year 2 (2016). Bars represent standard error (+SE).

6.3.3.2.2 Optimization of the pollen germination medium

The result of *in vitro* pollen germination (%) is summarised in Appendix 18. Medium 5 containing BK basic medium, 10% sucrose and 2.5% polyethylene glycol was selected as the optimal pollen germination medium because the highest germination (86.96%) was recorded on this medium (Figure 6.12). Medium 7 and 8 with a combination of sucrose and polyethylene glycol of (15%, 0) and (15%, 2.5%), respectively, were the second best options, providing pollen germination of 78.83% and 79.36%, respectively. Media with no sucrose addition showed the lowest pollen germination (media 1-3).

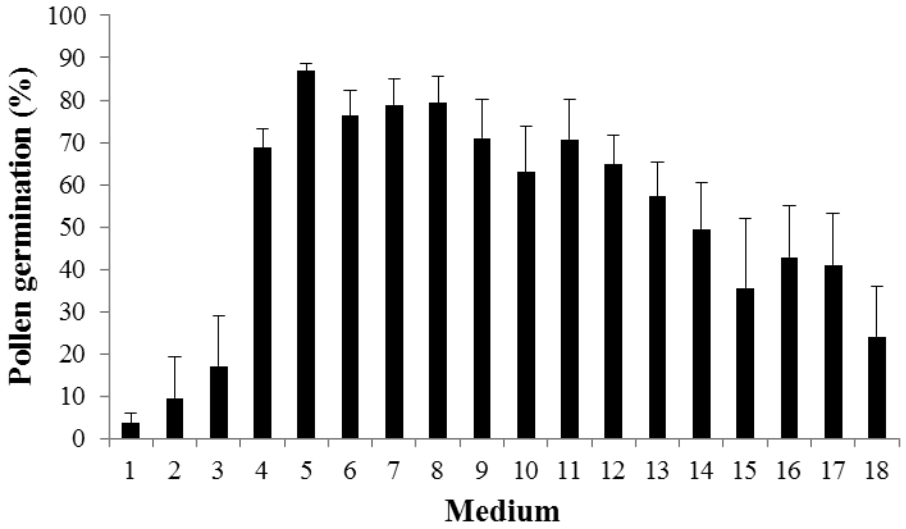

Figure 6.12 Average *in vitro* pollen germination (%) in 18 media (n=3) using basic BK medium mixed with different concentrations of sucrose (0, 10, 15, 20, 25 and 30%) and polyethylene glycol (0, 2.5 and 5%). Bars represent standard error (+SE).

6.3.3.2.3 Pollen viability (Pollen germination)

Pollen germination decreased significantly in plants grown under high temperature ($p<0.001$). Although there was significant genotypic variation ($p=0.016$), Genotype*Treatment interaction was not significant ($p=0.115$) (Table 6.5). Mean *in vitro* pollen germination of 46% was observed in the control compared to 14.5% under high temperature. As found in section 6.3.2, most of the heat treated pollen grains were deformed and flattened compared to the spherical shape of the control. Healthy pollen were characterized by the growth of multiple pollen tubes from each individual pollen grain (Figure 6.13).

Table 6.5 ANOVA for *in vitro* pollen germination for seven okra genotypes (n=3) in control and heat treatments in 2015.

		Pollen germination
Genotype	F	3.19
	P	0.016
Treatment	F	39.4
	P	<.001
Genotype*Treatment	F	1.91
	P	0.115

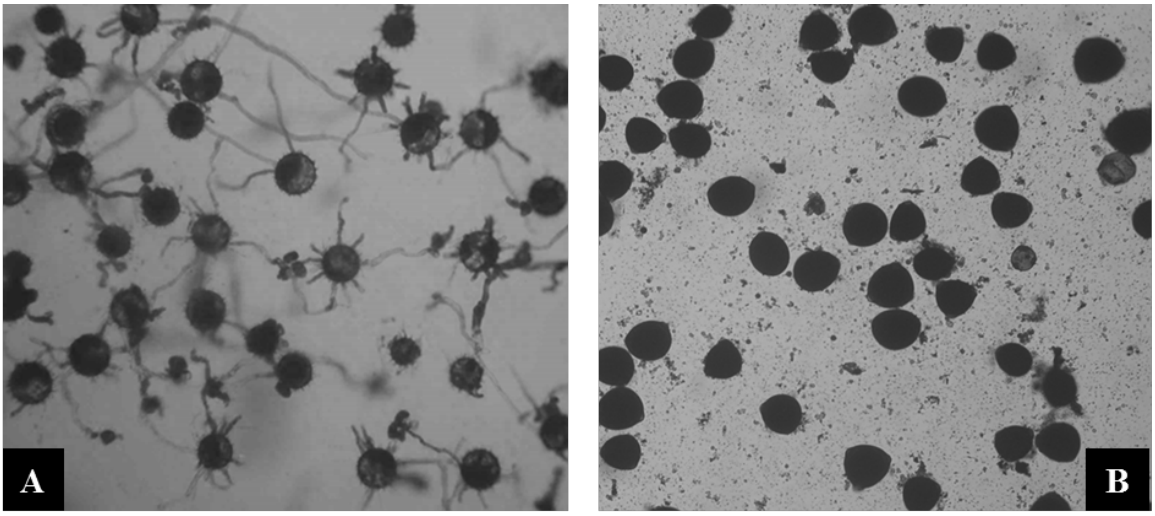

Figure 6.13 An example of *in vitro* pollen germination. A) Normal pollen grains with multiple pollen tubes in the control conditions (35°C). B) Deformed pollen grains from plants exposed to high temperature (45°C) with no germination.

6.3.3.2.4 Pollen germination sensitivity to different temperatures

Maximum pollen germination was observed at 28°C and 32°C in both genotypes (Figure 6.14). At 37°C, germination decreased significantly by around 17%. The lowest germination were observed at 45°C in both genotypes, however in genotype L2-11, germination was similar at 42 and 45°C. Figure 6.15 shows mean pollen germination at 28 and 32°C in contrast to mean germination at 42 and 45°C in both genotypes. Pollen germination dropped by 94.5% in L2-11 and 85.9% in L2-30 at 42°C or above. One hour exposure to high temperature inhibited pollen germination. Although pollen incubated at 42 and 45°C produced multiple tubes, none of the tubes elongated enough to be considered as germinated (Figure 6.16).

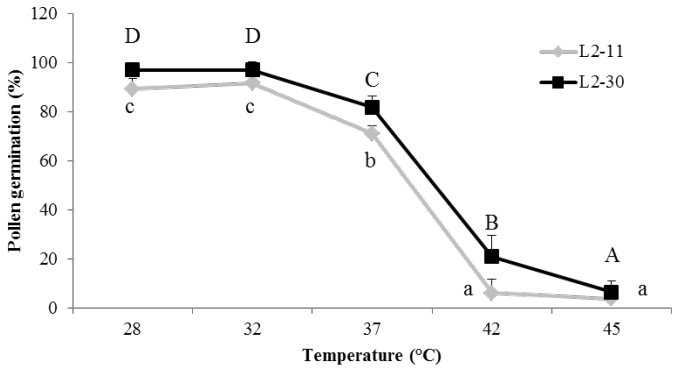

Figure 6.14 *In vitro* pollen germination (%) at different temperatures in genotypes L2-11 and L2-30. Bars represent standard error (+SE).

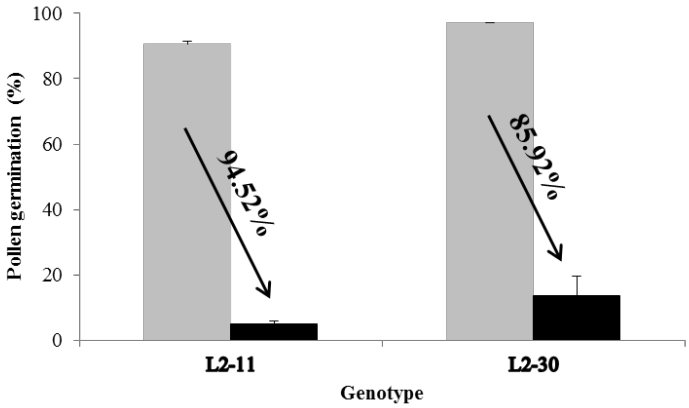

Figure 6.15 The effect of high temperature on pollen germination (%). Grey bars show average pollen germination at 28 and 32°C and black bars show average germination at 42 and 45°C. Bars represent standard error (+SE).

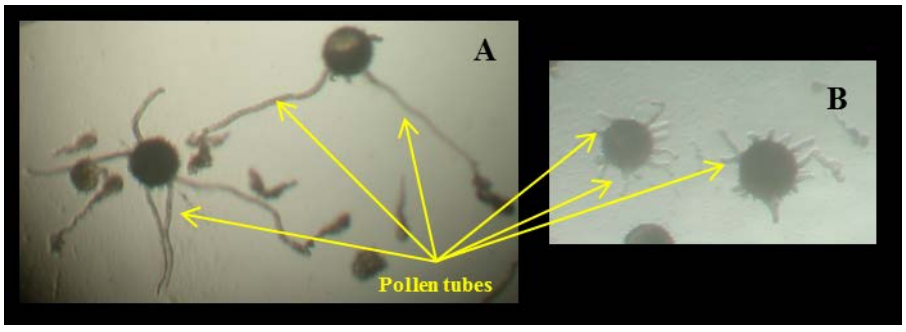

Figure 6.16 Pollen germination at 28°C (A) showing multiple elongated tubes per pollen grain and pollen grown at 42°C (B) with multiple short tubes (not considered as germinated).

6.4 Discussion

Heat stress had a significant negative effect on plant morphology. Under high temperature, flowers were smaller similar to cotton (Brown, 2008) with lower numbers of nodes; however, no difference was noted between base diameter of the main stem as well as the number of lateral shoots in either treatment. High temperature reduced seed-filling duration in cereals, resulting in the production of smaller seeds (Chowdhury and Wardlaw, 1978, Prasad et al., 2006b). Although okra plants appeared healthy under temperature stress, yield was reduced largely due to alterations in the reproductive tissue and poor fertilization. The reproductive tissues are complex and there are many processes involved in fruit setting, which makes it difficult to pin point any single process as the reason for low fruit setting (Dane et al., 1991).

Long term and short term heat stress can have negative effects on the reproductive tissues causing yield loss. In tomato, a severe reduction in pollen fertility occurs after prolonged periods of exposure to high temperature (Dane et al., 1991). Heat stress 10 days before anthesis was also observed to have a negative effect on pollen viability of green bean (Suzuki et al., 2001). Even a short period of high temperature (just before or after fertilization) can alter the function of reproductive tissue thus, impeding pollen tube growth on the stigma and the fertilization process (Snider et al., 2008, Zinn et al., 2010). In the current study, the effect of long-term (section 6.3.3.2.3) and short-term (section 6.3.3.2.4) heat stress on reproductive tissue was evaluated; specifically stigma receptivity, female structure, anther numbers and male structure were assessed under long-term and pollen germination were assessed under both long- and short-term heat stress. It was clear that male tissue (specifically pollen germination) was more sensitive to high temperature.

Reproductive tissue infertility could be a result of nutrient deficiency in both male and female organs resulting from a reduction in carbohydrate concentration following heat stress (Datta et al., 2001, Karni and Aloni, 2002, Pressman et al., 2002, Oosterhuis, 2011). In the male reproductive tissue, a reduction in carbohydrate reserves and degeneration of the tapetum layer was observed under high temperature in green bean and wheat (Saini et al., 1984, Suzuki et al., 2001); this likely reduced pollen mother cell

nourishment causing pollen sterility (Prasad et al., 2006b). The developing pollen grain is susceptible to high temperature at different stages of development spanning meiosis (Saini and Aspinall, 1982) to pollen maturation (Peet et al., 1998) and pollen tube growth (Dupuis and Dumas, 1990). However, a reduction in carbohydrate reserves and ATP have been also been observed in the female reproductive tissue of cotton (Snider et al., 2009) and tomato (Peet et al., 1997). High temperature reduced the stigma receptivity period, total number of ovules and increased ovary abnormalities and ovule abortion in other crops (Saini et al., 1983, Hedhly et al., 2005, Whittle et al., 2009). This contrasts with okra, as the microscopic analysis of the current study showed that the female structure was intact and no damage was observed during ovary development. This was supported by the stigma receptivity test which revealed no damage to the enzymatic activity of the stigmas in both the control and high-temperature treatments. Although damage to female tissues was observed in some crops such as canola and maize, the male tissues were more vulnerable to high temperature (Dupuis and Dumas, 1990, Young et al., 2004). In tomato, seed production was reduced by 37% when female tissue was affected by high temperature and by 88% when male tissue was damaged (Peet et al., 1998).

In the current study, male tissue alteration was observed 8 to 10 days prior to anthesis on the fourth bud above the opened flower under high temperature. A comparison between treatments showed that anthers were oriented normally around the staminal column of a flower in the control with larger pollen sac containing multiple round pollen grains, whereas in the heat there were lower numbers of anthers and anthers had smaller pollen sac sizes with fewer pollen grains which looked shrunken. The significant decrease in anther numbers under heat stress was less evident at bud initiation (albeit not statistically significant). Apart from anther number reduction in heat stress, reduced pollen dispersal on the stigma due to anther indehiscence additionally lowered observed fertilization rate. Similar findings have been published in bean, rice and tomato (Porch and Jahn, 2001, Matsui and Omasa, 2002, Sato et al., 2002).

Pollen quality can be assessed by *in vitro* pollen germination. *In vitro* pollen germination is an easy and rapid technique to test pollen viability. Basic BK medium was optimized for okra pollen germination with the addition of sucrose (10%) and

polyethylene glycol (2.5%). It was observed that additional sucrose improved *in vitro* pollen germination and pollen tube growth in cotton (Burke et al., 2004) which agrees with our study. Sucrose acts as an osmoticum, a substrate for respiration and an energy source for germination (Hong-Qi and Croes, 1982) and polyethylene glycol is not easily metabolized in plants and can promote pollen tube growth (Hong-Qi and Croes, 1982). Okra pollen grains are porous and produce multiple tubes (Thakur and Arora, 1986); however, only one of these tubes carries a nucleus and reaches the ovule (Dhankhar and Singh, 2009). This study clearly found a reduction in *in vitro* pollen germination under high temperature; however, the pollen from the control treatment did not have particularly high percentage of germination (46%). This could be due to loss of pollen viability during the experimental period and it was reported that okra pollen viability reduced by 50% one hour after anthesis (Patil et al., 2013). Nevertheless, high temperature strongly reduced anther numbers and *in vitro* pollen germination compared to the control, and caused pollen grain deformation including shrunken cytoplasmic contents similar to that observed in tomato and wheat (Sugiyama et al., 1965, Saini and Aspinall, 1982, Peet et al., 1998).

The study was extended to determine if loss of pollen viability in ambient temperature (*in vitro* incubation of 26°C) was due to the time lag between anthesis and *in vitro* germination or due to the temperature effect. In the process, the most effective temperatures for high and low germination were identified. The optimal temperature of 32°C produced 90% germination; however, at 37°C this dropped to 17%. The lowest germination was observed above 42°C. Clearly, okra pollen has sensitivity limits that are similar to cotton (Burke et al., 2004). Even one hour exposure to high temperature (42°C) was enough to inhibit pollen germination in okra.

In the current study, high temperature caused abnormality in pollen structure, lower pollen production and lower pollen germination which consequently reduced yield and increased the production of seedless fruit. Similar observations have been reported in sorghum where the maximum pollen production, pollen viability, *in vitro* pollen germination and seed set were observed at 32°C. However, the increase in temperature from 32 to 36°C reduced pollen germination and seed setting by 26% and 30%

respectively (Prasad et al., 2006b). More research is needed to quantify the effect of high temperature on okra female tissue to answer the questions: How long does the sigma stay receptive? Does the style provide enough nutrition to support pollen tube growth? Which stages of bud development have the most impact on the male tissue development? Moreover, the processes involved in pollen infertility needs to be explored further.

6.5 Conclusion

High temperature stress caused alteration of the reproductive tissue of okra. Plants under heat stress produced smaller flowers, however the stigmas remained receptive. The shape, orientation, curvature and structure of the ovules did not change with heat stress. Although there was no damage found in female tissues, the male tissues were severely damaged by heat. High temperature reduced anther numbers and dehiscence resulting in poor pollen dispersal. Under high temperature, pollen structure was altered in the early stages of bud development and *in vitro* pollen germination was significantly reduced. This disturbance in the fertilization process produced seedless fruit.

7 General discussion, recommendations and conclusion

7.1 Background and scope of the current study

Global food security is under threat by an increasing population and the effects of global warming. High temperature is a major environmental stress which inhibits plant growth and development and reduces plant productivity at different growth stages (Wahid et al., 2007). Temperature has been predicted to increase 1°C to 4°C in the twenty first century (IPCC, 2007) and understanding heat tolerance mechanisms in plants plays an important role in breeding thermo-tolerant crop varieties.

There are many mechanisms involved in yield loss, such as adverse effects of high temperature on physiology and metabolic pathways, as well as reproductive tissues. Photosynthesis is highly sensitive to high temperature and inhibits before other physiological functions (Camejo et al., 2005) and both short- and long-term heat stress can have a negative impact on the photosynthetic apparatus (Sinsawat et al., 2004, Zhang and Sharkey, 2009, Martinazzo et al., 2012, Ding et al., 2016). The most heat sensitive compartment in the cell is the chloroplast; within the chloroplast, thylakoid membranes and stroma, which are the sites where the photosystem is located and carbon metabolism takes place, are the main regions damaged by high temperature (Fahad et al., 2017). Membranes also become leaky which causes ion imbalances and disturbs cellular function (Mc Daniel, 1982, Yamane et al., 1997). In addition, heat denatures proteins situated inside the membranes which lead to changes in carbohydrate synthesis or cleavage and consequently impairs carbon metabolism (Schöffl et al., 1999). This impairment includes sugar mobilization to sink tissues, such as reproductive tissues, and results in low energy levels for development and functions of these tissues and consequently yield reduction (Ribeiro et al., 2012, Bhandari et al., 2016).

Physiological, morphological, phenological, metabolic and agronomic responses to heat have been studied in various crops such as cotton, wheat, rice and chickpea; however such information about okra is limited (Sharma et al., 2005, Weerakoon et al., 2008, Cottee et al., 2010, Kaushal et al., 2013). Okra is a valuable summer vegetable crop

which is well adapted to warm regions such as Africa and Asia; nevertheless high temperature has a negative effect on its productivity. In the current study, okra performed poorly under temperatures of 45°C and its yield reduced severely. Hence its response to heat was investigated from different aspects to establish the cause of yield reduction.

Initially, okra was evaluated for its physiological responses and adaptation to a short-term heat shock in a controlled environment and then compared with its responses under long-term heat stress in an outdoor production setting. In addition, okra reproductive organs were assessed to evaluate the sensitivity of both male and female tissues under high temperature. Finally, adverse effects of high temperature on morphology and productivity were evaluated, as well as okra's sensitivity to heat at two different stages of development (flowering and bud initiation) investigated. At the same time, one of the aims in the current study was to ensure genetic diversity in the genotypes which were evaluated for heat-sensitivity. This was achieved by optimising a CTAB method to amplify high quality DNA using SSR markers and this procedure generally is difficult in okra due to high mucilage content of the tissue. I was able to demonstrate that eight markers showed polymorphism in okra, with the highest PIC value for marker 13 (AVRDC-Okra64) which was also used by Yildiz et al. (2015) and (Schafleitner et al., 2013). The screening with SSR markers allowed the generation of a dendrogram which helped to select diverse genotypes.

These different techniques helped to select genotypes with contrasting responses, evaluate the usefulness of the employed methods and techniques for the future work and detect the most adverse temperatures for vegetative and reproductive growth. In addition, a potential tolerant genotype was detected (L4-48).

7.2 Physiological responses of okra to high temperature

Okra's photosynthetic apparatus showed sensitivity to a 6h heat shock where there was a significant reduction in F_v'/F_m' in many genotypes. At the same time, contrasting g_s and A were observed which suggested potentially different mechanisms of tolerance among genotypes. Under environmental stress, plants display short-term and

long-term responses and acclimation can be observed where an adjustment to short-term environmental changes occurs (Ruban, 2009). After growing for 2 weeks in the heat, acclimated okra increased g_s with increasing temperature in a controlled environment from 36°C onwards, resulting in constant *A* even at 45°C, whereas non-acclimated plants closed their stomata. Additionally, acclimation was evident in the constant F_v'/F_m' response between 33 and 45°C in the controlled environment, as well as in comparable values between control and heat in the outdoor setting. Comparison between the fluorescence parameters F_m', F_o' and F_s showed that F_o' was more sensitive to heat shock as it continued to increase in acclimated plants even after the temperature was reduced from 45°C to 30°C in the controlled environment setting. In contrast, F_s was more sensitive to long-term heat stress in the field setting, where after 15 weeks of heat treatment, F_s stayed higher in the heat compared to the control. This resulted in a reduction of Φ_{PSII} and *q*P which is an indication of a decline in photochemical capacity of PSII which in turn may decrease *A*, which was corroborated by a linear relationship between Φ_{PSII} and photosynthetic rate (Maxwell and Johnson, 2000). Nevertheless, Φ_{PSII} does not show true functionality of PSII as it is influenced by PSI fluorescence emission and an alteration in plastoquinone quenching (Baker, 2008).

At the beginning of the tunnel house experiment, *A* reduced in the heat, but then increased over time. This response may have partially resulted from higher g_s under high temperature, although there was a stronger relationship between *A* and fluorescence parameters under high temperature, rather than with g_s. In okra, g_s increased as a heat avoidance mechanism and acclimation response, and it successfully reduced leaf temperature by 10°C.

Another important parameter for imparting tolerance is membrane stability. Ristic et al. (2007) reported loss of chlorophyll under high temperature, which resulted from degradation of protein due to an increase in the activity of proteolytic enzymes, a phenomenon closely correlated with thylakoid damage. Heat increases the instability of the phospholipid bilayer of membranes which leads to ion leakage and disturbance to PSII activity (Mc Daniel, 1982, Pilon et al., 2016). Nevertheless, a 6h heat shock did not show evidence for damaged membranes in most of the okra genotypes. Additionally, in

the outdoor setting, okra exposed to high temperature showed constant EL. These results indicated that okra has stable membrane structure and is capable of membrane restoration under high temperature, and it has been classified as relatively tolerant with regard to its leaf-tissue injury (Kuo et al., 1992). In heat-acclimated potato, membrane stability is ensured by accumulation of zeaxanthin in leaves (Havaux and Gruszecki, 1993, Havaux and Tardy, 1996, Camejo et al., 2005); however these antioxidants were not assessed in the current study.

High temperature reduced root mass in okra, and reduced root mass and root conductance was demonstrated in sugarcane, resulting in water and nutrient uptake inhibition (Wahid and Close, 2007). High E and k_{leaf} in okra, however, suggested efficient water movement from roots to leaves in the heat. CO_2 and H_2O availability were not reduced in okra due to a dramatic increase in g_s and k_{leaf} under high temperature; yet sugar content decreased significantly. This reduction was most severe in the shoots which may have resulted from a reduction in phloem loading rate as well as reduced sucrose transport to the reproductive tissues (Li et al., 2011, Ribeiro et al., 2012, Bhandari et al., 2016). Heat can also alter the activities of sucrose cleavage and synthesis enzymes (Rufty et al., 1985, Holaday et al., 1992, Kaushal et al., 2013, Bhandari et al., 2016), but higher concentration of sugars in okra leaves and roots in the heat suggested the activity of those enzymes were not inhibited. In both control and heat, sucrose had the highest concentration followed by fructose and glucose in okra roots and leaves, which was similar to Li et al. (2006), but Roitsch and González (2004) reported higher concentration of fructose compared to glucose at ambient temperature. Similar concentration of sucrose to the other sugars in the shoots suggests a sustained activity of sucrose cleavage enzymes in both control and heat.

In summary, temperature above 39°C in a controlled environment had adverse effects on the photosynthetic apparatus in non-adapted plants. Okra can physiologically tolerate high temperature of 45°C by increasing g_s, E, k_{leaf} and maintaining constant EL to improve photosynthetic activity. Carbohydrate levels in leaves and roots increased rather than decreased in the heat, hence sucrose cleavage and synthesis enzymes seemed minimally affected by heat. In contrast, shoot carbohydrate content dramatically

decreased which may have resulted from modified phloem loading, and is an area for further investigation.

7.3 Biomass and yield of okra in response to high temperature

In the heat, okra plants were shorter with a lower number of nodes, but after a 15-week heat treatment, there was no significant effect of high temperature on main stem diameter, number of lateral shoots and above-ground dry weight which may have resulted from the recovery of *A* in heat-adapted okra after 4 weeks. Root dry matter decreased under high temperature, but interpretation of below-ground biomass is difficult in this study, since root growth was limited by pot size. Despite relatively stable vegetative growth and dry matter production, fruit yield decreased dramatically. Okra's photosynthetic apparatus acclimatized and membrane stability was maintained under high temperature which confirmed that it can survive in the heat, similar to cotton (Hasanuzzaman et al., 2013, Abro et al., 2015). In spite of this, productivity can be impaired, and several studies reported that reproductive tissues are more sensitive than vegetative tissues under high temperature (Hatfield et al., 2011).

In summary okra can maintain vegetative growth and dry matter (above ground) in high temperature, but below ground dry matter production is impaired. Below ground production needs to be evaluated with the crops planted in the ground to assess how much root growth and root functions can contribute to defend plants against heat by accessing deep water.

7.4 Response of okra reproductive tissues to high temperature

Reproductive development has a lower optimum temperature compared to vegetative development; for example, optimum temperature for vegetative growth of cotton is 37°C, whereas for reproductive growth it is 28-30°C (Ketring, 1984, Hatfield et al., 2011). Yield loss under high temperature is more related to damage in reproductive tissues compared to vegetative tissues, and Ketring (1984) reported 33% yield loss in peanut, when temperature increased from 30°C to 35°C during reproductive

development. Okra yield loss was due to a severe alteration to reproductive tissues. Similar to groundnut, okra produced flowers in the heat but they were infertile (Vara Prasad et al., 2000). Heat reduces fertilization efficiency of male and female organs; however, the most heat sensitive stage in most plants is pollen development (Driedonks et al., 2016). As a result, morning temperature has a stronger effect than afternoon temperature in self-pollinated species, since pollination occurs in the morning even before anthesis (Vara Prasad et al., 2000, Dhankhar and Singh, 2009). Pollen can be damaged early-on during meiosis, but continues to be sensitive to the latest stage of pollen germination and fertilization of an ovule (Saini and Aspinall, 1982, Dupuis and Dumas, 1990). In the outdoor environment, okra pollen proved vulnerable to high temperature from the early stage of bud development, where damage to pollen structure was observed c. 8 to 10 days prior to anthesis. In addition, anther numbers and *in vitro* pollen germination reduced. Similar to cotton (Hatfield et al., 2011), okra showed maximum *in vitro* pollen germination at temperatures between 28°C and 32°C, while the lowest germination was observed at 42°C and above. In okra, fertilization occurs c. 11 to 12 h after pollination (Dhankhar and Singh, 2009) and pollination and fertilization are more sensitive to high temperature than post-fertilization events such as embryo formation (Vara Prasad et al., 2000). Although okra was able to develop flowers in the heat which had receptive stigmata, pollen dispersal onto the stigma was reduced due to anther indehiscence. Seedless okra fruit in the heat indicated that fertilization was unsuccessful; however the structure of ovules did not change in the heat. The nutritional status of reproductive tissues was not assessed, yet this infertility may have resulted from deficient nutrition in reproductive tissues which may have arisen from reduced shoot sugar content and potential inhibition of sugar movement to reproductive tissues. Since sugar concentration in the medium of the *in vitro* pollen germination was kept constant, other factors likely played a role in fertilization, including enzyme activities, such as activity of hydrolases enzymes on pollen tubes which are responsible for availability of sugar for pollen tube growth (Wu et al., 1995).

In summary, high temperature severely affected male reproductive tissue with reduced anther and pollen numbers, as well as decreased or completely inhibited pollen

germination. The exact cause for the unsuccessful fertilization in okra remains unclear and is an area for future investigation.

7.5 Effect of high temperature on developmental stages in okra

Okra was more vulnerable when exposed to high temperature at an early stage of development. In both heat and control, plants at bud initiation had lower F_v'/F_m', qP, Φ_{PSII}, and A compared to plants at flowering. In contrast g_s and E was similar at both stages, where under high temperature an increase was observed in these two parameters. Additionally, EL was higher at bud initiation in the heat whereas in the control EL was higher at flowering at week 2 and 4, but at week 6 EL was similar between stages.

In the control, higher concentration of fructose and glucose in shoots were observed at bud initiation where plants needed more energy for growth. Under high temperature, on average, there was higher sugar reduction at bud initiation compared to flowering, probably due to lower A and fluorescence parameters; this corroborates that okra is more sensitive to high temperature at early developmental stages. The only exception to these results was the higher number of anthers which was observed at bud initiation.

In summary, high temperature had a stronger effect on okra when it was at an early stage of development; nevertheless, okra still showed tolerance when it was exposed to heat stress at bud initiation stage as photosynthetic parameters increased over time.

7.6 Genotypic differences in heat-tolerance in okra

Generally, no significant differences could be found between genotypes for many of the physiological measurements (e.g. fluorescence parameters, A and g_s) in the long-term heat treatment; however, in a 6-hour heat shock, some variation was observed among genotypes. EL varied more strongly among genotypes in the long-term heat treatment: L4-48 had the highest EL in both control and heat, whereas L2-11, L2-30 and L3-1 showed similarity after 6 weeks of heat treatment. Yet, these genotypes did not

show the same result in the 6-hour heat shock, where in the control, L2-11 had the highest EL followed by L3-1, and L2-30 had the lowest value.

Among all genotypes, L4-48 performed better under high temperature in both the controlled and outdoor environments, where it showed similar responses in F_v'/F_m', A, g_s between control and 6h heat treatment and higher F_v'/F_m', A, g_s after 15 weeks of outdoor heat treatment compared to the control. Genotype L4-48 was the shortest among 8 genotypes tested in the field which produced higher below ground dry matter both in the control and heat compared to the other genotypes. In the control, this genotype had the highest *in vitro* pollen germination followed by L2-11 and the second highest yield production after L2-11 in the outdoor setting. These traits demonstrate that this genotype could have potential to be used as a breeding line; it not only physiologically adapted to high temperature, but perhaps has higher root biomass production and may be able to limit drought stress by accessing deep water. A detailed study on reproductive tissue and determining threshold temperatures for yield loss in this genotype would be beneficial to breed an economically heat-tolerant genotype.

In summary, genetic variation was not strongly pronounced in this study, but slight different responses to high temperature among genotypes, identified a potential genotype for further studies.

7.7 Limitation and future work

There were some limitations in the current study which may have reduced the significance of some findings and will be addressed in this section. Additionally, areas for future work will be identified.

Firstly, temperature was recorded at 2.5 m height in the tunnel houses, which was not necessarily representative of canopy temperature, and branches lower to the ground or shorter genotypes may have been exposed to cooler temperatures.

Yield was found to reduce severely due to heat damage of reproductive tissues, but the precise stage of development which is sensitive to high temperature was beyond the scope of this study. Sugar imbalances in the plant may have played a role in the reduction of yield. Therefore, carbohydrate status of reproductive tissues needs to be evaluated,

coupled to the study of activity of sucrose synthesis and cleavage enzymes and gene expression, as was done in tomato (Li et al., 2011). Additionally, due to space limitation and a large number of genotypes, fruit production was not assessed in the controlled environment; therefore there was no estimation over yield loss in a short-term heat shock.

Heat damage is more severe along with drought; however, the effect of water limitation was not considered here. In order to increase food security, breeding genotypes for both heat and drought tolerance would be a better strategy. Since okra shows the tendency to open stomata in the heat, a functioning water transport system is needed. Evaluating the effect of high temperature on leaf and root structure, as well as water and nutrition movement through xylem, phloem and leaf veins will shed light on the adaptation of the water conductivity system, as well as the efficiency of nutrient delivery to the reproductive organs. Other methods such as measuring chlorophyll content and the activity of the mitochondrial electron transport chain by chlorophyll meter and triphenyl tetrazolium chloride (TTC) respectively, could be useful tools for screening as shown for wheat (Ristic et al., 2007). Evaluating chlorophyll content in regard to loss or reduction in chlorophyll biosynthesis could be also an effective screening tool (Ristic et al., 2007, Fahad et al., 2017).

In addition to identifying suitable heat-adapted genotypes, some management approaches such as forwarding sowing date and avoiding heat stress which occurs later in the season or shifting crop cultivation geographically (Driedonks et al., 2016) are important strategies to help increase plant productivity.

Finally, okra has been used for other purposes rather than human food, for example stems are used for paper production (Lamont, 1999, Benchasri, 2012), brightening agents in the electro-deposition of metals (Kumar et al., 2010), as a fuel source (Ahiakpa et al., 2013) or as cattle feed (Benchasri, 2012). This thesis demonstrated that high temperature had no significant effect on vegetative growth of okra; consequently, varieties which are used for above purposes can be grown in a warmer regions and varieties which are grown for fruit production can be shifted to cooler regions.

7.8 Conclusion

In okra, high temperature caused severe damage to the reproductive system which led to dramatic yield loss. This may have been due to imbalance in sugar content and inhibition of sugar movement to reproductive tissues. Under high temperature, sugar production was altered which may have partially been caused by a slight reduction in *A* due to damage to PSII, as there was a tendency of lower Φ_{PSII} and F_v'/F_m'. This thesis demonstrated that okra is more vulnerable at the early stage of growth and development, but it tolerated high temperature and could survive. The okra male organ was the most sensitive tissue and was affected by high temperature from early stages of bud development. Pollen sensitivity was reported above 32ºC where the germination reduced. This study nominated genotype L4-48 (e.g. Name: Cajun jewel, AVRDC No.: 056456, Origin: USA) for breeding tolerant varieties based on its physiological performance under high temperature and its high productivity in control conditions.

References

Abro, S., Rajput, M. T., Khan, M. A., Sial, M. A. & Tahir, S. S. 2015. Screening of cotton (*Gossypium Hirsutum* L.) genotypes for heat tolerance. *Pakistan Journal of Botany,* 47**,** 2085-2091.

Adeboye, O. & Oputa, C. 1996. Effects of galex on growth and fruit nutrient composition of okra (*Abelnurschus esculentus*). *Ife Journal of Agriculture,* 18**,** 1-9.

Adekalu, K. O., Ogunjimi, L. a. O., Olaosebikan, F. O. & Afolayan, S. O. 2008. Response of okra to irrigationand mulching. *International Journal of Vegetable Science,* 14**,** 339-350.

Ahiakpa, J., Kaledzi, P., Adi, E., Peprah, S. & Dapaah, H. 2013. Genetic diversity, correlation and path analyses of okra (*Abelmoschus spp*.(L.) Moench) germplasm collected in Ghana. *International Journal of Development and Sustainability,* 2**,** 1396-1415.

Ahmad, N. M., Martin, P. M. & Vella, J. M. 2009. Embryology of the dioecious Australian endemic *Lomandra longifolia* (*Lomandraceae*). *Australian Journal of Botany,* 56**,** 651-665.

Akash, M. W., Shiyab, S. M. & Saleh, M. I. 2013. Yield and AFLP analyses of inter-landrace variability in okra (*Abelmoschus esculentus* L.). *Life Science Journal,* 10**,** 771-9.

Aladele, S. E. 2009. Morphological distinctiveness and metroglyph analysis of fifty accessions of West African okra (*Abelmoschus caillei*)(A. Chev.) Stevels. *Journal of Plant Breeding and Crop Science,* 1**,** 273-280.

Aladele, S. E., Ariyo, O. & De Lapena, R. 2008. Genetic relationships among West African okra (*Abelmoschus caillei*) and Asian genotypes (*Abelmoschus esculentus*) using RAPD. *African Journal of Biotechnology,* 7**,** 1426.

Ariyo, O. 1993. Genetic diversity in West African okra (*Abelmoschus caillei*)(A. Chev.) Stevels—Multivariate analysis of morphological and agronomic characteristics. *Genetic Resources and Crop Evolution,* 40**,** 25-32.

Arulrajah, T. & Ormrod, D. 1973. Responses of okra (*Hibiscus esculentus* L.) to photoperiod and temperature. *Annals of Botany,* 37**,** 331-340.

Atteya, A. 2003. Alteration of water relations and yield of corn genotypes in response to drought stress. *Bulgarian Journal of Plant Physiology.,* 29**,** 63-76.

Azhar, F., Ali, Z., Akhtar, M., Khan, A. & Trethowan, R. 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). *Plant Breeding,* 128**,** 356-362.

Baghizadeh, A. & Hajmohammadrezaei, M. 2011. Effect of drought stress and its interaction with ascorbate and salicylic acid on okra (*Hibiscus esculents* L.) germination and seedling growth. *The Journal of Stress Physiology & Biochemistry,* 7**,** 55-65.

Baghizadeh. A., Ghorbanli. M., Haj Mohammad Rezaei, M. & Mozafari. H. 2009. Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on some of physiological and biochemical parameters in okra (*Hibiscus esculentus* L.). *Research Journal of Biological Sciences,* 4**,** 380-387.

Baker, N. R. 2008. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annu. Rev. Plant Biol.,* 59**,** 89-113.

Battisti, D. S. & Naylor, R. L. 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. *science,* 323**,** 240-244.

Benchasri, S. 2012. Okra (*Abelmoschus esculentus* (L.) Moench) as a valuable vegetable of the world. *Ratarstvo i povrtarstvo,* 49**,** 105-112.

Berry, S., Klara, C., Scchmul, P., Kulkami, S., Skhuir, K., Arora, S. & Sherma, G. 1988. Quality characteristics of seeds of five okra (*A. esculentus*) J. *Food Science and Technology,* 2**,** 303-307.

Bhandari, K., Siddique, K. H., Turner, N. C., Kaur, J., Singh, S., Agrawal, S. K. & Nayyar, H. 2016. Heat stress at reproductive stage disrupts leaf carbohydrate metabolism, impairs reproductive function, and severely reduces seed yield in lentil. *Journal of Crop Improvement,* 30**,** 118-151.

Bibi, A., Oosterhuis, D. & Gonias, E. 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. *Journal of Cotton Science,* 12**,** 150-159.

Bisht, I. & Bhat, K. 2006. Okra (*Abelmoschus spp.*). *CRC Press. Genetic resources, chromosome engineering, and crop improvement, Vegetable Crops,* 3**,** 147-183.

Blum, A. & Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Science,* 21**,** 43-47.

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics,* 32**,** 314.

Brake, V., Irwin, J. & Park, R. 2001. Genetic variability in Australian isolates of *Puccinia coronata* f. sp. avenae assessed with molecular and pathogenicity markers. *Australasian Plant Pathology,* 30**,** 259-266.

Brewbaker, J. L. & Kwack, B. H. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany***,** 859-865.

Brodribb, T. & Holbrook, N. 2003. Changes in leaf hydraulic conductance during leaf shedding in seasonally dry tropical forest. *New Phytologist,* 158**,** 295-303.

Brown, P. 2008. Cotton heat stress. *College of Agriculture and Life Sciences, University of Arizona (Tucson, AZ)***,** 10.

Bukhov, N. G., Wiese, C., Neimanis, S. & Heber, U. 1999. Heat sensitivity of chloroplasts and leaves: leakage of protons from thylakoids and reversible activation of cyclic electron transport. *Photosynthesis Research,* 59**,** 81-93.

Burke, J. J., Velten, J. & Oliver, M. J. 2004. *In vitro* analysis of cotton pollen germination. *Agronomy journal,* 96**,** 359-368.

Burkill, I. H., Birtwistle, W., Foxworthy, F., Scrivenor, J., Watson, J. & Burkhill, I. 1936. A dictionary of the economic products of the Malay Peninsula. JSTOR.

Camejo, D. 2013. High temperature effect on tomato (*Lycopersicon esculentum*) pigment and protein content and cellular viability. *Cultivos Tropicales,* 22**,** 13-17.

Camejo, D., Rodríguez, P., Morales, M. A., Dell'amico, J. M., Torrecillas, A. & Alarcón, J. J. 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Journal of plant physiology,* 162**,** 281-289.

Castro, M. M., Godoy, A. R. & Cardoso, A. I. I. 2008. Okra seed quality as a function of age and fruit post harvest rest. *Ciência e Agrotecnologia,* 32**,** 1491-1495.

Charles, W. & Harris, R. 1972. Tomato fruit-set at high and low temperatures. *Canadian Journal of Plant Science,* 52**,** 497-506.

Chauhan, K. & Bhandari, Y. 1971. Pod development and germination studies in okra (*Abelmoschus esculentus* (L.) Moench). *Indian Journal of Agricultural Sciences.*

Chen, L.-S. & Cheng, L. 2009. Photosystem 2 is more tolerant to high temperature in apple (*Malus domestica* Borkh.) leaves than in fruit peel. *Photosynthetica,* 47**,** 112-120.

Chowdhury, S. & Wardlaw, I. 1978. The effect of temperature on kernel development in cereals. *Crop and Pasture Science,* 29**,** 205-223.

Cottee, N., Tan, D., Bange, M., Cothren, J. & Campbell, L. 2010. Multi-level determination of heat tolerance in cotton (*Gossypium hirsutum* l.) under field conditions. *Crop Science,* 50**,** 2553-2564.

Cottee, N. S., Bange, M. P., Wilson, I. W. & Tan, D. K. 2012. Developing controlled environment screening for high-temperature tolerance in cotton that accurately reflects performance in the field. *Functional Plant Biology,* 39**,** 670-678.

Cui, L., Li, J., Fan, Y., Xu, S. & Zhang, Z. 2006. High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. *Botanical studies,* 47**,** 61-69.

Dafni, A. & Maués, M. M. 1998. A rapid and simple procedure to determine stigma receptivity. *Sexual plant reproduction,* 11**,** 177-180.

Dane, F., Hunter, A. G. & Chambliss, O. L. 1991. Fruit set, pollen fertility, and combining ability of selected tomato genotypes under high-temperature field conditions. *Journal of the American Society for Horticultural Science,* 116**,** 906-910.

Datta, R., Chourey, P. S., Pring, D. R. & Tang, H. V. 2001. Gene-expression analysis of sucrose-starch metabolism during pollen maturation in cytoplasmic male-sterile and fertile lines of sorghum. *Sexual plant reproduction,* 14**,** 127-134.

Demir, I. 2001. The effects of heat treatment on hardseededness of serially harvested okra seed lots at optimum and low temperatures. *Scientia Horticulturae,* 89**,** 1-7.

Dhankhar, B. S. & Singh, R. 2009. *Okra Handbook: Global Production, Processing, and Crop Improvement.*, HNB Publishing.

Dhankhar, S. & Singh, S. 2013. Thermal requirements for flowering and fruit yield attainment in advance lines of okra. *Journal of Agrometeorology,* 15(1)**,** 39-42.

Diizyaman, E. 1997. Okra: Botany and Horticulture, In: J. Janick (eds). *Horticultural Reviews,* 21**,** 41-72.

Ding, X., Jiang, Y., Hao, T., Jin, H., Zhang, H., He, L., Zhou, Q., Huang, D., Hui, D. & Yu, J. 2016. Effects of heat shock on photosynthetic properties, antioxidant enzyme activity, and downy mildew of cucumber (*Cucumis sativus* L.). *PloS one,* 11**,** e0152429.

Djanaguiraman, M., Prasad, P. & Al-Khatib, K. 2011. Ethylene perception inhibitor 1-MCP decreases oxidative damage of leaves through enhanced antioxidant defense mechanisms in soybean plants grown under high temperature stress. *Environmental and Experimental Botany,* 71**,** 215-223.

Downs, C. A., Ryan, S. L. & Heckathorn, S. A. 1999. The chloroplast small heat-shock protein: evidence for a general role in protecting photosystem II against oxidative stress and photoinhibition. *Journal of plant physiology,* 155**,** 488-496.

Driedonks, N., Rieu, I. & Vriezen, W. H. 2016. Breeding for plant heat tolerance at vegetative and reproductive stages. *Plant reproduction,* 29**,** 67-79.

Dupuis, I. & Dumas, C. 1990. Influence of temperature stress on in vitro fertilization and heat shock protein synthesis in maize (*Zea mays* L.) reproductive tissues. *Plant Physiology,* 94**,** 665-670.

Essmann, J., Schmitz-Thom, I., Schön, H., Sonnewald, S., Weis, E. & Scharte, J. 2008. RNA interference-mediated repression of cell wall invertase impairs defense in source leaves of tobacco. *Plant Physiology,* 147**,** 1288-1299.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S. & Saud, S. 2017. Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science,* 8.

Fang, G., Hammar, S. & Grumet, R. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biotechniques,* 13**,** 52-4, 56.

Fao 1968. Food composition table for use in Africa. *Rome, Italy,* 306**,** 335.

Fao 2015. Agriculture Organization of the United Nations, Statistics Division. *Agri-Environmental Indicators/Pesticides.*

Faralli, M., Lektemur, C., Rosellini, D. & Gürel, F. 2015. Effects of heat shock and salinity on barley growth and stress-related gene transcription. *Biologia Plantarum,* 59**,** 537-546.

Fedoroff, N., Battisti, D., Beachy, R., Cooper, P., Fischhoff, D., Hodges, C., Knauf, V., Lobell, D., Mazur, B. & Molden, D. 2010. Radically rethinking agriculture for the 21st century. *Science,* 327**,** 833-834.

Feller, U., Crafts-Brandner, S. J. & Salvucci, M. E. 1998. Moderately high temperatures inhibit ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. *Plant Physiology,* 116**,** 539-546.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution,* 39**,** 783-791.

Fougat, R. S., Purohit, A. R., Kumar, S., Parekh, M. J. & Kumar, M. 2015. SSR based genetic diversity in *Abelmoschus* species. *Indian Journal of Agricultural Sciences,* 85**,** 1223-1228.

Gawronska, H., Thornton, M. K. & Dwelle, R. B. 1992. Influence of heat stress on dry matter production and photo-assimilate partitioning by four potato clones. *American Journal of Potato Research,* 69**,** 653-665.

Geigenberger, P., Geiger, M. & Stitt, M. 1998. High-temperature perturbation of starch synthesis is attributable to inhibition of ADP-glucose pyrophosphorylase by decreased levels of glycerate-3-phosphate in growing potato tubers. *Plant Physiology,* 117**,** 1307-1316.

Grubben, G. J. 1977. Tropical vegetables and their genetic resources. IBPGR, Rome.

Guilioni, L., Wery, J. & Tardieu, F. 1997. Heat stress-induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs? *Annals of Botany,* 80**,** 159-168.

Gulsen, O., Karagul, S. & Abak, K. 2007. Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. *Biologia,* 62**,** 41-45.

Gunawardhana, M. & De Silva, C. 2012. Impact of temperature and water stress on growth yield and related biochemical parameters of okra. *Tropical Agricultural Research,* 23(1)**,** 77-83.

Gutierrez, M., Patto, M. V., Huguet, T., Cubero, J., Moreno, M. & Torres, A. 2005. Cross-species amplification of *Medicago truncatula* microsatellites across three major pulse crops. *Theoretical and Applied Genetics,* 110**,** 1210-1217.

Hamon, S. & Koechlin, J. 1991a. The reproductive biology of okra. 1. Study of the breeding system in four Abelmoschus species. *Euphytica,* 53**,** 41-48.

Hamon, S. & Koechlin, J. 1991b. The reproductive biology of okra. 2. Self-fertilization kinetics in the cultivated okra (Abelmoschus esculentus), and consequences for breeding. *Euphytica,* 53**,** 49-55.

Hamon, S. & Noirot, M. 1991. Some proposed procedures for obtaining a core collection using quantitative plant characterization data. *Report of an international workshop on okra genetic resources***,** 89-94.

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R. & Fujita, M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences,* 14**,** 9643-84.

Hatfield, J. L., Boote, K. J., Kimball, B., Ziska, L., Izaurralde, R. C., Ort, D., Thomson, A. M. & Wolfe, D. 2011. Climate impacts on agriculture: implications for crop production. *Agronomy journal,* 103**,** 351-370.

Havaux, I. 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant, Cell & Environment,* 16**,** 461-467.

Havaux, M. 1996. Short-term responses of photosystem I to heat stress. *Photosynthesis Research,* 47**,** 85-97.

Havaux, M. & Gruszecki, W. I. 1993. Heat-and light-induced chlorophyll a fluorescence changes in potato leaves containing high or low levels of the carotenoid zeaxanthin: Indications of a regulatory effect of zeaxanthin on thylakoid membrane fluidity. *Photochemistry and photobiology,* 58**,** 607-614.

Havaux, M. & Tardy, F. 1996. Temperature-dependent adjustment of the thermal stability of photosystem II *in vivo*: possible involvement of xanthophyll-cycle pigments. *Planta,* 198**,** 324-333.

Hawker, J. & Jenner, C. 1993. High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. *Functional Plant Biology,* 20**,** 197-209.

Haytowitz, D. B. & Matthews, R. H. 1984. Composition of foods: vegetables and vegetable products: raw, processed, prepared. *Agriculture handbook (USA).*

Hedhly, A., Hormaza, J. & Herrero, M. 2005. The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology,* 7**,** 476-483.

Herrero, M. & Arbeloa, A. 1989. Influence of the pistil on pollen tube kinetics in peach (Prunus persica). *American Journal of Botany***,** 1441-1447.

Herrero, M. P. & Johnson, R. 1980. High temperature stress and pollen viability of maize. *Crop Science,* 20**,** 796-800.

Holaday, A. S., Martindale, W., Alred, R., Brooks, A. L. & Leegood, R. C. 1992. Changes in activities of enzymes of carbon metabolism in leaves during exposure of plants to low temperature. *Plant Physiology,* 98**,** 1105-1114.

Hong-Qi, Z. & Croes, A. 1982. A new medium for pollen germination *in vitro*. *Acta botanica neerlandica,* 31**,** 113-119.

Ibpgr 1990. (International Bureau for Plant Genetic Resources). Report on international workshop on okra genetic resources. *the National Bureau for Plant Genetic Resources. New Delhi, India.*

Ipcc 2007. Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the *Intergovernmental Panel on Climate Change, UK, Cambridge University Press*.

Iremiren, G. & Okiy, D. 1986. Effects of sowing date on the growth, yield and quality of okra (*Abelmoschus esculentus* (L.) Moench.) in southern Nigeria. *The Journal of Agricultural Science,* 106**,** 21-26.

Iremiren, G., Osara, A. & Okiy, D. 1991. Effects of age of harvesting after pod set on the growth, yield and quality of okra (*Abelmoschus esculentus*). *Experimental Agriculture,* 27**,** 33-37.

Ismail, A. M. & Hall, A. E. 1999. Reproductive-stage heat tolerance, leaf membrane thermostability and plant morphology in cowpea. *Crop Science,* 39**,** 1762-1768.

Jamala, G., Boni, P., Abraham, P. & Musa, A. 2011. Soil status and yield response of different varieties of okra (*Abelmoschus esculentus* (L.) Moench) grown at Mubi floodplain, North Eastern, Nigeria. *Journal of Agricultural Biotechnology and Sustainable Development,* 3**,** 120-125.

John, M. E. 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Research,* 20**,** 2381.

Kakani, V., Reddy, K., Koti, S., Wallace, T., Prasad, P. V., Reddy, V. & Zhao, D. 2005a. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Annals of Botany,* 96**,** 59-67.

Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V., Reddy, V. R. & Zhao, D. 2005b. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Annals of Botany,* 96**,** 59-67.

Kalloo, G. & Bergh, B. 2012. *Genetic improvement of vegetable crops,* Newnes.

Kankwatsa, P. 2016. Genetic studies of rust resistance in the *Triticum spp.*, and *Puccinia graminis* f. sp. tritici pathosystem. *University of Sydeney, Faculty of Agriculture and Environment*.

Karni, L. & Aloni, B. 2002. Fructokinase and hexokinase from pollen grains of bell pepper (*Capsicum annuum* L.): possible role in pollen germination under conditions of high temperature and CO_2 enrichment. *Annals of Botany,* 90**,** 607-612.

Kaur, K., Pathak, M., Kaur, S., Pathak, D. & Chawla, N. 2013. Assessment of morphological and molecular diversity among okra [*Abelmoschus esculentus* (L.) Moench.] germplasm. *African Journal of Biotechnology,* 12.

Kaushal, N., Awasthi, R., Gupta, K., Gaur, P., Siddique, K. H. & Nayyar, H. 2013. Heat-stress-induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. *Functional Plant Biology,* 40**,** 1334-1349.

Ketring, D. 1984. Temperature effects on vegetative and reproductive development of peanut1, 2. *Crop Science,* 24**,** 877-882.

Khan, A. A. & Azmat, M. A. 2013. Assessment of genetic diversity in okra (*Abelmoschus esculentus* L.) using RAPD markers. *Pakistan Journal of Agricultural Sciences,* 50.

Khayat, E. & Zieslin, N. 1987. Effect of night temperature on the activity of sucrose phosphate synthase, acid invertase, and sucrose synthase in source and sink tissues of *Rosa hybrida* cv Golden Times. *Plant Physiology,* 84**,** 447-449.

Kirda, C. 2002. Deficit irrigation scheduling based on plant growth stages showing water stress tolerance. *Food and Agricultural Organization of the United Nations, Deficit Irrigation Practices, Water Reports,* 22**,** 102.

Krasensky, J. & Jonak, C. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of experimental botany,* 63**,** 1593-1608.

Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D. & Kouame, C. 2010. Okra (*Abelmoschus spp*.) in West and Central Africa: potential and progress on its improvement. *African Journal of Agricultural Research,* 5**,** 3590-3598.

Kumar, S., Parekh, M. J., Fougat, R. S., Patel, S. K., Patel, C. B., Kumar, M. & Patel, B. R. 2017. Assessment of genetic diversity among okra genotypes using SSR markers. *Journal of Plant Biochemistry and Biotechnology,* 26**,** 172-178.

Kuo, C., Chen, H. & Sun, H. 1992. Membrane thermostability and heat tolerance of vegetable leaves. *Kuo CG (ed), Adaptation of food crops to temperature and water stress. Proc Int Symp.* **,** 160-168.

Lafta, A. M. & Lorenzen, J. H. 1995. Effect of high temperature on plant growth and carbohydrate metabolism in potato. *Plant Physiology,* 109**,** 637-643.

Lafuente, M. T., Belver, A., Guye, M. G. & Saltveit, M. E. 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons possible role of abscisic acid and heat shock proteins. *Plant Physiology,* 95**,** 443-449.

Laing, W. A., Ogren, W. L. & Hageman, R. H. 1974. Regulation of soybean net photosynthetic CO_2 fixation by the interaction of CO_2, O_2, and ribulose 1, 5-diphosphate carboxylase. *Plant Physiology,* 54**,** 678-685.

Lambers, H., Chapin Iii, F. S. & Pons, T. L. 1998. Photosynthesis, respiration, and long-distance transport. *Plant physiological ecology.* Springer.

Lamont, W. J. 1999. Okra—A versatile vegetable crop. *HortTechnology,* 9**,** 179-184.

Law, R. D. & Crafts-Brandner, S. J. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1, 5-bisphosphate carboxylase/oxygenase. *Plant Physiology,* 120**,** 173-182.

Leal, N., Amaral Jr, A., Pereira, M., Daher, R. & Martinello, G. Comparison of morphological characteristics and RAPD for estimating genetic diversity in Abelmoschus spp. International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture 546, 2000. 101-104.

Lengsfeld, C., Titgemeyer, F., Faller, G. & Hensel, A. 2004. Glycosylated compounds from okra inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. *Journal of Agricultural and Food Chemistry,* 52**,** 1495-1503.

Leveitt, J. 1980. Responses of plants to environmental stresses: Chilling, Freezing and High Temperature stresses (Vol. 1). Academic press, New York.

Lewis, H. 2000. Environmental regulation of yield and quality components in American upland cotton. *Proceedings conference,* Genetic control of fiber and seed quality, 2000. 8-36.

Li, T., Liu, Q.-H., Ohsugi, R., Yamagishi, T. & Sasaki, H. 2006. Effect of high temperature on sucrose content and sucrose cleaving enzyme activity in rice grain during the filling stage. *Rice Science,* 13**,** 205-210.

Li, Z., Palmer, W. M., Martin, A. P., Wang, R., Rainsford, F., Jin, Y., Patrick, J. W., Yang, Y. & Ruan, Y.-L. 2011. High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. *Journal of experimental botany,* 63(3)**,** 1156-1166.

Liu, X. & Huang, B. 2000. Carbohydrate accumulation in relation to heat stress tolerance in two creeping bentgrass cultivars. *Journal of the American Society for Horticultural Science,* 125**,** 442-447.

Lobell, D. B. & Gourdji, S. M. 2012. The influence of climate change on global crop productivity. *Plant Physiology,* 160**,** 1686-1697.

Locke, A. M., Sack, L., Bernacchi, C. J. & Ort, D. R. 2013. Soybean leaf hydraulic conductance does not acclimate to growth at elevated [CO_2] or temperature in growth chambers or in the field. *Annals of Botany***,** mct143.

Lu, Z., Radin, J. W., Turcotte, E. L., Percy, R. & Zeiger, E. 1994. High yields in advanced lines of Pima cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. *Physiologia Plantarum,* 92**,** 266-272.

Mangrich, M. E. & Saltveit, M. E. 2000. Heat shocks reduce chilling sensitivity of cotton, kenaf, okra, and rice seedling radicles. *Journal of the American Society for Horticultural Science,* 125**,** 377-382.

Manoj-Kumar, A., Reddy, K. N., Manjulatha, M. & Blanco, L. 2012. Polysaccharide-free nucleic acids and proteins of *Abelmoschus esculentus* for versatile molecular studies. *Molecular Biology,* 46**,** 535-541.

Maréchal-Drouard, L. & Guillemaut, P. 1995. A powerful but simple technique to prepare polysaccharide-free DNA quickly and without phenol extraction. *Plant molecular biology reporter,* 13**,** 26-30.

Martin, F. & Rhodes, A. 1983. Seed characteristics of okra and related *Abelmoschus* species. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum),* 33**,** 41-49.

Martinazzo, E. G., Ramm, A. & Bacarin, M. A. 2012. The chlorophyll a fluorescence as an indicator of the temperature stress in the leaves of Prunus persica. *Brazilian Journal of Plant Physiology,* 24**,** 237-246.

Martinello, G., Leal, N., Amaral Jr, A., Pereira, M. & Daher, R. 2000. Comparison of morphological characteristics and RAPD for estimating genetic diversity in *Abelmoschus* spp. *Acta Horticulture.* (ISHS) 546, 2000. 101-104.

Matsui, T. & Omasa, K. 2002. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: anther characteristics. *Annals of Botany,* 89**,** 683-687.

Matsui, T., Omasa, K. & Horie, T. 2001. The difference in sterility due to high temperatures during the flowering period among japonica-rice varieties. *Plant Production Science,* 4**,** 90-93.

Maxwell, K. & Johnson, G. N. 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany,* 51**,** 659-668.

Mbagwu, J. & Adesipe, F. 1987. Response of three okra (*Abelmoschus esculentus* L. Moench) cultivars to irrigation at specific growth stages. *Scientia Horticulturae,* 31**,** 35-43.

Mc Daniel, R. 1982. The physiology of temperature effects on plants. *Breeding plants for less favorable environments***,** 13-45.

Mc Laughlin, J. E. & Boyer, J. S. 2004. Sugar-responsive gene expression, invertase activity, and senescence in aborting maize ovaries at low water potentials. *Annals of Botany,* 94**,** 675-689.

Merchant, A., Tausz, M., Arndt, S. K. & Adams, M. A. 2006. Cyclitols and carbohydrates in leaves and roots of 13 eucalyptus species suggest contrasting physiological responses to water deficit. *Plant, Cell & Environment,* 29**,** 2017-2029.

Misra, A. N., Misra, M. & Singh, R. 2012. *Chlorophyll fluorescence in plant biology,* INTECH

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends in plant science,* 11**,** 15-19.

Moekchantuk, T. & Kumar, P. 2004. Export okra production in Thailand. *Inter-country programme for vegetable IPM in South & SE Asia phase II Food & Agriculture Organization of the United Nations, Bangkok, Thailand***,** 56.

Murchie, E. H. & Lawson, T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany,* 64**,** 3983-3998.

Nagy, S., Poczai, P., Cernák, I., Gorji, A. M., Hegedűs, G. & Taller, J. 2012. PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. *Biochemical Genetics,* 50**,** 670-672.

Nath, P. 1976. *Vegetables for the tropical region*, Indian Council of Agricultural Research Krishi Anusandhan Bhavan Pusa; New Delhi.

Naveed, A., Khan, A., Rauf, S. & Athar, M. 2010. Assessment of germplasm and development of breeding populations of Okra (*Abelmoschus esculentus*) for drought tolerance. *Journal of Applied Botany and Food Quality,* 83**,** 137-147.

Naveed, A., Khan, A. A. & Rauf, S. 2012. The potential of breeding okra (*Abelmoschus esculentus* L.) for water stress tolerance. *Crop Production for Agricultural Improvement.* Springer.

Nguyen-Quoc, B. & Foyer, C. H. 2001. A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. *Journal of Experimental Botany,* 52**,** 881-889.

Nwangburuka, C., Kehinde, O., Ojo, D., Denton, O. & Popoola, A. 2011. Morphological classification of genetic diversity in cultivated okra, *Abelmoschus esculentus* (L) Moench using principal component analysis (PCA) and single linkage cluster analysis (SLCA). *African Journal of Biotechnology,* 10**,** 11165-11172.

Onwueme, I. 1975. Enhancement of okra (*Hibiscus esculentus*) seedling growth and emergence by heat stress conditions. *The Journal of Agricultural Science,* 85**,** 233-239.

Oosterhuis, D. 2011. *Stress physiology in cotton,* U.S.A., The Cotton Foundation.

Oosterhuis, D. M. 1999. Yield response to environmental extremes in cotton. *Special Reports-University of Arkansas Agricultural Experiment Station,* 193**,** 30-38.

Oppong-Sekyere, D. 2012. Evaluation of some okra (*Abelmoschus spp* L.) germplasm in Ghana. *African Journal of Plant Science,* 6.

Oppong-Sekyere, D., Akromah, R., Nyamah, E., Brenya, E. & Yeboah, S. 2011. Characterization of okra (*Abelmoschus spp*. L.) germplasm based on morphological characters in Ghana. *Journal of Plant Breeding and Crop Science,* 3**,** 368-379.

Osawaru, M., Ogwu, M. & Dania-Ogbe, F. 2013. Morphological assessment of the genetic variability among 53 Accessions of West African okra (*Abelmoschus caillei* (A. Chev.) Stevels) from South Western Nigeria. *Nigerian Journal of Basic and Applied Sciences,* 21**,** 227-238.

Osmond, C. & Grace, S. 1995. Perspectives on photoinhibition and photorespiration in the field: quintessential inefficiencies of the light and dark reactions of photosynthesis? *Journal of experimental botany***,** 1351-1362.

Patil, P., Malik, S. K., Negi, K. S., John, J., Yadav, S., Chaudhari, G. & Bhat, K. V. 2013. Pollen germination characteristics, pollen–pistil interaction and reproductive behaviour in interspecific crosses among *Abelmoschus esculentus* Moench and its wild relatives. *Grana,* 52**,** 1-14.

Peet, M., Sato, S. & Gardner, R. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. *Plant, Cell & Environment,* 21**,** 225-231.

Peet, M. M., Willits, D. & Gardner, R. 1997. Response of ovule development and post-pollen production processes in male-sterile tomatoes to chronic, sub-acute high temperature stress. *Journal of Experimental Botany,* 48**,** 101-111.

Pilon, C., Snider, J. L., Oosterhuis, D. M. & Loka, D. 2016. The effects of genotype and irrigation regime on PSII heat tolerance in cotton. *Sciencedomain International***,** 1-11.

Porch, T. & Jahn, M. 2001. Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. *Plant, Cell & Environment,* 24**,** 723-731.

Porebski, S., Bailey, L. G. & Baum, B. R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter,* 15**,** 8-15.

Porter, J. R. & Gawith, M. 1999. Temperatures and the growth and development of wheat: a review. *European Journal of Agronomy,* 10**,** 23-36.

Prasad, P., Boote, K., Allen, L., Sheehy, J. & Thomas, J. 2006a. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research,* 95**,** 398-411.

Prasad, P. V., Boote, K. J. & Allen, L. H. 2006b. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum (*Sorghum bicolor* (L.) Moench) are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agricultural and forest meteorology,* 139**,** 237-251.

Premachandra, G. S., Hahn, D. T., Rhodes, D. & Joly, R. J. 1995. Leaf water relations and solute accumulation in two grain sorghum lines exhibiting contrasting drought tolerance. *Journal of Experimental Botany,* 46**,** 1833-1841.

Pressman, E., Peet, M. M. & Pharr, D. 2002. The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Annals of Botany,* 90**,** 631-636.

Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V. & Jackson, R. B. 2011. *Campbell biology*, Pearson Boston.

Ribeiro, R. V., Machado, E. C., Espinoza-Núñez, E., Ramos, R. A. & Machado, D. F. S. P. 2012. Moderate warm temperature improves shoot growth, affects carbohydrate status and stimulates photosynthesis of sweet orange plants. *Brazilian Journal of Plant Physiology,* 24**,** 37-46.

Ristic, Z., Bukovnik, U. & Prasad, P. V. 2007. Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Science,* 47**,** 2067-2073.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S. & Mittler, R. 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. *Plant Physiology,* 134**,** 1683-1696.

Roessner, U., Wagner, C., Kopka, J., Trethewey, R. N. & Willmitzer, L. 2000. Simultaneous analysis of metabolites in potato tuber by gas chromatography–mass spectrometry. *The Plant Journal,* 23**,** 131-142.

Roháček, K., Soukupová, J. & Barták, M. 2008. Chlorophyll fluorescence: a wonderful tool to study plant physiology and plant stress. *Plant Cell Compartments-Selected Topics. Research Signpost, Kerala, India***,** 41-104.

Rohlf, F. 1998. NTSYS-pc Numerical taxonomy and multivariate analysis system. version 2.02 e. EXETER Software Setauket. *New York.*

Roitsch, T., Balibrea, M., Hofmann, M., Proels, R. & Sinha, A. 2003. Extracellular invertase: key metabolic enzyme and PR protein. *Journal of Experimental Botany,* 54**,** 513-524.

Roitsch, T. & González, M.-C. 2004. Function and regulation of plant invertases: sweet sensations. *Trends in plant science,* 9**,** 606-613.

Rolland, F., Baena-Gonzalez, E. & Sheen, J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review Plant Biology,* 57**,** 675-709.

Ruban, A. V. 2009. Plants in light. *Communicative and Integrative Biology,* 2**,** 50-55.

Rufty, T. W., Huber, S. C. & Kerr, P. S. 1985. Association between sucrose-phosphate synthase activity in leaves and plant growth rate in response to altered aerial temperature. *Plant science,* 39**,** 7-12.

Ruzin, S. E. 1999. *Plant microtechnique and microscopy*, Oxford University Press New York.

Sack, L. & Holbrook, N. M. 2006. Leaf hydraulics. *Annual Review Plant Biology,* 57**,** 361-381.

Sack, L. & Scoffoni, C. 2013. Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist,* 198**,** 983-1000.

Saini, H. & Aspinall, D. 1982. Abnormal sporogenesis in wheat (*Triticum aestivum* L.) induced by short periods of high temperature. *Annals of Botany,* 49**,** 835-846.

Saini, H., Sedgley, M. & Aspinall, D. 1983. Effect of heat stress during floral development on pollen tube growth and ovary anatomy in wheat (*Triticum aestivum* L.). *Functional Plant Biology,* 10**,** 137-144.

Saini, H., Sedgley, M. & Aspinall, D. 1984. Development anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. *Functional Plant Biology,* 11**,** 243-253.

Salem, M. A., Kakani, V. G., Koti, S. & Reddy, K. R. 2007. Pollen-based screening of soybean genotypes for high temperatures. *Crop Science,* 47**,** 219-231.

Sass, J., E. 1958. Botanical microtechnique. 3rd ed. USA; UK: The Iowa State College Press; Constable & Co.

Sato, S., Peet, M. M. & Thomas, J. F. 2002. Determining critical pre-and post-anthesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures. *Journal of Experimental Botany,* 53**,** 1187-1195.

Savello, P. A., Martin, F. W. & Hill, J. M. 1980. Nutritional composition of okra seed meal. *Journal of Agricultural and Food Chemistry,* 28**,** 1163-1166.

Sawadogo, M., Ouedraogo, J. T., Balma, D., Ouedraogo, M., Gowda, B. S., Botanga, C. & Timko, M. P. 2009. The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso. *African Journal of Biotechnology,* 8.

Schafleitner, R., Kumar, S., Lin, C. Y., Hegde, S. G. & Ebert, A. 2013. The okra (*Abelmoschus esculentus*) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. *Gene,* 517**,** 27-36.

Schöffl, F., Prandl, R. & Reindl, A. 1999. Molecular responses to heat stress. *Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants, R.G. Landes Co., Austin, Texas***,** 81-98.

Schrader, S., Wise, R., Wacholtz, W., Ort, D. & Sharkey, T. 2004. Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. *Plant, Cell & Environment,* 27**,** 725-735.

Sellin, A. & Kupper, P. 2007. Temperature, light and leaf hydraulic conductance of little-leaf linden (*Tilia cordata*) in a mixed forest canopy. *Tree Physiology,* 27**,** 679-688.

Shao, H. B., Chu, L. Y., Jaleel, C. A. & Zhao, C. X. 2008. Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies,* 331**,** 215-25.

Sharma, K., Pannu, R. & Behl, R. 2005. Effect of early and terminal heat stress on biomass portioning, chlorophyll stability and yield of different wheat genotypes. Proceedings of the international conference on sustainable crop production in stress environments: Management and genetic options, 2005. Jabalpur, MP, India., 9-12.

Shehata, A. I., Al-Ghethar, H. A. & Al-Homaidan, A. A. 2009. Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. *Saudi journal of biological sciences,* 16**,** 57-62.

Siebert, S., Ewert, F., Rezaei, E. E., Kage, H. & Graß, R. 2014. Impact of heat stress on crop yield—on the importance of considering canopy temperature. *Environmental Research Letters,* 9**,** 044012.

Siemonsma, J. & Kouame, C. 2004. Vegetable. *Plant Resource of Tropical Africa,* 2**,** 21-29.

Simonin, K. A., Burns, E., Choat, B., Barbour, M. M., Dawson, T. E. & Franks, P. J. 2014. Increasing leaf hydraulic conductance with transpiration rate minimizes the water potential drawdown from stem to leaf. *Journal of experimental botany***,** eru481.

Singh, R. P., Prasad, P. V., Sunita, K., Giri, S. & Reddy, K. R. 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. *Advances in Agronomy,* 93**,** 313-385.

Sinsawat, V., Leipner, J., Stamp, P. & Fracheboud, Y. 2004. Effect of heat stress on the photosynthetic apparatus in maize (*Zea mays* L.) grown at control or high temperature. *Environmental and Experimental Botany,* 52**,** 123-129.

Sneath, P. H. & Sokal, R. R. 1973. *Numerical taxonomy. The principles and practice of numerical classification*.

Snider, J. L., Oosterhuis, D. M., Skulman, B. W. & Kawakami, E. M. 2009. Heat stress-induced limitations to reproductive success in Gossypium hirsutum. *Physiologia Plantarum,* 137**,** 125-138.

Snider, J. L., Oosterhuis, D. M., Skulman, B. W., Kawakami, E. M. & Storch, D. K. 2008. Effect of High Temperature on Pollen Tube Growth and Energetics in the Cotton Pistil. *Summaries of Arkansas cotton research***,** 52.

Stewart, J. 1986. Integrated events in the flower and fruit. *Cotton physiology,* 1**,** 261-300.

Strasser, R. J., Tsimilli-Michael, M. & Srivastava, A. 2004. Analysis of the chlorophyll a fluorescence transient. *Chlorophyll a Fluorescence***,** 321-362.

Sugiyama, T., Iwahori, S. & Takahashi, K. Effect of high temperature on fruit setting of tomato under cover. Symposium on Vegetable Growing under Glass 4, 1965. 63-69.

Sulikiri, G. & Swamy Rao, T. 1972. Studies on floral biology and fruit formation in okra (*Abelmoschus esculentus* (L.) Moench varieties. *Progress Horticulture,* 4**,** 71.

Sullivan, C. Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. *Sorghum in Seventies. Oxford & IBH Pub. Co***,** 247.

Suzuki, K., Tsukaguchi, T., Takeda, H. & Egawa, Y. 2001. Decrease of pollen stainability of green bean at high temperatures and relationship to heat tolerance. *Journal of the American Society for Horticultural Science,* 126**,** 571-574.

Taha El-Katib, M. 1947. Value of adding cotton okra fenugreek seed to maize flour. *Nature,* 156**,** 716-722.

Taiz, L. & Zeiger, E. 2002. Plant Physiology. Sunderland, MA. Sinauer Associates, Inc.

Tamura, K., Nei, M. & Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America,* 101**,** 11030-11035.

Thakur, M. & Arora, S. 1986. Okra. *Vegetable Crops in India, ed. TK Bose and Ma Som, Naya Prokash, Calcutta,* 606.

Tóth, S. Z., Schansker, G., Garab, G. & Strasser, R. J. 2007. Photosynthetic electron transport activity in heat-treated barley leaves: the role of internal alternative electron donors to photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics,* 1767**,** 295-305.

Tripathi, K., Govila, O., Warrier, R. & Ahuja, V. 2011. Biology of *Abelmoschus esculentus* L. (Okra). *Govt. of India, Department of Biotechnology, New Delhi*.

Turgeon, R. & Wolf, S. 2009. Phloem transport: cellular pathways and molecular trafficking. *Annual review of plant biology,* 60**,** 207-221.

Tyree, M. T. 1997. The cohesion-tension theory of sap ascent: current controversies. *Journal of experimental botany,* 48**,** 1753-1765.

Ur Rahman, H., Malik, S. A. & Saleem, M. 2004. Heat tolerance of upland cotton during the fruiting stage evaluated using cellular membrane thermostability. *Field Crops Research,* 85**,** 149-158.

Usda. 2017. *Classification for Kingdom Plantae Down to Species Abelmoschus esculentus (L.) Moench* [Online]. United State Department of Agriculture. Available: https://plants.usda.gov/java/ClassificationServlet?source=display&classid=ABES.

Van Oldenborgh, G. J., Philip, S., Kew, S., Van Weele, M., Uhe, P., Otto, F., Singh, R., Pai, I. & Achutarao, K. 2017. Extreme heat in India and anthropogenic climate change. *Natural Hazards and Earth System Scienced*.

Vara Prasad, P. V., Craufurd, P. Q., Summerfield, R. J. & Wheeler, T. R. 2000. Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypogaea* L.). *Journal of experimental botany,* 51**,** 777-784.

Velayudhan, K. & Upadhyay, M. 1994. Collecting okra, eggplant and their wild relatives in Nepal. *Pl. Genet. Resource. Newsl***,** 55-57.

Venkataramani, K. 1953. Some observations on blossom biology and fruit formation in *Hibiscus esculentus*. *Journal of the Madras University. Section B,* 23**,** 1-14.

Vieira, M. L. C., Santini, L., Diniz, A. L. & Munhoz, C. D. F. 2016. Microsatellite markers: what they mean and why they are so useful. *Genetics and molecular biology,* 39(3)**,** 312-328.

Vinocur, B. & Altman, A. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current opinion in biotechnology,* 16**,** 123-132.

Vollenweider, P. & Günthardt-Goerg, M. S. 2005. Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environmental Pollution,* 137**,** 455-465.

Wahid, A. & Close, T. 2007. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biologia Plantarum,* 51**,** 104-109.

Wahid, A., Gelani, S., Ashraf, M. & Foolad, M. 2007. Heat tolerance in plants: An overview. *Environmental and Experimental Botany,* 61**,** 199-223.

Weaver, J. E. & Bruner, W. E. 1927. *Root Development Of Vegetable Crops Ist Edn,* Mcgraw-Iill Book Co,; London.

Weerakoon, W., Maruyama, A. & Ohba, K. 2008. Impact of humidity on temperature-induced grain sterility in rice (*Oryza sativa* L). *Journal of Agronomy and Crop Science,* 194**,** 135-140.

Whittle, C., Otto, S., Johnston, M. O. & Krochko, J. 2009. Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana Botany,* 87(6)**,** 650-657.

Wise, R., Olson, A., Schrader, S. & Sharkey, T. 2004. Electron transport is the functional limitation of photosynthesis in field-grown pima cotton plants at high temperature. *Plant, Cell and Environment,* 27**,** 717-724.

Wu, H.-M., Wang, H. & Cheung, A. Y. 1995. A pollen tube growth stimulatory glycoprotein is deglycosylated by pollen tubes and displays a glycosylation gradient in the flower. *Cell,* 82**,** 395-403.

Yamane, Y., Kashino, Y., Koike, H. & Satoh, K. 1995. Effects of high temperatures on photosynthetic systems in higher plants. 1. Causes of the increase in the fluorescence F_0 level. *Photosynthesis: from light to biosphere,* 4**,** 849-852.

Yamane, Y., Kashino, Y., Koike, H. & Satoh, K. 1997. Increases in the fluorescence Fo level and reversible inhibition of photosystem II reaction center by high-temperature treatments in higher plants. *Photosynthesis Research,* 52**,** 57-64.

Yamane, Y., Shikanai, T., Kashino, Y., Koike, H. & Satoh, K. 2000. Reduction of QA in the dark: another cause of fluorescence Fo increases by high temperatures in higher plants. *Photosynthesis Research,* 63**,** 23-34.

Yildiz, M., Koçak, M. & Baloch, F. 2015. Genetic bottlenecks in Turkish okra germplasm and utility of iPBS retrotransposon markers for genetic diversity assessment. *Genetics and Molecular Research,* 14**,** 10588-10602.

Young, L. W., Wilen, R. W. & Bonham-Smith, P. C. 2004. High temperature stress of Brassica napus during flowering reduces micro-and megagametophyte fertility, induces fruit abortion, and disrupts seed production. *Journal of Experimental Botany,* 55**,** 485-495.

Zhang, J. 2012. Effect of moderately-high temperature stress on photosynthesis and carbohydrate metabolism in tomato (Lycopersico esculentum L.) leaves. *African Journal of Agricultural Research,* 7.

Zhang, R. & Sharkey, T. D. 2009. Photosynthetic electron transport and proton flux under moderate heat stress. *Photosynthesis Research,* 100**,** 29-43.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., Huang, M., Yao, Y., Bassu, S. & Ciais, P. 2017. Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences***,** 201701762.

Zinn, K. E., Tunc-Ozdemir, M. & Harper, J. F. 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of experimental botany,* 61**,** 1959-1968.

Zlatev, Z. S. & Yordanov, I. T. 2004. Effects of soil drought on photosynthesis and chlorophyll fluorescence in bean plants. *Bulgarian Journal of Plant Physiology.,* 30**,** 3-18.

Appendix

Appendix 1 Okra genotypes from the Vegetable Research Institute (VRI) (List No.2) and the Asian Vegetable Research and Development Center (AVRDC) (List 3 and 4). No.: genotype number in each list, VINo.: AVRDC ID number, Org.: origin of the genotype.

List	No.	Name	VI No	List	No.	Name	VI No
2	**1**	Sabzpari		**3**	**14**	Lady's finger	VI033773
2	**5**	Green Wounder		**3**	**15**	Local 5-angled okra	VI033784
2	**6**	OH-152		**3**	**16**	Malaysia okra 5-angled	VI033786
2	**8**	China Red		**3**	**17**		VI033791
2	**11**	Oh-139		**3**	**19**		VI033810
2	**12**	OH-713		**3**	**22**		VI036215
2	**14**	OH-597		**3**	**23**	Krachiapkhieo	VI046536
2	**15**	OH-2324		**3**	**24**	Krachiapkhieo	VI046537
2	**19**	Okra-1548		**3**	**26**		VI046554
2	**21**	Okra-7080		**3**	**27**		VI046556
2	**22**	Kiran		**3**	**31**	Deshi dheleosh	VI047808
2	**23**	Sitara-9101		**3**	**32**		VI048154
2	**24**	Okra-7100		**3**	**33**		VI048596
2	**25**	Click-5769		**3**	**34**		VI050145
2	**26**	Laxmy		**3**	**35**	Okra	VI051038
2	**27**	JKOH-456		**3**	**36**		VI051039
2	**28**	JK Tetra-6		**3**	**37**	Okra	VI051042
2	**29**	Lush Green		**3**	**38**		VI051047
2	**30**	Rama Krishna		**3**	**39**		VI051048
2	**31**	Garnier		**3**	**40**	Okra	VI051062
2	**32**	Tokita		**3**	**42**	Utong	VI051114
2	**33**	Green Gold		**3**	**44**	Okra	VI054562
3	**1**	Choitali	VI039622	**3**	**46**	Okra	VI054565
3	**2**	Local	VI039638	**3**	**47**		VI054566
3	**3**	Deshi	VI039643	**3**	**50**		VI056402
3	**4**	Local	VI039651	**3**	**51**		VI056404
3	**5**	Krachiapkhieo	VI040649	**3**	**52**		VI056407
3	**6**	Krachiapkhieo	VI040770	**3**	**53**		VI056447
3	**7**	Krachiapkhieo	VI040865	**3**	**54**		VI057245
3	**8**	Thua lea	VI041139	**3**	**55**		VI057249
3	**10**		VI044241	**3**	**56**	Gombo paysan	VI060131
3	**11**		VI037997	**3**	**57**	Gancr/08	VI060132
3	**12**		VI037995	**3**	**58**	Gancr/CS0708	VI060133
3	**13**		VI037994	**3**	**59**	Bhendi	VI060206

Appendix 1. Continued

List	No.	Name	VI No.	List	No.	Name	VI No.
3	**60**	Tz-smn-86	VI060313	**4**	**23**	Mahnco arka abhhay	VI049961
3	**61**	Tz-smn-9-5	VI060314	**4**	**24**		VI050150
3	**62**	Tz-smn-98	VI060315	**4**	**25**		VI050170
3	**63**	Tz-smn-103	VI060316	**4**	**26**		VI050549
3	**64**	Rca 1	VI060317	**4**	**27**		VI055017
4	**1**	P.s.type	VI039652	**4**	**28**		VI055018
4	**2**		VI041215	**4**	**29**	Kacang li (lendir)	VI055110
4	**3**		VI041461	**4**	**30**		VI055119
4	**4**	Sel-2	VI041763	**4**	**31**	Kacang bendi	VI055219
4	**5**		VI044233	**4**	**32**	Kacang bendi	VI055220
4	**6**		VI044244	**4**	**33**	Lein meuanang	VI055578
4	**7**	Mk be 1	VI033775	**4**	**34**		VI055582
4	**8**	Bendir lima segi	VI033781-A	**4**	**36**	Phak pang	VI055996
4	**9**	Bendir lima segi	VI033781-B	**4**	**37**		VI056069
4	**10**	Malaysia okra 5-angled	VI033785	**4**	**38**		VI056079
4	**11**		VI033803	**4**	**39**		VI056081
4	**12**		VI033824	**4**	**40**	Cowhorn	VI056448
4	**13**		VI036201	**4**	**41**	Burgundy	VI056449
4	**14**	Okrang tagalog	VI036203	**4**	**42**	Clemson spineless 80	VI056450
4	**15**		VI036211	**4**	**43**	Lee dwarf	VI056451
4	**16**	Ok5	VI046566	**4**	**44**	Silver queen	VI056452
4	**17**		VI047672	**4**	**45**	Choppee	VI056453
4	**18**	Dheros/vendi	VI047751	**4**	**46**	Café quetzal	VI056454
4	**19**		VI048291	**4**	**47**	Yuma red	VI056455
4	**21**		VI049632	**4**	**48**	Cajun jewel	VI056456
4	**22**	Haritha	VI049954				

Appendix 2 Stomatal conductance (g_s) and chlorophyll fluorescence (in the dark, F_v/F_m and in the light F_v'/F_m') for119 genotypes (n=1) in the control and heat, measured with the porometer and LI-COR respectively.

Genotype No.	g_s (mmol m^{-2} s^{-1})		Chlorophyll fluorescence			
	Control	Heat	Control dark (F_v/F_m)	Control light (F_v'/F_m')	Heat dark (F_v/F_m)	Heat light (F_v'/F_m')
L2-1	303.5	364.0	0.81	0.72	0.80	0.74
L2-5	140.2	27.9	0.80	0.74	0.80	0.67
L2-6	396.7	351.7	0.81	0.72	0.80	0.72
L2-8	87.7	100.6	0.81	0.75	0.79	0.58
L2-11	243.9	210.6	0.82	0.74	0.81	0.74
L2-12	359.3	259.8	0.81	0.74	0.81	0.74
L2-14	272.9	189.4	0.81	0.74	0.81	0.74
L2-15	330.4	258.4	0.81	0.73	0.79	0.73
L2-19	451.3	371.5	0.81	0.72	0.80	0.72
L2-21	426.7	207.1	0.82	0.73	0.81	0.75
L2-22	278.4	256.9	0.81	0.74	0.81	0.72
L2-23	344.9	409.5	0.82	0.74	0.81	0.73
L2-24	319.4	387.7	0.82	0.72	0.80	0.72
L2-25	344.4	305.4	0.82	0.74	0.79	0.71
L2-26	146.7	158.2	0.81	0.73	0.81	0.73
L2-27	351.1	471.7	0.81	0.73	0.81	0.73
L2-28	337.4	393.4	0.82	0.73	0.81	0.74
L2-29	114.0	21.8	0.81	0.72	0.81	0.65
L2-30	397.6	112.6	0.82	0.74	0.80	0.72
L2-31	253.9	264.1	0.81	0.73	0.80	0.74
L2-32	526.8	268.0	0.82	0.73	0.81	0.73
L2-33	371.9	239.0	0.82	0.73	0.81	0.73
L3-1	258.2	47.1	0.81	0.74	0.80	0.65
L3-2	91.6	58.8	0.80	0.72	0.81	0.72
L3-3	75.0	100.9	0.82	0.74	0.80	0.72
L3-4	128.3	124.0	0.81	0.74	0.79	0.72
L3-5	153.4	12.8	0.81	0.74	0.81	0.58
L3-6	150.5	55.9	0.81	0.75	0.79	0.71
L3-7	141.5	84.7	0.81	0.75	0.80	0.62
L3-8	319.9	237.1	0.81	0.74	0.81	0.74
L3-10	85.5	166.9	0.81	0.75	0.79	0.61
L3-11	122.1	48.5	0.81	0.75	0.80	0.72
L3-12	30.1	11.6	0.82	0.75	0.81	0.66
L3-13	206.3	12.7	0.82	0.75	0.82	0.64
L3-14	126.8	39.0	0.80	0.74	0.78	0.59
L3-15	57.9	26.2	0.81	0.74	0.79	0.69
L3-16	87.2	19.7	0.80	0.74	0.80	0.71

Appendix 2 Continued.

Genotype No.	g_s (mmol m^{-2} s^{-1})		Chlorophyll fluorescence			
	Control	Heat	Control dark (F_v/F_m)	Control light (F_v'/F_m')	Heat dark (F_v/F_m)	Heat light (F_v'/F_m')
L3-17	107.4	339.4	0.79	0.73	0.80	0.73
L3-19	117.1	40.3	0.82	0.74	0.81	0.68
L3-22	83.1	129.8	0.81	0.75	0.81	0.72
L3-23	366.3	59.6	0.82	0.73	0.81	0.73
L3-24	102.2	28.2	0.80	0.74	0.81	0.73
L3-26	68.3	80.0	0.81	0.72	0.81	0.74
L3-27	112.8	82.0	0.82	0.73	0.81	0.72
L3-31	227.9	237.8	0.82	0.72	0.81	0.72
L3-32	246.8	14.1	0.82	0.72	0.80	0.71
L3-33	443.7	122.5	0.80	0.73	0.80	0.73
L3-34	371.2	153.7	0.82	0.74	0.81	0.73
L3-35	339.9	129.0	0.82	0.75	0.82	0.72
L3-36	291.6	100.8	0.82	0.74	0.82	0.69
L3-37	267.7	44.6	0.82	0.69	0.82	0.72
L3-38	227.1	22.6	0.81	0.74	0.82	0.72
L3-39	445.3	68.0	0.82	0.74	0.79	0.73
L3-40	188.3	26.7	0.80	0.75	0.81	0.74
L3-42	334.1	179.7	0.81	0.74	0.82	0.71
L3-44	235.2	99.3	0.77	0.74	0.82	0.73
L3-46	207.2	101.1	0.82	0.74	0.81	0.71
L3-47	273.6	268.8	0.80	0.73	0.81	0.71
L3-50	435.1	31.2	0.82	0.75	0.82	0.73
L3-51	283.0	89.5	0.82	0.74	0.80	0.63
L3-52	395.0	240.3	0.82	0.73	0.81	0.73
L3-53	296.8	354.6	0.82	0.74	0.81	0.71
L3-54	337.8	78.3	0.82	0.73	0.77	0.70
L3-55	168.0	66.7	0.81	0.64	0.78	0.62
L3-56	136.8	34.9	0.79	0.75	0.81	0.71
L3-57	207.1	47.6	0.82	0.73	0.81	0.75
L3-58	126. 5	75.0	0.80	0.75	0.81	0.73
L3-59	77.0	11.9	0.82	0.74	0.82	0.73
L3-60	131.7	44.3	0.80	0.74	0.82	0.73
L3-61	303.9	95.8	0.81	0.74	0.81	0.74
L3-62	149.2	370.7	0.81	0.74	0.81	0.74
L3-63	119.8	194.0	0.81	0.74	0.81	0.75
L3-64	243.0	121.3	0.80	0.74	0.81	0.72
L4-1	258.4	54.3	0.81	0.63	0.80	0.70
L4-2	349.9	43.4	0.78	0.71	0.81	0.70
L4-3	321.4	167.2	0.73	0.72	0.80	0.74

Appendix 2 Continued.

Genotype No.	g_s (mmol m^{-2} s^{-1})		Chlorophyll fluorescence			
	Control	Heat	Control dark (F_v/F_m)	Control light (F_v'/F_m')	Heat dark (F_v/F_m)	Heat light (F_v'/F_m')
L4-4	324.0	41.4	0.77	0.71	0.80	0.71
L4-5	224.2	77.2	0.74	0.68	0.79	0.72
L4-6	469.5	152.7	0.80	0.72	0.81	0.72
L4-7	207.6	58.2	0.73	0.66	0.76	0.72
L4-8	292.7	111.6	0.69	0.66	0.77	0.71
L4-9	282.9	104.1	0.77	0.71	0.81	0.73
L4-10	358.4	99.6	0.77	0.70	0.78	0.70
L4-11	128.2	125.7	0.81	0.74	0.80	0.73
L4-12	306.5	279.3	0.81	0.67	0.77	0.71
L4-13	305.8	104.1	0.80	0.74	0.81	0.71
L4-14	479.8	74.7	0.81	0.74	0.77	0.73
L4-15	57.4	139.2	0.81	0.74	0.79	0.74
L4-16	408.2	62.2	0.80	0.73	0.80	0.71
L4-17	208.9	131.1	0.81	0.72	0.78	0.72
L4-18	190.5	191.1	0.79	0.72	0.79	0.68
L4-19	183.8	158.9	0.79	0.72	0.79	0.72
L4-21	164.0	115.4	0.81	0.70	0.80	0.73
L4-22	430.6	248.6	0.74	0.71	0.80	0.60
L4-23	342.2	122.1	0.79	0.72	0.71	0.66
L4-24	509.9	37.9	0.73	0.72	0.77	0.70
L4-25	330.4	151.5	0.81	0.73	0.80	0.70
L4-26	105.2	223.7	0.81	0.72	0.81	0.72
L4-27	330.2	464.1	0.82	0.75	0.81	0.74
L4-28	276.2	292.4	0.82	0.74	0.81	0.73
L4-29	271.8	557.5	0.82	0.74	0.81	0.73
L4-30	268.0	763.8	0.81	0.74	0.81	0.73
L4-31	183.1	497.3	0.82	0.74	0.81	0.71
L4-32	257.4	409.7	0.82	0.73	0.81	0.72
L4-33	220.0	499.0	0.81	0.73	0.80	0.73
L4-34	364.5	417.0	0.80	0.73	0.79	0.73
L4-36	114.4	261.4	0.82	0.73	0.81	0.73
L4-37	159.0	451.6	0.81	0.74	0.81	0.75
L4-38	238.3	169.7	0.81	0.74	0.80	0.73
L4-39	166.4	56.3	0.81	0.65	0.81	0.67
L4-40	340.7	152.7	0.81	0.72	0.81	0.70
L4-41	467.2	422.0	0.80	0.72	0.80	0.73
L4-42	479.3	451.9	0.82	0.74	0.82	0.73
L4-43	483.4	555.7	0.82	0.74	0.80	0.74
L4-44	315.3	343.8	0.82	0.72	0.82	0.74

Appendix 2 Continued.

Genotype No.	g_s (mmol m^{-2} s^{-1})		Chlorophyll fluorescence			
	Control	Heat	Control dark (F_v/F_m)	Control light (F_v'/F_m')	Heat dark (F_v/F_m)	Heat light (F_v'/F_m')
L4-45	556.9	688.9	0.81	0.74	0.80	0.73
L4-46	238.2	545.8	0.82	0.75	0.81	0.73
L4-47	52.1	190.6	0.82	0.71	0.81	0.74
L4-48	87.8	103.0	0.81	0.73	0.80	0.73

Appendix 3 List of 107 genotypes collected from VRI (1-19) and AVRDC (20-107) and their origin. No.: isolate number.

No.	Genotype	Origin	No.	Genotype	Origin	No.	Genotype	Origin
1	L2-1	Unknown	**37**	L3-23	Thailand	**73**	L4-8	Malaysia
2	L2-6	Unknown	**38**	L3-24	Thailand	**74**	L4-9	Malaysia
3	L2-8	Unknown	**39**	L3-26	Thailand	**75**	L4-10	Malaysia
4	L2-11	Unknown	**40**	L3-27	Thailand	**76**	L4-11	Philippines
5	L2-12	Unknown	**41**	L3-31	Bangladesh	**77**	L4-12	Philippines
6	L2-14	Unknown	**42**	L3-32	Bangladesh	**78**	L4-13	Philippines
7	L2-15	Unknown	**43**	L3-33	Taiwan	**79**	L4-14	Philippines
8	L2-19	Unknown	**44**	L3-34	Taiwan	**80**	L4-15	Philippines
9	L2-21	Unknown	**45**	L3-35	Philippines	**81**	L4-16	Thailand
10	L2-22	Unknown	**46**	L3-36	Philippines	**82**	L4-17	Bangladesh
11	L2-23	Unknown	**47**	L3-37	Philippines	**83**	L4-18	Bangladesh
12	L2-24	Unknown	**48**	L3-42	Philippines	**84**	L4-19	Bangladesh
13	L2-25	Unknown	**49**	L3-44	Philippines	**85**	L4-21	Thailand
14	L2-27	Unknown	**50**	L3-46	Philippines	**86**	L4-22	Thailand
15	L2-28	Unknown	**51**	L3-47	Philippines	**87**	L4-23	India
16	L2-30	Unknown	**52**	L3-50	Philippines	**88**	L4-24	Taiwan
17	L2-31	Unknown	**53**	L3-51	Philippines	**89**	L4-25	Taiwan
18	L2-32	Unknown	**54**	L3-53	Philippines	**90**	L4-26	Thailand
19	L2-33	Unknown	**55**	L3-54	Cambodia	**91**	L4-29	Malaysia
20	L3-1	Bangladesh	**56**	L3-55	Cambodia	**92**	L4-30	Myanmar
21	L3-2	Bangladesh	**57**	L3-56	Mali	**93**	L4-31	Malaysia
22	L3-3	Bangladesh	**58**	L3-57	Mali	**94**	L4-32	Malaysia
23	L3-4	Bangladesh	**59**	L3-58	Mali	**95**	L4-33	Lao
24	L3-5	Thailand	**60**	L3-59	Australia	**96**	L4-34	Lao
25	L3-7	Thailand	**61**	L3-60	Tanzania	**97**	L4-36	Lao
26	L3-8	Thailand	**62**	L3-61	Tanzania	**98**	L4-37	Cambodia
27	L3-10	Philippines	**63**	L3-62	Tanzania	**99**	L4-38	Cambodia
28	L3-11	Thailand	**64**	L3-63	Tanzania	**100**	L4-39	Cambodia
29	L3-12	Thailand	**65**	L3-64	Tanzania	**101**	L4-40	USA
30	L3-13	Thailand	**66**	L4-1	Bangladesh	**102**	L4-41	USA
31	L3-14	Malaysia	**67**	L4-2	Philippines	**103**	L4-42	USA
32	L3-15	Malaysia	**68**	L4-3	Philippines	**104**	L4-43	USA
33	L3-16	Malaysia	**69**	L4-4	India	**105**	L4-44	USA
34	L3-17	Malaysia	**70**	L4-5	Philippines	**106**	L4-47	USA
35	L3-19	Philippines	**71**	L4-6	Philippines	**107**	L4-48	USA
36	L3-22	Philippines	**72**	L4-7	Malaysia			

Appendix 4 Protocol for DNA extraction using ISOLATE Plant DNA mini kit (Bioline, Cat number: 52069).

ISOLATE Plant DNA Mini Kit

DNA ISOLATION FROM PLANT TISSUE

1. Homogenize 50-100mg plant tissue under liquid N_2 using mortar and pestle. Use 120-180mg wet starting material.
2. Transfer homogenized material to 1.5-2.0ml tube.
3. Add 400µl Lysis Buffer PD.
4. Add 3µl RNase A (100mg/ml). Vortex for 10 sec.
5. Incubate at 65°C until sample is lysed. Vortex.
6. Add 100µl Precipitation Buffer, vortex for 5 sec.
7. Incubate on ice for 5 min.
8. Spin: max. speed for 5 min.
9. Transfer supernatant to Spin Column PD1 placed in Collection Tube.
10. Spin: 10,000 x g for 1 min. Discard Spin Column PD1 and SAVE THE FILTRATE.
11. Add 0.5 volume Binding Buffer PD to filtrate. Mix by pipetting.
12. Transfer sample to Spin Column PD2 placed in Collection Tube.
13. Spin: 10,000 x g for 2 min. Discard Collection Tube, place Spin Column PD2 in new Collection Tube.
14. Add 700µl Wash Buffer PD.
15. Spin: 10,000 x g for 1 min. Discard filtrate, reuse Collection Tube.
16. Repeat steps 14 and 15.
17. Spin: max. speed for 2 min. Discard Collection Tube.
18. Place Spin Column PD2 in 1.5ml Elution Tube.
19. Add 200µl Elution Buffer directly to Spin Column membrane.
20. Incubate at RT for 1 min.
21. Spin: 6000 x g for 1 min. to elute DNA.
22. The isolated DNA is ready for use in downstream applications.

Appendix 5 Thirty five SSR primers, forward (F) and reverse (R) with their repeated motif and primer sequences from *A. esculentus* (No. 1-19) and *Medicago truncatula* (No. 20-35) were used to amplify DNA of 107 okra genotypes.

No	Name (Marker)	ID	Repeated motif	Primer sequences (5'-3')
1	AVRDC-Okra1	5200	(AAG)13	F:ATGGAGTGATTTTTGTGGAG
				R:GACCCGAACTCACGTTACTA
2	AVRDC-Okra8	128713	(AAG)8	F:TGCTGTGGAAGGTTTTTACT
				R:ATGACGAAAGTGGTGAAAAG
3	AVRDC-Okra9	89235	(AAT)12	F:ACCTTGAACACCAGGTACAG
				R:TTGCTCTTATGAAGCAGTGA
4	AVRDC-Okra17	8461	(AGA)7	F:ACGAGAGTGAAGTGGAACTG
				R:CTCCTCTTTCCTTTTTCCAT
5	AVRDC-Okra21	43380	(AGA)9	F:TCATGTCTTTCCACTCAACA
				R:CCAAACAAAATATGCCTCTC
6	AVRDC-Okra28	151529	(ATT)8	F:CCTCTTCATCCATCTTTTCA
				R:GGAAGATGCTGTGAAGGTAG
7	AVRDC-Okra39	51708	(AG)16	F:TGAGGTGATGATGTGAGAGA
				R:TTGTAGATGAGGTTTGAACG
8	AVRDC-Okra52	126731	(CAT)8-(TCA)9	F:AACACATCCTCATCCTCATC
				R:ACCGGAAGCTATTTACATGA
9	AVRDC-Okra54	87311	(GAA)10	F:CGAAAAGGAAACTCAACAAC
				R:TGAACCTTATTTTCCTCGTG
10	AVRDC-Okra56	21030	(GAA)44	F:GGCAACTTCGTAATTTCCTA
				R:TGAGTAAAAGTGGGGTCTGT
11	AVRDC-Okra57	151995	(GAA)9-(GAG)7	F:CGAGGAGACCATGGAAGAAG
				R:ATGAGGAGGACGAGCAAGAA
12	AVRDC-Okra63	34632	(TCT)12	F:GTGTTTGAAAGGGACTGTGT
				R:CTTCATCAAAACCATGCAG

Appendix 5 Continued

No	Name (Marker)	ID	Repeated motif	Primer sequences (5'-3')
13	AVRDC-Okra64	5886	(TCT)22	F:AAGGAGGAGAAAGAGAAGGA
				R:ATTTACTTGAGCAGCAGCAG
14	AVRDC-Okra66	20291	(TTC)12-(TTC)13	F:CACCAGAATTTCCCTTTTG
				R:ACTGTTGTTTGGCTTATGCT
15	AVRDC-Okra70	89044	(TC)11	F:GTAGCTGAACCCTTTGCTTA
				R:CTATCATGGCGGATTCTTTA
16	AVRDC-Okra77	152270	(GAAATA)4-(GAAACA)7	F:CTGTTTGTTCGTCGTAATCA
				R:AAAGTTTCTTCCTTTCCACC
17	AVRDC-Okra78	122488	(TAT)11-(TATTGT)4-(TATCGT)4	F:CTCCGACAATTCAAGAAAAG
				R:CACCCAATCAAGCTATGTTA
18	AVRDC-Okra86	461	(AGC)8	F:ATGCAAACAAGCTAGTGGAT
				R:ATTCTCTTCAGGGTTTCCTC
19	AVRDC-Okra89	129459	(AGC)8	F:TTTGAGTTCTTTCGTCCACT
				R:GTATTTGGACATGGCGTTAT
20	3		(AAC)5	F:TGGTGACGACATACAAGAAAAGA
				R:CCCGGTGGTTTAGGAAGTTT
21	7		(AAC)6	F:ACCACTTCTCCATCCATCCA
				R:AGCTTGCTGCATGAGTGCT
22	8		(AAC)5-(AAC)6	F:CAAAGGCACTTCATCAGCAA
				R:GTGAGCGTCAATGTTGGATG
23	20		(AAG)5	F:TGAAGGTCAAATTGCCAAGA
				R:TCCTTGTTTTTGAAGGTCACG
24	27		(AAG)6	F:CGATCGGAACGAGGACTTTA
				R:CCCCGTTTTTCTTCTCTCCT
25	35		(AAG)8	F:GAAGAAGAAAAAGAGATAGATCTGTGG
				R:GGCAGGAACAGATCCTTGAA

Appendix 5 Continued

No	Name (Marker)	ID	Repeated motif	Primer sequences (5'-3')
26	55		(AAG)6	F:CAGTTCGGGAAGAGGACAAA
				R:ATCCCAAACCAGGTTCTTCA
27	62		(AT)10	F:TTCCGCCCATAGTCTTTGAC
				R:TGAAAGGGCTTAGAGGGTTTT
28	74		(AT)16	F:GGTGGAAGGAACAACTCTGG
				R:CCGGCATGATTAAGACACAC
29	82		(TC)11	F:CACTTTCCACACTCAAACCA
				R:GAGAGGATTTCGGTGATGT
30	95		(TCC)6	F:AAAGGTGTTGGGTTTTGTGG
				R:AGGAAGGAGAGGGACGAAAG
31	96		(TCC)6	F:CCAGTGGCAGCTACGGTACTA
				R:GAGACGGAGGAGAAGTTGCTT
32	103		(TG)5	F:TGGGTTGTCCTTCTTTTTGG
				R:GGGTGCAGAAGTTTGACCA
33	107		(AC)5	F:CAAACCATTTCCTCCATTGTG
				R:TACGTAGCCCCTTGCTCATT
34	135		(AG)10	F:GCTGACTGGACGGATCTGAG
				R:CCAAAGCATAAGCATTCATTCA
35	136		(AT)5	F:TTTGTGTCGAGAGATGCACA
				R:CTTGAAACTTCAACGGCATT

Appendix 6 Fertilizer composition in 1000L of water

Components	Quantity
Calcium Nitrate	125 Kg
Iron Chelate	2 Kg
Ammonium Nitrate	2 L
Potassium Nitrate	75 Kg
MKP	1 Bag
Potassium Sulphate	15 Kg
Magnesium Sulphate (Epsom Salt)	2 Bags (25 Kg/Bag)
BMX	2 Kg
Borax	800 g
Phosphoric Acid	1.5 L
Sodium Molybdate	25 g

Appendix 7 Average chlorophyll fluorescence (in the light F_v'/F_m'), maximum fluorescence (F_m'), efficiency of the open reaction center (Φ_{PSII}) and photochemical quenching (qP) for eight okra genotypes (n=3) in the control and heat (Year 1) measured by LICOR. Values are presented with ±SE.

Genotype	F_v'/F_m'		F_m'		Φ_{PSII}		qP	
	Control	Heat	Control	Heat	Control	Heat	Control	Heat
L2-11	0.56 ± 0.003	0.56 ± 0.007	1284±31	1263±19	0.37 ± 0.005	0.34 ± 0.02	0.67 ± 0.006	0.61 ± 0.029
L2-30	0.55 ± 0.004	0.55 ± 0.003	1300±18	12667±53	0.35 ± 0.035	0.34 ± 0.037	0.65 ± 0.055	0.60 ± 0.053
L2-32	0.57 ± 0.004	0.56 ± 0.022	1162±27	1168±81	0.42 ± 0.012	0.37 ± 0.032	0.73 ± 0.014	0.67 ± 0.052
L3-1	0.58 ± 0.020	0.57 ± 0.012	1219±75	12228±73	0.40 ± 0.031	0.37 ± 0.035	0.71 ± 0.032	0.66 ± 0.053
L3-58	0.58 ± 0.015	0.54 ± 0.000	1226±24	1110±37	0.41 ± 0.019	0.31 ± 0.009	0.71 ± 0.016	0.55 ± 0.024
L3-59	0.53 ± 0.001	0.52 ± 0.036	1097±7	1132±80	0.37 ± 0.023	0.36 ± 0.007	0.70 ± 0.043	0.59 ± 0.050
L4-31	0.56 ± 0.002	0.55 ± 0.015	1168±109	1303±50	0.35 ± 0.028	0.31 ± 0.075	0.67 ± 0.004	0.56 ± 0.075
L4-48	0.51 ± 0.007	0.55 ± 0.003	1128±57	1271±41	0.33 ± 0.030	0.39 ± 0.002	0.66 ± 0.033	0.71 ± 0.002

Appendix 8 Average photosynthesis (*A*), stomatal conductance (g_s) and electrolyte leakage (EL) for eight okra genotypes (n=3) in the control and heat (Year 1). *A* and g_s were measured by LICOR and EL with conductivity meter. Values are presented with ±SE and least significant differences (LSD) of means for Genotype*Treatment interaction, when the interaction was significant at p<0.05.

Genotype	*A* (µmol m^{-2} s^{-1})		g_s (mol m^{-2} s^{-1})		EL (%)	
	Control	Heat	Control	Heat	Control	Heat
L2-11	42.04 ± 1.42	37.17 ± 1.06	0.65 ± 0.08	1 ± 0.06	30 ± 0.72	38 ± 1.85
L2-30	36.82 ± 4.87	33.32 ± 3.99	0.53 ± 0.10	0.95 ± 0.18	30 ± 1.98	30 ± 1.49
L2-32	42.92 ± 2.03	37.13 ± 3.54	0.57 ± 0.07	0.74 ± 0.09	29 ± 1.88	36 ± 1.99
L3-1	40.34 ± 6.75	35.44 ± 4.80	0.61 ± 0.14	0.88 ± 0.16	32 ± 2.79	36 ± 0.67
L3-58	42.03 ± 3.26	29.11 ± 0.54	0.63 ± 0.09	1.1 ± 0.070	33 ± 1.65	40 ± 1.82
L3-59	33.84 ± 1.20	30.98 ± 4.85	0.48 ± 0.02	1.04 ± 0.15	35 ± 1.26	46 ± 1.95
L4-31	28.10 ± 7.73	30.66 ± 4.58	0.46 ± 0.13	1.2 ± 0.050	30 ± 2.29	48 ± 0.36
L4-48	22.83 ± 5.61	36.66 ± 1.03	0.33 ± 0.09	0.98 ± 0.09	37 ± 0.86	39 ± 1.32
LSD					4.84	

Appendix 9 Average dry matter (g) and yield (number of fruits) for eight okra genotypes (n=3) in the control and heat (Year 1). Values are presented with ±SE. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test.

Genotype	Dry matter (g)		Yield	
	Control	Heat	Control	Heat
L2-11	514 ± 13.5 abcd	549 ± 55.5 d	46.0 ± 13.8	19.3 ± 5.4
L2-30	372 ± 13.9 abc	355 ± 39.8 ab	10.7 ± 2.4	12.0 ± 6.2
L2-32	480 ± 18.5 abcd	455 ± 15.3 bcd	10.3 ± 2.6	16.7 ± 6.7
L3-1	262 ± 24.4 a	407 ± 57.8 abcd	21.3 ± 1.9	19.3 ± 2.0
L3-58	488 ± 63.4 bcd	517 ± 37.4 bcd	7.0 ± 1.7	1.3 ± 0.3
L3-59	478 ± 50.0 bcd	500 ± 47.7bcd	2.3 ± 1.2	1.0 ± 1.0
L4-31	433 ± 89.4 abcd	537 ± 84.2 cd	3.7 ± 2.3	1.3 ± 0.9
L4-48	419 ± 8.6 abcd	361 ± 39.4 ab	33.7 ± 0.7	16.0 ± 7.9

Appendix 10 Average chlorophyll fluorescence (F_v'/F_m'), efficiency of the open reaction centre (Φ_{PSII}) and photochemical quenching (qP) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage) measured by LICOR at week (W) 2, 4 and 6 (Year 2). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test ($p<0.001$).

W	DS	Genotype	F_v'/F_m'		Φ_{PSII}		qP	
			Control	Heat	Control	Heat	Control	Heat
2	F	L2-11	0.60 ± 0.005 cd	0.58 ± 0.003 bc	0.43 ± 0.004 ef	0.36 ± 0.030 cd	0.71 ± 0.009 gh	0.65 ± 0.036 cdef
2	F	L2-30	0.60 ± 0.008 cd	0.60 ± 0.009 cd	0.41 ± 0.001 de	0.41 ± 0.016 def	0.68 ± 0.007 defg	0.68 ± 0.016 efg
2	F	L3-1	0.60 ± 0.007 cd	0.61 ± 0.015 cd	0.45 ± 0.010 f	0.43 ± 0.011 ef	0.75 ± 0.007 h	0.70 ± 0.003 gh
2	F	L4-48	0.61 ± 0.005 d	0.62 ± 0.003 d	0.42 ± 0.014 ef	0.42 ± 0.005 ef	0.69 ± 0.018 fg	0.69 ± 0.006 fg
2	B	L2-11	0.55 ± 0.007 a	0.55 ± 0.008 a	0.35 ± 0.005 bc	0.30 ± 0.034 a	0.63 ± 0.003 cdef	0.56 ± 0.046 a
2	B	L2-30	0.54 ± 0.008 a	0.54 ± 0.017 a	0.34 ± 0.006 bc	0.31 ± 0.023 ab	0.64 ± 0.002 cdef	0.57 ± 0.027 ab
2	B	L3-1	0.54 ± 0.010 a	0.55 ± 0.006 a	0.34 ± 0.014 abc	0.33 ± 0.015 abc	0.62 ± 0.019 bcd	0.60 ± 0.022 abc
2	B	L4-48	0.56 ± 0.005 ab	0.55 ± 0.014 a	0.36 ± 0.007 c	0.34 ± 0.010 bc	0.64 ± 0.008 cdef	0.63 ± 0.011 cde
4	F	L2-11	0.59 ± 0.013 cdef	0.61 ± 0.017 f	0.42 ± 0.012 e	0.40 ± 0.021 cde	0.72 ± 0.005 e	0.65 ± 0.018 bcd
4	F	L2-30	0.60 ± 0.009 def	0.61 ± 0.007 def	0.42 ± 0.001 e	0.41 ± 0.007 cde	0.70 ± 0.011 de	0.68 ± 0.004 cde
4	F	L3-1	0.57 ± 0.014 bcdef	0.60 ± 0.019 def	0.40 ± 0.023 cde	0.42 ± 0.023 e	0.69 ± 0.024 de	0.69 ± 0.017 de
4	F	L4-48	0.58 ± 0.015 bcdef	0.61 ± 0.006 ef	0.40 ± 0.012 cde	0.42 ± 0.008 de	0.68 ± 0.013 de	0.68 ± 0.006 cde
4	B	L2-11	0.54 ± 0.010 ab	0.54 ± 0.018 ab	0.34 ± 0.016 bc	0.32 ± 0.016 ab	0.63 ± 0.022 abc	0.59 ± 0.010 a
4	B	L2-30	0.56 ± 0.008 abcd	0.57 ± 0.007 a	0.35 ± 0.008 bcde	0.35 ± 0.022 a	0.63 ± 0.018 abc	0.60 ± 0.031 ab
4	B	L3-1	0.54 ± 0.011 abc	0.56 ± 0.009 abcde	0.34 ± 0.007 bc	0.34 ± 0.027 bc	0.62 ± 0.006 ab	0.60 ± 0.039 ab
4	B	L4-48	0.55 ± 0.005abc	0.57 ± 0.005 abcdef	0.34 ± 0.011 bcd	0.36 ± 0.009 bcde	0.62 ± 0.026 ab	0.63 ± 0.011 abc
6	F	L2-11	0.60 ± 0.007 de	0.59 ± 0.006 cde	0.44 ± 0.005	0.43 ± 0.011	0.74 ± 0.000	0.72 ± 0.012
6	F	L2-30	0.58 ± 0.012 cde	0.61 ± 0.001 e	0.42 ± 0.008	0.46 ± 0.000	0.71 ± 0.009	0.76 ± 0.002
6	F	L3-1	0.59 ± 0.006 cde	0.59 ± 0.007 cde	0.44 ± 0.009	0.42 ± 0.016	0.74 ± 0.020	0.71 ± 0.018
6	F	L4-48	0.59 ± 0.007 cde	0.60± 0.007 e	0.41 ± 0.006	0.43 ± 0.011	0.70 ± 0.004	0.71 ± 0.010
6	B	L2-11	0.54 ± 0.016 a	0.56 ± 0.002 bc	0.36 ± 0.004	0.39 ± 0.003	0.68 ± 0.021	0.69 ± 0.009
6	B	L2-30	0.52 ± 0.006 a	0.57 ± 0.005 bcd	0.36 ± 0.006	0.40 ± 0.004	0.70 ± 0.008	0.7 ± 0.0010

Appendix 10 Continued

W	DS	Genotype	F_v'/F_m'		Φ_{PSII}		*q*P	
			Control	**Heat**	**Control**	**Heat**	**Control**	**Heat**
6	B	L3-1	0.53 ± 0.028 a	0.54 ± 0.002 ab	0.36 ± 0.021	0.35 ± 0.008	0.67 ± 0.010	0.64 ± 0.018
6	B	L4-48	0.54 ± 0.007 ab	0.54 ± 0.009 ab	0.37 ± 0.007	0.35 ± 0.010	0.68 ± 0.018	0.65 ± 0.009
LSD*								0.055

* LSD for significant Developmental Stage*Week interaction

Appendix 11 Average photosynthesis (A) and stomatal conductance (g_s) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage). A and g_s were measured by LICOR at week (W) 2, 4 and 6 (Year 2). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test ($p<0.001$).

W	DS	Genotype	A (µmol m^{-2} s^{-1})		g_s (mol m^{-2} s^{-1})	
			Control	Heat	Control	Heat
2	F	L2-11	37.52 ± 1.72 fg	28.26 ± 3.34 abc	0.77 ± 0.14	0.89 ± 0.05
2	F	L2-30	35.14 ± 2.04 efg	31.99 ± 1.61 bcdef	0.68 ± 0.18	0.76 ± 0.15
2	F	L3-1	38.19 ± 0.19 g	34.15 ± 2.12 cdefg	0.69 ± 0.03	0.88 ± 0.17
2	F	L4-48	38.36 ± 2.09 g	35.37 ± 0.96 efg	0.80 ± 0.08	0.81 ± 0.07
2	B	L2-11	34.96 ± 0.83 defg	25.47 ± 2.81 a	0.71 ± 0.03	0.69 ± 0.09
2	B	L2-30	34.98 ± 0.71 defg	29.80 ± 3.47 abcde	0.83 ± 0.01	1.01 ± 0.07
2	B	L3-1	32.62 ± 1.06 bcdefg	27.65 ± 0.82 ab	0.68 ± 0.06	0.64 ± 0.09
2	B	L4-48	34.03 ± 1.06 cdefg	29.06 ± 3.24 abcd	0.62 ± 0.03	0.75 ± 0.21
4	F	L2-11	34.40 ± 3.09 bc	34.15 ± 2.00 bc	0.52 ± 0.11	1.16 ± 0.15
4	F	L2-30	37.03 ± 1.30 bc	35.34 ± 0.78 bc	0.69 ± 0.12	1.06 ± 0.13
4	F	L3-1	36.16 ± 1.90 bc	33.18 ± 2.37 bc	0.82 ± 0.06	1.02 ± 0.12
4	F	L4-48	38.03 ± 0.54 c	34.97 ± 1.06 bc	0.79 ± 0.03	1.13 ± 0.02
4	B	L2-11	32.15 ± 1.50 bc	28.90 ± 2.40 ab	0.56 ± 0.09	0.83 ± 0.08
4	B	L2-30	36.91 ± 1.39 bc	32.99 ± 2.80 a	0.88 ± 0.06	1.20 ± 0.00
4	B	L3-1	35.01 ± 0.07 bc	30.99 ± 2.02 abc	0.84 ± 0.02	1.18 ± 0.06
4	B	L4-48	33.11 ± 0.12 bc	31.74 ± 1.51 abc	0.64 ± 0.07	0.96 ± 0.08
6	F	L2-11	37.55 ± 2.56 fg	35.06 ± 1.83 cdefg	0.58 ± 0.13	1.03 ± 0.05
6	F	L2-30	38.19 ± 2.20 g	35.53 ± 1.26 defg	0.67 ± 0.12	0.99 ± 0.05
6	F	L3-1	38.88 ± 1.07 g	35.36 ± 1.83 cdefg	0.71 ± 0.11	1.14 ± 0.08
6	F	L4-48	36.53 ± 1.19 efg	31.47 ± 2.35 bcdef	0.64 ± 0.04	0.92 ± 0.04
6	B	L2-11	29.12 ± 2.83 abc	34.38 ± 0.45 bcdefg	0.33 ± 0.10	1.01 ± 0.11
6	B	L2-30	28.14 ± 2.43 a	34.94 ± 0.71 cdefg	0.25 ± 0.06	1.23 ± 0.05
6	B	L3-1	33.58 ± 4.49 bcdefg	29.31 ± 0.20 abcd	0.61 ± 0.19	0.95 ± 0.07
6	B	L4-48	30.28 ± 0.86 abcde	28.21 ± 0.73 ab	0.37 ± 0.03	0.83 ± 0.06
LSD*					0.109	

* LSD for significant Treatment*Week interaction

Appendix 12 Average transpiration (*E*) and electrolyte leakage (EL) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage). *E* was measured by LICOR and EL with conductivity meter at week (W) 2, 4 and 6 (Year 2). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test ($p<0.001$).

W	DS	Genotype	*E* (mmol m^{-2} s^{-1})		EL (%)	
			Control	Heat	Control	Heat
2	F	L2-11	9.66 ± 0.41 a	20.34 ± 0.28 bcd	42 ± 9.3	34 ± 1.9
2	F	L2-30	9.02 ± 0.98 a	19.54 ± 1.66 bcd	43 ± 1.7	34 ± 1.4
2	F	L3-1	9.73 ± 0.27 a	20.41 ± 1.43 bcd	41 ± 0.9	34 ± 0.8
2	F	L4-48	9.94 ± 0.16 a	21.44 ± 0.87 d	44 ± 1.7	38 ± 3.4
2	B	L2-11	9.18 ± 0.40 a	18.33 ± 0.81 bc	37 ± 1.6	34 ± 1.3
2	B	L2-30	9.79 ± 0.26 a	20.99 ± 0.42 cd	38 ± 1.6	36 ± 0.3
2	B	L3-1	9.50 ± 0.52 a	17.85 ± 1.33 b	37 ± 2.4	33 ± 1.8
2	B	L4-48	9.11 ± 0.06 a	18.49 ± 1.80 bc	38 ± 1.7	38 ± 1.6
4	F	L2-11	12.22 ± 2.01 ab	23.79 ± 1.02 d	29 ± 1.1	29 ± 4.1
4	F	L2-30	14.30 ± 1.88 abc	22.71 ± 0.84 d	36 ± 1.9	30 ± 0.4
4	F	L3-1	15.33 ± 0.53 c	22.85 ± 1.36 d	38 ± 0.6	32 ± 1.9
4	F	L4-48	15.02 ± 0.43 bc	22.91 ± 0.26 d	37 ± 3.8	36 ± 1.6
4	B	L2-11	12.00 ± 0.96 a	21.17 ± 0.39 d	35 ± 2.1	37 ± 3.5
4	B	L2-30	15.42 ± 0.91 c	18.38 ± 5.27 d	31 ± 0.4	38 ± 8.6
4	B	L3-1	15.13 ± 0.70 c	23.27 ± 0.42 d	34 ± 0.3	36 ± 2.3
4	B	L4-48	13.48 ± 0.73 abc	21.75 ± 0.36 d	37 ± 2.4	34 ± 2.8
6	F	L2-11	11.08 ± 2.00 bcd	25.44 ± 1.20 ef	26 ± 2.2	30 ± 2.2
6	F	L2-30	12.03 ± 1.74 cd	26.41 ± 1.01 ef	31 ± 1.8	31 ± 1.2
6	F	L3-1	13.07 ± 0.71 d	27.06 ± 1.56 f	26 ± 3.1	30 ± 1.1
6	F	L4-48	12.49 ± 0.28 cd	25.18 ± 0.49 ef	38 ± 1.3	36 ± 3.1
6	B	L2-11	8.17 ± 1.67 ab	23.98 ± 0.59 ef	29 ± 2.0	33 ± 2.7
6	B	L2-30	6.84 ± 1.23 a	25.41 ± 0.55 ef	27 ± 3.3	29 ± 4.0
6	B	L3-1	11.35 ± 2.32 bcd	25.13 ± 0.48 ef	29 ± 1.6	33 ± 0.2
6	B	L4-48	9.26 ± 0.71 abc	22.91 ± 0.37 e	38 ± 1.4	42 ± 1.9
LSD*			1.301		2.713	
LSD**					2.215	
LSD***					3.837	

* LSD for significant Treatment*Week interaction, ** LSD for significant Treatment*Developmental Stage interaction, ***LSD for significant Genotype*Week interaction.

Appendix 13 Average dry matter (above and below the soil) and yield (No. of fruits) for four okra genotypes (n=3) in the control and heat (Year 2) at flowering (F) and bud initiation (B) stages (DS: developmental stage). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test ($p<0.001$).Genotypes with no fruit in the heat left blank.

DS	Genotype	Dry matter above the soil (g)		Dry matter below the soil (g)		Yield	
		Control	Heat	Control	Heat	Control	Heat
F	L2-11	271 ± 37 cd	192 ± 49 abcd	37 ± 4 efg	23 ± 4 abcd	10.7 ± 1.5	
F	L2-30	287 ± 62 d	208 ± 26 abcd	31 ± 6 cdefg	25 ± 6 abcde	8.0 ± 1.2	1.5 ± 0.4
F	L3-1	287 ± 40 d	195 ± 37 abcd	41 ± 8 g	17 ± 2 ab	16.0 ± 1.5	4.3 ± 1.2
F	L4-48	251 ± 7 bcd	197 ± 8 abcd	56 ± 5 h	37 ± 1 efg	16.0 ± 0.6	1.0 ± 0.0
B	L2-11	239 ± 19 bcd	114 ± 37 a	34 ± 4 defg	14 ± 3 a	4.7 ± 0.9	
B	L2-30	186 ± 14 abc	127 ± 58 a	29 ± 2 bcdefg	20 ± 9 abc	3.7 ± 0.3	
B	L3-1	169 ± 22 ab	161 ± 10 ab	26 ± 2 abcdef	14 ± 3 a	5.3 ± 0.3	1.0 ± 0.0
B	L4-48	179 ± 9 abc	115 ± 10 a	38 ± 2 fg	25 ± 2 abcde	3.0 ± 0.6	
LSD*						1.714	
LSD**						1.714	
LSD***						1.212	

* LSD for significant Genotype*Treatment interaction, ** LSD for significant Genotype*Developmental Stage interaction, ***LSD for significant Treatment* Developmental Stage interaction.

Appendix 14 Average leaf sugar concentrations (Fructose, Glucose and sucrose) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage) at week 2 (Year 2). Values are presented with ±SE. Missing data left blank.

DS	Genotype	Fructose		Glucose		Sucrose	
		Control	Heat	Control	Heat	Control	Heat
F	L2-11	203 ± 38	466 ± 100	216 ± 76	485 ± 105	1080 ± 202	1451 ± 288
F	L2-30	359 ± 52	395 ± 103	395 ± 10	591 ± 270	1516 ± 34	2225 ± 578
F	L3-1	204 ± 26	372 ± 40	166 ± 32	451 ± 55	834 ± 179	1354 ± 57
F	L4-48	91 ± 31	137 ± 37		143 ± 33	877 ± 155	705 ± 130
B	L2-11	123 ± 8	505 ± 51	94 ± 15	521 ± 74	1029 ± 114	1044 ± 78
B	L2-30	239 ± 68	293 ± 47	203 ± 51	292 ± 79	1156 ± 42	986 ± 97
B	L3-1	154 ± 18	485 ± 68	142 ± 28	516 ± 47	1150 ± 111	1240 ± 120
B	L4-48	80 ± 17	275 ± 8	66 ± 34	306 ± 62	693 ± 244	1105 ± 69

Appendix 15 Average leaf sugar concentrations (Fructose, Glucose and sucrose) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage) at week 6 (Year 2). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$.

DS	Genotype	Fructose		Glucose		Sucrose	
		Control	Heat	Control	Heat	Control	Heat
F	L2-11	302 ± 28	509 ± 34	194 ± 20	508 ± 196	1777 ± 240	1987 ± 274
F	L2-30	401 ± 166	1143 ± 245	280 ± 123	823 ± 193	1601 ± 327	2083 ± 295
F	L3-1	379 ± 68	864 ± 196	249 ± 54	559 ± 146	1588 ± 368	2233 ± 69
F	L4-48	417 ± 83	534 ± 26	322 ± 87	340 ± 9	1866 ± 464	1821 ± 197
B	L2-11	348 ± 67	641 ± 74	294 ± 55	527 ± 148	1821 ± 340	1576 ± 115
B	L2-30	348 ± 24	1240 ± 327	275 ± 31	913 ± 270	1834 ± 146	1572 ± 249
B	L3-1	553 ± 203	696 ± 145	439 ± 206	469 ± 45	2402 ± 730	1471 ± 24
B	L4-48	202 ± 45	481 ± 76	188 ± 61	318 ± 42	1316 ± 235	1711 ± 185
LSD*		291.9		262.4			

* LSD for significant Genotype*Treatment interaction

Appendix 16 Average shoot sugar concentrations (Fructose, Glucose and sucrose) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage) at week 6 (Year 2). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$.

DS	Genotype	Fructose		Glucose		Sucrose	
		Control	Heat	Control	Heat	Control	Heat
F	L2-11	2786 ± 446	757 ± 64	2345 ± 338	888 ± 44	3557 ± 142	573 ± 58
F	L2-30	3718 ± 238	1014 ± 93	3104 ± 269	1235 ± 63	4109 ± 156	686 ± 178
F	L3-1	3148 ± 142	693 ± 62	2848 ± 179	734 ± 267	3572 ± 139	688 ± 229
F	L4-48	3182 ± 231	321 ± 12	2683 ± 143	475 ± 7	3892 ± 288	381 ± 117
B	L2-11	3339 ± 259	647 ± 56	2649 ± 94	829 ± 100	3037 ± 397	419 ± 26
B	L2-30	4819 ± 246	343 ± 89	4037 ± 163	433 ± 87	3610 ± 90	275 ± 130
B	L3-1	3723 ± 27	456 ± 61	3180 ± 35	587 ± 105	3161 ± 47	233 ± 35
B	L4-48	3602 ± 303	428 ± 156	3182 ± 195	484 ± 185	2710 ± 896	366 ± 156
LSD*		415.4		352.3			
LSD**		293.7		249.1			

* LSD for significant Genotype*Treatment interaction, **LSD for Treatment*Developmental Stage interaction

Appendix 17 Average root sugar concentrations (Fructose, Glucose and sucrose) for four okra genotypes (n=3) in the control and heat at flowering (F) and bud initiation (B) stages (DS: developmental stage) at week 6 (Year 2). Values are presented with ±SE and least significant differences (LSD) of means for significant interaction at $p<0.05$. Missing data left blank.

DS	Genotype	Fructose		Glucose		Sucrose	
		Control	Heat	Control	Heat	Control	Heat
F	L2-11	456 ± 193	513 ± 204	174 ± 78	178 ± 33	878 ± 112	948 ± 212
F	L2-30	211 ± 56	905 ± 141	81 ± 27	359 ± 51	671 ± 81	1158 ± 94
F	L3-1	244 ± 26	245 ± 124	90 ± 7	110 ± 76	645 ± 137	648 ± 215
F	L4-48	275 ± 29	386 ± 122	131 ± 25	180 ± 70	768 ± 53	929 ± 467
B	L2-11	49 ± 4	189 ± 30	12 ± 2	75 ± 14	258 ± 87	485 ± 101
B	L2-30	201 ± 35	423 ± 84	86 ± 15	254 ± 84	242 ± 85	821 ± 159
B	L3-1	77 ± 33	177 ± 35	21 ± 13	79 ± 17	154 ± 60	417 ± 208
B	L4-48	93 ± 39		35 ± 21		228 ± 89	
LSD*		198.9		91.8			

* LSD for significant Genotype*Treatment interaction.

Appendix 18 Three replication (P1, P2, P3) of pollen germination (%) in 18 medium containing basic BK medium with different concentration of sucrose and polyethylene glycol. Different letters denote significant differences between genotypes, based on a Fisher's unprotected LSD test ($p<0.001$).

Medium	Sucrose (%)	Polyethylene glycol (%)	Germination (%)			Average (%)
			P1	P2	P3	
1	0	0	2.83	0	8.24	3.69 a
2	0	2.5	0.00	0	28.93	9.64 ab
3	0	5	0.00	11.43	39.89	17.11 abc
4	10	0	61.54	67.65	76.87	68.68 ghij
5	10	2.5	88.89	83.33	88.65	86.96j
6	10	5	68.33	72.73	87.88	76.31 hij
7	15	0	66.67	84.00	85.83	78.83 ij
8	15	2.5	66.88	83.51	87.70	79.36 ij
9	15	5	52.70	77.78	82.55	71.01 hij
10	20	0	72.07	75.47	41.26	62.94 fghij
11	20	2.5	52.52	75.00	84.66	70.73 hij
12	20	5	50.89	71.19	72.17	64.75 fghij
13	25	0	45.86	73.21	52.70	57.26 efghi
14	25	2.5	50.00	68.18	30.43	49.54 defgh
15	25	5	36.71	63.24	6.96	35.63 bcde
16	30	0	51.90	57.69	19.12	42.90 cdefg
17	30	2.5	20.44	62.26	40.34	41.01 cdef
18	30	5	10.17	47.83	14.48	24.16 abcd