THE BASIS OF CHICKPEA HEAT TOLERANCE UNDER SEMI-ARID ENVIRONMENTS

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STATEMENT OF AUTHENTICATION

The work presented in this thesis, to the best of my knowledge and belief, is original except as acknowledged in the text. I declare that I have not submitted this material, either in whole or in part, for a degree at this or any other institution.

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Abstract

Chickpea (*Cicer arietinum* L.) is an important grain legume. Global warming and changes in cropping systems are driving chickpea production to relatively warmer growing conditions. Studies on the impact of climate change on chickpea production highlighted the effect of warmer temperatures on crop development and subsequent chickpea yield. For example, the yield of chickpea declined by up to 301 kg/ha per 1˚C increase in mean seasonal temperature in India. Assessment of whole plant response, particularly flowering and grain filling in warmer environments, in the field is generally an effective screening method. The identification of heat tolerant genotypes can help adapt chickpea to the effects of warmer temperatures.

In this study, 167 chickpea genotypes were screened in heat stressed (late season) and non-stressed (normal season) conditions in the field during 2009-10 (year 1) and 2010-11 (year 2) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. The aim of these experiments was to screen chickpea germplasm in contrasting chickpea growing seasons for high temperature tolerance. Plant phenology (days to first flowering, days to 50% flowering, days to first pod, and days to maturity), growth (plant height, plant width and biomass at harvest) and grain yield including pod number per plant, filled pod number per plant and seed number per plant were recorded in both seasons. There was large and significant variation for phenology, growth, grain yield and yield traits. Pod numbers per plant and harvest index are the two key traits that can be used in selection for breeding programs. The genetic variation was also confirmed by canopy temperature depression and the Heat Tolerance Index (HTI). Furthermore, using daily maximum and minimum temperature during the growing period, temperature for chickpea developmental stages (vegetative, flowering and grain filling phases) was calculated for both seasons to understand genotype × environment (G × E) interaction.

In addition, sensitivity of male and female reproductive tissues to high temperature is important to explain the effect of heat stress on the reproductive phase. Therefore, field experiment was conducted at ICRISAT under stressed condition (late season) during 2011. The aim of these experiments was to study genetic variation in male reproductive tissue (anther, pollen), its function (pollen germination and tube growth) and pod set. Pollen fertility, *in vitro* pollen germination, *in vivo* pollen germination and pod set was examined under different temperatures. The field

experiment was compared with controlled environments (stressed and non-stressed conditions). Both anthers and pollen grains showed more structural abnormalities such as changes in anther locule number, anther epidermis wall thickening and pollen sterility, rather than function (e.g. *in vivo* pollen tube growth). Clearly, chickpea pollen grains are more sensitive to high temperature than the stigma in both the field and controlled environments. Both studies suggested that the critical temperature for pod set was ≥37˚C in heat tolerant genotypes (ICC 1205; ICC 15614 and ICCV 92944) and ≥33˚C for heat sensitive genotypes (ICC 4567; ICC 10685 and ICC 5912).

Implementation of molecular breeding in chickpea improvement program depends on the understanding of genetic diversity. Diversity Array Technology (DArT) is a micro-array based method allowing for finding of DNA polymorphism at several thousand loci in a single assay. The aim of this research was to investigate the genetic diversity between the167 chickpea genotypes using DArT markers. Based on 359 polymorphic DArT markers, 153 genotypes showed polymorphism. A dendrogram derived from cluster analysis based on the genetic similarity coefficient matrix for the 153 genotypes was constructed. There were nine groups (group 1-9) identified from dendrogram. The genotypes were collected from 36 countries and ICRISAT breeding lines were also included in the germplasm. Based on eleven quantitative traits (days to first flowering, days to 50% flowering, days to first pod, days to physiological maturity, plant height, plant width, plant biomass, pod number per plant, filled pod number per plant, seed number per plant and grain yield) observed in the field, the diversity groups were arranged under stressed and non-stressed conditions for two years and their relationship of origin was also studied. The group 9 (ICRISAT breeding lines) produced highest grain yield under non-stressed and heat stressed followed by group 3. Those breeding lines were crossbreeds from the ICRISAT's breeding programs and released in different countries at different times. Furthermore, characterisation of ICRISAT screening environments using 29 years of temperature data was done to understand the chickpea growing season for future breeding programs.

Association analysis was conducted on chickpea genotypes evaluated in the field screening for high temperature tolerance. Eleven quantitative traits observed in the field under heat stressed and non-stressed conditions were analysed to understand the genetic control of heat tolerance through marker-trait association. Under heat stress, 44 DArT markers were associated with grain yield and pod characteristics such as total pod number, filled pod number and seed number. A DArT marker was associated with three or four traits and may be efficiently used in improvement of more than one trait at a time. The associated markers for the traits like plant height, plant width, pod number and grain yield were found in the genomic regions of previously reported QTLs. In addition, many genomic regions for phenology, biomass and grain yield under heat stressed and non-stressed conditions. The number of markers significantly associated with different traits was higher under heat stress, suggesting that many genes are present that control plant response to high temperature in chickpea.

Four populations, ICC 1356 x ICC 15614; ICC 10685 x ICC 15614; ICC 4567 x ICC 15614 and ICC 4567 x ICC 1356 of F_1s, F_2s along with their parents were assessed in the field in 2011 at heat stressed condition (late season). The objective of this experiment was to study the inheritance of heat tolerance. Days to first flowering (DFF), pod number per plant (TNP), filled pod number per plant (NFP), seed number per plant (NS) and grain yield per plant (GY) was recorded. Estimates of broad sense heritability for the traits DFF, TNP, NFP, NS and GY were calculated for all four crosses. In this study, parents were heterogeneous for heat response. At extreme high temperature (>40˚C) the population, especially ICC 4567 x ICC 15614, set pods and gave higher grain yield compared with other crosses. The adaptation of chickpea to high temperature may also be improved using more exotic parents to combine allelic diversity for flowering time, pod number, filled pod number, seed number per plant and grain yield.

High temperature clearly has an influence on plant growth, development and grain yield. The research has identified heat tolerant sources of chickpea and also found the impact of high temperature on the male reproductive tissue. Studying genetic diversity using DArT markers and understanding diversity group with agronomic traits provided the basis of chickpea response to high temperature. Further research is needed from populations of chickpea crosses using late generations. This will enable the development of heat tolerant chickpea cultivar.

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Abbreviation

AA	Association anaysis
cpPb	Chickpea probe
CTD	Canopy temperature depression
D50%F	Days to 50% flowering
DArT	Diversity array technology
DFF	Days to first flowering
DFP	Days to first podding
DPM	Days to physiological maturity
GY	Grain yield
HI	Harvest index
HS	Heat stressed
HTI	Heat tolerance index
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
NFP	Number of filled pods
NS	Non-stressed
PH	Plant height
PW	Plant width
QTL	Quantitative trait loci
SN	Seed number
TNP	Total number of pods

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Peer-reviewed publications arising from this thesis

Chapter 2

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Chapter 4

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Chapter 5

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Devasirvatham V, Gaur PM, Mallikarjuna N, Raju TN, Trethowan RM, Tan DKY (2013) Reproductive biology of chickpea response to heat stress in the field is associated with the performance in controlled environments. *Field Crops Research* **142**, 9-19.

Poster presentation

Devasirvatham V, Trethowan RM, Gaur PM, Raju TN, Tan DKY (2012) Breeding targets to improve heat tolerance in chickpea (*Cicer arietinum* L.). In 'Plant Breeding for Future Generations'. 19th EUCARPIA General Congress. 21st-24th May. Budapest, Hungary.

Chapter 1: Introduction

Chickpea is an important cool season grain legume with indeterminate growth habit. Chickpea seeds are classified into desi and kabuli types and are a good source of protein, carbohydrate, fibre and minerals. Abiotic and biotic stresses interludes in chickpea production environments. Among the abiotic stress, high temperature is a major factor, associated with yield reduction (Summerfield *et al*. 1990). Predicted climate change, particularly high temperature will reduce grain yield in chickpea. For example, the yield of chickpea declined by up to 301 kg/ha per 1˚C increase in mean seasonal temperature in India (Karla *et al*. 2010). India and Australia ranks first and second in global chickpea production, respectively (FAO, 2010). In both countries, chickpea have been exposed to high temperature stress in the growing season, mainly in reproductive phase. Chickpea production mostly occurs in residual soil moisture under rainfed conditions, where terminal drought and heat stresses are major limitations to chickpea grain yield (Summerfield *et al*. 1990). These rainfed regions are accompanied by variable rainfall pattern (Basu *et al*. 2009). Therefore isolation of heat stress from the drought is a major challemge in chickpea production. Although recent research on chickpea field screening for heat tolerance (Krishnamurthy *et al*. 2011; Upadhyaya *et al*. 2011) identified sources for heat tolerance, there has been little attempt to extrapolate these findings to the world's chickpea production areas. The determination of physiological response and reproductive tissues response to heat stress is vital. However, the research on the impact of high temperature in chickpea is not extensive.

The chickpea grain yield is related to its phenology which is influenced by temperature. The timing and duration of flowering has an important role in determining crop duration and grain yield at high temperature. The crop is forced into maturity under hot and dry condition (>30ºC) by reducing the crop duration (Summerfield *et al*. 1984). The different developmental stages of chickpea are affected by high temperature. Finally, the grain yield is reduced. High temperature also affects physiological traits such as canopy temperature in chickpea plants (Rosyara *et al*. 2010). Canopy temperature reflects the interactions between plants, soil and atmosphere and canopy temperature depression (CTD[1]) can be used to predict the performance of genotypes under heat stressed environments. Therefore,

[1] CTD is calculated difference from canopy and air temperatures (Rosyara *et al*. 2010).

understanding of the effect of high temperature on plant response under stressed and non-stressed environments is needed for germplasm screening. This will help to identify the sources of heat tolerance in chickpea.

The factors that affect crop yield in chickpea during the reproductive development are flower abortion due to pollen sterility, lack of pollination, stigma receptivity and pod abortion. Failure of any of these functions can decrease pod formation, number of seeds and grain yield (Vara Prasad *et al*. 2001). Although the male and female parts are sensitive to high temperature, pollen can be used as a trait to estimate the genetic variability to high temperature during the reproductive phase. Therefore it is essential to study the effect of high temperature during reproductive phase.

Previous studies developed screening methods for heat tolerance and identified sources for heat tolerance in chickpea (Krishnamurthy *et al*. 2011). However, there is no published report on the inheritance study of heat tolerance in chickpea. Molecular studies using different markers for heat tolerance are also not available at present for chickpea germplasm. Thudi *et al*. (2011) constructed a comprehensive genetic map comprising 253 simple sequence repeat (SSR) markers and 675 DArT markers for chickpea. Therefore basic information of molecular markers in chickpea is increasing. Coping with the consequences of high temperature on chickpea production will require better knowledge of the genomics approach. This approach can enable the identification and selection of chromosome regions harbouring genes or QTLs (Quantitative Trait Loci) controlling quantitative traits and yield in chickpea under high temperature. Association mapping is a better approach to identify genotype-phenotype association i.e. molecular marker-trait association. This has been studied in chickpea drought tolerance using DArT markers (Nayak 2010).

The broad aim of this project was to develop an improved understanding of genetic diversity of chickpea genotypes using molecular markers, the effects of high temperature on chickpea growth and yield and identifying traits that can be potentially exploited for future breeding programs for heat tolerance in chickpea. The information on agronomic and physiological traits, inheritance of heat tolerance, and identification of genetic basis of heat adaptive traits through marker-trait association will help to develop breeding strategies to improve heat tolerance in chickpea. The key research question was: Is there any genetic variation for heat tolerance in chickpea?
The specific objectives of this research were

1) To assess genetic diversity of chickpea germplasm using DArT markers

2) To assess genetic variability for heat tolerance of 167 chickpea genotypes by field screening in heat stressed (late season) and non-stressed (normal season) environments.

- Determination of phenology, plant growth, grain yield and physiological trait (CTD) responses
- Classification of genotypes based on heat tolerance index
- Assess maximum and minimum temperatures at different developmental stages (vegetative, flowering and grain filling phases) of chickpea to find out the variation pattern of genotypes and temperature

3) To determine genetic variation of male reproductive tissue under heat stressed and non-stressed environments

- Develop screening procedure and determine the genetic variation of reproductive biology of chickpea genotypes to heat stress under controlled environments
- Determine the reproductive biology of chickpea response to heat stress in both field and controlled environments

4) To dissect the genetic basis of heat adaptive traits and grain yield of 167 genotypes evaluated under heat stressed and non-stressed environments using association analysis

5) To study inheritance of four chickpea populations (ICC 1356 x ICC 15614; ICC 10685 x ICC 15614; ICC 4567 x ICC 15614 and ICC 4567 x ICC 1356) with parents, F_1 and F_2 under heat stressed environment.

Chapter 2: Review of literature

2.1. Introduction

Chickpea is a major grain legume used for food from ancient days. It is one of the essential semi-arid tropical (SAT) legume crop. Chickpea is either grown during the post-rainy season on stored soil moisture (South Asia and spring-sown Mediterranean) or as a Mediterranean winter crop on in-season rainfall; in both instances the crop is exposed to terminal drought which is accompanied by rising temperatures. The South Asian crop may also experience high temperatures in the seedling phase if planted early (Berger and Turner 2007). Chickpea productivity is constrained by several abiotic stresses (Singh *et al*. 1994; Gaur *et al*. 2007) and temperature is one of the most important determinants of crop growth over a range of environments (Summerfield *et al*. 1990) and may limit chickpea yield (Basu *et al*. 2009).

The effects of heat stress during the vegetative and reproductive growth stages using agronomic, phenological, morphological and physiological assessment has been studied in various crops such as wheat (Sharma *et al*. 2005), rice (Weerakoon *et al*. 2008) and cotton (Cottee *et al*. 2010) whilst only limited research has been conducted in chickpea (Wang *et al*. 2006). The detrimental effects of high temperature on various growth and reproductive stages are difficult to assess when growing conditions are favourable in the short term (few days) as the plant continues vegetative growth but sets fewer pods because of indeterminate plant type and plasticity (Liu *et al*. 2003). The relatively narrow genetic base of cultivated chickpea is another reason why high temperature has such a detrimental effect on growth and reproductive physiology (Abbo *et al*. 2003a). For these reasons chickpea tends to be sensitive to high temperature during the growth and reproductive stages. In general, the cool season food legumes (peas, lentil, chickpea and faba bean) are more sensitive to heat than warm season legumes (cowpea, soybean, groundnut, pigeonpea, and mung bean). Among cool season legumes, chickpea is less sensitive to high temperature (Wery *et al*. 1993; McDonald and Paulsen 1997). Although chickpea is exposed to warm temperature (> 30ºC) in certain regions, limited yield loss was found at 30ºC which is higher than other cool season legumes such as field peas, faba bean and lentil (Summerfield *et al*. 1984; Erskine *et al*. 1994; McDonald and Paulsen 1997; Patrick and Stoddard 2010). Therefore a base level of heat tolerance is found in chickpea. However, there is no clear evidence to show the mechanism of heat tolerance. This review outlines the occurrence of high temperature stress, the state of chickpea production, the effects of high temperature on growth and physiology of chickpea, and explores strategies to improve chickpea breeding for heat tolerance.

2.2. State of chickpea production

Climates favourable for chickpea production fall into two general groupings; Mediterranean and summer dominant rainfall semi-arid subtropical climates (Berger and Turner 2007). Chickpea production is also grouped into three regions globally: West Asia and North Africa (WANA), the Indian subcontinent region and recently emerged regions. The details of these regions, their climate and relative intensity of the principle stresses are discussed by Berger and Turner (2007). Chickpea is extensively cultivated in the Mediterranean climate regions of northern Pakistan, Iran, Iraq, Turkey, southern and south western Australia and the Mediterranean basin. In these areas, chickpea is widely sown in winter at a maximum air temperature of 10ºC (Berger, 2007) and high temperature occasionally occurs during reproductive development in the spring (Iliadis 1990).

In the Indian subcontinent region and recently emerged regions (e.g. eastern, northern and southern Australia), the crop experiences cool (5 to10ºC) and frosty nights (0 to -1ºC) in the early vegetative stage and warm (20 to 27ºC) to hot (> 30ºC) air temperature during the day over the reproductive phase (Summerfield *et al*. 1984; Summerfield *et al*. 1990; Berger and Turner 2007). During the last two decades, south Indian and eastern Australian late-sown chickpea has been exposed to heat stress in the growing season, mainly in reproductive phase. In south India, if the rainy season (*kharif*) is extended, then the chickpea sowing in the *rabi* season will be delayed (Ali 2004). This delay exposes the crop to high temperature during the reproductive stage. In Australia, particularly in northern NSW and depending on the climatic conditions, sowing can be delayed until last week of June to reduce the incidence of *Ascochyta* blight (Moore and Knights 2009). However, late sown crops may experience high temperatures during the reproductive phase. Berger and Turner (2007) and Berger *et al*. (2011) described the global chickpea distribution based on climate analysis and current production trends. The climate analysis showed that the current chickpea growing area is under threat from increasing temperature and production may extend to cooler regions.

2.3. The nature of heat stress and plant response

High temperature often occurs in combination with high solar irradiance, drought, and strong wind, all of which can aggravate plant injury even in well watered plants (Hall 1992). Heat stress is a function of plant genotype, high temperature, water status and soil type. The occurrence and severity of heat stress varies in different regions from year to year. Depending on timing, duration and interaction, observed heat stress can be grouped into chronic and acute, each of which involve different coping mechanisms, adaptation strategies and ultimately, breeding techniques (Blum 1988; Wery *et al*. 1993). Chronic heat stress occurs at any stage of crop growth and generally results in substantial yield loss and even crop failure. Acute heat stress of relatively short duration can occur at any stage of crop growth, often leading to lower yield. Acute heat stress is more prevalent than chronic heat stress in the spring sown chickpea regions of WANA (e.g. Turkey) and the Indian subcontinent region. In the spring crop, the mean seed yield of 1627kg/ha decreased compared with the autumn crop due to seasonal temperature fluctuation (26 to 38˚C) during the reproductive stage (Ozdemir and Karadavut 2003). In north India, chickpea grain yield decreased by 53kg/ha in Uttar Pradesh and 301kg/ha in Haryana per 1ºC increase in seasonal temperature (Kalra *et al*. 2008). Pod development is clearly impaired at above optimum (> 30ºC) temperatures (Summerfield *et al*. 1984). Nevertheless, different genotypes have a range of tolerance or resistance mechanisms that help them cope.

2.4. Genotypic variability for heat tolerance

During the Greek and Roman period, chickpea was grown as a summer crop (sown in March/April and harvested in June/July) (Kumar and Abbo 2001). In the Mediterranean and near-eastern gene pools, the wild *Cicer reticulatum* germinates after autumn rain and the crop matures in spring. During the spring the crop is exposed to rising temperature which influences the flowering period, accelerates maturity and may limit yield. However, the shift of chickpea sowing from autumn to spring occurred early in the crop's history (Abbo *et al*. 2003b) and was driven by high fungal disease (*Ascochyta* blight) incidence in the autumn sown crop (Kumar and Abbo 2001). This shift of season has likely caused a genetic bottleneck which narrowed the genetic diversity (Abbo *et al*. 2003a) and most probably genetic variation for heat tolerance. Therefore, the origin and diversity of genetic resources must be considered when screening germplasm for heat tolerance if the plant breeder is to improve the temperature tolerance of modern chickpea in the target environments.

A decade ago, the heat tolerant genotypes ICCV 88512 and ICCV 88513 were identified from among 25 genotypes (Dua 2001). Recently, a reference collection of 280 diverse chickpea germplasm was screened in the field for heat tolerance in two locations (Patancheru and Kanpur) in India during the post-rainy season (optimum) and summer season (late heat). Based on a Heat Tolerance Index (≥ 1.00), ICC 3362, ICC 6874 and ICC 12155 were identified as heat tolerant lines. ICC 16374, ICC 4567 and ICC 10685 were classified as heat sensitive lines based on low HTI (negative values) (Krishnamurthy *et al*. 2011). Upadhyaya *et al*. (2011) identified ICC 14346 as a heat tolerant genotype among 35 early maturing germplasm under ideal crop management (irrigation, nitrogen application) conditions in field screening at Patancheru based on yield (kg ha^{-1}). At present, genotypic diversity of chickpea global germplasm collections and chickpea production environments, particularly those affected by high temperature have not been amply studied. The heat tolerance of chickpea is likely to be multi-genic and the components of heat tolerance are probably controlled by different sets of genes (Upadhyaya *et al*. 2011).

2.5. Plant responses to heat

2.5.1. Effect of heat stress on crop establishment (germination and crop development)

Heat stress at sowing directly affects crop germination and crop establishment. Chickpea seed germination decreases at supra-optimum temperatures (Singh and Dhaliwal 1972; Ellis *et al*. 1986). Ellis *et al*. (1986) indicated that the optimal temperature for germination is 10 -15˚C and noted that high germination temperature is considered to be 22 - 35˚C. Covell *et al*. (1986) showed that germination was faster at higher temperatures between 31.8 and 33ºC. However, at high temperature the mobilisation of cotyledon reserves and embryo growth are adversely affected. Whilst chickpea showed genotypic variation in the rate of germination under various temperatures (Ellis *et al*. 1986), the germination percentage of chickpea was zero when temperature ranged between 45 to 48ºC (Singh and Dhaliwal 1972). High mean maximum temperature and low relative humidity can have a marked influence on seedlings (Saxena 1987). Low photosynthetic rates and high transpiration rates occur during high temperature stress and tend to reduce plant establishment in chickpea

(Singh and Dhaliwal 1972). Recent climate data from field experiments in south India (Patancheru – 18˚N, 78˚E) during sowing time after the rainy season (last week of October) showed a temperature range of 28 - 31˚C (Upadhyaya *et al*. 2011). Sowing temperature is an important determinant of yield, and will become a significant constraint should predicted climate change lead to higher future sowing temperatures.

Though early phenology was mentioned in the heat escape mechanism section, the importance of early phenology in chickpea breeding for heat tolerance will be discussed in greater detail in this section. Higher temperature and photoperiod can modify plant phenology (e.g. opening of first flower), particularly if crops are exposed to warming temperatures and long days in summer (Summerfield *et al*. 1984; van der Maesen 1972). An understanding of these effects and their interactions with genotype are needed in field screening under stress. Breeders generally use days to first flowering as an indicator of crop duration (Anbessa *et al*. 2006). The photoperiod sensitive genotype (Chafa) produced flowers in 25 days under optimum temperature (26ºC) and 15 h photoperiod compared with 52 days in the late flowering genotypes (K 850, G 130) (ICRISAT 1979). The flowering model studies of Summerfield *et al*. (1985) indicated that the rate of progress towards flowering was a linear function of mean temperature and there was no interaction between photoperiod and mean temperature. However, the linear development of the plant through to flowering only occurs within a defined range above which the rate of crop development declines. These critical temperatures vary among genotypes with tolerant lines having higher optimum temperature compared to sensitive genotypes.

2.5.2. Effect of heat stress on reproductive development and yield

Chickpea has small flowers and the stamens are diadelphous (9+1 anthers). Self pollination takes place before the flower opens and pods form within five to six days (Singh 1997). Heat stress during the reproductive phase in legumes is generally allied with lack of pollination, abscission of flower buds, flowers and pods with substantial yield loss (Nakano *et al*. 1997, 1998). Hot (> 30ºC) and dry atmospheric conditions lead to profligate loss of flower buds and open flowers in chickpea (Sinha 1977).

High temperature after flower opening decreases chickpea seed yield by reducing the number of seeds per plant and weight per seed (Wang *et al*. 2006). In chickpea, Summerfield *et al*. (1984) suggested that the longer the exposure during reproductive development to a high day temperature of 35°C, the lower the yield. Most chickpea genotypes do not set pods when temperatures reach > 35ºC (Basu *et al*. 2009). However, there is considerable variation among genotypes for response to high temperature. The period of anthesis and seed set are clearly critical stages for exposure to heat stress (Gross and Kigel 1994). Nayyar *et al*. (2005) suggested that the development of male (pollen, anthers) and female (stigma-style, ovary) parts are the most sensitive organs to abiotic stress in reproductive biology. Therefore pollen viability, stigma receptivity and ovule viability are useful indicators of sensitivity to abiotic stress (Nayyar *et al*. 2005). However, the effect of stress on either male or female organs depends upon the stage of sporogenesis (micro or mega). Due to heat stress, meiosis and pollen development are the most affected part in micro-sporogenesis. Megaspore formation in the ovule and fertilisation are the most important events in mega-sporogenesis under high temperature stress (Gross and Kigel 1994).

High temperature effects on pre-anthesis are related to anther development, pollen sterility and pollen production. The study of pollen may help to predict genetic variation among genotypes for reproductive phase heat tolerance. Pollen sterility is one of the key factors limiting legume yield under high temperature (Porch and Jahn 2001). Eight stages of chickpea pollen development, from pollen mother cell development to mature pollen can be distinguished (Fig 2.1). Two of the stages, microspore mother cell meiosis and mature microspores at anthesis, appear to be detrimentally affected by high temperature (Iwahori 1965; Ahmed *et al*. 1992). The microspore mother cell meiosis, particularly early meiosis I, II is also sensitive to high temperature (Iwahori 1965). Ahmed *et al*. (1992) reported that tapetal cells (meiosis II) did not become binucleate and the locular cavity was less developed in anthers under high temperatures (33°C day/ 30°C night) resulting in premature pollen development. Such information is lacking in chickpea at meiosis stage. Most of the pollen studies in chickpea have focused on cold tolerance (Srinivasan *et al*. 1999; Clarke *et al*. 2004) and the meiosis stage (9 days to 5-6 days before anthesis (DBA)) of chickpea was found to be sensitive to cold (< 3º C) (Clarke and Siddique 2004).

Reduced pollen viability is common in legumes during pre-anthesis. In chickpea, 80-90 % pollen germination occurs in the range 7-25ºC (assessed after 4 h incubation *in vitro)*. During germination pollen hydration is inhibited by low temperature (Clarke and Siddique 2004).

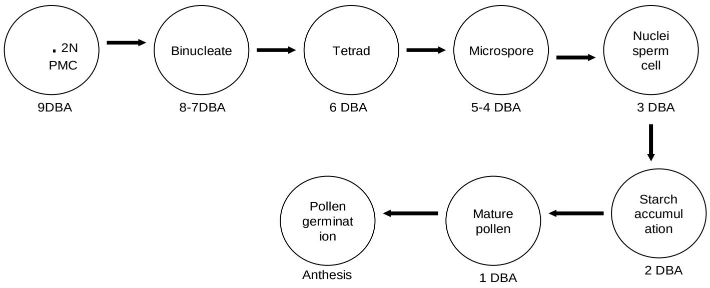

Fig 2.1 Sequences of chickpea pollen development

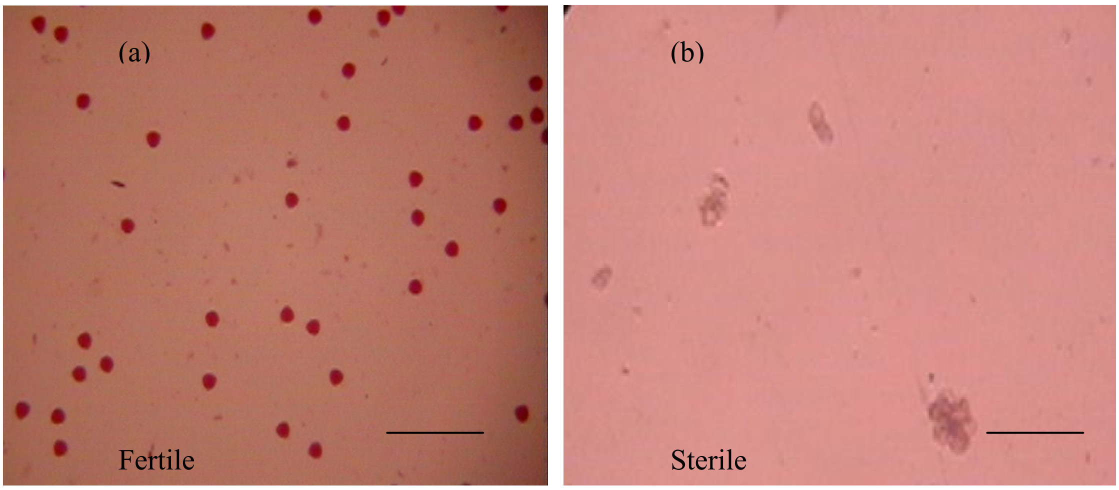

Fig 2.2 Fertile and sterile pollen of chickpea at 35/20ºC (pollen grains stained with 2% acetocarmine) (a) Fertile pollen of ICCV 92944 (picked up stain); (b) Sterile pollen of ICC 5912 (no stain) (Devasirvatham *et al*. 2010) (Bars = 10 µm)

In vitro pollen germination after 60 min incubation was higher (61 %) at 25ºC in chickpea compared with 45ºC (33 %) (Jaiwal and Mehta 1983). Therefore both high and low temperatures reduce pollen germination. Pollen abnormalities were observed in cowpea at 33/30ºC when plants were exposed to heat 3 DBA (Ahmed *et al*. 1992). Anther indehiscence in bean occurred at 32/27ºC when subjected to heat stress during the period 9 -13 DBA (Porch and Jahn 2001; Gross and Kigel 1994).

Pollen production was reduced about 30-50 % at 38/30ºC compared with 30/22ºC in soybean (Koti *et al*. 2005). Therefore pre-anthesis flower abortion is caused by male sterility resulting from abnormal pollen development and anther indehiscence (Warrag and Hall 1984). In chickpea there is genotypic variability for high temperature sensitivity. At 35/20ºC day/night exposure for 24 h before anthesis, the chickpea genotype ICC 5912 became sterile, while pollen from the genotype ICCV 92944 was fertile (Devasirvatham *et al*. 2010) (Fig 2.2).

High temperature effects post-anthesis are associated with loss of stigma receptivity (Kakani *et al*. 2002), poor pollen germination, pollen tube growth on the stigma (Talwar and Yanagihara 1999) and failure of pollen fertilisation and ovule formation (Ormrod *et al*. 1967). Heat stress sometimes has combined effect on male and female parts, thus creating asynchrony between male and female organs (Zinn *et al*. 2010). There is a lack of information about asynchrony in chickpea under heat stress. Nevertheless, progress has been made in chickpea reproductive biology under cold stress. The stigma receptivity is reported to be low at low temperature (12/7ºC) in chickpea (Nayyar *et al*. 2005). At 12/7ºC, decline in pollen germination and pollen tube growth on the stigma may be associated with stigma receptivity (Clarke and Siddique 2004). This might occur due to low amounts of exudates on the stigma (Nayyar *et al*. 2005). Lack of pollen germination and tube growth in the style was found in the heat sensitive genotype ICC 5912 at 35/20ºC due to sterile pollen (Devasirvatham *et al*. 2010). In this study, stigma receptivity was not affected by high temperature stress. However, the observed reduction in pollen germination at high temperature on the stigma is not clear. Therefore it is essential that the effect of heat stress on pollen function (pollen germination and tube growth) and stigma receptivity of genotypes in the field be studied.

Pre-anthesis heat stress resulted in flower abortion indicating that this stress limits pods formation. In addition, the number of days of exposure to high post-anthesis temperatures is important in legumes. The timing of pre- and post-anthesis heat stress was studied in cowpea (Hall 1992) and groundnut (Vara Prasad *et al*. 1998). A combination of pre- and post-flowering stress reduced pod set in bean at 34/29ºC (Agtunong *et al*. 1992). The maximum sensitivity to hot day temperatures (38°C) in groundnut occurred anywhere between 6 days before to 15 days after flowering which can reduce the fruit set, i.e. the proportion of flowers producing pegs or pods (Vara Prasad *et al*. 1998; 1999). These examples indicate that the period of anthesis (pre-anthesis; anthesis and post-anthesis) and number of days of exposure to heat during flowering play an essential role in the development of reproductive organs i.e. pods.

Generally, the responses of plants to high temperature are assessed under high day temperature. However, high night temperature might also play a significant role in legumes and can influence seed set. Anthers failed to dehisce and pod development was affected by a high night temperature of 27ºC in bean (Konsens *et al*. 1991). In cowpea, high night temperature (33/30 ºC) increased the occurrence of small and shrunken pollen leading to zero pod set compared with low night temperature (33/20ºC) (Ahmed *et al*. 1992). Therefore diurnal temperatures play an important role in legume male reproductive organs (Ahmed *et al*. 1992). However, little is known about these effects in chickpea.

Seed development in legumes is a function of the rate and duration of embryo growth, which is in turn influenced by abiotic stress that may lead to embryo abortion (Warrag and Hall 1983), or small endosperms (Davies *et al*. 1999). Finally endosperm filling in the seed is affected by high temperature resulting in small or wrinkled seeds (Egli *et al*. 2005). The probable reason for small endosperm or smaller seed size after post-anthesis heat stress is that the remobilisation of photosynthates to the grain is reduced. A large proportion of carbohydrate is generally utilised to fill the grain in legumes (Davies *et al*. 1999), thus influencing seed weight and number. Under heat stress seeds are not fully develop in sensitive genotypes at agronomic maturity (Sivakumar and Singh 1987). More research is needed to address the remobilisation of the photosynthates to chickpea seeds under heat stress.

Seed quality (uniformity of seed size, shape, colour and texture of the seed coat) is important for grain marketing and is subjected to genotypic × environmental (G × E) effects that seem to be related to abiotic stress, particularly in kabuli chickpea (Sivakumar and Singh 1987; Leport *et al*. 1999). Whilst heat stress is expected to play a role here, its effects have not been well studied.

In summary, available evidence indicates that chickpea crop establishment (seedling growth) has a lower supra-optimal temperature (20 to 24˚C) than pollen germination or pollen tube growth (25˚C) (Table 2.1). However, high temperature frequently occurs during the reproductive stage in chickpea production areas. Heat stress has important effects on the reproductive period that influence time to pod set, seed set and yield including: (1) flowering time (2) asynchrony of male and female organ development and (3) impairment of male and female organs (Craufurd and

Wheeler 2009; Zinn *et al*. 2010). Therefore, improved understanding of chickpea response to heat stress (both day and night temperatures) combined with the timing and duration (short or acute/ long or chronic) of heat stress is important for chickpea breeding.

Table 2.1 Summary of findings on the effect of high temperature on germination, growth and development and flowering of chickpea

Crop stage	Optimum temperature	Detrimental high temperature	References
Germination			
Soil temperature	15 to 34ºC	≥ 35ºC	Singh and Dhaliwal (1972)
Air temperature	31.8 to 33ºC	≥ 35ºC	Covell *et al*. (1986)
Growth and development			
Seedling growth	20 to 24ºC	≥ 28ºC	Sivaprasad and Sundrasarma (1987)
Leaf growth	10 to 25ºC	≥ 27ºC	Khanna-Chopra and Sinha (1987)
Early growth	20 to 26ºC	≥ 27ºC	Van derMaesen (1972)
Flowering			
In vitro Pollen germination	25ºC	35ºC	Jaiwal and Mehta (1983)
In vitro Pollen tube growth	25ºC	45ºC	Jaiwal and Mehta (1983)
Flowering and pod development	20 to 26ºC	≥ 30ºC	Summerfield *et al*. (1980)

2.5.3. Effect of heat stress on physiology

Photosynthetic rate and chlorophyll content are important physiological parameters in plants. Heat stress directly affects photosynthesis including photosystem II (PS II) in chickpea (Srinivasan *et al*. 1996). The rate of photosynthesis has a negative linear relationship with temperature (Grace 1988). Peak photosynthetic rate was observed at sub-optimal temperatures (22ºC) in chickpea under controlled environments (Singh *et al*. 1982). At Hissar in north India, the net photosynthetic rate at 25˚C was linearly related to photon flux density and reduced at ≥ 28˚C (Singh *et al*. 1987). Singh *et al*. (1987) also reported that transpiration efficiency (photosynthesis/ transpiration) of chickpea decreases with increasing temperature. Photosynthetic rates are higher during 50% flowering to pod formation than the vegetative stage in chickpea. Photosynthetic duration is controlled by the requirement of assimilates in the growing organs (e.g. leaves) and the reproductive organs (e.g. pods) (Singh *et al*. 1987) and also by the environment.

Membrane stability of leaf tissue can be used as another physiological indicator of heat stress, and is determined by electrolyte leakage measured as electrical conductivity (Stoddard *et al*. 2006). Genotypic variation for heat tolerance in chickpea was evaluated during vegetative, flowering and pod filling stages by testing cell membrane thermostability (using electrolyte leakage). The chickpea lines Annigeri, ILC 482 and ICCV 10 were more thermostable at 45ºC than K 850 and injury decreased with crop development (Srinivasan *et al*. 1996). However, there is no evidence of a relationship between cellular integrity under heat stress and grain yield. Tongden *et al*. (2006) used cell membrane stability as a screening technique at the seedling stage of chickpea to identify heat tolerant and sensitive cultivars.

The plant is unlikely to show any significant yield difference due to short duration (acute) heat stress and distinguishing genotypes may be difficult. Under such situations, testing membrane stability and photosynthesis may be more suitable screening techniques if they can be linked with injury to tissue or physiological process.

The rate of assimilate partitioning and leaf senescence are important physiological responses that influence pod set and yield. Carbohydrates (accumulated at the time of photosynthesis) supplied to the reproductive organs (e.g. flowers and pods) directly influence grain filling (Hendrix 2001). The rapid growth and development of reproductive organs arises through partitioning of a large proportion of the net accumulated biomass from leaves under heat stress (Evans 1993).). However, there are limited physiological studies on chickpea heat tolerance and the interaction between germplasm and environments.

In general, the success of heat screening physiological techniques depends on the frequency of heat stress in the field and the relevance of managed screening techniques to the target environment (Wery *et al*. 1994). Limited screening techniques have been developed for heat tolerance in chickpea (Singh *et al*. 1994) because significant G × E interaction and differences in phenology make screening difficult (Wery *et al*. 1994). Hence, the improvement of heat tolerance in chickpea is dependent upon access to reliable and accurate phenotyping procedures.

2.6. Effect of heat stress on nitrogen fixation

High temperatures affect nitrogen fixation and symbiosis in chickpea (Rodrigues *et al*. 2006). Generally, high temperature reduces nodule formation, impairs nodule function and affects nodule structure (Roughley and Dart 1970; Kurdali 1996). Detrimental effect on nodule formation and nitrogen fixation efficiency of chickpea was observed in continuous warm days of 30/18ºC day/ night temperatures (Minchin *et al*. 1980). Slightly increased day temperature (32.5ºC) delayed nodulation, decreased total plant nitrogen fixation and longevity of the symbiotically active nodule population (Rawsthorne *et al*. 1985). Nodules were not formed at > 32ºC soil temperature and recovery of nitrogenase activity failed after plant roots were exposed to 35ºC. The optimum soil temperatures for chickpea growth lie between 18 and 22ºC for nodulation and nitrogen fixation (Dart *et al*. 1975). When the chickpea cultivar ILC 482 was inoculated with *Rhizobium leguminosarum L*., strain CP 37A, the initial growth rate was encouraged at 40/25ºC under controlled conditions (Laurie and Stewart 1993). However, the effect of heat stress during nitrogen fixation could vary in different genotypes (Summerfield *et al*. 1981). Thus, further investigation of heat stress and rhizobium culture in chickpea is needed.

Most nitrogen fixation in chickpea occurs during the vegetative phase (biomass accumulation) and declines after pod filling. Most of the spring-sown chickpea is exposed to warm temperature during flowering. But in south Asia (north, central and south India), the vegetative phase is subjected to high maximum temperature of 31 to 33˚C (Berger *et al*. 2011). From the available data, it is clear that temperature > 30˚C has detrimental effect on nitrogen fixation. Therefore, particular consideration is needed in these regions.

The heritability of nitrogen fixation traits, under heat stress may be important to obtaining higher, more sustainable yields in hot environments. However, most research of this nature has focussed on water stress and very little has been published on heat stress. There is a need for greater knowledge of plant physiological response to nitrogen fixation by different rhizobial strains under heat stress.

2.7. Adaptation mechanism

Chickpea performance over different environments under high temperature has been covered in the previous sections. The adaptive strategies to high temperature stress are classified into the following three groups (Wery *et al*. 1993):

2.7.1. Adaptation mechanisms of crop plants to high temperatures

i. *Heat escape*: Plants can escape heat stress with early phenology. Though flower initiation is sensitive to rising temperature in chickpea (Toker and Canci 2006), early flowering and maturity is a heat escape mechanism (Toker *et al*. 2007) particularly in the Mediterranean spring-sown environments and south Indian germplasm (Berger *et al*. 2011).

ii. *Heat avoidance*: Leaf reflectance, reduction of non-photosynthetic energy intercepted by the canopy and transpiration are important physiological components of heat avoidance. Leaves play a vital role in heat avoidance by changing their orientation, transpiration rate and reflectance (Wery *et al*. 1993). The mechanism of heat avoidance has not been studied in chickpea and screening germplasm for heat avoidance may lead to improve productivity in heat stressed environments.

iii. *Heat tolerance*: Heat tolerance is linked to membrane stability, alteration of membrane lipid composition, accumulation of heat shock proteins and specific solutes (proline and glycine) particularly in pollen (Blum 1988). The role of protein functional properties (e.g. heat shock proteins) has not been studied in chickpea and their assessment may assist plant breeders in the development of heat tolerant cultivars.

2.8. Strategies to improve breeding for heat tolerance in chickpea

Visual selection, selection for physiological traits linked to plant response to high temperature, empirical selection for yield and marker assisted selection (MAS) are four important selection methods used to improve heat tolerance through breeding (Howarth 2005). However, the first step in the breeding process is identification of genetic diversity for economically important traits. Genetic diversity can be measured by quantifying variation in morphological characters that are targeted for selection for adaptation to heat stress. This approach has been used in south India (Krishnamurthy *et al*. 2011; Upadhaya *et al*. 2011). Genetic diversity is also assessed using an eco-geographic approach to select chickpea germplasm for crossing (Berger 2007). In addition, new DNA based fingerprinting technologies can be used to quantify the extent of diversity among potential parental lines (Lin *et al*. 2008).

The next step in the breeding process is selection of superior heat tolerant germplasm from the progeny of each cross. A suitable screening environment is essential. Some breeders use late planting to induce high levels of heat stress from anthesis through the grain filling period (Krishnamurthy *et al*. 2011). Others use more sophisticated techniques such as field based heat chambers or controlled environment chambers (Cottee *et al*. 2010). The primary consideration when choosing a screening method is relevance to the target environment. If quantitative trait loci (QTL) linked to superior heat tolerance has been identified then molecular markers associated with these QTL can be used at any time during the selection process to conserve these chromosomal regions in the progeny. While molecular markers for heat tolerance have been identified in rice (Ying-hui *et al*. 2011) and wheat (Al-Doss *et al*. 2010), there are currently no effective markers available in chickpea. Nevertheless, contrasting parents of chickpea for heat tolerance were crossed and used to develop recombinant inbred lines (Krishnamurthy *et al*. 2011) which will later be assessed for heat tolerance and QTL mapping.

2.9. Conclusions and presumption for the future

Although classification of heat responses of chickpea has been documented (Krishnamurthy *et al*. 2011; Upadhaya *et al*. 2011), there has been little attempt to extrapolate these findings across the world's chickpea production areas. The determination of a heat response phenotype through screening is vital if the genetic control of heat tolerance in chickpea is to be understood and significant progress made through plant breeding. Clearly, the research under high temperature stress shows that early phenology is the most important mechanism and pod set the primary yield component to be considered in heat tolerance breeding. Overall, the heat stress can be studied using a holistic approach that integrates genetic and physiological characterisation of plant response to help define plant breeding targets. These combined approaches which include molecular tools and agronomic practices, will be pivotal to developing improved heat tolerant chickpea cultivars. However research gaps include:

- Development of simple screening methods to identify heat tolerance in chickpea genotypes relevant to the target environment
- Determination of the physiological response of chickpea to heat stress across a range of concurrent factors such as moisture availability and evaporative demand and the underlying genetic control of these traits
- Classification of genetic material to determine diversity groupings and establishment of genetic correlations between the traits linked to heat stress response
- Identification of molecular markers linked to major QTLs that explain a significant portion of the variation in heat tolerance

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Chapter 3: Genetic diversity in chickpea germplasm and characterisation of screening environments

3.1. Introduction

Progress in chickpea improvement depends on the knowledge of germplasm diversity and identification of genotypes better adapted to their environment. Investigation of genetic diversity and characterisation of screening environments have a significant impact on crop improvement. Access to diversity for economically important traits is the first step in plant breeding and the expression of these traits in the target environment determines the emphasis given by plant breeders. Morphological traits and molecular markers can be used to study the genetic diversity and genetics and the relationship between and among the germplasm. However, molecular markers offer the advantage of interpreting genetic diversity free from confounding environmental effects. In chickpea, genetic diversity has been studied using microsatellite-anchored fragment length polymorphism (MFLP) (Lin *et al*. 2008); randomly amplified polymorphic DNA (RAPDs) (Singh *et al*. 2003) and simple sequence repeats (SSRs) (Udupa *et al*. 1999; Upadhyaya *et al*. 2008). Diversity array technology (DArT) markers have also been used in several crops. This technique is based on isolating a random set of cloned DNA fragments from a pooled DNA sample. This technique provides extensive genome coverage and DArT markers have been used to assess genetic diversity and construct of genetic map for cereals (wheat – Akbari *et al*. 2006) and legumes (pigeonpea – Yang *et al*. 2011). Genetic diversity was also studied using DArT markers in oats (Tinker *et al*. 2009). In chickpea, DArT markers have also been used to construct a genetic consensus map together with SSRs (Thudi *et al*. 2011).

Characterisation of screening environments is vital if genotype response it to be understood. The characterisation of screening environments can be based on the whole cropping system (climate, soil, cropping practices and crop rotation), in a particular geographic region targeted by a breeding programme, or may represent carefully managed environments where environmental conditions are controlled. An example of the later is screening materials under a rain shelter in the field to reduce the confounding effects of variable rainfall. The characterisation of screening environments has been conducted using seasonal data to establish patterns (Chapmen *et al*. 2000a), physiological traits (Krishnamurthy *et al*. 2011) and the calculation of stress indices using models (Chapman *et al*. 200b). Traditionally, breeders mostly depend on local knowledge, historical multi-environment trial data, soil type, rainfall, and latitude to characterise the environment.

In India, the chickpea production area is increasing in the south and tending to decrease in the north although there is an increase in chickpea production in the northesteast of India. In southern India the crop duration is short (100 days) and the cropping season temperature is higher compared to northern India. Decreases in chickpea yield of 53 kg/ha was observed in India per 1ºC increase in seasonal temperature (Kalra *et al*. 2008). In the last 50 years global temperature has increased by 0.13ºC per decade and is expected to increase another 1.1 by the end of this century (Craufurd and Wheeler *et al*. 2009). The temperature increase will impact on future global chickpea production including India. Therefore, there is a need to screen and identify germplasm with high temperature tolerance for future crop improvement. This study examines the genetic diversity of chickpea genotypes using DArT markers and the expression of quantitative traits under heat stressed and non-stressed conditions. The genotype response is then explained in terms of the screening environment at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India.

3.2. Materials and methods

Germplasm, DNA isolation and DArT markers

A set of 191 chickpea genotypes was selected from the ICRISAT gene bank to study genetic diversity using DArT markers. The germplasm consisted of 167 genotypes selected for heat tolerance breeding, 8 genotypes selected for drought tolerance, 11 genotypes from disease screening and 5 Australian cultivars. The origin of the genotypes, name of the variety, year and country of release are summarised in Appendix 1. Young leaf tissue of each genotype was collected from greenhouse grown plants and stored in a 96 well plate. A high-throughput DNA isolation method described by Mace *et al*. (2003) was followed to isolate DNA from leaf tissue in the 96 well plate. A chickpea DArT array was developed by Diversity Arrays Technology Pty Ltd and DArTsoft software used to score each marker. Markers were scored 1 for presence, 0 for absence of each clone. A total of 359 markers were polymorphic on 176 genotypes. The marker name and polymorphism details are shown in Appendix 11. Fifteen genotypes could not be scored reliably and were removed to avoid reducing data quality. This chapter focuses on the subset of 167 genotypes which were selected for heat tolerance studies. Of the 167 genotypes a total of 153 genotypes were polymorphic.

Screening environments

The ICRISAT, Patancheru research station (17.53°N; 78.27°E; 545 m) in India was the site used to conduct the heat tolerance experiments. The average daily maximum and minimum temperature over 29 years (1982 – 2011) were collected from the meteorology department at ICRISAT to predict the pattern of maximum and minimum temperatures during the chickpea growing season. Three years of crop history for the experimental site at Patancheru, ICRISAT was also collected (Table 5).

Field experiments

Field experiments were conducted at ICRISAT for two seasons (normal – non-stressed and late – heat stressed) in two years (2009-10 and 2010-11). Eleven quantitative traits (days to first flower, days to 50% flower, days to first pod, days to physiological maturity, plant height, plant width, plant biomass, pod number per plant, filled pod number per plant, seed number per plant and grain yield) were studied. The details of field experiments are explained in the chapter 4.

3.3. Statistical analysis

The DArT marker scores were subjected to cluster analysis using Genstat 12th Ed. VSN International Ltd to visualise the genetic relationships among genotypes. ANOVA was performed for eleven quantitative traits under heat stressed and non-stressed environments for two years using Genstat 12th Ed. VSN International Ltd. Means and standard errors of eleven quantitative traits are presented in Table 4.

3.4. Results

Genetic diversity among chickpea genotypes revealed using DArT

The polymorphic information content (PIC) values were relatively low. Only 11.4% of DArT markers had PIC values of 0.4 – 0.5, whereas 63.2% of markers showed PIC values ≤0.1 (Table 3.1). The average PIC value was 0.15. Out of 359 polymorphic markers in the germplasm analysed, there are 41 markers in the PIC value range of 0.4-0.5.

Genetic relationship and diversity among genotypes

A dendrogram derived from cluster analysis based on the genetic similarity coefficient matrix for the 153 polymorphic genotypes was constructed (Fig 3.1). The genetic similarity coefficient for all genotypes ranged from 0.6 to 1.0. There were nine groups or clusters (group 1-9) identified from the dendrogram. The genotypes were collected from 36 countries and included with ICRISAT breeding lines; these are presented in Appendix 1. The breeding lines developed at ICRISAT clustered groups 1, 2, 3, 4 and 9. Of the 153 genotypes tested, 80% were located in groups 1, 2 and 3. Thirty four countries were listed in groups 1, 2 and 3 with the exception of Hungary and Uganda (Table 3.2). Group 5 is not discussed because those genotypes were not selected for heat tolerance. The mean performance of all eight groups (1, 2, 3, 4, 6, 7, 8 and 9) was calculated for eleven traits means and the standard errors presented (Table 3.3).

Table 3.1 Polymorphism information content (PIC) values for 359 polymorphic DArT markers

Range of PIC values	No of markers	% of markers
0-0.1	227	63.2
0.1-0.2	54	15
0.2-0.3	20	5.6
0.3-0.4	17	4.7
0.4-0.5	41	11.4

Distribution patterns of genotypes into groups based on the 11 traits studied indicated the presence of considerable genetic diversity. Under normal season condition, group 9 had the highest mean grain yield followed by groups 4 and 1. In late season, the groups 9, 4 and 3 had higher grain yield respectively (Table 3.3). Group 9 also had the highest mean grain yield in both years (808 and 2476 kg/ha for late season sowing). Seven ICRISAT breeding lines were clustered in the high-yielding group 9. Of these, ICCV 94954 (JG 130); ICCL 87207 (Vishal) and ICCX 820065 (GG2) are released as varieties in India. ICCV 97105 (Ukiringuru) was released in Tanzania. The genotype ICCX 820065 is a desi variety of medium duration, bold seeded with medium heat tolerance.

The genotypes in group 4 ranked second in grain yield under heat stress (671 and 2085 kg/ha for late and normal sowing) (Table 3.3). Only seven genotypes clustered in group 4 including ICCV 07113, ICCV 118 and genotypes collected from Malawi, Sudan, Tanzania and Myanmar. Twelve ICRISAT breeding lines clustered with 10 Indian genotypes in group 3. The remaining genotypes in this group originated from Iran, Iraq, Israel, Jordan, Lebanon, Syria, Peru, Malawi, Morocco, Spain, Tunisia and Yugoslavia. This group produced mean grain yield of 559 and 2253 kg/ha in both years under heat stress (Table 3.3). The genotype ICCV 92944 (JG 14 in India and Yezin 6 in Myanmar) is a well-known short duration, heat tolerant desi variety found in group 3. One of the parents of ICCV 92944 (ICCL 83149) was released as Barichhola 6 in Bangladesh during 1996. However, ICCL 83149 was clustered into group 2 in this study.

Fig 3.1 Dendogram for 176 genotypes of chickpea using similarity coefficient based on 359 DArT markers

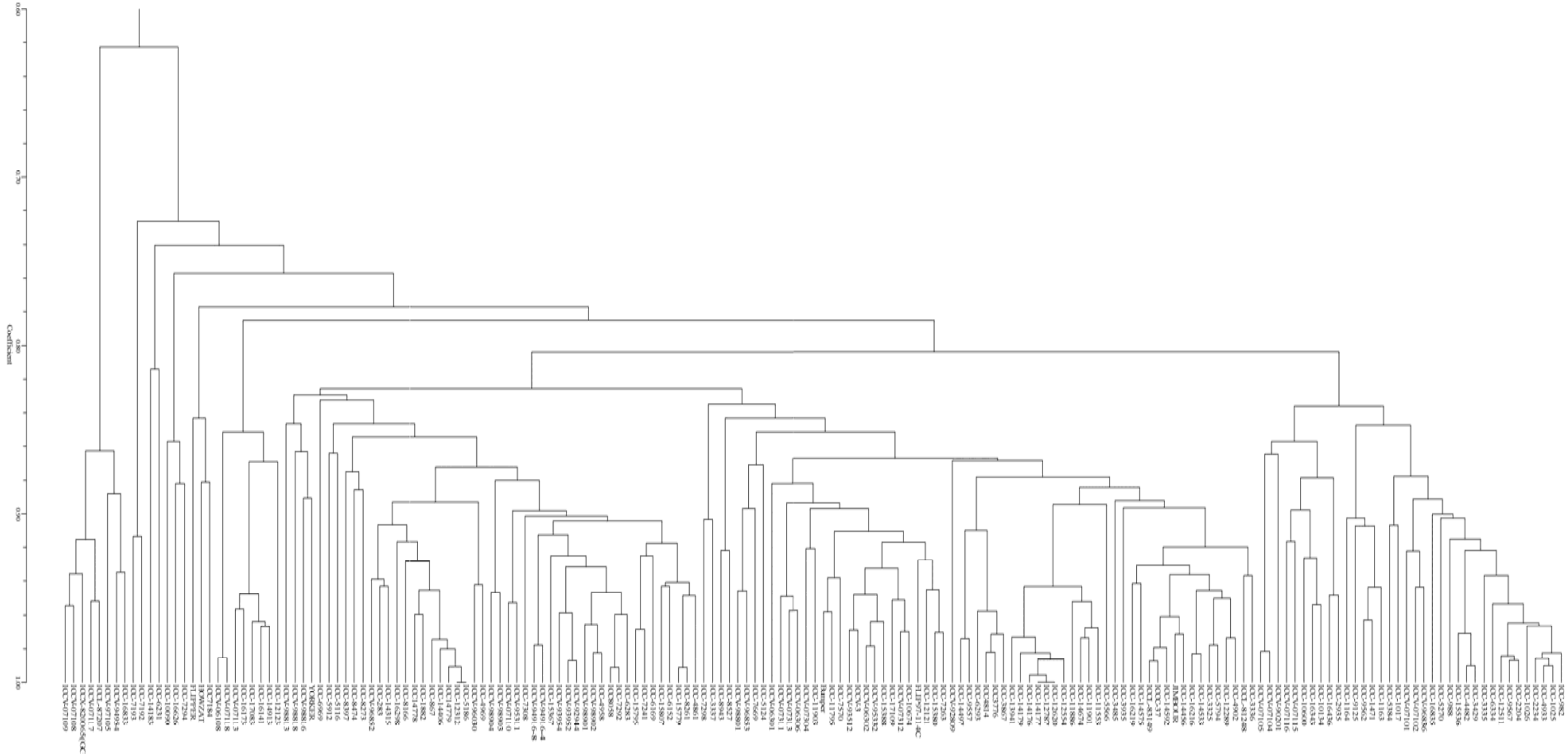


Table 3.2 Number of genotypes/ total number of genotypes in each group listed based on their country of origin

Country	Group 1	Group 2	Group 3	Group 4	Group 6	Group 7	Group 8	Group 9
Algeria	2/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Bangladesh	0/36	4/57	0/39	0/7	0/2	0/2	0/2	0/8
Breeding line developed at ICRISAT	8/36	14/57	12/39	2/7	0/2	0/2	0/2	7/8
Bulgaria	0/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Chile	0/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Cyprus	1/36	2/57	0/39	0/7	0/2	0/2	0/2	0/8
Egypt	3/36	2/57	0/39	0/7	0/2	0/2	0/2	0/8
Ethiopia	0/36	5/57	0/39	0/7	0/2	0/2	0/2	0/8
France	1/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Germany	0/36	3/57	0/39	0/7	0/2	1/2	0/2	0/8
Greece	0/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Hungary	0/36	0/57	0/39	0/7	0/2	0/2	2/2	0/8
India	5/36	2/57	10/39	0/7	0/2	0/2	0/2	0/8
Iran	2/36	2/57	2/39	0/7	0/2	0/2	0/2	0/8
Iraq	2/36	0/57	1/39	0/7	0/2	0/2	0/2	0/8
Israel	1/36	1/57	1/39	0/7	0/2	0/2	0/2	0/8
Italy	0/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Jordan	0/36	1/57	1/39	0/7	0/2	0/2	0/2	0/8
Lebanon	0/36	0/57	2/39	0/7	0/2	0/2	0/2	0/8
Malawi	0/36	1/57	1/39	1/7	0/2	0/2	0/2	0/8
Mexico	1/36	3/57	0/39	0/7	0/2	0/2	0/2	0/8
Morocco	1/36	2/57	1/39	0/7	0/2	0/2	0/2	0/8
Myanmar	0/36	2/57	0/39	2/7	0/2	0/2	0/2	0/8
Nepal	0/36	1/57	0/39	0/7	0/2	0/2	0/2	0/8
Nigeria	2/36	0/57	0/39	0/7	0/2	0/2	0/2	0/8
Pakistan	2/36	0/57	0/39	0/7	1/2	0/2	0/2	0/8
Peru	0/36	0/57	2/39	0/7	0/2	0/2	0/2	0/8
Spain	0/36	0/57	1/39	0/7	0/2	1/2	0/2	0/8
Sri Lanka	3/36	0/57	0/39	0/7	0/2	0/2	0/2	0/8
Sudan	0/36	2/57	0/39	1/7	0/2	0/2	0/2	0/8
Syria	0/36	0/57	3/39	0/7	0/2	0/2	0/2	0/8
Tanzania	0/36	0/57	0/39	1/7	0/2	0/2	0/2	0/8
Tunisia	0/36	1/57	1/39	0/7	1/2	0/2	0/2	0/8
Turkey	1/36	2/57	0/39	0/7	0/2	0/2	0/2	0/8
Uganda	0/36	0/57	0/39	0/7	0/2	0/2	0/2	1/8
USA	1/36	0/57	0/39	0/7	0/2	0/2	0/2	0/8
Yugoslavia (Former)	0/36	1/57	1/39	0/7	0/2	0/2	0/2	0/8

Table 3.3 Mean and standard error of chickpea genotypes for 11 traits under heat stressed (late) and non-stressed conditions (normal)

Group no	Group 1				Group 2			
	Normal		Late		Normal		Late	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
DFF	57±2.2	56±1.4	55±1.6	52±3.8	49±1.8	46±3.2	46±2.3	43±2.6
D50%F	60±2.3	61±1.3	59±1.8	55±7.8	52±1.8	53±3.4	50±2.3	48±4.8
DFP	63±2.3	64±1.8	61±1.8	59±3.4	56±1.9	55±3.5	52±2.3	50±2.7
DPM	115±3.8	108±1.7	94±2.3	103±3.6	110±5.4	107±2.2	90±2.4	102±3.1
PH	41.6±3.1	50.2±2.5	29.3±2.7	42.6±2.7	45.7±3.5	51.2±4.3	30.8±2.8	42.2±2.7
PW	36.7±5.8	33.5±3.9	22.4±3.3	44.1±4.4	41.6±5.2	37.1±5.9	23.7±4.3	40.7±4.2
Biomass	23.6±5.2	27.7±5.4	8.6±2.5	25.2±4.9	23±4.6	27.3±5.8	9.7±3.1	27.1±7.8
TNP	62±16	80±14.5	11±5.2	66±15.3	55±12.9	66±14.1	20±8.7	62±17.2
NFP	58±15.9	77±14.3	10±4.9	63±14.9	51±12.8	63±13.8	18±8.4	59±16.4
NS	72±22.8	99±19.7	12±8.6	83±21.3	66±20.1	78±19.7	23±12.1	79±23.8
GY	1782±472	2330±410	233±121	1882±503	1790±390	2073±524	475±247	1954±556

Group no	Group 3				Group 4			
	Normal		Late		Normal		Late	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
DFF	46±2.9	45±1.3	44±2.9	42±2.7	45±1	41±1.2	41±1.3	39±1.6
D50%F	51±2.7	51±1.9	48±2.7	47±2.3	49±1	47±1.3	46±1.1	43±2.1
DFP	53±2.9	54±1.9	51±2.8	49±2.8	52±1.3	51±2.1	47±1.5	45±1.7
DPM	110±4.9	108±1.8	90±2.2	100±3	109±5	106±3.1	88±2.7	101±3.9
PH	43.7±4	50±3.8	29.8±2.6	40.5±2.3	41.8±3.6	48.1±2.8	31.5±1.8	38.7±2.2
PW	39.3±6.1	38.1±5.1	22.5±3.7	39.7±5.6	36.6±2.1	40.5±5	23.8±3.1	37.5±4.1
Biomass	23.9±4.9	28.2±5	10.4±2.9	28.4±5.4	20.3±2.2	26.9±4.1	11.4±3.4	23.4±7.6
TNP	51±12.7	64±17.4	20±6.1	65±11.2	54±11.1	80±13.1	26±7	67±17.6
NFP	45±11.1	59±16.4	18±5.2	59±11.2	48±10.2	77±12	23±6.7	64±18.6
NS	51±12.2	66±17.8	22±7.4	72±13.2	60±14.6	91±16.8	30±7.1	79±24.3
GY	1758±442	2194±441	559±204	2253±426	1596±367	2310±280	671±261	2085±652

Group no	Group 6				Group 7			
	Normal		Late		Normal		Late	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
DFF	61±3.2	62±0.4	62±1.4	55±5.7	56±1.8	56±1.4	54±2.4	49±7.4
D50%F	64±3.2	68±0.7	69±2.1	63±2.1	59±2.1	61±1.3	58±2.1	54±10
DFP	68±4.2	69±0.4	0	62±3.2	62±0.4	64±1.8	62±0.7	56±8.8
DPM	120±0.4	113±0.4	107±3.5	110±1.4	119±4.2	108±1.7	98±1.1	105±5
PH	50±4.5	59±2.6	34.3±4	47.3±1	53.2±1.9	50.2±2.5	36.3±1	46.9±5.9
PW	39±2.8	34.8±1	28.2±2.4	53.8±4	40.4±1.6	33.5±3.9	30.2±3.2	41.9±9.4
Biomass	30.3±3.2	20.9±0.7	9.5±0.7	25.9±4.5	30.1±3.7	27.7±5.4	11.1±2.1	33±4.8
TNP	39±12.7	34±11.9	0	33±11.4	36±6.4	80±14.5	5±2.5	53±15.2
NFP	36±12.4	32±12	0	29±10.1	30±5.3	77±14.2	4±2.8	49±16
NS	43±17.3	27±3.1	0	39±10.8	34±1.8	99±19.7	7±0	55±17.1
GY	1570±640	1124±260	0	1365±300	1259±92	2330±410	133±118	1940±485

Table 3.3 Continued

Group no	Group 8				Group 9			
	Normal		Late		Normal		Late	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
DFF	70±4.6	67±1.4	66±2.8	63±2.8	44±2.4	41±1.8	41±1.6	39±5.6
D50%F	72±4.6	73±1.8	72±0.7	0	48±1.4	47±1.7	45±1.8	44±6.2
DFP	75±3.8	74±1.8	0	69±2.8	51±2.9	50±2.2	47±1.6	47±5.8
DPM	125±3.5	116±0.7	103±2.5	112±2.5	104±4.4	105±1.6	87±2.5	98±4.1
PH	51.1±4.1	69.4±3.7	30.7±2.7	53.3±4.2	40.2±3.4	46±4.3	30.4±2.4	38.1±1.1
PW	28.9±4.9	36.6±3.9	22.9±0.4	46.4±9.6	38.8±5	35.3±6.8	22±3.2	32.9±5.5
Biomass	17.4±3.6	25±0.4	8.9±1.3	10.8±7.2	23.3±3.1	31.4±7.3	12.3±2.9	25.4±8.8
TNP	13±3.8	29±1.6	0	9±3.9	49±8.4	71±11.9	23±7	56±15.3
NFP	12±3.2	28±1.9	0	9±3.9	44±9.7	65±12.7	21±6.1	53±14.5
NS	16±2.5	34±1.8	0	10±6.4	57±6.3	77±18.7	27±9.3	69±17.2
GY	519±187	1148±7.8	0	273±195	2134±246	2664±552	808±349	2476±803

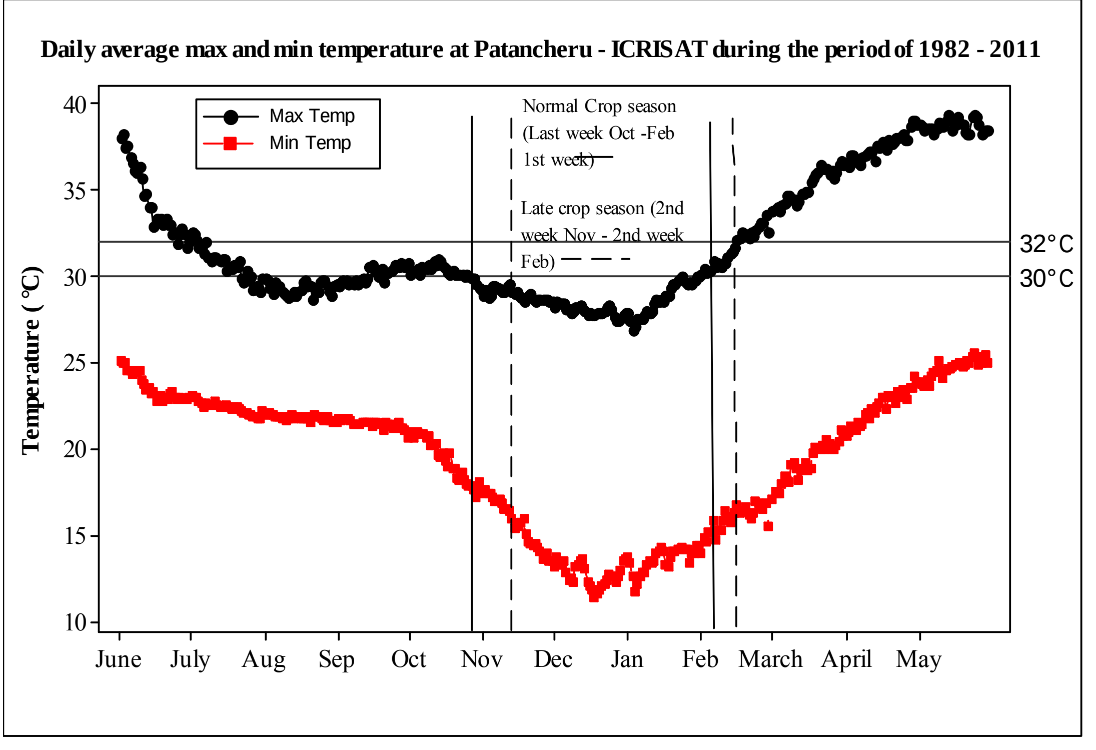
(DFF – days to first flower; D50F – days to 50% flowering; DFP – days to first pod; DPM – days to physiological maturity; PH – plant height (cm); PW – plant width (cm); TNP – total number of pods/plant; NFP – number of filled pods per plant; NS – number of seeds/plant; GY – grain yield kg/ha)

Table 3.4 Three years of crop history in the experimental site at Patancheru – ICRISAT

Field no.	Year	Season	Crop	Soil type	Fertilizer used	Herbicide used
BP 08B	2009	Post rainy	Chickpea	Black	DAP @ 100 kg/ha	Nil
(Year 1)	2009	Rainy	Fallow			
	2008	Post rainy	Fallow			
	2008	Rainy	Sorghum			
	2007	-	Fallow			
	2006	Post rainy	Fallow			
	2006	Rainy	Maize			
BP 11B	2010	Post rainy	Chickpea	Black	DAP @ 100 kg/ha	Nil
(Year 2)	2010	Rainy	Fallow			
	2009	Post rainy	Fallow			
	2009	Rainy	Sorghum			
	2008	Post rainy	Chickpea			
	2008	Rainy	Fallow			
	2007	Post rainy	Fallow			
	2007	Rainy	Sorghum			

The genotypes in groups 1, 2 and 7 had moderate heat tolerance. Mean group 2 yield was 41% and 21% lower than the highest yielding group 9 genotypes under heat stressed condition for year 1 and 2, respectively. Similarly, group1 and 7 mean grain yields were 71% and 83% lower for year 1 and 24% and 22% lower for year 2 respectively, compared with group 9 under heat stress. Groups 6 and 8 consisted mostly of heat sensitive genotypes. Grain yield and pod traits (pod number per plant; filled pod number per plant and seed number per plant) were lower in these groups under heat stress. During year 1, genotypes in group 6 showed zero DFP values indicating that the plants did not set pods under heat stress. However, in the milder year 2 the genotypes produced 33 pods/ plant under heat stress. Similarly, plants did not produce any pods in group 8 in year 1 and in year 2, most plants did not attain 50% flowering under heat stress. The mean yield under heat stress of this cluster was lower than all other groups, therefore group 8 was considered to have the most heat sensitive genotypes.

Fig 3.2 Daily average maximum and minimum temperatures at Patancheru – ICRISAT during the period of 1982 - 2011



Characterisation of screening environments

Experimental sites were rotated with sorghum and maize (Table 3.4). The site in year 1 was comparatively lower yielding than in year 2 based on the grain yield under non-stressed conditions, even though both sites received equal amounts of fertiliser. Generally, chickpea grows with available soil moisture during post-monsoon i.e. normal season. The soil depth of the field at ICRISAT was ≥ 1.2 m and known to retain about 230 mm of plant available water (Krishnamurthy *et al*. 2011). At ICRISAT, the normal season is sown during the last week of October with a post-sowing irrigation applied to facilitate uniform germination. If the rainy period is extended, the sowing will take place during the second week of November. Long term temperature data were analysed to identify the distribution of high temperatures during the normal chickpea season. The sowing temperature in the normal season (October sowing) is usually 30-31˚C (Fig 3.2). Similarly, the normal season crop is exposed to 30-32˚C during late grain filling and at maturity. If sowing is delayed two weeks the late grain filling period is exposed to 32-33˚C. Previous experience at ICRISAT suggested that exposing late season chickpea to >35˚C will facilitate the identification of heat tolerant germplasm for breeding programs. The long term data showed that sowing chickpea during early February exposes the plants to >35˚C during reproductive development (Fig 3.2). Therefore, late sown experiments in the current study were sown in February to expose genotypes to maximum heat stress. The average maximum day temperature varied between 35 and 39˚C and night temperature varied between 23 and 25˚C during the flowering and grain filling periods. This high temperature induced differences between the genotypes based on changes in phenology, pod characteristics and grain yield.

3.5. Discussion

Chickpea production relies on stored soil moisture and increasing temperature (up to 32˚C) during the grain filling period. Due to climate change the grain filling temperature is expected to increase thus reducing grain yield. Studies on the impact of climate change on chickpea grain yield highlighted the importance of changes in crop development at warmer temperatures (Ozdemir and Karadavut 2003; Kalra *et al*. 2008). For example, the grain yield of chickpea declined by 53-301 kg/ha per 1˚C rise in mean seasonal temperature in India (Kalra *et al*. 2008). In Turkey, the spring sown crop produced mean grain yield of 1600 kg/ha compared to 3227 kg/ha when sown in the autumn. In the spring, the crop was exposed to temperatures ranging from 26 to 38˚C during the reproductive period (Ozdemir and Karadavut 2003). This shows the importance of seasonal temperature fluctuation on the grain yield in chickpea.

In current study, changes in temperature at different phenological stages affect grain yield. Generally, the timing of flowering and grain maturity is accelerated under heat stress, thus shortening the duration of crop development and reducing grain yield (Table 3.3). High temperature stress also reduced plant biomass. The reduction in grain yield was related to the impact of heat stress on pod characteristics such as pod number and filled pod number per plant. These findings agree with Krishnamurthy *et al*. (2011). The brief episodes of high temperature stress (32-35˚C) in chickpea reduce pod set and hence grain yield in the controlled environments (Basu *et al*. 2009;

Devasirvatham *et al*. 2010). However, for the purpose of crop improvement large numbers of genotypes must be screened and the critical temperature for pod set determined. In reality, screening should be conducted in the field on relevant soil types where larger numbers can be accommodated. Using historical weather data, the chickpea growing environment and temperature-stress patterns occurring at ICRISAT were identified. Since the environment contributed more to grain yield than genotype, this approach is useful for identifying and managing large G × E effects. The characterisation of environment using similar techniques was attempted in world wetland rice production (Garrity 1984), Indian chickpea cropping systems (Berger *et al*. 2006) and wheat cropping areas of north-eastern Australia (Chenu *et al*. 2011). Garritty (1984) used this method to identify similar environments to enhance exchange of genetic material by including information on each environment. Chickpea germplasm from southern and central India were characterised by early phenology and conferring with drought escape and genotypes in north India have a prolonged vegetative period to escape low temperature (Berger *et al*. 2006). Chenu *et al*. (2011) identified that the drought stress pattern was more common in wheat production areas of north-eastern Australia. The present study showed the temperature-stress pattern occurring at ICRISAT which is useful for future chickpea breeding programs.

Recent advances in molecular biology have reduced the cost and increased the effectiveness of genotyping. However, cultivated chickpea has a relatively low level of polymorphism compared with other crops. Microsatellite markers (e.g. Simple Sequence Repeats-SSR) have been used to investigate genetic diversity in breeding studies (Gupta and Varshney, 2004). Chickpea genetic diversity was studied using SSR markers to understand the genetic structure and allelic richness among the 2915 germplasm and suggested that these accessions are ideal set for the development of cultivars in applied breeding under diverse environments (Upadhyaya *et al*. 2008).

Later a reference set (~ 300) selected from the 2915 accessions was screened for heat tolerance in the field under multi-environmental trials in India to study genetic variation with respect to seven quantitative traits (days to 50% flowering, days to physiological maturity, shoot biomass, pod number per plant, seed number per plant, 100 seed weight and grain yield) (Krishnamurthy *et al*. 2011). The days to 50% flowering were delayed and maturity was accelerated under heat stress and indicated that the vegetative period was longer than grain filling period. These genotypes represent ideal materials for further characterisation of underlying mechanisms of tolerance involved (Krishnamurthy *et al*. 2011). In the current study, the DArT markers confirmed the similar results of Upadhyaya *et al*. (2008) and demonstrated significant genetic variation among 167 chickpea lines based on eleven quantitative traits under heat stressed and non-stressed environments. Furthermore, both phenology and maturity was accelerated under high temperature.

The DArT markers used in the current study were effective in grouping genotypes based on their genetic diversity. The genotypes across groups were then classified by country of origin. The breeding lines developed at ICRISAT represented relatively diversity as they were distributed five groups out of seven. Groups 2 and 3 have greater similarity in their countries of origin indicating the probable existence of two different environment types in these regions. In general, the absence of a clear relationship between geographic origin and the grouping pattern of the different genotypes suggests that chickpea spreads from its centre of origin in the Near East over different historical periods (Abbo *et al*. 2003). However, the Hungarian genotypes (group 8) are isolated from the rest with medium grain yielding under normal season and greater sensitivity to heat. This distinctness of Hungarian materials was also reported by Iruela *et al*. (2002) using RAPD and ISSR markers. It is likely that chickpea introduction into Hungary initially involved a very narrow germplasm and limited subsequent germplasm exchange and a benign production environment has resulted in a unique gene pool.

Furthermore, when the diversity groups based on DArT were summarised using eleven quantitative traits under heat stressed and non-stressed conditions, there were significant differences among the individual groups. The highest yielding group 9 is based on new ICRISAT germplasm indicating the importance of modern plant breeding and empirical selection in elevating grain yield in both non-stressed and heat stressed conditions. These materials were screened in mulit-environment trials and it is likely that the selected materials represent broad adaptation. This is evidenced by the release of some of these crossbreeds in different countries at different times. Some of these genotypes are crosses between desi and kabuli types and have consistently produced high yielding progenies (Yadav *et al*. 2004). The short duration chickpea varieties ICCV 2, ICCC 37 (Kranthi) and ICCV 93954 (JG 11) were developed by ICRISAT's breeding programmes (Gaur *et al*. 2007) and have been extensively as parents for the introgression of genetic diversity from landraces (Narsingpur bold and

Dahod local). Apart from the short duration varieties, a drought tolerant genotype ICC 4958 was developed by ICRISAT breeding program (Saxena *et al*. 1993) and is another important parent used to develop four of the tested breeding lines (ICCVs 98901; 98902; 98903 and 98904). These breeding lines are clustered in the high yielding group 9. These results suggest that the mechanism of heat tolerance may be similar to that of drought tolerance in chickpea. These results also present evidence of increasing overall genetic diversity in chickpea.

3.6. Conclusions

The DArT markers used in this study identified significant genetic diversity among the 167 genotypes tested for high temperature response. There were significant differences in the responses of the various diversity groups, clustered based on these DArT profiles, in both heat stressed and non-stressed environments. The two chickpea growing environments studied have different temperature stress patterns and water availability and germplasm differed in response to these conditions. It is clear that field based phenotyping strategies that optimize heat stress during reproductive development should be developed. If materials are screened under both heat stress and normal conditions it should also be possible to identify broadly adapted germplasm such as those found in group 9. In addition, extending environmental characterisation to other chickpea growing areas in India, Australia, Ethiopia, Myanmar and Canada could help identify globally adapted materials. The genotypes evaluated in this study is a useful resource for association mapping analysis to identify genomic regions linked to yield and adaptation and for the selection of traits linked to heat stress response. These materials are also potential parents for developing mapping populations in chickpea.

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Chapter 4: Field response of chickpea to heat stress

4.1. Introduction

High temperature during the reproductive period can limit chickpea grain yield. High temperature (>30˚C) regulates flower initiation and grain yield in chickpea (Summerfield *et al*. 1984). At present, chickpea is generally produced in warm temperatures (Devasirvatham *et al*. 2012) in rotation with cereals. There is potential to increase the area of chickpea rotation in future, especially in the warmer areas of India, Australia and Myanmar (Subbarao *et al*. 2001; Gentry 2011; Than *et al*. 2006). Furthermore, heat stress is expected to increase due to predicted climate change further impacting on chickpea production and productivity.

A threshold day/ night temperature for chickpea growth and reproductive development is between 29/21˚C and 21/15˚C (Imtiaz *et al*. 2011). However, most of the chickpea growing regions experience >30˚C during the reproductive period (Devasirvatham *et al*. 2012). Grain yield reduction was observed at high temperature (≥35˚C) during flowering and pod development (Wang *et al*. 2006) linked to reduced pollen viability (Chapter 5). Stigma receptivity was also affected at high temperature (≥40/30˚C) through oxidative stress in the leaves which causes failure of fertilisation (Kumar *et al*. 2012a). Therefore, the mechanism of heat stress tolerance is related to growth, seed set and grain yield. The response to heat stress in chickpea is also governed by abscisic acid (ABA) (Kumar *et al*. 2012b) and high temperature can affect root nodulation and nitrogen fixation (Saxena *et al*. 1988).

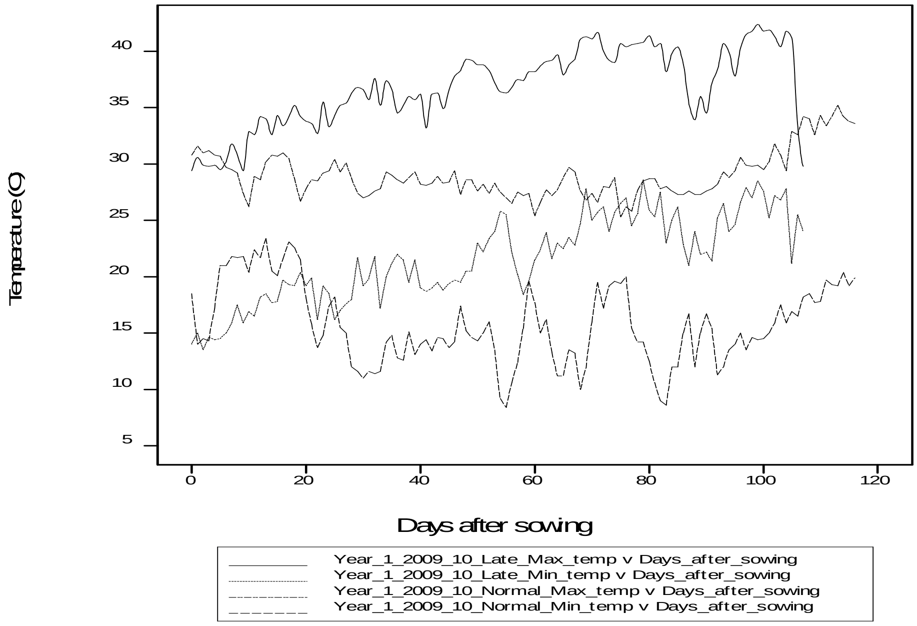
Generally, assessment of whole plant response to heat stress in the field is an effective screening method. The chickpea plant response was studied by comparing two growing environments (cool and warm regions) using available cultivars in Kenya and identified ICCV 92318 as a heat tolerant cultivar (Kaloki 2010). A farmer’s field survey concluded that chickpea yielded better in warmer environments than bean, cowpea, green gram and maize in Kenya (Kaloki 2010). The whole plant response of chickpea was observed in the field using different sowing dates and temperatures (normal and late seasons) at ICRISAT (Krishnamurthy *et al*. 2011; Upadhyaya *et al*. 2011). Krishnamurthy *et al*. (2011) identified new sources of heat tolerance from the reference collection of chickpea germplasm with potential for use in future breeding programs for heat tolerance. Early maturing heat tolerant chickpea genotypes suitable for semi-arid environments were identified by Upadhyaya *et al*. (2011). Grain yield loss varied from 10 to 15% among early maturing genotypes for 1˚C rise from optimum temperature (Upadhyaya *et al*. 2011).

The identification of heat tolerant genotypes can help to adapt chickpea to warmer temperature. In future, not only chickpea must withstand drier and warmer conditions, but also more variable seasonal growing conditions (Imtiaz *et al*. 2011). Experiments were conducted to investigate the field response of chickpea to heat stress. The objective of this research was to assess genetic variability for heat tolerance in chickpea by screening germplasm in heat stressed (late season) and non-stressed (normal season) environments.

4.2. Materials and methods

4.2.1. Experiments design and management

One hundred and sixty seven chickpea genotypes were obtained from the International Crops Research Institute for the Semi-arid Tropics (ICRISAT), gene bank for field evaluation under high temperature. A randomised complete block design with two replications was used for field experiments during year 1 (2009-10) and year 2 (2010-11) at ICRISAT on a Vertisol soil. The ICRISAT site (Patancheru) is located approximately 30 km west of Hyderabad, south India (17.53^{o}N; 78.27^{o}E; 545 m). At ICRISAT, the field used for the experiments was solarised using polythene mulch during the preceding summer to sanitise the field, mainly to eradicate wilt causing fungus *Fusarium oxysporum* f. sp. *ciceri*. After soil solarisation, the field was kept fallow. Both normal and late planting experiments were sown in the ridges and furrows with inter- and intra- row spacing of 60 × 10 cm. A 4 m long row was considered as a replication plot. Seeds were treated with 0.5% Benlate® (E.I. DuPont India Ltd., Gurgaon, India) + Thiram® (Sudhama Chemicals Pvt. Ltd., Gujarat, India) mixture in both the sowings. The normal sown crop was supported with post-sowing irrigation. Whilst the late sown crop received irrigation 0, 20, 28, 35, 45, 55, 65 and 75 days after sowing to minimise drought stress. Two seeds per hill were sown and later thinned to one seedling. The experiments were maintained weed free by manual weeding. Insecticide was sprayed to control pod borer (*Helicoverpa armigera*) based on need.



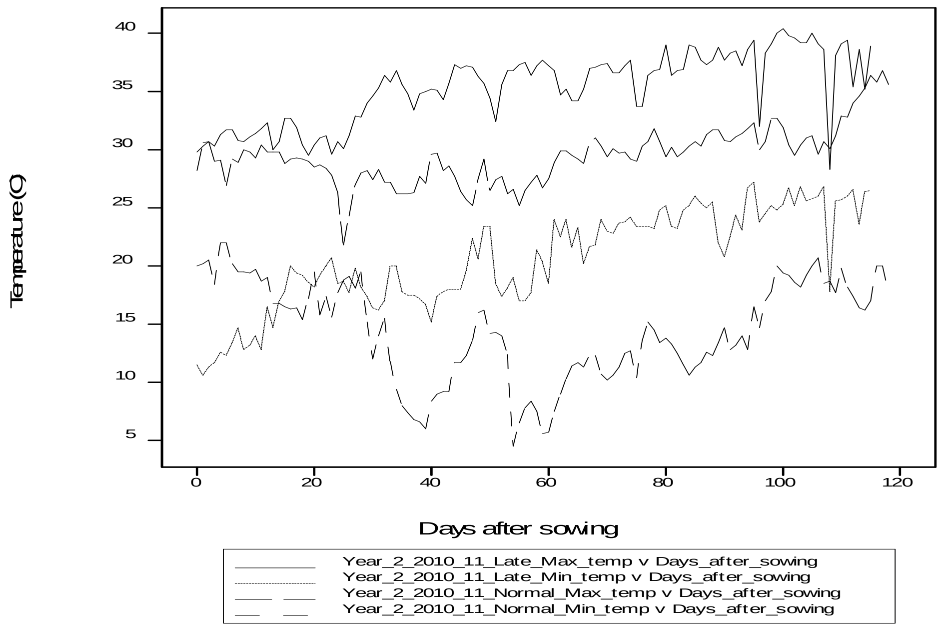


Fig 4.1 Maximum and minimum temperatures of non-stressed (normal season) and stressed environments (late season) during 2009-10 (year 1) and 2010-11 (year 2) at ICRISAT

4.2.2. Weather data

Daily maximum and minimum temperatures were recorded in both seasons (Fig 4.1).

4.2.3. Measurements of growth and yield

Crop Phenology

Days to first flowering (DFF), days to 50% flowering (D50%F), days to first pod formation (DFP) and days to physiological maturity (DPM – the date when 80% of the mature pods in a plot) were recorded for each genotype.

Plant height and canopy width

Before harvest, at physiological maturity, five plants were randomly selected and measured plant height (cm) and width (cm) was measured for each genotype. An average of five plants were calculated and recorded.

Harvest and yield components

At physiological maturity, the aerial parts of the plants from 2 m row length of each genotype was harvested, air dried at 38ºC for 48 h and total shoot dry weight was recorded. At harvest, five plants were randomly collected and yield components per plant (pod number, filled pod number, unfilled pod number, seed number per plant and grain yield – g/plant) were recorded. Harvest Index (%) was calculated as (grain yield/total shoot dry weight) x 100.

Canopy temperature depression

Canopy temperature depression (CTD) is an indicator of the difference between the plant canopy and air temperature. The plants continue to transpire through the open stomata and the canopy temperature is maintained at a metabolically comfortable range. However, plants close stomata for considerable periods due to stress, and this is known to increase canopy temperature (Kashiwagi *et al*. 2008). The canopy temperature in a plot was captured using thermal images during the reproductive stage. The thermal images were captured from 50% flowering to two weeks before physiological maturity. These observations were recorded on six different days in heat stressed environments in both years. An infrared camera IR FLEXCAM (Infrared Solutions, Inc, USA) was used to capture the images between 1300 and 1500 h. As maximum plant height of chickpea was approximately 40 cm, the top view of the thermal images was captured. The target area of the image captured was about 30 cm × 20 cm at the centre of each plot, and the images were captured from the north to avoid shading of the target area. The images could be easily classified into 7 colours, viz, white (very hot temperature = VH, ca. ≥40.0°C), red (hot temperature = H, ca. 35.9°C to 39.9°C), yellow (relatively hot temperature = RH, ca. 32.6°C to 35.8°C), green (moderate temperature = MD, ca. 32.2°C to 32.5°C), light blue (relatively cool temperature = RC, ca. 29.1°C to 32.1°C), blue (cool temperature = C, ca. 25.5°C to 29.0°C), and black (very cool temperature = VC, ca. ≤25.4°C). The modified thermal images were analysed using colour analysis function of the image analysis software Smart View 2.1 (Fluke Thermography, USA) to estimate the canopy temperature of plant canopy area occupied by each colour. In each image five spots were selected from which average canopy temperature was calculated. Finally the average of five spots was calculated as an average temperature of that genotype. Then canopy temperature depression (CTD) was calculated as the difference between ambient and canopy temperature for each genotype.

4.2.4. Estimation of heat tolerance

Heat Tolerance Index (HTI)

The grain yield under stressed and non-stressed conditions was used to predict the stress tolerance of genotypes. However, there were higher yields in short duration genotypes compared to long duration materials under heat stress. To eliminate the differences in crop phenology and heat escape, the multiple regression approach of Bidinger *et al*. (1987) under abiotic stress was used. This approach has shown that the residual yield after removal of heat escape (early flowering) and yield potential (fully irrigated yield) of a genotype gave a good indication of heat stress of that genotype (Krishnamurthy *et al*. 2011). Briefly this approach considers grain yield under heat stress condition (Ys) as a function of yield potential (Yp), time to 50% flowering (F), and a Heat Tolerance Index (HTI) such that the yield of a genotype can be expressed as follows:

$$Ysi = a + bYp + cFi + HTIi + E,$$

Where E is random error with zero mean and variance σ. The difference between the estimated late season grain yield and estimated normal season grain yield plus standardised residuals from regression analysis indicated the heat tolerance of genotypes. Heat Tolerance Index (HTI) was calculated for each genotype. One way Analysis of Variance (ANOVA) was conducted to identify the genotypes differences. This approach was used in chickpea to identify tolerant and sensitive genotypes for heat tolerance (Krishnamurthy *et al*. 2011).

4.2.5. Temperature at different developmental stages of chickpea

The plant growing days at different developmental stages (vegetative, flowering and grain filling period) were calculated. Vegetative period (V) was defined as the number of days from sowing to one day before flowering date. The days from first flower to first pod was considered the flowering period (F). The grain filling period (GF) was defined as the number of days from first pod to maturity. Then, the average maximum and minimum temperatures were calculated at different developmental stages (V_{Max}; V_{Min}; F_{Max}; F_{Min}; GF_{Max} and GF_{Min}) (Appendix 6, 7, 8 and 9). Grain yield was considered as the dependant variable and the influence of temperature determined from different developmental stages (Vargas *et al*. 1998). Partial least squares (PLS) were used to show the main variation pattern of the independent variables genotypes and temperature (V_{Max}; V_{Min}; F_{Max}; F_{Min}; GF_{Max} and GF_{Min}). This method was used in wheat to explain physiological basis of G × E (Reynolds *et al*. 2002). The PLS data were presented as biplots to show the relationship of genotypes with temperature in heat stressed and non-stressed environments.

4.2.6. Statistical Analysis

One way Analysis of Variance (ANOVA) was conducted for 11 traits assessed on 167 genotypes with two replications over two years. Two way analysis (genotype × season) was conducted for all traits for heat stressed and non-stressed environments. The correlation of 11 traits for both environments and years was also studied. One way analysis of CTD was calculated for six days in stressed environments. The relationship of chickpea grain yield and average CTD during the reproductive period under heat stressed environment for both years was identified using regression analysis. GenStat 12th version from VSN International Ltd was used for all statistical analyses.

4.3. Results

4.3.1. High temperature effects on phenology, growth and yield of chickpea

Significant differences in crop phenology were observed among the 167 genotypes of chickpea in both environments (stressed and non-stressed) and years. ANOVA revealed a large treatment difference between stressed and non-stressed conditions in DFF, D50%F, DFP and DPM in both years (Table 4.1; Appendix 2 and 3). There were 4-5 days differences in crop phenological duration. The overall crop cycle was reduced in the stressed environment in both years. The variation in crop maturity was 19 days in year 1 and 6 days in year 2 (Table 4.1). This was associated with high temperature in the stressed environment. In heat stressed environments, DFF, D50%F and DFP had significant ($R^2 \geq 0.50$) negative association with maximum high temperature during the first year (Fig 4.2). In year 2, DFF and D50%F were negatively associated with high temperature and DFP was positively associated (Fig 4.3). The Figs 4.2 and 4.3

show that the experiments were exposed to temperatures up to 42˚C in year 1 and 38˚C in year 2.

Significant variation in plant height, plant width and plant biomass at harvest were also observed in both environments and these are considered as growth parameters. At high temperature plant growth was significantly affected (Table 4.1). During year 1, the plant biomass at high temperature was less than half (9.7 g/plant) that of non-stressed (23.3 g/plant) materials whilst, fewer differences were observed in biomass in year 2. The interaction of environment and genotypes was significant in year 1 for all three growth parameters and significant for plant biomass and plant height in year 2. There was significant variation in grain yield and pod number, filled pod number and seed number per plant in both environments and years. In year 1, high temperature reduced pods from 50 (normal season) to 14 per plant ($P<0.001$). Similarly grain yield was also reduced from 10.6 to 2.4 g/plant ($P<0.001$). In year 2, the grain yield difference between non-stressed and stressed conditions was only 0.17 g/plant. The interaction was not significant in year 2 (Table 4.1).

4.3.2. Contribution of yield and its components to heat stress

Yield components such as pod number per plant, filled pod number per plant, seed number per plant and harvest index were regressed against grain yield in both environments and years. Among yield components, pod number per plant and harvest index were most related to grain yield under heat stress ($R^2>0.5$) (Figs 4.4 and 4.5). Phenology (DFF, D50%F and DFP) was negatively associated with grain yield (data not shown), highlighting the advantages of early flowering and shorter crop cycles in heat stressed environments.

4.3.3. Correlation among various traits measured in the field

Correlation coefficients in non-stressed and heat stressed environments are presented in Tables 4.2, 4.3, 4.4 and 4.5 for both years. In year 1, among phenological traits DFF and DPM showed significant and negative correlation under heat stress (Table 4.3). Plant biomass, pod number, filled pod number and seed number per plant were positively correlated with grain yield ($P<0.001$) (Table 4.3). Similarly, crop phenology (DFF, D50%F, DFP) showed significant negative correlation with grain yield in year 2. Plant width, plant biomass, pod number, filled pod number and seed number per plant were positively correlated with grain yield ($P<0.001$) (Table 4.5). Similar trends were observed in non-stressed environment in both years (Tables 4.2 and 4.4).

Table 4.1 Stressed environments (non-stressed (normal) and heat stressed (late)) means and interaction of chickpea in the field experiments during 2009-10 (year 1) and 2010-11 (year 2) at ICRISAT (Significant difference at ***P<0.001; *P<0.05 and NS-Non significant)

Year	Season	DFF	D50%F	DFP	DPM	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	Pod number/ plant	Filled pod number/ plant	Seed number/ plant	Grain yield (g/ plant)	HI
1	Normal	50	54	57	111	23.33	44.27	39.45	54	50	61	10.60	0.46
	LSD(*P*=0.05)	(6.7) ***	(6.6) ***	(6.9) ***	(13.2) ***	NS	(9.9) ***	NS	(35.8) ***	(33.8) ***	(48.8) ***	(7.1) **	(0.13) ***
1	Late	46	47	48	92	9.65	30.14	23.12	15	14	18	2.40	0.17
	LSD(*P*=0.05)	(7.7) ***	(19.5) ***	(25.3) ***	(7.4) ***	(7.9) ***	(8.3) ***	(11.3) ***	(19) ***	(17) ***	(25.5) ***	(1.8) ***	(0.2) ***
1	Normal x Late	49	50	52	102	16.49	37.20	31.29	35	31	39	6.49	0.32
	Season	(0.6) ***	(1.1) ***	(1.5) ***	(0.8) ***	(0.9) ***	(0.7) ***	(1.1) ***	(2.2) ***	(2.1) ***	(3.1) ***	(0.4) ***	(0.01) ***
	Genotype effect	(5.1) ***	(10.2) ***	(13.07) ***	(1.2) ***	NS	(6.4) ***	(9.4) ***	(20.1) ***	(18.9) ***	(27.4) ***	(4.0) ***	(0.12) ***
	Season x Genotype	(7.2) ***	(14.5) ***	(18.5) ***	(10.7) ***	(11.3) ***	(9.1) ***	NS	(28.4) ***	(26.8) ***	(38.8) ***	(5.6) ***	(0.17) ***
2	Normal	49	55	57	108	27.88	50.74	36.50	68	64	78	13.17	0.46
	LSD(*P*=0.05)	(6.48) ***	(6.89) ***	(7.4) ***	(5.47) ***	(15.01) ***	(10.45) ***	(14.85) ***	(41.45) ***	(40.13) ***	(51.66) ***	(7.88) ***	(0.1) ***
2	Late	46	49	52	102	26.30	41.99	41.24	61	58	75	12.00	0.45
	LSD(*P*=0.05)	(8.96) ***	(15.85) ***	(8.77) ***	(9.03) ***	(18.79) *	(7.21) ***	(13.49) ***	(43.24) ***	(41.91) ***	(57.19) ***	(9.00) ***	(0.14) ***
2	Normal x Late	47	52	55	105	34.93	45.99	31.40	64	61	76	12.59	0.46
	Season	(0.6) ***	(0.94) ***	(0.63) ***	(0.58) ***	(0.91) ***	(0.93) ***	(1.31) ***	(3.27) ***	(3.16) ***	(4.2) NS	(0.65) ***	(0.01) ***
	Genotype	(5.51)	(8.16)	(5.73)	(5.26)	(8.31)	(8.5)	(11.93)	(29.84)	(28.91)	(38.39)	(5.96)	(0.09)

effect	***	***	***	***	***	***	NS	***	***	***	***	***
Season x Genotype	(7.79) *	(12.18) ***	(8.1) *	(7.44) NS	(11.75) ***	(12.02) ***	(16.87) NS	(42.2) NS	(40.88) NS	(54.29) NS	(8.42) NS	(0.12) ***

DFF-Days to first flower; D50%F-Days to 50% flowering; DFP-Days to first pod formation; DPM-Days to physiological maturity

Table 4.2 Correlation of various traits for non-heat stressed condition (normal) during 2009-10 (year 1) at ICRISAT (Significant difference at ***P<0.001 and*P<0.05)

	DFF	D50F	DFP	DPM	PH	PW	Biomass	NP	NFP	No seeds	GY	HI
DFF												
D50F	0.989***											
DFP	0.981***	0.977***										
DPM	0.639***	0.640***	0.641***									
PH	0.175*	0.176*	0.174*	0.272***								
PW	-0.052	-0.057	-0.049	0.023	0.598***							
Biomass	0.187***	0.194***	0.179	0.291***	0.491***	0.437						
NP	0.065	0.060	0.073	0.059	-0.079	0.140	0.475***					
NFP	0.076	0.073	0.087	0.045	-0.095	0.108	0.435***	0.967***				
No seeds	0.097	0.090	0.108	0.041	-0.073	0.138	0.391***	0.889***	0.880***			
GY	0.207***	-0.198***	-0.206***	-0.088	0.135	0.358***	0.641***	0.686***	0.676***	0.665***		
HI	0.571***	-0.578***	-0.566***	-0.555***	-0.384***	-0.023	-0.186*	0.327***	0.347***	0.339***	0.477***	

Table 4.3 Correlation of various traits for heat stressed condition (late) during 2009-10 (year 1) at ICRISAT (Significant difference at ***P<0.001 and*P<0.05)

	DFF	D50F	DFP	DPM	PH	PW	Biomass	NP	NFP	No seeds	GY	HI
DFF												
D50F	0.989***											
DFP	0.981***	0.977***										
DPM	0.639***	0.640*	0.641									
PH	0.187	0.193	0.178	0.290								
PW	0.174	0.176	0.174	0.271	0.491***							
Biomass	-0.052	-0.056	-0.049	0.023***	0.436***	0.597***						
NP	0.065***	0.060	0.072	0.059***	0.474	-0.078	0.139***					
NFP	0.075***	0.072	0.087	0.045***	0.434	-0.095	0.107***	0.966***				
No seeds	0.096***	0.090	0.108	0.041***	0.391	-0.072	0.138***	0.888***	0.880***			
GY	-0.206***	-0.197	-0.205	-0.087***	0.641	0.134	0.358***	0.685***	0.675***	0.664***		
HI	-0.570***	-0.577	-0.566	-0.555***	-0.185	-0.384	0.022***	0.327***	0.347***	0.338***	0.477***	

DFF-Days to first flower; D50F-Days to 50% flowering; DFP-Days to first pod; DPM-Days to physiological pods; PH-Plant height; PW-Plant width; NP-Number of pods/plant; NFP-Number of filled pods/plant; GY-Grain yield; HI-Harvest index

Table 4.4 Correlation of various traits for non-heat stressed condition (normal) during 2010-11 (year 2) at ICRISAT (Significant difference at ****P*<0.001 and**P*<0.05)

DFF												
D50F	0.962***											
DFP	0.971***	0.967***										
DPM	0.613***	0.636***	0.637***									
PH	0.337***	0.354***	0.336***	0.435***								
PW	-0.125	-0.079	-0.110	0.052	0.172*							
Biomass	-0.021	0.014	0.006	0.079	0.155	0.011						
NP	-0.120	-0.095	-0.097	-0.255***	-0.259***	-0.095	0.466***					
NFP	-0.099	-0.071	-0.079	-0.241***	-0.254***	-0.078	0.465***	0.992***				
No seeds	-0.056	-0.039	-0.051	-0.253***	-0.276***	-0.092	0.411***	0.917***	0.929***			
GY	-0.305***	-0.289***	-0.283***	-0.299***	-0.198***	-0.052	0.722***	0.721***	0.711***	0.673***		
HI	-0.482***	-0.499***	-0.483***	-0.604***	-0.519***	-0.102	-0.014	0.519***	0.516***	0.512***	0.578***	
	DFF	D50F	DFP	DPM	PH	PW	Biomass	NP	NFP	No seeds	GY	HI

Table 4.5 Correlation of various traits for heat stressed condition (late) during 2010-11 (year 2) at ICRISAT (Significant difference at ****P*<0.001 and**P*<0.05)

DFF												
D50F	0.690***											
DFP	0.980***	0.692***										
DPM	0.525***	0.368***	0.543***									
PH	0.590***	0.494***	0.590***	0.538***								
PW	0.0421	0.170	0.037	0.240***	0.312***							
Biomass	0.548***	0.426***	0.553***	0.549***	0.586***	0.152*						
NP	-0.274***	-0.131	-0.274***	-0.127	-0.050	0.527***	-0.354***					
NFP	-0.292***	-0.148	-0.292***	-0.150***	-0.073***	0.519***	-0.374***	0.984***				
No seeds	-0.251***	-0.117	-0.249***	-0.149*	-0.062	0.486***	-0.375***	0.933***	0.949***			
GY	-0.401***	-0.226***	-0.408***	-0.168	-0.127	0.726***	-0.305***	0.738***	0.761***	0.708***		
HI	-0.726***	-0.471***	-0.736***	-0.670***	-0.637***	-0.057	-0.739***	0.440***	0.477***	0.446***	0.546***	
	DFF	D50F	DFP	DPM	PH	PW	Biomass	NP	NFP	No seeds	GY	HI

DFF-Days to first flower; D50F-Days to 50% flowering; DFP-Days to first pod; DPM-Days to physiological pods; PH-Plant height; PW-Plant width; NP-Number of pods/plant; NFP-Number of filled pods/plant; GY-Grain yield; HI-Harvest index

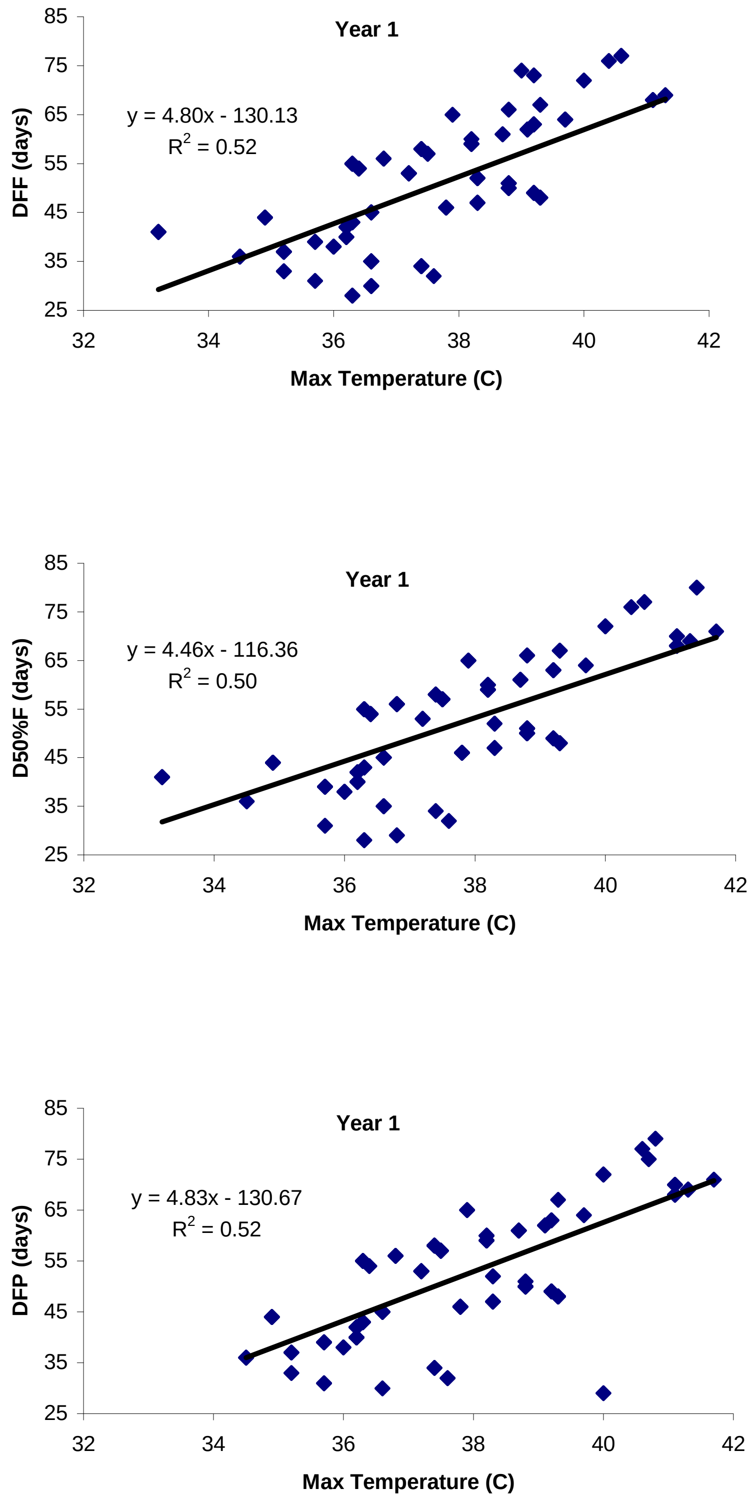
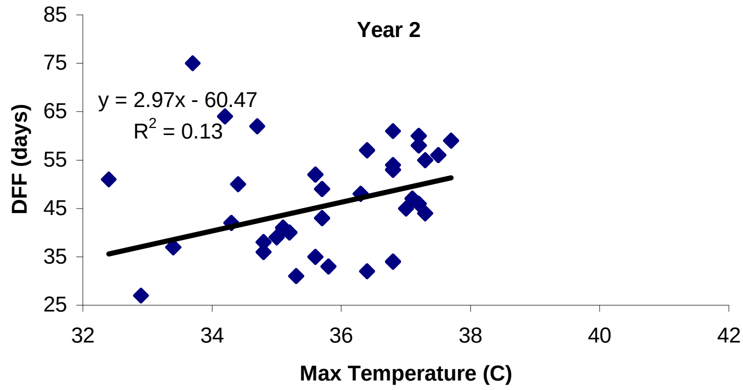
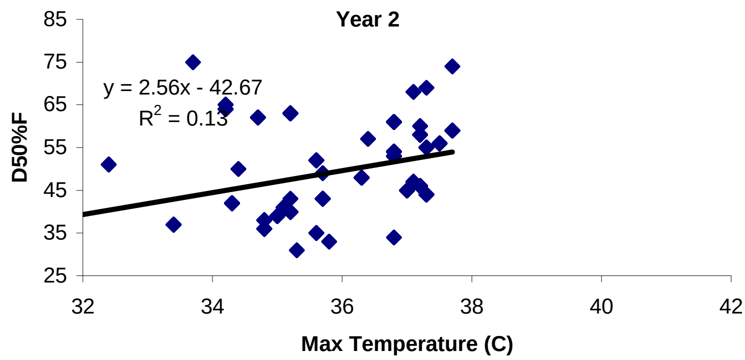


Fig 4.2 Relationship of phenology with maximum temperature during year 1 at heat stressed environment (late) (2009-10) (X-axis represents Tmax for particular phenological stage e.g. DFF, D50%F, DFP; Y-axis represents number of days for particular phenological stage; Genotypes = 167)





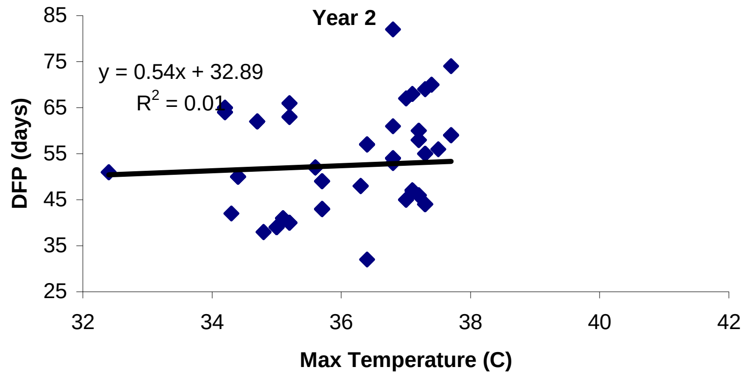


Fig 4.3 Relationship of phenology with maximum temperature during year 2 at heat stressed environment (late) (2010-11) (X-axis represents Tmax for particular phenological stage e.g. DFF, D50%F, DFP; Y-axis represents number of days for particular phenological stage; Genotypes = 167)

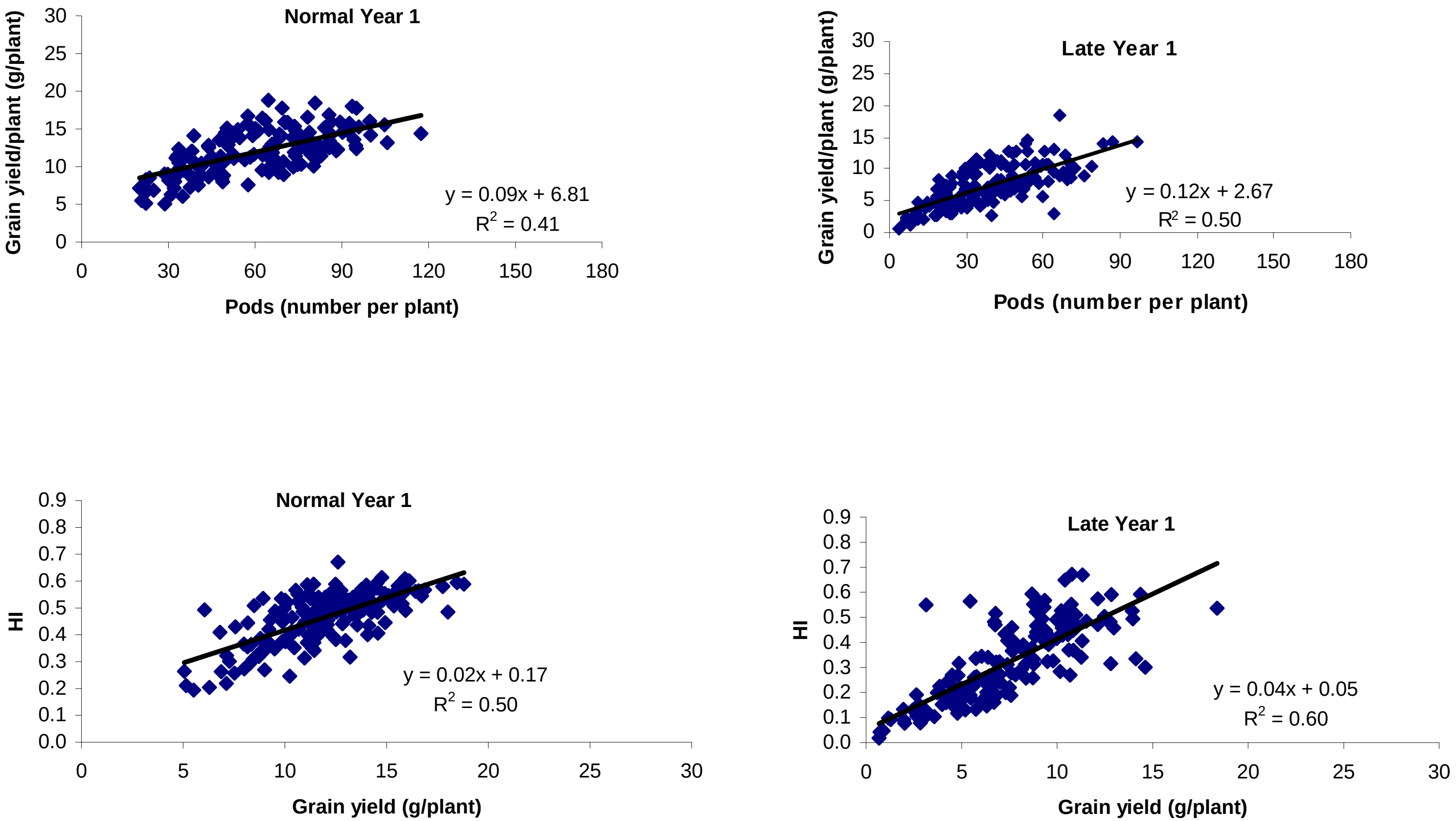


Fig 4.4 Relationship of pod number per plant with grain yield (g/plant) and harvest index (HI %) at heat stressed (late) and non-stressed (normal) during year 1 (2009-10)

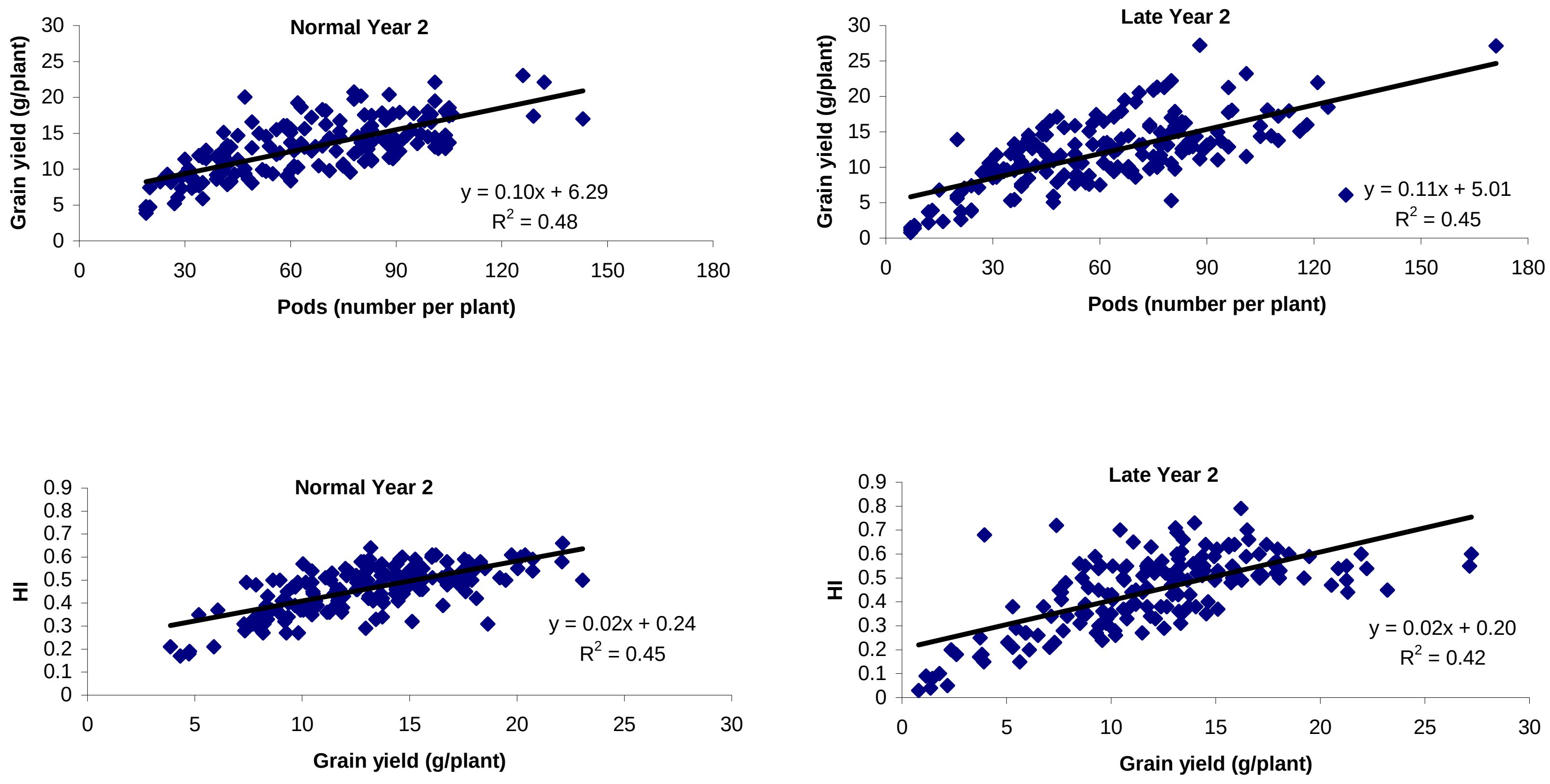


Fig 4.5 Relationship of pod number per plant with grain yield (g/plant) and harvest index (HI %) at heat stressed (late) and non-stressed (normal) during year 2 (2010-11)

4.3.4. Classification of heat response

There was a significant difference between genotypes and years for heat response (Appendix 4). The hierarchical cluster analysis classified the genotypes into five groups. Based on means, the groups were classified into (1) stable heat tolerant (>0.5), (2) moderate heat tolerant (0.1-0.49), (3) stable heat sensitive (-ve values), (4) heat tolerant to moderately sensitive (-0.10 to 1) and (5) heat sensitive to moderately tolerant (-0.5 to 0.4) (Table 4.6). The most representative stable heat tolerant, moderate tolerant and stable sensitive genotypes were 12, 24 and 44 respectively. Seven genotypes were classified as heat tolerant to moderately sensitive. Heat sensitive to moderately tolerant genotypes comprised 79 genotypes out of 167 genotypes tested. The most stable heat tolerant genotypes are ICCV 95311, ICCV 98902, ICCV 07109, ICCV 92944 and ICC 12312. The most heat sensitive genotypes included ICCV 07117, ICC 5566, ICC 7570 and ICC 5912.

4.3.5. Canopy temperature depression

The CTD reading at 50% flowering in both years did not differ significantly among genotypes. Among six observations, only two showed significant differences among genotypes under stressed environments in both years (Appendix 5 and 6). The CTD of selected genotypes were arranged based on their response to high temperature (Tables 4.7 and 4.8). In both years, the high temperature reduced the CTD values. The sensitive genotypes had lower CTD than tolerant genotypes. The stable heat tolerant genotypes ICCVs 95311, 98902, 07109 and 92944 recorded higher level of CTD in the significant observations compared with stable sensitive genotypes (Tables 4.7 and 4.8). Some of the sensitive genotypes ICCV 07116, ICCV 07117 and ICC 14592 produced negative CTD values (i.e., no temperature depression) further highlighting their sensitivity to heat stress (Table 4.7). The average CTD of 167 genotypes regressed against grain yield under heat stressed environment showed significant relationship in year 1 ($P<0.001$) (Fig 4.6), but in year 2 the relationship was non-significant (Fig 4.7).

4.3.6. Effect of temperature at different developmental stages of chickpea

High temperature reduced the vegetative, flowering and grain filling periods of chickpea. High temperature for each developmental stage is shown in Appendix 7, 8, 9 and 10. In both years the vegetative and grain filling periods were significantly reduced in the stressed environment compared to non-stressed conditions (Table 4.9). In year 1, the overall grain filling period was reduced from 53 days to 38 days at high temperature (*P*<0.001) (Table 4.9), but in year 2 the difference was much less. This indicates the importance of average maximum and minimum temperatures at each growth stage in both environments. The calculated variables V_{Max}, F_{Max} and GF_{Max} were comparatively higher in year 1 compared with year 2 (Table 4.10). The GF experienced 39.2/ 24.2˚C of maximum/minimum temperature in year 1 and 37.1/23.2˚C in year 2. This is the main reason for yield reduction in year 1 compared with year 2. Overall, in the normal season the GF_{Max} temperature varied between 29.1 and 30.3˚C. A minimum low temperature (≤14˚C) was observed during the flowering period (Table 4.10).

Temperature at different developmental stages measured for each genotype explained some of the variability in grain yield. The temperature variables were helpful in explaining G × E interaction. In the biplots generated using Genstat the temperature variables are shown as vectors and genotypes as points (Fig 4.8). The significance of each temperature variable on G × E is related to distance from the origin. The longest vectors are the most significant. In year 1 normal and late seasons, GF_{Max} had the greatest influence on G × E. In the year 2 non-stressed environment, V_{Max} was an important variable whereas V_{Min} was the dominant factor in the stressed environment (Fig 4.8). PCA demonstrated temperature differences (stressed and non-stressed) very effectively. Under stressed conditions, experiments in both years, the mean maximum temperatures in different growing periods explained >73% of total variance in two components (PC1, PC2) (Fig 4.8). However the relationship between temperature variables and yield can be identified using simple linear regression based on % variance. The order of temperature variables in each year and environment is presented in Table 4.10. In the non-stressed environment in both years, GF_{Max} and GF_{Min} significantly influenced grain yield. In contrast, in stressed environment experiments in both years GF_{Min} and V_{Max} played important roles in the grain yield (Table 4.10).

Table 4.6 Heat response classification of chickpea genotypes

No	Stable heat tolerant	HTI Year 1	HTI Year 2
1	ICCV 95311	0.89	1.19
2	ICCV 98902	0.97	1.15
3	ICCV 07109	0.96	0.78
4	ICCV 92944	1.49	0.43
5	ICC 6969	0.45	1.25
6	ICCV 07108	0.48	0.74
7	ICCV 98903	0.56	0.6
8	ICCV 96836	0.23	0.82
9	ICC 14406	1.06	0.28
10	ICC 16173	0.92	0.33
	Stable moderate tolerant		
1	ICCX 820065(GG2)	0.54	0.34
2	ICCL 87207	0.6	0.28
3	ICC 4902	0.62	0.18
4	ICC 14315	0.62	0.12
5	ICCV 89314	0.61	0.12
6	ICC 3935	0.5	0.2
7	ICC 13941	0.4	0.26
8	ICC 14497	0.42	0.21
9	ICC 16181	0.4	0.22
	Stable sensitive		
1	ICC 988	-0.24	-0.47
2	ICC 8261	-0.25	-0.45
3	ICC 10090	-0.27	-0.47
4	ICC 7294	-0.21	-0.4
5	ICCV 07117	-0.21	-0.43
6	ICC 6231	-0.22	-0.56
7	ICC 7292	-0.21	-0.53
8	ICC 16453	-0.18	-0.49
9	ICC 5912	-0.3	-0.51
10	ICC 7308	-0.26	-0.54
11	ICC 5566	-0.22	-0.92
12	ICC 7570	-0.3	-0.87
	Tolerant to moderate sensitive		
1	ICC 1017	-0.19	0.94
2	ICCV 94916-8	-0.24	0.92
3	ICC 9125	-0.15	0.88
4	ICC 12169	-0.12	1.05
	Sensitive to moderate tolerant		
1	ICC 982	-0.32	0.05
2	ICC 16298	-0.31	0.01
3	ICC 14183	-0.26	0.1
4	ICCV 07116	0.03	0.1
5	ICC 14592	-0.24	0.19
6	ICC 1025	-0.27	0.47

Table 4.7 Canopy temperature depression (CTD - ˚C) of selected chickpea genotypes grown in the field under heat stressed condition (late) during 2010 (year 1)

Genotype No	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day6
Stable heat tolerant							
1	ICCV 95311	1.9	6.1	3.3	8.5	8.1	6.5
2	ICCV 98902	3.6	2.6	4.8	5.0	10.3	6.3
3	ICCV 07109	4.2	4.5	2.3	8.6	7.3	7.2
4	ICCV 92944	4.7	6.2	8.2	9.6	10.9	9.3
5	ICC 6969	2.1	4.8	2.7	6.2	5.4	5.1
6	ICCV 07108	1.9	4.9	1.6	7.2	7.2	5.6
7	ICCV 98903	2.0	2.8	3.0	4.4	6.0	4.9
8	ICCV 96836	1.5	4.0	3.0	8.3	8.9	5.5
9	ICC 14406	1.0	4.1	4.2	3.6	4.1	2.0
10	ICC 16173	2.3	5.4	2.0	9.3	7.4	5.3
Moderate heat tolerant							
1	ICCX 820065(GG2)	2.2	4.3	1.9	7.6	8.6	6.4
2	ICCL 87207	3.5	2.8	3.0	4.7	6.8	5.1
3	ICC 4902	1.6	5.4	1.0	6.9	6.8	5.5
4	ICC 14315	1.8	1.5	1.8	9.o	8.9	7.2
5	ICCV 89314	1.6	5.6	5.1	7.4	8.3	6.9
Stable heat sensitive							
1	ICC 988	2.3	3.8	0.8	3.6	5.9	5
2	ICC 8261	1.4	3.4	0.4	2.5	4	3.5
3	ICC 10090	0.5	2.7	1.95	2.5	5.8	4.9
4	ICC 7294	1.4	3.1	2.2	4.6	3.4	3.9
5	ICCV 07117	1.4	5.2	-0.7	6.5	3.3	5.5
Tolerant to moderate sensitive							
1	ICC 1017	3.6	5.9	3.8	8.1	8.8	5.9
2	ICCV 94916-8	1.1	2.2	0	8.1	4.7	5.2
3	ICC 9125	2.4	4.1	2.7	8.3	7.6	7.3
4	ICC 12169	2.2	2.15	3.9	6.9	4.5	5.6
Sensitive to moderate tolerant							
1	ICC 982	1.5	4.5	0.6	4.5	5.6	5.1
2	ICC 16298	2.5	2.5	2.5	2.2	4.3	3.7
3	ICC 14183	1.5	1.9	1.5	2.6	5.2	5.5
4	ICCV 07116	2	1.6	-2.1	5.4	4.8	3.7
5	ICC 14592	0.9	5.8	-0.8	5.2	4.2	5.3
6	ICC 1025	2.4	6.6	2.9	9.7	7.5	6.0

Table 4.8 Canopy temperature depression (CTD - ˚C) of selected chickpea genotypes grown in the field under heat stressed condition (late) during 2011 (year 2)

Genotype No	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day6
Stable heat tolerant							
1	ICCV 95311	3.0	5.7	9.3	4.8	5.8	6.8
2	ICCV 98902	3.4	5.3	5.8	5.5	5.2	5.9
3	ICCV 07109	3.7	5.3	7.0	5.4	6.5	8.6
4	ICCV 92944	3.0	6.4	7.3	4.7	5.4	6.7
5	ICC 6969	5.2	5.3	8.2	6.0	6.1	6.2
6	ICCV 07108	4.4	5.6	6.9	5.9	6.6	6.7
7	ICCV 98903	4.6	5.4	7.9	6.3	7.4	6.3
8	ICCV 96836	4.2	4.8	6.8	5.6	5.9	7.8
9	ICC 14406	4.4	3.8	8.3	5.2	5.0	7.2
10	ICC 16173	5.2	8.5	10.6	7.0	8.0	7.3
Moderate heat tolerant							
1	ICCX 820065(GG2)	4.2	7.2	8.4	6.4	7.7	7.6
2	ICCL 87207	4.4	7.4	8.1	5.8	7.5	7.9
3	ICC 4902	4.6	6.3	7.6	6.8	7.3	7.6
4	ICC 14315	5.1	7.2	8.6	7.1	7.9	9.8
5	ICCV 89314	3.8	4.4	8.9	5.4	5.7	6.8
Stable heat sensitive							
1	ICC 988	3.0	4.5	6.7	5.1	5.1	4.2
2	ICC 8261	3.8	6.6	7.9	6.5	6.3	8.2
3	ICC 10090	4.9	6.1	6.0	7.2	6.3	7.4
4	ICC 7294	2.1	3.4	6.6	5.2	5.3	7.7
5	ICCV 07117	4.1	6.2	9.3	6.2	6.1	8.8
Tolerant to moderate sensitive							
1	ICC 1017	3.6	7.2	9.7	5.5	7.1	8.2
2	ICCV 94916-8	4.4	4.1	7.7	5.0	5.5	5.7
3	ICC 9125	5.3	5.5	8.5	6.1	6.5	7.6
4	ICC 12169	3.4	6.9	10.3	6.6	6.7	10.3
Sensitive to moderate tolerant							
1	ICC 982	3.2	4.6	7.1	4.6	5.0	5.6
2	ICC 16298	4.5	6.8	8.7	6.4	8.5	8.5
3	ICC 14183	4.7	3.2	5.1	5.4	4.0	5.0
4	ICCV 07116	3.1	5.3	7.0	5.9	6.3	7.3
5	ICC 14592	3.8	7.1	9.4	6.5	8.5	8.0
6	ICC 1025	3.4	6.8	8.1	5.8	6.1	7.2

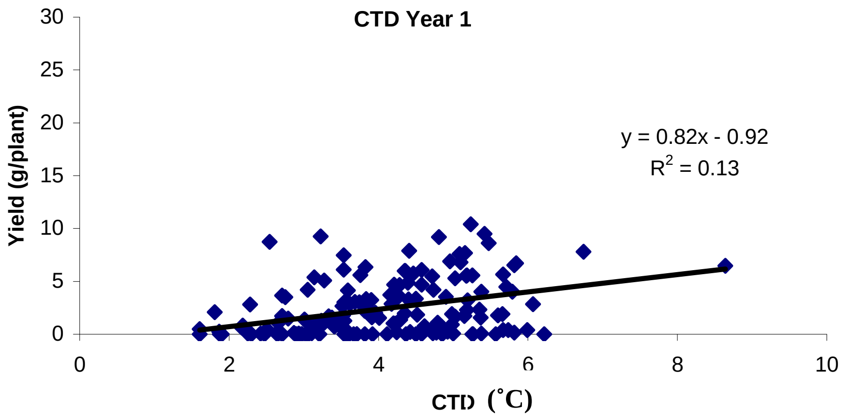


Fig 4.6 The relationship (R^2=0.13) of chickpea grain yield and average canopy temperature depression in reproductive period under heat stressed condition (late) during 2010 (year 1) (*P*<0.001)

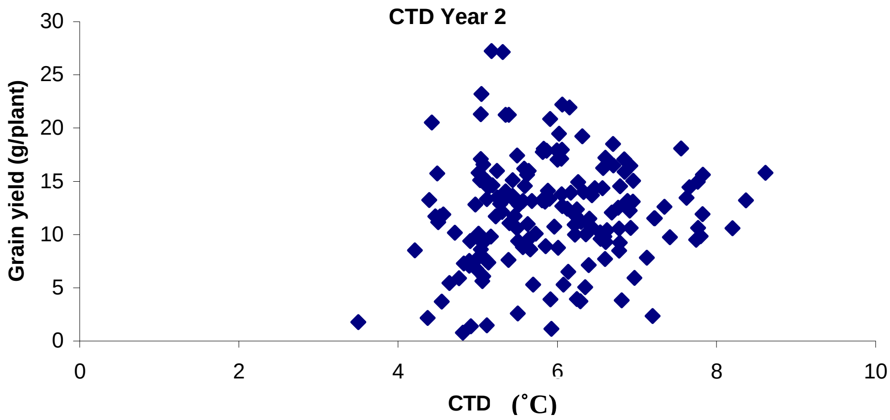


Fig 4.7 The relationship (R^2=0.01) of chickpea grain yield and average canopy temperature in reproductive period under heat stressed condition (late) during 2011 (year 2) (*P*>0.05)

4.3.7. Crop phenology, growth and yield of selected chickpea genotypes

The HTI and biplots showed similar results. The most heat tolerant genotypes were not affected by temperature variables i.e. they were not linked to any temperature variable in the biplots. The most heat sensitive genotypes were linked to temperature variables. The most heat tolerant (ICCV 98902) and sensitive genotypes (ICC 5566 and ICC 7570) were selected from the biplots to illustrate their phenology, growth and yield (Table 4.11). The most heat tolerant genotype was ICCV 98902 which had the highest grain yield among the 167 genotypes in both years during the late season. In year 2, ICCV 98902 had higher grain yield (27 g/plant) compared with year 1 (10 g/plant) (Table 4.11). The average maximum temperature (≥39˚C) was a contributing factor to lower yield during the late season in year 1 across all genotypes. In year 2 the average maximum temperature only reached 37˚C (Table 4.10) during the late season. The plant biomass difference in the two seasons clearly explained the yield difference (Table 4.11). The difference between the yield and plant biomass of heat tolerant and sensitive genotypes was explained by growing season temperatures. The maximum temperature during F and GF was >39˚C and 35.7 - 37.5˚C during the late season in year 1 and year 2, respectively, and the tolerant genotypes produced the highest grain yield (Table 4.11). Overall, the duration of GF was reduced by 4-19 days in the sensitive genotypes grown in the stressed environment. The heat tolerant genotype, ICCV 98902, had similar GF (60 days) in both stressed and non-stressed environments. However, at 39.4˚C the GF period of ICCV 98902 was reduced by 30 days and plant biomass was reduced by 50% (Table 4.11). Therefore, a maximum temperature ≥38˚C was the critical temperature for yield reduction in the field for this genotype.

4.4. Discussion

Field screening demonstrated that delayed sowing is a successful strategy to detect the heat tolerance in chickpea. These results confirmed the earlier studies of Krishnamurthy *et al*. (2011) and Upadhyaya *et al*. (2011) in semi-arid environments. Using delayed sowing, Canci and Toker (2009) studied the combined effect of drought and heat in the Mediterranean environment. They used visual scoring on a 1-9 scale to screen 377 lines in the field.

Table 4.9 Number of days in different developmental stages of chickpea under non-stressed (normal) and heat stressed (late) conditions (Significant difference at ***P<0.001 and NS-Not significant)

Year/ season	Vegetative period (V)	Flowering period (F)	Grain filling period (GF)
Year 1			
Normal	49	8	53
LSD (P=0.05)	6.7***	2.9***	14.4*
Late	46	6	38
LSD (P=0.05)	7.1***	3.4NS	9.4***
Year 2			
Normal	48	10	51
LSD (P=0.05)	6.5***	4.7NS	8.3***
Late	45	8	50
LSD (P=0.05)	8.9***	3.8NS	12***

V= one day before first flower – sowing day; F= days to first pod – days to first flower; GF= harvest date – one day after pod formation

Table 4.10 Developmental stages sensitive to max and min high temperatures identified by PLS analysis and simple linear regression based on % of variance considering G × E among 167 chickpea genotypes grain yield at ICRISAT – India during 2009-10 (year 1) and 2010-11 (year 2) (non-stressed (normal) and heat stressed (late) conditions)

Temperature/ Growth stage	% variance				Temperature (˚C) Mean*			
	Year 1 Normal	Year 1 Late	Year 2 Normal	Year 2 Late	Year 1 Normal	Year 1 Late	Year 2 Normal	Year 2 Late
V_{Max}	4.2^{3}	39.1^{2}	8.5^{4}	10.6^{1}	28.9± 0.23	34.1±0.16	28.1±0.27	32.6±0.36
F_{Max}	0.2^{5}	28.5^{5}	8.5^{4}	8.1^{4}	27.8± 0.23	37.4±0.16	27.9±0.27	35.9±0.36
GF_{Max}	10.7^{1}	18.6^{6}	17.2^{2}	7.2^{5}	29.1± 0.23	39.2±0.16	30.3±0.27	37.1±0.36
V_{Min}	3.5^{4}	32.3^{3}	9.6^{3}	10.2^{2}	16.5± 0.23	18.3±0.16	15.5±0.27	16.7±0.36
F_{Min}	0	31.8^{4}	0.3^{5}	10.1^{3}	14± 0.23	21.5±0.16	10.4±0.27	19.2±0.36
GF_{Min}	8.8^{2}	44.8^{1}	18.3^{1}	10.2^{2}	15± 0.23	24.2±0.16	13.7±0.27	23.2±0.36
% variance	10	50	21	12				

*Max and min temperatures were calculated for each genotype during the vegetative (V); flowering (F) and grain filling (GF) periods. Numbers $^{1-6}$ (in superscript) in vertical order refer to factors explaining significant amounts of G x E rank.

Krishnamurthy *et al*. (2011) used HTI as a tool to identify the most heat tolerant and sensitive genotypes among 280 genotypes. Later Upadhyaya *et al*. (2011) found a correlation between climatic factors and plant traits. This research used plant growth and yield traits, plant physiological traits (CTD), a stress index (HTI) and temperature prediction at different developmental stages of chickpea (V_{Max}; V_{Min}; F_{Max}; F_{Min}; GF_{Max} and GF_{Min}) as tools for heat tolerance screening.

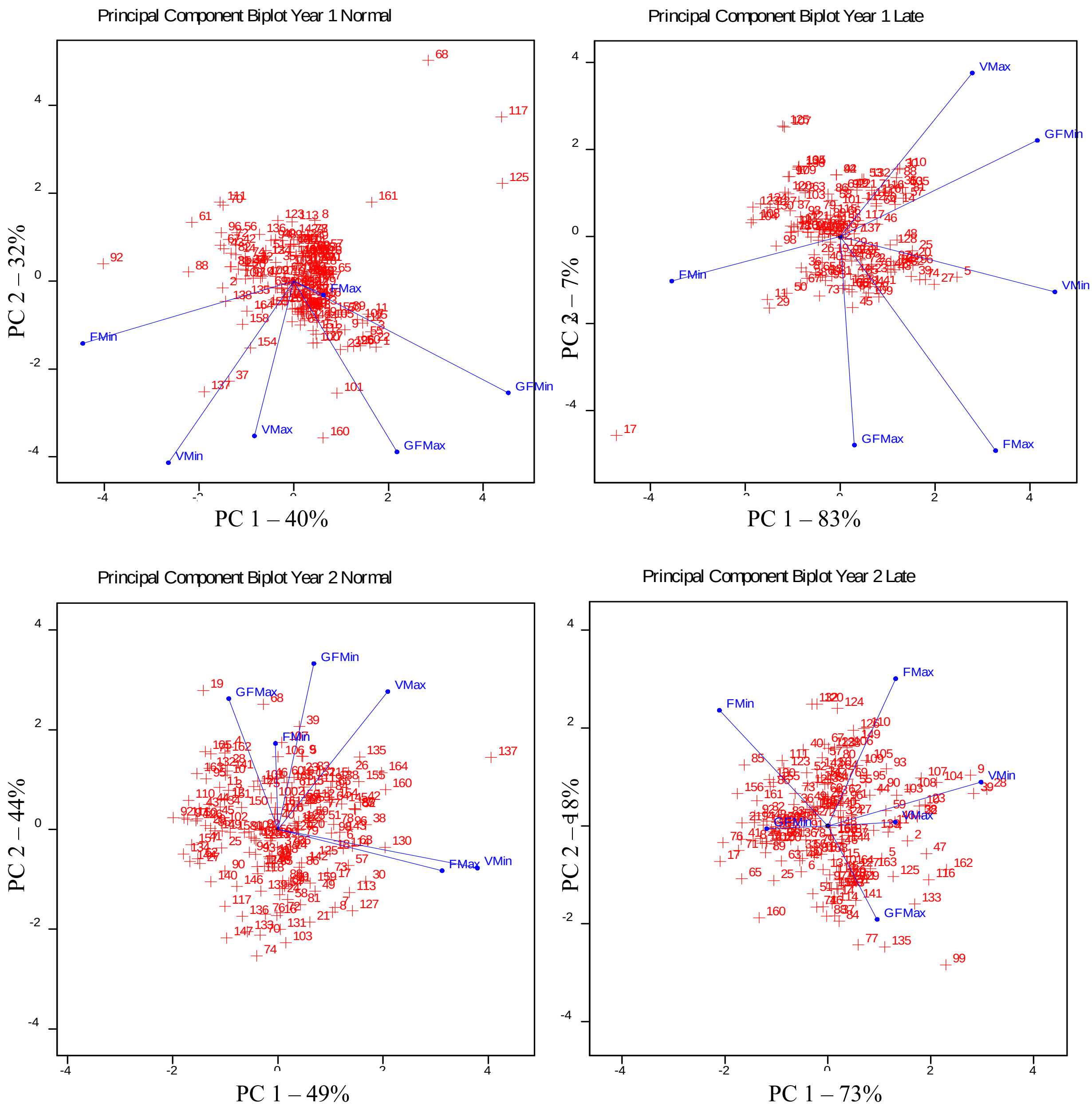


Fig 4.8 Biplot based on PLS analysis of G × E for 167 chickpea genotypes showing the relationship with temperature and yield (g/plant) at ICRISAT – India during 2009-10 and 2010-11 (non-stressed (normal) and heat stressed (late) conditions)

These data showed that grain filling period was most affected under high temperature (Table 4.11).Crop phenological duration (DFF, D50%F and DFP) was negatively related to high temperature (Figs 4.2 and 4.3) and shortened period of phenology and/ development is the main reason for yield reduction at high temperature (Tables 4.1 and 4.9). Krishnamurthy *et al*. (2011) suggested that D50%F was delayed and DPM was hastened at high temperature, but Upadhyaya *et al*. (2011) observed early flowering and forced maturity. However, the present study and previous studies observed a shortened period of grain filling due to accelerated rate of plant development (Gan *et al*. 2004).

Table 4.11 The most heat tolerant and heat sensitive chickpea genotypes phenology, biomass and yield and the maximum and minimum temperatures (˚C) of chickpea developmental stages (genotypes data were obtained from the ANOVA table of two years, 2009-10 (Year 1) and 2010-11 (Year 2) and two environments (non-stressed (normal) and heat stressed (late))[a]

	DFF (days)	D50F (days)	DFP (days)	DPM (days)	Plant Biomass (g/plant)	Grain Yield (g/plant)	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
Heat tolerant - ICCV 98902												
Yr1												
Normal	36	42	44	100	21.1	11.0	29.2	17.5	28.5	13.8	27.9	14.2
Late	46	50	53	82	14.0	9.5	33.9	18.2	38.5	21.6	39.4	24.1
Yr2												
Normal	36	45	47	106	37.5	19.2	28.3	17.3	27.4	9.3	29.7	13.0
Late	35	38	42	102	46.5	27.2	32.1	16.5	35.7	17.3	36.7	22.3
Heat tolerant - ICCV 95311												
Yr1												
Normal	44	48	52	113	21.0	8.5	29.1	16.8	28.2	15.0	28.9	14.8
Late	40	44	46	87	15.2	4.5	33.7	18.0	35.9	19.2	39.1	23.8
Yr2												
Normal	40	47	49	108	36.6	18.6	28.1	16.2	27.8	11.7	29.8	13.1
Late	43	46	48	103	40.0	20.7	31.6	16.1	35.2	18.1	36.8	22.2
Heat sensitive - ICC 5566												
Yr1												
Normal	64	67	72	125	28.4	8.5	28.6	16	28.0	13.7	30.7	16.1
Late	66	70	72	106	5.1	0	35.2	19.3	40.5	25.1	39.5	25.3
Yr2												
Normal	61	68	68	113	23.0	6.1	27.9	14.2	29.7	10.7	30.9	15.0
Late	64	75	74	115	18.4	1.8	32.3	16.5	36.4	19.0	36.9	22.7
Heat sensitive - ICC 7570												
Yr1												
Normal	60	63	66	116	34.6	13.7	28.8	16.1	27.0	14.0	29.6	15.5
Late	55	59	60	100	8.8	0	34.6	18.8	37.4	21.2	39.5	24.9
Yr2												
Normal	66	69	74	115	26.5	4.3	28.0	13.9	29.9	11.6	31.3	15.6
Late	60	63	66	111	24.9	1.5	33.4	17.3	35.7	22.0	37.5	24.4

a –Max and min temperatures data for each geneotype are avialble in Appendix 6, 7, 8 and 9.

Controlled environment studies also suggested that high temperature (35˚C) during the grain filling period reduced grain yield more significantly than during early flowering (Wang *et al*. 2006; Summerfield *et al.* 1984). Under controlled conditions, high temperature accelerated the rate of senescence and shortened the duration of the reproductive period (Wang *et al*. 2006; Summerfield *et al.* 1984). Therefore the grain yield under heat stress was reduced due to lack of assimilate partitioning from leaves to seed (Wardlaw 1974).

The yield related traits most affected by temperature stress were pod number per plant and harvest index. These observations support the findings of Krishnamurthy *et al*. (2011). The advantage of earliness and the link between pod and seed number with eventual yield under heat stress suggests that manipulation of these traits will further improve yield in warmer environments. It is widely accepted that the genetic structure of grain yield can be better understood by studying linked quantitative traits (Guler *et al*. 2001), such as those identified in the current study.

Generally, high temperature reduces grain yield. High temperature during the grain filling period can reduce the individual seed size at maturity which may lower grain yield per plant (Ong 1983). Grain yield was reduced by 53-330 kg/ha for every 1˚C seasonal temperature rise in India (Kalra *et al*. 2008). In spring sown crops, the mean grain yield decreased compared with autumn sown materials due to seasonal temperature fluctuations (26-38˚C) during the reproductive stage (Ozdemir and Karadavut 2003). In Bangladesh, a six week delay in sowing from the optimum period was observed to reduce the grain yield by 40% and flowering and maturity was also accelerated (Ahmed *et al*. 2011).

Heat tolerant genotypes produced more grain yield than sensitive genotypes (Appendix 2 and 3). The HTI was high in heat tolerant genotypes which have advantages in earliness and yield potential under stress. Some of the released cultivars listed in Table 7 (ICCVs 98902, 98903, 95311, 92944, 07109, 07108 and 96836) are good sources of heat tolerance for crop improvement. ICCV 92944 is known to have heat tolerance from a previous study (Gaur *et al*. 2010). This line can be either deployed directly in farmers' fields or used as a parent in a plant breeding program. ICC 6969, ICC 14406 and ICC 16173 are potential new sources of heat tolerance from among the 167 genotypes tested.

Canopy temperature depression was used as a method of screening for heat tolerance in cereals (Rosyara *et al*. 2010). Canopy temperature was also used to identify a relationship between canopy conductance and transpiration rate under drought in chickpea (Zaman-Allah *et al*. 2011). Heat tolerant genotypes showed some degree of temperature depression. However, all stable and moderate heat tolerant genotypes did not show higher CTD than the stable heat sensitive lines in all the observations in both years (Tables 4.7 and 4.8). A similar response was found in wheat screened for heat tolerance in the field (Rosyara *et al*. 2010). The current experiment received frequent irrigation to avoid moisture stress which may have dispersed some of the heat load from the leaves.

Earlier work reported a lack of significant differences among chickpea genotypes for CTD under high temperature (Ibrahim 2011). In the current study, those genotypes which had lower CTD (1-3˚C) showed lower grain yield compared with higher canopy depression lines (>4˚C) (Figs 4.6 and 4.7). Generally CTD measurements require clear weather conditions and this is considered as a limitation for use in large scale field measurements.

Temperatures at different developmental stages of chickpea played an important role in crop performance under heat stress. Generally, grain filling period was reduced at high temperature. Maximum temperature during the vegetative and grain filling periods play an important role in the performance of chickpea genotypes under heat stress (Table 4.10). Overall, the stressed environment maximum temperatures varied 34-39˚C for year 1 and 32-37˚C for year 2. Clearly, temperatures >37˚C are the main reason for grain yield reduction. The maximum temperature also determines the critical temperature for each individual genotype. Table 4.12 showed that the critical temperature of two heat tolerant genotypes (ICCVs 98902 and 95311) was ≥38˚C. At and above this temperature, both genotypes suffered an average yield reduction of 50%. Generally, chickpea is a cool season legume and has a base level of heat tolerance compared with other cool season legumes (Malhotra and Saxena 1993). This experiment confirmed the findings of Malhotra and Saxena (1993) and validates the need to breed for heat tolerance to provide food security in warmer environments.

4.5. Conclusions

This study identified genetic variation in chickpea for a range of traits under temperature stress including crop phenology, plant growth and yield traits. Earlier developing genotypes tended to yield more under heat stress indicating the importance of heat escape among other possible mechanisms of tolerance. Generally, heat stress reduced plant biomass and grain yield. The most affected traits at high temperature were pod number per plant and harvest index. Temperature measured at different developmental stages explained the variability in grain yield. This research identified a group of heat tolerant and sensitive genotypes for further evaluation and use in chickpea improvement. The most heat tolerant genotype had a critical temperature of ≥38˚C, which indicates that chickpea is more heat tolerant than previously thought. In summary, chickpea improvement programs should consider plant growth characteristics, grain yield, various physiological traits including CTD and a stress index to improve selection for heat tolerance.

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Chapter 5: Reproductive biology of chickpea response to heat stress in controlled environments and in the field

5.1. Introduction

Whilst chickpea is considered to be a cool season legume, it is often grown where temperature exceeds >30˚C during the reproductive stage, which can limit yield (Summerfield *et al*. 1990). The negative effect of high temperature on grain yield is expected to increase due to global warming. A minimum decrease of 53 kg/ha of chickpea yield was observed in India per 1˚C increase in seasonal temperature (Kalra *et al*. 2008).

Heat stress during reproductive development in legumes is generally allied with lack of pollination and abscission of flower buds, flowers and pods, leading to substantial yield loss (Nakano *et al*. 1997, 1998; Duthion and Pigeaire 1991). For example, short periods (10 day) of high temperatures (≥ 35˚C) during early flowering and pod development of chickpea are known to cause significant reduction in pod number, seed set and grain yield (Wang *et al*. 2006). Grain yield reduction was due to high temperature effects on pre-anthesis, post-anthesis development and pollination. Nayyar *et al*. (2005) suggested that the male (pollen, anthers) and female (stigma-style, ovary) organs in chickpea are most sensitive to temperature stress. Therefore the period of anthesis and seed set may be critical with respect to high temperature tolerance (Summerfield and Wien 1980; Gross and Kigel 1994).

Male reproductive development and fertility are sensitive to heat (Sakata and Higashitani 2008). Anthers can be influenced by high temperature. Anther indehiscence occurs in cowpea (*Vigna unguiculata* L.) due to heat stress (33/30˚C) and is associated with degeneration of tapetal layer (Ahmed *et al*. 1992). The degeneration of tapetum cells was also found in common bean (*Phaseolus vulgaris* L.) at 33/29˚C (Suzuki *et al*. 2001), resulting in premature pollen development within the anther during early development. High temperature (33/27˚C) before anthesis can also cause anther indehiscence and pollen sterility in common beans (Gross and Kigel, 1994). In legumes, reductions in pollen production and fertility, and increases in pollen abnormalities (small, shrunken and empty pollen grains) occur during pre-anthesis at high temperatures. Warm night temperatures (28˚C) reduce pollen production in groundnut (*Arachic hypogaea* L.) (Prasad *et al*. 1999b) and associated with yield loss (Prasad *et al*. 1999a). Chickpea genotype ICC 5912 became sterile showing heat sensitivity with exposure at 35/20˚C a day before anthesis (1 DBA), but the pollen of chickpea genotype ICCV 92944 pollen was fertile at the same temperature (Devasirvatham *et al*. 2010). Halterlein *et al*. (1980) reported that pollen viability in common bean decreased when temperatures were held at 35/20˚C or 35˚C for a 24 h period just before anthesis. Shrunken pollen was observed at 38/30˚C in heat tolerant soybean (*Glycine max* L.) (Koti *et al*. 2005).

In legumes, post-anthesis high temperature effects are associated with poor pollen germination on the stigma and reduced pollen tube growth in the style (Talwar and Yanagihara 1999), failure of fertilisation (Ormrod *et al*. 1967) and embryo abortion (Gross and Kigel 1994). Chickpea pods will generally form 5-6 days after pollination in the field (Singh and Diwakar 1995), and on the 5th day in controlled environments (Bassiri *et al*. 1987). Generally the peak grain filling period is 20 days after end of flowering (Ozalkan *et al*. 2010) but varies with genotype and environment. However there is no evidence of parthenocarpic pod formation under abiotic stress in chickpea. Chickpea genotypes differ in response to heat based on physiological (photosynthesis and membrane stability) and biochemical (enzyme) mechanisms. For example membrane stability in chickpea was higher (> 40˚C) than pollen viability (≥ 35˚C) (Basu *et al*. 2009) at high temperature. To date, there is no published data linking these mechanisms to reproductive stage tolerance such as pollen viability, pod set, seed set and grain yield. Whilst under cool conditions the mechanism of pollen development and fertilisation in chickpea has been elucidated (Srinivasan *et al*. 1999; Clarke *et al*. 2004), it is not well understood at high temperatures. Recent study revealed that oxidative stress expressed as lipid peroxidation and hydrogen peroxide content in the leaves of heat sensitive chickpea genotypes creates failure of fertilization in controlled environments (Kumar *et al*. 2012).

Heat tolerant genotypes of chickpea were identified from field screening in India (Krishnamurthy *et al*. 2011; Upadhyaya *et al*. 2011), and heat tolerance in ICCV 92944 has been observed under field conditions (Gaur *et al*. 2010). Therefore ICCV 92944 was selected as a heat tolerant source and ICC 5912 as a relatively sensitive genotype. The development of pollen viability screens for high temperature stress has provided a useful tool for breeding temperature tolerant chickpea varieties. Two experiments were conducted to study the effect of high temperature on reproductive biology of chickpea. The aim of experiment – 1 was to determine if differences in high temperature effects on pollen viability, pollen germination, pollen tube growth and pod set can explain the relative heat tolerance/ sensitivity of ICCV 92944 and ICC 5912 chickpea genotypes in controlled environments.

Under semi-arid field conditions in south India, chickpea anthers dehisce between 08:00 and 10:00 h, followed by pollination and fertilization (Singh and Diwakar 1995). Therefore, high temperature during anthers dehiscence, pollen release and germination may severely affect male reproductive tissue more than ovules (Peet and Willits 1998). High temperature during flowering was also caused more sterility in male rather than female structures in other legumes (cowpea, bean and groundnut) (Hall 2004). However, the relationship between chickpea response to high temperature in the field and controlled environments is currently unknown. The aim of experiment – 2 was to determine chickpea response to high temperature by studying pollen viability, *in vitro* pollen germination, *in vivo* pollen germination, tube growth and pod set under high ambient temperature stress during the reproductive stage in the field and in the controlled environments and to detect genotypic differences.

5.2. Experiment – 1 (controlled environments only)

5.2.1. Materials and methods

Two controlled environment experiments were conducted with two chickpea genotypes (ICCV 92944 and ICC 5912) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India in 2010 and 2011. Plants of the two genotypes were grown in a controlled environment with five replications. Each replication has five plants i.e. one plant per pot. Three seeds of each genotype were sown in pots (2.4 L volume) containing a mixture of black vertisol soil, sand and vermi-compost (4:2:1 by volume), later seedlings were thinned to one per pot. Because the phenology of the two genotypes differs, they were sown on different dates to synchronise anthesis. The plants were grown at 27/16˚C in a greenhouse and transferred to a growth room to expose them to high temperature at the first appearance of flowers. The plants used as a non-stressed control continued to grow in the glasshouse at 27/16˚C under natural light. The temperature in the growth room was increased daily by 1˚C, e.g. 28 to 40˚C during the day and 16 to 25˚C during night (Table 5.1) in a square wave form. Therefore, the plants were exposed to a gradual increase in temperature to identify the critical temperature above which reproductive development begins to fail. The temperature was constantly maintained in the growth chamber with a 15 min transition period from day to night temperature and vice versa. The growth room had 72% input wattage of 1500-mA cool white fluorescent and 28% input wattage of Sylvania 50W-277V incandescent lighting. The light intensity (quantum) was about 320 µmol s^{-1} m^{-2} (Light meter model LI-189 from Li-Cor; USA) during 12 h photoperiod (08 to 20 h) and relative humidity was 75-80% in the growth chamber. Careful watering ensured that moisture was not a limiting factor in either temperature regime (day/night).

5.2.2. Pollen fertility studies

The effects of a one day exposure to day/night temperatures ranging from 31/16˚C to 40/25˚C one day before anthesis (termed pre-anthesis period) were studied to determine the critical temperature for pollen viability. Five flower buds were collected between 08:00 and 08:15 h from 31/16˚C to 40/25˚C to examine pollen viability during pre-anthesis. Non-dehiscent anthers were stained with Alexander's stain procedure-3 (Alexander 1969). The samples were examined under a compound microscope. The fertile pollen grains inside the anthers were red in colour whilst the sterile pollen grains were green. The differentiation of fertile and sterile pollen grain was found to depend on the pollen wall thickness and the chemical composition and pH of the stain. Malachite green was used to stain the pollen grain wall. Therefore, sterile pollen grains appeared green in colour. The protoplasm in the pollen grain was stained by acid fusion used in the Alexander's stain and hence it coloured the fertile pollen grain red to deep red (Alexander 1969). ICCV 92944 was examined at 40/25˚C (extreme temperature) for pollen viability. At 40/25˚C, the pollen viability in all 10 anthers of the heat tolerant genotype was observed using 2% acetocarmine stain and replicated three times. Each anther was squashed and mounted on a slide. Stained (fertile) and non-stained (sterile) pollen grains were counted and percentage of pollen fertility was determined.

5.2.3. *In vitro* pollen germination

In vitro pollen germination and tube growth were assessed using pollen germination medium (Mallikarjuna *et al*. 2007). Pollen germination (i.e. fertile and sterile pollen per flower) was counted at 35/20˚C and compared with the control at 27/16˚C. Two flowers per temperature treatment were collected the next morning between 08:00 and 08:15 h and each flower considered a replication. The available pollen grains in a flower were carefully transferred to the slide. The number of pollen grains (germinated and non-germinated) was counted in horizontal microscopic observation field using all pollen grains in the microscope field. Fifty such observations were made per replication in high temperature treatments. One hundred observations were made per replication (flower) in the control treatments. Therefore the average from the 50 or 100 observations per flower was considered to be a replication. The pollen production per flower was determined by summing the fertile (germinated) and sterile (non-germinated) pollen grains.

To evaluate the effect of different temperature regimes (33/18˚C to 40/25˚C) on pollen viability, the percentage of pollen germination was calculated. Under the high temperature treatment in pollen grains, the flower buds (one day before anthesis) were exposed from 33/18˚C to 38/23˚C and the flowers were collected the next day morning between 08:00 and 08:15 h and *in vitro* pollen germination and pollen tube growth determined. Two flowers per temperature treatment were collected and each flower considered a replication. The available pollen grains in a flower were carefully transferred to the slide. The *in vitro* pollen germination was terminated after 60 min incubation by adding a drop of 2% acetocarmine stain. Pollen grains were counted as germinated when pollen tubes were at least equal to the diameter of the pollen grain by the random microscopic field observation method. Percent pollen germination was determined by using all pollen grains in a microscope field as a pseudo replication; 15 such observations were made. The average from the 15 observations per flower was considered to be a replication. Therefore two replications per temperature treatment were used to calculate pollen germination.

5.2.4. *In vivo* pollen germination and tube growth

Similar to experiment-1 the temperature was increased daily by 1˚C, e.g. 28 to 35˚C during the day and 16 to 20˚C during night (Table 1). Hand pollination was carried out between 08:00 and 08:30 h at 35/20˚C and 27/16˚C to examine *in vivo* pollen germination and tube growth. Stressed stigma x stressed pollen and stressed stigma x non-stressed pollen crossing was done in the growth chamber. Non-stressed stigma x stressed pollen crossing was done in the glasshouse. Reciprocal crosses (stressed x non-stressed; non-stressed x stressed) were carried out to determine the site of sensitivity; whether it was the pollen or the stigma responsible for high temperature stress susceptibility. The following crosses were made

i. Stressed stigma (35/20˚C) x stressed pollen (35/20˚C) within the same genotypes
ii. Stressed stigma (35/20˚C) x stressed pollen (35/20˚C) between the genotypes
iii. Non-stressed stigma (27/16˚C) x stressed pollen (35/20˚C) between the genotypes
iv. Non-stressed stigma (27/16˚C) x stressed pollen (35/20˚C) within the same genotypes
v. Stressed stigma (35/20˚C) x non-stressed pollen (27/16˚C) within the same genotypes

Seven flowers were pollinated per one crossing combination and each flower was considered a replication. Each stigma (flower) was pollinated with pollen grains from one flower. Therefore seven flowers per one crossing combination were pollinated with seven different flowers from the male parent (i.e. different plants). The flower samples were collected 15 min and 30 min after pollination to observe pollen germination on the stigma and pollen tube growth down the style. The flowers were fixed for 24 h in 80% alcohol. The pistils (styles and ovary) were removed from the flowers, cleared with 6 N NaOH for 48 h and thoroughly rinsed with water. The pistils were stained with aniline blue and observed under a fluorescence microscope.

5.2.5. Pod set

All open flowers in the high temperature treatments (32/17˚C to 40/25˚C) were tagged to observe the pod set. Pod formation and withered pods were recorded on 7th day from tagging of the open flower.

5.2.6. Statistical analysis

Analysis of variance (ANOVA) was performed for flower and anther data, pollen germination count and percentage of different temperature regimes using Genstat 12th Ed. VSN International Ltd. One way ANOVA was conducted at for ICCV 92944 at 40/25˚C for all 10 anthers with three replications. Two way ANOVA (genotype × temperature) was conducted with two replications for pollen production per flower and pollen germination (%). For floral morphology (%) and pod characters, two way ANOVA (genotype × temperature) was performed with five replications to study the difference between heat stressed and non-stressed conditions.

5.3. Experiment – 2 (field and controlled environments)

5.3.1. Materials and methods

Field

Two field experiments were conducted with four chickpea genotypes, two heat tolerant (ICC 1205 and ICC 15614) and two heat sensitive (ICC 4567 and ICC 10685) at ICRISAT, Patancheru, India in 2011. The genotypes were selected based on the grain yield and heat tolerance index (HTI) from the reference collection of 280 genotypes for heat tolerance screening by Krishnamurthy *et al*. (2011). A randomised complete block design with four replications was used in the late season planting (Feb) on a Vertisol soil to utilise the high temperature stress at the reproductive stage. Seed treatment and experimental plot maintanence was explained in chapter 4. Seeds of the four genotypes were sown with a plant spacing of 60 × 15 cm. A 4 m long row was considered as a replication plot. The phenology of the four genotypes was different. To overcome this issue, two staggered sowings (14 days apart) were planted. The crop received irrigation on 0, 20, 28, 35, 45, 55 and 65 days after sowing.

The samples for pollen fertility and *in vivo* pollen germination were collected between 50% of flowering stage and 10 consecutive days of maximum reproductive period. Pod set was subsequently recorded. The maximum and minimum temperature of the growing period was recorded daily. The vegetative period was exposed to respective maximum and minimum temperatures of 30-35˚C and 17-20˚C. The maximum and minimum temperature varied between 35-40.4˚C and 17-27˚C during the reproductive period, respectively. The procedures of sample collection, pollen staining to identify fertile pollen grains, *in vitro* pollen germination and *in vivo* pollen germination used to assess the field experiments were similar to those used under controlled conditions.

Controlled environments

Two controlled environment experiments were conducted with four chickpea genotypes (ICC 4567; ICC 10685; ICC 1205 and ICC 15614) at ICRISAT, Patancheru, India in 2010 and 2011 with five replications. Soil preparation, sowing, maintenance of the controlled environment growth room, and the movement of plants from the control to the high temperature growth room including high temperature treatments was explained in Experiment – 1.

5.3.2. Pollen fertility studies

Field

The effect of high temperature (38/25.2˚C) was studied in flower buds (1 DBA) (pre-anthesis) and open flowers (anthesis) with Alexander's stain to determine pollen fertility. Five flower buds and five open flowers were collected between 08:00 and 08:15 h to examine pollen fertility. Anthers were stained with Alexander's stain (Alexander, 1969) and examined under a compound microscope. The fertile pollen grains inside the anthers were stained red whilst the sterile pollen grains remained green. To confirm the fertility, the anthers were squashed from 1 DBA flower buds and stained with 2% acetocarmine. Pollen grains from open flower samples were stained with 2% acetocarmine (data not shown).

Controlled environments

The effects of one day exposure to day/ night temperatures of 31/16˚C and 40/25˚C one day prior to anthesis were studied to determine the critical temperature. Five flower buds were collected between 08:00 and 08:15 h to examine pre-anthesis pollen fertility. Anthers were stained with Alexander's stain and examined under a compound microscope.

5.3.3. *In vitro* pollen germination and tube growth

Field

The flower samples were collected at 35.2/24˚C; 36.4/23.4˚C; 37.1/21.8˚C and 38/25.2˚C day/ night temperatures. Open flowers were collected in the morning between 08:00 and 08:15 h to examine *in vitro* pollen germination and pollen tube growth. The *in vitro* pollen germination was terminated after 60 min incubation by adding a drop of 2% acetocarmine stain. Two replications per temperature treatment were used to calculate pollen germination.

Controlled environments

The percentage of germinated pollen grains was calculated to estimate the effect of temperature regime (33/18˚C to 40/25˚C) on pollen viability. The flowers were collected daily between 08:00 and 08:15 h from the 33/18˚C and 40/25˚C treatments to examine *in vitro* pollen germination and pollen tube growth.

5.3.4. *In vivo* pollen germination and tube growth

Field

Hand pollinations were carried out to obtain *in vivo* pollen germination and tube growth. Crosses (stressed stigma x stressed pollen) were conducted at 38/25.2˚C and 39/25.2˚C day/ night temperature treatments. Reciprocal crosses (stressed stigma x non-stressed pollen 40.2/25.5˚C x 27/16˚C) were carried out to determine the stigma receptivity for high temperature stress. In summary, the following crosses were made

vi. Stressed stigma x stressed pollen within the same genotype
vii. Stressed stigma x non-stressed pollen within the same genotype

Seven flowers were collected 30 min after pollination to observe pollen germination on the stigma and pollen tube growth down the style. The flowers were fixed for 24 h in 80% alcohol.

Controlled environments

Hand pollination was carried out at 35/20˚C day/night temperature to obtain *in vivo* pollen germination and tube growth. Reciprocal crosses (stressed x non-stressed; non-stressed x stressed) were carried out to determine which tissue; pollen or stigma is most responsive to high temperature stress. The following crosses were made

viii. Stressed stigma (35/20˚C) x stressed pollen (35/20˚C) within the same genotype
ix. Non-stressed stigma (27/16˚C) x stressed pollen (35/20˚C) within the same genotype
x. Stressed stigma (35/20˚C) x non-stressed pollen (27/16˚C) within the genotype

A similar procedure of *in vivo* pollen germination to that used in the field was followed in the controlled environments.

5.3.5. Pod set

Field

Pod set was observed at 50% of flowering, and for 10 consecutive days post this date by tagging 20 flowers daily. Pod set was counted for each flower seven days after anthesis.

Controlled environments

Flowers grown between 32/17˚C and 40/25˚C were tagged to observe the pod set.

5.3.6. Ovule observation

The numbers of ovules in an ovary were counted in the field at 39.4/27.2˚C and compared with the non-stressed treatment (27/16˚C) of the controlled environment experiment.

5.3.7. Statistical analysis

Analysis of variance (ANOVA) was performed for flower and pod data, pollen germination percentage of different temperature regimes using Genstat 12th Ed. VSN International Ltd. For both experiments, two way ANOVA (genotype × temperature) was conducted with two replications for pollen germination (%). Similarly two way ANOVA (genotype × temperature) was performed for floral morphology (%) with five replications to study the difference between temperature treatments in the controlled environments. For grain yield, pod characters and plant biomass at harvest in the field was studied with four replications using one way ANOVA. A curve relating % pod set to high temperatures (day/night) was generated.

5.4. Results

5.4.1. Experiment – 1 (controlled environments only)

5.4.1.1. Pollen fertility studies

The one day exposure of flower buds to high temperature influenced pollen fertility. In ICC 5912, the pollen grains inside the anthers were fertile up to 34/19˚C (red colour pollen grains) (Fig 5.1a). However, at 34/19˚C, anthers showed abnormalities (Fig 5.2). Increased numbers of locules in the anther indicated heat damage. At 34/19˚C, the sensitive genotype ICC 5912 had more than three locules (Figs 5.2a, b, and c). High temperature affected the pollen sterility (Figs 5.2a, b).

The anthers contained a mixture of fertile and sterile pollen grains (partial sterility) and viable pollen within the anther was found at 34/19˚C (Figs 5.2e, f). However, at 35/20˚C, pollen grains inside the anther of ICC 5912 became sterile (Fig 5.1b). The sterile pollen grains were uniformly distributed in all 10 anthers of a flower in ICC 5912 at 35/20˚C. At 40/25˚C, the anthers were devoid of pollen grains (Fig 5.1c). In addition, there was no pod set in ICC 5912 at 35/20˚C, indicating that this is the critical temperature for cessation of pod set in this genotype.

Table 5.1 Details of temperature regime, pre-anthesis, and post-anthesis of chickpea flower collection under controlled environments

Days	Temp regime (day and night - °C)	Flower buds collected one day before anthesis to check critical temperature (pollen viability)	Pollen germination and tube growth using medium	Post-anthesis (pod set) observation	Hand pollination to study pollen germination and pollen tube growth
Day 1	29/16	-	-	-	-
Day 2	30/16	-	-	-	-
Day 3	31/16	-	-	-	-
Day 4	32/17	-	-	√	-
Day 5	33/18	√	√	√	-
Day 6	34/19	√	√	√	-
Day 7	35/20	√	√	√	√
Day 8	36/21	√	√	√	-
Day 9	37/22	√	√	√	-
Day 10	38/23	√	√	√	-
Day 11	39/24	√	-	-	-
Day 12	40/25	√	-	-	-

Note: The symbol √ indicates the day of sample collection

Pollen grains of ICCV 92944 remained fertile at 36/21˚C (Fig 5.3c). Though ICCV 92944 had reduced the pod set, pollen fertility was observed up to 38/23˚C (Fig 5.3e). However, at 36/21˚C, few anthers showed indehiscence due to anther wall thickness (Figs 5.3c, d, and e). At 40/25˚C, the pollen grains inside the anther were partially sterile in ICCV 92944 as observed with Alexander's stain (Fig 5.3f). Among 10 anthers observed, two were completely sterile (Table 5.2). The sterility in the remaining eight anthers ranged from 85 to 97%. The temperature rise from 35/20˚C to 40/25˚C led to complete pollen grain sterility (P<0.05). ICCV 92944 ceased to flower, moved into a pod development phase and showed symptoms of leaf yellowing (maturity), while the heat sensitive genotype ICC 5912 retained green leaves and continued to flower without pod setting.

5.4.1.2. *In vitro* pollen germination and tube growth

High temperature (35/20˚C) reduced pollen production and pollen fertility (Tables 5.3 and 5.4). Generally at high temperature (38/23˚C) the sterile pollen was high (91%) (Table 5.4). In ICCV 92944, pollen germination (%) and pollen production at 35/20˚C was reduced by more than 50% compared with the optimum temperature (27/16˚C). At 35/20˚C in ICC 5912, the percentage of pollen germination was zero; only sterile pollen was produced (Table 5.4). Therefore high temperature influenced pollen fertility. At 34/19˚C, ICC 5912 had both normal (straight, smooth and cylindrical) (Fig 5.4a) and stressed/ abnormal (zigzag) tubes (Fig 5.4b), which suggested inhibition of pollen tube growth at high temperature. Pollen germination within the anther may be a sign of heat stress (Fig 5.4c) and was observed before reaching the critical temperature (35/20˚C). 39% sterile pollen of ICC 5912 was found at 34/19˚C (Table 5.4; Figs 5.4d, e, and f).

The pollen germination (%) in ICCV 92944 declined from 64% at 33/18˚C to 19% at 38/23˚C (P<0.001) (Table 5.4; Figs 5.5a to f). Overall pollen germination was 9% at 38/23˚C (Table 5.4). However, length of pollen tube growth varied from 35/20˚C (Fig 5.5c) and bursting of pollen grains including release of protoplasm occurred at 38/23˚C (Fig 5.5f). Therefore the high temperature of 38/23˚C was not conducive for pollen tube growth.

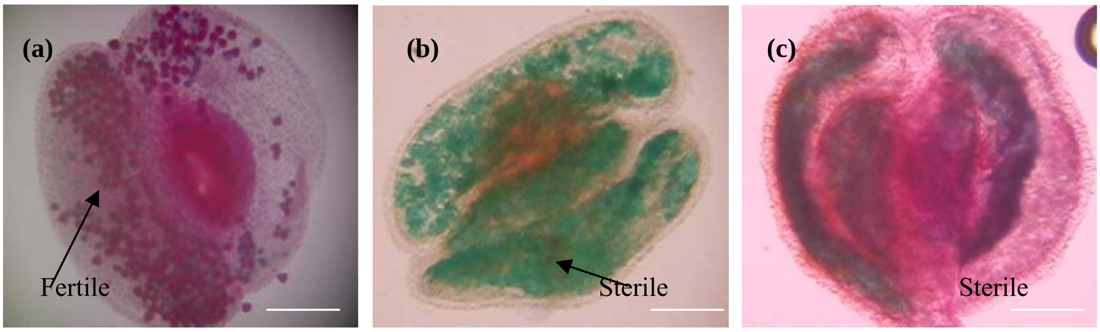

Fig 5.1 Anthers of ICC 5912 (a) 34/19˚C (pollen grains are fertile) (b) 35/20ºC (pollen grains are sterile) (c) 40/25˚C (pollen grains are sterile) (Fertile pollen grains are red; Sterile pollen grains are green) (Bars = 10 µm)

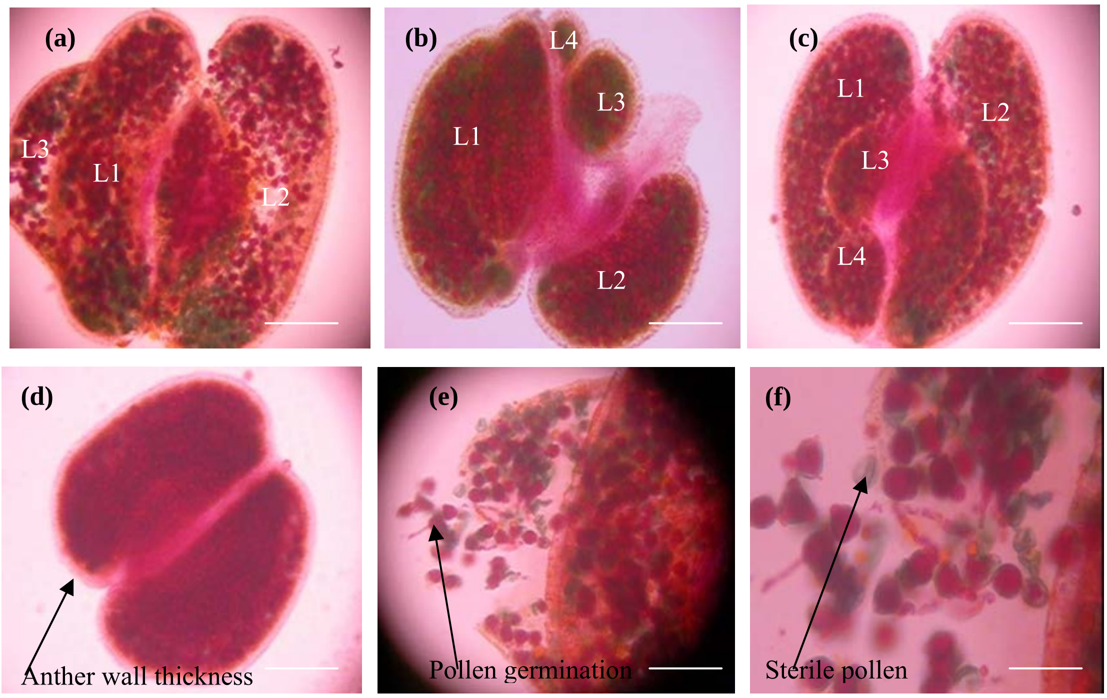

Fig 5.2 ICC 5912 – 34/19˚C (a), (b), (c)-Abnormalities of anthers at 34/19˚C – changes in number of locules (d)-Anther wall is thick (e) and (f)-Pollen germination within the anther without germination medium, L-Locules. (Bars = 10 µm)

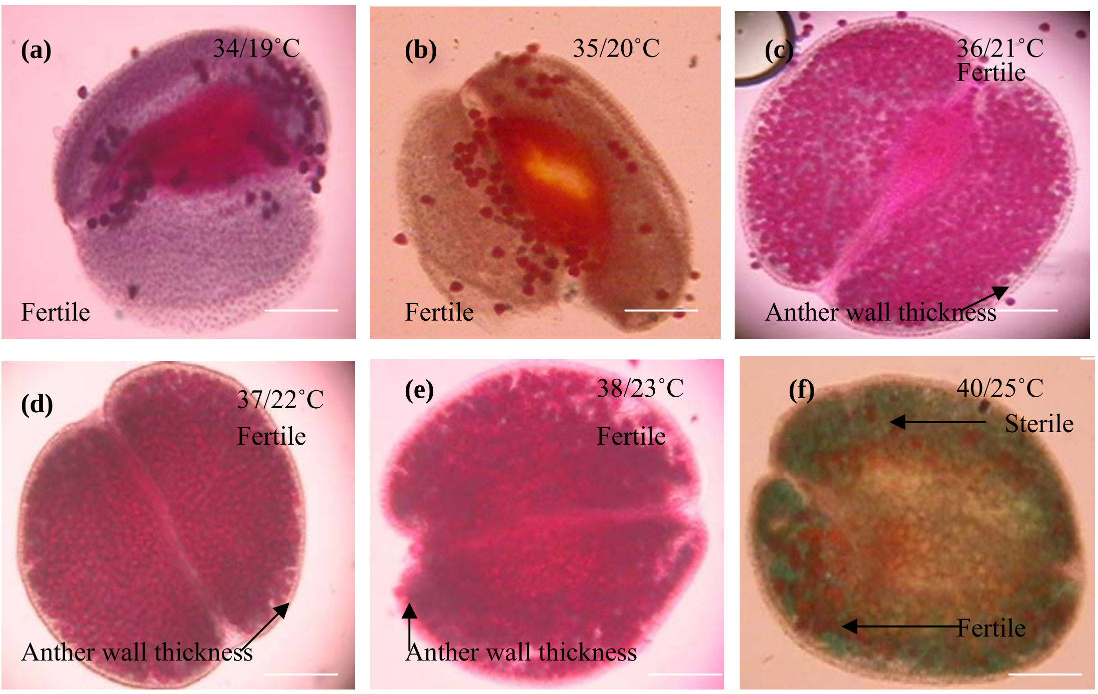

Fig 5.3 Anthers of ICCV 92944 (a) 34/19˚C (pollen grains are fertile). (b) 35/20˚C (pollen grains are fertile. (c) 36/21˚C (pollen grains are fertile). (d) 37/22˚C (pollen grains are fertile). (e) 38/23˚C (pollen grains are fertile). (f) 40/25˚C (pollen grains are fertile and sterile) (Bars = 10 µm)

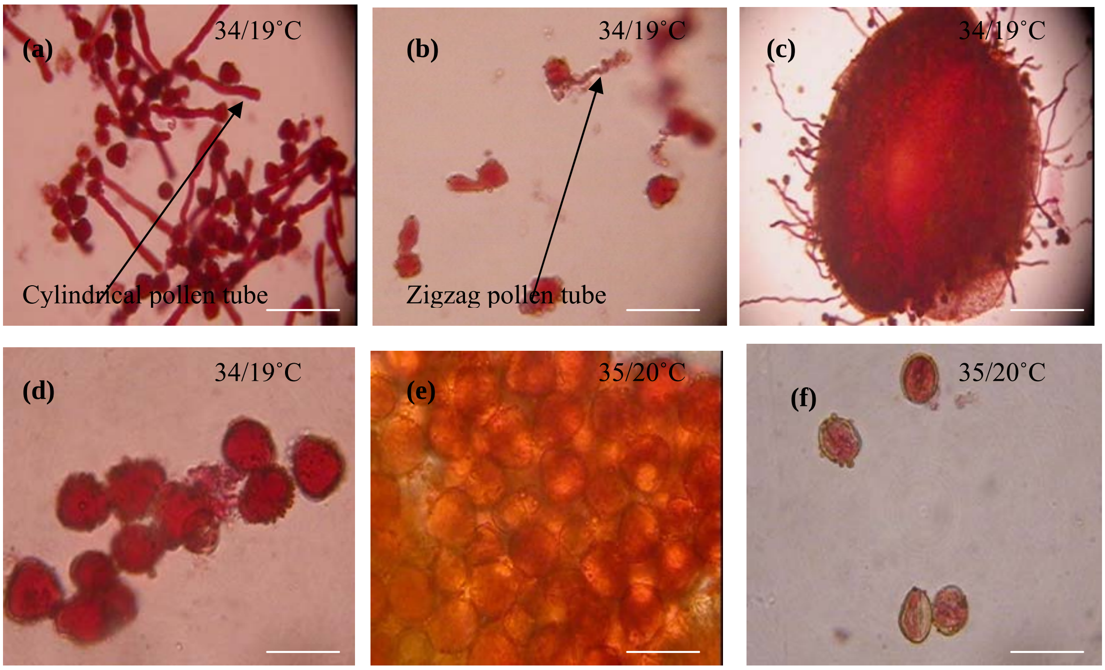

Fig 5.4 ICC 5912 – in vitro pollen germination with medium after 60 min incubation (a) Normal (cylindrical) pollen tube growth at 34/19˚C (b) Abnormal (zigzag) pollen tube growth at 34/19˚C (c) Germinated pollen grains in the anther at 34/19˚C (indehiscence of anther) (d) Non- germinated pollen grains at 34/19˚C (e) and (f) Non-germinated pollen grains at 35/20˚C (Bars = 10 µm)

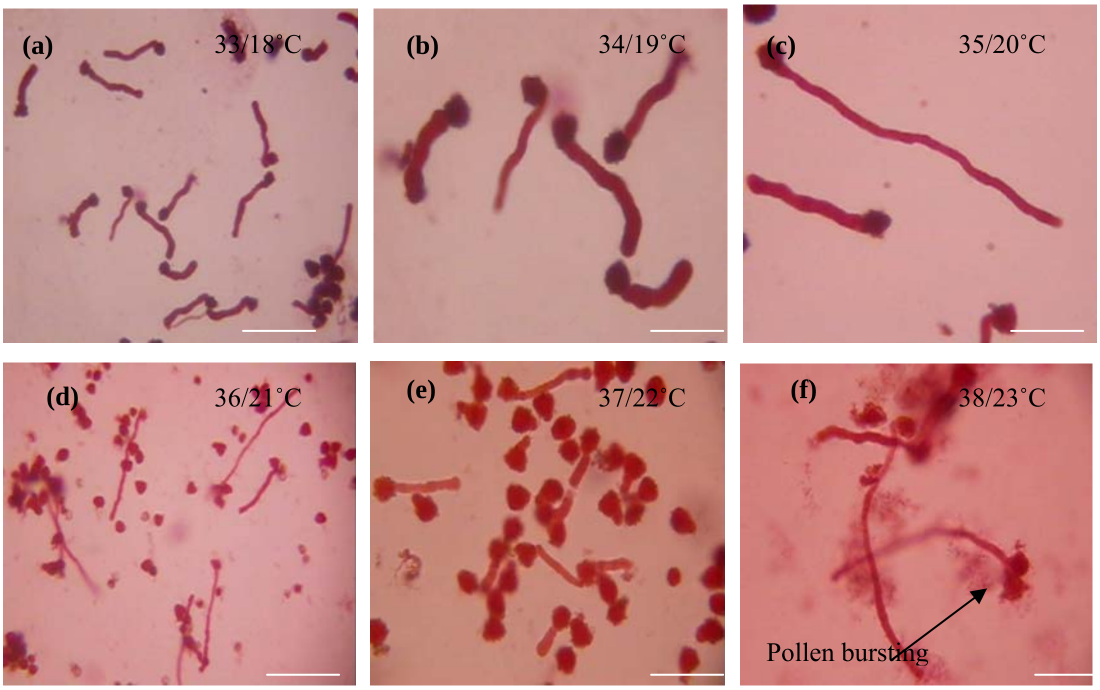

Fig 5.5 ICCV 92944 – in vitro pollen germination with medium after 60 min incubation (a) Germination at 33/18˚C (b) Germination at 34/19˚C (c) Germination at 35/20˚C (d) Germination at 36/21˚C (e) Germination at 37/22˚C (f) Germination at 38/23˚C (Bars = 10 µm)

Table 5.2 Effect of high temperature on pollen viability (%) within the anther (using 2% Acetocarmine stain) for two chickpea genotypes under controlled environments

Genotypes	Temperature (day /night) ˚C	Anther-1		Anther-2		Anther-3		Anther-4		Anther-5		Anther-6		Anther-7		Anther-8		Anther-9		Anther-10	
		F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S
ICCV 92944	35/20	86	14	93	7	91	9	91	9	93	8	94	6	93	7	90	11	95	9	91	9
ICCV 92944	40/25	30	70	26	74	15	85	13	87	5	95	12	88	0	100	5	95	10	90	0	100
ICC 5912	35/20	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100
ICC 5912	40/25	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Genotype effect																					
ICCV 92944		58	42	59	41	53	47	52	48	49	51	53	47	47	54	48	53	52	48	46	55
ICC 5912		0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Temperature effect																					
35/20		43	57	46	54	46	54	46	55	46	54	47	58	47	54	45	55	48	53	46	55
40/25		15	85	13	87	8	93	7	94	3	98	6	94	0	100	3	98	5	95	0	100
LSD(*P*=0.05)																					
Genotype		27.9*	10.1*	19.2*	19.2*	17.9*	6.5*	12.8***	12.4***	8.5***	8.5***	9***	9***	2.8***	2.8***	8.9***	8.5***	14.8*	14.8*	11.1***	11.1***
Temperature		NS	NS	19.2*	19.2*	17.9*	6.5*	12.8*	12.4***	8.5***	8.5***	9***	9***	2.8***	2.8***	8.9***	8.5***	14.8***	14.8***	11.1***	11.1***
Genotype x Temperature		NS	NS	27.1*	27.1*	25.3*	9.2*	18.1*	17.5***	12.0***	12.0***	12.7***	12.7***	3.9***	3.9***	12.6***	12.0***	20.9*	20.9*	15.8***	15.8***

Note: F-Fertile; S-Sterile (***, * significant at $P<0.001$, $P<0.05$ and NS-Not Significant)

Table 5.3 Evaluation of high and optimum temperature on pollen germination count (total pollen production per flower) for two chickpea genotypes under controlled environments

Genotype	Germinated pollen		Sterile pollen		Mean of genotype for germinated pollen	Mean of genotype for sterile pollen
	35/20˚C	27/16˚C	35/20˚C	27/16˚C		
ICCV 92944	2569	4850	124	582	3709	353
ICC 5912	0	3450	2254	411	1725	1332
Mean of temperature	1284	4150	1189	496		
LSD (*P*=0.05)						
Temperature	1299.8*		542*			
Genotype	1299.8*		542*			
Temperature x Genotype	NS		767*			

(* significant at *P*<0.05 and NS-Not Significant)

Table 5.4 Pollen germination (%) of two chickpea genotypes at different high temperature regimes under controlled environments

Temperature Regimes (˚C)	Pollen Germinated (PG)		Pollen Non-germinated (PNG)		Mean	
	ICCV 92944	ICC 5912	ICCV 92944	ICC 5912	PG	PNG
27/16	89	85	11	15	87	13
33/18	64	65	36	35	65	35
34/19	60	39	40	61	50	50
35/20	41	0	59	100	20	80
36/21	26	0	74	100	13	87
37/22	22	0	78	100	11	89
38/23	19	0	81	100	9	91
Mean	46	27	54	73		
LSD(*P*<0.05)						
Temperature	11.86 ***		3.55 ***			
Genotype	6.34 ***		1.90 ***			
Temperature x Genotype	16.77 *		16.77 *			

(*** significant at *P*<0.001 and NS-Not Significant)

5.4.1.3. Flower production and pod set in different temperature regimes

Flower formation was reduced at high temperature compared with optimum temperature with the number of flowers reduced (*P*<0.001) from 10 (27/16˚C) to three (38/23˚C). ICC 5912 did not set pods at 35/20˚C (Table 5.5). The seed set % was also zero in ICC 5912 at 35/20˚C. In contrast, ICCV 92944 continued to set pods up to 38/23˚C, but % pod set in ICCV 92944 was reduced along with flower formation. Therefore the seed was low in ICCV 92944 at high temperature.

Table 5.5 Floral morphology (%) of two chickpea genotypes at different high temperature regimes under controlled environments

Temperature Regimes(˚C)	**No of flowers (FL)**		**% of dry flowers (DF)**		**% of pod set (PS)**		**% of seed set**		**Mean of temperature regimes**		
	ICCV92944	ICC5912	ICCV92944	ICC5912	ICCV92944	ICC5912	ICCV92944	ICC5912	DF	PS	F
27	11	10	4	4	96	96	77	87	4	96	10
32	14	4	18	29	82	71	60	55	24	76	9
33	11	4	10	58	91	42	75	42	34	66	7
34	6	5	28	60	72	40	45	50	44	56	5
35	4	5	29	100	71	0	71	0	65	35	4
36	6	4	82	100	18	0	18	0	90	9	5
37	5	3	80	100	20	0	20	0	90	10	4
38	2	4	50	100	50	0	25	0	75	25	3
Mean of genotype	7	5	38	69	62	31	49	29			
LSD(P<0.05)											
Temperature	2.00***		8.83***		8.83***		20.8***				
Genotype	1.00***		4.41***		4.41***		10.4*				
Temp x Genotype	2.82NS		12.49***		12.49***		29.4NS				

(*** significant at P<0.001; * significant at P<0.05and NS-Not Significant)

5.4.1.4. Pod characters and plant biomass under high temperature

At high temperature stress, number of pods/plant, filled pods/plant and seeds/plant was reduced (*P*<0.001) from 41, 37, 37 (27/16˚C) to 15, 11 and 13 (heat stress) respectively (Table 5.6). Despite seeds being formed at high temperature stress, most of the filled pods or seeds were formed before the temperature reached 35/20˚C. Generally, the flower production was reduced after 36/21˚C. The pollen grains of ICC 5912 were fertile at 35/20˚C. The plant biomass was also reduced from 15.3 (27/16˚C) to 10.9 g/plant under heat stress. Therefore high temperature reduced grain yield and biomass accumulation.

5.4.1.5. *In vivo* pollen germination and tube growth

i. Stressed stigma (35/20˚C) x stressed pollen (35/20˚C) within the same genotype

Pollen germination and pollen tube growth was found in ICCV 92944 at 35/20˚C (Figs 5.6 panel-1 a, b). Though the pollen tube had callose[2] formation, the tube reached the base of the style at 30 min (Fig 5.6 panel-1 c). However there was no pollen germination on the stressed stigma of ICC 5912 at 35/20˚C.

ii. Stressed stigma (35/20˚C) x stress pollen (35/20˚C) between the genotypes

Stressed pollen from ICC 5912 did not germinate on the stigma of ICCV 92944 (Fig 5.6 panel-2 d), but stressed pollen grains from ICCV 92944 germinated on the stressed stigma of ICC 5912 and formed uneven pollen tube growth after large callose formation under stress (35/20˚C) (Fig 5.6 panel-2 e). After 30 min the callose deposition was reduced in the style (Fig 5.6 panel-2 f).

iii. Non-stressed stigma (27/16˚C) x stressed pollen (35/20˚C) between the genotypes

The stressed pollen from ICC 5912 did not germinate on the non-stressed stigma (27/16˚C) of ICCV 92944 (Fig 5.6 panel-3 g). However, the stressed pollen from ICCV 92944 germinated on the non-stressed stigma of ICC 5912 (Figs 5.6 panel-3 h, i). After 30 min reduced callose formation was found in the non-stressed style (27/16˚C) of ICC 5912.

iv. Non-stressed stigma (27/16˚C) x stressed pollen (35/16˚C) within the same genotype

In ICCV 92944, the stressed pollen (35/20˚C) was germinated (Fig 5.6 panel-4 j) on the non-stressed stigma (27/16˚C) with smooth tube growth 30 min after pollination

[2] Callose, an amorphous, colourless substance, is a β-1, 3-polyglucan composed of β-D glucopyranose residues. It is insoluble in water and ethanol. Callose forms along the inner pollen tube membrane and restricts tube cytoplasm (Stanley and Linskens, 1974).

(Fig 5.6 panel-4 k). However, in ICC 5912 the stressed pollen (35/20˚C) did not germinate on its non-stressed stigma (27/16˚C) (Fig 5.6 panel-4 l).

v. Stressed stigma (35/20˚C) x non-stressed pollen (27/16˚C) within the same genotype

In ICCV 92944, the non-stressed pollen (27/16˚C) was germinated on the stressed stigma (35/20˚C) (Fig 5.6 panel-5 m). Similar germination was found on the non-stressed ICC 5912 stigma (Fig 5.6 panel-5 n). The pollen tube growth was smooth with little callose deposition near the ovary (Fig 5.6 panel-5 o).

Table 5.6 Pod characters (per plant) and biomass (per plant) of two chickpea genotypes at high temperature stress under controlled environments

Genotypes	Treatment	Total no of pods	No filled pods	No of seeds	Biomass (g/plant)
ICCV 92944	Control	38	33	35	13.1
ICCV 92944	Heat stress	15	14	15	6.3
ICC 5912	Control	44	40	39	17.4
ICC 5912	Heat stress	14	8	11	15.6
Mean Control		41	37	37	15.3
Mean Heat stress		15	11	13	10.9
LSD (*P*=0.05)					
Temperature		9.2***	7.8***	8.4***	2.8*
Genotype		9.2NS	7.8NS	8.4NS	2.8***

(*** significant at *P*<0.001; * significant at *P*<0.05and NS-Not Significant)

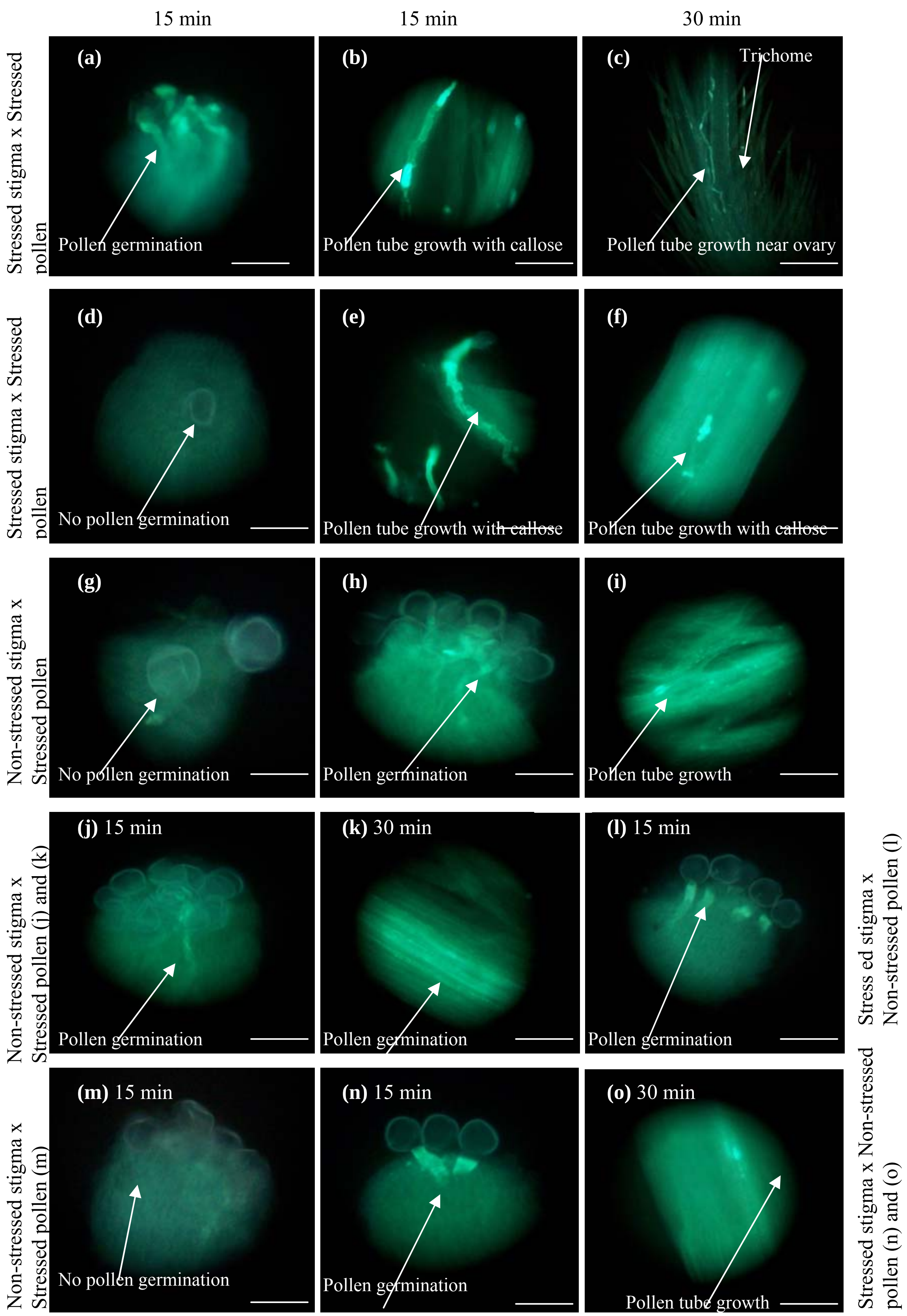

Fig 5.6 Effect of high temperature on pollen germination and tube growth panel 1 - ICCV 92944; panels 2 (d) and 3 (g) - ICC 92944 x ICC 5912; panel 2 (e); (f) and panel 3 (h) and 3(i) - ICC 5912 x ICC 92944; panel 4 - ICCV 92944 and panel 5 - ICC 5912 (Panel was created in horizontal direction) (Bar=10 µm)

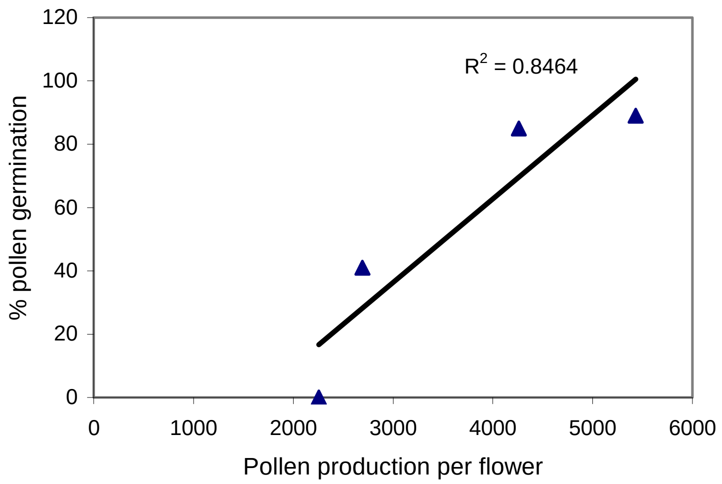

Fig 5.7 Relationship between pollen production per flower and % pollen germination (pooled data of 27/16˚C and 35/20˚C day/night of ICCV 92944 and ICC 5912 from Table 3 and Table 4)

5.4.2. Experiment – 2 (field and controlled environments)

5.4.2.1. Field response

Pollen fertility studies

The genotype ICC 4567 exhibited abnormal anthers when exposed to 38/25.2˚C before anthesis (Fig 5.8a) and during anthesis (Figs 5.8e, f). Increased numbers of locules were observed before anthesis (Fig 5.8a) and at anthesis (Fig 5.8e). Fig 5.8a and Figs 5.8e, f showed the epidermis wall thickening in response to increasing temperature. The epidermis wall thickening was a likely cause of indehiscence and partial dehiscence at anthesis (Fig 5.8a). A mixture of fertile and sterile pollen grains was observed after exposure to high temperature during pre-anthesis (Fig 5.8a) and this coincides with appearance of pronounced anther indehiscence at anthesis (Fig 5.8e). However, dehiscent anthers mixed with fertile and sterile pollen grains were observed in ICC 4567 during anthesis at 38/25.2˚C. ICC 10685 produced fertile pollen grains (Fig 5.8b) but epidermis wall thickening and reduced pollen number was observed after high (38/25.2˚C) pre-anthesis temperature. On the other hand, the line ICC 10685 produced dehiscent anthers at anthesis (Fig 5.8g). Fertile pollen grains were found in the anthers of ICC 1205 (Fig 5.8c) and these dehisced at 38/25.2˚C during anthesis (Fig 5.8h). Although the anthers of ICC 15614 dehisced at 38/25.2˚C during anthesis (Fig 5.8i), the anther had few sterile pollen grains. With acetocarmine stain all genotypes showed similar results to those observed using Alexander's stain (data not shown). In addition, the pollen cytoplasm of ICC 10685 appeared to be released at 38/25.2˚C during anthesis (data not shown).

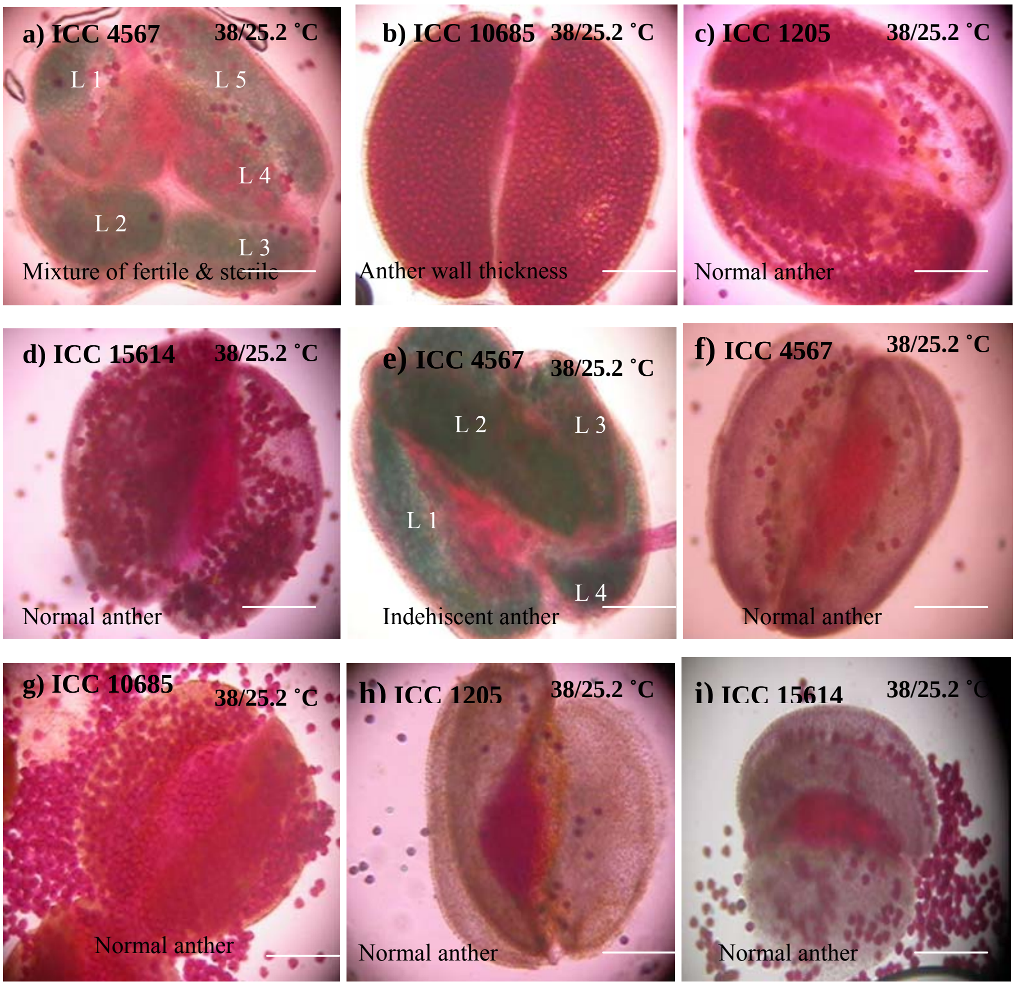

Fig 5.8 Anthers respond to 38/25.2˚C in the field (Sample were stained with Alexander's stain). Figs (a) to (d) are high pre-anthesis temperature and (e) to (i) are high anthesis temperature (open flowers) (L-Locules) (Bar=10 µm)

In vitro pollen germination and tube growth

Pollen fertility was observed at 35.2/24˚C; 36.4/23.4˚C; 37.1/21.8˚C and 38/25.2˚C day/ night temperatures. Increasing temperature gradually reduced pollen fertility (%) (Table 5.7). Pollen fertility in ICC 4567 was 7%, 6% and 0% at 35.2/24˚C, 36.4/23.4˚C and 37.1/21.8˚C, respectively (Fig 5.9). There were also changes in pollen grain size observed and the stain entered into the structurally abnormal (size variation) pollen grain. In ICC 10685 fertility decreased from 16% (35.2/24˚C) to 11% (38/25.2˚C). In ICC 1205 and ICC 15614 pollen fertility was 55% at 35.2/24˚C, however, this decreased to 33% in ICC 1205 and 22% in ICC 15614 at 38/25.2˚C. Percentage pollen germination across genotypes (fertility) was lower ($P < 0.001$) with every 1˚C increase ($P < 0.05$), but no difference was measured between genotypes and temperature when samples were examined at 60 min incubation period.

Pod set

Genotypic differences observed for pollen fertility under high temperature were consistent in pod set (Figs 5.10 and 5.11). The temperature range in staggered sowing-1 was 35.6/17.4 to 37.5/17˚C (day/night). The tolerant genotypes ICC 1205 and ICC 15614 produced 65% and 41% of mean pod set respectively. In contrast, the sensitive genotypes ICC 4567 and ICC 10685 produced 4% and 1%, respectively. The temperature range in staggered sowing-2 was 33.7/23.4 to 39/25.2˚C day/night temperatures. Within this range, ICC 1205 produced 50% pod set, followed by ICC 15614 (39%). Pod set on the sensitive genotypes ICC 4567 and ICC 10685 was 3% and 1%, respectively. In both sowings, ICC 1205 set more pods (65%; 50%) than other genotypes followed by ICC 15614 (41%; 39%). The sensitive genotype ICC 4567 produced a pod set of 4% and 3%, for the first and second sowing, respectively. ICC 10685 was the most heat sensitive genotype which with 1% pod set in both sowings.

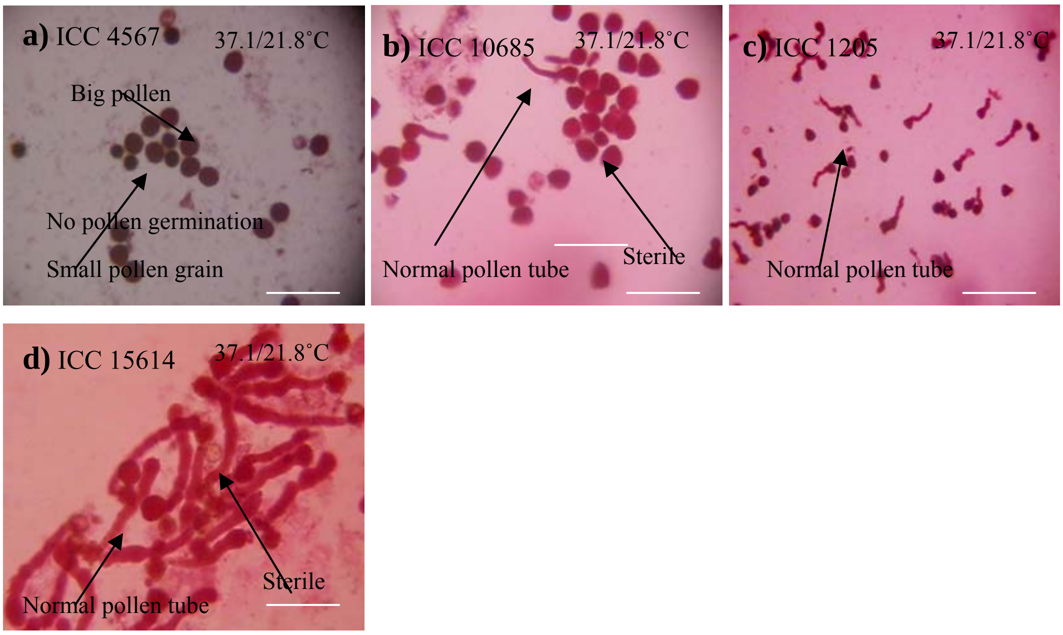

Fig 5.9 Field response: *In vitro* pollen germination (anthesis) at 37.1/21.8˚C a) ICC 4567 – no pollen germination (changes in pollen grain size) b) ICC 10685 – few pollen grains germination c) ICC 1205 – mixture of germinated and non-germinated d) ICC 15614 –germinated pollen grain (Bars = 10 µm)

***In vivo* pollen germination and tube growth**

vi. Stressed stigma x stressed pollen within the same genotype (38/25.2 x 38/25.2˚C; 39/25.2 x 39/25.2˚C)

Thirty minutes after hand pollination at 38/25.2˚C in the field the pollen of ICC 4567 did not germinate on the stigma (Fig 5.12a). At 39/25.2˚C the style of the same genotype was shortened (Fig 5.12h). The pollen of ICC 10685 germinated at 38/25.2˚C and 39/25.2˚C but had more callose formation in the style tissue nearest the stigma (Fig 5.12c) and less callose development at the base of the style (Figs 5.12i). In contrast, pollen germinated and had smooth pollen tube growth at the base of the style in ICC 1205 under both temperature regimes (38/25.2˚C; 39/25.2˚C) (Figs 5.12d and 5.12j). Similar responses were observed in ICC 15614 (Figs 5.12f and 5.12l).

vii. Stressed stigma x non-stressed pollen within the same genotype (40.2/25.5˚C x 27/16˚C)

In three genotypes (ICC 10685; ICC 1205 and ICC 15614), the non-stressed pollen (27/16˚C) germinated on the stressed stigma at 40.2/25.5˚C and the pollen tube reached the base of the style with little callose development on the style (Figs 5.12m, 5.12n and 5.12o). The stressed stigma was clearly receptive in all three genotypes. There was no sample for ICC 4567.

Table 5.7 Pollen germination (%) of chickpea under different temperature regimes (day/ night) in the field

Temperature regimes (˚C)	Relative Humidity (%)	ICC 4567		ICC 10685		ICC 1205		ICC 15614		Mean	
		PG	PNG	PG	PNG	PG	PNG	PG	PNG	PG	PNG
35.2/24	67/27	7	93	16	84	55	45	55	46	33	67
36.4/23.4	85/41	6	95	12	88	50	50	42	59	27	73
37.1/21.8	76/31	0	100	11	89	46	55	26	77	20	80
38/25.2	72/26	0	100	11	89	33	67	22	78	17	83
Genotype		3	97	13	87	46	54	35	65		
LSD(*P*<0.05)											
Genotype		11***									
Temperature		11*									

Relative humidity (day/ night) was not statistically analysed. PG - Pollen germinated; PNG - Pollen not germinated. (*** significant at P < 0.001; * significant at P < 0.05)

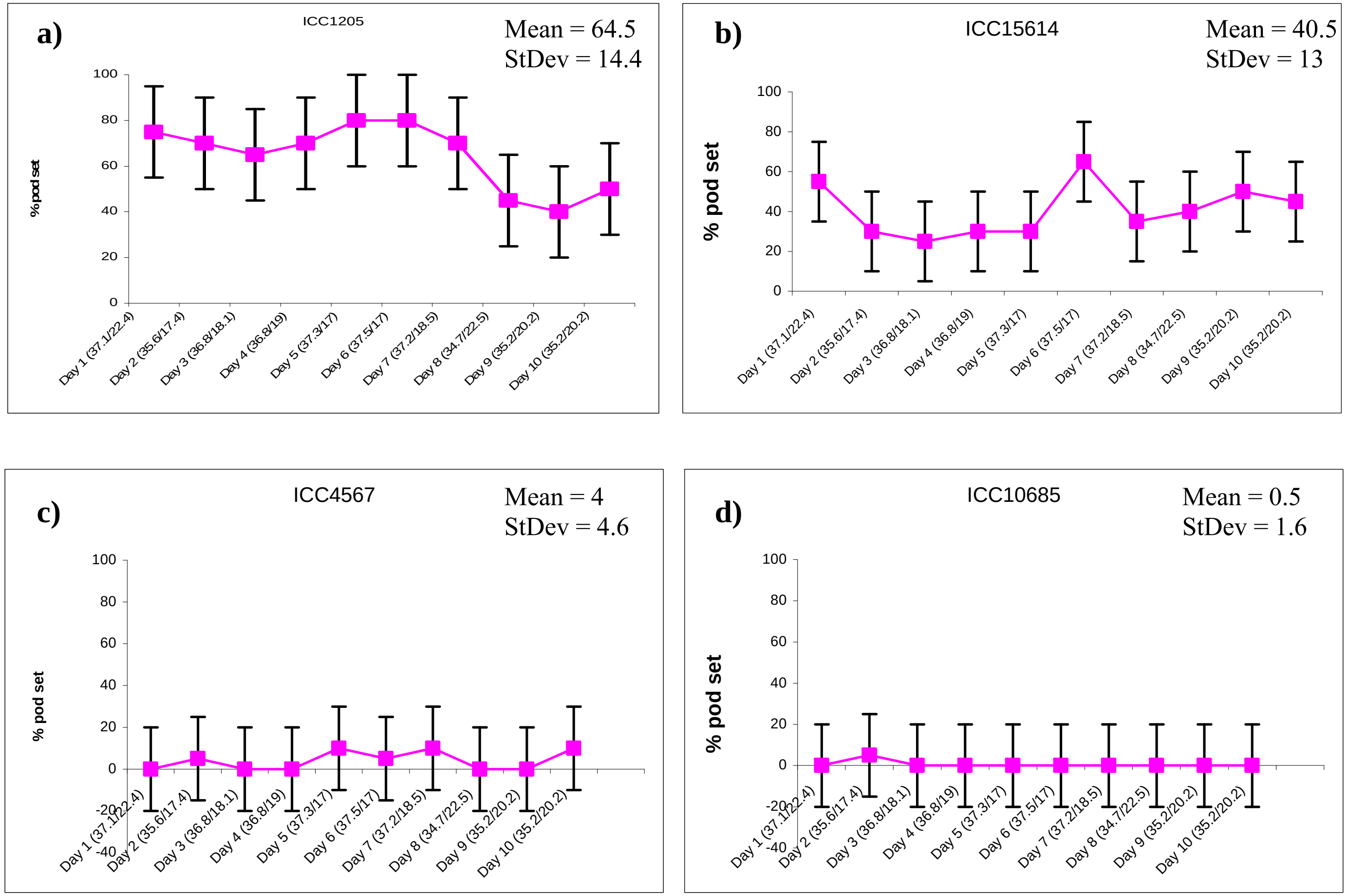

Fig 5.10 Daily pod set during maximum active period (10 days) of reproductive stage (observation were recorded from 50% flowering date) in the field – Staggered sowing -1 (date of sowing – 4/2/2011; date of harvest – 20/5/2011) Values are mean ± SE (n = 20)

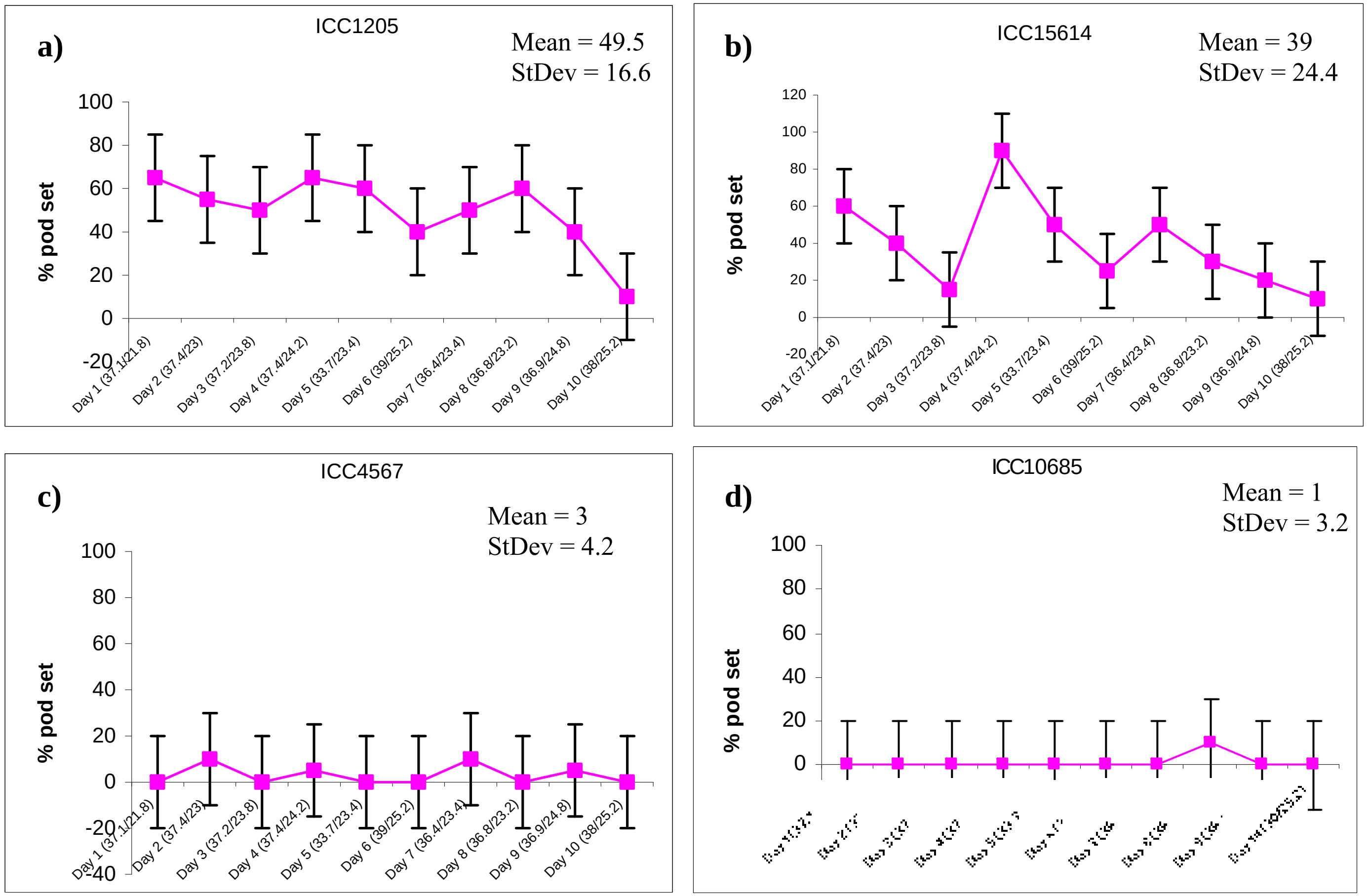

Fig 5.11 Daily pod set during maximum active period (10 days) of reproductive stage (observation commenced from 50% flowering date) in the field – Staggered sowing -2 (date of sowing – 18/2/2011; date of harvest – 30/5/2011) Values are mean ± SE (n = 20)

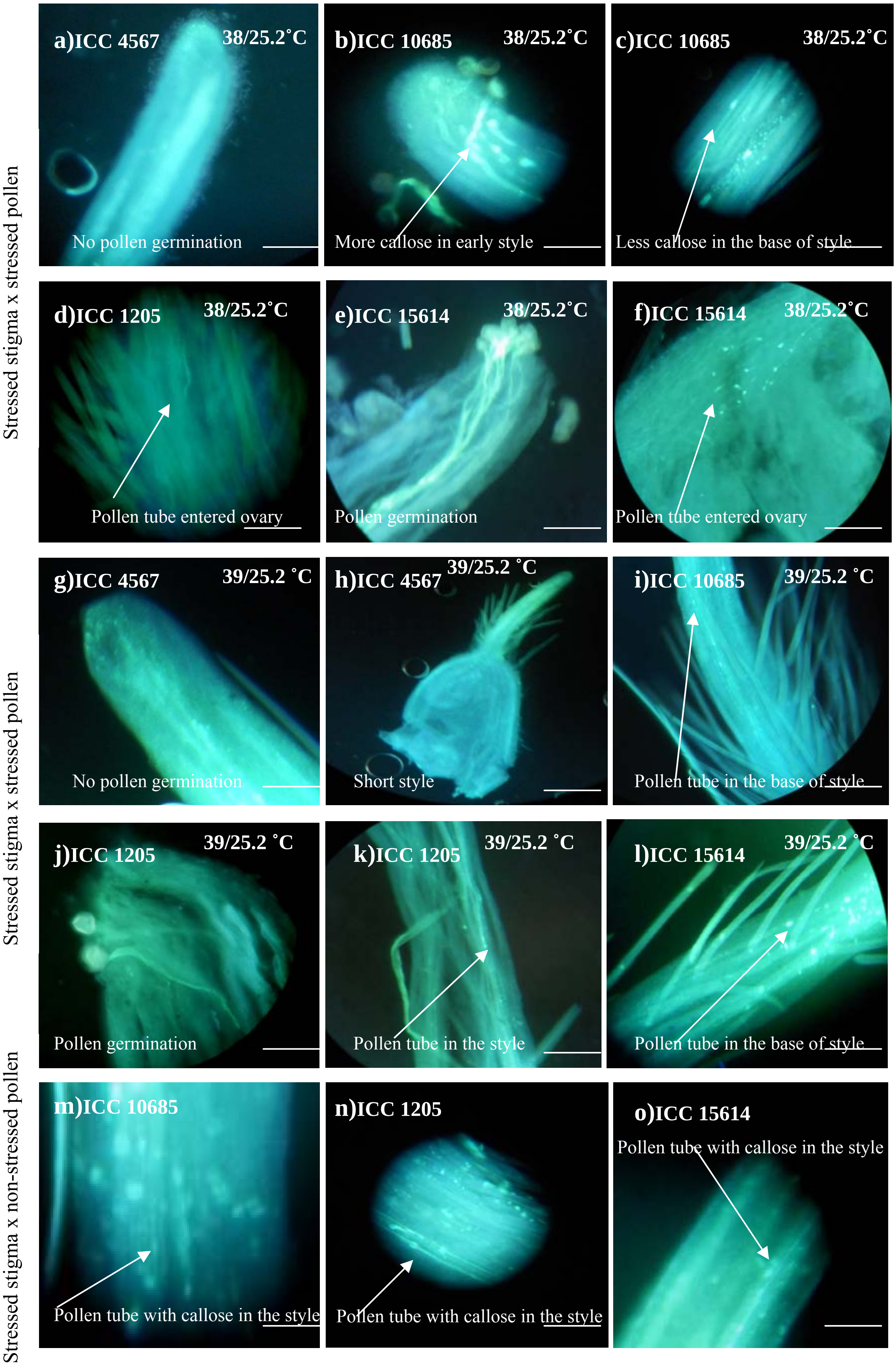

Fig 5.12 ***In vivo*** **pollen germination and tube growth in the field under heat stress** (Bars = 10 µm)

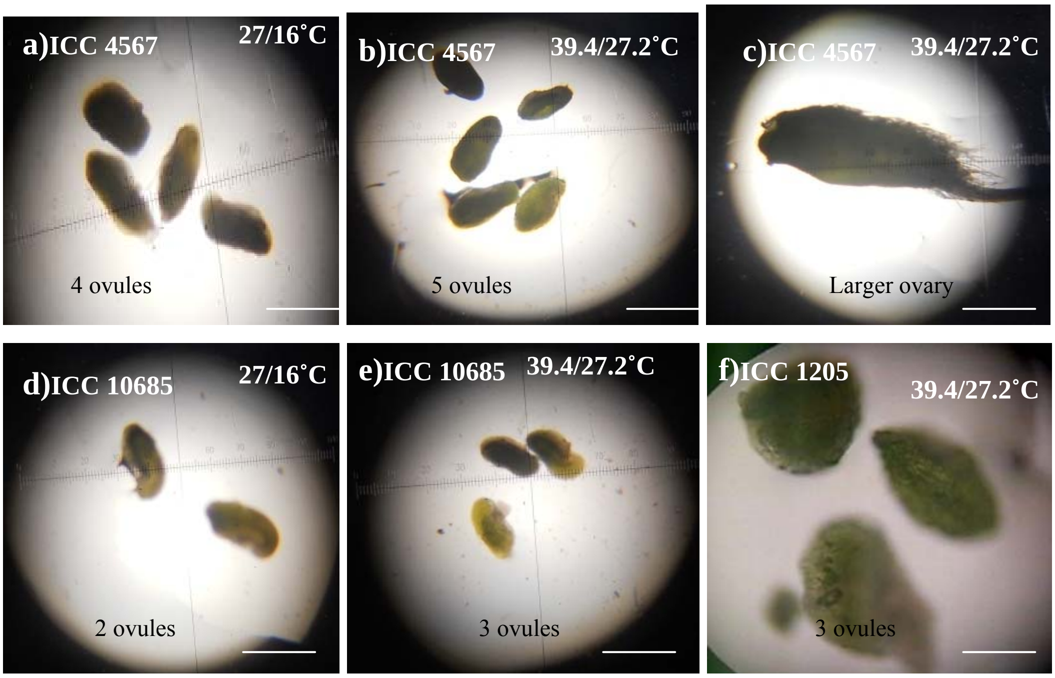

Fig 5.13 Numbers of ovules under heat stress (39.4/27.2˚C) in the field compared with optimum temperature (27/16˚C) under the controlled environments. a) ICC 4567 – 4 ovules at 27/16˚C b) ICC 4567 – 5 ovules at 39.4/27.2˚C c) ICC 4567 – larger size ovary at 39.4/27.2˚C (Ovule size was not measured) d) ICC 10685 – 2 ovules at 27/16˚C e) ICC 10685 – 3 ovules at 39.4/27.2˚C f) ICC 1205 – 3 ovules at 39.4/27.2˚C (Bars = 10 µm)

Ovule observation

The number of ovules were counted at high temperature (39.4/27.2˚C) and compared with the non-stressed treatment (27/16˚C) of the controlled environment experiment. ICC 4567 had five ovules in an ovary (Fig 5.13b) under high temperature stress whereas this genotype normally produced four ovules under non-stressed conditions (27/16˚C) (Fig 5.13a). The size of the ovary was therefore larger than other genotypes (Fig 5.13c). Other genotypes (ICC 10685; ICC 1205 and ICC 15614) produced three ovules in an ovary in the stressed treatment, compared with only two ovules in the non-stressed treatment (Figs 5.13d, e, f).

Grain yield and biomass at high temperature in the field

High temperature significantly influenced pod number, filled pod number, seed number and grain yield per plant (P<0.001). Significant differences among genotypes were observed at high temperature (Table 5.8). The results showed that ICC 1205 and ICC 15614 were heat tolerant and the genotypes ICC 4567 and ICC 10685 more heat sensitive. However, the genotypes did not show any significant difference in biomass at maturity.

Table 5.8 Grain yield (g/plant), pod characters (per plant) and biomass (g/plant) of chickpea genotypes at high temperature in the field

Genotypes	Total pod number	Filled pod number	Seed number	GY	Biomass
ICC 4567	10^{C}	3^{C}	4^{C}	0.4^{C}	23.0^{a}
ICC 10685	2^{d}	1^{d}	2^{d}	0.2^{d}	25.2^{a}
ICC 1205	69^{a}	65^{a}	87^{a}	11.7^{a}	21.9^{a}
ICC 15614	66^{b}	62^{b}	88^{b}	10.5^{b}	20.4^{a}
Mean ± s.e.	37±7.9	33±7.6	45±9.3	5.7±1.2	22.6±5.4
CV%	43.6	46.4	41.4	42.2	47.5

Means within the column followed by different letters are significantly different (P<0.001)

5.4.2.3. Controlled environments response

The overall summary of the pre-anthesis pollen fertility, *in vitro*, *in vivo* pollen germination, and pod set is presented in Table 5.11 and a more complete description of the responses is available in the following section:

Pollen fertility studies

In ICC 4567, a mixture of fertile and sterile pollen grains was observed in the anther at 35/20˚C and 36/21˚C and all pollen grains became completely sterile at 37/22˚C. Epidermis wall thickening occurred from 35/20˚C. Then pollen grains of ICC 10685 were fertile at 36/21˚C but the pollen germinated within the anther. At 37/22˚C, the pollen grains were completely sterile. ICC 1205 pollen grains were fertile at 38/23˚C. A mixture of fertile and sterile pollen grains was observed at 39/24˚C and 40/25˚C. ICC 1205 showed epidermis wall thickening at 38/23˚C. In ICC 15614, pollen grains were fertile and the anther dehisced at 39/24˚C. The number of flowers produced by this genotype reduced after 36/21˚C.

***In vitro* pollen germination and tube growth**

High temperature reduced pollen germination % (pollen fertility). At 37/22˚C, pollen germination was zero in ICC 4567, ICC 10685 and ICC 15614. ICC 1205 also produced completely sterile pollen at 38/23˚C. Differences were observed in pollen germination % between genotypes ($P < 0.05$) and between temperature regimes ($P < 0.001$). However, the genotype and temperature regimes interaction was non significant (Table 5.9).

Genetic variation in pollen tube growth among the genotypes was determined. At 35/20˚C, ICC 4567 had abnormal zigzag pollen tubes. The stain entered into the shrunken and structurally abnormal pollen and the release of protoplasm was observed in this genotype at 35/20˚C. ICC 10685 produced abnormal tubes at 36/21˚C. At 37/22˚C, intact pollen grain (cell walls intact) was found but the contents of pollen started to stream out through an aperture which resembled a pollen tube. Pseudo-germination, wrinkled pollen grain and tri-apertures germination were typical abnormalities under high temperature stress (38/23˚C). ICC 1205 pollen germinated at 37/22˚C and anthers were indehiscent. The release of protoplasm and tri-apertures germination was found at 38/23˚C. ICC 15614 was observed to have a bulbous pollen tube tip at 36/21˚C compared to a cylindrical tip in the control temperature (27/16˚C). Shrunken and round, fully and partially stained pollen grains were observed at 37/22˚C in ICC 15614. Indehiscent anthers were also found in this genotype at 37/22˚C and tri-aperture germination was observed at 38/23 ˚C.

Table 5.9 Pollen germination (%) of chickpea genotypes at different temperature regimes (day/night) under controlled environments (*** significant at $P < 0.001$; * significant at $P < 0.05$)

Temperature regime (˚C)	**ICC4567**		**ICC10685**		**ICC1205**		**ICC15614**		**Mean**	
	PG	PNG	PG	PNG	PG	PNG	PG	PNG	PG	PNG
27/16	93	7	92	8	89	12	88	12	90	10
33/18	44	56	60	40	76	24	72	29	63	37
34/19	28	77	65	35	60	41	62	39	54	48
35/20	5	95	44	56	33	68	43	58	31	69
36/21	1	99	22	78	19	82	22	79	16	84
37/22	0	100	0	100	2	98	0	100	1	99
38/23	0	100	0	100	0	100	0	100	0	100
Mean	24	76	41	59	40	60	41	59		
LSD (P<0.05)	PG	PNG								
Temperature	11.63 ***	11.41 ***								
Genotype	8.79 *	8.63 ***								
Temperature x Genotype	NS	NS								

PG - Pollen germinated; PNG - Pollen not germinated

In vivo pollen germination and tube growth

viii. Stressed stigma (35/20˚C) x stressed pollen (35/20˚C) within the same genotype

At 35/20˚C, stressed pollen germinated on the stressed stigma in four genotypes and the pollen tube grew to the base of the style. ICC 15614 produced more callose deposition in the stigma.

ix. Non-stressed stigma (27/16˚C) x stressed pollen (35/20˚C) within the same genotype

In two genotypes (ICC 4567 and ICC 10685) stressed pollen (35/20˚C) did not germinate on the non-stressed stigma (27/16˚C). In ICC 1205 the stressed pollen germinated and produced a smooth pollen tube in the non-stressed stigma. However, the pollen of ICC 15614 germinated and more callose deposition was noted in the style tissue close to the stigma. The pollen tube in this genotype entered the ovary.

Table 5.10 Floral morphology (%) of chickpea at different high temperature regimes (day/night) under controlled environments (*** significant at *P* < 0.001)

Temperature regime (˚C)	**ICC4567**			**ICC10685**			**ICC1205**			**ICC15614**			**Mean**		
	NF	%DF	%PS	NF	%DF	%PS	NF	%DF	%PS	NF	%DF	%PS	NF	%DF	%PS
27/16	10	12	89	10	25	75	10	10	90	11	9	91	10	14	86
32/17	15	53	47	25	94	7	17	33	67	32	78	22	22	64	36
33/18	7	55	45	16	77	23	11	64	37	6	75	25	10	68	32
34/19	12	74	26	14	100	0	18	85	15	8	67	33	13	81	19
35/20	18	89	12	12	100	0	11	81	19	6	82	19	11	88	12
36/21	13	100	0	9	100	0	23	83	17	4	88	13	12	93	7
37/22	3	100	0	7	100	0	11	96	5	2	100	0	5	99	1
38/23	0	0	0	5	100	0	5	100	0	0	0	0	2	50	0
Mean	9	60	27	12	87	13	13	69	31	8	62	25			
LDS (P<0.05)	NF	%DF	%PS												
Temperature	1.32 ***	7.62 ***	7.63 ***												
Genotype	0.94 ***	5.39 ***	5.39 ***												
Temperature x genotype	2.64 ***	15.24 ***	15.26 ***												

NF - Number of flowers; %DF - % of dry flowers; %PS - % of pod set

Table 5.11 Summary of findings on the effect of high temperature on pre-anthesis pollen fertility, *in vitro*, *in vivo* pollen germination and pod set of chickpea under controlled environments

Observations	**ICC 4567**	**ICC 10685**	**ICC 1205**	**ICC 15614**
Pre-anthesis pollen fertility was found up to	35/20˚C	36/21˚C	38/23˚C	38/23˚C
Abnormalities during pre-anthesis at high temperature	Mixture of fertile and sterile pollen grains	Pollen germination within the anther	Anther wall thickness	Mixture of fertile and sterile pollen grains
In vitro pollen germination was found up to	35/20˚C	36/21˚C	38/23˚C	38/23˚C
Abnormalities during *in vitro* pollen germination at high temperature	Zigzag tube growth and leakage of protoplast	Intact pollen tube growth, pseudo germination and wrinkled sterile pollen grain	Indehiscent anthers	Bulbous tip in the pollen tube
In vivo pollen germination at 35/20˚C: stressed stigma x stressed pollen and non-stressed stigma x stressed pollen confirmed the results	No pollen germination on the stigma	No pollen germination on the stigma	Pollen germination and tube growth on the style	Pollen germination and tube growth on the style
Stigma receptivity observed through stressed stigma (35/20˚C) x non-stressed pollen (27/16˚C)	Mostly stigma was receptive. However, short style was observed.	Stigma receptive	Stigma receptive	Stigma receptive
Critical temperature for pod set	34/19˚C	34/19˚C	38/22˚C	37/21˚C

x. Stressed stigma (35/20˚C) x non-stressed pollen (27/16˚C) within the same genotype

The non-stressed pollen germinated in the stressed stigma and produced smooth pollen tube growth in all four genotypes. The high temperature stress reduced the style length in the genotype ICC 4567 at 35/20˚C.

Pod set

Compared to the non-stressed control, the high temperature treatments reduced flower number and % pod set. The number of flowers was reduced from 22 at 32/ 17˚C to 2 at 38/23˚C ($P < 0.001$) (Table 5.10). No pods were set at 36/21˚C in ICC 4567 and at 34/19˚C in ICC 10685. In ICC 1205, 5% pod set occurred at 37/22˚C and was zero at 38/23˚C. 13% pod set occurred at 36/21˚C and became zero at 37/22˚C in ICC 15614. Overall, % pod set was reduced from 86% (27/16˚C) to 1% (37/22˚C). Differences ($P < 0.001$) were found in genotypes, temperature regimes and there was an interaction between temperature and genotypes (Table 5.10).

Fig 5.14 was replotted from pod set (data not shown) to identify the critical temperature for % pod set in chickpea under controlled environments. The critical temperature for pod set was 34/19˚C because pod set was reduced by 13% at this temperature compared to 33/18˚C. A 67% of reduction in pod set occurred at 34/19˚C compared to the optimum temperature (27/16˚C). Overall, there was a strong negative relationship ($R^2 = 0.95$) between pod set and high temperature, such that pod set was reduced by 39% per 1˚C above the threshold temperature.

Comparison of field and controlled environment pod set at different temperature

The adverse effect of high temperature on % pod set was compared in both field and controlled environments (Fig 5.15). The aim was to identify the critical temperature for pod set in chickpea under high temperature. Therefore Fig 8 was replotted from pod set from controlled environment, Figs 3 and 4. The critical temperature for pod set was ≥38˚C in ICC 1205; ≥37˚C in ICC 15614; ≥34˚C in ICC 4567 and ≥33˚C in ICC 10685. There were clearly differences among genotypes in the critical temperature for pod set with temperatures ranging from 33 to 38˚C.

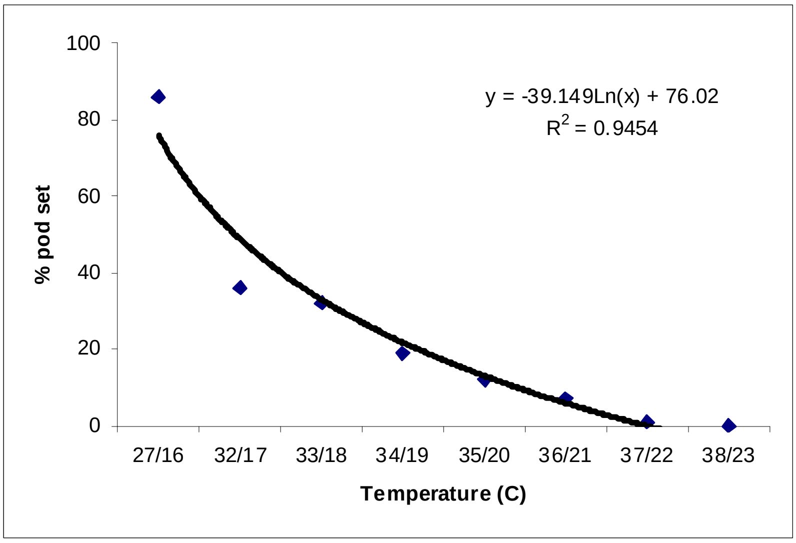

Fig 5.14 Relationship between temperature and pod set in the controlled environments (Table 4 mean values % pod set were replotted to predict the reduction by every temperature increase by 1˚C using logarithmic regression)

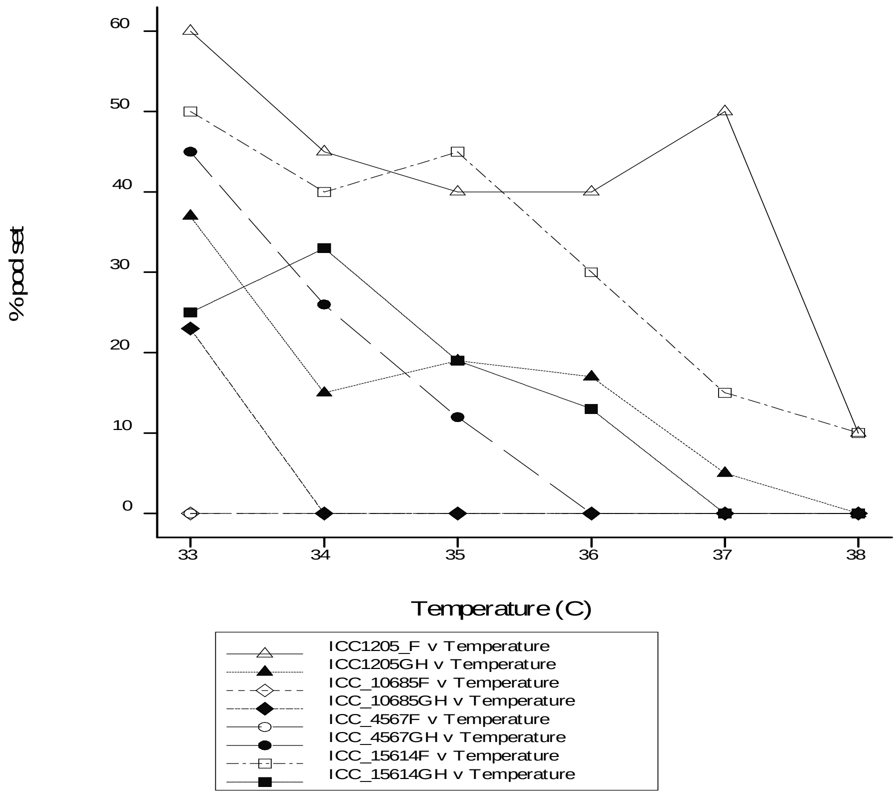

Fig 5.15 Comparison of the pod set in the field (F) and the controlled environments (GH) under high day temperature

5.5. Discussion

5.5.1. Experiment – 1

This study demonstrated that a heat-tolerant and a heat-sensitive genotype in chickpea varied in pollen viability before anthesis, pollen germination at anthesis, and pollen germination and pollen tube growth on the stigma when exposed to high temperature (day/night). High temperature caused three types of damage: an increase in locule number; partial sterility, and pollen germination within the anther. These were observed in the heat sensitive genotype ICC 5912 at 34/19˚C while partial sterility was the only damage observed in heat tolerant genotype ICCV 92944 at 40/25˚C. This study confirmed that pre-anthesis heat stress resulted in anthers with changed locule numbers in the sensitive genotype. In ICC 5912, the abnormal anther (Fig 5.2b) was larger in size (length and width) than the normal anther (Fig 5.2d) at 34/19˚C because of increased numbers of locules. Normal anthers (Fig 5.1a and Fig 5.3a) have two locules but the high temperature stressed anthers produced more than three locules (Figs 5.2a, b and c). This observation may reflect changes in anther development phase-1 (changes in anther cell differentiation) and anther development phase-2, especially changes in stomium which is responsible for anther dehiscence (Goldberg *et al*. 1993). Changes in locules number were also reported in cowpea at 33/30˚C (day/night) that was exposed to heat 5-7 DBA (Warrag and Hall 1984). Changes in anther shape and stomium opening at 32/27˚C (day/night) was found in a heat tolerant common bean genotype after 13 days of pre-anthesis heat treatment (Porch and Jahn, 2001).

The temperature sensitive genotype ICC 5912 showed a mixture of fertile and sterile pollen grains (partial sterility) in the anther at 34/19˚C, but only partial sterility was found in the heat tolerant genotype ICCV 92944 at 40/25˚C. Partial sterility (a mixture of fertile and sterile pollen grains) occurred in wheat (*Triticum aestivum* L.) at exposure to 30˚C (day/night) for three days pre-anthesis (Saini *et al*. 1984). Variation in pollen fertility occurred among the flowers and among the anthers within the flower in ICC 5912 at 34/19˚C and in ICCV 92944 at 40/25˚C (Table 5.2). The pollen germination within the anther before anthesis was observed without any artificial supplementary nutrients (e.g. sucrose) in the sensitive genotype at 34/19˚C. Generally, the Fabaceae family (e.g. chickpea) has 1-10 min lag period before pollen germination because the pollen is equipped with fully developed mitochondria at anther dehiscence (Hoekstra

1979; Hoekstra 1983). It may be possible to utilise fully developed mitochondria to conduct protein synthesis which is essential for germination and tube growth within the anther (Hoekstra and Bruinsma 1979). Both functions were attributed with promotion of germination within the anther prior to dehiscence. However, this had been occurred pre-anthesis at just 1˚C below critical temperature. It may be an indication of the critical temperature. The three types of damage were observed in the sensitive genotype ICC 5912 five days after exposure to high temperature stress.

The number of pollen grains per flower in the heat tolerant and heat sensitive genotypes was reduced with increasing temperature, but pollen grains of the sensitive genotype ICC 5912 only produced sterile pollen at 35/20˚C. The variation in pollen production per flower observed under optimum temperature is likely due to genotype differences (Palmer *et al*. 1978). High temperature (35/20˚C) clearly reduced pollen production and pollen fertility. To confirm this statement, pollen number and % pollen viability were re-plotted in Fig 5.7. There was a strong positive relationship (R^2 = 0.85) between pollen production and % of pollen fertility which showed that reduced pollen production was associated with low % pollen fertility regardless of genotype. A decline in pollen fertility with reduced pollen production per flower was also confirmed in groundnut high temperature (De Beer 1963).

The sensitive genotype showed normal and abnormal pollen tubes at 34/19 ˚C during *in vitro* germination. Indehiscent anthers were observed in ICC 5912 during pollen collection at 34/19˚C (Fig 5.4c). At anthesis, the relative humidity around the anthers might be reduced and the locules may lose more water by evaporation (Keijzer 1983). This can result in indehiscence due to the wall thickening mechanism of the endothecium (Keijzer 1983). ICCV 92944 showed release of protoplasm by bursting at 38/23 ˚C. We assume that this negative result happened due to low pollen population and medium. Similar results were experienced in many plant species (Brewbaker and Kwack 1963). Mature pollen grains in Fabaceae generally show higher osmotic potential when desiccated (Baker and Baker, 1979; Shivanna, 2003). Due to higher osmotic potential, the high temperature (38/23˚C) stressed pollen burst with 20% sucrose medium (Fig 5.5f). Higher sucrose concentration (> 20%) in the media may help to prevent pollen bursting.

High temperature clearly affects flower production and pod set. The reduction in flower production at high temperature is likely due to slower rates of flower bud initiation, flower bud development and flower bud abortion. ICCV 92944 had fewer flowers with lower pod set (50%) at 38/23˚C. No pod set at 35/20˚C was observed in ICC 5912. These findings confirm that pollen production per flower, pollen viability, pollen germination and pod set was reduced by high temperature. The lack of fertile pollen and lack of pod set at 35/20˚C in ICC 5912 indicates this temperature as the threshold for infertility.

In general, the number of pods/plant, filled pods/plant and seeds/plant and plant biomass/plant were reduced under high temperature stress. This reduction may be due to the limited supply of exogenous nutrients to the heat-stressed plants. Wang *et al*. (2006) also noted that chickpea plant biomass and number of seeds/ plant were reduced at 35/16˚C compared with 28/16˚C. Flower number and pod number were also reduced in groundnut at high temperature (> 36˚C) (Prasad *et al*. 2000). In the current study, pod set was associated with heat stress effects on anther development, pollen development and pollen release by anther dehiscence. Studies on cowpea (Ahmed *et al*. 1992), common bean (Gross and Kigel, 1994) and groundnut (Prasad *et al*. 2001) also found that high temperature reduces pod set.

The failure of pod set was apparently related to male sterility rather than stigma receptivity. Stressed pollen (35/20˚C) from ICC 5912 did not germinate on either stressed or non-stressed stigmas of the same genotype or ICCV 92944. Non-stressed pollen of ICC 5912 germinated on the stressed stigmas of ICC 5912, indicating that while the pollen was sterile at 35/20˚C, the stigma of ICC 5912 was receptive. Peet *et al*. (1998) reported that tomato (*Lycopersicon esculentum* Mil.) pollen was sterile while stigma was receptive during reciprocal crossing (29˚C x 25˚C; 25˚C x 29˚C). However, the response of ICCV 92944 was different. Stressed pollen germinated on stressed and unstressed stigmas on both ICCV 92944 and ICC 5912, showing that pollen was fertile and stigmas receptive at 35/20˚C.

Overall, the pollen tubes in the non-stressed stigma were smooth but pollen tubes in the stressed stigma grew with callose deposition, although they continued to grow down to the end of the style at 30 min after pollination. High temperature produced a series of plugs resembling a ladder, along the pollen tube in ICCV 92944 (Figs 5.6 panel 1b, 2e). Turgor pressure in the pollen tube may be maintained by the formation of a series of callose plugs[3]. Pressure is needed for tube penetration into the pistil, which provides the pathway for the tubes because new plugs would restore

[3] Callose plug, which form in the back of extending pollen tube, thus limit and contain the path of the cytoplasmic stream inside the tube (Stanley and Linskens, 1974).

pressure (Dumas and Knox 1983). These plugs function to prevent the backflow of cytoplasm and nuclei in the long pollen tubes (Iwanami *et al.* 1988), but physical stress is known to induce callose formation (Aist 1976).

The experiment – 1 conclude that pollen is more sensitive to high temperature than the stigma in chickpea. Consequently, there is a potential for developing screening techniques for heat tolerance in chickpea breeding programs using differences in pollen viability. There is also a possibility of using a pollen selection method in breeding for heat tolerance in chickpea. Its success will depend on genotypic variation in high temperature sensitivity of pollen.

5.5.2. Experiment – 2

The data showed that % pod set in chickpea was reduced at high temperatures (day/night). Factors controlling lower pod set were the influence of high temperatures on the flower production, anther dehiscence, pollen viability, pollen germination and pollen tube growth. An increased number of locules, epidermis wall thickening, and a mixture of fertile and sterile pollen grains were observed under high pre-anthesis temperature stress in the heat sensitive genotypes (ICC 4567 and ICC 10685) in the field. At anthesis, sensitive genotypes produced indehiscent anthers, epidermis wall thickening of anthers, released protoplasm from the pollen grain and pollen germination within the anther. Further investigation of indehiscent anthers should consider factors affecting microclimatic conditions within the anther. Warrag and Hall (1984) and Porch and Jehn (2001) reported changes in the locule numbers and epidermis wall thickening in anthers due to failure of stomium opening and anther cell differentiation under high temperature stress in cowpea and bean.

Genetic variation in fertility (% pollen germination) was observed under high temperature stress. In all genotypes, shrunken and structurally abnormal deformities of pollen occurred in both the field and controlled environment experiments at high temperature. ICC 10685 produced intact pollen grain however, pollen tubes were abnormal (zigzag growth) and pseudo-germination was observed. The pollen grains were wrinkled and tri-aperture germination occurred at high temperature under controlled conditions. The genotype ICC 4567 showed abnormal tubes (zigzag growth) with protoplasm starting to stream out of the pollen grains due to osmosis (Kroon *et al.* 1974). At high temperature, i*n vitro* pollen tube abnormalities such as thinner, zigzag tubes with stunted growth was also observed in *Brassica napus*

(Young *et al*. 2004), and in groundnut (Prasad *et al*. 2001). Reduced *in vitro* pollen germination (44%) at > 35˚C was also reported in groundnut (Kakani *et al*. 2002); 50% reduction at 38˚C in soybean (Koti *et al*. 2005) and 52% reduction in bean at 32/27˚C (Porch and Jehn 2001). These data suggest that pollen viability is the main cause of sterility in chickpea under high temperature stress at anthesis.

The critical temperature for pod set in heat tolerant and sensitive genotypes were clearly identified. Generally the pod set was reduced under high temperatures. Devasirvatham *et al*. (2010) found that ~ 50% of pod set reduction in chickpea at 35/20˚C compared to the optimum temperature (28/16˚C). Wang *et al*. (2006) calculated that pod production was decreased by 34% for Myles and 22% for Xena chickpea cultivars at 35/16˚C compared with the control (20/16˚C). Therefore, hot days (≥ 34˚C) combined with warm nights (≥ 19˚C) can potentially reduce pod set in chickpea. These findings are similar to those of Prasad *et al*. (2001) who showed that the critical pre-anthesis flower bud temperature in groundnut was ≥ 33˚C, above which pod set reduced by 6% pod set per 1˚C. Similarly, an increase in the seasonal temperature of 1˚C reduced chickpea yield by 53 – 300 kg/ha in different regions of India (Kalra *et al*. 2008).

In our study high temperature decreased flower production and increased flower abortion. This is consistent with an earlier report of flower abortion in chickpea at > 30˚C (Sinha, 1977). Although chickpea is an indeterminate grain legume and plant growth continues after flowering, given sufficient moisture flower number reduced in our study under high temperature. Flower number was also reduced in groundnut under high temperature (Prasad *et al*. 1999).

In the field, stressed pollen did not germinate on the stressed stigmas of ICC 4567. Though, stressed pollen was observed to germinate on the stressed stigmas of ICC 1205 and ICC 15614 indicating better heat tolerances in these genotypes. The non-stressed pollen grains of ICC 10685; ICC 4567 and ICC 15614 germinated and produced smooth pollen tube growth on the stressed stigmas (40.2/25.5˚C) of the same genotypes. Clearly the stigmas remain receptive at high temperature in chickpea.

However, short style was observed in heat sensitive genotypes such as ICC 4567 in both field (39˚C) and controlled environments (35˚C). Plants were continuously stressed for more than 10 days in the field and seven days in the controlled environments during flowering. At 39˚C in field this genotype also showed ovule and ovary abnormalities. These results suggested that high temperature initially reduces pollen fertility (≥3 days) and extended exposure to high temperature (≥7 days) eventually effects the stigma and ovule. Female abnormalities were observed in wheat at 30˚C over 3 continuous days (Saini *et al*. 1983). However, deformity of male and female reproductive tissue depends up on the critical temperature of the crop and the level of heat tolerance among genotypes.

In the controlled environment at 35/20˚C the stressed pollen grains of ICC 4567 and ICC 10685 did not show consistency in germination on the stressed or non-stressed stigmas due to low pollen fertility (%). However, pollen germination on the stigma of the tolerant genotypes (ICC 1205 and ICC 15614) was much higher and more consistent. Therefore, these genotypes have stable heat tolerance. Similarly, the stigmas of bean (Gross and Kigel, 1994) and tomato (Peet *et al*. 1998) remain receptive at high temperature. Overall, the pollen grain of chickpea was more sensitive to high temperature than the stigma in both the field and controlled environments.

5.6. Conclusions

These data suggest that pollen grains, *in vitro* pollen germination, *in vivo* pollen germination and pollen tube growth are negatively affected by high temperature stress thus reducing subsequent pod set. The male flower structures (anther and pollen) showed more abnormalities such as changes in the number of locules per anther, anther epidermis wall thickening and pollen sterility than pollen function (*in vivo* pollen tube growth). The threshold temperature for pod set was ≥ 34/19˚C. Among the four genotypes tested, ICC 1205 was the most heat tolerant genotype and ICC 4567 the most heat sensitive. ICC 10685 was also classified as sensitive but was low yielding in the optimal growing conditions due to a genetically inherent problem. In conclusion, the heat tolerant genotypes identified in this study are suitable for inclusion in breeding programs targeting warmer areas. The materials are also potential parents for developing genetic mapping populations to identify QTLs (Quantitative Trait Loci) linked to heat tolerance.

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Chapter 6: Association analysis to identify genomic regions linked to heat tolerance in chickpea

6.1. Introduction

Most agronomic traits and grain yield are controlled by quantitative trait loci (QTL) and multi genes in chickpea (Gowda *et al*. 2011). Clearly, improved understanding of the genetic control of these characters will facilitate their use in breeding programs. Improved chickpea cultivars more resilient to temperature fluctuations are needed to cope with the consequences of a warming climate. Identification of the main traits underlying the adaptive response of chickpea across a broad range of temperatures is an important first step to effective breeding and selection. Generally, a genomics approach identifies marker alleles in chromosome regions associated with expression of the target trait. Association analysis (AA) is a one such approach that can be used to identify marker-trait (phenotype-genotype) associations (Painter *et al*. 2011). This approach is complementary to traditional linkage mapping but does not rely on the construction of a defined population derived from a single cross.

In earlier studies, association mapping and analyses were conducted in the mapping population using whole-genome wide and/or candidate gene association (Zhu *et al*. 2008). Later, germplasm were included in association studies to target various traits using whole-genome wide association (Zhu *et al*. 2008). Meaningful AA depends on linkage disequilibrium (LD), which assumes non-random association of alleles at different loci. In other words, there is an assumption that many generations of meiosis have removed associations between QTL and any markers not tightly linked to the QTL. A genetic marker close to and in LD with a targeted trait will show significant allele frequencies in the genome (Painter *et al*. 2011). These genome-wide scanning approaches allow researchers to search the genome for genetic variation for targeted traits (Alonso-Blanco *et al*. 2009); in particular, complex traits such as drought, high temperature and salinity tolerance. Further, AA can increase mapping resolution or polymorphism detection at the sequence level and therefore enhances the efficiency of gene discovery and subsequent marker assisted selection in plant breeding (Gupta *et al*. 2005). Here, AA refers to the significant association of DArT markers with traits involved in the adaptive response to heat stress in chickpea.

AA of sequenced polymorphism and phenotypic variability in agronomic traits has been published in rice, wheat, barley, maize and cotton (Li *et al*. 2012; Yao *et al*. 2009;

Wang *et al*. 2012; Lu *et al*. 2009; Abdurakhmonov *et al*. 2009). The association between SSR markers and QTLs was also identified for seed protein content in soybean (Jun *et al*. 2008). Similarly, the association between SSR markers and rice sheath blight resistance QTLs was studied (Jia *et al*. 2012). Generally, these AA studies have been reported on experiments grown under favourable conditions. The large AA study on wheat by Crossa *et al*. (2007) identified DArT marker associations with resistance to stem rust, leaf rust, yellow rust and powdery mildew and grain yield. The results of Crossa *et al*. (2007) reported many new chromosome regions associated with disease resistance and grain yield, and some of these regions did not contain previously identified genes. An exception is a durum wheat study that targeted drought adaptive traits such as plant height, heading date, peduncle length, grain yield, kernels m^{-2}, thousand kernel weight and test weight using AA (Maccaferri *et al*. 2011). These authors concluded that major loci for phenology were responsible for the control of drought response. Using DArT markers, the physiological traits and associated markers for drought tolerance and avoidance in barley was identified (Mohamed 2008). The key traits studied were proline content, root shoot ratio, wilting score, relative water content and root length. However, at present there are no AA studies reported for high temperature tolerance in any crop.

In chickpea, the first AA published with a candidate gene sequencing approach (i.e. phenotype linked with a region of the genome) for fusarium wilt using RFLP markers (Huettel *et al*. 2002). Later, AA based on candidate gene sequencing showed the association of two genes (*ASR* and *CAP2* promoter) with drought related traits in chickpea (Nayak 2010). However, this method is worked based on prior knowledge from biochemical pathway or linkage analysis of the trait of interest and misses the link of unknown loci (Zhu *et al*. 2008). In a more comprehensive study, using 724 DArT markers in chickpea, 26 markers associated with drought adaptive traits such as shoot-dry weight, rooting depth, root dry weight, root length, root length density, root surface area, root dry weight: total dry weight ratio and root volume were identified (Nayak 2010).

Effective AA is based on linkage disequilibrium (LD) which tends to be maintained between loci (Neumann *et al*. 2011). However, there is the possibility of false positive correlation between a marker and a trait resulting in bias and meaningless associations (Neumann *et al*. 2011). Hence, to separate LD from physical linkage, population structure is important and its estimation is a prerequisite in association analysis (Crossa *et al*. 2007). These methods were used in AA in chickpea for drought tolerance (Nayak 2010; Kebede 2012).

Chickpea is mostly grown in the semi-arid regions of the world (Berger and Turner 2007) and crops are often exposed to high temperature (≥35˚C) stress during reproductive development. Heat stress reduces pod number per plant and thus limits grain yield (Krishnamurthy *et al*. 2011). Under such conditions, adaptative mechanisms based on traits that allow the plant to escape (e.g. flowering date), avoid (e.g. transpiration rate) and/ or tolerate (e.g. abscisic acid) the negative effects of heat stress either in the vegetative or reproductive stages are critical for maintenance of grain yield (Devasirvatham *et al*. 2012; Kumar *et al*. 2012). The aim of this study was to identify chromosomal regions and linked markers that contribute to heat tolerance in chickpea using association analysis.

6.2. Materials and methods

Plant material, phenotypic analysis and genotyping with DArT markers

A total of 167 chickpea genotypes, including ICRISAT breeding lines developed under various breeding programs were selected for analyses. The method of DNA extraction and the genetic diversity of these 167 genotypes were explained in chapter 3. Field evaluations of 11 traits (days to first flowering-DFF; days to 50% flowering- D50F; days to first podding-DFP; days to physiological maturity-DPM; biomass-Bio; plant height-PH; plant width-PW; total number of pods per plant-TNP; number of filled pods per plant-NFP; seed number-SN; and grain yield-GY) was conducted for 167 chickpea genotypes in two environments (heat stressed and non-stressed) at ICRISAT, Patancheru, India during 2009-10 (year 1) and 2010-11 (year 2). The details of field experiments and correlations between traits have been explained in chapter 4.

DArT markers and genetic map

DArT markers were generated by Diversity Arrays Technology Pty Ltd (www.diversityarrays.com). An integrated DArT map developed from inter-specific chickpea cross (ICC 4958 × PI 489777) was used to assign DArT markers to chromosomes (Thudi *et al*. 2011). In this study, 359 DArT markers were assessed of which 107 markers were identified as polymorphic. However, only 21 of these were located on the only available genetic map of chickpea.

Statistical analysis

The marker-trait association was analysed using R software 2.13.0 (Bansal *et al*. unpublished wheat rust data; Wimmer *et al*. 2011). The R software was used to run pattern analyses of phenotypic and marker data and can provide an estimate of the convergence between phenotype and marker data (www.R-project.org; Guillot *et al*. 2012). This analysis provided single marker-trait association analysis based on the *t* test. The significance of association between a marker and a trait was indicated by *P* value ($P<0.05$). The significance value of marker-trait association was ≥3.0. Markers significantly associated with traits were detected for loci on the chromosome. Finally the heat map of markers and traits was generated using R software. The association between markers and traits is indicated by the intensity of colour on the heat map. Dark blue indicates a significant positive association whereas a red colour indicates a significant negative association between the markers and the target trait. However, for the disease data analysis the colour difference is the opposite as low trait values are favoured over high values.

Linkage disequilibrium

Estimating LD between markers evaluates marker segregation. The R software was used to estimate the LD parameter r^2 among loci. The r^2 parameter was estimated for unlinked loci and for loci on the same chromosome. *Lewontin's D* is another summary statistic for LD that is commonly used and describes the difference between the coupling gamete frequencies and repulsion gamete frequencies at two loci. From *D*, a second measure of LD, that is, normalised *D´* can also be estimated. It is important to estimate the rate of decay of LD with physical distance, to be able to extrapolate information gathered from a small collection of sampled loci to the whole genome investigated (Nayak 2010).

Population structure

As an alternative to graphical clustering, a possible population sub-structuring was investigated using R software (Arief *et al*. 2009). Based on traits observed in both heat stressed and non-stressed conditions population sub-structuring/clustering was grouped to evaluate the clusters and to assign each individual to a corresponding subgroup without using the predefined information. In this AA study data was standardised, then similarity matrix was converted into dissimilarity matrix based on Gower's equation (Gower 1971). Then clusters were generated using Ward's method and an optimised dendrogram was generated.

6.3. Results

Linkage disequilibrium among DArT markers was investigated in the entire set of genotyped population. The scatter plot of r^2 values also revealed linear arrangement of LD between polymorphic sites of two loci in the genomic regions (Fig 6.1). Since r^2 revealed both recombination and mutation history, we considered pairs of loci which revealed greater r^2 value (>0.2) as in the linkage disequilibrium stage. Few loci represented r^2 value >0.2 in Fig 6.1.

Table 6.1 Association of DArT markers with agronomic traits under heat stressed and non-stressed conditions based on genome-wide association analysis

Significant markers	Heat stressed (late season)				Non-stressed (normal season)			
cpPb.678546	GY							
cpPb.678822	GY							
cpPb.322806	DFF	D50F	PW					
cpPb.489318	D50F	DFP	PW		DFF	D50F	DFP	PH
cpPb.676219	Biomass	NFP	GY		TNP	NFP	GY	
cpPb.682393	Biomass	GY			GY			
cpPb.675277	TNP	NFP	NS	GY	TNP	NFP	NS	
cpPb.675905	TNP	NFP	NS	GY	TNP	NFP	NS	GY
cpPb.677822	TNP	NFP	NS	GY	TNP	NFP	NS	GY
cpPb.172290					PW			
cpPb.490210	GY							
cpPb.491194	TNP	GY						
cpPb.171485	TNP	NFP	NS		TNP	NFP	NS	
cpPb.171608	DPM							
cpPb.172052	GY							
cpPb.172145					GY			
cpPb.172207					TNP	NFP	NS	
cpPb.172238	DPM							
cpPb.172931	PH	TNP	NFP	NS				
cpPb.173065	TNP	NFP	NS		NS			
cpPb.173294	TNP	NFP			TNP	NFP	NS	
cpPb.173309	DFF	D50F	DPM					
cpPb.173435					PH			
cpPb.173437	D50F				D50F	DPM		
cpPb.322752	DPM							
cpPb.323506	PH				DPM	PH	PW	
cpPb.349899	PH				DPM	PH	PW	
cpPb.350495	GY							
cpPb.350553	TNP	NFP	NS	GY	TNP	NFP	NS	GY
cpPb.488661	TNP	NFP			TNP	NFP	NS	
cpPb.488664					GY			
cpPb.488820					GY			
cpPb.489118					NS			
cpPb.489266	DPM							
cpPb.489372					GY			
cpPb.490632					NS			
cpPb.490834					PH	PW	GY	
cpPb.490878	GY							
cpPb.490962	NFP	NS			TNP	NFP	NS	
cpPb.491458	TNP	NFP	NS	GY	TNP	NFP	NS	GY
cpPb.491461					PW			
cpPb.675652	TNP	NFP	NS					
cpPb.676058					NS	GY		
cpPb.676076	DFF	D50F	DPM					
cpPb.676142	DPM							

DFF-days to first flower; D50F-days to 50% flower; DFP-days to first pod; DPM-days to physiological maturity; PH-plant height; PW-plant width; TNP-total number of pos; NFP-number of filled pods; NS-Number of seeds; GY-grain yield. Markers used for DArT analysis are named as chickpea probe (cpPb)

Table 6.1 (continued.) Association of DArT markers with agronomic traits under heat stressed and non-stressed conditions based on genome-wide association analysis

Significant markers	Heat stressed (late season)				Non-stressed (normal season)				
cpPb.676446					NS				
cpPb.676606	NFP	NS							
cpPb.676729					NS	GY			
cpPb.676765	TNP	NFP	NS		TNP	NFP	NS	GY	
cpPb.676860	PH				PH	PW			
cpPb.676902	DFF	D50F	DPM						
cpPb.677056	TNP	NFP	NS		TNP	NFP	NS		
cpPb.677063	NS								
cpPb.677249					TNP	NFP			
cpPb.677314	NFP	NS			TNP	NFP	NS		
cpPb.677490	TNP	NFP	NS	GY					
cpPb.677529	PH				DPM	PH			
cpPb.677672	TNP	NFP	NS		NS	N	NFP		TNP
cpPb.677690	PH				PH	PW			
cpPb.677783	TNP	NFP	NS		TNP	NFP	NS	GY	
cpPb.677798	TNP	NFP	NS		NS	NFP	TNP		
cpPb.678269					NS	GY			
cpPb.678344	DFF	D50F	DPM						
cpPb.678527	GY				NS				
cpPb.678561					NS	GY			
cpPb.678752	PH	TNP	NFP	NS					
cpPb.678785					NS				
cpPb.679133	DFF	D50F	DFP		DFF	D50F	DFP	DPM	
cpPb.679142					Biomass				
cpPb.679216	PH				PH	PW			
cpPb.679354	TNP	NFP	NS		NS				
cpPb.679404	DPM								
cpPb.679444	NS	GY							
cpPb.679544	NFP	NS	GY						
cpPb.679611	TNP	NFP							
cpPb.679623					NS				
cpPb.679660	TNP	NFP		NS	TNP	NFP	NS	GY	
cpPb.679806					NS	GY			
cpPb.679894	NS	GY							
cpPb.679915	PH				PH	PW			
cpPb.680077					Biomass				
cpPb.680218	TNP	NFP	NS	GY	TNP	NFP	NS	GY	
cpPb.680276	NFP	NS	GY						
cpPb.680354	DPM								
cpPb.680385					NS	GY			
cpPb.680501					NS				
cpPb.680572	PH				PH				
cpPb.680609					NS				
cpPb.680647					NS				
cpPb.680656	TNP	NFP	NS	GY					

DFF-days to first flower; D50F-days to 50% flower; DFP-days to first pod; DPM-days to physiological maturity; PH-plant height; PW-plant width; TNP-total number of pos; NFP-number of filled pods; NS-Number of seeds; GY-grain yield. Markers used for DArT analysis are named as chickpea probe (cpPb)

Table 6.1 (continued.) Association of DArT markers with agronomic traits under heat stressed and non-stressed conditions based on genome-wide association analysis

Significant markers	Heat stressed (late season)				Non-stressed (normal season)	
cpPb.680657	TNP	NFP	NS		GY	
cpPb.680737	Biomass					
cpPb.680770					NS	GY
cpPb.681126	TNP	NFP	NS	GY		
cpPb.681206	PH					
cpPb.681233					NS	GY
cpPb.681254	GY					
cpPb.681286	GY					
cpPb.682050					NS	
cpPb.682106	PH				PH	PW
cpPb.682120					NS	GY
cpPb.682212	DFF	D50F	DPM			
cpPb.682354					NS	
cpPb.682410					NS	GY
cpPb.682533					NS	GY
cpPb.682683	PH	TNP	NFP	NS		
cpPb.682733	TNP	NS	GY		TNP	NFP

DFF-days to first flower; D50F-days to 50% flower; DFP-days to first pod; DPM-days to physiological maturity; PH-plant height; PW-plant width; TNP-total number of pos; NFP-number of filled pods; NS-Number of seeds; GY-grain yield. Markers used for DArT analysis are named as chickpea probe (cpPb)

Table 6.2 Association of unmapped DArT markers with disease resistance based on genome-wide association analysis

Trait	DArT markers
Blight	322652; 350149; 488895; 489449; 491447; 491634; 678036; 678476; 678810; 679196; 680572; 680811; 680963; 681156; 681286; 681330; 681331; 681332
Wilt	171267; 171280; 171297; 171311; 171324;171327; 171351; 171372; 171398; 171444; 171462; 171511; 171609; 172047; 172087; 172098; 172103; 172105; 172123; 172157; 172172; 172175; 172214; 172220; 172241; 172249; 172318; 72327; 172338; 172342; 172351; 172388; 172844; 172847; 172882; 172893; 172970; 172986; 173010; 173030; 173057; 173076; 173078; 173102; 173105; 173141; 173159; 173192; 173214; 173253; 173335; 173393; 173406; 173439; 173445; 173446; 173510; 173554; 322934; 323720; 323735; 323988; 324078; 325805; 325866; 325891; 325985; 326002; 326063; 326066; 326587; 327629; 327643; 327689; 327838; 350147; 350201; 350267; 350376; 350432; 350524; 350549; 489144; 489561; 490180; 675517; 675583; 675600; 675695; 676060; 676266; 676884; 676935; 676970; 677540; 677562; 677624; 677797; 678166; 678321; 680014; 680528; 681083; 681402; 681975

In population structure analysis using R software a dendrogram was generated to show the relationship among lines. Four clusters of genotypes were found based on traits observed (Fig 6.2). Group 1 and 2 contained 46 stable heat sensitive genotypes and 16 moderate heat sensitive genotypes respectively. High yielding genotypes under normal conditions and heat tolerant genotypes were clustered in group 3. Fourteen moderate heat tolerant genotypes were presented in group 4.

A total of 359 DArT markers were studied in association analysis. Of these, 107 markers were linked with agronomic traits under heat stressed and non-stressed conditions. All eleven traits assessed were linked in both conditions. The mapped and unmapped DArT markers that significantly associated with the 11 traits are summarised in Table 6.1. The markers 489318 and 679133 were linked to phenology (DFF; D50F and DFP) under both heat stressed and non-stressed conditions. Similarly, five markers (675905; 677822; 350553; 491458; 680218) were linked with the pod characteristics TNP; NFP; NS and grain yield in both conditions. At high temperature only, DPM was linked with 172238; 489266; 676142; 679404; and 680354. Whilst in non-stressed conditions DPM was linked with markers present in regions that control phenology.

Plant growth parameters such as biomass, plant height and plant width were linked with DArT markers either individually or in combination with pod characters in both conditions (Table 6.1). Plant height was linked with 10 DArT markers at high temperature only compared with the same materials grown without stress. A maximum of four traits (TNP, NFP, NS, GY) were linked with the same DArT markers in both heat stressed and non-stressed environments. However, the marker associations were more complex for grain yield. Nine markers (678546, 678822, 490210, 172052, 350495, 490878, 678527,681254 and 681286) were linked to GY under heat stress only and a different set of five markers were linked to GY in non-stressed conditions. In total, grain yield and associated pod characters were linked with 44 markers at high temperature compared to 41 in non-stressed conditions. Nine markers were linked to variation in phenology.

The mapped and unmapped DArT markers and their significant associations with traits are listed in Appendix 11. In heat stressed environments, the probability values ranged from 3 to 43.7 compared with non-stressed conditions (3 to 23.65) (Appendix 11). Higher probability values indicate higher levels of significance and the marker-trait associations assessed were generally more significant under heat stress than non-stressed conditions. Of the 359 DArT markers, only 12 DArT markers were mapped in the inter-specific reference genetic map of chickpea developed by Thudi *et al*. (2011). Four DArT markers were located on each of chromosomes 2 and 4. Phenology, growth and yield traits were positioned on the chromosome 2 (Fig 6.3). Whereas on chromosome 4 the primary characters were grain yield and pod characters under both heat stressed and non-stressed conditions (Fig 6.3). Under heat stress regions linked to grain yield were found on chromosome1, 6 and 7 (Fig 6.1). No marker-trait association was located on chromosome 8.

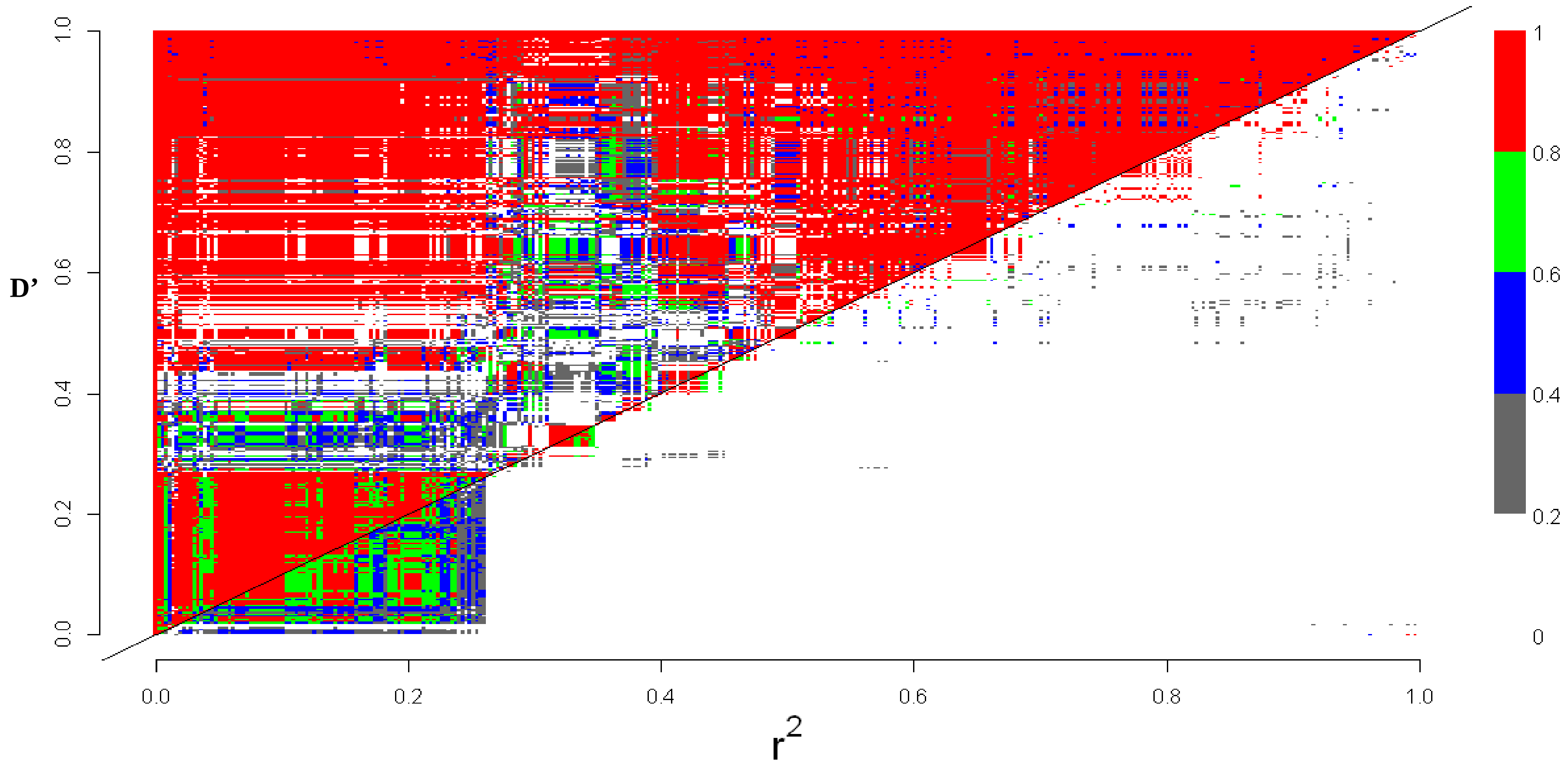

Fig 6.1 LD plot showing polymorphic sites of loci

Fig 6.2 Analysis of population structure showing clustering of genotypes based on traits observed in both heat stressed and non-stressed conditions

Clusters from above Fig 6.2 explained in this table

Clusters	Genotypes
1 (46)	ICC 3337 ICC 3867 ICC 4861 ICC 5116 ICC 5124 ICC5270 ICC 5566 ICC 5912 ICC 6152 ICC 6169 ICC 6231 ICC 6283 ICC 6293 ICC 7192 ICC 7193 ICC 7241 ICC 7263 ICC 7292 ICC 7294 ICC 7298 ICC 7308 ICC 7570 ICC 7669 ICC 8273 ICC 8397 ICC 8527 ICC 8943 ICC 9557 ICC 10090 ICC 10134 ICC 10600 ICC 10674 ICC 11795 ICC 11886 ICC 11903 ICC 12121 ICC 12123 ICC 14183 ICC 15367 ICC 15388 ICC 15795 ICC 15807 ICC 16626 ICCV 3 ICCV 92809 ICC 8261
2 (16)	ICC 982 ICC 988 ICC 1017 ICC 1025 ICC 1026 ICC 2204 ICC 2234 ICC 2935 ICC 3335 ICC 3429 ICC 4482 ICC 4933 ICC 4969 ICC 5384 ICC 16343 ICC 16436
3 (77)	ICC 1163 ICC 1164 ICC 1471 ICC 3336 ICC 3485 ICC 3935 ICC 4902 ICC 5186 ICC 5794 ICC 6334 ICC 6969 ICC 8166 ICC 8474 ICC 9125 ICC 9562 ICC 9567 ICC 11553 ICC 11901 ICC 12289 ICC 12312 ICC 12511 ICC 12554 ICC 12620 ICC 12787 ICC 13941 ICC 14176 ICC 14177 ICC 14179 ICC 14315 ICC 14406 ICC 14456 ICC 14497 ICC 14533 ICC 14575 ICC 14592 ICC 14674 ICC 14913 ICC 15380 ICC 16141 ICC 16173 ICC 16216 ICC 16219 ICC 16298 ICC 16833 ICC 16835 ICC 17083 ICCC 37 ICCL 81248 ICCL 83149 ICCL 87207 ICCV 90201 ICCV 92944 ICCV 93512 ICCV 93952 ICCV 93954 ICCV 94954 ICCV 95311 ICCV 96836 ICCV 97105 ICCV 07101 ICCV 07102 ICCV 07104 ICCV 07105 ICCV 07108 ICCV 07109 ICCV 07113 ICCV 07116 ICCV 07117 ICCV 07118 ICC 283 ICC 1882 ICC 4958 ICCV 94916-8 ICCV 98901 ICCV 98902 ICCV 98903 ICCV 98904
4 (14)	ICCV 95332 ICCX 820065 ICCV 96030 ICC 17109 ICCV 07110 ICCV 07115 ICCV 06301 ICCV 06302 ICCV 06306 ICCV 07304 ICCV 07311 ICCV 07312 ICCV 07313 ICCV 94916-4

Number in parenthesis indicates total number of genotypes in that cluster.

Table 6.3a Location of significant DArT markers associated with phenology under heat stressed and non-stressed conditions for each chromosome. Other reported genes and QTLs on the respective chromosomes are listed

Chr	DArT marker	DFF	Chr	DArT marker	D50F	Chr	DArT marker	DFP	Chr	DArT marker	DPM	Flower colour gene
1	-	-	1	-	-	1	-	-	1	-	QTL[a]	B/b[c]
2	322806 489318	-	2	322806 489318	-	2	489318	-	2	-	QTL[a]	-
3		QTL[d] QTL[e]	3	-	QTL[f]	3	-	-	3	-	-	-
4	-	-	4	-	-	4	-	-	4	-	-	-
5	-	-	5	-	-	5	-	-	5	-	-	-
6	-	-	6	-	-	6	-	-	6	-	-	-
7	-	-	7	-	-	7	-	-	7	-	-	-
8	-	-	8	-	-	8	-	-	8	-	-	-

Table 6.2a and b: [a]Gowda *et al*. (2011); [b]Cobos *et al*. (2007); [c]Cobos *et al*. (2005); [d]Aryamanesh *et al*. (2010); [e]Hossain *et al*. (2010); [f]Cho *et al*. (2002)
(DFF-days to first flower; D50F-days to 50% flower; DFP-days to first pod; DPM-days to physiological maturity; TNP-total number of pods; NFP-no of filled pods; NS-seed number; GY-grain yield)

Table 6.3b Location of significant DArT markers associated with pod characters and grain yield under heat stressed and non-stressed conditions for each chromosome. Other reported genes and QTLs on the respective chromosomes are listed

Chr	DArT marker	TNP	Chr	DArT marker	NFP	Chr	DArT marker	NS	Chr	DArT marker	GY	Double podding gene	Seed coat thickness gene
1			1		-	1	-	-	1	678546 678822	QTL[a]	-	Tt/tt[e]
2	676219	QTL[a]	2	676219	-	2	-	-	2	676219 682393	QTL[a]	-	-
3			3		-	3	-	-	3	-	-	-	-
4	675277 675905 677822	QTL[a]	4	675277 675905 677822	-	4	675277 675905 677822	QTL[f]	4	675277 675905 677822	QTL[b]	-	-
5	-	-	5	-	-	5	-	-	5	-	-	-	-
6	-	-	6	-	-	6	-	-	6	-	-	*s*[f]	-
7	491194	-	7	-	-	7	-	-	7	490210 491194	QTL[a]	-	-
8	-	-	8	-	-	8	-	-	8	-	-	-	-

Table 6.3c Location of significant DArT markers associated with plant growth under heat stressed and non-stressed conditions for each chromosome. Other reported genes and QTLs on the respective chromosomes are listed

Chr	DArT marker	Biomass	Chr	DArT marker	PH	Chr	DArT marker	PW	Plant growth habit gene	Stem pigment gene
1	-	-	1	-	QTL[a]	1		QTL[a]	-	-
2	676219 682393	-	2	489318	QTL[a]	2	322806 489318	QTL[a]	-	-
3	-	-	3	-	-	3		-	*Prostrate*[b]	-
4	-	-	4	-	QTL[a]	4		QTL[a]	-	-
5	-	-	5	-	-	5		-	-	-
6	-	-	6	-	-	6	172290	-	-	-
7	-	-	7	-	-	7		-	-	-
8	-	-	8	-	-	8		-	-	*C*[c]

[a]Gowda *et al*. (2011); [b]Aryamanesh *et al*. (2010); [c]Cho *et al*. (2002) (PH-plant height; PW-plant width)

Table 6.3d Location of significant DArT markers associated with disease resistance for each chromosome. Other reported genes and QTLs on the respective chromosomes are listed

Chr	DArT marker	Blight	Chr	DArT marker	Wilt	Chr	DArT marker	BGM
1	-	-	1	-	-	1	-	-
2	-	QTL[1] *ar1*, *ar2a*[2]	2	-	-	2	-	-
3	-	-	3	681290	$Foc0_1$/$foc0_1$[3]	3	-	QTL[8]
4	327672	QTL[4, 5, 6, 7] *ar2b*[9]	4	489107, 489311 675277, 677636	-	4	-	-
5	-	-	5	681450	-	5		-
6	-	QTL[4, 6]	6	172879	-	6		QTL[8]
7	-	-	7		-	7	490874 682790	-
8	-	-	8		-	8		-

[1]Cobos *et al*. (2006); [2]Udupa and Baum (2003); [3]Cobos *et al*. (2005); [4]Anbessa *et al*. (2009); [5]Aryamanesh *et al*. (2010); [6]Taran *et al*. (2007); [7]Kottapalli *et al*. (2009); [8]Anuradha *et al*. (2011) (BGM – Botrytis grey mould)

Table 6.2e Location of reported genes and QTLs for salt and drought tolerance on the respective chromosomes are listed

Chr	DArT marker	Salt	Chr	DArT marker	Drought
1	-	Salt	1	-	QTL-GY, HI, DTI, PH, CTD[2]
2	-	QTL-shoot dry weight[1]	2	-	-
3	-	-	3	-	-
4	-	-	4	-	QTL-flowering time[2]
5	-	QTL-flowering time[1]	5	-	-
6	-	-	6	-	QTL-DTI, PH[2] QTL-SDW, LDW, RLD, RSA[3]
7	-	QTL- pod and seed number, 100 seed weight[1]	7	-	QTL-DTI, DM, SC[2]
8	-	QTL-seed number[1]	8	-	QTL-GY, HI, DTI, PH, CTD[2]-

[1]Vadez *et al*. (2012); [2]Rehman (2009); [3]Nayak (2010); (GY-Grain yield; HI-Harvest Index; DTI-Drought tolerance index; PH-Plant height; CTD-Canopy temperature depression; SDW-Shoot dry weight; LDW-Leaf dry weight; RLD-Root length density; RSA-Root surface area; DM-days to maturity; SC-Stomatal conductance)

In addition, the published information on the chromosomal locations of genes and QTL for phenology, growth and yield traits are presented in Tables 6.3a, b, c including genes controlling flower colour (*B/b*), plant growth habit (*prostrate*), stem pigmentation (*C*), double podding (*s*) and seed coat thickness (*Tt/tt*). In this study, grain yield and pod characters were reported on chromosomes 2 and 4. The published QTL reports for grain yield and pod traits showed a similar result. QTL controlling phenology under non-stressed and abiotic stress (salt and drought) conditions was reported in chromosome 3 and 4 respectively, in the previous studies (Aryamanesh *et al*. 2010; Vadez *et al*. 2012; Rehman 2009). The unmapped markers associated with ascochyta blight and fusarium wilt are shown in Table 6.2. Botrytis grey mould (BGM) was associated only with DArT markers 490874 and 682790 on chromosome 7 (Fig 6.3). The genomic region resistant to blight was found on chromosome 4 and genomic regions linked to wilt resistance were found on chromosomes 4, 5 and 6 (Fig 6.3). However, in this study the phenology-marker associations under heat stress were located on chromosome 2. In general, no QTLs were reported for any traits on chromosomes 5 and 8 under either normal or stressed conditions. At present, no QTLs were found for DFP, NFP and biomass. The current study identified new genomic regions for DFF, D50F, DFP, TNP, NFP, biomass and plant width not linked to previously reported QTL. All significant marker-trait associations identified in this study generated using R software are listed in Appendix 12. However, the information on markers mapped to specific chromosomes is limited.

6.4. Discussion

The chickpea genotypes used in this study for field screening at high temperature were compiled for association analysis and to the best of my knowledge this is the first association analysis in chickpea (including other crops) under high temperature. The experiments were conducted in a semi-arid environment under two conditions (heat-stressed and non-stressed), thus allowing the measurement of different traits over a range of temperatures. The observed traits and number of lines tested in this study are greater than previously reported in many association studies in other crops (Ravel *et al*. 2005; Breseghello and Sorrells 2006; Maccaferri *et al*. 2011). A higher number of agronomic traits (12) were reported in rice in the association analysis followed by wheat and maize (9 traits) (Al-Maskri *et al*. 2012). The present study reports 11 agronomic traits of chickpea in both heat stressed and non-stressed conditions. Providing intrinsic nature of exploiting germplasm from different environments, the present chickpea association analysis provides marker-assisted selection for future plant breeding for heat tolerance.

Many significant mapped markers associated in genomic regions in the current study align with previously reported QTLs that influence traits such as plant height, plant width, pod number and grain yield (Tables 6.1 and 6.3a, b, c; Fig 6.3). In addition, markers not linked to previously reported QTL for grain yield under heat stressed and non-stressed conditions were identified. There were also new regions accounting for biomass, plant height, phenology (DFF; D50F; DFP) and seed number under heat stress (Table 6.1). Grain yield under heat stress significantly associated with markers 678546 and 678822 located on chromosome 1. Grain yield under drought linked to a genomic region of TA8 reported by Rehman (2009) is located 25 cM from the DArT markers associated with grain yield under heat stress on chromosome 1 (Fig 6.3). This yield effect may be linked to a QTL for plant height found on the same chromosome under normal conditions (Gowda *et al*. 2011). Gene *s* responsible for double podding associated with STMS markers (TA80 and closely linked with TA176) located on the chromosome 6 is not far from the DArT marker (172290) which is significant for plant width under normal conditions (Cho *et al*. 2002; Fig 6.1).

Similarly, new DArT marker associations with agronomic traits were identified on the B genome of wheat and AA is a first step in discovering the genetic variants underlying these complex traits (Yu *et al*. 2012).

The double podding gene (*s*) was useful for breeding high yielding chickpea cultivars and double pod chickpea mutants (Ali *et al*. 2010). On chromosome 4, DArT markers associated with pod characters and grain yield under both heat stressed and normal conditions are located 10 cM from STMS marker TA130 in Fig 6.1. TA130 is linked to a seed size QTL under normal conditions (Cobos *et al*. 2007). Rehman (2009) identified a close relationship between TA21 and the genomic region controlling reproductive period (number of days between flowering and maturity) under drought in chickpea (Table 6.3e). The current AA found that the genomic region controlling reproductive period under drought is closely linked with DArT markers associated with total pod number and grain yield under heat stress (Fig 6.3) on chromosome 7. This is most likely distinguishing the genomic regions for drought and heat stress.

The present study also incorporated genotype responses to three major diseases of chickpea (Raju – unpublished data; Table 6.2). The AA identified chromosomal regions where QTL linked to blight (chromosomes 2 and 4) (Table 6.2c) has been identified (Cobos *et al*. 2006; Anbessa *et al*. 2009). However, nineteen unmapped markers were also significantly associated with blight resistance and 106 unmapped markers associated with wilt resistance across the genome (Table 6.2). Fusarium wilt resistance gene ($Foc0_1/foc0_1$) was already reported in chromosome 3 (Cobos *et al*. 2005; Table 6.3d), a new genomic region associated with fusarium wilt was found in chromosome 5 from present AA (Fig 6.3). Botrytis grey mould (BGM) association was also found and was unlinked to previous mapping studies (Fig 6.3). Similar associations for disease resistance were reported in wheat (Crossa *et al*. 2007).

The markers associated with more than one trait may be efficiently utilised in the improvement of more than one trait at a time in chickpea under heat stressed and non-stressed conditions through marker assisted selection. New chromosome regions of phenology and grain yield may provide increases in grain yield not only under optimum conditions, but also with environmental interactions i.e. high temperature. The flowering time is a major component of crop adaptation and grain yield in chickpea (Kumar and Abbo 2001).

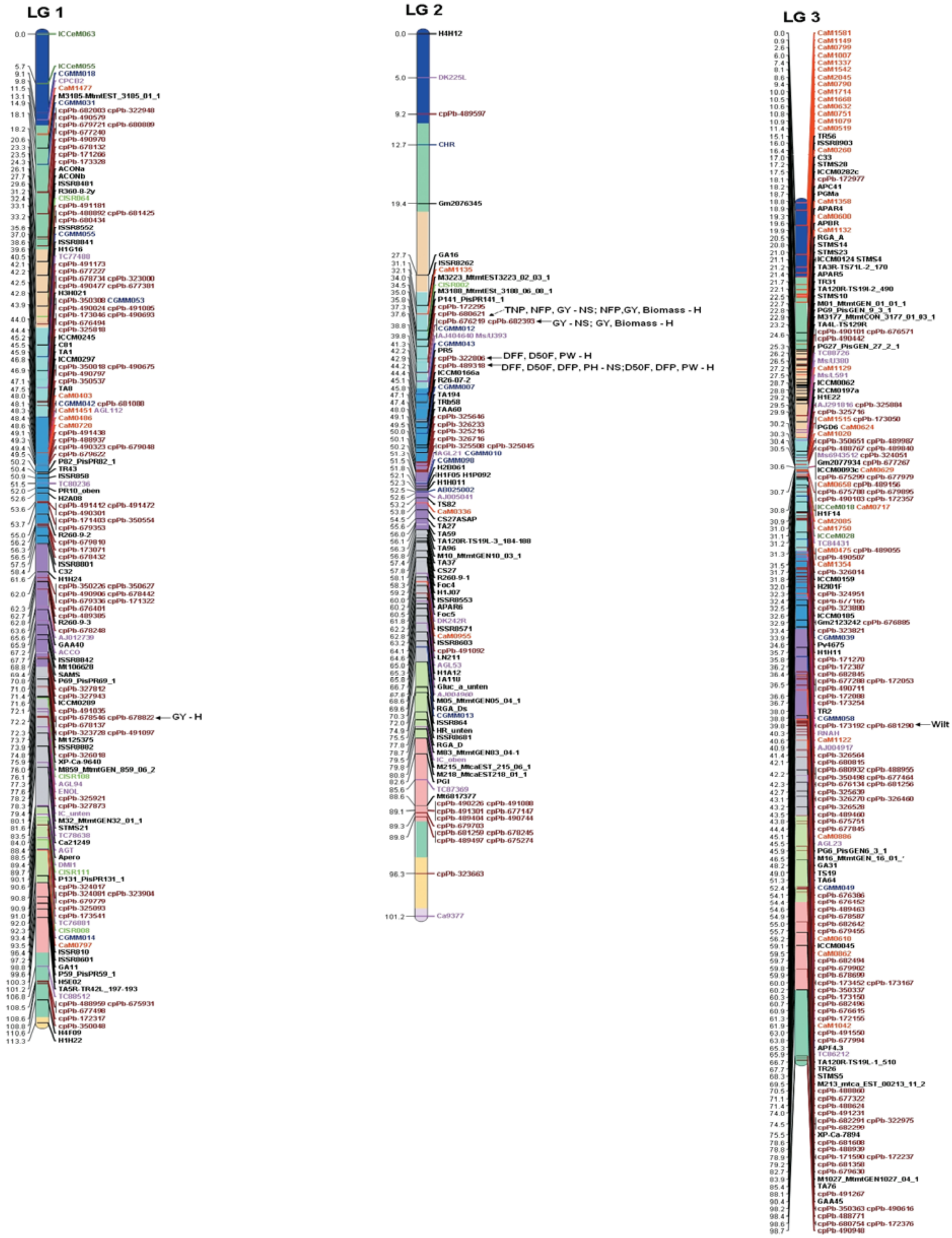

Fig 6.3 Chromosome locations of the significant marker-trait association have been reported on chickpea consensus map (Thudi *et al*. 2011)

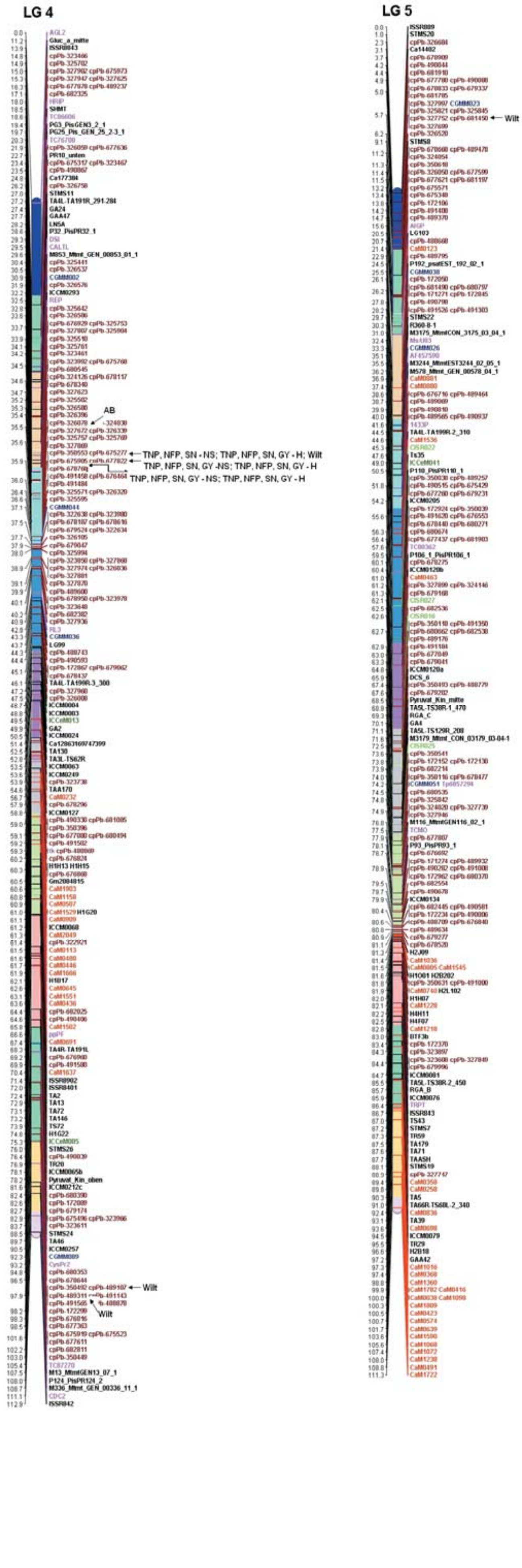

Fig 6.3 continued.

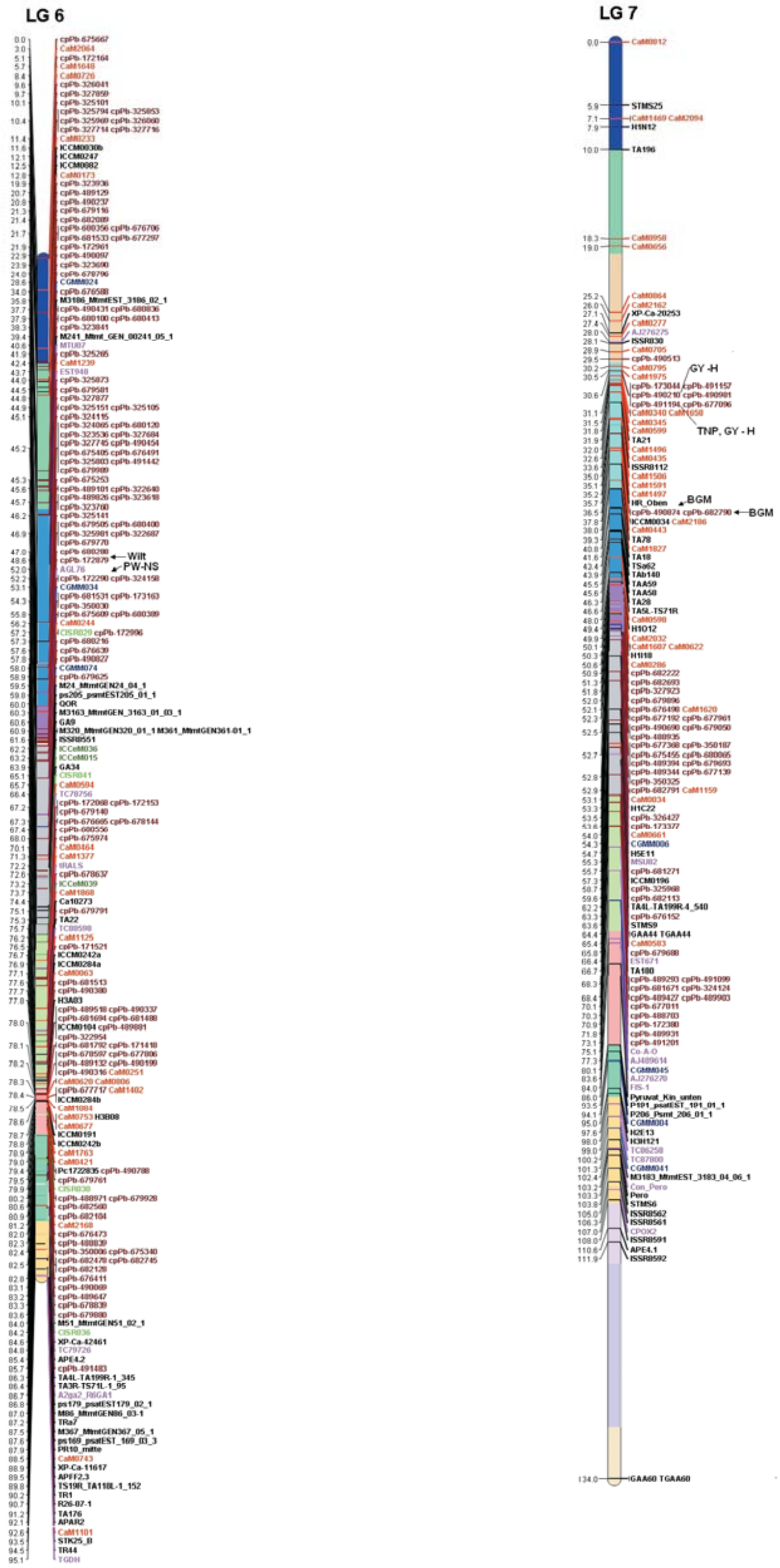

Fig 6.3 continued.

6.5. Conclusions

Many new genomic regions were found for phenology and grain yield under heat stress and normal conditions. This demonstrates the feasibility of applying association analysis to explore complex traits in future. The number of markers significantly associated with grain yield was higher in heat stressed environments, suggesting that many genes are present that control plant response to high temperature. Therefore, this study identified genomic regions associated with heat tolerance in chickpea and identification of the genes or QTLs linked to this response is the obvious next step.

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Chapter 7: Inheritance of heat tolerance in chickpea

7.1. Introduction

High temperature is an important abiotic stress in chickpea, which cause significant yield losses and limits the area of production (Gaur *et al*. 2007). Generally high temperature occurs during reproductive development in chickpea, especially in the Indian subcontinent and recently emerged regions like Australia (Berger *et al*. 2011). Mean global temperature is expected to increase 1.1 – 6.4˚C by the end of this century, accompanied by an increasing frequency of hot extremes and heavy precipitation (IPCC 2007a, b). Such climate change is expected to have an impact on cropping systems and grain yield.

In chickpea, high temperature stress causes flower abortion, thus reducing the grain yield (Kaloki 2010). Early maturing varieties tend to have reasonable grain yield under high temperature stress, but lower biomass and a shortened total photosynthetic duration (IIPR 2010). Flowering and podding in chickpea are very sensitive to high temperature. Grain yield is reduced when plants are exposed to 35˚C during flowering and early pod development (Wang *et al*. 2006). High temperature of 35˚C can also affect pollen fertility, pollination and pod set in chickpea (Devasirvatham *et al*. 2010). Therefore, the identification of sources of heat tolerance in chickpea is as important as understanding the mechanism of heat tolerance. In Kenya, chickpea growth and yield was compared in cool and warm growing regions leading to the identification of heat tolerant genotypes (Kaloki 2010). However, there is a lack of efficient field screening techniques for heat tolerance. Krishnamurthy *et al*. (2011) used delayed sowing in the field (late season) to expose plants to high temperature during reproductive development and compared the growth and grain yield to normally sown materials. This technique identified the sources of heat tolerance in chickpea in a semi-arid environment. Later, Upadhyaya *et al*. (2011) used a similar technique to identify short duration heat tolerant chickpea genotypes in a semi-arid environment. Krishnamurthy *et al*. (2011) suggested that pod number per plant is a key trait for selection of heat tolerant genotypes.

The duration of crop maturity in chickpea in any given environment is a function of phenological and morphological variables (Kumar *et al*. 1996). However, recording days to maturity under heat stress is difficult due to forced maturity and many plant breeders use days to first flowering as a key indicator of crop maturity (Kumar and Abbo 2001). Despite the recognition of the importance of identifying sources of heat tolerance, large-scale improvement of heat tolerance in chickpea is limited by access to genetic diversity and knowledge of the genetic control of heat tolerance. Nevertheless, significant genetic diversity was observed for heat tolerance in the reference collection of chickpea germplasm (Krishnamurthy *et al*. 2011). Heat tolerant and sensitive genotypes were selected from this reference collection to study the inheritance of heat tolerance in chickpea. The genetic basis of heat tolerance in chickpea has not been published. However, the genetics of heat tolerance was studied in bean and cowpea (Marsh *et al*. 1985; Dickson and Petzoldt 1989; Marfo and Hall 1992; Rainey and Griffiths 2005). The present study was conducted to investigate the genetic control of heat tolerance in chickpea at reproductive development (flowering, podding and seed set) at high temperature (33-41˚C). The study hypothesised that improved heat tolerance during reproductive development could lead to improve grain yield under heat stress.

7.2. Materials and methods

Two heat tolerant genotypes ICC 1356 and ICC 15614 and two heat sensitive genotypes ICC 4567 and ICC 10685 were selected from the reference collection of chickpea germplasm based upon the previous study (Krishnamurthy *et al*. 2011). These parents are desi type. Four crosses were made viz. ICC 1356 x ICC 15614; ICC 10685 x ICC 15614; ICC 4567 x ICC 15614 and ICC 4567 x ICC 1356. In the non-stressed condition (normal season) the parents and F_1 were screened in the field. In the stressed condition (late season) parents, F_1 and F_2 were screened in the field by exposure to high temperature during reproductive development. A separate replicated study was conducted both non-stressed (normal) and stressed (late) conditions to observe the phenology and grain yield of the parents. The experiments were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India.

The four parents were grown in a 4 m row in the field with two replications to study phenology and grain yield under normal and late season sowing. The normal season crop was sown on 13th Nov 2010 and the late season crop was planted on 18th Feb 2011.

The parents and F_1 were grown in an un-replicated trial in the normal season. During the stressed season (late), parents, F_1 and F_2 seeds were grown in an un-replicated trial. Parents and F_1s were sown in single row plots of 4 m length in both seasons and the number of plants in the F_2 population varied among the crosses in the late season. The normal season experiment was sown on 13th Nov 2010 and the late season experiment was sown on 9th March 2011.

For both seasons, including the population study and parental screening, seeds were sown manually on the ridges of beds by maintaining 15 cm spacing between the plants. Seed treatment, irrigation, maintenance of field was explained in chapter 3. A total of 16.2 mm and 5.8 mm of rain was received during the normal and late seasons, respectively.

In the stressed (late) season, twenty plants were tagged in the parents and F_1s for observation. In the F_2 population, 100 plants were tagged in the cross of ICC 1356 x ICC 15614. In the remaining three crosses, 300 plants were tagged for observation during the late season. Plant phenology of the parents was assessed by recording days to first flower (DFF), days to 50% flower (D50%F) and days to first pod (DFP) in both seasons. During the normal season, DFF and grain yield were recorded in the F_1s. In the late season, DFF, pod number per plant (TNP), filled pod number per plant (NFP), seed number per plant (NS) and grain yield per plant (GY) was recorded.

The maximum and minimum temperatures of the growing period during the normal and late seasons were recorded. The temperature data for parents during both seasons are presented in Fig 7.1. The temperature data for parents, F_1 and F_2 in the late season experiment is presented in Fig 7.2.

Statistical analysis

The late season data were analysed using Genstat 12th version to calculate the range and mean of traits. Broad sense heritability (h^2) was calculated for each population according to Burnette and White (1985):

Heritability (h^2) = $[V_{F2} - (V_{P1} + V_{P2} + V_{F1})/3]/V_{F2}$

Where V = generation variance

Then the distribution pattern of all traits (DFF, TNP, NFP, NS and GY) under heat stress for parents, F_1 and F_2 were plotted using MiniTab version 15.

7.3. Results

7.3.1. Phenology and yield of parents

The phenology and grain yield of heat stressed (late season) and non-stressed (normal season) experiments are shown in Table 7.1. The crop phenology (DFF, D50%F and DFP) was earlier under heat stress. Both heat tolerant and sensitive genotypes matured earlier in the late sown trials. The earliness, determined as the difference in number of days between heat stressed and non-stressed experiments, was high in the sensitive genotypes (20 days) compared with tolerant genotypes (5-6 days) (Table 7.1). In sensitive genotypes the grain yield was less than 2 g per plant under heat stress which was significantly ($P<0.05$) lower than heat tolerant genotypes.

7.3.2. Response of populations to high temperature

Significant differences were detected among F_1s and F_2s under heat stress. F_1 plants were screened under both heat stressed (Table 7.3) and non-stressed (Table 7.2) conditions. The F_2s were screened at high temperature along with their parents to observe difference in crop phenology and grain yield. In all crosses under high temperature, the range in number of days to first flower in the F_1s was longer than under normal temperature (Tables 7.2 and 7.3). The maximum temperature during the flowering in F_1s varied between 33.9-41.4˚C (Table 7.4) in the stressed season whilst in the non-stressed season, it varied between 21-25˚C (Fig 7.1). The F_2s were exposed to a maximum temperature range of 33.9-41.7˚C and minimum temperature of 21-28.6˚C during flowering (Table 7.4).

At high temperature, the F_1 and F_2 of the cross ICC 1356 x ICC 15614 flowered at 53-61 and 41-64 DAS, respectively (Table 7.3). The mean DFF of the F_2 was 14 days earlier than the mid-parental (55 days), indicating the earliness is dominant at high temperature. Similarly, the F_2 of the cross ICC 10685 x ICC 15614 flowered earlier (14 days) than the mid-parent value. The F_2 of ICC 4567 x ICC 1356 flowered earlier (36 days) than other crosses and had a mid-parent value of 63 days. The DFF mean was 27 days earlier than the mid-parent indicating that the F_2 plants were escaping from heat stress. The F_2 and F_1s of ICC 4567 x ICC 15614 flowered at 50-67 and 53-67 DAS with a mean of 56 and 57 DAS, respectively. In this cross, the F_2 mean was similar and the difference between the F_2 mean and the mid-parent (58 days) was lower than all other crosses. There appeared to be no dominance of earliness at high temperature in this cross. The DFF of the F_2s in ICC 4567 x ICC 15614 were within the parental ranges at high temperature and no transgressive segregation was observed.

Table 7.1 Phenology and grain yield of parents under normal (non-stress) and late (heat stress) seasons at ICRISAT[a] (***, ** and * at *P*<0.001; *P*<0.01 *P*<0.05)

Genotypes	Origin	Season	DFF	D50%F	DFP	Yield (g/plant)
ICC 4567	India	Normal	63	66	70	17.5
		Late	48	51	54	1.8
ICC 10685	Turkey	Normal	62	65	68	12.7
		Late	48	52	65	0.8
ICC 15614	Tanzania	Normal	53	57	60	28.6
		Late	48	52	53	14.25
ICC 1356	India	Normal	52	55	58	34.9
		Late	46	49	52	11.7
LSD (*P*=0.05)		Season	2.6***	2.3**	3.9*	3***
		Genotypes	3.7*	3.3*	5.6*	4.3**
		Genotypes x Season	5.3*	4.6*	NS	NS

[a]Date of sowing for normal=13[th] Nov 2010 and late=18[th] Feb 2011

Table 7.2 Days to first flower and grain yield of F_1 under normal season (non-stress)[a]

Crosses	No of plants	DFF		Grain yield g/plant	
		Range	Mean± S.E	Range	Mean± S.E
F1 ICC 1356-HT x ICC 15614-HT	22	54-58	55±1.3	3.1-67.2	43.4±14.5
F1 ICC 10685-HS x ICC 15614-HT	17	50-53	51±1.1	5.1-138.5	60.5±36.5
F1 ICC 4567-HS x ICC 15614-HT	7	62-63	62±0.5	51.3-147.8	103.8±38.2
F1 ICC 4567-HS x ICC 1356-HT	6	54-55	54±0.5	50.3-199.3	107.4±58.9

[a]Date of sowing for normal=13[th] Nov 2010

The heat sensitive parents (ICC 4567 and ICC 10685) did not produce any pods at high temperature whereas the heat tolerant parents produced pods (Table 7.3). Among the tolerant parents ICC 15614 produced more pods per plant (13-61 pods) than ICC 1356 (2-63 pods). Among the F_2s of the four crosses, ICC 1356 x ICC 15614 and ICC 4567 x ICC 15614 produced more pods (100 and 112 respectively). These two crosses had a higher number of heat tolerant plants in the F_2 (Table 7.3). The F_2 plants in ICC 4567 x ICC 15614 did not produce any unfilled pods at high temperature (33.9-41.7˚C/ 21-28.6˚C) during flowering. The F_2 plants in this cross produced higher grain yield (0.03-21.7 g) compared with the F_1 plants (0.15-5.54 g) and all other crosses under high temperature.

Table 7.3 Parental, F_1 and F_2 mean values of days to first flower (DFF), total number of pods (TNP), number of seeds and grain yield under heat stress at ICRISAT[a]

Lines	No of plants tagged	DFF		TNP		NFP		No of seeds		Grain yield (g)	
		Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.
ICC 4567 – HS	20 (0)	49-69	62±1.5	0		0		0		0	
ICC 10685 – HS	20 (0)	48-65	57±1.3	0		0		0		0	
ICC 1356 – HT	20 (20)	47-64	56± 1.0	2-63	28±4.3	2-62	26±3.9	2-79	33±5.2	0.34-10.5	4.44±0.7
ICC 15614 – HT	20 (20)	51-56	53±0.3	13-61	31±2.9	13-61	28±2.8	15-74	35±3.9	1.68-8.2	3.87±0.5
F1 ICC 1356-HT x ICC 15614-HT	20 (17)	53-61	57±0.7	2-70	38±5.2	1-66	36±5.0	1-86	49±5.9	0.18-9.3	5.50±0.7
F2 ICC 1356-HT x ICC 15614-HT	100 (83)	41-64	54±0.4	1-100	33±2.5	1-97	31±2.5	1-115	38±3.1	0.22-15.8	4.5±0.4
F1 ICC 10685-HS x ICC 15614-HT	20 (15)	53-64	58±0.7	1-35	17±2.6	4-31	17±2.3	6-46	22±3.0	0.15-4.79	2.26±0.4
F2 ICC 10685-HS x ICC 15614-HT	300 (167)	41-67	56±0.3	1-65	17±1.0	1-61	17±1.0	1-90	22±1.4	0.06-8.85	2.25±1.8
F1 ICC 4567-HS x ICC 15614-HT	20 (16)	53-67	57±0.8	1-29	10±2.2	1-29	8±2.1	1-35	11±2.7	0.15-5.54	1.53±0.4
F2 ICC 4567-HS x ICC 15614-HT	300 (178)	50-67	56±0.2	1-112	17±1.3	1-112	17±1.3	1-148	22±1.7	0.03-21.7	2.99±0.2
F1 ICC 4567-HS x ICC 1356-HT	20 (13)	48-65	60±1.5	3-103	30±9.4	1-86	20±7.4	1-111	26±9.6	0.14-11.6	2.89±1.0
F2 ICC 4567-HS x ICC 1356-HT	300 (156)	36-67	60±0.3	1-62	12±0.9	1-55	10±0.8	1-71	12±1.0	0.02-7.94	1.29±0.1

Note: The values in the parenthesis were heat tolerant plants. The sensitive plants did not flower and/or set pods. [a]Date of sowing = 9th March 2011

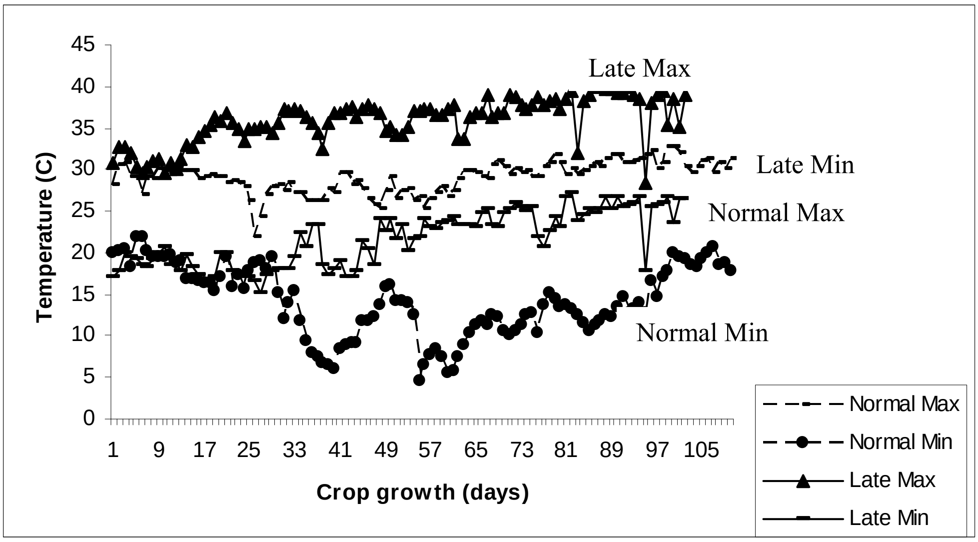

Fig 7.1 Daily maximum and minimum temperature of parent's crop growth during normal (non-stress) and late seasons (heat stress) (Normal season=13th Nov 2010 to 2nd March 2011; Late season=18th Feb to 30th May 2011)

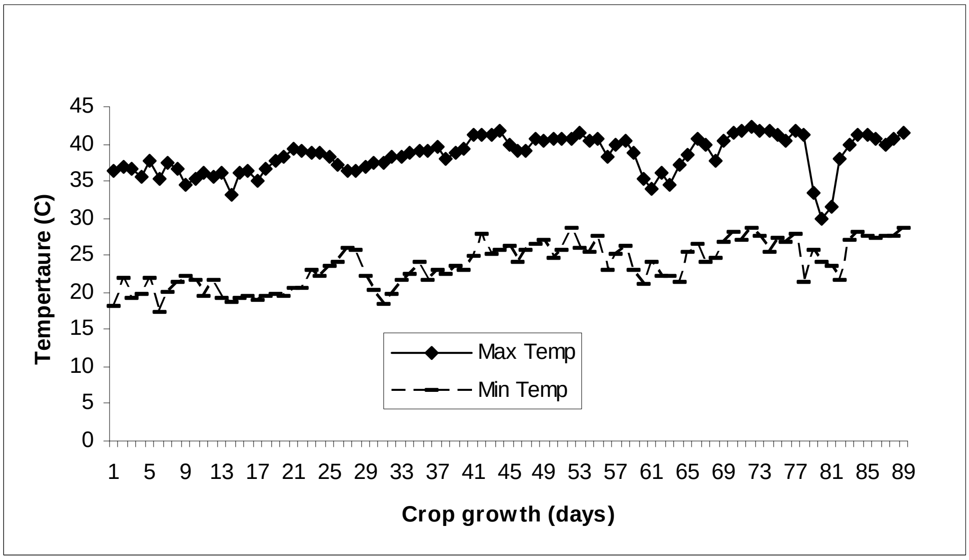

Fig 7.2 Daily maximum and minimum temperature of crop growth (parents, F_1 and F_2) during the late season (heat stress) (Crop growth period = 9th March to 30th May 2011)

Estimates of broad sense heritability for the traits DFF, TNP, NFP, NS and GY were calculated for all four crosses. Number of seeds (NS) showed higher heritability (0.90) compared with other traits. The pod traits such as TNP (0.70), NFP (0.73), NS (0.90) and GY (0.48) showed higher heritability in ICC 4567 x ICC 15614 (HS x HT) compared with other crosses (Table 7.3). The heritability of ICC 4567 x ICC 1356 was zero for all traits observed in this study indicating that both parents likely carry the same or similar genes. In contrast, the F_1 and F_2 mean for all observed traits for the other three crosses (ICC 1356 x ICC 15614; ICC 10685 x ICC 15614 and ICC 4567 x ICC 15614) always exceeded the parental mean, indicating possible dominance effects associated with heat tolerance.

Table 7.4 Range of maximum and minimum temperature during the flowering time in parents, F_1 and F_2s of chickpea under heat stress at ICRISAT[a]

Parents/Crosses	DFF	Range Maximum temperature	Range Minimum temperature
ICC 4567 – HS	49-69	33.9-41.4	21-28.6
ICC 10685 – HS	48-65	33.9-41.4	21-28.6
ICC 1356 – HT	47-64	33.9-41.4	21-28.6
ICC 15614 – HT	51-56	38.2-41.4	23-28.6
F1 ICC 1356-HT x ICC 15614-HT	53-61	33.9-41.4	21-27.5
F2 ICC 1356-HT x ICC 15614-HT	41-64	33.9-41.7	21-28.6
F1 ICC 10685-HS x ICC 15614-HT	53-64	33.9-41.4	21-27.5
F2 ICC 10685-HS x ICC 15614-HT	41-67	33.9-41.7	21-28.6
F1 ICC 4567-HS x ICC 15614-HT	53-67	33.9-41.4	21-27.5
F2 ICC 4567-HS x ICC 15614-HT	50-67	33.9-41.4	21-28.6
F1 ICC 4567-HS x ICC 1356-HT	48-65	33.9-41.4	21-28.6
F2 ICC 4567-HS x ICC 1356-HT	36-67	33.9-41.7	21-28.6

[a]Date of sowing = 9^{th} March 2011

Table 7.5 Broad sense heritability of chickpea crosses under heat stress

Crosses	DFF	TNP	NFP	No seeds	Grain Yield
ICC 1356-HT x ICC 15614-HT	0.80	0.39	0.41	0.40	0.37
ICC 10685 -HS x ICC 15614 -HT	0.44	0.49	0.55	0.57	0.40
ICC 4567 -HS x ICC 15614 -HT	0.25	0.70	0.73	0.90	0.48
ICC 4567 -HS x ICC 1356 -HT	N/A	N/A	N/A	N/A	N/A

7.3.3. Frequencies of traits in the population at high temperature

Plants from the population (F_1 and F_2) and parents were distributed across the full range of frequencies between tolerant and sensitive plants for DFF, TNP, NFP, NS and GY. The segregation of tolerant and sensitive plants was identified for DFF for all crosses. In ICC 1356 x ICC 15614, both parents were tolerant to heat but the F_1 and F_2 produced more sensitive plants which did not flower at high temperature (Appendix 10). Of the remaining three crosses, all F_1 plants in ICC 4567 x ICC 15614 were tolerant to heat and had fewer sensitive F_2 plants compared with ICC 10685 x ICC 15614 and ICC 4567 x ICC 1356 (Fig 7.3a; Appendix 10). The pod traits such as TNP, NFP and NS had zero values in the range of 120-140 plants in the F_2 of ICC 4567 x ICC 15614, ICC 10685 x ICC 15614 and ICC 4567 x ICC 1356 (Figs 7.3b, 7.3c and Appendix 11, 12 and 13). This range of zero values indicates the number of heat sensitive plants in the F_1. Among the heat tolerant plants, a higher (45) range of TNP, NFP and NS was observed in the F_2 in ICC 4567 x ICC 15614. A similar pattern was reflected in the grain yield (Appendix 14). Therefore, ICC 4567 x ICC 15614 was the best cross at high temperature.

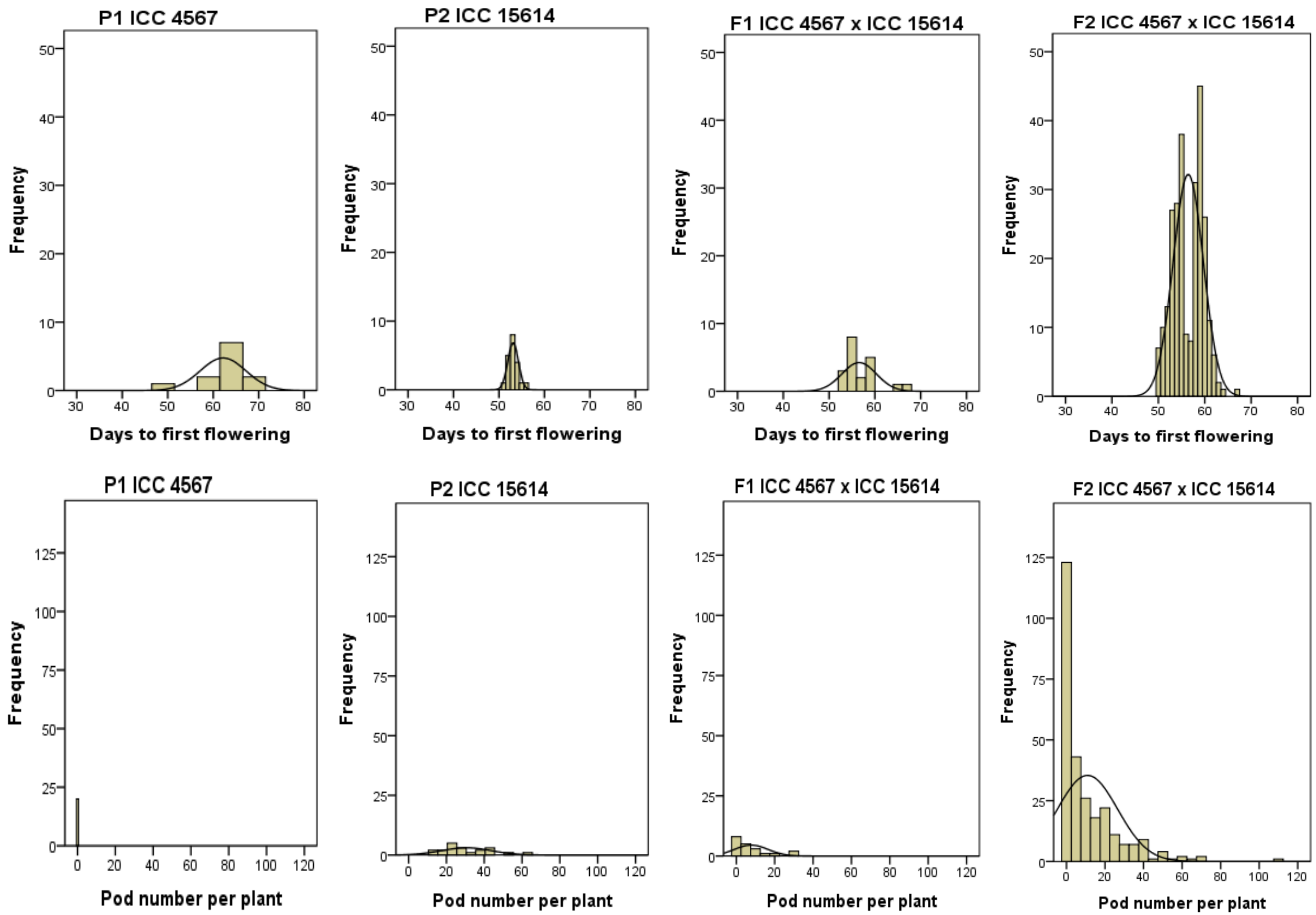

Fig 7.3a Frequency distribution of ICC 4567 × ICC 15614

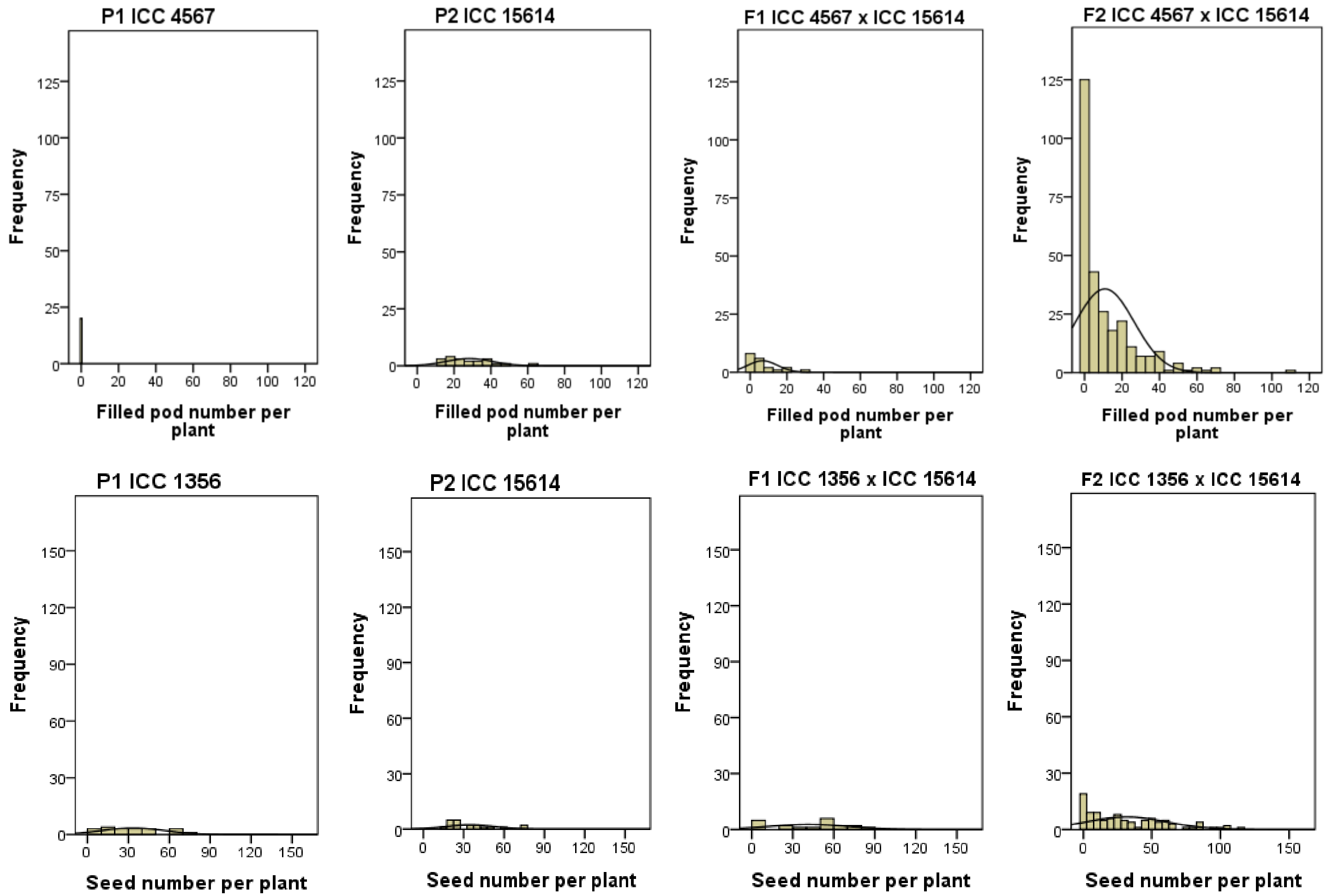

Fig 7.3b Frequency distribution of ICC 4567 × ICC 15614

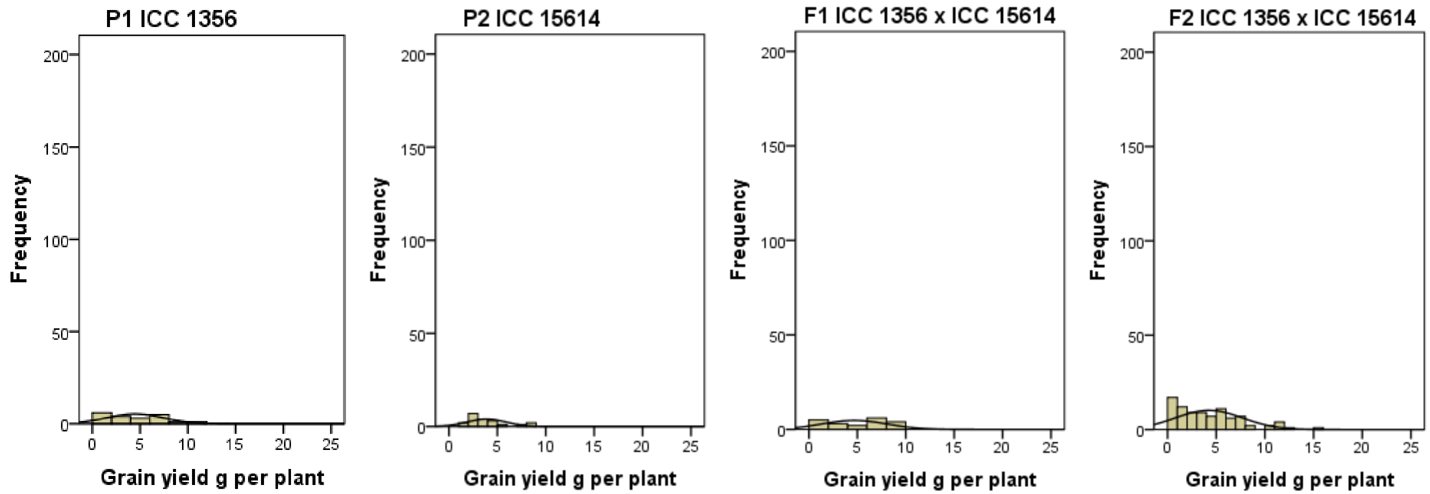

Fig 7.3c Frequency distribution of ICC 4567 × ICC 15614

7.4. Discussion

Exposure to high temperature especially during reproductive development results in yield loss in chickpea. Reduced pod set has been recorded under high temperature (>35˚C) in chickpea (Wang *et al*. 2006; Krishnamurthy *et al*. 2011; Upadhyaya *et al*. 2011) and attributed to impaired pollen fertility (chapter 5). Early flowering also influences heat tolerance (Table 7.1). At high temperature, heat sensitive genotypes (ICC 4567 and ICC 10685) were generally infertile and subsequent grain yield very low.

Sensitivity to heat stress was visible by flower abortion. Other pulse crops such as mung bean, cowpea and bean tend to drop flowers at high temperature due to changed metabolic activity (Marfo and Hall 1992; Shonnard and Gepts 1994; Khattak *et al*. 2009). However, chickpea appears more susceptible to pollen sterility which makes the breeding program easier to target than other pulse crops. The parents in this study were pre-screened in the field and controlled environments using pollen as a trait. The critical temperature for pod set was ≥37˚C for the heat tolerant genotype ICC 15614 and 33˚C for heat sensitive genotype ICC 4567 (chapter 5).

In this study, parents were heterogeneous for heat response. This is evident from the range of responses of individual plant within parents such as ICC 1356 and ICC 10685, that frequently mimic the variances observed in their derived F_2 (Fig 7.3a). At extreme high temperature (>40˚C) the population, especially ICC 4567 x ICC 15614, set pods and gave higher grain yield compared with other crosses. The remaining three crosses tended to flower earlier at high temperature. The timing of flowering is important in conferring crop adaptation to high temperature (Subbarao *et al*. 1995). The experiments in the current study were mostly affected by high temperature at flowering and pod set. Generally flowering time is mainly affected by polygenes and including those controlling photoperiod response (PPD) (Kumar and Abbo 2001). However, Roberts *et al*. (1985) suggested that the photoperiod response loci are independent from the temperature response loci.

In this study, the respective heritability of DFF was high (0.80 and 0.44) in two crosses (ICC 1356 x ICC 15614 and ICC 10685 x ICC 15614) demonstrating that the time of flowering under high temperature was affected by flowering genes, not just the environment. The presumption in this study is that flowering time under high temperature indirectly affects the crop maturity and/or seed yield is controlled by polygenes. In ICC 4567 x ICC 15614, the DFF was not affected by high temperature and flowering date was not earlier than normal. The heritability of seed number was also high in this cross. In previous work under non-stressed conditions (normal season), additive gene action controlled DFF and pods per plant and grain yield tended to be associated with non-additive effects (Singh *et al.* 1992). However, gene response to high temperature in chickpea has not been previously reported.

The lower heritability of ICC 4567 x ICC 1356 indicates that these two parents are generally similar for response to heat stress. Dickson and Petzoldt (1989) also found zero heritability for pod number and seed number in bean due to heat stress probably reflecting a similar lack of diversity among the parents used. Although a normal distribution was found for segregating lines, it is likely that large environmental variance obscured any genetic effects in their study. Later, the genetics of heat tolerance was extensively studied in bean and cowpea (Marfo and Hall 1992; Shonnard and Gepts 1994). Shonnard and Gepts (1994) suggested that a single gene controlling growth habit was linked to heat tolerance during flower bud formation in bean. They also identified that additive gene effects were involved in bud abortion at high temperature. Rainey and Griffiths (2005) also identified an association between a flower abscission gene and genes that control pod number in beans.

The present study showed that the pattern of inheritance of heat tolerance in chickpea is complex. Clearly, selection for increased heat tolerance in the early generation, apart from removing sterile plants is not viable. To better understand the genetic control of tolerance it may be better to develop fixed line materials for screening and evaluation. It may be possible to use new molecular breeding strategies such as marker assisted recurrent selection (MARS) where quantitative trait loci are estimated in every cross and recombined by crossing progeny from the same cross (Trethowan and Mahmood 2011). Adaptation for specific environments and the geographic distribution of germplasm also determines the level of genetic diversity within and between populations (Tuwafe *et al.* 1988). The countries India and Turkey lie in same genetic diversity group but India and Tanzania are in different diversity groups (Tuwafe *et al.* 1988; Chapter 3). This may in part explain the success of the cross ICC 4567 x ICC 15614 which combined parents from India and Tanzania.

7.5. Conclusions

The F_1 and F_2s in ICC 4567 x ICC 1356 produced higher range of pods (1-112) with high number of filled pods, seed number and grain yield at high temperature range of 33.9-41.7˚C/ 21-28.6˚C during the flowering. Therefore, the adaptation of chickpea to high temperature may also be improved using more exotic parents to combine allelic diversity for flowering time, pod number, filled pod number, seed number per plant and grain yield.

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Chapter 8: General discussion

Introduction

Chickpea is sensitive to high temperature (>30˚C) and high temperature has negative effects on plant growth and grain yield (Summerfield *et al*. 1984). Recent research on chickpea summarised the adverse effects of high temperature on phenology, flower abortion, pollen fertility, stigma receptivity, yield components and grain yield (Kaloki 2010; Krishnamurthy *et al*. 2011; Upadhyaya *et al*. 2011; Kumar *et al*. 2012). This research evaluated genetic variation for heat tolerance in chickpea using plant growth and yield traits, plant physiological traits (CTD), a stress index (HTI) and temperature prediction at different developmental stages of chickpea (V_{Max}; V_{Min}; F_{Max}; F_{Min}; GF_{Max} and GF_{Min}) as tools for heat tolerance screening.

This thesis has contributed to the understanding of genetic diversity of 167 chickpea germplasm including characterisation of screening environments (chapter 3), the effect of high temperature on phenology, growth, yield traits, CTD, including heat stress index and maximum and minimum temperatures of crop developmental stages of 167 genotypes (chapter 4), the effect of high temperature on reproductive biology of chickpea (chapter 5), identification of molecular markers linked with genomic regions associated with heat tolerance i.e. association mapping in chickpea across a broad range of temperatures (chapter 6) and inheritance of heat tolerance in chickpea (chapter 7).

Genetic diversity

One hundred and sixty seven chickpea genotypes were assayed in this study using DArT molecular markers to study genetic diversity among these germplasm (chapter 3). Although DArT markers had not been extensively used in chickpea in the past, their use to construct a high density genetic map based on RIL population ICC 4958 (*C. aritetinum*) × PI 489777 (*C. reticulatum*) has been validated (Thudi *et al*. 2011). Genetic similarities were observed and nine groups (1-9) of germplasm identified from a dendrogram were assessed with their centres of origin (chapter 3). ICRISAT breeding lines produced higher grain yield under heat stressed and non-stressed conditions. One of the parents ICC 4958 is drought tolerant and has been used to develop breeding lines. Therefore, the mechanism of heat tolerance may be similar to drought tolerance in chickpea. Kaloki (2010) also identified heat tolerant ICRISAT breeding lines in Kenya from field screening.

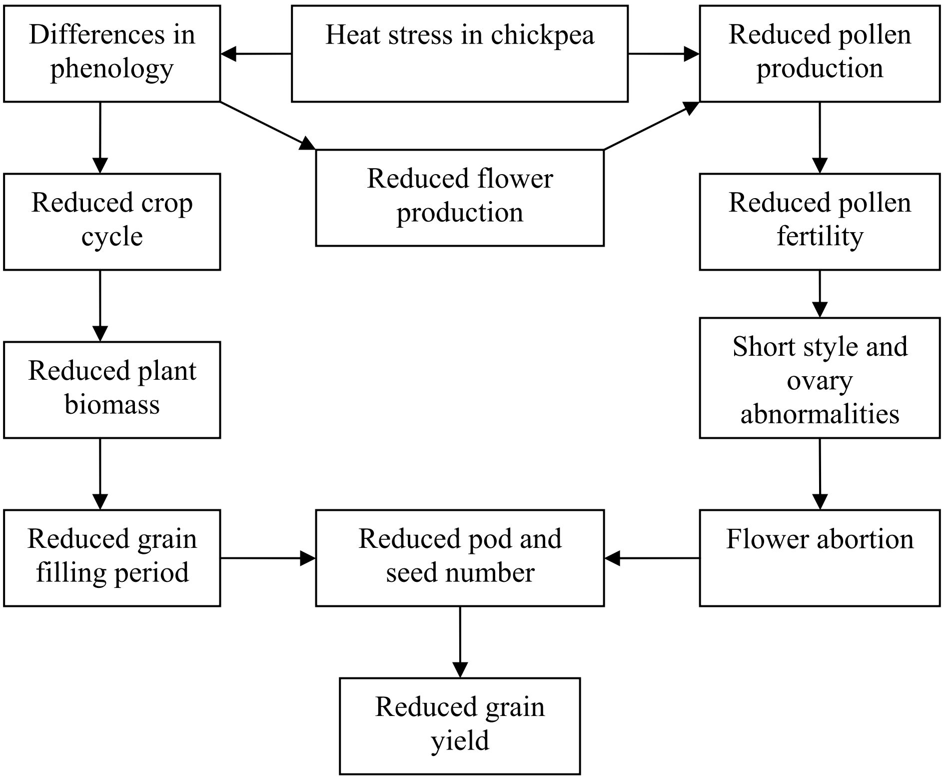


Fig 8.1 Sequential effects of high temperature on chickpea growth and yield (chapter 4 and 5)

Chickpea growth, yield and physiological traits response to heat stress

In this study, 167 genotypes were evaluated in the field for two years (chapter 4) to assess genetic diversity for heat tolerance. Previous studies (Krishnamurthy *et al*. 2011; Upadhyaya *et al*. 2011) assessed the germplasm based on growth, yield traits and a heat tolerance index. The current study further evaluated the above traits along with canopy temperature depression (CTD) and temperature prediction at different developmental stages of chickpea (V_{Max}; V_{Min}; F_{Max}; F_{Min}; GF_{Max} and GF_{Min}) (chapter 4) and found a large genetic diversity. The genotypes that showed lower canopy depression had lower grain yield compared to those with higher CTD values. However, the degree of canopy depression differed in both years for all genotypes. This finding provides new information on the relationship of physiological traits and grain yield under high temperature. Similarly, a significant relationship between CTD and grain yield under high temperature was observed in wheat (Rosyara *et al*. 2010). Clearly, CTD can potentially be applied in an integrative indirect selection scheme to improve heat tolerance in chickpea.

Generally, high temperature reduces grain yield by reducing the crop cycle (left side in Fig 8.1). This indicates that developmental stages of chickpea were reduced due to high temperature. The most affected developmental stage was grain filling period. The heat stressed environment maximum temperature varied 34-39˚C in year 1 and 32-37˚C in year 2. The critical temperature for individual genotypes and genotype performance for all developmental stages was analysed based on grain yield. Using this pattern, chickpea breeders may be able to select genotypes under normal season conditions without significant negative effects on yield. Selected heat tolerant chickpea genotypes developed by ICRISAT's various breeding programs can provide an incremental increase in yield without penalty in grain yield up to 37˚C.

It is a challenge to select chickpea genotypes that can achieve increased and/or maintain grain yield under high temperature stress >37˚C. There is a need under semi-arid environments in India and Australia to increase grain yield of chickpea under high temperature stress as these conditions are likely to be more prevalent in future (Upadhyaya *et al*. 2011; Moore and Knights 2009).

Effect of high temperature on the reproductive phase

Potential traits for reproductive phase heat tolerance evaluation in chickpea are pollen viability, *in vitro* pollen germination and *in vivo* pollen germination. Generally, pollen parameters are directly related to pod set (Vara Prasad *et al*. 1999). In the current study, pollen viability declined at high temperature in controlled environments and in the field (chapter 5). Pollen viability showed genotypic differences in both environments (field and controlled conditions). The sequential effect of high temperature on pollen viability, stigma and ovule abnormalities was illustrated in the right side of Fig 8.1. Therefore, pollen is more sensitive to high temperature than the stigma. The stigma was receptive at 35/20˚C in controlled environments and at 40.2/25.2˚C in the field. My study agrees with recent work showing that short periods (2 days) of high temperature stress reduced pollen fertility and stigma receptivity under controlled conditions (Kumar *et al*. 2012). The stigma was still receptive at the temperatures experienced in my study, and higher temperatures will need to be trialled to discover the limits of stigma receptivity. My work provides clear understanding of the effect of heat stress on reproductive development and will be useful in setting breeding targets for the improvement of heat stress tolerance in chickpea.

Association analysis

Understanding the genetic control of quantitative traits such as heat tolerance is major challenge in plant breeding. Association analysis is an approach that can be used in crop improvement to identify chromosomal regions associated with the expression of complex traits and ultimately the genes or QTLs within these regions (Al-Maskri *et al.* 2012). High temperature tolerance in chickpea was associated with several genomic regions based on association analysis using DArT markers (chapter 6). While QTL for grain yield had been previously identified in these regions there is ample scope to explore other components of yield such as biomass, pod number and number of filled pods where no QTL have been previously detected. The number of traits examined in the current association study is more than previously reported in chickpea (Nayak 2010). The identified marker-trait associations from this study can be used to identify genes and QTLs within these chromosome regions to facilitate marker-assisted selection for heat tolerance. Hence, the disparate loci spread across the genome that potentially have an additive effect on yield under heat stress can be accumulated into a single genotype using these molecular tags.

Inheritance of heat tolerance

The genetic basis of chickpea heat tolerance has not been published. In the current study, the genetic control of F_1, F_2s for heat tolerance during reproductive development was investigated at high temperature (33-41˚C) using parental, F_1 and F_2 responses (chapter 7). The parents were heterogeneous in this study for heat response and it was therefore difficult to estimate gene number as the parental and F_1 variances were larger than expected. Among the four crosses, ICC 4567 × ICC 15614 showed no dominance of earliness in flowering and produced more grain yield at high temperature. The remaining three crosses were likely influenced by large environmental factors that concealed genetic effects. Time to flowering in chickpea is a function of temperature interacting with major genes (Kumar and Abbo 2001). Selection for combined traits such as flowering time, pod number, filled pod number, seed number and grain yield may assist the improvement of heat tolerance in chickpea, but additional genetic gain is possible by combining all traits into a single genotype. Similarly, Shonnard and Gepts (1994) also suggested that improving heat tolerance in bean is possible by combining genetic effects for flower bud initiation and filled pod.

Suggested future research

This study evaluated genetic diversity using molecular markers, field screening for heat tolerance at the whole plant level, reproductive biology under heat stress, association mapping to analyse the genetic basis of heat adaptive traits and inheritance of heat tolerance. However, much more needs to be done and the opportunities for future research are summarised below:

- Genetic diversity of chickpea genotypes using DArT markers had not been published in the past. A genetic map of chickpea using DArT markers is currently under development. This study is the first step in towards map based cloning of targeted genes for heat tolerance in chickpea.
- Genotypes tested in this study were screened in the field in one location (although at two sowing times). These genotypes should be further tested in multi location environments to validate their response to high temperature.
- The genotypes studied for reproductive biology response to heat stress are potential sources for developing genetic mapping populations.
- The selected population developed from ICC 4567 × ICC 15614 for the inheritance study should be further developed to produce a random inbred line population which can be used for QTL analysis following genotyping and phenotyping for heat stress response.
- QTLs and ultimately genes in the genomic regions identified using association analysis. These regions are indicative of the presence of QTLs and genes and are the first step in gene discovery; particularly in those regions where no previous QTL have been reported.

APPENDICES

Appendix 1 Country of origin, name of variety, country and year of release of chickpea germplasm

Group No.	Pedigree	Origin	Name of variety, country and year of release
1	ICC 982	Sri Lanka	
	ICC 1025	Algeria	
	ICC 4933	Sri Lanka	
	ICC 2234	Iraq	
	ICC 1026	Iraq	
	ICC 2204	Sri Lanka	
	ICC 9567	Israel	
	ICC 12511	India	
	ICC 6334	Egypt	
	ICC 3335	Cyprus	
	ICC 3429	Egypt	
	ICC 4482	Turkey	
	ICC 15536	Morocco	
	ICC 988	Mexico	
	ICC 5270	India	
	ICC 16835	France	
	ICCV 96836	Breeding line developed at ICRISAT	Genesis 836 - Australia (2005)
	ICCV 07102	Breeding line developed at ICRISAT	
	ICCV 07101	Breeding line developed at ICRISAT	
	ICC 1017	Egypt	
	ICC 5384	India	
	ICC 1163	Nigeria	
	ICC 1471	India	
	ICC 9562	Algeria	
	ICC 9125	Iran	
	ICC 1164	Nigeria	
	ICC 2935	Iran	
	ICC 16436	Pakistan	
	ICC 10134	India	
	ICC 16343	USA	
	ICC 10600	Pakistan	
	ICCV 07115	Breeding line developed at ICRISAT	
	ICCV 07116	Breeding line developed at ICRISAT	
	ICCV 90201	Breeding line developed at ICRISAT	Himchana 2 - India (2003)
	ICCV 07104	Breeding line developed at ICRISAT	
	ICCV 07105	Breeding line developed at ICRISAT	
2	ICC 3336	Cyprus	
	ICCL 81248	Breeding line developed at ICRISAT	Nabin - Bangladesh (1987)
	ICC 4902	Turkey	
	ICC 12289	Nepal	
	ICC 5794	India	
	ICC 14533	Bangladesh	
	ICC 16216	Mynamar	
	ICC 14456	Yogoslavia (Former)	

Group No.	Pedigree	Origin	Name of variety, country and year of release
	ICC 14592	Bangladesh	
	ICCC 37	Breeding line developed at ICRISAT	Kranthi - India (1989)
	ICCL 83149	Breeding line developed at ICRISAT	Barichhola 6 - Bangladesh (1996)
	ICC 14575	Bangladesh	
	ICC 16219	Mynamar	
	ICC 3935	Iran	
	ICC 3485	Jordan	
	ICC 5566	Mexico	
	ICC 11553	Egypt	
	ICC 11901	Sudan	
	ICC 14674	India	
	ICC 11886	Sudan	
	ICC 12554	Ethiopia	
	ICC 12620	Ethiopia	
	ICC 12787	Ethiopia	
	ICC 14177	Germany	
	ICC 14176	Ethiopia	
	ICC 14179	Germany	
	ICC 13941	Ethiopia	
	ICC 3867	Iran	
	ICC 6293	Italy	
	ICC 9557	Mexico	
	ICC 14497	Bangladesh	
	ICCV 92809	Breeding line developed at ICRISAT	Myles - USA (1994)
	ICC 7263	France	
	ICC 15380	Morocco	
	ICC 12121	Malawi	
	ICC 10674	Turkey	
	ICCV 07312	Breeding line developed at ICRISAT	
	ICC 17109	Mexico	
	ICC 15388	Morocco	
	ICCV 95332	Breeding line developed at ICRISAT	JGK 2 - India (2006)
	ICCV 06302	Breeding line developed at ICRISAT	
	ICCV 3	Breeding line developed at ICRISAT	Yezin 5 - Myanmar (2004)
	ICCV 93512	Breeding line developed at ICRISAT	Shasho - Ethiopia (2000)
	ICC 7570	Bulgaria	
	ICC 11795	Chile	
	ICC 11903	Germany	
	ICCV 07304	Breeding line developed at ICRISAT	
	ICCV 06306	Breeding line developed at ICRISAT	
	ICCV 07313	Breeding line developed at ICRISAT	
	ICCV 07311	Breeding line developed at ICRISAT	
	ICCV 06301	Breeding line developed at ICRISAT	
	ICC 5124	Israel	
	ICC 7669	Greece	
	ICC 8527	Algeria	
	ICC 8943	Egypt	
	ICC 3337	Cyprus	
	ICC 7298	Tunisia	

Group No.	Pedigree	Origin	Name of variety, country and year of release
3	ICC 4861	Yogoslavia (Former)	
	ICC 15779	Syria	
	ICC 6152	Jordan	
	ICC 15807	Syria	
	ICC 6169	Iraq	
	ICC 7241	Lebanon	
	ICC 15795	Syria	
	ICC 6283	Peru	
	ICC 7292	Tunisia	
	ICC 4958	India	
	ICCV 98902	Breeding line developed at ICRISAT	
	ICCV 98901	Breeding line developed at ICRISAT	
	ICCV 92944	Breeding line developed at ICRISAT	Yezin 6 - Myanmar (2004), JG 14 - India (2009)
	ICCV 93952	Breeding line developed at ICRISAT	JAKI 9218 - India (2006)
	ICCV 93954	Breeding line developed at ICRISAT	JG 11 - India (1999)
	ICC 15367	Morocco	
	ICCV 94916-4	Breeding line developed at ICRISAT	
	ICCV 94916-8	Breeding line developed at ICRISAT	
	ICC 7308	Peru	
	ICCV 95311	Breeding line developed at ICRISAT	Vihar - India (2002)
	ICCV 07110	Breeding line developed at ICRISAT	
	ICCV 98903	Breeding line developed at ICRISAT	
	ICCV 98904	Breeding line developed at ICRISAT	
	ICC 4969	India	
	ICCV 96030	Breeding line developed at ICRISAT	
	ICC 5186	India	
	ICC 12312	India	
	ICC 14406	India	
	ICC 1882	India	
	ICC 8166	Iran	
	ICC 16298	Malawi	
	ICC 14315	India	
	ICC 283	India	
	ICC 8273	Lebanon	
	ICC 8474	Spain	
	ICC 8397	India	
	ICC 5116	Israel	
	ICC 5912	India	
	ICC 6969	Iran	
4	ICC 12123	Malawi	
	ICC 14913	Sudan	
	ICC 16141	Mynamar	
	ICC 17083	Tanzania	
	ICC 16173	Mynamar	
	ICCV 07113	Breeding line developed at ICRISAT	
	ICCV 07118	Breeding line developed at ICRISAT	
6	ICC 7294	Tunisia	
	ICC 16626	Pakistan	

Group No.	Pedigree	Origin	Name of variety, country and year of release
7	ICC 6231	Spain	
	ICC 14183	Germany	
8	ICC 7192	Hungary	
	ICC 7193	Hungary	
9	ICC 16833	Uganda	
	ICCV 94954	Breeding line developed at ICRISAT	JG 130 - India (2000)
	ICCV 97105	Breeding line developed at ICRISAT	Ukiriguru 1 - Tanzania (2011)
	ICCL 87207	Breeding line developed at ICRISAT	Vishal - India (1995)
	ICCV 07117	Breeding line developed at ICRISAT	
	ICCX 820065	Breeding line developed at ICRISAT	GG 2 - India (1998)
	ICCV 07108	Breeding line developed at ICRISAT	
	ICCV 07109	Breeding line developed at ICRISAT	

Appendix 2. Effect of heat stress and non-stress on chickpea phenology and yield parameters in year 1 (2009-2010) (Significant difference at **P<0.01; ***P<0.001 and NS-Not significant)

Geno type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
Normal Season													
1	ICC 982	64	66	70	117	17.69	44.50	36.30	49	47	52	6.82	0.38
2	ICC 988	69	71	76	127	32.07	47.90	37.20	100	99	109	10.68	0.32
3	ICC 1017	63	66	69	120	18.78	37.20	32.40	64	59	72	9.40	0.52
4	ICC 1025	67	69	73	120	23.34	41.00	38.00	78	77	89	9.71	0.43
5	ICC 1026	68	70	74	120	29.21	39.70	33.60	81	78	83	10.03	0.34
6	ICC 1163	50	53	57	114	22.88	36.50	38.20	72	66	73	12.35	0.54
7	ICC 1164	51	55	59	115	21.56	38.90	40.60	72	68	77	8.15	0.49
8	ICC 1471	49	52	55	105	21.09	32.80	29.70	55	50	59	12.75	0.59
9	ICC 2204	65	68	71	117	21.65	41.50	35.80	54	51	56	9.41	0.44
10	ICC 2234	59	62	65	116	37.34	44.40	36.50	77	75	90	13.55	0.35
11	ICC 2935	62	65	68	117	19.18	43.10	34.70	50	47	67	8.27	0.46
12	ICC 3335	63	66	69	116	18.74	47.60	41.30	52	50	65	7.92	0.42
13	ICC 3336	52	56	60	113	23.94	37.20	43.20	71	65	98	11.01	0.51
14	ICC 3337	56	59	62	120	13.09	45.70	43.70	44	38	54	8.24	0.38
15	ICC 3429	63	66	69	118	28.96	39.20	35.20	83	78	124	14.33	0.51
16	ICC 3485	40	45	49	113	19.34	37.00	32.30	81	71	81	10.98	0.53
17	ICC 3867	51	54	58	112	32.94	59.10	51.60	84	52	118	14.53	0.45
18	ICC 3935	49	52	58	103	24.17	36.10	40.10	87	88	101	14.05	0.59
19	ICC 4482	68	70	75	119	16.57	38.10	31.10	55	52	65	7.61	0.49
20	ICC 4861	52	57	61	125	23.48	51.40	40.10	36	34	37	7.35	0.28
21	ICC 4902	50	54	57	113	19.24	39.90	37.70	109	103	148	16.72	0.60
22	ICC 4933	65	67	70	119	21.85	36.50	28.90	52	50	71	7.53	0.39
23	ICC 4969	65	67	71	119	15.13	36.90	29.60	55	54	71	6.75	0.44
24	ICC 5116	65	68	73	120	26.41	50.20	43.00	39	35	52	8.64	0.31

Geno type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
25	ICC 5124	57	60	62	117	20.17	48.60	36.90	33	31	43	6.95	0.37
26	ICC 5186	48	52	55	113	18.08	32.90	35.80	50	48	57	8.75	0.39
27	ICC5270	59	62	65	121	24.48	52.50	42.20	49	48	53	7.89	0.37
28	ICC 5384	65	68	72	123	25.78	39.00	32.70	89	83	109	10.99	0.39
29	ICC 5566	64	67	72	125	28.44	54.90	43.10	47	46	54	8.46	0.24
30	ICC 5794	49	52	56	114	26.20	41.00	43.50	93	83	102	15.13	0.54
31	ICC 5912	54	58	61	126	44.29	49.50	40.20	129	88	100	12.98	0.31
32	ICC 6152	51	56	60	122	28.80	47.40	46.20	47	43	45	9.97	0.41
33	ICC 6231	58	61	64	120	34.52	57.00	45.70	31	25	29	7.51	0.21
34	ICC 6283	54	57	59	116	23.66	51.20	47.50	36	33	38	10.02	0.39
35	ICC 6293	51	54	57	113	18.63	53.40	44.70	50	48	61	7.34	0.39
36	ICC 6969	48	52	56	115	24.05	36.60	34.10	67	64	78	9.99	0.55
37	ICC 7192	63	65	69	122	14.00	53.10	24.60	10	10	14	2.33	0.18
38	ICC 7193	77	80	82	128	20.71	49.10	33.20	17	15	18	3.96	0.14
39	ICC 7241	59	62	65	117	28.82	54.70	45.50	37	34	38	9.68	0.34
40	ICC 7263	54	58	61	117	24.73	61.10	45.80	29	24	32	8.35	0.29
41	ICC 7292	55	59	61	122	30.77	55.30	44.50	31	28	31	9.76	0.33
42	ICC 7294	63	67	71	125	31.92	50.50	42.00	27	24	27	6.69	0.20
43	ICC 7298	59	62	65	120	28.84	50.70	43.40	34	28	35	8.28	0.36
44	ICC 7308	50	53	57	110	24.66	48.60	42.90	48	44	47	11.39	0.44
45	ICC 7570	60	63	66	116	34.62	61.40	48.60	55	51	59	13.70	0.38
46	ICC 7669	66	69	73	125	31.55	63.90	37.40	39	39	31	7.84	0.29
47	ICC 8166	45	49	53	106	27.18	42.00	49.90	68	62	73	12.85	0.56
48	ICC 8273	64	66	70	125	20.38	47.20	38.60	31	28	27	4.92	0.18
49	ICC 8397	53	56	59	115	36.49	51.20	41.80	82	68	85	11.60	0.33
50	ICC 8474	35	42	42	99	30.46	43.50	44.40	60	56	70	17.95	0.57
51	ICC 8527	49	52	56	112	22.70	52.60	37.30	25	19	27	10.49	0.43
52	ICC 8943	59	62	65	120	30.48	51.00	45.30	52	51	76	12.18	0.41

Geno type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
53	ICC 9125	62	65	68	119	32.21	36.10	42.40	82	63	68	14.75	0.50
54	ICC 9557	58	61	64	116	30.10	48.90	42.90	58	51	62	11.51	0.35
55	ICC 9562	42	47	51	100	8.42	38.30	37.20	40	39	49	10.08	0.51
56	ICC 9567	58	61	64	115	22.78	47.00	43.00	74	66	121	14.45	0.52
57	ICC 10090	49	54	57	117	21.98	50.20	46.40	68	58	66	8.19	0.36
58	ICC 10134	63	65	69	117	22.30	44.90	45.50	71	64	76	11.22	0.51
59	ICC 10600	72	75	78	125	27.28	40.00	31.90	60	53	63	6.42	0.29
60	ICC 10674	52	56	58	117	16.70	56.40	44.20	27	23	24	6.83	0.31
61	ICC 11553	48	52	57	115	24.18	49.90	39.40	90	82	135	13.37	0.52
62	ICC 11795	65	68	72	129	30.60	52.80	38.80	23	21	24	6.28	0.19
63	ICC 11886	43	46	51	96	21.96	58.00	39.60	63	56	66	9.53	0.47
64	ICC 11901	44	48	58	107	23.21	51.30	51.30	92	91	103	11.79	0.50
65	ICC 11903	54	57	61	124	28.01	56.40	48.10	21	18	21	5.22	0.25
66	ICC 12123	60	63	65	118	32.34	56.10	47.10	71	69	87	14.34	0.40
67	ICC 12169	55	58	61	110	23.12	36.00	40.40	83	73	93	13.17	0.58
68	ICC 12289	56	59	63	115	23.09	38.80	38.90	68	64	89	12.72	0.57
69	ICC 12511	56	59	64	115	29.30	32.60	35.60	61	57	74	8.85	0.32
70	ICC 12554	42	46	51	107	27.82	47.60	42.60	89	88	116	15.53	0.57
71	ICC 12620	47	51	55	107	17.52	43.50	37.40	47	47	89	10.13	0.55
72	ICC 12787	47	51	54	104	28.46	47.80	48.70	86	68	113	14.79	0.52
73	ICC 13941	47	50	54	104	25.69	45.70	40.40	78	70	120	13.09	0.52
74	ICC 14176	38	46	50	104	16.70	44.80	44.40	57	52	56	8.43	0.55
75	ICC 14177	43	47	51	102	17.92	49.10	43.30	62	60	79	9.99	0.57
76	ICC 14179	48	52	57	106	18.36	46.40	43.30	61	55	63	9.11	0.47
77	ICC 14183	54	57	60	119	25.78	49.30	35.00	41	35	39	7.76	0.35
78	ICC 14315	34	41	42	108	15.61	38.60	29.90	63	54	65	8.32	0.61
79	ICC 14406	44	48	52	110	22.83	35.40	44.20	58	54	73	12.86	0.54
80	ICC 14456	56	59	62	116	22.14	36.60	42.40	93	84	111	11.86	0.52

Geno type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
81	ICC 14497	42	45	49	103	21.22	41.40	36.80	74	74	93	12.21	0.56
82	ICC 14533	50	53	56	107	31.78	41.20	49.20	98	90	107	17.10	0.57
83	ICC 14575	59	62	60	112	21.96	31.60	31.70	76	70	89	10.15	0.51
84	ICC 14592	50	54	58	107	28.09	33.90	34.10	95	89	130	15.71	0.57
85	ICC 14674	43	47	51	107	24.00	43.20	40.40	68	65	68	14.64	0.61
86	ICC 14913	48	52	55	117	20.32	48.00	47.10	60	49	82	9.46	0.45
87	ICC 15367	60	63	60	117	25.82	58.10	48.50	42	35	37	12.27	0.46
88	ICC 15380	48	51	55	120	36.78	42.90	38.00	57	54	68	14.55	0.40
89	ICC 15388	51	55	59	111	23.42	46.20	41.90	21	20	23	6.58	0.33
90	ICC 15536	63	66	69	116	19.54	37.80	32.50	65	58	46	9.39	0.51
91	ICC 15779	55	58	63	115	16.90	40.20	34.20	31	28	28	6.10	0.27
92	ICC 15795	57	60	64	116	22.31	48.20	38.10	14	11	23	6.50	0.22
93	ICC 15807	49	53	57	117	37.34	51.10	41.60	90	86	75	18.09	0.44
94	ICC 16141	43	47	52	111	10.20	32.80	30.80	32	23	34	4.38	0.47
95	ICC 16173	41	44	48	117	26.54	41.10	35.90	79	67	65	10.62	0.43
96	ICC 16181	38	42	47	111	18.77	44.00	36.30	66	55	82	10.43	0.59
97	ICC 16216	44	48	52	108	15.89	38.50	43.10	32	28	82	8.23	0.51
98	ICC 16219	50	53	56	111	14.76	41.20	38.40	53	51	59	7.88	0.52
99	ICC 16298	48	52	56	117	26.55	45.60	40.90	88	65	95	12.74	0.50
100	ICC 16343	61	64	67	119	25.67	44.40	38.00	49	46	60	12.08	0.49
101	ICC 16376	65	68	73	125	28.13	58.00	44.70	25	25	25	6.43	0.22
102	ICC 16436	64	66	69	118	19.24	40.60	36.50	52	43	78	8.11	0.43
103	ICC 16453	57	60	64	121	21.88	52.40	41.30	21	21	21	6.46	0.22
104	ICC 16528	60	62	65	115	24.04	49.00	39.00	57	53	50	10.05	0.47
105	ICC 16626	59	62	65	116	28.66	49.30	36.50	50	48	59	12.34	0.42
106	ICC 16774	54	58	61	110	31.51	56.30	44.20	16	13	14	4.99	0.17
107	ICC 16820	43	46	48	121	31.28	55.70	44.90	25	23	28	9.55	0.34
108	ICC 16833	51	54	58	101	18.92	41.00	39.40	50	46	62	9.02	0.54

Geno type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
109	ICC 16835	47	51	54	107	16.98	36.40	35.20	52	49	61	9.00	0.54
110	ICC 17083	45	48	53	98	13.23	37.90	30.70	50	44	49	6.11	0.50
111	ICCC 37	44	47	51	98	32.02	39.20	38.70	81	77	85	19.36	0.60
112	ICCL 83149	44	48	52	115	15.86	36.70	35.00	42	39	44	9.71	0.64
113	ICCL 87207	48	51	56	104	24.96	40.30	37.10	45	37	46	13.38	0.56
114	ICCV 3	52	55	59	118	25.31	40.90	37.40	58	49	53	15.07	0.55
115	ICCV 88202	43	47	51	98	16.78	44.50	42.80	35	33	53	10.27	0.59
116	ICCV 89314	48	51	54	99	25.36	41.00	41.50	67	64	82	15.46	0.55
117	ICCV 89509	49	53	56	108	28.82	45.80	44.60	61	51	65	12.97	0.47
118	ICCV 90201	59	62	65	112	25.58	46.80	40.20	61	59	68	12.11	0.51
119	ICCV 92809	63	65	69	117	21.72	46.00	39.40	47	46	48	9.27	0.46
120	ICCV 92944	36	41	44	90	10.04	31.60	26.70	30	28	32	5.70	0.54
121	ICCV 93512	50	53	57	115	24.16	48.40	45.50	37	30	38	10.66	0.51
122	ICCV 93952	39	42	44	108	22.45	39.10	39.50	65	51	56	12.15	0.52
123	ICCV 93954	35	44	40	105	24.41	41.70	35.00	58	53	53	15.62	0.55
124	ICCV 94954	45	49	52	98	28.09	43.30	43.70	37	34	50	11.65	0.56
125	ICCV 95311	44	48	52	113	20.99	40.80	36.80	33	30	32	8.47	0.44
126	ICCV 95332	37	40	45	94	20.81	43.50	45.50	42	40	43	10.85	0.53
127	ICCV 96836	46	49	52	114	25.96	53.00	39.90	64	62	71	11.67	0.50
128	ICCX 820065(GG2)	31	38	37	96	20.84	38.30	35.30	38	35	38	11.13	0.61
129	ICCV 97105	59	61	65	110	21.00	36.00	33.70	63	56	90	13.39	0.53
130	ICCV 96030	23	30	29	87	8.54	39.10	38.20	29	28	30	4.26	0.51
131	ICC 17109	30	38	38	103	9.54	44.40	33.10	13	11	12	5.26	0.49
132	ICCV 07101	43	47	52	108	22.38	41.30	34.80	52	50	59	11.45	0.54
133	ICCV 07102	44	48	52	106	16.27	45.90	32.20	39	37	47	8.10	0.51
134	ICCV 07104	44	48	52	104	22.07	46.30	32.50	53	52	62	13.31	0.62
135	ICCV 07105	45	48	52	102	26.57	48.40	35.00	61	56	81	14.78	0.57

Geno-type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
136	ICCV 07108	40	46	47	114	21.93	37.70	37.40	46	40	48	13.00	0.64
137	ICCV 07109	40	44	48	108	25.12	40.30	39.10	56	42	50	12.82	0. 52
138	ICCV 07110	34	42	39	104	19.01	39.70	40.80	45	42	41	10.36	0.57
139	ICCV 07113	44	48	51	105	18.35	38.20	33.80	47	46	58	9.82	0.77
140	ICCV 07115	38	42	46	103	22.09	40.90	41.50	49	45	54	12.78	0.62
141	ICCV 07116	42	46	49	113	25.15	38.50	49.70	53	55	58	15.49	0.67
142	ICCV 07117	39	46	45	104	31.76	40.50	42.40	61	57	80	19.29	0.66
143	ICCV 07118	34	42	44	99	21.13	38.40	30.70	41	40	44	13.02	0.59
144	ICCV 06301	37	41	44	91	13.83	35.20	40.00	32	31	32	7.44	0.53
145	ICCV 06302	38	42	45	98	18.53	38.10	44.80	40	37	38	11.37	0.58
146	ICCV 06306	41	45	49	95	17.09	45.30	37.60	37	34	38	10.68	0.57
147	ICCV 07304	32	35	38	118	15.02	31.50	44.40	31	30	31	8.17	0.51
148	ICCV 07311	32	38	39	104	23.97	42.70	39.60	37	36	43	13.28	0.50
149	ICCV 07312	45	49	52	104	14.98	43.00	45.60	27	24	27	8.44	0.62
150	ICCV 07313	45	48	51	91	19.87	48.10	48.50	27	24	28	9.58	0.53
151	ICC 283	55	57	60	112	23.49	32.70	34.00	73	66	78	12.84	0.56
152	ICC 1882	46	50	53	110	20.28	35.60	43.80	70	64	67	11.40	0.59
153	ICC 4958	38	45	52	116	23.50	41.10	42.00	45	37	40	14.19	0.56
154	ICC 8261	52	55	58	120	23.12	50.10	40.80	23	21	24	6.16	0.31
155	ICCV 94916-4	42	46	50	101	24.28	53.50	46.60	35	34	37	10.51	0.58
156	ICCV 94916-8	41	44	48	100	27.47	48.60	37.40	59	55	42	12.75	0.49
157	ICCV 98901	32	40	44	106	24.52	35.80	36.40	66	59	64	15.23	0.59
158	ICCV 98902	36	42	44	100	21.04	42.40	32.40	39	36	37	11.02	0.57
159	ICCV 98903	35	41	44	96	19.26	43.90	34.10	36	33	25	9.97	0.55
160	ICCV 98904	36	41	43	97	21.01	41.80	24.60	47	42	45	12.13	0.58
Mean of genotype effect		50	54	57	111	23.33	44.27	39.45	54	50	61	10.6	0.46
	LSD(*P*=0.05)	(6.7) ***	(6.6) ***	(6.9) ***	(13.2) ***	NS	(9.9) ***	NS	(35.8) ***	(33.8) ***	(48.8) ***	(7.1) **	(0.13) ***

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
	Late Season												
1	ICC 982	77	0	0	102	6.74	25.90	18.70	0	0	0	0	0
2	ICC 988	63	68	70	97	8.60	30.80	22.40	0	0	0	0	0
3	ICC 1017	63	67	69	98	10.20	29.00	24.80	4	2	3	0.40	0.04
4	ICC 1025	64	68	70	100	13.21	34.00	32.60	1	0	0	0.15	0.01
5	ICC 1026	76	0	0	100	14.05	22.20	20.90	0	0	0	0	0
6	ICC 1163	55	60	61	83	3.62	24.45	19.05	5	4	4	0.25	0.01
7	ICC 1164	59	63	65	86	11.33	25.10	19.30	15	13	17	1.85	0.09
8	ICC 1471	52	56	58	89	6.92	22.60	17.20	18	14	16	1.48	0.18
9	ICC 2204	68	0	0	100	7.76	25.00	20.70	1	1	0	0	0
10	ICC 2234	59	63	68	106	8.07	33.60	25.20	2	1	2	0.19	0.01
11	ICC 2935	0	0	0	100	4.30	25.50	20.40	0	0	0	0	0
12	ICC 3335	69	72	77	110	7.31	26.10	24.60	0	0	0	0	0
13	ICC 3336	51	54	56	87	13.48	23.50	21.80	40	34	41	0.91	0.18
14	ICC 3337	53	56	59	101	18.62	34.30	16.90	18	16	18	1.78	0.08
15	ICC 3429	73	76	38	110	8.48	27.50	26.00	0	0	0	0	0
16	ICC 3485	38	41	46	90	7.00	29.45	14.05	23	20	21	1.77	0.19
17	ICC 3867	41	45	47	90	4.00	32.40	24.20	3	2	2	0.64	0.12
18	ICC 3935	47	50	54	87	10.74	23.30	22.30	36	35	47	4.01	0.40
19	ICC 4482	0	0	0	95	8.73	23.40	19.00	0	0	0	0	0
20	ICC 4861	52	55	58	93	13.48	37.40	35.80	17	16	18	2.29	0.12
21	ICC 4902	44	48	49	86	14.71	30.00	28.10	50	44	64	5.75	0.37
22	ICC 4933	62	66	34	105	9.88	32.00	31.60	0	0	0	0.40	0.01
23	ICC 4969	61	65	67	95	6.38	28.00	16.35	1	0	0	0.05	0
24	ICC 5116	56	60	62	95	7.47	36.70	31.40	10	9	10	1.09	0.09
25	ICC 5124	67	71	72	103	3.50	34.30	19.25	2	2	3	0.19	0.02
26	ICC 5186	47	50	53	89	11.68	28.30	25.70	29	25	30	3.37	0.26
27	ICC 5270	64	68	71	102	6.40	29.60	19.80	0	0	0	0	0

Geno-type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
28	ICC 5384	68	72	75	100	4.77	18.20	12.10	0	0	0	0	0
29	ICC 5566	66	70	72	106	5.08	27.50	16.40	0	0	0	0	0
30	ICC 5794	50	54	57	83	12.44	26.10	26.40	50	46	55	6.12	0.33
31	ICC 5912	49	48	59	97	8.52	28.70	18.50	0	0	0	0	0
32	ICC 6152	51	54	57	90	5.52	32.10	28.60	2	1	1	1.08	0.10
33	ICC 6231	59	64	68	104	9.16	38.90	31.40	1	0	0	0.23	0.01
34	ICC 6283	54	57	61	100	5.00	24.40	13.50	1	1	1	0.07	0.01
35	ICC 6293	53	53	55	93	7.58	35.70	30.40	4	4	5	0.75	0.03
36	ICC 6969	46	49	50	82	13.88	26.30	20.90	46	44	56	5.98	0.39
37	ICC 7192	77	80	0	105	15.08	35.75	28.85	0	0	0	0	0
38	ICC 7193	54	63	32	102	2.83	25.70	16.90	0	0	0	0	0
39	ICC 7241	64	0	0	100	3.37	27.80	30.45	0	0	0	0	0.10
40	ICC 7263	55	59	0	100	9.09	36.20	23.05	0	0	0	0	0.03
41	ICC 7292	53	57	59	97	16.18	40.70	40.00	9	7	10	1.98	0.07
42	ICC 7294	61	67	33	104	9.22	29.80	22.90	1	1	1	0.05	0.01
43	ICC 7298	58	0	0	94	9.01	27.20	25.40	2	2	2	0.19	0.01
44	ICC 7308	57	61	63	98	3.92	30.60	13.20	0	0	0	0	0.02
45	ICC 7570	55	59	60	100	8.81	39.30	36.40	0	0	0	0	0
46	ICC 7669	69	0	0	94	9.73	29.10	19.30	0	0	0	0	0
47	ICC 8166	46	50	52	84	9.06	23.70	16.50	13	13	14	1.60	0.19
48	ICC 8273	52	54	57	91	7.41	31.50	26.10	1	1	1	0.22	0.03
49	ICC 8397	58	0	0	93	5.47	26.70	19.50	0	0	0	0	0
50	ICC 8474	35	39	40	86	12.62	30.80	25.00	26	24	31	6.72	0.46
51	ICC 8527	52	55	57	96	3.72	41.80	29.75	1	1	2	0.25	0.01
52	ICC 8943	58	61	65	105	8.95	31.00	23.20	1	0	0	0.09	0.01
53	ICC 9125	55	59	61	91	16.57	33.60	28.90	18	16	21	0.07	0.10
54	ICC 9557	54	58	59	87	6.12	28.40	18.90	2	1	1	0.12	0.01
55	ICC 9562	45	48	50	90	16.37	32.10	22.90	42	38	39	4.21	0.32

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
56	ICC 9567	62	66	68	99	10.34	30.80	30.90	7	6	8	0.74	0.06
57	ICC 10090	52	55	60	97	6.68	29.40	20.40	2	1	1	1.26	0.03
58	ICC 10134	74	77	79	104	5.13	29.80	22.20	0	0	0	0	0
59	ICC 10600	0	0	0	108	6.24	28.10	25.00	0	0	0	0	0
60	ICC 10674	59	29	36	95	7.22	33.70	27.20	1	1	1	0.07	0
61	ICC 11553	47	51	57	89	7.79	36.60	21.40	13	13	14	1.37	0.1
62	ICC 11795	63	68	69	99	11.68	35.30	30.40	1	0	1	0.15	0.01
63	ICC 11886	47	51	57	100	8.63	41.20	27.10	1	1	1	0.08	0.01
64	ICC 11901	44	47	50	89	8.66	33.50	23.40	28	27	31	3.33	0.29
65	ICC 11903	62	28	0	105	10.76	38.00	30.70	0	0	0	0	0
66	ICC 12123	51	56	57	100	9.11	32.70	26.50	2	1	1	0.12	0.02
67	ICC 12169	53	56	59	88	9.89	23.00	19.15	15	14	16	1.03	0.12
68	ICC 12289	50	53	58	95	7.97	30.00	27.30	14	13	20	1.57	0.11
69	ICC 12511	53	57	59	99	11.59	34.20	26.90	17	15	20	1.91	0.10
70	ICC 12554	41	45	46	87	7.43	34.10	24.80	20	19	24	2.02	0.27
71	ICC 12620	40	44	47	84	9.10	35.00	26.70	27	24	36	4.14	0.32
72	ICC 12787	48	52	53	86	10.37	32.90	28.00	25	23	30	3.31	0.28
73	ICC 13941	42	46	48	89	12.62	29.90	26.50	35	34	48	4.87	0.34
74	ICC 14176	39	43	45	85	13.96	29.30	25.70	29	29	43	3.59	0.33
75	ICC 14177	47	50	52	85	8.34	34.00	28.30	11	10	13	1.06	0.13
76	ICC 14179	48	51	53	90	8.60	32.90	31.60	28	24	37	3.06	0.19
77	ICC 14183	49	52	55	92	13.10	33.70	29.10	10	7	13	1.38	0.02
78	ICC 14315	33	38	40	84	12.21	28.50	19.50	51	46	56	5.28	0.49
79	ICC 14406	45	48	50	88	19.11	27.35	30.25	45	41	57	5.39	0.38
80	ICC 14456	52	55	57	97	8.45	23.80	23.65	25	22	32	2.84	0.22
81	ICC 14497	41	45	47	85	11.63	29.70	25.90	54	48	63	6.79	0.38
82	ICC 14533	52	54	56	96	10.44	26.40	23.30	22	19	26	1.10	0.13
83	ICC 14575	53	56	58	88	8.62	23.20	23.60	24	21	27	1.27	0.16

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
84	ICC 14592	53	57	59	92	5.29	25.10	22.70	7	6	9	0.72	0.06
85	ICC 14674	42	46	49	85	9.55	26.30	19.60	17	16	21	2.66	0.23
86	ICC 14913	45	49	53	88	12.62	35.00	30.50	18	14	20	1.91	0.15
87	ICC 15367	0	0	0	100	3.23	21.70	16.60	0	0	0	0	0
88	ICC 15380	51	54	56	87	10.73	28.10	22.20	20	19	22	2.42	0.19
89	ICC 15388	54	58	60	97	2.63	27.90	13.20	3	2	3	0.31	0.09
90	ICC 15536	65	69	72	95	8.31	25.80	18.80	0	0	0	0	0
91	ICC 15779	60	28	29	94	8.88	29.70	22.60	11	10	9	1.01	0.04
92	ICC 15795	52	56	31	85	5.93	27.95	15.10	0	2	2	0.09	0
93	ICC 15807	57	60	62	100	4.02	29.10	25.80	2	0	0	0	0.02
94	ICC 16141	40	45	46	85	10.39	28.80	21.30	35	32	39	1.58	0.37
95	ICC 16173	36	42	45	92	12.63	30.30	24.20	26	23	42	5.57	0.53
96	ICC 16181	34	38	42	84	11.29	27.20	20.60	32	31	41	4.21	0.50
97	ICC 16216	37	41	45	83	13.34	24.40	22.70	59	56	89	6.91	0.49
98	ICC 16219	47	50	53	83	13.40	30.50	23.50	35	32	43	4.70	0.23
99	ICC 16298	56	59	62	97	7.00	24.90	17.90	2	2	2	0.04	0
100	ICC 16343	64	35	36	100	6.58	27.90	17.30	0	0	0	0	0
101	ICC 16376	72	0	40	102	5.29	33.80	23.10	0	0	0	0	0
102	ICC 16436	68	31	31	103	9.87	29.00	27.90	1	1	1	0.05	0.01
103	ICC 16453	56	61	63	97	4.04	31.80	21.10	0	0	0	0	0.03
104	ICC 16528	0	0	0	84	6.98	24.20	15.90	0	0	0	0	0
105	ICC 16626	63	70	0	109	9.84	38.80	33.50	0	0	0	0	0.01
106	ICC 16774	62	65	69	99	12.02	33.30	24.70	2	2	2	1.20	0
107	ICC 16820	55	58	60	99	6.24	36.00	28.20	1	0	0	0.03	0
108	ICC 16833	48	51	53	91	10.41	27.70	18.40	14	12	16	1.48	0.15
109	ICC 16835	53	57	60	97	4.71	24.10	15.80	13	11	13	1.09	0.08
110	ICC 17083	45	50	50	84	9.90	28.70	21.70	40	35	40	4.03	0.42
111	ICCC 37	39	44	48	89	9.20	28.30	26.00	21	19	22	2.20	0.21

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
112	ICCL 83149	36	42	43	85	18.53	28.10	23.20	47	45	56	10.39	0.60
113	ICCL 87207	36	40	43	83	12.27	32.90	21.80	15	14	23	4.65	0.28
114	ICCV 3	46	55	53	96	7.40	25.80	24.00	5	4	6	0.82	0.10
115	ICCV 88202	35	40	42	83	16.69	32.00	30.00	28	27	39	7.79	0.39
116	ICCV 89314	45	53	50	93	14.73	29.00	24.60	41	39	45	6.54	0.38
117	ICCV 89509	52	55	58	97	10.95	35.20	32.90	9	8	10	1.53	0.03
118	ICCV 90201	59	63	64	103	7.38	33.30	26.60	6	6	9	1.65	0.12
119	ICCV 92809	63	29	30	97	8.45	35.40	29.30	5	4	3	0.38	0.02
120	ICCV 92944	32	35	37	85	12.40	30.20	20.30	30	27	31	6.46	0.55
121	ICCV 93512	41	46	45	88	19.16	37.80	29.00	32	29	33	7.90	0.16
122	ICCV 93952	37	42	44	85	11.50	28.10	21.20	27	25	30	5.53	0.28
123	ICCV 93954	35	41	44	89	9.24	27.40	19.70	21	19	20	9.25	0.21
124	ICCV 94954	42	45	48	88	10.88	28.80	20.15	23	21	29	5.07	0.22
125	ICCV 95311	40	44	46	87	15.02	32.50	19.20	34	30	33	4.47	0.44
126	ICCV 95332	34	39	42	81	10.54	31.50	24.40	19	18	22	5.57	0.44
127	ICCV 96836	36	43	42	91	10.94	38.70	34.00	26	24	33	3.20	0.25
128	ICCX 820065(GG2)	31	35	36	85	15.81	34.00	23.00	32	25	31	7.69	0.49
129	ICCV 97105	54	59	61	97	9.28	32.80	28.30	16	14	19	1.71	0.11
130	ICCV 96030	28	32	32	83	5.79	26.90	14.80	21	19	22	2.34	0.42
131	ICC 17109	37	40	43	90	9.04	34.90	22.10	8	7	8	2.86	0.16
132	ICCV 07101	30	40	39	89	7.28	29.10	18.70	22	19	28	2.86	0.17
133	ICCV 07102	47	51	51	84	6.74	27.20	16.00	19	16	20	1.69	0.20
134	ICCV 07104	43	46	49	84	7.12	35.00	19.90	17	15	25	3.25	0.34
135	ICCV 07105	43	46	48	83	10.71	33.50	18.30	34	30	40	4.70	0.43
136	ICCV 07108	40	44	46	84	14.05	30.30	20.80	30	25	35	5.47	0.42
137	ICCV 07109	37	42	44	87	11.79	26.90	20.40	27	27	30	5.65	0.52
138	ICCV 07110	34	40	43	84	9.46	31.30	22.30	20	19	21	3.02	0.42

Geno-type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
139	ICCV 07113	39	44	43	85	8.09	33.40	18.60	21	20	25	3.73	0.34
140	ICCV 07115	41	46	48	86	8.10	27.20	20.30	10	8	10	0.92	0.06
141	ICCV 07116	37	41	44	84	11.43	23.70	16.10	39	36	46	8.74	0.45
142	ICCV 07117	38	42	45	85	13.77	29.60	23.10	32	31	38	7.46	0.28
143	ICCV 07118	34	38	39	83	16.77	31.80	24.10	38	35	44	9.20	0.49
144	ICCV 06301	35	43	42	84	6.88	25.30	15.00	11	10	12	3.01	0.28
145	ICCV 06302	33	38	39	86	12.91	29.30	27.00	10	9	20	6.09	0.27
146	ICCV 06306	35	39	40	90	5.45	31.60	19.80	10	9	9	1.73	0.36
147	ICCV 07304	35	39	40	95	5.14	25.80	13.70	10	9	10	2.09	0.28
148	ICCV 07311	28	34	34	80	7.23	30.00	14.00	14	13	18	3.51	0.41
149	ICCV 07312	44	47	48	86	13.89	39.10	24.80	20	18	19	5.98	0.47
150	ICCV 07313	35	39	39	82	16.93	35.00	26.70	22	21	25	8.61	0.45
151	ICC 283	49	53	55	84	13.45	25.90	20.00	41	36	35	5.58	0.43
152	ICC 1882	37	51	46	100	5.65	19.65	12.10	15	8	8	0.49	0.05
153	ICC 4958	35	40	40	89	7.25	28.80	19.30	17	17	19	2.81	0.36
154	ICC 8261	50	54	55	88	7.48	32.50	17.75	3	3	3	0.45	0.03
155	ICCV 94916-4	45	49	51	88	14.96	35.20	27.00	18	16	18	3.55	0.19
156	ICCV 94916-8	53	57	60	98	6.08	32.80	20.65	5	5	6	1.28	0.13
157	ICCV 98901	30	36	38	84	12.20	24.90	21.95	41	40	49	7.58	0.62
158	ICCV 98902	46	50	53	82	13.96	34.30	28.35	46	44	51	9.51	0.47
159	ICCV 98903	30	35	37	85	14.40	38.10	23.50	24	22	28	6.36	0.47
160	ICCV 98904	38	45	46	85	9.43	23.40	16.10	15	15	18	3.65	0.30
Mean of genotype effect		48	47	46	92	9.65	30.14	23.12	15	14	18	2.40	0.17
	LSD(*P*=0.05)	7.7 ***	19.5 ***	25.3 ***	7.4 ***	7.9 ***	8.3 ***	11.3 ***	19 ***	17 ***	25.5 ***	1.8 ***	0.2 ***

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
Normal X Late Season													
1	ICC 982	70	0	0	109	12.22	35.20	27.50	25	24	26	3.41	0.19
2	ICC 988	66	69	73	112	20.33	39.35	29.80	50	50	55	5.34	0.16
3	ICC 1017	63	66	69	109	14.49	33.10	28.60	34	30	37	4.90	0.28
4	ICC 1025	65	69	71	110	18.27	37.50	35.30	39	38	45	4.93	0.22
5	ICC 1026	72	0	0	110	21.63	30.95	27.25	41	39	42	5.01	0.17
6	ICC 1163	52	56	59	99	13.25	30.47	28.63	38	35	38	6.30	0.28
7	ICC 1164	55	59	62	101	16.44	32.00	29.95	44	40	47	5.00	0.29
8	ICC 1471	50	54	56	97	14.01	27.70	23.45	36	33	38	7.11	0.38
9	ICC 2204	66	34	36	109	14.70	33.25	28.25	27	25	28	4.70	0.22
10	ICC 2234	59	63	66	111	22.70	39.00	30.85	39	38	46	6.87	0.18
11	ICC 2935	31	32	34	108	11.74	34.30	27.55	25	23	33	4.13	0.23
12	ICC 3335	66	69	73	113	13.02	36.85	32.95	26	25	33	3.96	0.21
13	ICC 3336	51	55	58	100	18.71	30.35	32.50	56	50	69	5.96	0.34
14	ICC 3337	54	58	60	110	15.86	40.00	30.30	31	27	36	5.01	0.23
15	ICC 3429	68	71	83	114	18.72	33.35	30.60	42	39	62	7.16	0.25
16	ICC 3485	39	43	47	101	13.17	33.22	23.18	52	46	51	6.37	0.36
17	ICC 3867	46	49	52	101	18.47	45.75	37.90	44	27	59	7.58	0.28
18	ICC 3935	48	51	56	95	17.45	29.70	31.20	61	60	74	9.03	0.49
19	ICC 4482	34	35	37	107	12.65	30.75	25.05	28	26	33	3.80	0.24
20	ICC 4861	52	56	59	109	18.48	44.40	37.95	27	25	27	4.82	0.20
21	ICC 4902	47	51	53	99	16.97	34.95	32.90	79	74	106	11.23	0.48
22	ICC 4933	63	66	52	112	15.87	34.25	30.25	26	25	36	3.96	0.20
23	ICC 4969	63	66	69	107	10.75	32.45	22.98	28	27	35	3.40	0.21
24	ICC 5116	60	64	67	107	16.94	43.45	37.20	24	22	31	4.86	0.20
25	ICC 5124	62	65	67	110	11.83	41.45	28.08	17	16	23	3.57	0.19
26	ICC 5186	47	51	54	101	14.88	30.60	30.75	40	36	43	6.06	0.32

Geno-type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
27	ICC 5270	61	65	68	112	15.44	41.05	31.00	24	24	27	3.94	0.19
28	ICC 5384	67	70	73	111	15.27	28.60	22.40	44	41	54	5.49	0.19
29	ICC 5566	65	68	72	115	16.76	41.20	29.75	24	23	27	4.26	0.12
30	ICC 5794	49	53	56	98	19.32	33.55	34.95	71	64	78	10.62	0.43
31	ICC 5912	51	53	60	111	26.40	39.10	29.35	65	44	50	6.49	0.15
32	ICC 6152	51	55	58	106	17.16	39.75	37.40	24	22	23	5.52	0.25
33	ICC 6231	58	62	66	112	21.84	47.95	38.55	16	13	15	3.57	0.11
34	ICC 6283	54	57	60	108	14.33	37.80	30.50	18	17	19	5.04	0.20
35	ICC 6293	52	54	56	103	13.11	44.55	37.55	27	26	33	4.04	0.21
36	ICC 6969	47	50	53	99	18.97	31.45	27.50	57	54	67	7.99	0.47
37	ICC 7192	70	73	35	113	14.54	44.42	26.73	5	5	7	1.16	0.09
38	ICC 7193	65	71	57	115	11.77	37.40	25.05	8	8	9	1.98	0.07
39	ICC 7241	62	31	33	109	16.09	41. 25	37.98	18	17	19	4.84	0.22
40	ICC 7263	55	58	30	109	16.91	48.65	34.43	15	12	16	4.17	0.16
41	ICC 7292	54	58	60	110	23.47	48.00	42.25	20	18	20	5.87	0.20
42	ICC 7294	62	67	52	114	20.57	40.15	32.45	14	12	14	3.37	0.10
43	ICC 7298	59	31	33	107	18.92	38.95	34.40	18	15	19	4.23	0.18
44	ICC 7308	53	57	60	104	14.29	39.60	28.05	24	22	23	5.69	0.23
45	ICC 7570	58	61	63	108	21.71	50.35	42.50	28	26	29	6.85	0.19
46	ICC 7669	67	35	37	109	20.64	46.50	28.35	20	19	15	3.92	0.15
47	ICC 8166	45	49	52	95	18.12	32.85	33.20	40	37	43	7.22	0.37
48	ICC 8273	58	60	63	108	13.90	39.35	32.35	16	14	14	2.57	0.11
49	ICC 8397	56	28	30	104	20.98	38.95	30.65	41	34	43	5.80	0.17
50	ICC 8474	35	40	41	92	21.54	37.15	34.70	43	40	50	12.33	0.51
51	ICC 8527	50	53	56	104	13.21	47.20	33.53	13	10	14	5.37	0.23
52	ICC 8943	58	61	65	113	19.71	41.00	34.25	26	25	38	6.13	0.21
53	ICC 9125	58	62	64	105	24.39	34.85	35.65	50	40	45	7.41	0.30
54	ICC 9557	56	59	61	101	18.11	38.65	30.90	30	26	32	5.81	0.18

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
55	ICC 9562	44	47	51	95	12.39	35.20	30.05	41	38	44	7.14	0.41
56	ICC 9567	60	64	66	107	16.56	38.90	36.95	40	36	65	7.60	0.29
57	ICC 10090	50	54	58	107	14.33	39.80	33.40	35	30	33	4.72	0.20
58	ICC 10134	68	71	74	110	13.72	37.35	33.85	36	32	38	5.61	0.25
59	ICC 10600	36	37	39	117	16.76	34.05	28.45	30	26	32	3.21	0.14
60	ICC 10674	55	42	47	106	11.96	45.05	35.70	14	12	12	3.45	0.15
61	ICC 11553	48	52	57	102	15.98	43.25	30.40	51	48	75	7.31	0.31
62	ICC 11795	64	68	54	114	21.14	44.05	34.60	12	10	12	3.21	0.10
63	ICC 11886	45	48	70	98	15.29	49.60	33.35	32	28	33	4.81	0.24
64	ICC 11901	44	47	54	98	15.94	42.40	37.35	60	59	67	7.56	0.39
65	ICC 11903	58	42	30	115	19.38	47.20	39.40	11	9	11	2.61	0.12
66	ICC 12123	55	59	61	109	20.72	44.40	36.80	37	35	44	7.23	0.21
67	ICC 12169	54	57	60	99	16.51	29.50	29.78	49	43	55	7.10	0.35
68	ICC 12289	53	56	60	105	15.53	34.40	33.10	41	38	55	7.15	0.34
69	ICC 12511	54	58	61	107	20.44	33.40	31.25	39	36	47	5.38	0.21
70	ICC 12554	41	45	48	97	17.63	40.85	33.70	54	53	70	8.77	0.42
71	ICC 12620	43	47	51	95	13.31	39.25	32.05	37	36	62	7.13	0.43
72	ICC 12787	47	51	53	95	19.41	40.35	38.35	55	45	71	9.05	0.40
73	ICC 13941	44	48	51	96	19.15	37.80	33.45	56	52	84	8.98	0.43
74	ICC 14176	38	45	48	94	15.33	37.05	35.05	43	40	49	6.01	0.44
75	ICC 14177	45	48	51	93	13.13	41.55	35.80	37	35	46	5.52	0.35
76	ICC 14179	48	52	55	98	13.48	39.65	37.45	44	39	50	6.08	0.33
77	ICC 14183	51	54	58	105	19.44	41.50	32.05	25	21	26	4.57	0.18
78	ICC 14315	33	39	41	96	13.91	33.55	24.70	57	50	60	6.79	0.54
79	ICC 14406	44	48	51	99	20.97	31.37	37.23	52	47	65	9.12	0.46
80	ICC 14456	54	57	59	106	15.29	30.20	33.03	59	53	71	7.35	0.37
81	ICC 14497	41	45	48	94	16.43	35.55	31.35	64	61	78	9.50	0.47
82	ICC 14533	51	53	56	101	21.11	33.80	36.25	60	55	66	9.10	0.35

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
83	ICC 14575	56	59	59	100	15.29	27.40	27.65	50	45	58	5.71	0.33
84	ICC 14592	51	55	58	99	16.69	29.50	28.40	51	47	69	8.21	0.31
85	ICC 14674	43	46	50	96	16.77	34.75	30.00	42	41	44	8.65	0.42
86	ICC 14913	46	50	54	102	16.47	41.50	38.80	39	32	51	5.68	0.30
87	ICC 15367	30	31	30	109	14.53	39.90	32.55	21	18	18	6.13	0.23
88	ICC 15380	49	53	55	103	23.76	35.50	30.10	38	36	45	8.49	0.29
89	ICC 15388	52	56	59	104	13.03	37.05	27.55	12	11	13	3.44	0.21
90	ICC 15536	64	67	70	106	13.92	31.80	25.65	33	29	23	4.69	0.25
91	ICC 15779	57	43	46	104	12.89	34.95	28.40	21	19	18	3.55	0.15
92	ICC 15795	54	58	47	101	14.12	38.07	26.60	8	6	12	3.29	0.11
93	ICC 15807	53	57	59	109	20.68	40.10	33.70	45	43	38	9.04	0.23
94	ICC 16141	41	46	49	98	10.29	30.80	26.05	33	28	36	2.98	0.42
95	ICC 16173	38	43	46	104	19.58	35.70	30.05	52	45	54	8.10	0.48
96	ICC 16181	36	40	44	97	15.03	35.60	31.85	49	43	62	7.32	0.54
97	ICC 16216	40	44	48	95	14.61	31.45	30.55	45	42	86	7.57	0.50
98	ICC 16219	48	52	54	97	14.08	35.85	29.90	44	41	51	6.29	0.37
99	ICC 16298	52	56	59	107	16.77	35.25	29.40	45	33	49	6.39	0.25
100	ICC 16343	62	49	51	110	16.13	36.15	27.65	25	23	30	6.04	0.24
101	ICC 16376	68	34	56	114	16.71	45.90	33.90	13	12	13	3.21	0.11
102	ICC 16436	66	49	50	110	14.55	34.80	32.20	26	22	39	4.08	0.22
103	ICC 16453	57	60	63	109	12.96	42.10	31.20	11	10	10	3.32	0.12
104	ICC 16528	30	31	33	100	15.51	36.60	27.45	29	26	25	5.02	0.23
105	ICC 16626	61	66	33	113	19.25	44.05	35.00	25	24	30	6.17	0.21
106	ICC 16774	58	61	65	104	21.76	44.80	34.45	9	7	8	3.10	0.08
107	ICC 16820	49	52	54	110	18.76	45.85	36.55	13	11	14	4.79	0.17
108	ICC 16833	50	53	55	96	14.66	34.35	28.90	32	29	39	5.25	0.34
109	ICC 16835	50	54	57	102	10.85	30.25	25.50	32	30	37	5.04	0.31

Geno-type No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
110	ICC 17083	45	49	51	91	11.56	33.30	26.20	45	39	44	5.07	0.46
111	ICCC 37	41	46	49	94	20.61	33.75	32.35	51	48	53	10.78	0.40
112	ICCL 83149	40	45	47	100	17.19	32.40	29.10	45	42	50	10.05	0.62
113	ICCL 87207	42	45	49	93	18.61	36.60	29.45	30	25	34	9.01	0.42
114	ICCV 3	49	55	56	107	16.35	33.35	30.70	32	27	29	7.94	0.32
115	ICCV 88202	39	43	46	91	16.73	38.25	36.40	31	30	46	9.02	0.49
116	ICCV 89314	47	52	52	96	20.05	35.00	33.05	54	52	63	11.00	0.47
117	ICCV 89509	50	54	57	102	19.88	40.50	38.75	35	29	38	7.25	0.25
118	ICCV 90201	59	62	65	107	16.48	40.05	33.40	33	32	38	6.88	0.31
119	ICCV 92809	63	47	49	107	15.08	40.70	34.35	26	25	25	4.82	0.24
120	ICCV 92944	34	38	41	88	11.22	30.90	23.50	30	28	31	6.08	0.54
121	ICCV 93512	45	50	51	101	21.66	43.10	37.25	34	30	36	9.28	0.33
122	ICCV 93952	38	42	44	96	16.98	33.60	30.35	46	38	43	8.84	0.40
123	ICCV 93954	35	42	42	97	16.82	34.55	27.35	40	36	36	12.43	0.38
124	ICCV 94954	43	47	50	93	19.49	36.05	31.93	30	28	39	8.36	0.39
125	ICCV 95311	42	46	49	100	18.00	36.65	28.00	33	30	32	6.47	0.46
126	ICCV 95332	35	40	43	88	15.67	37.50	34.95	31	29	33	8.21	0.49
127	ICCV 96836	41	46	47	102	18.45	45.85	36.95	45	43	52	7.43	0.37
128	ICCX 820065(GG2)	32	37	36	91	18.32	36.15	29.15	35	30	35	9.41	0.55
129	ICCV 97105	56	60	63	103	15.14	34.40	31.00	39	35	54	7.55	0.32
130	ICCV 96030	25	31	30	85	7.16	33.00	26.50	25	23	26	3.30	0.47
131	ICC 17109	33	39	40	96	9.29	39.65	27.60	10	9	10	4.06	0.32
132	ICCV 07101	36	43	45	99	14.83	35.20	26.75	37	34	44	7.15	0.35
133	ICCV 07102	45	49	51	95	11.51	36.55	24.10	29	26	33	4.90	0.35
134	ICCV 07104	43	47	50	94	14.59	40.65	26.20	35	33	44	8.27	0.48
135	ICCV 07105	44	47	50	92	18.64	40.95	26.65	47	43	60	9.74	0.50
136	ICCV 07108	40	45	46	99	17.99	34.00	29.10	38	32	41	9.24	0.53

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
137	ICCV 07109	39	43	47	97	18.45	33.60	29.75	42	34	40	9.24	0.52
138	ICCV 07110	34	41	41	94	14.23	35.50	31.55	32	30	31	6.69	0.46
139	ICCV 07113	42	46	47	95	13.22	35.80	26.20	34	33	41	6.77	0.56
140	ICCV 07115	39	44	47	95	15.09	34.05	30.90	29	26	32	6.85	0.33
141	ICCV 07116	40	44	46	98	18.29	31.10	32.90	46	43	52	12.12	0.53
142	ICCV 07117	38	44	45	95	22.76	35.05	32.75	46	44	59	13.37	0.44
143	ICCV 07118	34	40	41	91	18.95	35.10	27.40	40	37	44	11.11	0.54
144	ICCV 06301	36	42	43	87	10.35	30.25	27.50	21	20	22	5.22	0.40
145	ICCV 06302	35	40	42	92	15.72	33.70	35.90	25	23	29	8.73	0.42
146	ICCV 06306	38	42	44	93	11.27	38.45	28.70	23	21	23	6.21	0.46
147	ICCV 07304	33	37	39	107	10.08	28.65	29.05	21	19	20	5.13	0.39
148	ICCV 07311	30	36	36	92	15.60	36.35	26.80	25	24	30	8.39	0.46
149	ICCV 07312	44	48	50	95	14.43	41.05	35.20	23	21	23	7.21	0.54
150	ICCV 07313	40	43	45	87	18.40	41.55	37.60	25	22	26	9.09	0.49
151	ICC 283	52	55	57	98	18.47	29.30	27.00	57	51	57	9.21	0.49
152	ICC 1882	42	51	49	105	12.97	27.62	27.95	42	36	38	5.95	0.32
153	ICC 4958	36	42	46	103	15.37	34.95	30.65	31	27	29	8.50	0.46
154	ICC 8261	51	54	57	104	15.30	41.30	29.28	13	12	13	3.31	0.17
155	ICCV 94916-4	44	47	50	95	19.62	44.35	36.80	26	25	27	7.03	0.34
156	ICCV 94916-8	47	51	54	99	16.78	40.70	29.03	32	30	24	7.01	0.31
157	ICCV 98901	31	38	41	95	18.36	30.35	29.18	54	49	57	11.40	0.60
158	ICCV 98902	41	46	48	91	17.50	38.35	30.38	42	40	44	10.27	0.52
159	ICCV 98903	32	38	40	91	16.83	41.00	28.80	30	27	26	8.16	0.51
160	ICCV 98904	37	43	45	91	15.22	32.60	20.35	31	28	31	7.89	0.44
Mean of genotype effect		49	50	52	102	16.49	37.20	31.29	35	31	39	6.49	0.32
	Seasonal effect												
	Normal	50	54	57	111	23.33	44.27	39.45	54	49	61	10.57	0.46

Late	48	47	46	92	9.65	30.14	23.12	15	14	18	2.40	0.17
LSD(P=0.05)												
Season	(0.6) ***	(1.1) ***	(1.5) ***	(0.8) ***	(0.9) ***	(0.7) ***	(1.1) ***	(2.2) ***	(2.1) ***	(3.1) ***	(0.4) ***	(0.01) ***
Genotype effect	(5.1) ***	(10.2) ***	(13.07) ***	(1.2) ***	NS	(6.4) ***	(9.4) ***	(20.1) ***	(18.9) ***	(27.4) ***	(4.0) ***	(0.12) ***
Season x Genotype	(7.2) ***	(14.5) ***	(18.5) ***	(10.7) ***	(11.3) ***	(9.1) ***	NS	(28.4) ***	(26.8) ***	(38.8) ***	(5.6) ***	(0.17) ***

Note: Values in parenthesis indicate the LSD at *P*=0.05

Appendix 3. Effect of heat stress and non-stress on chickpea phenology and yield parameters of chickpea genotypes under semi-arid environment of India in year 2 (2010-2011) (Significant difference at **P*<0.05; ***P*<0.01; ****P*<0.001 and NS-Not significant)

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
Normal Season													
1	ICC 982	64	68	70	107	25.18	49.50	31.80	88	86	92	11.61	0.46
2	ICC 988	67	69	74	113	24.78	48. 9	28.30	71	59	78	14.34	0.44
3	ICC 1017	63	66	71	109	30.35	48.70	38.00	100	98	141	16.51	0.51
4	ICC 1025	64	70	73	116	34.84	48.80	39.70	99	98	127	14.49	0.45
5	ICC 1026	66	70	74	111	24.23	50.10	27.70	68	66	81	10.49	0.45
6	ICC 1163	50	60	62	108	24.72	48.40	34.20	82	77	78	13.93	0.52
7	ICC 1164	50	54	60	107	23.39	46.40	31.00	89	85	97	12.02	0.55
8	ICC 1471	50	56	59	107	25.66	44.10	37.20	82	80	91	15.26	0.59
9	ICC 2204	66	70	74	112	31.53	49.80	37.90	89	103	110	13.66	0.44
10	ICC 2234	64	68	71	111	33.96	55.80	25.10	82	101	115	15.60	0.46
11	ICC 2935	62	67	69	111	27.84	47.90	30.00	89	77	101	11.48	0.44
12	ICC 3335	64	69	70	109	23.91	47.80	29.40	82	79	111	12.80	0.51
13	ICC 3336	51	57	62	108	32.96	43.40	49.50	129	125	151	17.37	0.52
14	ICC 3337	54	59	63	109	25.73	49.90	36.10	55	53	79	9.37	0.40
15	ICC 3429	65	69	71	110	25.71	48.80	33.80	73	71	103	12.53	0.46
16	ICC 3485	38	41	46	100	24.40	42.10	29.30	96	91	101	13.57	0.56
17	ICC 3867	50	59	58	107	30.80	53.70	37.80	87	85	114	13.73	0.42
18	ICC 3935	43	51	53	106	33.92	39.90	38.30	86	82	95	17.80	0.54
19	ICC 4482	64	68	73	110	17.50	46.00	38.90	80	78	120	13.70	0.57
20	ICC 4861	56	63	66	112	25.99	48.80	36.70	52	49	51	9.92	0.37
21	ICC 4902	49	56	57	105	24.65	42.00	32.20	101	99	134	14.42	0.57
22	ICC 4933	65	69	71	111	31.45	52.40	31.40	101	99	110	13.11	0.43
23	ICC 4969	66	70	73	110	20.37	48.60	35.20	60	58	87	8.39	0.43
24	ICC 5116	56	61	63	114	26.15	50.00	31.70	43	40	45	8.22	0.31
25	ICC 5124	58	64	65	111	24.91	61.80	51.10	32	30	30	7.33	0.28

Genotype No	Genotypes	Days to First Flowering	Days to 50% Flowering	Days to First Podding	Days to Physiological Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
26	ICC 5186	44	50	50	108	32.37	41.10	44.60	104	101	114	18.09	0.55
27	ICC5270	60	65	67	114	26.24	53.90	41.90	49	47	52	8.08	0.36
28	ICC 5384	64	69	73	109	33.90	46.50	34.50	102	99	127	13.78	0.40
29	ICC 5566	61	68	68	113	23.02	60.10	41.50	28	26	38	6.07	0.37
30	ICC 5794	46	55	56	106	29.85	44.20	31.90	92	88	100	14.27	0.51
31	ICC 5912	55	60	62	113	38.88	62.00	43.20	82	79	98	13.43	0.33
32	ICC 6152	55	63	63	111	25.35	44.00	38.50	35	35	37	8.11	0.28
33	ICC 6169	52	62	61	113	29.85	57.60	46.40	35	32	32	11.65	0.41
34	ICC 6231	58	63	68	113	43.73	72.50	29.60	49	46	46	12.96	0.29
35	ICC 6283	52	59	60	111	45.47	55.10	36.80	70	67	67	18.12	0.42
36	ICC 6293	53	60	61	111	23.92	57.40	30.90	47	45	84	9.35	0.34
37	ICC 6334	52	59	60	110	29.58	50.20	23.50	86	84	98	14.28	0.48
38	ICC 6969	45	52	56	108	27.35	44.90	38.60	132	123	156	22.12	0.66
39	ICC 7192	67	72	73	116	23.14	65.30	31.50	39	37	50	9.19	0.32
40	ICC 7193	67	75	75	115	26.86	73.50	41.80	20	18	18	4.73	0.18
41	ICC 7241	58	65	67	113	23.58	44.50	32.10	23	21	21	8.26	0.35
42	ICC 7263	46	52	54	109	24.97	60.20	38.90	42	39	40	10.63	0.41
43	ICC 7292	59	65	69	113	22.21	65.80	35.80	34	32	23	7.69	0.31
44	ICC 7294	63	69	69	113	22.20	60.60	35.10	35	32	21	5.89	0.21
45	ICC 7298	57	64	66	111	24.58	46.10	24.90	41	38	42	9.67	0.39
46	ICC 7308	37	43	47	107	29.50	62.80	40.10	42	40	42	11.15	0.36
47	ICC 7570	66	69	74	115	26.50	60.20	33.50	19	18	18	4.33	0.17
48	ICC 7669	65	69	70	115	30.60	80.90	30.70	26	24	30	8.17	0.27
49	ICC 8166	43	48	52	103	23.26	39.10	48.40	66	63	80	12.54	0.49
50	ICC 8273	60	64	68	112	17.45	54.40	32.10	27	24	24	5.19	0.35
51	ICC 8397	49	58	59	111	29.07	53.40	50.50	83	78	92	11.27	0.36
52	ICC 8474	34	38	43	105	29.43	44.20	38.30	56	54	55	15.50	0.52
53	ICC 8527	42	50	53	110	30.69	54.30	36.00	40	38	38	11.84	0.36

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
54	ICC 8943	53	58	60	109	19.00	57.70	46.40	43	41	46	8.32	0.39
55	ICC 9125	63	66	70	107	25.59	51.60	32.90	64	61	74	13.04	0.50
56	ICC 9557	53	60	62	111	34.89	66.00	46.10	74	72	76	14.46	0.41
57	ICC 9562	45	53	56	109	33.91	53.10	27.30	99	96	102	18.09	0.55
58	ICC 9567	54	62	61	108	31.36	49.90	35.10	105	103	155	17.42	0.47
59	ICC 10090	47	57	58	110	25.77	49.50	35.10	62	60	74	10.26	0.38
60	ICC 10134	66	69	73	113	24.33	52.30	39.00	61	60	85	12.57	0.46
61	ICC 10600	68	73	75	114	27.45	48.40	34.60	75	72	89	10.70	0.39
62	ICC 10674	58	65	66	114	44.30	67.70	31.00	41	37	37	15.12	0.32
63	ICC 11553	45	53	55	107	22.89	53.60	29.80	75	72	93	10.51	0.44
64	ICC 11795	45	52	53	115	31.02	57.20	42.20	19	16	16	4.75	0.19
65	ICC 11886	44	53	52	109	32.83	65.60	37.50	104	102	117	14.71	0.44
66	ICC 11901	41	50	51	108	33.67	57.50	36.00	143	140	140	16.99	0.50
67	ICC 11903	55	62	64	109	31.87	59.70	38.60	60	57	75	9.83	0.27
68	ICC 12121	49	65	67	108	28.59	51.70	33.30	41	37	38	10.46	0.35
69	ICC 12123	54	59	61	110	21.59	54.60	45.00	59	56	59	9.07	0.41
70	ICC 12169	52	59	60	106	21.50	37.80	31.90	81	77	103	11.08	0.51
71	ICC 12184	62	67	70	111	29.56	50.50	26.00	79	76	103	14.55	0.48
72	ICC 12289	52	61	60	106	35.61	47.80	38.10	100	97. 0	162	17.62	0.49
73	ICC 12312	44	51	54	105	27.44	40.40	38.10	83	77	91	13.73	0.34
74	ICC 12511	54	61	63	103	23.45	50.40	36.20	79	74	114	12.51	0.52
75	ICC 12554	37	48	46	107	21.09	44.80	31.00	83	80	98	11.18	0.51
76	ICC 12620	39	45	48	100	25.01	51.20	34.80	103	97	135	14.27	0.56
77	ICC 12787	43	51	52	107	29.73	45.90	42.20	100	99	129	16.74	0.51
78	ICC 13941	46	51	52	105	23.51	49.30	32.00	91	89	109	12.51	0.52
79	ICC 14176	41	51	50	105	36.93	48.20	42.10	106	103	167	17.61	0.45
81	ICC 14179	42	52	54	104	24.41	51.00	35.10	75	73	88	10.45	0.49
82	ICC 14183	51	59	59	111	23.55	50.50	44.80	39	36	42	8.58	0.37

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
83	ICC 14315	36	42	46	106	24.39	51.20	44.50	77	75	97	9.55	0.47
84	ICC 14406	43	50	50	107	20.98	42.80	44.60	61	59	66	10.45	0.54
85	ICC 14456	45	52	55	108	25.38	45.50	36.50	62	59	65	13.05	0.48
86	ICC 14497	41	47	49	102	23.73	42.30	32.90	89	86	120	12.75	0.58
87	ICC 14533	45	52	55	108	26.85	42.90	46.80	82	80	103	14.38	0.53
88	ICC 14575	55	60	62	108	21.11	44.80	28.00	71	69	90	9.79	0.49
89	ICC 14592	47	53	56	105	26.09	40.50	34.20	97	95	122	14.88	0.53
90	ICC 14674	40	45	47	102	30.74	44.10	33.70	80	79	101	12.90	0.53
91	ICC 14913	38	46	51	106	25.58	53.40	44.40	104	97	120	12.86	0.51
92	ICC 15367	59	65	71	116	30.78	55.60	40.60	34	31	31	11.88	0.38
93	ICC 15380	51	55	60	109	17.83	46.90	43.60	48	45	44	8.63	0.50
94	ICC 15388	51	60	59	113	29.61	60.50	44.50	36	34	36	11.45	0.38
95	ICC 15536	64	69	71	109	24.70	47.40	32.80	60	57	113	13.68	0.54
96	ICC 15779	45	54	55	108	27.32	48.70	32.00	63	60	60	13.60	0.48
97	ICC 15795	59	65	68	113	29.16	53.30	40.60	31	29	29	10.05	0.37
98	ICC 15807	44	51	56	114	24.90	54.10	44.40	39	36	40	11.75	0.46
99	ICC 16141	41	45	51	105	27.27	41.60	38.80	93	89	108	14.68	0.51
100	ICC 16173	38	46	47	106	23.54	46.10	45.50	69	67	88	13.21	0.56
101	ICC 16181	37	44	45	107	21.66	47.30	30.90	89	84	119	14.55	0.59
102	ICC 16216	40	45	48	104	19.35	41.50	34.30	67	63	88	13.13	0.59
103	ICC 16219	48	56	59	104	28.08	50.80	34.90	78	75	79	12.16	0.54
104	ICC 16298	51	59	62	108	26.20	51.10	41.00	102	100	103	12.88	0.58
105	ICC 16343	63	67	73	111	33.39	49.10	36.40	60	58	70	15.61	0.50
106	ICC 16376	66	70	75	113	20.78	63.80	34.90	19	14	15	3.87	0.21
107	ICC 16436	66	69	74	110	31.30	50.70	32.80	81	79	107	14.38	0.46
108	ICC 16453	53	60	60	112	27.60	56.00	36.20	29	25	25	7.29	0.31
109	ICC 16528	65	68	70	107	22.42	55.50	41.50	41	40	54	11.93	0.43
110	ICC 16626	62	68	69	113	19.50	57.70	34.50	33	31	33	7.73	0.33

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
111	ICC 16774	59	63	68	113	31.30	63.40	44.90	25	24	24	9.26	0.27
112	ICC 16820	42	49	55	113	31.68	55.60	38.90	42	38	39	13.30	0.41
113	ICC 16833	46	53	57	104	40.23	47.70	28.70	101	96	120	19.48	0.50
114	ICC 16835	45	50	56	106	19.56	48.40	32.30	54	52	71	13.19	0.64
115	ICC 17083	44	49	52	108	35.38	45.30	37.80	98	96	116	16.75	0.48
116	ICCC 37	39	44	46	106	29.99	46.90	42.90	81	78	99	17.55	0.59
117	ICCL 81248	43	48	54	102	29.81	45.50	35.30	95	92	131	17.76	0.58
118	ICCL 83149	38	46	46	102	39.49	49.50	40.60	101	97	123	22.10	0.58
119	ICCL 87207	41	47	47	109	21.50	42.70	33.70	57	51	52	12.26	0.53
120	ICCV 3	48	55	59	108	34.78	48.60	26.50	59	56	59	15.63	0.51
121	ICCV 88202	37	39	44	106	24.01	40.00	35.90	56	53	81	12.07	0.52
122	ICCV 89314	47	57	58	108	30.37	47.40	43.40	89	86	103	17.68	0.58
123	ICCV 89509	48	56	59	107	46.28	48.40	42.10	126	124	137	23.05	0.50
124	ICCV 90201	52	59	60	107	21.64	56.60	38.70	53	50	65	9.68	0.47
125	ICCV 91302	50	59	61	108	30.36	54.30	30.10	60	58	59	15.10	0.55
126	ICCV 92809	57	62	66	109	20.31	57.80	33.80	44	42	47	9.31	0.45
127	ICCV 92944	35	38	43	98	29.00	44.30	30.90	87	72	79	16.82	0.53
128	ICCV 93512	53	57	62	109	35.44	41.10	29.40	51	48	50	15.00	0.48
129	ICCV 93952	41	46	50	105	31.11	51.20	28.30	83	73	75	17.46	0.54
130	ICCV 93954	32	36	40	101	25.89	51.10	40.30	83	78	81	16.05	0.61
131	ICCV 94954	42	47	51	100	35.62	44.00	27.40	78	74	89	19.75	0.61
132	ICCV 95311	40	47	49	108	36.58	47.90	35.70	63	60	62	18.64	0.31
133	ICCV 95332	38	41	46	98	23.19	47.40	41.50	45	42	48	14.67	0.6
134	ICCV 96836	60	64	67	107	35.91	68.60	28.90	94	90	109	15.48	0.46
135	ICCX 820065(GG2)	35	38	44	111	19.70	44.80	43.90	40	34	34	10.16	0.49
136	ICCV 97105	52	61	61	105	31.39	48.00	35.50	74	67	76	15.26	0.51
137	ICCV 96030	26	29	32	93	16.68	43.60	33.80	42	39	51	7.85	0.48

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
138	ICC 17109	34	38	44	107	19.29	52.60	33.10	33	16	16	8.37	0.33
139	ICCV 07101	39	45	47	101	31.33	52.30	33.70	88	84	112	20.37	0.61
140	ICCV 07102	40	45	47	101	35.26	56.00	32.70	80	76	114	20.17	0.6
141	ICCV 07104	40	45	47	107	26.49	50.90	30.00	70	67	90	16.22	0.61
142	ICCV 07105	43	48	51	103	33.87	56.90	33.20	78	75	120	20.73	0.59
143	ICCV 07108	34	38	41	103	30.63	45.30	41.30	58	49	60	16.06	0.51
144	ICCV 07109	40	44	51	103	34.61	47.90	32.30	91	82	98	17.90	0.50
145	ICCV 07110	34	37	41	106	28.36	53.10	32.00	45	42	62	11.32	0.50
146	ICCV 07113	39	47	51	102	28.57	44.70	36.30	83	81	85	15.41	0.57
147	ICCV 07115	37	40	44	99	14.88	44.10	41.10	47	46	53	10.04	0.57
148	ICCV 07116	39	45	45	102	28.55	37.40	32.40	74	69	76	16.74	0.58
149	ICCV 07117	40	45	48	104	37.13	47.60	39.30	69	66	86	18.30	0.58
150	ICCV 07118	37	40	45	105	26.25	50.90	35.40	59	56	59	16.04	0.60
151	ICCV 06301	49	58	58	111	26.56	51.00	43.70	29	25	26	8.90	0.36
152	ICCV 06302	36	39	42	109	21.98	43.80	36.90	36	36	36	12.60	0.50
153	ICCV 06306	38	41	46	104	15.10	51.60	36.70	31	30	30	8.96	0.50
154	ICCV 07304	34	38	43	105	35.40	51.00	40.20	47	45	55	20.03	0.55
155	ICCV 07311	31	35	41	103	20.81	43.00	38.10	30	29	40	11.39	0.53
156	ICCV 07312	42	46	51	106	23.29	58.60	42.10	41	39	39	12.64	0.51
157	ICCV 07313	40	45	49	104	14.64	50.30	39.20	20	18	20	7.41	0.49
158	ICC 283	51	59	62	107	26.35	47.70	32.80	82	80	85	14.27	0.52
159	ICC 1882	44	47	55	105	25.39	40.90	37.70	105	92	105	13.67	0.52
160	ICC 4958	33	36	42	105	28.94	49.70	38.10	64	59	66	15.62	0.55
161	ICC 8261	47	55	56	113	39.09	57.30	37.50	49	43	48	16.55	0.39
162	ICCV 94916-4	40	48	47	108	28.51	49.20	43.40	53	51	53	14.56	0.49
163	ICCV 94916-8	40	51	50	109	26.66	51.80	46.40	43	41	36	13.09	0.42
164	ICCV 98901	32	37	41	106	33.38	47.60	31.80	105	87	95	18.52	0.55
165	ICCV 98902	36	45	47	106	37.45	47.10	37.40	62	58	59	19.21	0.51

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
166	ICCV 98903	38	43	46	104	32.67	48.70	33.30	66	55	58	17.23	0.49
167	ICCV 98904	36	39	44	106	35.61	45.80	30.30	78	70	76	20.74	0.54
Genotype effect		49	55	57	108	27.88	50.74	36.50	68	64	78	13.17	0.46
	LSD(*P*=0.05)	(6.48) ***	(6.89) ***	(7.4) ***	(5.47) ***	(15.01) ***	(10.45) ***	(14.85) ***	(41.45) ***	(40.13) ***	(51.66) ***	(7.88) ***	(0.1) ***
Late Season													
1	ICC 982	57	61	63	103	23.55	41.50	49.30	68	66	93	10.07	0.41
2	ICC 988	62	69	68	110	20.92	51.40	54.90	47	44	58	5.90	0.27
3	ICC 1017	57	60	63	100	28.56	41.10	48.70	86	81	124	12.93	0.43
4	ICC 1025	60	64	67	106	29.10	44.60	58.60	76	71	91	10.00	0.35
5	ICC 1026	60	64	66	109	27.13	42.80	53.40	74	71	92	9.80	0.34
6	ICC 1163	42	47	49	99	33.57	41.10	46.80	110	100	104	17.22	0.51
7	ICC 1164	48	52	54	102	21.10	32.70	33.00	72	71	100	11.77	0.56
8	ICC 1471	43	46	49	98	26.57	34.00	34.60	77	73	88	14.93	0.59
9	ICC 2204	64	68	70	111	25.86	41.90	40.60	57	54	75	8.83	0.35
10	ICC 2234	59	63	65	104	17.33	45.30	47.10	60	59	74	7.51	0.45
11	ICC 2935	60	65	65	103	14.55	44.30	42.90	35	33	44	5.29	0.38
12	ICC 3335	47	54	53	102	23.46	44.20	46.80	65	63	95	10.03	0.43
13	ICC 3336	42	47	51	101	20.90	29.40	45.60	80	79	105	10.58	0.50
14	ICC 3337	42	48	52	107	34.32	49.30	48.00	72	69	95	13.23	0.36
15	ICC 3429	57	61	63	100	19.04	38.90	39.40	50	47	75	8.91	0.46
16	ICC 3485	36	39	43	104	31.48	36.90	40.10	116	114	129	15.10	0.53
17	ICC 3867	41	46	47	102	35.56	53.00	44.90	88	78	103	11.17	0.39
18	ICC 3935	43	48	50	99	18.29	30.00	33.70	69	69	87	9.51	0.55
19	ICC 4482	56	61	62	103	21.61	40.60	49.00	63	59	84	9.84	0.43
20	ICC 4861	44	47	50	103	27.15	46.00	45.60	53	50	65	10.59	0.37
21	ICC 4902	44	48	50	97	22.89	33.90	28.50	83	82	121	12.08	0.52
22	ICC 4933	59	63	65	103	39.13	44.00	49.60	94	91	134	13.69	0.38

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
23	ICC 4969	59	63	66	107	23.95	39.40	51.10	70	63	90	8.60	0.35
24	ICC 5116	56	24	39	51	28.42	45.20	48.40	38	34	54	7.29	0.23
25	ICC 5124	57	62	62	110	8.34	48.10	34.50	21	20	24	3.74	0.25
26	ICC 5186	38	44	46	102	28.72	35.20	39.50	77	67	76	13.10	0.53
27	ICC5270	58	61	62	111	30.00	46.50	51.80	64	63	92	12.12	0.33
28	ICC 5384	58	62	64	105	29.90	40.30	56.50	81	79	93	9.76	0.31
29	ICC 5566	64	75	74	115	18.44	57.20	47.80	8	8	11	1.79	0.10
30	ICC 5794	42	46	47	100	29.03	35.90	40.40	108	99	119	14.45	0.53
31	ICC 5912	49	52	56	111	33.20	42.40	46.90	129	65	82	6.09	0.20
32	ICC 6152	48	53	54	103	27.79	42.40	48.20	58	53	74	13.21	0.43
33	ICC 6169	48	53	54	101	32.13	45.60	43.70	33	28	32	9.80	0.33
34	ICC 6231	49	54	57	105	22.36	47.80	41.70	38	35	37	7.62	0.41
35	ICC 6283	53	56	58	103	38.36	45.90	41.10	57	51	56	15.10	0.37
36	ICC 6293	52	56	58	105	27.20	50.20	50.50	53	48	70	7.70	0.28
37	ICC 6334	49	54	55	95	15.63	41.50	41.00	54	53	70	8.76	0.55
38	ICC 6969	37	40	47	103	30.08	36.80	44.00	121	120	170	21.95	0.6
39	ICC 7192	62	36	69	110	14.45	57.10	45.70	12	12	13	2.17	0.05
40	ICC 7193	64	0	70	114	7.13	49.50	47.10	7	6	6	1.14	0.09
41	ICC 7241	55	58	60	102	32.96	45.60	38.50	40	38	43	14.04	0.38
42	ICC 7263	46	52	53	101	25.77	48.20	47.00	33	31	34	9.41	0.30
43	ICC 7292	49	56	58	108	28.07	50.30	44.20	21	20	23	6.50	0.26
44	ICC 7294	52	64	58	113	26.01	48.80	51.50	20	18	21	5.63	0.15
45	ICC 7298	57	61	63	107	41.92	44.10	45.60	42	39	46	10.20	0.26
46	ICC 7308	45	51	51	100	14.52	48.50	42.20	31	29	42	8.62	0.5
47	ICC 7570	60	33	66	111	24.87	52.70	50.80	7	6	8	1.46	0.08
48	ICC 7669	59	68	65	115	17.45	59.80	48.90	21	10	12	2.60	0.18
49	ICC 8166	43	48	49	101	26.55	38.70	38.20	20	85	100	13.93	0.56
50	ICC 8273	50	55	55	103	28.95	44.90	47.20	54	52	66	10.75	0.33

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
51	ICC 8397	53	56	59	102	25.60	42.70	44.40	80	65	82	5.30	0.21
52	ICC 8474	33	37	39	94	18.61	36.90	29.40	35	33	44	11.91	0.63
53	ICC 8527	49	56	54	101	33.44	51.60	44.60	44	40	42	12.38	0.38
54	ICC 8943	49	54	55	104	30.44	50.20	50.70	45	44	63	9.29	0.27
55	ICC 9125	53	56	59	101	31.42	41.70	39.60	84	78	114	16.24	0.49
56	ICC 9557	55	61	62	108	39.97	52.50	58.50	65	56	70	12.53	0.29
57	ICC 9562	40	43	47	101	19.24	39.70	36.70	55	51	63	10.62	0.49
58	ICC 9567	52	56	58	103	23.55	40.70	39.20	85	83	94	12.79	0.51
59	ICC 10090	43	53	50	98	21.42	46.80	54.80	47	35	44	5.06	0.23
60	ICC 10134	61	65	67	107	14.07	42.70	51.30	16	14	19	2.35	0.2
61	ICC 10600	75	40	82	114	8.86	45.20	48.20	7	7	8	0.79	0.03
62	ICC 10674	56	60	62	109	25.80	49.80	45.30	22	19	16	7.06	0.21
63	ICC 11553	40	46	52	100	18.60	42.30	34.30	57	54	61	7.62	0.44
64	ICC 11795	58	61	63	108	32.13	52.70	53.10	13	12	15	3.91	0.15
65	ICC 11886	41	46	50	105	16.91	60.00	42.70	36	34	43	5.45	0.29
66	ICC 11901	39	45	48	100	25.56	48.40	37.50	96	93	129	12.88	0.47
67	ICC 11903	47	58	57	108	35.15	50.50	52.40	36	33	37	9.57	0.24
68	ICC 12121	46	57	52	107	30.62	47.70	49.80	30	29	35	10.17	0.28
69	ICC 12123	55	58	61	100	14.44	47.70	48.30	24	23	28	3.82	0.18
70	ICC 12169	45	53	52	99	25.76	34.10	43.30	83	78	132	12.60	0.52
71	ICC 12184	58	62	64	101	22.44	42.90	37.50	68	63	73	9.40	0.45
72	ICC 12289	45	50	52	99	26.73	31.80	37.20	53	51	124	13.21	0.51
73	ICC 12312	41	45	47	106	24.31	39.00	45.70	71	70	85	13.15	0.51
74	ICC 12511	49	53	55	103	21.61	43.00	44.00	77	68	99	10.98	0.44
75	ICC 12554	40	43	47	93	24.89	42.80	42.30	85	83	126	13.31	0.55
76	ICC 12620	34	38	40	96	29.24	38.20	36.40	113	109	161	17.95	0.62
77	ICC 12787	40	43	46	100	31.42	38.30	35.30	105	100	132	15.81	0.50
78	ICC 13941	41	48	48	101	27.17	40.00	41.40	105	100	122	14.35	0.51

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
79	ICC 14176	34	41	44	107	29.34	40.00	41.60	110	107	133	13.77	0.43
80	ICC 14177	40	44	47	99	21.33	40.00	35.30	77	73	101	11.71	0.55
81	ICC 14179	42	46	50	96	22.82	40.40	34.60	89	81	128	12.42	0.57
82	ICC 14183	52	55	59	102	34.01	46.90	37.50	53	47	56	11.88	0.34
83	ICC 14315	34	37	40	94	19.46	37.70	33.20	91	89	121	13.44	0.66
84	ICC 14406	42	46	48	104	30.97	34.70	30.80	74	73	107	15.98	0.53
85	ICC 14456	47	54	54	106	27.47	53.10	43.40	30	29	40	8.51	0.31
86	ICC 14497	35	39	43	97	25.21	36.90	32.00	90	87	124	13.15	0.6
87	ICC 14533	44	47	49	101	48.12	37.00	50.70	171	158	234	27.13	0.55
88	ICC 14575	48	51	54	95	31.31	33.30	44.60	118	117	169	15.98	0.50
89	ICC 14592	39	47	47	100	25.91	32.40	38.00	75	73	111	11.52	0.44
90	ICC 14674	34	38	41	98	23.54	35.80	41.50	80	77	100	15.06	0.62
91	ICC 14913	38	42	45	97	14.26	39.60	33.10	56	51	72	7.82	0.48
92	ICC 15367	53	59	62	103	31.07	50.70	54.70	37	33	38	11.49	0.27
93	ICC 15380	40	44	46	103	52.66	37.90	46.50	101	96	123	23.20	0.45
94	ICC 15388	47	53	57	106	22.97	44.40	38.90	26	23	29	7.13	0.34
95	ICC 15536	54	58	62	99	27.08	43.00	60.20	77	73	90	11.18	0.45
96	ICC 15779	46	54	54	102	31.39	42.10	45.50	74	68	72	15.74	0.48
97	ICC 15795	58	62	64	105	40.96	49.00	51.20	36	34	38	13.32	0.31
98	ICC 15807	52	59	60	109	34.53	44.70	43.00	44	41	59	14.63	0.40
99	ICC 16141	37	40	43	101	32.43	37.00	40.20	107	97	113	18.08	0.53
100	ICC 16173	36	41	43	105	30.74	36.80	43.30	93	87	98	14.96	0.49
101	ICC 16181	31	36	41	102	10.63	35.30	35.60	79	77	110	13.15	0.69
102	ICC 16216	38	42	44	98	14.70	30.20	34.90	93	92	127	11.06	0.65
103	ICC 16219	37	40	44	104	33.48	37.20	42.20	80	76	126	17.02	0.51
104	ICC 16298	41	46	50	99	23.52	37.70	37.70	101	95	116	11.52	0.51
105	ICC 16343	60	63	68	101	28.01	42.50	42.40	85	77	80	13.64	0.49
106	ICC 16376	62	74	69	111	29.60	53.00	53.90	8	7	7	1.37	0.04

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
107	ICC 16436	58	61	64	104	23.67	43.20	50.00	53	52	79	8.77	0.39
108	ICC 16453	52	59	59	105	25.67	56.10	56.00	20	19	25	5.94	0.27
109	ICC 16528	60	63	65	107	32.36	46.60	52.40	41	38	60	12.66	0.38
110	ICC 16626	57	63	66	107	25.74	45.80	56.10	47	40	57	10.91	0.38
111	ICC 16774	57	61	62	110	16.74	55.30	46.90	12	10	11	3.70	0.17
112	ICC 16820	54	58	60	104	29.48	50.80	48.10	28	28	35	9.58	0.31
113	ICC 16833	43	48	50	93	20.84	33.80	23.10	61	57	78	10.62	0.54
114	ICC 16835	40	45	47	100	27.50	36.40	37.10	83	81	68	16.45	0.59
115	ICC 17083	37	41	43	101	16.82	38.40	35.50	64	64	75	9.39	0.54
116	ICCC 37	34	37	41	98	18.60	32.50	27.00	63	62	81	13.08	0.71
117	ICCL 81248	37	39	44	102	22.73	36.30	27.30	87	84	116	14.35	0.59
118	ICCL 83149	31	35	39	90	12.65	29.10	33.30	45	45	56	10.42	0.7
119	ICCL 87207	41	45	51	98	26.64	36.80	30.20	53	49	62	15.89	0.64
120	ICCV 3	49	55	57	102	44.78	37.50	44.60	71	67	73	20.53	0.47
121	ICCV 88202	33	36	38	99	20.88	34.10	30.70	39	38	57	10.08	0.55
122	ICCV 89314	40	44	47	102	31.49	33.90	34.00	96	91	113	17.75	0.56
123	ICCV 89509	45	49	51	101	23.91	39.30	38.00	78	69	92	14.11	0.52
124	ICCV 90201	56	59	62	100	29.83	43.20	40.00	82	80	118	15.04	0.51
125	ICCV 91302	48	52	54	100	18.91	44.10	40.30	38	37	47	10.74	0.55
126	ICCV 92809	56	64	62	106	30.33	44.90	46.00	48	47	64	7.89	0.34
127	ICCV 92944	32	36	38	88	19.06	33.20	26.90	58	56	66	16.20	0.79
128	ICCV 93512	53	56	59	104	48.87	43.50	50.40	76	75	87	21.31	0.44
129	ICCV 93952	35	38	41	101	27.09	35.10	34.80	68	65	73	14.43	0.55
130	ICCV 93954	34	37	41	99	21.35	36.10	26.80	61	58	63	13.37	0.61
131	ICCV 94954	35	39	43	98	17.85	36.60	27.80	40	38	45	8.48	0.56
132	ICCV 95311	43	46	48	103	40.04	41.90	39.80	75	70	74	20.85	0.54
133	ICCV 95332	33	36	39	100	24.41	37.40	34.60	44	43	53	15.62	0.64
134	ICCV 96836	52	55	57	95	36.56	51.70	33.80	97	92	131	18.04	0.50

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
135	ICCX 820065(GG2)	32	35	38	100	20.11	42.70	36.90	46	45	58	16.58	0.66
136	ICCV 97105	43	51	52	102	22.72	41.90	38.80	61	57	85	12.25	0.54
137	ICCV 96030	27	31	32	89	7.17	33.30	27.40	24	23	25	3.94	0.68
138	ICC 17109	38	42	43	101	14.62	47.70	38.00	15	13	15	6.77	0.38
139	ICCV 07101	38	42	47	106	27.66	44.50	35.60	45	43	68	11.72	0.38
140	ICCV 07102	52	56	57	112	25.16	55.40	47.20	62	61	63	13.49	0.36
141	ICCV 07104	39	43	46	105	33.83	42.80	35.10	81	76	95	17.88	0.52
142	ICCV 07105	40	45	47	103	38.38	44.20	35.90	96	91	118	21.25	0.49
143	ICCV 07108	34	38	43	100	32.20	36.60	31.80	67	64	72	19.47	0.59
144	ICCV 07109	50	55	57	104	41.11	41.40	45.50	80	77	87	22.22	0.54
145	ICCV 07110	34	38	41	98	25.64	35.60	35.10	59	56	62	17.42	0.64
146	ICCV 07113	36	40	43	102	31.67	38.30	34.00	66	64	96	17.90	0.57
147	ICCV 07115	40	46	47	98	23.85	44.20	38.20	49	47	63	11.71	0.46
148	ICCV 07116	37	41	44	99	21.64	35.40	35.20	42	41	53	13.22	0.58
149	ICCV 07117	39	43	45	92	21.87	35.30	29.40	40	39	63	14.52	0.64
150	ICCV 07118	36	39	41	101	23.74	32.90	28.30	61	58	72	16.50	0.70
151	ICCV 06301	51	55	57	106	41.33	50.60	51.80	45	44	51	14.55	0.35
152	ICCV 06302	33	36	40	100	21.20	34.30	29.30	29	28	34	10.57	0.50
153	ICCV 06306	33	36	40	99	20.43	40.10	27.90	38	37	41	12.81	0.51
154	ICCV 07304	33	37	39	100	28.98	39.20	37.80	48	47	62	17.13	0.52
155	ICCV 07311	34	40	40	93	7.53	34.70	24.10	24	22	30	7.38	0.72
156	ICCV 07312	38	42	45	101	31.71	37.90	28.80	48	46	51	17.10	0.50
157	ICCV 07313	38	42	43	90	15.04	36.40	28.70	27	26	27	9.23	0.59
158	ICC 283	41	45	47	99	31.20	34.10	35.40	81	80	103	15.79	0.55
159	ICC 1882	37	40	44	102	33.31	32.30	32.00	124	119	134	18.50	0.60
160	ICC 4958	33	36	39	90	28.90	37.30	30.20	50	48	53	15.63	0.63
161	ICC 8261	47	56	59	102	23.54	47.50	39.50	34	32	35	9.60	0.36

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
162	ICCV 94916-4	38	44	45	98	22.27	38.30	40.00	31	28	44	11.73	0.54
163	ICCV 94916-8	41	45	49	96	38.10	41.90	54.70	78	70	76	21.25	0.55
164	ICCV 98901	31	34	38	95	18.27	28.80	29.10	66	63	77	13.98	0.73
165	ICCV 98902	35	38	42	102	46.49	44.50	38.70	88	84	90	27.22	0.60
166	ICCV 98903	39	43	45	93	37.74	44.30	39.50	70	65	78	19.22	0.50
167	ICCV 98904	36	40	43	96	29.25	39.20	29.80	64	63	60	17.07	0.60
Genotype effect		46	49	52	102	26.30	41.99	41.24	61.2	57.6	74.7	12.00	0.45
	LSD(*P*=0.05)	(8.96) ***	(15.85) ***	(8.77) ***	(9.03) ***	(18.79) *	(7.21) ***	(13.49) ***	(43.24) ***	(41.91) ***	(57.19) ***	(9.00) ***	(0.14) ***
Normal x *Late Season*													
1	ICC 982	60	64	67	105	33.34	49.40	27.68	78	76	92	10.84	0.44
2	ICC 988	64	69	71	111	38.09	51.90	24.61	59	51	68	10.12	0.36
3	ICC 1017	60	63	67	104	35.72	48.70	33.28	93	90	132	14.72	0.47
4	ICC 1025	62	67	70	111	39.72	53.70	34.40	87	84	109	12.25	0.40
5	ICC 1026	63	67	70	110	33.51	51.75	27.42	71	68	86	10.14	0.40
6	ICC 1163	46	53	55	104	32.91	47.60	33.89	96	88	91	15.57	0.51
7	ICC 1164	49	53	57	104	28.04	39.70	26.05	80	78	99	11.89	0.55
8	ICC 1471	46	51	54	103	29.83	39.35	31.89	80	76	90	15.10	0.59
9	ICC 2204	65	69	72	111	36.71	45.20	31.88	81	79	92	11.24	0.39
10	ICC 2234	61	65	68	107	39.63	51.45	21.22	82	80	94	11.55	0.45
11	ICC 2935	61	66	67	107	36.07	45.40	22.28	57	55	73	8.39	0.41
12	ICC 3335	55	61	61	105	34.05	47.30	26.43	74	71	103	11.41	0.47
13	ICC 3336	46	52	56	104	31.18	44.50	35.20	105	102	128	13.97	0.51
14	ICC 3337	48	53	58	108	37.51	48.95	35.21	64	61	87	11.30	0.38
15	ICC 3429	61	65	67	105	32.30	44.10	26.42	61	59	89	10.72	0.46
16	ICC 3485	37	40	44	102	30.65	41.10	30.39	106	102	115	14.34	0.55
17	ICC 3867	46	52	53	105	41.90	49.30	36.68	87	81	109	12.45	0.40
18	ICC 3935	43	50	51	103	31.96	36.80	28.30	77	75	91	13.66	0.54

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
19	ICC 4482	60	64	68	107	29.05	47.50	30.26	71	68	102	11.77	0.50
20	ICC 4861	50	55	58	108	35.99	47.20	31.93	52	50	58	10.25	0.37
21	ICC 4902	46	52	54	101	29.27	35.25	27.55	92	90	127	13.25	0.54
22	ICC 4933	62	66	68	107	37.72	51.00	35.27	97	95	122	13.40	0.41
23	ICC 4969	63	67	70	108	29.88	49.85	29.58	65	60	89	8.49	0.39
24	ICC 5116	56	60	63	110	35.67	49.20	30.06	40	37	50	7.76	0.27
25	ICC 5124	57	63	63	110	36.50	48.15	29.72	26	25	27	5.53	0.26
26	ICC 5186	41	47	48	105	33.78	40.30	36.66	91	84	95	15.59	0.54
27	ICC5270	59	63	65	113	36.37	52.85	35.95	56	55	72	10.10	0.34
28	ICC 5384	61	65	68	107	37.10	51.50	32.20	91	89	110	11.77	0.36
29	ICC 5566	63	71	71	114	40.11	53.95	29.97	18	17	24	3.93	0.23
30	ICC 5794	44	50	52	103	32.87	42.30	30.47	100	93	109	14.36	0.52
31	ICC 5912	52	56	59	112	40.64	54.45	38.20	106	72	90	9.76	0.26
32	ICC 6152	51	58	58	107	33.87	46.10	33.15	47	44	55	10.66	0.36
33	ICC 6169	50	57	57	107	37.72	50.65	39.27	34	30	32	10.72	0.37
34	ICC 6231	53	58	62	109	45.76	57.10	25.98	44	40	41	10.29	0.35
35	ICC 6283	52	58	59	107	45.68	48.10	37.58	63	59	62	16.61	0.39
36	ICC 6293	53	58	60	108	37.06	53.95	29.05	50	46	77	8.53	0.31
37	ICC 6334	51	56	57	102	35.54	45.60	19.57	70	69	84	11.52	0.52
38	ICC 6969	41	46	51	105	32.07	44.45	34.34	127	122	163	22.04	0.63
39	ICC 7192	65	54	71	113	40.12	55.50	22.98	25	25	32	5.68	0.19
40	ICC 7193	66	37	72	115	38.18	60.30	24.47	13	12	12	2.93	0.13
41	ICC 7241	56	61	64	108	34.59	41.50	32.53	31	29	32	11.15	0.17
42	ICC 7263	46	52	54	105	36.58	53.60	32.34	37	35	37	10.02	0.36
43	ICC 7292	54	60	63	111	36.25	55.00	31.94	28	26	23	7.10	0.28
44	ICC 7294	57	66	64	113	35.50	56.05	30.56	28	25	21	5.76	0.18
45	ICC 7298	57	62	64	109	34.34	45.85	33.41	41	38	44	9.94	0.32
46	ICC 7308	41	47	49	103	39.00	52.50	27.31	37	35	42	9.88	0.43

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
47	ICC 7570	63	51	70	113	39.60	55.50	29.19	13	12	13	2.89	0.13
48	ICC 7669	62	68	68	115	45.20	64.90	24.08	18	17	21	5.39	0.22
49	ICC 8166	43	48	50	102	30.98	38.65	37.48	81	74	90	13.24	0.53
50	ICC 8273	55	59	61	107	31.17	50.80	30.53	40	38	45	7.97	0.34
51	ICC 8397	51	57	59	107	35.88	48.90	38.05	82	71	87	8.28	0.28
52	ICC 8474	33	37	41	99	33.16	36.80	28.46	45	43	49	13.70	0.57
53	ICC 8527	45	53	54	105	41.14	49.45	34.72	42	39	40	12.11	0.37
54	ICC 8943	51	56	57	106	34.60	54.20	38.42	44	42	55	8.81	0.33
55	ICC 9125	58	61	64	104	33.64	45.60	32.16	74	70	94	14.64	0.50
56	ICC 9557	54	60	62	109	43.69	62.25	43.04	69	64	73	13.49	0.35
57	ICC 9562	42	48	51	105	36.80	44.90	23.27	77	74	83	14.36	0.52
58	ICC 9567	53	59	59	105	36.03	44.55	29.33	95	93	125	15.11	0.49
59	ICC 10090	45	55	54	104	36.28	52.15	28.26	54	47	59	7.66	0.30
60	ICC 10134	63	67	70	110	33.51	51.80	26.54	39	37	52	7.46	0.33
61	ICC 10600	71	56	79	114	36.32	48.30	21.73	41	39	48	5.74	0.21
62	ICC 10674	57	62	64	111	47.05	56.50	28.40	31	28	27	11.09	0.27
63	ICC 11553	43	50	53	104	32.59	43.95	24.20	66	63	77	9.07	0.44
64	ICC 11795	51	57	58	111	41.86	55.15	37.17	16	14	15	4.33	0.17
65	ICC 11886	43	49	51	107	46.41	54.15	27.21	70	68	80	10.08	0.37
66	ICC 11901	40	47	50	104	41.03	47.50	30.78	120	117	135	14.94	0.48
67	ICC 11903	51	60	60	108	41.18	56.05	36.88	48	45	56	9.70	0.25
68	ICC 12121	48	61	59	107	38.14	50.75	31.96	36	33	37	10.31	0.32
69	ICC 12123	54	58	61	105	34.64	51.45	29.72	41	40	44	6.45	0.29
70	ICC 12169	48	56	56	102	27.80	40.55	28.83	82	78	118	11.84	0.52
71	ICC 12184	60	64	67	106	36.23	44.00	24.22	73	69	88	11.97	0.47
72	ICC 12289	49	55	56	102	33.70	42.50	32.42	77	74	143	15.41	0.50
73	ICC 12312	42	48	50	105	33.22	43.05	31.21	77	74	88	13.44	0.42
74	ICC 12511	51	57	59	103	33.22	47.20	28.91	78	71	106	11.74	0.48

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
75	ICC 12554	38	45	46	100	31.94	43.55	27.95	84	81	112	12.25	0.53
76	ICC 12620	37	41	44	98	31.60	43.80	32.02	108	103	148	16.11	0.59
77	ICC 12787	41	47	49	103	34.01	40.60	36.81	102	99	131	16.28	0.51
78	ICC 13941	44	49	50	103	31.75	45.35	29.59	98	94	116	13.43	0.52
79	ICC 14176	37	46	47	106	38.46	44.90	35.72	108	105	150	15.69	0.44
80	ICC 14177	40	47	49	104	35.92	42.95	32.97	101	98	132	14.44	0.55
81	ICC 14179	42	49	52	100	32.40	42.80	28.96	82	77	108	11.43	0.53
82	ICC 14183	51	57	59	106	35.22	44.00	39.41	46	41	49	10.23	0.35
83	ICC 14315	35	39	43	100	31.04	42.20	31.98	84	82	109	11.50	0.56
84	ICC 14406	43	48	49	105	27.84	36.80	37.79	68	66	87	13.21	0.54
85	ICC 14456	46	53	54	107	39.24	44.45	31.99	46	44	52	10.78	0.39
86	ICC 14497	38	43	46	100	30.31	37.15	29.06	90	87	122	12.95	0.59
87	ICC 14533	44	50	52	105	31.92	46.80	47.46	127	119	169	20.76	0.54
88	ICC 14575	51	56	58	102	27.20	44.70	29.66	94	93	130	12.88	0.50
89	ICC 14592	43	50	51	102	29.24	39.25	30.06	86	84	116	13.20	0.49
90	ICC 14674	37	41	44	100	33.27	42.80	28.62	80	78	100	13.98	0.58
91	ICC 14913	38	44	48	101	32.59	43.25	29.33	80	74	96	10.34	0.50
92	ICC 15367	56	62	66	109	40.74	55.15	35.84	36	32	35	11.69	0.32
93	ICC 15380	46	49	53	106	27.86	46.70	48.13	75	71	83	15.91	0.47
94	ICC 15388	49	56	58	109	37.00	49.70	33.74	31	29	33	9.29	0.36
95	ICC 15536	59	64	67	104	33.85	53.80	29.94	69	65	102	12.43	0.49
96	ICC 15779	45	54	54	105	34.71	47.10	31.70	69	64	66	14.67	0.48
97	ICC 15795	59	63	66	109	39.08	52.25	40.78	33	32	33	11.68	0.34
98	ICC 15807	48	55	58	111	34.80	48.55	39.47	41	39	50	13.19	0.43
99	ICC 16141	39	42	47	103	32.13	40.90	35.62	100	93	110	16.38	0.52
100	ICC 16173	37	43	45	106	30.17	44.70	38.12	81	77	93	14.09	0.52
101	ICC 16181	34	40	43	104	28.48	41.45	20.77	84	80	114	13.85	0.64
102	ICC 16216	39	43	46	101	24.77	38.20	24.50	80	78	108	12.09	0.62

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
103	ICC 16219	42	48	52	104	32.64	46.50	34.19	79	75	102	14.59	0.53
104	ICC 16298	46	53	56	104	31.95	44.40	32.26	102	97	110	12.20	0.49
105	ICC 16343	62	65	70	106	37.94	45.75	32.21	73	68	75	14.62	0.5
106	ICC 16376	64	72	72	112	36.89	58.85	32.25	14	10	11	2.62	0.12
107	ICC 16436	62	65	69	107	37.25	50.35	28.24	67	66	93	11.57	0.42
108	ICC 16453	52	59	59	109	41.85	56.00	30.94	25	22	25	6.62	0.29
109	ICC 16528	62	66	68	107	34.51	53.95	36.93	41	39	57	12.29	0.41
110	ICC 16626	60	65	67	110	32.65	56.90	30.12	40	36	45	9.32	0.36
111	ICC 16774	58	62	65	111	43.30	55.15	30.82	18	17	17	6.48	0.22
112	ICC 16820	48	54	58	108	41.24	51.85	34.19	35	33	37	11.44	0.36
113	ICC 16833	44	50	53	99	37.01	35.40	24.77	81	77	99	15.05	0.52
114	ICC 16835	42	48	51	103	27.98	42.75	29.90	68	66	69	14.82	0.62
115	ICC 17083	40	45	47	105	36.89	40.40	27.31	81	80	96	13.07	0.51
116	ICCC 37	36	40	43	102	31.24	36.95	30.75	72	70	90	15.32	0.65
117	ICCL 81248	40	44	49	102	33.05	36.40	29.02	91	88	124	16.05	0.59
118	ICCL 83149	34	40	42	96	34.29	41.40	26.63	73	71	89	16.26	0.64
119	ICCL 87207	41	46	49	103	29.15	36.45	30.17	55	50	57	14.07	0.59
120	ICCV 3	48	55	58	105	36.14	46.60	35.64	65	61	66	18.08	0.49
121	ICCV 88202	35	38	41	102	29.05	35.35	28.39	47	45	69	11.07	0.53
122	ICCV 89314	44	51	52	105	32.13	40.70	37.45	93	88	108	17.72	0.57
123	ICCV 89509	46	52	55	104	42.79	43.20	33.01	102	96	115	18.58	0.51
124	ICCV 90201	54	59	61	103	32.42	48.30	34.27	67	65	91	12.36	0.49
125	ICCV 91302	49	55	57	104	37.23	47.30	24.51	49	47	53	12.92	0.55
126	ICCV 92809	56	63	64	107	32.60	51.90	32.07	46	44	56	8.60	0.40
127	ICCV 92944	34	37	40	93	31.10	35.60	24.98	73	64	72	16.51	0.66
128	ICCV 93512	53	56	60	107	39.47	45.75	39.14	63	61	69	18.16	0.41
129	ICCV 93952	38	42	45	103	33.10	43.00	27.70	75	69	74	15.94	0.54
130	ICCV 93954	33	37	40	100	30.99	38.95	30.83	72	68	72	14.71	0.61

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
131	ICCV 94954	38	43	47	99	36.11	35.90	22.63	59	56	67	14.11	0.59
132	ICCV 95311	41	46	49	105	39.24	43.85	37.87	69	65	68	19.75	0.46
133	ICCV 95332	36	38	43	99	30.29	41.00	32.96	45	42	50	15.14	0.62
134	ICCV 96836	56	60	62	101	43.80	51.20	32.73	96	91	120	16.76	0.48
135	ICCX 820065(GG2)	33	36	41	105	31.20	40.85	32.01	43	39	46	13.37	0.58
136	ICCV 97105	48	56	56	103	36.64	43.40	29.11	68	62	80	13.75	0.53
137	ICCV 96030	27	30	32	91	24.99	35.50	20.49	33	31	38	5.89	0.58
138	ICC 17109	36	40	43	104	33.49	45.30	23.86	24	14	15	7.57	0.35
139	ICCV 07101	39	43	47	103	37.91	43.95	30.68	66	64	90	16.04	0.49
140	ICCV 07102	46	50	52	106	45.33	51.60	28.93	71	69	88	16.83	0.48
141	ICCV 07104	39	44	47	106	34.64	43.00	31.92	75	71	93	17.05	0.56
142	ICCV 07105	42	46	49	103	39.03	46.40	35.79	87	83	119	20.99	0.54
143	ICCV 07108	34	38	42	101	33.61	38.55	36.75	62	57	66	17.77	0.55
144	ICCV 07109	45	49	54	104	38.00	46.70	36.71	86	79	93	20.06	0.52
145	ICCV 07110	34	37	41	102	31.98	44.10	28.82	52	49	62	14.37	0.57
146	ICCV 07113	38	43	47	102	33.43	39.35	33.99	75	72	90	16.66	0.57
147	ICCV 07115	38	43	46	99	29.54	41.15	32.48	48	46	58	10.88	0.51
148	ICCV 07116	38	43	45	100	31.97	36.30	27.02	58	55	64	14.98	0.58
149	ICCV 07117	39	44	46	98	36.21	38.50	30.59	54	53	75	16.41	0.61
150	ICCV 07118	36	39	43	103	29.57	39.60	29.57	60	57	66	16.27	0.65
151	ICCV 06301	50	57	57	108	38.58	51.40	42.52	37	35	38	11.72	0.36
152	ICCV 06302	34	37	41	104	28.14	36.55	29.05	32	32	35	11.59	0.50
153	ICCV 06306	36	38	43	101	27.60	39.75	28.57	35	33	35	10.89	0.51
154	ICCV 07304	34	37	41	103	37.30	44.40	34.59	47	46	58	18.58	0.53
155	ICCV 07311	32	37	40	98	27.75	33.55	22.82	27	25	35	9.39	0.62
156	ICCV 07312	40	44	48	104	30.59	43.70	36.91	44	43	45	14.87	0.51
157	ICCV 07313	39	43	46	97	25.52	39.50	27.12	23	22	23	8.32	0.54

Genotype No	Genotypes	Days to First Flower-ing	Days to 50% Flower-ing	Days to First Podding	Days to Physiolo-gical Maturity	Plant Biomass (g/plant)	Plant height (cm)	Plant width (cm)	No of pods/ plant	No of filled pods/ plant	No of seeds/ plant	Seed yield (g/ plant)	HI
158	ICC 283	46	52	54	103	30.22	41.55	32.00	82	80	94	15.03	0.53
159	ICC 1882	40	43	49	104	28.84	36.45	35.51	114	105	120	16.08	0.56
160	ICC 4958	33	36	40	97	33.12	39.95	33.50	57	53	60	15.62	0.59
161	ICC 8261	47	55	58	108	43.29	48.40	30.52	41	37	42	13.08	0.38
162	ICCV 94916-4	39	46	46	103	33.40	44.60	32.84	42	39	49	13.14	0.52
163	ICCV 94916-8	41	48	49	102	34.28	53.25	42.25	60	55	56	17.17	0.48
164	ICCV 98901	31	35	39	101	31.09	38.35	25.04	86	75	86	16.25	0.64
165	ICCV 98902	36	42	44	104	40.97	42.90	41.95	75	71	75	23.21	0.56
166	ICCV 98903	39	43	45	99	38.48	44.10	35.52	68	60	68	18.22	0.49
167	ICCV 98904	36	39	43	101	37.40	37.80	29.78	71	67	68	18.90	0.57
	Mean of Genotype effect	47	52	55	105	34.93	45.99	31.40	64	61	76	12.59	0.46
	Seasonal effect												
	Normal	49	55	57	108	27.88	50.74	36.5	67.67	64.2	78	13.17	0.46
	Late	46	49	52	102	41.99	41.24	26.3	61.23	57.57	74.7	12	0.45
	LSD(*P*=0.05)												
	Season	(0.6) ***	(0.94) ***	(0.63) ***	(0.58) ***	(0.91) ***	(0.93) ***	(1.31) ***	(3.27) ***	(3.16) ***	(4.2) NS	(0.65) ***	(0.01) ***
	Genotype effect	(5.51) ***	(8.16) ***	(5.73) ***	(5.26) ***	(8.31) ***	(8.5) ***	(11.93) NS	(29.84) ***	(28.91) ***	(38.39) ***	(5.96) ***	(0.09) ***
	Season x Genotype	(7.79) *	(12.18) ***	(8.1) *	(7.44) NS	(11.75) ***	(12.02) ***	(16.87) NS	(42.2) NS	(40.88) NS	(54.29) NS	(8.42) NS	(0.12) ***

Note: Values in parenthesis indicate the LSD at *P*=0.05

Appendix 4. HTI, HSI and Grain yield response of chickpea genotypes under semi-arid environment of India in year 1 (2009-10) and year 2 (2010-11) (Significant difference at ***P<0.001) (- Data not available)

Genotype No	Genotype Name	HTI Year 1	HTI Year 2
1	ICC 982	-0.32	0.05
2	ICC 988	-0.24	-0.47
3	ICC 1017	-0.19	0.94
4	ICC 1025	-0.27	0.47
5	ICC 1026	-0.42	0.17
6	ICC 1163	-0.31	0.68
7	ICC 1164	-0.19	-0.14
8	ICC 1471	-0.22	0.35
9	ICC 2204	-0.43	0.23
10	ICC 2234	-0.29	-0.26
11	ICC 2935	-0.46	-0.41
12	ICC 3335	-0.25	0.00
13	ICC 3336	0.02	-0.08
14	ICC 3337	-0.21	0.73
15	ICC 3429	-0.25	0.22
16	ICC 3485	0.11	-0.11
17	ICC 3867	-0.26	0.04
18	ICC 3935	0.50	0.20
19	ICC 4482	-0.43	-0.02
20	ICC 4861	-0.04	0.38
21	ICC 4902	0.62	0.18
22	ICC 4933	-0.23	0.57
23	ICC 4969	-0.28	0.32
24	ICC 5116	-0.06	0.01
25	ICC 5124	-0.20	-0.90
26	ICC 5186	0.20	0.33
27	ICC5270	-0.29	0.25
28	ICC 5384	-0.26	-0.30
29	ICC 5566	-0.22	-0.92
30	ICC 5794	0.03	0.08
31	ICC 5912	-0.30	-0.51
32	ICC 6152	-0.18	0.65
33	ICC 6169	-	-0.06
34	ICC 6231	-0.22	-0.56
35	ICC 6283	-0.20	-0.10
36	ICC 6293	-0.23	-0.14
37	ICC 6334	-	0.52
38	ICC 6969	0.45	1.25
39	ICC 7192	-0.13	-1.13
40	ICC 7193	-0.19	-1.18
41	ICC 7241	-0.33	0.45
42	ICC 7263	-0.14	-0.71
43	ICC 7292	-0.21	-0.53
44	ICC 7294	-0.21	-0.40
45	ICC 7298	-0.43	-0.42
46	ICC 7308	-0.26	-0.54
47	ICC 7570	-0.30	-0.87
48	ICC 7669	-0.42	-0.39
49	ICC 8166	-0.17	-0.22

Genotype No	Genotype Name	HTI Year 1	HTI Year 2
50	ICC 8273	-0.14	0.18
51	ICC 8397	-0.44	-0.79
52	ICC 8474	0.46	0.05
53	ICC 8527	-0.25	0.43
54	ICC 8943	-0.26	-0.72
55	ICC 9125	-0.15	0.88
56	ICC 9557	-0.26	-0.19
57	ICC 9562	0.33	-0.27
58	ICC 9567	-0.18	0.43
59	ICC 10090	-0.27	-0.47
60	ICC 10134	-0.28	-0.65
61	ICC 10600	-0.39	-1.47
62	ICC 10674	-0.31	-0.89
63	ICC 11553	-0.26	-0.56
64	ICC 11795	-0.19	-0.33
65	ICC 11886	-0.30	-0.81
66	ICC 11901	0.17	-0.37
67	ICC 11903	-0.32	-0.12
68	ICC 12121	-	-0.20
69	ICC 12123	-0.28	-1.16
70	ICC 12169	-0.12	1.05
71	ICC 12184	-	-0.27
72	ICC 12289	-0.12	0.27
73	ICC 12312	0.44	1.33
74	ICC 12511	-0.07	-0.04
75	ICC 12554	-0.01	0.57
76	ICC 12620	0.22	0.10
77	ICC 12787	0.26	0.18
78	ICC 13941	0.40	0.26
79	ICC 14176	0.30	-0.10
80	ICC 14177	-0.12	0.25
81	ICC 14179	0.04	0.07
82	ICC 14183	-0.26	0.10
83	ICC 14315	0.62	0.12
84	ICC 14406	1.06	0.28
85	ICC 14456	0.13	-0.18
86	ICC 14497	0.42	0.21
87	ICC 14533	-0.16	1.70
88	ICC 14575	0.00	0.37
89	ICC 14592	-0.24	0.19
90	ICC 14674	0.17	0.25
91	ICC 14913	-0.09	-0.69
92	ICC 15367	-0.44	-0.34
93	ICC 15380	-0.12	1.13
94	ICC 15388	-0.16	-0.37
95	ICC 15536	-0.28	0.34
96	ICC 15779	-0.27	0.53
97	ICC 15795	-0.24	-0.11
98	ICC 15807	-0.31	0.59
99	ICC 16141	0.48	0.75
100	ICC 16173	0.92	0.33
101	ICC 16181	0.40	0.22
102	ICC 16216	0.58	-0.20

Genotype No	Genotype Name	HTI Year 1	HTI Year 2
103	ICC 16219	0.07	0.70
104	ICC 16298	-0.31	0.01
105	ICC 16343	-0.37	0.36
106	ICC 16376	-0.38	-0.93
107	ICC 16436	-0.34	-0.01
108	ICC 16453	-0.18	-0.49
109	ICC 16528	-0.47	0.38
110	ICC 16626	-0.30	0.54
111	ICC 16774	-0.20	-0.75
112	ICC 16820	-0.27	-0.58
113	ICC 16833	-0.13	-0.57
114	ICC 16835	-0.18	0.07
115	ICC 17083	0.54	-0.57
116	ICCC 37	-0.05	-0.61
117	ICCL 81248	-	0.52
118	ICCL 83149	1.28	-0.54
119	ICCL 87207	0.60	0.28
120	ICCV 3	-0.28	0.54
121	ICCV 88202	0.41	-0.42
122	ICCV 89314	0.61	0.12
123	ICCV 89509	-0.25	0.02
124	ICCV 90201	-0.06	0.91
125	ICCV 91302		-0.91
126	ICCV 92809	-0.33	-0.05
127	ICCV 92944	1.49	0.43
128	ICCV 93512	-0.01	0.51
129	ICCV 93952	0.14	0.13
130	ICCV 93954	-0.06	-0.44
131	ICCV 94954	0.30	-0.55
132	ICCV 95311	0.89	1.19
133	ICCV 95332	0.31	-0.57
134	ICCV 96836	0.23	0.82
135	ICCX 820065(GG2)	0.54	0.34
136	ICCV 97105	-0.05	-0.23
137	ICCV 96030	-0.08	-0.98
138	ICC 17109	-0.07	-0.63
139	ICCV 07101	-0.27	-0.98
140	ICCV 07102	-0.21	0.09
141	ICCV 07104	0.28	0.34
142	ICCV 07105	0.20	0.54
143	ICCV 07108	0.48	0.74
144	ICCV 07109	0.96	0.78
145	ICCV 07110	0.18	0.22
146	ICCV 07113	0.19	0.41
147	ICCV 07115	-0.31	-0.05
148	ICCV 07116	0.03	0.10
149	ICCV 07117	-0.21	-0.43
150	ICCV 07118	0.72	-0.49
151	ICCV 06301	0.21	0.54
152	ICCV 06302	0.56	-0.74
153	ICCV 06306	-0.03	-0.01
154	ICCV 07304	-0.17	-0.62

Genotype No	Genotype Name	HTI Year 1	HTI Year 2
155	ICCV 07311	-0.16	-0.92
156	ICCV 07312	0.64	0.00
157	ICCV 07313	0.47	-0.70
158	ICC 283	0.18	0.33
159	ICC 1882	-0.21	0.71
160	ICC 4958	-0.08	-0.05
161	ICC 8261	-0.25	-0.45
162	ICCV 94916-4	0.08	-0.21
163	ICCV 94916-8	-0.24	0.92
164	ICCV 98901	0.53	0.09
165	ICCV 98902	0.97	1.15
166	ICCV 98903	0.56	0.60
167	ICCV 98904	0.07	0.49
	Genotype effect *LSD*(*P*=0.05)	0.63***	1.2***

Appendix 5. Canopy temperature depression of 160 chickpea genotypes grown in the field under heat stress during 2010 (year 1) (Significant difference at *P<0.05 and NS-Not significant)

Genotype No	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day6
1	ICC 982	1.5	4.5	0.6	4.45	5.55	5.05
2	ICC 988	2.3	3.75	0.75	3.6	5.85	5
3	ICC 1017	3.55	5.9	3.75	8.1	8.75	5.9
4	ICC 1025	2.4	6.55	2.9	9.65	7.45	5.95
5	ICC 1026	0.9	4.25	0.9	5.5	5.6	6.35
6	ICC 1163	0.35	0.25	1.85	3.65	2.8	2.3
7	ICC 1164	2.3	5.85	1.8	5.05	5.15	6.95
8	ICC 1471	1.85	4.35	1.3	2.55	2.65	4.05
9	ICC 2204	1.75	1.9	1.2	3.75	5.15	3.6
10	ICC 2234	2.2	4.25	2.3	8.95	3.55	6.25
11	ICC 2935	0.25	5.35	1.4	4.85	6.75	4.3
12	ICC 3335	1.45	1.85	-1.1	6.05	5.6	4.5
13	ICC 3336	1.4	4.4	2.65	8.75	6.5	6.2
14	ICC 3337	0.5	2.8	2.3	3.25	6.65	5.65
15	ICC 3429	1.55	3.6	2.25	5.9	3.65	5.05
16	ICC 3485	4.35	5.2	4.3	8.45	6.15	5.15
17	ICC 3867	-0.9	-0.65	1.4	3.65	6.95	2.55
18	ICC 3935	1.15	7.15	5.4	7.85	6.7	6.5
19	ICC 4482	1.8	2.5	1.65	4.85	4.45	4
20	ICC 4861	1.5	2.75	4.8	8.5	7.85	5.7
21	ICC 4902	1.55	5.4	0.75	6.9	6.75	5.45
22	ICC 4933	2.65	4.85	3.8	9.7	6.3	7.1
23	ICC 4969	2.85	-1.15	1.9	3.55	4.8	2.6
24	ICC 5116	2.1	1.95	1.65	7.05	9.7	6.3
25	ICC 5124	-0.05	3.35	2.45	1.8	3.3	2.7
26	ICC 5186	0.35	4.75	1.65	6.9	8.45	4.9
27	ICC 5270	-0.15	2.6	0.9	3.9	3.85	3.75
28	ICC 5384	0.8	4.4	2.1	4.8	6.45	3.7
29	ICC 5566	-2.4	1.7	2.35	1.75	2.65	5.35
30	ICC 5794	1.05	2.6	1.3	5.3	5.6	5.35
31	ICC 5912	1.7	4.35	0.35	3.95	8.15	7.7
32	ICC 6152	2.1	1.65	1.6	3.65	5.8	4.05
34	ICC 6231	1.55	5	1.4	6.95	9.4	5.25
35	ICC 6283	2.25	0	1.2	5	6.05	3.1
36	ICC 6293	3.15	4.6	1.35	6.3	7.25	5.05
38	ICC 6969	2.05	4.75	2.65	6.2	5.4	5.1
39	ICC 7192	5.15	8.65	4.5	4.1	7.55	7.35
40	ICC 7193	2.15	2.8	2.9	3.05	4	2.9
41	ICC 7241	1.6	2.35	-0.15	2.25	4.7	3.05
42	ICC 7263	1.45	6.4	3.3	3.1	5.3	2.7
43	ICC 7292	0.85	2.95	4	7.1	4.85	6.35
44	ICC 7294	1.35	3.05	2.2	4.55	3.35	3.9
45	ICC 7298	1.95	2.3	1.75	7.9	6.3	5.25
46	ICC 7308	1.35	3.35	2.1	1.9	5.1	3.6
47	ICC 7570	0.75	5.3	3.4	7	7.35	3.2
48	ICC 7669	1.65	1	1.45	4.7	7.35	5.4
49	ICC 8166	2.35	3.75	2.15	3.3	6.6	5.3
50	ICC 8273	2.8	7.05	2.35	3.9	5.85	4.6
51	ICC 8397	1.2	2.5	1.8	1.7	7.25	3.4

Genotype No	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day6
52	ICC 8474	2.85	5.2	1.95	7.55	8.05	9.45
53	ICC 8527	1.15	1.3	4.2	4.65	7.6	2.55
54	ICC 8943	1.95	2.15	2.2	3.5	3.35	2.65
55	ICC 9125	2.35	4.1	2.65	8.25	7.6	7.3
56	ICC 9557	1.15	3.2	0	4.2	4.05	3.4
57	ICC 9562	-0.4	3.25	2	6.5	4.45	2.5
58	ICC 9567	0.55	6.4	3.25	5.8	6.45	6.6
59	ICC 10090	0.45	2.65	1.95	2.45	5.8	4.85
60	ICC 10134	-0.1	5.4	3.1	6.6	9.2	7.4
61	ICC 10600	1.4	2.8	2.2	4.3	8.55	5.45
62	ICC 10674	0.05	1.8	0.9	1.9	5.75	3.1
63	ICC 11553	2.55	5.25	1.3	3.8	6.1	6.75
64	ICC 11795	3.9	5.4	2.3	3.7	5.5	7.85
65	ICC 11886	2.7	5.45	2.55	5.1	6.45	6.1
66	ICC 11901	2.85	5.15	1.35	3.7	5.2	4.75
67	ICC 11903	1.65	-0.75	1	1.25	3.8	2.7
69	ICC 12123	1.8	1.15	0.45	2.9	7	3.9
70	ICC 12169	2.2	2.15	3.9	6.9	4.45	5.6
72	ICC 12289	4.35	7.1	1.8	7.05	5.65	6.25
74	ICC 12511	2	6.05	0.75	7.9	7.2	6
75	ICC 12554	3.2	3	2.45	5.2	6.35	3.55
76	ICC 12620	2.8	1.65	1.7	4.2	7.3	3.9
77	ICC 12787	2	3.5	1.25	5.9	9.45	4.3
78	ICC 13941	1.35	5.8	2.45	5.4	6.1	5.25
79	ICC 14176	1.9	5.25	1.65	5.85	6.8	4.2
80	ICC 14177	0.65	2.45	1.45	3.55	4	3.85
81	ICC 14179	1.1	5.25	1.05	3.8	4.25	6.65
82	ICC 14183	1.45	1.9	1.45	2.6	5.15	5.5
83	ICC 14315	1.8	1.45	1.8	9	8.9	7.2
84	ICC 14406	1	4.05	4.2	3.6	4.05	1.95
85	ICC 14456	2.25	7.25	2.75	5.6	10.45	8.1
86	ICC 14497	1.1	6.5	2.6	7.4	5.7	7.3
87	ICC 14533	-0.25	2	1.3	4.5	6.4	5.3
88	ICC 14575	1.4	4.85	1.65	4.2	4.7	2.6
89	ICC 14592	0.85	5.8	-0.8	5.15	4.15	5.3
90	ICC 14674	3.1	3.55	2.7	3.15	4.4	4.2
91	ICC 14913	0.55	5.5	3.35	7.1	9.7	7.75
92	ICC 15367	1.95	1.95	0.85	1.5	2.85	2.15
93	ICC 15380	1.7	4.7	1.65	5.95	4.3	4.8
94	ICC 15388	1.75	3.45	2.5	2.1	5.8	5.85
95	ICC 15536	3.05	4.2	3.75	7.75	6.7	6.1
96	ICC 15779	3	3.7	2.2	3.5	7.3	5.65
97	ICC 15795	-0.1	2.95	3.05	2.9	7.1	2.75
98	ICC 15807	2.15	2.95	3.65	2.2	5.7	1.5
99	ICC 16141	1.5	3.3	2.9	5.45	4.25	2.85
100	ICC 16173	2.25	5.35	1.95	9.3	7.4	5.3
101	ICC 16181	2.95	4.55	4.3	4.7	6.15	5.75
102	ICC 16216	0.45	5.7	1.65	9.15	8.15	4.65
103	ICC 16219	2.05	4.25	1.65	5.8	5.6	5.9
104	ICC 16298	2.45	2.5	2.5	2.2	4.3	3.65
105	ICC 16343	2.3	4.85	2.15	7.65	6.65	5.5
106	ICC 16376	1.15	4.8	1.9	4.2	6.35	3.15

Genotype No	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day6
107	ICC 16436	1.6	3.55	3.25	7.45	7.15	7
108	ICC 16453	0.15	3.15	2.1	3.3	3.45	3.75
109	ICC 16528	1.8	1.95	2.1	1.55	4.75	4.1
110	ICC 16626	1.55	7.15	2.6	6.95	8.25	6.9
111	ICC 16774	1.55	3.95	3.25	2.85	5.65	3.55
112	ICC 16820	1.9	6.1	2.7	4.3	8.4	4.05
113	ICC 16833	1	2.15	3.3	4.1	4.5	5.85
114	ICC 16835	3.25	2.25	3	5.1	2.7	3.35
115	ICC 17083	1	6.15	1.75	8.8	7.5	7.05
116	ICCC 37	2.85	4.65	2.15	4.2	4.3	4.75
118	ICCL 83149	2.95	4.15	1.55	8.3	8.25	6.2
119	ICCL 87207	3.45	2.8	2.95	4.7	6.75	5.05
120	ICCV 3	2	-0.95	0.75	3.55	2.85	4.9
121	ICCV 88202	3	5.05	5.4	10.15	10.45	6.4
122	ICCV 89314	1.6	5.6	5.1	7.4	8.3	6.9
123	ICCV 89509	2.1	1.8	2	5.15	6.55	6.45
124	ICCV 90201	2.4	5.85	1.9	3.8	9.5	6.7
126	ICCV 92809	2.65	6.2	3.15	7.2	8.7	6.1
127	ICCV 92944	4.7	6.15	8.15	9.6	10.95	9.3
128	ICCV 93512	1.05	6.45	1.7	6.75	6.8	3.7
129	ICCV 93952	2.3	5.5	2.65	8.2	5.2	7.2
130	ICCV 93954	2.85	1.25	4.35	2.15	4.5	4.25
131	ICCV 94954	1.5	4.35	2.15	5.65	4.3	1.7
132	ICCV 95311	1.9	6.05	3.3	8.45	8.05	6.5
133	ICCV 95332	2.55	3.85	2.55	5.65	7.65	8.85
134	ICCV 96836	1.5	4	3	8.3	8.85	5.5
135	ICCX 820065(GG2)	2.2	4.3	1.9	7.55	8.6	6.4
136	ICCV 97105	1.85	6.2	2.05	8.6	6.05	6.15
137	ICCV 96030	1.85	4.7	4.3	7.2	7	7.05
138	ICC 17109	2.05	4.8	2.2	4.85	6.85	4.3
139	ICCV 07101	2	2.7	0.05	4.9	6.25	5.8
140	ICCV 07102	2.35	3.9	2.2	3.4	4.55	3.6
141	ICCV 07104	2.9	3.7	0.85	6.8	4.35	4.8
142	ICCV 07105	2.65	4.15	1.2	5.15	8.25	6.05
143	ICCV 07108	1.85	4.9	1.55	7.2	7.2	5.6
144	ICCV 07109	4.2	4.45	2.3	8.6	7.3	7.15
145	ICCV 07110	1.65	1.6	1.6	5.45	7.45	4.7
146	ICCV 07113	2.05	2.05	2.6	4.6	6.55	7.1
147	ICCV 07115	2.25	1.2	1.55	5.75	5.75	4.3
148	ICCV 07116	2	1.55	-2.1	5.35	4.8	3.65
149	ICCV 07117	1.4	5.2	-0.65	6.5	3.3	5.45
150	ICCV 07118	1.2	7.45	2.6	8.35	5.75	3.5
151	ICCV 06301	2.55	3.8	1.8	3.8	6.1	3.2
152	ICCV 06302	2.25	6.85	1.5	7	4.15	5.7
153	ICCV 06306	0.8	3.05	1.6	2.85	5.05	2.9
154	ICCV 07304	2.25	0.05	-0.25	2.35	3.75	2.7
155	ICCV 07311	0.95	2.5	0.55	2.55	5.85	4.1
156	ICCV 07312	1.1	5.15	2.3	4.8	6.75	6
157	ICCV 07313	2.15	7.55	2.55	6.7	8.05	5.85
158	ICC 283	0.9	1.6	1.65	4.85	8.45	5.1
159	ICC 1882	0.15	2.3	1.9	2.9	1.65	0.75

Genotype No	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day6
160	ICC 4958	1	2.1	2.95	2.45	2.35	2.85
161	ICC 8261	1.35	3.35	0.4	2.45	4	3.5
162	ICCV 94916-4	0.35	3.8	2.05	10.25	6.95	6
163	ICCV 94916-8	1.1	2.2	0	8.1	4.7	5.15
164	ICCV 98901	1.05	3.7	0.15	10.35	7.25	8
165	ICCV 98902	3.6	2.6	4.75	5	10.25	6.25
166	ICCV 98903	1.95	2.8	3	4.35	5.95	4.9
167	ICCV 98904	1.45	0.45	0.45	3.3	5.2	5.4
	Mean	1.8	3.8	2.1	5.3	6.1	5
	LSD P=0.05	NS	4.6*	NS	4.9**	NS	NS

Appendix 6. Canopy temperature depression of 167 chickpea genotypes grown in the field under heat stress during 2011 (year 2) (Significant difference at ***P<0.001; * P<0.05 and NS-Not significant)

Genotype no	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day 6
1	ICC 982	3.21	4.56	7.09	4.64	4.98	5.59
2	ICC 988	3.03	4.5	6.65	5.13	5.07	4.21
3	ICC 1017	3.56	7.24	9.74	5.54	7.11	8.17
4	ICC 1025	3.44	6.79	8.07	5.76	6.07	7.21
5	ICC 1026	3.56	6.81	8.24	5.93	6.41	8.6
6	ICC 1163	4.92	6.71	7.54	5.77	7.03	7.65
7	ICC 1164	2.1	6.38	8.53	5.85	5.8	8.73
8	ICC 1471	3.92	6.34	7.98	5.69	6.52	7.12
9	ICC 2204	4.02	5.09	7.64	5.12	5.31	6.22
10	ICC 2234	3.19	3.79	6.28	5.01	4.15	6.93
11	ICC 2935	3.35	5.21	7.95	5.84	6.24	7.87
12	ICC 3335	3.89	6.27	8.89	5.04	6.84	7.22
13	ICC 3336	4.69	8.21	10.54	6.69	9.43	9.64
14	ICC 3337	3.28	4.27	6.07	4.46	3.71	4.56
15	ICC 3429	2.91	5.62	8.14	5.32	5.59	7.55
16	ICC 3485	3.44	4.5	6.03	5.18	4.83	6.21
17	ICC 3867	4.17	4.21	4.76	4.08	3.96	5.85
18	ICC 3935	4.99	6.76	8.78	7.34	9	9.6
19	ICC 4482	5.87	7.11	8.58	7.13	7.48	10.64
20	ICC 4861	3.79	5.3	7.17	5.34	5.28	6.08
21	ICC 4902	4.56	6.27	7.59	6.78	7.31	7.6
22	ICC 4933	3.68	6.16	7.18	5.67	7.05	8.86
23	ICC 4969	3.39	4.42	8.07	4.37	5.84	7.88
24	ICC 5116	2.84	5.43	6.14	4.54	3.69	6.31
25	ICC 5124	2.75	4.45	7.69	6.68	6.87	9.3
26	ICC 5186	3.08	4.8	7.08	6.11	6.85	7.14
27	ICC 5270	2.9	5.24	7.04	5.32	4.59	6.76
28	ICC 5384	4.19	7.23	9.45	7.41	7.64	8.57
29	ICC 5566	2.47	3.14	4.99	3.43	2.24	4.74
30	ICC 5794	5.48	7.15	9.48	7.53	7.75	8.57
31	ICC 5912	2.42	4.97	6.21	5.32	5.17	6.32
32	ICC 6152	2.56	4.98	7.48	5.08	4.76	7.06
33	ICC 6169	3.87	4.35	7.14	5.34	5.12	5.19
34	ICC 6231	3.66	4.43	7.9	4	5.05	7.29
35	ICC 6283	4.3	5.45	7.66	4.53	4.55	6.14
36	ICC 6293	5.07	4.89	6.93	7.47	8.42	6.81
37	ICC 6334	3.88	5.76	6.19	5.51	5.82	6.69
38	ICC 6969	5.16	5.31	8.17	6.01	6.1	6.17
39	ICC 7192	2.11	4.37	5.96	3.48	3.72	6.59
40	ICC 7193	3.63	5.28	6.08	6.79	5.74	8.03
41	ICC 7241	3.14	5.97	6.42	5.35	5.28	5.94
42	ICC 7263	3.43	5.98	5.85	4.6	4.46	6.23
43	ICC 7292	4.21	5.59	7.37	6.52	5.57	7.57
44	ICC 7294	2.14	3.38	6.62	5.23	5.32	7.67
45	ICC 7298	4.19	5.35	6.31	7.34	6.57	9.47
46	ICC 7308	3.61	3.44	7.01	4.61	3.89	7.68
47	ICC 7570	3.27	4.08	6.14	4.97	4.48	7.75
48	ICC 7669	2.93	4.65	6.34	5.7	5.67	7.73
49	ICC 8166	1.93	5.24	8.26	5.69	6.86	9.05

Genotype no	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day 6
50	ICC 8273	2.46	6.35	7.87	5.71	6.04	7.35
51	ICC 8397	5.66	5.2	6.1	5.09	4.83	7.31
52	ICC 8474	4.22	7.89	9.22	7.55	7.36	10.71
53	ICC 8527	4.79	6.22	8.09	5.87	5.34	7.18
54	ICC 8943	3.9	5.38	7.01	7.04	6.5	9.82
55	ICC 9125	5.33	5.47	8.46	6.12	6.52	7.55
56	ICC 9557	3.23	5.49	8.33	7.48	5.95	10.11
57	ICC 9562	4.33	6.82	8.62	5.91	8.71	7.14
58	ICC 9567	4.53	5.1	6.36	4.95	5.13	7.03
59	ICC 10090	4.99	6.12	6.04	7.2	6.31	7.44
60	ICC 10134	5.43	5.95	7.77	7.9	7.16	8.98
61	ICC 10600	4.42	4.86	6.8	3.43	3.37	6.01
62	ICC 10674	1.85	4.34	6.39	5.49	4.63	6.65
63	ICC 11553	2.42	4.81	6.62	5.64	5.23	5.9
64	ICC 11795	3.09	5.86	6.15	6.21	6.05	8.11
65	ICC 11886	2.75	3.35	6.99	4.33	3.22	7.24
66	ICC 11901	2.46	5.52	6.71	4.91	5.43	6.68
67	ICC 11903	3.8	6	5.13	4.52	4.18	6.48
68	ICC 12121	3.03	4.21	6.67	5.06	5.15	4.16
69	ICC 12123	4.84	6.87	7.95	6.19	6.62	8.38
70	ICC 12169	3.4	6.91	10.26	6.57	6.66	10.28
71	ICC 12184	1.55	5.39	8.41	4.92	5.26	7.51
72	ICC 12289	5.27	7.43	10.9	7.92	9.01	9.7
73	ICC 12312	4.3	6.69	7.99	6.39	7.68	8.24
74	ICC 12511	4.6	5.01	6.56	5.38	6.2	6.03
75	ICC 12554	4.13	4.73	6.26	5.54	6.01	6.04
76	ICC 12620	3.11	5.88	6.7	6.343	5.91	8.4
77	ICC 12787	4.43	3.71	6.88	5.07	3.04	6.93
78	ICC 13941	5.41	6.03	7.39	6.27	5.93	8.38
79	ICC 14176	3.71	5.45	7.2	5.96	5.71	8.29
80	ICC 14177	4.07	1.38	5.97	4.65	3.18	7.57
81	ICC 14179	3.52	5.67	6.71	5.88	6.46	8.52
82	ICC 14183	4.71	3.17	5.08	5.44	4.04	4.97
83	ICC 14315	5.1	7.2	8.55	7.14	7.94	9.82
84	ICC 14406	4.44	3.81	8.31	5.09	4.99	7.21
85	ICC 14456	4.74	4.23	4.61	3.84	2.42	5.42
86	ICC 14497	3.22	4.71	6.85	5.41	5.7	8.2
87	ICC 14533	3.39	4.28	6.97	4.74	5.24	7.26
88	ICC 14575	2.37	4.28	7.95	5.65	6.23	4.97
89	ICC 14592	3.83	7.08	9.43	6.48	8.45	8.04
90	ICC 14674	4.5	7.05	9.66	5.86	6.88	7.76
91	ICC 14913	6.07	6.46	8.55	7.3	6.55	7.82
92	ICC 15367	4.5	4.89	6.78	8.54	6.31	7.4
93	ICC 15380	4.5	4.37	6.9	4.99	5.07	4.44
94	ICC 15388	4.04	5.21	6.56	6.96	6.58	9.02
95	ICC 15536	3.52	6.4	7.75	5.96	6.28	7.88
96	ICC 15779	3	3.5	6.82	3.78	4.51	5.35
97	ICC 15795	3.29	5.1	6.84	4.82	4.79	5.85
98	ICC 15807	1.47	4.26	7.16	5.21	6.48	6.54
99	ICC 16141	5.27	7.14	8.09	6.38	7.94	10.51
100	ICC 16173	5.16	8.52	10.62	6.98	7.99	7.31
101	ICC 16181	3.53	4.87	7.3	5.05	5.69	6.97

Genotype no	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day 6
102	ICC 16216	2.88	4.55	7.55	5.05	5.86	6.51
103	ICC 16219	4.54	6.5	7.68	5.44	6.18	5.66
104	ICC 16298	4.48	6.75	8.74	6.37	8.48	8.54
105	ICC 16343	4.18	4.24	7.95	4.58	4.78	6.9
106	ICC 16376	2.59	4.08	5.28	4.54	5.25	7.75
107	ICC 16436	3.95	4.29	7.93	5.83	6.27	7.78
108	ICC 16453	4.17	6.4	8.67	7.43	7.22	7.93
109	ICC 16528	3.05	5.57	7.43	5.93	5.65	8.74
110	ICC 16626	2.98	5.1	7.12	6.33	6.65	9.12
111	ICC 16774	4.56	4.8	5.73	4.35	2.83	5.02
112	ICC 16820	3.61	5.1	7.69	5.14	5.69	6.6
113	ICC 16833	6.07	8.62	10.51	6.22	7.32	7.86
114	ICC 16835	2.49	6.25	9.78	7.48	7.31	8.2
115	ICC 17083	3.77	3.95	7.39	4.04	4.64	5.65
116	ICCC 37	3.33	7.67	9.74	5.65	8.68	6.63
117	ICCL 81248	2.83	6.33	8.58	6.29	6.4	8.39
118	ICCL 83149	3.5	4.98	9.38	5.94	6.81	9.13
119	ICCL 87207	4.42	7.39	8.1	5.84	7.46	7.86
120	ICCV 3	3.01	3.17	5.84	4.57	4.82	5.13
121	ICCV 88202	1.78	5.31	7.5	6.82	6.82	6.13
122	ICCV 89314	3.81	4.36	8.93	5.35	5.68	6.78
123	ICCV 89509	2.11	4.74	7.47	6.01	6.84	8.11
124	ICCV 90201	2.43	5.24	6.07	5.65	5.72	5.55
125	ICCV 91302	6.08	6.66	7.82	5.76	5.88	6.35
126	ICCV 92809	4.29	4.75	5.57	3.84	4.44	7.2
127	ICCV 92944	3.04	6.38	7.3	4.71	5.39	6.69
128	ICCV 93512	3.88	4.63	5.94	5.34	4.31	6.12
129	ICCV 93952	3.93	3.8	6.5	4.65	4.94	6.99
130	ICCV 93954	3.48	6.06	8.03	5.35	5.44	7.05
131	ICCV 94954	5.41	5.2	9.81	6.95	7.01	6.28
132	ICCV 95311	3	5.73	9.24	4.84	5.81	6.84
133	ICCV 95332	4.23	4.76	7.8	4.67	5.27	6.99
134	ICCV 96836	4.22	4.76	6.76	5.56	5.92	7.75
135	ICCX 820065(GG2)	2.96	5.29	7.11	4.88	4.84	5.35
136	ICCV 97105	4.21	7.17	8.36	6.43	7.72	7.57
137	ICCV 96030	2.44	4.39	8.54	6.67	6.32	9.13
138	ICC 17109	3.29	3.73	7.39	4.5	4.25	6.72
139	ICCV 07101	1.14	3.81	6.84	4.42	5.04	5.93
140	ICCV 07102	2.95	5.54	6.95	4.22	5.32	6.49
141	ICCV 07104	3.65	5	7.4	5.79	6.19	7.15
142	ICCV 07105	3.47	4.43	7.45	4.62	5.65	6.49
143	ICCV 07108	4.41	5.6	6.9	5.92	6.64	6.66
144	ICCV 07109	3.7	5.26	6.98	5.37	6.48	8.57
145	ICCV 07110	2.61	3.81	8.61	5.76	6.26	5.92
146	ICCV 07113	3.5	5.43	8.87	5.2	5.75	7.2
147	ICCV 07115	2.7	5.51	6.68	5.05	5.18	6.23
148	ICCV 07116	3.05	5.34	7.02	5.86	6.25	7.33
149	ICCV 07117	4.09	6.22	9.29	6.21	6.12	8.81
150	ICCV 07118	5.2	6.55	8	5.97	7.45	7.06
151	ICCV 06301	3.21	4.78	6.74	5.42	4.67	8.74
152	ICCV 06302	3.35	5.76	8.31	6.67	7.28	9.28

Genotype no	Genotype name	CTD Day1	CTD Day2	CTD Day3	CTD Day4	CTD Day5	CTD Day 6
153	ICCV 06306	3.24	3.31	6.91	4.93	4.81	6.62
154	ICCV 07304	3.38	5.21	8.52	5.66	6.06	7.48
155	ICCV 07311	2.41	5.06	7.24	4.56	4.28	7.26
156	ICCV 07312	3.21	3.88	6.77	4.76	4.73	6.87
157	ICCV 07313	5.32	5.94	9.02	6.03	5.99	8.4
158	ICC 283	5.3	7.89	11.59	6.99	9.13	10.8
159	ICC 1882	4.23	6.9	8.85	5.62	6.14	8.47
160	ICC 4958	5.84	7.81	9.74	7.4	7.57	8.61
161	ICC 8261	3.78	6.64	7.92	6.53	6.25	8.15
162	ICCV 94916-4	3.39	5.78	6.06	5.13	4.87	7.55
163	ICCV 94916-8	4.39	4.1	7.67	5.01	5.5	5.69
164	ICCV 98901	3.54	5.36	8.52	5.51	7.62	7.44
165	ICCV 98902	3.41	5.28	5.79	5.53	5.15	5.89
166	ICCV 98903	4.59	5.39	7.9	6.29	7.43	6.29
167	ICCV 98904	4.97	6.22	8.5	6.65	7.25	7.46
	Mean	3.7	5.4	7.5	5.6	5.9	7.3
	LSD P=0.05	NS	NS	2.7***	NS	3.1*	NS

Appendix 7. Temperature for different developmental stages of chickpea during 2009-10 in normal season (year 1)

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
1	28.6	16	28.2	12.4	29.7	15.8
2	28.6	15.7	27.1	17.9	31.2	16.1
3	28.7	16.1	28.2	12.0	30.0	15.9
4	28.6	15.8	27.7	15.3	30.2	15.8
5	28.6	15.8	27.6	16.2	30.3	15.7
6	29	16.6	27.5	12.4	29.1	15.2
7	29	16.5	27.4	13.3	29.3	15.3
8	29	16.6	27.8	13.0	28.2	14.4
9	28.6	16	28	13.1	29.9	15.8
10	28.8	16	27.3	15.0	29.6	15.4
11	28.7	16.1	28.3	12.7	29.7	15.6
12	28.6	16.1	28.2	12.0	29.7	15.7
13	29	16.5	27.1	13.6	29.1	15.1
14	28.9	16.2	26.9	15.3	29.8	15.4
15	28.7	16.1	28.2	12.0	29.9	15.8
16	29.2	17.1	28.4	14.6	28.9	14.8
17	29	16.5	27.5	12.6	29.0	15.1
18	29	16.6	27.6	12.9	28.1	14.6
19	28.6	15.8	27.3	16.6	30.2	15.6
20	29	16.5	27.1	13.8	30.2	15.7
21	29	16.6	27.5	12.4	29.0	15.1
22	28.6	16	28.3	12.6	30.0	15.9
23	28.6	16	28.0	13.6	30.0	15.9
24	28.6	16	28.0	14.6	30.2	15.8
25	28.8	16.1	27.0	16.1	29.6	15.3
26	29	16.7	27.9	13.3	29.0	14.9
27	28.8	16	27.3	14.9	30.0	15.6
28	28.6	16	28.0	14.1	30.5	15.9
29	28.6	16	28.0	13.7	30.7	16.1
30	29	16.6	27.6	12.7	29.1	15.2
31	28.9	16.4	26.9	13.5	30.3	15.8
32	29	16.5	27.2	13.8	29.9	15.5
33	28.8	16.0	27.0	15.5	29.9	15.5
34	28.8	16	27.0	15.5	29.9	15.5
35	28.9	16.4	27.2	12.6	29.3	15.3
36	29	16.5	27.5	12.2	29.0	15.2
37	26.6	15	27.7	16.2	28.2	15
38	29	16.7	27.7	13.0	29.2	15.3
39	28.6	16.1	28.2	12.0	30.2	15.9
40	28.5	15.9	27.9	12.7	31.8	16.4
41	28.8	16	27.3	14.9	29.7	15.5
42	28.9	16.4	26.9	13.5	29.5	15.3
43	28.9	16.3	26.8	14.2	29.9	15.5

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
44	28.6	16.1	27.9	13.3	30.7	16.1
45	28.8	16	27.3	14.9	29.9	15.6
46	29	16.6	27.5	12.4	28.7	14.9
47	28.8	16.1	27.0	14.0	29.6	15.5
48	28.6	15.9	27.9	15.1	30.8	16.0
49	29.1	16.8	28.2	14.9	28.3	14.3
50	28.6	16	28.2	12.4	30.6	16.1
51	28.9	16.5	27.3	12.7	29.3	15.2
52	29.2	17.6	28.6	13.8	27.9	14.2
53	29	16.6	27.6	12.7	28.9	15.0
54	28.8	16	27.3	14.9	29.9	15.6
55	28.7	16.1	28.3	12.7	29.9	15.8
56	28.8	16	27.0	15.5	29.6	15.2
57	29.1	16.9	28.4	14.7	27.9	14.1
58	28.8	16	27.0	15.5	29.5	15.3
59	29	16.6	27.6	12.7	29.3	15.4
60	28.6	16.1	28.2	12.0	29.7	15.8
61	28.6	15.7	27.1	17.9	31.2	15.8
62	29	16.5	27.3	12.2	29.4	15.4
63	29	16.7	27.7	12.9	29.2	15.3
64	28.6	16	28.0	14.1	31.1	16.3
65	29.1	16.9	28.4	14.8	27.7	14.1
66	29.1	16.8	27.9	13.6	28.5	14.7
67	28.9	16.4	26.9	13.5	30.1	15.6
68	26.5	10.6	27.0	16.1	29.8	15.4
69	28.8	16.1	27.2	14.1	29.8	15.6
70	28.9	16.3	26.8	14.2	28.9	14.8
71	26.6	15	28.9	12.3	30.1	15.7
72	28.9	16.2	26.9	15.0	29.5	15.2
73	29.1	16.6	27.7	13.4	28.3	14.6
74	28.9	16.2	27.0	14.6	29.5	15.3
75	29.1	16.9	28.4	14.7	28.4	14.4
76	29.1	16.6	27.8	13.7	28.4	14.6
77	29.1	16.6	27.9	14.4	28.1	14.3
78	29.1	16.6	27.9	14.4	28.1	14.3
79	29.2	17.2	28.4	14.5	28.1	14.3
80	29.1	16.9	28.4	14.8	28.0	14.2
81	29	16.7	27.7	12.9	28.3	14.7
82	28.9	16.4	26.9	13.3	29.6	15.4
83	29.2	17.7	28.7	13.8	28.4	14.5
84	29.1	16.8	28.2	15.0	28.6	14.6
85	28.9	16.2	26.9	15.3	29.5	15.3
86	29.1	16.9	28.5	14.7	28.1	14.2
87	29	16.6	27.5	12.4	28.4	14.7
88	28.8	16	26.4	17.7	29.0	15.0

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
89	29	16.6	27.5	12.8	28.5	14.7
90	29.1	16.9	28.4	14.8	28.4	14.4
91	29	16.7	27.9	13.3	29.3	15.3
92	28.8	16.1	25.4	17.7	29.4	15.3
93	29	16.7	27.9	13.3	29.5	15.4
94	29	16.5	27.4	13.3	28.9	14.9
95	28.6	16.1	28.2	12.0	29.7	15.8
96	28.9	16.3	26.9	14.3	29.5	15.2
97	28.8	16.1	27.1	15.1	29.6	15.4
98	29	16.6	27.6	12.7	29.3	15.4
99	29.1	16.9	28.3	15.0	28.7	14.7
100	29.1	17	28.4	14.7	29.1	15.1
101	29.2	17.2	28.5	14.4	28.7	15.1
102	29.1	16.8	28.2	15.0	28.5	14.5
103	29	16.6	27.5	12.4	28.8	15.0
104	29	16.7	27.7	13.0	29.3	15.4
105	28.7	16.1	28.1	13.4	29.9	15.7
106	28.6	16	28.0	14.6	30.8	16.0
107	28.6	16	28.3	11.8	29.9	15.8
108	28.8	16.1	27.1	15.1	30.0	15.6
109	28.8	16.1	27.2	14.1	29.5	15.4
110	28.8	16	27.3	14.9	29.6	15.5
111	28.9	16.4	26.9	13.5	28.9	14.8
112	29.1	16.9	28.5	14.9	29.4	15.2
113	29	16.5	27.5	12.6	28.0	14.4
114	29.1	16.6	27.9	14.4	28.4	14.5
115	29.1	16.8	28.2	14.9	27.8	14.1
116	29.1	16.8	28.3	14.9	27.8	14.1
117	29.4	14.2	28.0	15.1	28.1	14.2
118	29.1	16.8	28.2	15.0	29.1	15.0
119	29	16.7	27.7	13.0	28.1	14.5
120	29	16.5	27.4	13.1	29.5	15.5
121	29.1	16.9	28.4	14.8	27.8	14.1
122	29	16.7	28.0	14.0	27.9	14.2
123	29	16.6	27.6	12.7	28.5	14.6
124	28.8	16	27.3	14.9	29.2	15.1
125	29.4	14.2	27.9	14.4	29.2	15.2
126	28.6	16.1	28.2	12.0	29.7	15.8
127	29.2	17.5	28.5	13.8	27.6	14.3
128	29	16.6	27.5	12.4	29.2	15.3
129	29.2	17.2	28.5	14.0	28.4	14.5
130	29.2	17.6	28.7	13.7	28.2	14.4
131	29.1	16.8	28.2	15.1	27.8	14.1
132	29.1	16.8	28.2	15.0	28.9	14.8
133	29.2	17.4	28.5	13.9	27.7	14.2

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
134	29.1	16.7	28.2	15.2	29.0	14.9
135	29.4	18.3	28.3	12.7	27.9	14.2
136	28.8	16	27.3	14.9	29.0	14.9
137	29.5	19.6	29.2	14.9	27.8	14.0
138	29.5	18.5	28.2	12.8	28.1	14.3
139	29.1	16.9	28.3	15.0	28.5	14.5
140	29.1	16.8	28.2	15.0	28.3	14.3
141	29.1	16.8	28.2	15.0	28.1	14.2
142	29.1	16.8	28.2	15.1	28.0	14.1
143	29.2	17.1	28.4	14.5	28.9	14.9
144	29.2	17.1	28.4	14.6	28.4	14.5
145	29.2	17.7	28.9	13.8	28.1	14.3
146	29.1	16.8	28.3	14.9	28.2	14.3
147	29.2	17.2	28.6	14.1	28.1	14.3
148	29.1	16.9	28.5	14.7	28.9	14.8
149	29.2	17.2	28.5	14.0	28.1	14.3
150	29.2	17.7	28.6	14.0	27.9	14.2
151	29.2	17.4	28.5	14.0	27.7	14.3
152	29.2	17.2	28.5	14.0	27.9	14.2
153	29.1	17	28.4	14.7	27.7	14.1
154	29.3	18.1	28.5	13.2	29.1	15.0
155	29.3	18.1	28.6	13.2	28.1	14.3
156	29.1	16.8	28.2	15.1	28.1	14.2
157	29.1	16.8	28.3	14.9	27.5	14.2
158	28.9	16.3	26.8	14.0	29.0	15.4
159	29.1	16.7	28.2	15.0	28.6	14.6
160	29.2	17.2	28.4	14.6	29.1	15.5
161	29	15	27.3	12.2	29.6	15.5
162	29.1	16.9	28.4	14.7	27.9	14.1
163	29.1	17	28.4	14.7	27.9	14.1
164	29.3	18.1	28.5	13.6	28.3	14.4
165	29.2	17.5	28.5	13.8	27.9	14.2
166	29.2	17.6	28.6	13.9	27.8	14.2
167	29.2	17.5	28.6	13.8	27.9	14.2

Appendix 8. Temperature for different developmental stages of chickpea during 2009-10 in late season (year 1)

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
2	35.1	19.2	39.8	23.9	39.2	25.0
3	35.1	19.2	39.6	23.7	39.4	25.1
4	35.1	19.2	39.9	24.2	39.5	25.3
6	34.6	18.8	37.6	21.4	40	24.9
7	34.8	19.0	38.7	22.1	40.1	25.3
8	34.5	18.5	37.1	22.8	39.3	24.2
10	34.8	19.0	39	22.6	39.7	25.4
12	35.4	19.5	40.4	25.6	38.9	25.1
13	34.4	18.4	37.3	23.9	39.5	24.7
14	34.5	18.6	37.1	22.2	39.5	24.8
15	35.7	19.9	40.0	25.5	38.9	25.1
16	33.5	17.9	35.9	19.5	38.8	23.7
17	33.7	18.1	36.2	19.2	38.9	23.8
18	34.0	18.2	38.3	22.4	39.4	24.1
20	34.5	18.5	37.1	22.8	39.2	24.3
21	33.8	18.1	37.7	19.7	39.3	24.1
22	35.0	24.1	39.3	22.8	39.8	25.2
23	34.9	19.1	39.0	22.8	39.3	24.9
24	34.6	18.9	38.0	21.2	39.2	24.6
25	35.2	19.4	40.8	25.4	39.6	25.3
26	34.0	18.2	38.6	21.9	39.0	24.1
27	35.1	19.2	40.1	24.4	39.5	25.3
28	35.3	19.4	40.5	25.7	39.3	25.3
29	35.2	19.3	40.5	25.1	39.5	25.3
30	34.3	18.3	37.5	23.3	39.8	24.2
32	34.4	18.4	37.3	23.3	39.1	24.0
34	34.8	19.0	39.0	22.6	39.8	25.5
35	34.6	18.7	37.4	22	39.5	24.9
36	34.5	18.6	36.6	25.1	39.0	24.0
38	33.9	18.2	38.7	20.6	39.3	24.1
43	34.5	18.6	37.1	22.2	39.3	24.4
44	34.9	19.1	39.0	22.6	39.7	25.1
46	34.7	19.0	38.3	21.1	39.4	24.8
47	34.6	18.8	37.4	21.2	39.5	24.9
49	33.9	18.2	38.6	21.3	39.3	24.2
50	34.5	18.5	37.1	23.5	39.1	24.0
52	33.4	17.6	35.7	20.8	38.8	23.2
53	34.5	18.5	37.1	23.5	39.1	24.2
54	34.7	19.0	38.6	21.6	39.7	25.3
55	34.6	18.8	37.6	21.4	39.2	24.5
56	34.6	18.7	37.1	22.0	39.7	24.6
57	33.8	18.1	38.3	20.4	38.8	24.0
58	35.0	19.1	39.3	22.9	39.5	25.3
59	34.5	18.5	37.4	22.3	39.3	24.6
60	35.7	19.9	40.4	26.3	39.5	25.4
63	34.0	18.2	37.9	22.4	39.3	24.2
64	35.0	19.2	39.6	23.7	39.5	25.2
65	34.0	18.2	37.9	22.4	39.4	24.5
66	33.8	18.1	37.8	20.2	39.0	24.0
69	39.0	18.4	37.3	23.3	39.4	24.5
70	34.5	18.6	37.1	22.2	39.5	24.6

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
72	34.3	18.3	37.5	22.7	39.1	24.1
73	33.8	18.1	38.3	20.4	39.0	24.1
74	34.5	18.6	37.1	22.2	39.4	24.7
75	33.7	18.1	35.8	19.2	39.1	23.8
76	33.7	18.0	36.2	19.2	39.2	23.9
77	34.1	18.2	38.6	22.3	39.4	24.2
78	33.7	18.1	37.1	19.5	39.0	23.9
79	33.6	18.0	35.6	19.4	39.2	23.7
80	34.0	18.2	38.8	21.5	39.3	24.3
81	34.1	18.2	38.6	22.3	38.9	24.0
82	34.2	18.3	37.9	23.5	39.0	23.9
83	33.2	17.5	35.9	20.2	38.8	23.2
84	33.8	18.1	38.3	20.4	39.0	24.6
85	34.5	18.5	37.1	23.5	39.2	24.1
86	33.7	18.1	36.2	19.2	39.3	23.7
87	34.5	18.5	37.0	24.2	39.1	24.1
88	34.5	18.6	36.9	22.8	39.5	24.4
89	34.5	18.6	37.1	22.2	39.2	24.4
90	33.7	18.1	37.3	19.6	39.3	24.1
91	33.8	18.1	38.3	21.4	39.1	24.1
93	34.4	18.4	37.3	23.9	39.5	24.1
94	34.6	19.0	37.3	21.9	39.3	24.7
95	35.2	19.3	40.2	24.8	39.0	24.7
97	34.5	18.5	37.8	23.7	39.4	24.3
98	34.7	19.0	38.2	21.0	39.5	25.0
99	33.7	18.0	35.9	19.2	39.3	23.9
100	33.5	17.7	35.5	19.9	38.8	23.6
101	33.3	17.5	35.7	20.3	38.9	23.4
102	33.5	17.8	35.6	19.7	39.1	23.7
103	34.0	18.2	38.6	21.9	39.4	24.2
104	34.6	18.9	38.0	21.2	39.3	24.7
108	34.6	18.9	38.1	21.2	39.3	24.7
111	35.0	19.1	39.6	23.7	39.5	25.2
112	34.6	18.8	37.4	21.2	39.5	24.7
113	34.1	18.2	38.6	22.6	38.8	24.0
114	34.5	18.6	37.3	22.2	39.8	24.3
115	33.8	18.1	38.3	20.4	39.3	24.1
116	33.6	18.0	36.5	19.6	39.0	23.9
118	33.5	17.7	35.4	20.1	39.0	23.5
119	33.5	17.7	35.4	20.1	38.9	23.4
120	33.9	18.2	38.5	21.6	38.9	24.1
121	33.4	17.6	35.5	20.3	38.9	23.3
122	33.8	18.1	38.3	20.4	38.8	24.0
123	34.5	18.5	37.1	22.8	39.2	24.4
124	34.8	19.0	38.9	22.0	39.7	25.2
127	33.1	17.4	36.1	20.6	38.6	23.0
128	33.7	18.1	35.4	19.1	38.9	23.7
129	33.5	17.8	35.5	19.7	39.2	23.6
130	33.4	17.6	35.5	20.1	38.8	23.6
131	33.7	18.1	37.1	19.5	39.0	24.0
132	33.7	18.0	35.9	19.2	39.1	23.8
133	33.3	17.5	35.7	20.3	38.8	23.2
134	33.5	17.7	35.3	20.2	38.6	23.2
135	33.0	17.3	36.2	20.3	38.6	23.0

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
136	34.6	18.7	37.4	22.0	39.3	24.8
137	32.6	17.1	36.6	20.1	38.3	22.7
138	33.5	17.8	35.5	19.8	38.6	23.3
139	32.9	17.3	36.1	20.4	38.5	23.1
140	34.0	18.2	38.9	21.1	39.3	24.1
141	33.8	18.1	37.5	19.7	39.2	24.1
142	33.8	18.1	37.2	19.6	39.2	24.0
143	33.7	18.0	35.9	19.2	39.2	23.7
144	33.5	17.8	35.5	19.7	39.1	23.6
145	33.3	17.5	35.7	20.2	39.0	23.5
146	33.6	18.0	35.5	19.5	39.0	23.5
147	33.7	18.1	36.6	19.4	39.3	24.0
148	33.5	17.8	35.5	19.7	39.0	23.6
149	33.5	17.9	35.6	19.4	39.2	23.7
150	33.3	17.5	35.9	21.0	38.7	23.0
151	33.4	17.6	35.5	20.3	38.9	23.4
152	33.2	17.5	35.8	20.4	38.7	23.1
153	33.4	17.6	35.7	20.8	38.5	23.1
154	33.4	17.6	35.7	19.6	38.5	23.3
155	32.6	17.1	36.5	19.7	38.3	22.7
156	33.8	18.1	37.4	19.6	39.3	24.0
157	33.4	17.6	35.6	21.1	38.7	23.0
158	34.2	18.3	37.6	23.5	39.6	24.1
159	33.5	17.8	35.8	19.7	39.1	24.1
160	33.4	17.6	35.7	20.8	38.6	23.2
161	34.3	18.3	37.6	24.0	39.3	24.1
162	33.8	18.1	38.4	20.7	39.0	24.1
163	34.5	18.6	37.3	22.2	39.3	24.7
164	32.9	17.3	36.1	20.1	38.6	23.0
165	33.9	18.2	38.5	21.6	39.4	24.1
166	32.9	17.3	36.1	20.3	38.6	23.0
167	33.5	17.9	35.9	19.5	39.3	23.9

Appendix 9. Temperature for different developmental stages of chickpea during 2010-11 in normal season (year 2)

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
1	27.9	14.0	29.8	11.4	30.7	14.7
2	28.0	13.9	30.0	11.6	31.1	15.5
3	27.9	14.0	29.9	11.2	30.7	15.0
4	27.9	14.0	29.8	11.4	31.0	15.6
5	28.0	13.9	29.9	11.6	30.9	15.5
6	28.1	15.3	27.2	9.0	30.5	14.2
7	28.1	15.3	26.8	9.2	30.5	13.7
8	28.1	15.3	26.8	9.5	30.4	13.5
9	28.0	13.9	29.9	11.6	30.9	15.5
10	27.9	14.0	29.9	11.3	30.8	15.2
11	27.9	14.1	29.9	11.1	30.7	15.0
12	27.9	14.0	29.8	11.4	30.7	14.9
13	28.0	15.3	27.3	8.6	30.5	14.2
14	28.0	15.2	27.6	7.3	30.5	14.4
15	28.0	13.9	29.9	11.3	30.7	15.1
16	28.2	16.7	27.9	9.3	29.6	12.3
17	28.1	15.3	26.8	10.0	30.3	13.5
18	28.2	15.7	27.1	13.2	30.0	12.8
19	27.9	14.0	29.8	14.0	30.8	15.3
20	28.0	14.9	28.4	8.7	30.8	14.7
21	28.1	15.3	26.9	10.9	30.3	13.1
22	28.0	13.9	29.9	11.3	30.8	15.1
23	28.0	13.9	30.0	11.4	30.8	15.3
24	28.0	14.9	28.1	7.7	30.9	14.7
25	27.9	14.6	28.7	8.6	30.7	14.7
26	28.2	15.6	26.9	13.7	29.9	13.1
27	27.9	14.4	29.3	9.9	31.0	14.9
28	27.9	14.0	29.8	11.4	30.7	15.1
29	27.9	14.2	29.7	10.7	30.9	15.0
30	28.2	15.4	26.7	12.0	30.3	13.2
31	28.0	15.0	27.5	7.3	30.8	14.6
32	28.0	15.0	27.7	7.6	30.6	14.7
33	28.0	15.3	27.0	8.0	30.8	14.5
34	27.9	14.6	28.2	9.5	30.9	15.0
35	28.0	15.3	27.0	8.0	30.6	14.2
36	28.0	15.3	26.7	7.3	30.6	14.4
37	28.0	15.3	27.0	8.0	30.5	14.2
38	28.2	15.5	26.7	12.0	30.2	13.4
39	28.0	13.9	30.1	11.4	30.8	15.6
40	28.0	13.9	29.9	11.5	31.4	15.7
41	27.9	14.6	28.9	9.2	30.9	14.9
42	28.2	15.4	26.9	13.1	30.1	13.3
43	27.9	14.5	29.3	9.8	30.9	15.1
44	27.9	14.0	29.9	11.4	30.9	15.1
45	27.9	14.7	28.5	8.8	30.7	14.8
46	28.2	17.0	27.5	9.5	29.7	13.1
47	28.0	13.9	29.9	11.6	31.3	15.6
48	28.0	13.9	29.9	11.4	31.2	15.2
49	28.2	15.7	27.2	13.3	29.9	12.4
50	27.9	14.4	29.5	10.2	30.8	14.9
51	28.1	15.3	27.0	10.1	30.5	14.1

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
52	28.4	17.8	27.6	8.0	29.5	12.8
53	28.2	15.9	27.2	12.9	30.1	13.2
54	28.0	15.3	26.7	7.3	30.5	14.0
55	27.9	14.0	29.8	11.3	30.7	14.7
56	28.0	15.3	27.3	7.5	30.6	14.5
57	28.2	15.5	26.7	12.0	30.5	13.5
58	28.0	15.2	27.1	6.7	30.5	14.1
59	28.1	15.3	26.9	11.3	30.4	13.8
60	28.0	13.9	30.0	11.4	31.0	15.5
61	28.0	13.8	29.8	11.3	31.2	15.7
62	27.9	14.6	28.7	9.1	31.0	14.9
63	28.2	15.5	26.7	12.3	30.2	13.2
64	28.2	15.5	26.9	13.9	30.4	13.5
65	28.2	15.6	27.0	13.8	30.0	13.1
66	28.2	16.0	27.5	12.5	29.9	13.0
67	28.0	15.0	27.9	8.0	30.6	14.4
68	28.1	15.3	28.0	13.8	30.6	14.5
69	28.0	15.2	27.1	6.7	30.6	14.3
70	28.0	15.3	26.8	8.0	30.5	13.6
71	27.9	14.1	29.8	11.0	30.8	15.3
72	28.0	15.1	26.8	8.0	30.5	13.8
73	28.2	15.6	26.9	12.9	30.1	12.8
74	28.0	15.2	27.6	7.3	30.5	13.7
75	28.2	17.0	27.7	9.1	29.7	13.1
76	28.2	16.5	27.6	10.7	29.7	12.2
77	28.2	15.7	27.2	13.3	29.9	12.9
78	28.2	15.4	27.0	14.4	29.9	12.7
79	28.2	16.0	27.5	12.3	29.8	12.7
80	28.2	16.0	27.5	12.5	29.9	13.0
81	28.2	15.9	27.1	12.3	30.0	12.5
82	28.0	15.3	26.8	9.0	30.5	14.1
83	28.3	17.3	27.6	8.9	29.7	13.2
84	28.2	15.7	27.1	13.1	29.8	12.9
85	28.2	15.5	26.7	12.3	30.1	13.3
86	28.2	16.0	27.4	12.1	29.7	12.4
87	28.2	15.5	26.7	12.3	30.1	13.3
88	28.0	15.0	27.5	7.4	30.5	14.2
89	28.1	15.3	26.8	12.0	30.2	13.0
90	28.1	16.2	27.6	10.6	29.7	12.5
91	28.2	16.7	27.6	11.3	29.9	12.8
92	27.9	14.5	29.4	9.9	31.3	15.5
93	28.0	15.3	26.9	8.7	30.5	14.0
94	28.0	15.3	26.8	9.0	30.7	14.2
95	27.9	14.0	29.9	11.3	30.7	15.0
96	28.2	15.5	26.7	12.3	30.2	13.3
97	27.9	14.5	29.2	9.7	30.9	15.0
98	28.2	15.6	26.8	11.9	30.6	13.8
99	28.2	16.0	27.5	12.5	29.9	12.7
100	28.2	16.7	27.6	9.8	29.7	13.0
101	28.2	17.0	27.9	8.7	29.6	13.1
102	28.1	16.2	27.6	11.2	29.7	12.7
103	28.1	15.3	27.0	10.6	30.4	13.1
104	28.0	15.3	27.3	8.6	30.5	14.2
105	27.9	14.0	29.8	11.3	30.9	15.4

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
106	28.0	13.9	29.8	11.4	31.1	15.7
107	28.0	13.9	29.9	11.6	30.8	15.4
108	28.0	15.3	26.7	7.3	30.6	14.3
109	28.0	13.9	29.9	11.4	30.7	14.7
110	27.9	14.1	29.9	11.1	30.9	15.1
111	27.9	14.5	29.2	9.7	30.9	15.0
112	28.2	15.9	27.0	11.8	30.4	13.7
113	28.2	15.4	26.7	11.7	30.2	13.0
114	28.2	15.5	26.7	12.0	30.2	13.2
115	28.2	15.6	27.0	13.8	29.9	13.0
116	28.2	16.5	27.9	9.7	29.7	13.0
117	28.1	15.7	27.1	13.3	30.0	12.3
118	28.2	16.7	27.9	9.3	29.6	12.5
119	28.2	16.0	27.4	11.0	29.8	13.2
120	28.1	15.3	27.0	10.6	30.4	13.8
121	28.2	17.0	28.1	8.4	29.5	13.0
122	28.1	15.3	26.9	11.3	30.3	13.6
123	28.1	15.3	27.0	10.8	30.3	13.7
124	28.0	15.3	26.8	8.0	30.5	14.0
125	28.1	15.3	27.0	9.0	30.5	14.0
126	27.9	14.7	28.5	8.8	30.6	14.6
127	28.4	17.5	27.7	7.8	29.3	12.0
128	28.0	15.3	27.3	7.5	30.5	14.3
129	28.2	16.0	27.5	12.3	29.8	12.7
130	28.5	18.0	27.1	8.9	29.4	12.2
131	28.2	15.9	27.2	12.8	29.8	12.0
132	28.1	16.2	27.8	11.7	29.8	13.1
133	28.2	16.7	27.9	9.3	29.5	12.0
134	27.9	14.4	29.3	9.9	30.6	14.4
135	28.4	17.5	27.7	8.2	29.7	13.4
136	28.0	15.3	27.0	8.0	30.5	13.7
137	28.8	18.4	27.2	16.2	28.8	11.0
138	28.4	17.8	27.6	8.3	29.6	13.0
139	28.2	16.5	27.6	10.1	29.7	12.4
140	28.1	16.2	27.6	10.6	29.7	12.4
141	28.1	16.2	27.6	10.6	29.7	13.1
142	28.2	15.7	27.1	13.2	29.8	12.4
143	28.4	17.8	27.4	7.7	29.4	12.5
144	28.1	16.2	27.6	12.2	29.8	12.4
145	28.4	17.8	27.4	7.7	29.5	12.8
146	28.2	16.5	27.6	11.7	29.8	12.3
147	28.2	17.0	28.1	8.4	29.4	11.9
148	28.2	16.5	28.2	9.3	29.5	12.5
149	28.1	16.2	27.6	10.7	29.7	12.7
150	28.2	17.0	27.9	8.7	29.6	12.9
151	28.0	15.3	27.0	10.6	30.4	13.9
152	28.3	17.3	27.8	7.6	29.5	13.1
153	28.2	16.7	27.9	9.3	29.6	12.7
154	28.4	17.8	27.6	8.0	29.5	12.8
155	28.5	18.2	27.4	9.4	29.4	12.5
156	28.2	15.9	27.2	12.8	29.8	12.8
157	28.1	16.2	27.8	11.7	29.8	12.6
158	28.0	15.3	27.3	9.0	30.5	14.1
159	28.2	15.6	26.9	12.3	30.2	12.9

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
160	28.5	18.0	27.4	8.3	29.5	12.8
161	28.1	15.3	26.8	12.0	30.5	13.8
162	28.1	16.2	27.6	10.6	29.7	13.2
163	28.1	16.2	27.8	12.0	29.8	13.0
164	28.5	18.0	27.3	8.9	29.5	12.8
165	28.3	17.3	27.4	9.3	29.7	13.0
166	28.2	16.7	27.9	9.3	29.6	12.7
167	28.3	17.3	27.9	8.3	29.5	13.0

Appendix 10. Temperature for different developmental stages of chickpea during 2010-11 in late season (year 2)

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
1	33.3	17.2	36.5	21.2	37.4	24.0
2	33.6	17.4	35.4	22.2	37.6	24.4
3	33.3	17.2	36.5	21.2	37.1	23.8
4	33.5	17.3	35.6	22.0	37.7	24.4
5	33.5	17.3	35.4	22.0	37.5	24.3
6	32.3	16.5	36.3	20.1	36.8	22.7
7	32.9	16.8	35.4	20.1	37.2	23.3
8	32.4	16.5	36.6	20.0	36.8	22.7
9	33.7	17.6	36.1	22.2	37.7	24.5
10	33.4	17.3	35.7	22.0	37.6	24.1
11	33.5	17.3	35.4	22.3	37.5	24.1
12	32.8	16.7	35.5	20.5	37.2	23.2
13	32.3	16.5	35.9	20.0	37.1	22.9
14	32.3	16.5	35.9	19.7	37.4	23.4
15	33.3	17.2	36.5	21.2	37.1	23.8
16	32.0	16.4	34.8	17.2	37.1	22.7
17	32.3	16.4	36.2	18.7	37.0	22.9
18	32.4	16.5	36.3	20.4	36.9	22.7
19	33.3	17.2	36.8	20.2	37.3	24.0
20	32.5	16.5	36.4	20.8	37.1	23.0
21	32.5	16.5	36.4	20.8	36.8	22.6
22	33.4	17.3	35.7	22.0	37.5	24.1
23	33.4	17.3	35.7	21.8	37.8	24.3
24	33.3	17.2	36.5	20.8	37.3	24.1
25	32.1	16.4	35.7	17.5	37.0	22.8
26	33.4	17.2	36.7	21.4	37.4	24.2
27	33.4	17.2	36.1	21.8	37.5	24.2
28	33.7	17.6	36.4	22.7	37.7	24.7
29	32.3	16.5	36.4	19.0	36.9	22.7
30	32.9	16.9	35.8	19.2	37.3	23.8
31	32.9	16.8	35.4	20.1	37.2	23.3
32	32.9	16.8	35.4	20.1	37.1	23.1
33	32.9	16.9	35.9	19.1	37.3	23.8
34	33.0	17.1	37.0	18.4	37.2	23.8
35	33.0	17.1	36.8	18.2	37.3	23.9
36	32.9	16.9	35.6	19.5	37.0	23.1
37	32.0	16.4	35.6	18.0	37.0	22.9
38	33.6	17.4	35.6	22.4	37.6	24.4
39	33.7	17.6	36.1	22.2	37.6	24.6
40	33.2	17.2	37.2	18.7	37.2	23.9
41	32.7	16.6	35.7	20.4	37.1	23.1
42	32.9	16.9	36.0	19.3	37.2	23.8
43	33.0	17.1	36.8	18.2	37.3	24.0
44	33.3	17.2	36.5	21.2	37.5	24.2
45	32.6	16.6	35.7	20.8	37.0	22.8
46	33.5	17.3	35.4	22.0	37.6	24.3
47	33.4	17.3	35.7	22.0	37.5	24.4
48	32.4	16.5	36.6	20.0	36.9	22.8
49	33.0	17.0	35.6	18.9	37.2	23.5
50	33.0	17.1	37.1	18.7	37.2	23.8
51	31.6	16.1	35.2	18.1	36.6	21.8

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
52	32.9	16.9	36.2	20.0	37.1	23.2
53	32.9	16.9	35.6	19.5	37.3	23.5
54	33.0	17.1	37.1	18.7	37.1	23.8
55	33.2	17.2	36.9	19.8	37.3	24.1
56	32.2	16.5	36.1	18.3	36.9	22.8
57	33.0	17.1	36.8	18.2	37.2	23.7
58	32.4	16.5	36.3	20.4	36.8	22.7
59	33.6	17.3	35.3	22.5	37.8	24.5
61	33.3	17.2	36.8	20.2	37.2	24.1
62	32.2	16.5	35.6	19.2	37.1	23.0
63	33.4	17.2	36.5	21.8	37.3	24.1
64	32.3	16.4	36.0	19.9	37.2	23.1
65	32.1	16.5	36.0	18.4	36.9	22.8
66	32.8	16.7	36.0	19.5	37.2	23.8
67	32.7	16.6	35.5	20.8	37.4	23.4
68	33.2	17.2	37.2	19.4	37.1	23.8
69	32.6	16.6	35.7	20.4	37.0	22.9
70	33.4	17.2	36.1	21.8	37.3	23.9
71	32.6	16.6	35.7	20.4	37.0	22.9
72	32.3	16.4	36.2	18.7	37.1	23.1
73	32.9	16.9	35.6	19.5	37.2	23.5
74	32.2	16.5	36.1	18.3	36.7	22.3
75	31.7	16.2	35.1	17.4	36.6	22.1
76	32.2	16.5	36.0	17.7	36.9	22.7
77	32.3	16.4	36.3	18.6	36.9	22.9
78	31.7	16.2	35.3	17.6	37.2	23.0
79	32.2	16.5	36.1	18.3	36.8	22.7
80	32.3	16.5	36.1	20.1	36.7	22.6
81	33.0	17.1	36.9	18.5	37.2	23.8
82	31.7	16.2	35.1	17.4	36.6	21.8
83	32.3	16.5	36.4	19.2	37.1	23.0
84	32.8	16.7	35.6	20.4	37.4	23.5
85	31.8	16.3	34.9	17.2	36.7	22.4
86	32.5	16.5	36.8	20.3	37.0	22.8
87	32.9	16.8	35.4	20.1	37.0	23
88	32.1	16.5	36.0	18.1	36.9	22.7
89	31.7	16.2	35.1	17.4	36.7	22.3
90	32.1	16.4	35.6	17.3	36.7	22.5
91	33.0	17.1	36.8	19.6	37.3	24.0
92	32.2	16.5	36.0	17.7	37.0	22.9
93	32.8	16.7	36.0	19.5	37.4	23.7
94	33.1	17.2	36.8	19.7	37.1	23.8
95	32.7	16.6	35.8	20.3	37.2	23.3
96	33.4	17.2	36.1	21.8	37.5	24.2
97	33.0	17.1	36.9	18.5	37.2	24.1
98	32.0	16.4	34.8	17.1	36.9	22.6
99	32.0	16.4	34.8	17.2	37.1	22.8
100	31.3	16.1	35.3	17.5	36.9	22.5
101	32.1	16.4	35.3	17.2	36.8	22.5
102	32.0	16.4	35.1	17.2	37.1	22.8
103	32.3	16.4	36.0	19.9	36.8	22.7
104	33.5	17.3	35.7	22.0	37.5	24.3
105	33.6	17.4	35.6	22.4	37.7	24.5
106	33.4	17.2	36.1	21.8	37.5	24.1

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
107	33.0	17.1	36.9	18.5	37.3	23.9
108	33.5	17.3	35.4	22.3	37.7	24.3
109	33.3	17.2	36.0	21.4	37.7	24.4
110	33.3	17.2	36.7	20.8	37.3	24.1
111	33.1	17.2	37.2	18.7	37.3	24.0
112	32.4	16.5	36.3	20.4	36.7	22.3
113	32.2	16.5	36.1	18.3	36.9	22.7
114	32.0	16.4	34.8	17.1	36.9	22.6
115	31.7	16.2	35.1	17.4	36.7	22.3
116	32.0	16.4	35.1	17.2	37.0	22.7
117	31.3	16.1	35.3	17.8	36.5	21.6
118	32.3	16.4	35.7	19.7	36.9	22.7
119	32.9	16.9	35.9	19.1	37.2	23.7
120	31.6	16.1	35.2	18.3	36.7	22.0
121	32.2	16.5	36.1	18.3	37.0	22.2
122	32.6	16.6	35.7	20.8	37.1	22.9
123	33.3	17.2	36.8	20.2	37.1	23.9
124	32.9	16.8	35.4	20.1	37.1	23.1
125	33.3	17.2	36.8	19.8	37.5	24.1
126	31.4	16.1	35.4	18.1	36.4	21.6
127	33.0	17.1	37.1	18.7	37.3	23.9
128	31.8	16.3	34.8	17.0	36.9	22.4
129	31.7	16.2	35.1	17.4	36.8	22.3
130	31.8	16.3	34.9	17.2	36.8	22.4
131	32.4	16.5	36.8	19.4	37.0	23.0
132	31.6	16.1	35.2	18.1	36.8	22.2
133	33.0	17.1	36.7	17.7	37.0	23.4
134	31.4	16.1	35.4	18.1	36.7	22.1
135	32.4	16.5	35.9	19.9	37.2	23.1
136	31.0	15.8	34.3	17.5	36.3	21.2
137	32.1	16.4	35.0	17.1	36.9	22.6
138	32.1	16.4	35.9	18.0	37.1	23.1
139	33.0	17.1	36.7	17.7	37.3	24.0
140	32.1	16.5	35.9	18.1	37.1	23.0
141	32.2	16.5	36.1	18.3	37.0	22.9
142	31.7	16.2	34.9	17.5	36.9	22.5
143	33.0	17.0	35.9	18.5	37.3	23.8
144	31.7	16.2	35.1	17.4	36.7	22.3
145	32.0	16.4	34.8	17.2	37.0	22.6
146	32.2	16.5	36.1	18.3	36.7	22.6
147	32.0	16.4	35.1	17.3	36.8	22.5
148	32.1	16.5	35.7	17.3	36.7	22.3
149	32.0	16.4	34.7	16.9	36.9	22.4
150	33.0	17.1	36.1	17.8	37.4	23.9
151	31.6	16.1	35.2	17.7	36.8	22.3
152	31.6	16.1	35.2	17.7	36.7	22.2
153	31.6	16.1	35.2	17.8	36.8	22.2
154	31.7	16.2	35.1	17.4	36.6	21.9
155	32.1	16.4	35.6	17.3	36.9	22.7
156	32.1	16.4	35.0	17.1	36.6	22.0
157	32.3	16.4	36.2	18.7	36.8	22.7
158	32.0	16.4	35.1	17.2	37.0	22.7
159	31.6	16.1	35.2	17.8	36.5	21.8
160	32.8	16.7	36.2	19.7	37.2	23.8

Genotype no	V_{Max}	V_{Min}	F_{Max}	F_{Min}	GF_{Max}	GF_{Min}
161	32.1	16.4	35.6	17.3	36.2	22.6
162	32.3	16.4	36.2	19.5	36.7	22.6
163	31.3	16.1	35.4	17.9	36.6	21.8
164	31.8	16.3	34.8	17.1	37.0	22.6
165	32.1	16.5	35.7	17.3	36.7	22.3
166	32.0	16.4	34.8	17.2	36.7	22.3
167	32.0	16.4	34.8	17.2	36.7	22.3

Appendix 11. Probability values from association analysis of DArT markers and agronomic traits

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Days to first flower	cpPb.679133		3.13
	cpPb.679133		3.16
	cpPb.322806	3.53	
	cpPb.489318	3.95	
	cpPb.679133	3.72	
	cpPb.173309	19.02	
	cpPb.676076	19.02	
	cpPb.676902	18.05	
	cpPb.678344	18.83	
	cpPb.679133	3.54	
	cpPb.682212	19.02	
Days to 50% flower	cpPb.173437		3.1
	cpPb.489318		3.09
	cpPb.679133		3.66
	cpPb.173437	3.03	
	cpPb.679133	3.89	
	cpPb.173309	19.48	
	cpPb.322806	3.28	
	cpPb.489318	3.64	
	cpPb.676076	19.48	
	cpPb.676902	19.44	
	cpPb.678344	19.34	
	cpPb.682212	19.48	
Days to first pod	cpPb.489318		3.1
	cpPb.679133		3.02
	cpPb.679133	3.22	
	cpPb.489318	3.18	
Days to physiological maturity	cpPb.679133		4.72
	cpPb.173437		4.56
	cpPb.323506		3.3
	cpPb.349899		3.12
	cpPb.677529		4.32
	cpPb.679133		4.98
	cpPb.171608	3.04	
	cpPb.172238	3.04	
	cpPb.173309	14.84	
	cpPb.322752	3.04	
	cpPb.489266	3.04	
	cpPb.676076	14.84	
	cpPb.676142	3.05	
	cpPb.676902	15.07	
	cpPb.678344	14.93	
	cpPb.679404	3.04	
	cpPb.680354	3.04	
	cpPb.682212	14.84	
Biomass	cpPb.679142		5.68
	cpPb.680077		3.5
	cpPb.676219	3.38	
	cpPb.682393	3.58	
	cpPb.680737	38.15	

Appendix 11 continued.

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Plant height	cpPb.173435		3.29
	cpPb.323506		6.25
	cpPb.323506		5.08
	cpPb.349899		6.11
	cpPb.349899		5.38
	cpPb.489318		3.08
	cpPb.490834		11.6
	cpPb.676860		5.24
	cpPb.676860		4.74
	cpPb.677529		5.46
	cpPb.677690		4.52
	cpPb.677690		4.7
	cpPb.679216		4.52
	cpPb.679216		4.7
	cpPb.679915		4.23
	cpPb.679915		4.55
	cpPb.680572		3.96
	cpPb.682106		4.52
	cpPb.682106		4.7
	cpPb.172931	7.17	
	cpPb.323506	6.04	
	cpPb.349899	5.4	
	cpPb.676860	4.76	
	cpPb.677529	6.17	
	cpPb.677690	3.32	
	cpPb.677690	4.53	
	cpPb.678752	7.17	
	cpPb.679216	3.32	
	cpPb.679216	4.53	
	cpPb.679915	3.31	
	cpPb.679915	4.17	
	cpPb.680572	3.73	
	cpPb.680572	3.41	
	cpPb.681206	7.44	
	cpPb.682106	4.53	
	cpPb.682683	7.17	
Plant width	cpPb.172290		3.85
	cpPb.323506		5.08
	cpPb.349899		5.38
	cpPb.490834		11.6
	cpPb.491461		3.23
	cpPb.676860		4.74
	cpPb.677690		4.7
	cpPb.679216		4.7
	cpPb.679915		4.55
	cpPb.682106		4.7
	cpPb.322806	3.24	
	cpPb.489318	3.29	

Appendix 11 continued.

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Total no of pods	cpPb.171485		6.69
	cpPb.171485		10.07
	cpPb.172207		5.31
	cpPb.173294		6.7
	cpPb.350553		8.2
	cpPb.488661		6.57
	cpPb.490962		5.65
	cpPb.490962		8.24
	cpPb.491458		8.26
	cpPb.675277		3.69
	cpPb.675905		8.2
	cpPb.676219		4.05
	cpPb.676765		5.13
	cpPb.676765		10.39
	cpPb.677056		6.8
	cpPb.677056		9.01
	cpPb.677249		4.23
	cpPb.677314		4.61
	cpPb.677314		9.82
	cpPb.677672		6.69
	cpPb.677672		10.07
	cpPb.677783		7.91
	cpPb.677783		12.71
	cpPb.677798		6.69
	cpPb.677798		10.07
	cpPb.677822		8.2
	cpPb.679660		5.24
	cpPb.679660		10.06
	cpPb.680218		8.17
	cpPb.682393		4.01
	cpPb.682733		3.6
	cpPb.171485	3.59	
	cpPb.172931	3.48	
	cpPb.173065	3.46	
	cpPb.173294	18.46	
	cpPb.350553	15.77	
	cpPb.350553	12.84	
	cpPb.488661	18.37	
	cpPb.490962	3.26	
	cpPb.491194	3.38	
	cpPb.491458	15.8	
	cpPb.491458	12.95	
	cpPb.675277	23.15	
	cpPb.675277	4.38	
	cpPb.675652	14.88	
	cpPb.675905	15.77	
	cpPb.675905	12.84	
	cpPb.676765	3.32	
	cpPb.677056	3.59	

Appendix 11 continued.

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Total no of pods	cpPb.677490	13.96	
	cpPb.677672	3.59	
	cpPb.677783	5.55	
	cpPb.677798	3.59	
	cpPb.677822	15.77	
	cpPb.677822	12.84	
	cpPb.678752	3.48	
	cpPb.679354	3.53	
	cpPb.679611	3.99	
	cpPb.679660	3.28	
	cpPb.679660	3.31	
	cpPb.680218	15.89	
	cpPb.680218	12.8	
	cpPb.680656	13.96	
	cpPb.680657	13.96	
	cpPb.681126	14.88	
	cpPb.682393	3.1	
	cpPb.682683	3.48	
	cpPb.682733	3.28	
Number of filled pods	cpPb.171485		8.5
	cpPb.171485		11.33
	cpPb.172207		5.29
	cpPb.173294		13.86
	cpPb.350553		7.97
	cpPb.488661		13.73
	cpPb.490962		7.64
	cpPb.490962		9.5
	cpPb.491458		8.03
	cpPb.675277		3.65
	cpPb.675905		7.97
	cpPb.676219		4.05
	cpPb.676765		6.26
	cpPb.676765		11.1
	cpPb.677056		8.58
	cpPb.677056		10.15
	cpPb.677249		4.25
	cpPb.677314		5.84
	cpPb.677314		10.55
	cpPb.677672		8.5
	cpPb.677672		11.33
	cpPb.677783		8.41
	cpPb.677783		13.26
	cpPb.677798		8.5
	cpPb.677798		11.33
	cpPb.677822		7.97
	cpPb.679660		6.3
	cpPb.679660		10.64
	cpPb.680218		7.94
	cpPb.682393		3.97

Appendix 11 continued.

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Number of filled pods	cpPb.682733		3.6
	cpPb.171485	4.94	
	cpPb.172931	3.18	
	cpPb.173065	3.63	
	cpPb.173294	19.42	
	cpPb.350553	17	
	cpPb.488661	19.35	
	cpPb.490962	4.72	
	cpPb.491458	17.15	
	cpPb.675277	14.02	
	cpPb.675277	4.73	
	cpPb.675652	14.43	
	cpPb.675905	17	
	cpPb.676219	3.38	
	cpPb.676606	7.54	
	cpPb.676765	4.4	
	cpPb.677056	4.95	
	cpPb.677314	3.59	
	cpPb.677490	4.29	
	cpPb.677672	4.94	
	cpPb.677783	5.84	
	cpPb.677798	4.94	
	cpPb.677822	17	
	cpPb.678752	3.18	
	cpPb.679354	3.7	
	cpPb.679544	17.71	
	cpPb.679611	3.62	
	cpPb.679660	3.2	
	cpPb.679660	4.38	
	cpPb.680218	16.95	
	cpPb.680276	17.75	
	cpPb.680656	4.29	
	cpPb.680657	4.29	
	cpPb.681126	4.22	
	cpPb.682393	3.34	
	cpPb.682683	3.18	
Number of seeds	cpPb.171485		12.93
	cpPb.171485		15.74
	cpPb.172207		3.68
	cpPb.172207		5.75
	cpPb.173065		3.49
	cpPb.173294		22.59
	cpPb.350553		5.97
	cpPb.488661		22.63
	cpPb.489118		4.43
	cpPb.490632		4.44
	cpPb.490962		11.44
	cpPb.490962		13.74
	cpPb.491458		6

Appendix 11 continued.

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Number of seeds	cpPb.675277		4.43
	cpPb.675905		5.97
	cpPb.676058		5.63
	cpPb.676446		4.41
	cpPb.676729		5.59
	cpPb.676765		6.86
	cpPb.676765		11.91
	cpPb.677056		12.71
	cpPb.677056		13.96
	cpPb.677063		4.43
	cpPb.677314		6.33
	cpPb.677314		10.77
	cpPb.677672		12.93
	cpPb.677672		15.74
	cpPb.677783		9.55
	cpPb.677783		16.17
	cpPb.677798		12.93
	cpPb.677798		15.74
	cpPb.677822		5.97
	cpPb.678269		5.59
	cpPb.678527		3.16
	cpPb.678561		5.71
	cpPb.678785		4.43
	cpPb.679354		3.58
	cpPb.679444		3.16
	cpPb.679623		4.43
	cpPb.679660		7.07
	cpPb.679660		11.61
	cpPb.679806		5.59
	cpPb.680218		5.97
	cpPb.680385		5.67
	cpPb.680501		4.43
	cpPb.680609		4.43
	cpPb.680647		4.43
	cpPb.680770		5.63
	cpPb.681233		5.64
	cpPb.682050		4.43
	cpPb.682120		5.59
	cpPb.682354		3.45
	cpPb.682410		5.63
	cpPb.682533		5.67
	cpPb.171485	8.1	
	cpPb.172931	4.08	
	cpPb.173065	3.87	
	cpPb.350553	7.19	
	cpPb.350553	20.57	
	cpPb.490962	7.9	
	cpPb.491458	7.25	
	cpPb.491458	20.72	

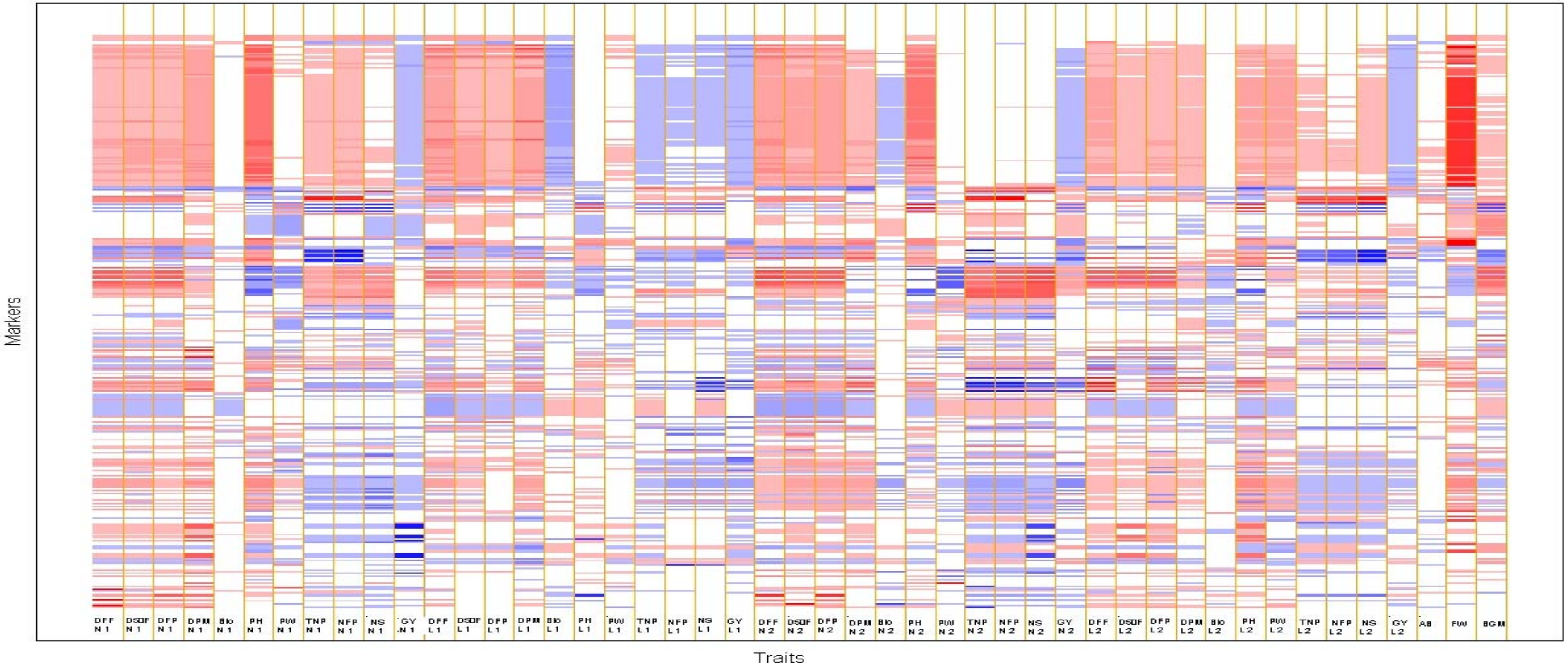
Appendix 11 continued.

Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Number of seeds	cpPb.675277	16.99	
	cpPb.675277	5.95	
	cpPb.675652	18.97	
	cpPb.675905	7.19	
	cpPb.675905	20.57	
	cpPb.676606	5.32	
	cpPb.676765	3.73	
	cpPb.676765	5.67	
	cpPb.677056	7.99	
	cpPb.677314	3.57	
	cpPb.677314	4.73	
	cpPb.677490	3.42	
	cpPb.677672	8.1	
	cpPb.677783	3.42	
	cpPb.677783	7.83	
	cpPb.677798	8.1	
	cpPb.677822	7.19	
	cpPb.677822	20.57	
	cpPb.678752	4.08	
	cpPb.679354	3.95	
	cpPb.679544	43.61	
	cpPb.679660	3.73	
	cpPb.679660	5.56	
	cpPb.679894	17.98	
	cpPb.680218	7.25	
	cpPb.680218	20.53	
	cpPb.680276	43.73	
	cpPb.680656	3.42	
	cpPb.680657	3.42	
	cpPb.681126	3.38	
	cpPb.682683	4.08	
	cpPb.682733	3.06	
Grain yield	cpPb.172145		3.02
	cpPb.350553		4.86
	cpPb.488664		3.18
	cpPb.488820		3.21
	cpPb.489372		3.41
	cpPb.490834		23.65
	cpPb.491458		4.86
	cpPb.675905		4.86
	cpPb.676058		7.64
	cpPb.676219		5.2
	cpPb.676729		7.63
	cpPb.676765		3.5
	cpPb.677783		5.02
	cpPb.677822		4.86
	cpPb.678269		7.63
	cpPb.678561		7.62
	cpPb.679660		3.59

Appendix 11 continued.

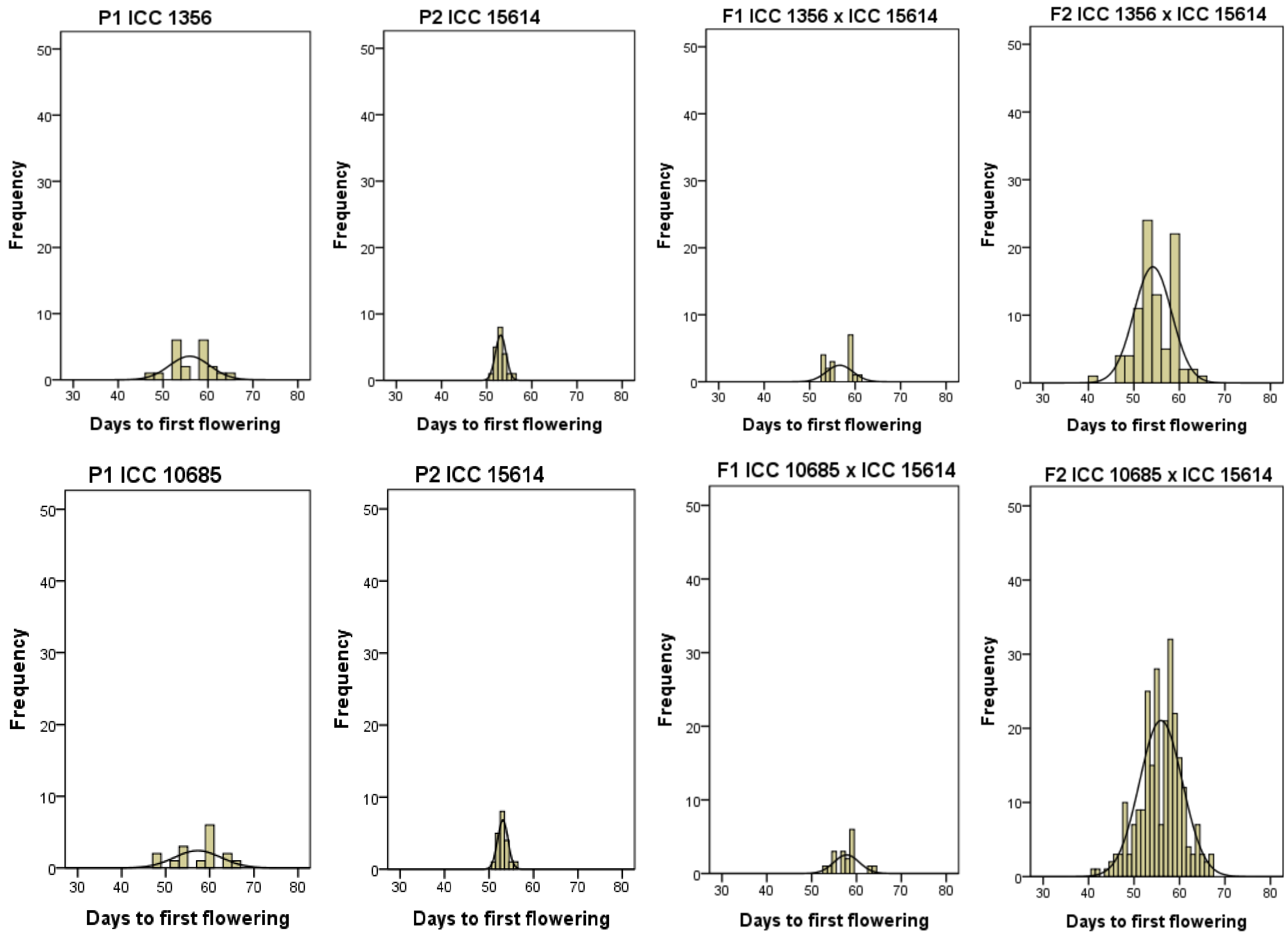
Trait	Marker	Heat stressed (*P* value)	Non-stressed (*P* value)
Grain yield	cpPb.679806		7.63
	cpPb.680218		4.87
	cpPb.680385		7.68
	cpPb.680770		7.63
	cpPb.681233		7.69
	cpPb.682120		7.63
	cpPb.682393		3.03
	cpPb.682393		5.43
	cpPb.682410		7.63
	cpPb.682533		7.69
	cpPb.172052	3.1	
	cpPb.350495	3.21	
	cpPb.350553	13.54	
	cpPb.490210	4.67	
	cpPb.490878	5.14	
	cpPb.491194	5.93	
	cpPb.491458	13.57	
	cpPb.675277	20.43	
	cpPb.675277	3.02	
	cpPb.675905	13.54	
	cpPb.676219	3.38	
	cpPb.676219	3.94	
	cpPb.677490	9.93	
	cpPb.677822	13.54	
	cpPb.678527	3.09	
	cpPb.678546	3.79	
	cpPb.678822	3.65	
	cpPb.679444	3.09	
	cpPb.679544	16.07	
	cpPb.679894	12.61	
	cpPb.680218	13.62	
	cpPb.680276	16.03	
	cpPb.680656	9.93	
	cpPb.680657	9.93	
	cpPb.681126	9.81	
	cpPb.681254	3.6	
	cpPb.681286	3.45	
	cpPb.682393	3.65	
	cpPb.682393	4.2	
	cpPb.682733	5.36	
	cpPb.682733	3.03	

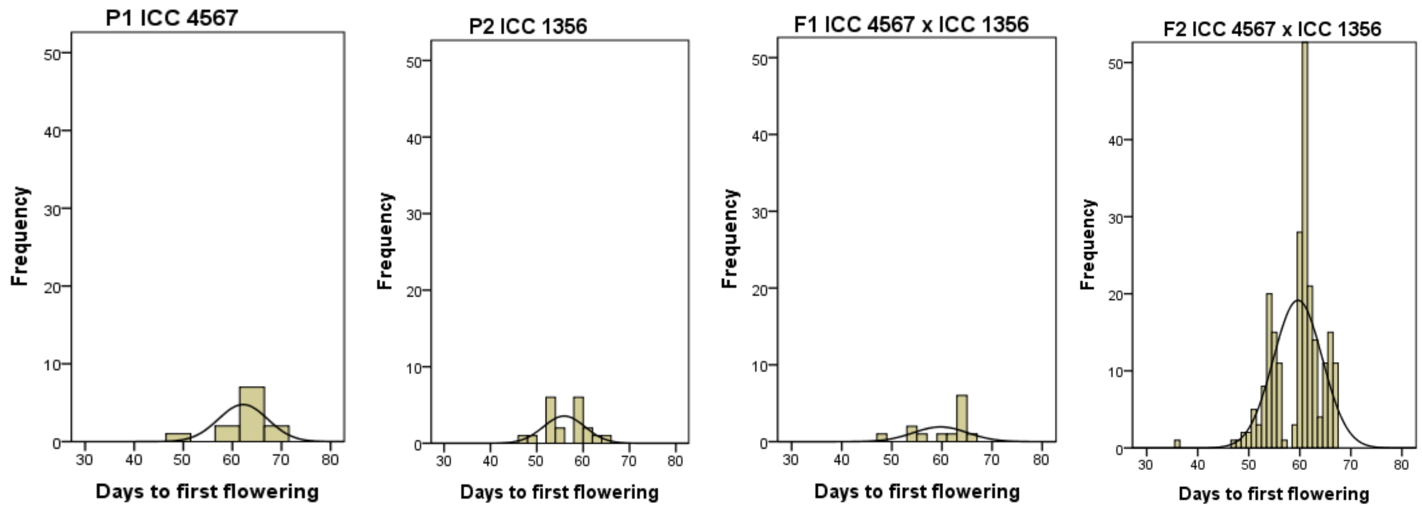
Appendix 12. Marker-trait association of chickpea under heat stressed and non-stressed conditions



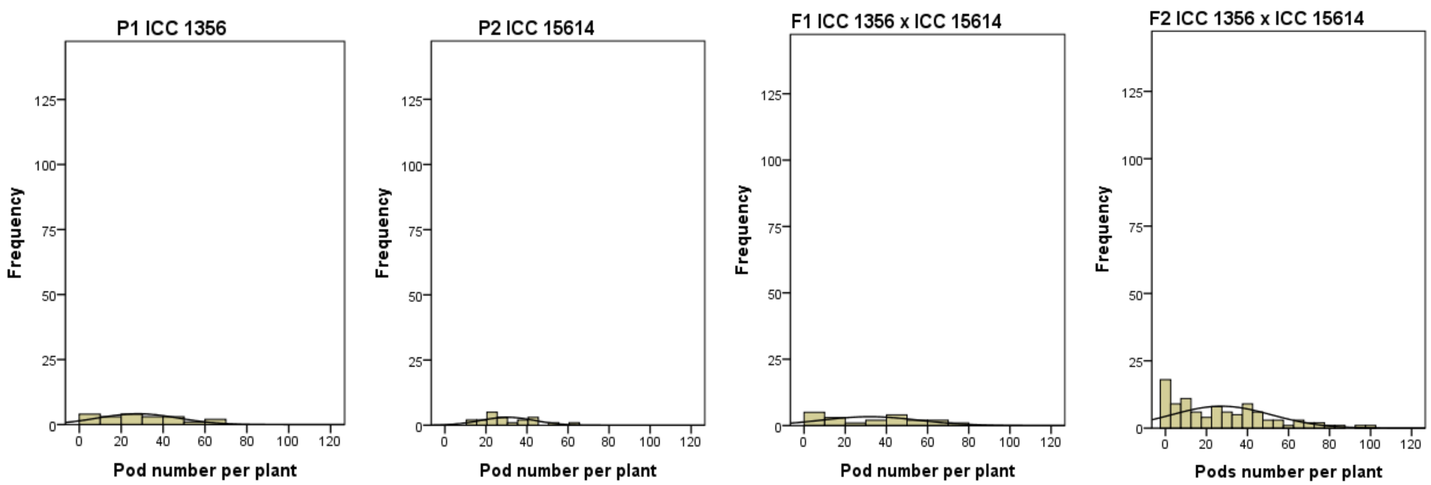
DFF-days to first flower; D50F-days to 50% flower; DFP-days to first pod; DPM-days to physiological maturity; Bio-biomass; PH-plant height; PW-plant width; TNP-total number of pods; NFP-number of filled pods; NS-number of seeds; GY-grain yield; AB-Ascochyta blight; FS-Fusarium wilt and BGM-Botrytis grey mould (N-normal season (non-stressed); L-late season (heat stressed); 1-year1; 2-year2)

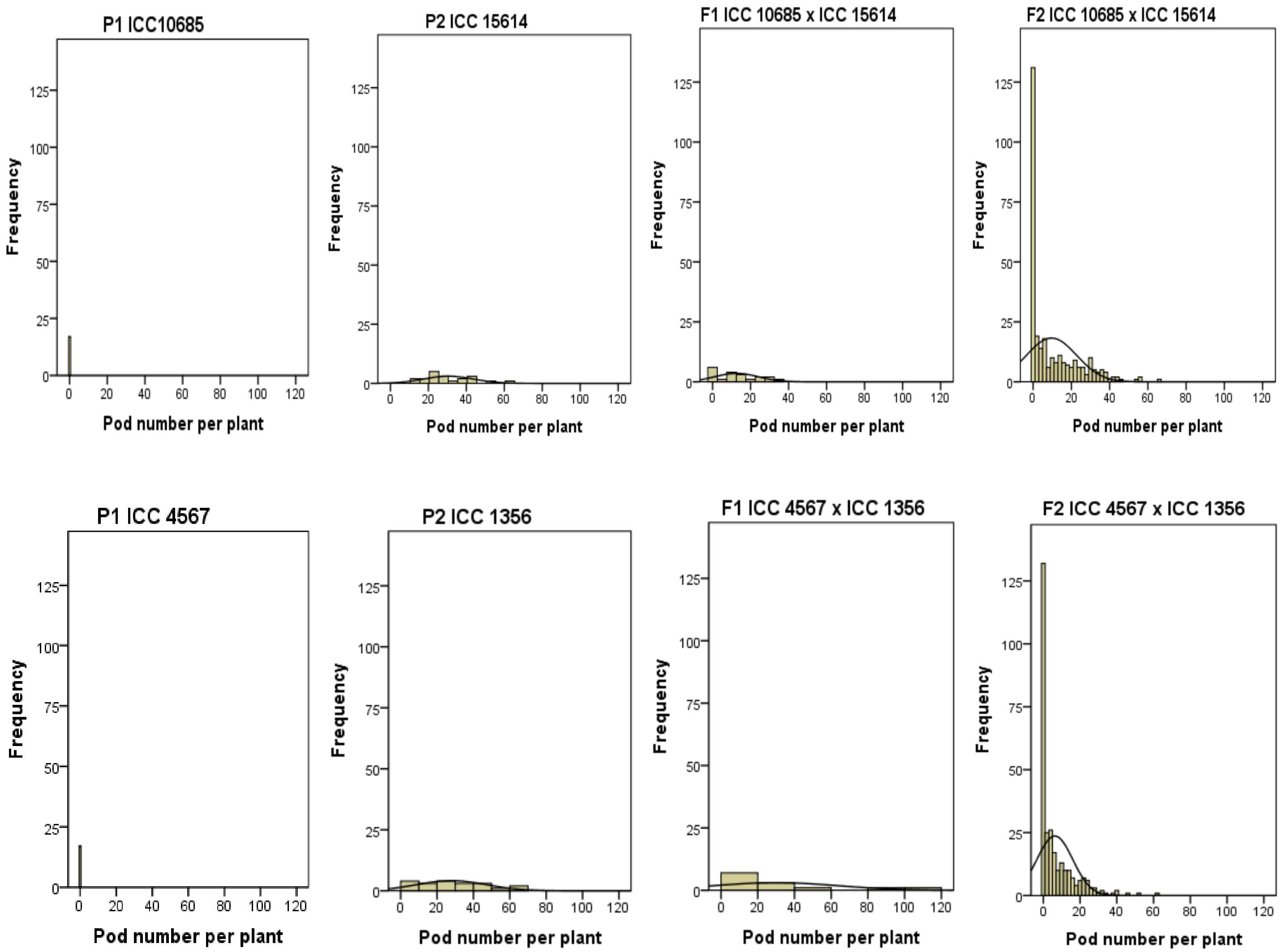
Appendix 13. Frequency distribution of days to first flowering at high temperature





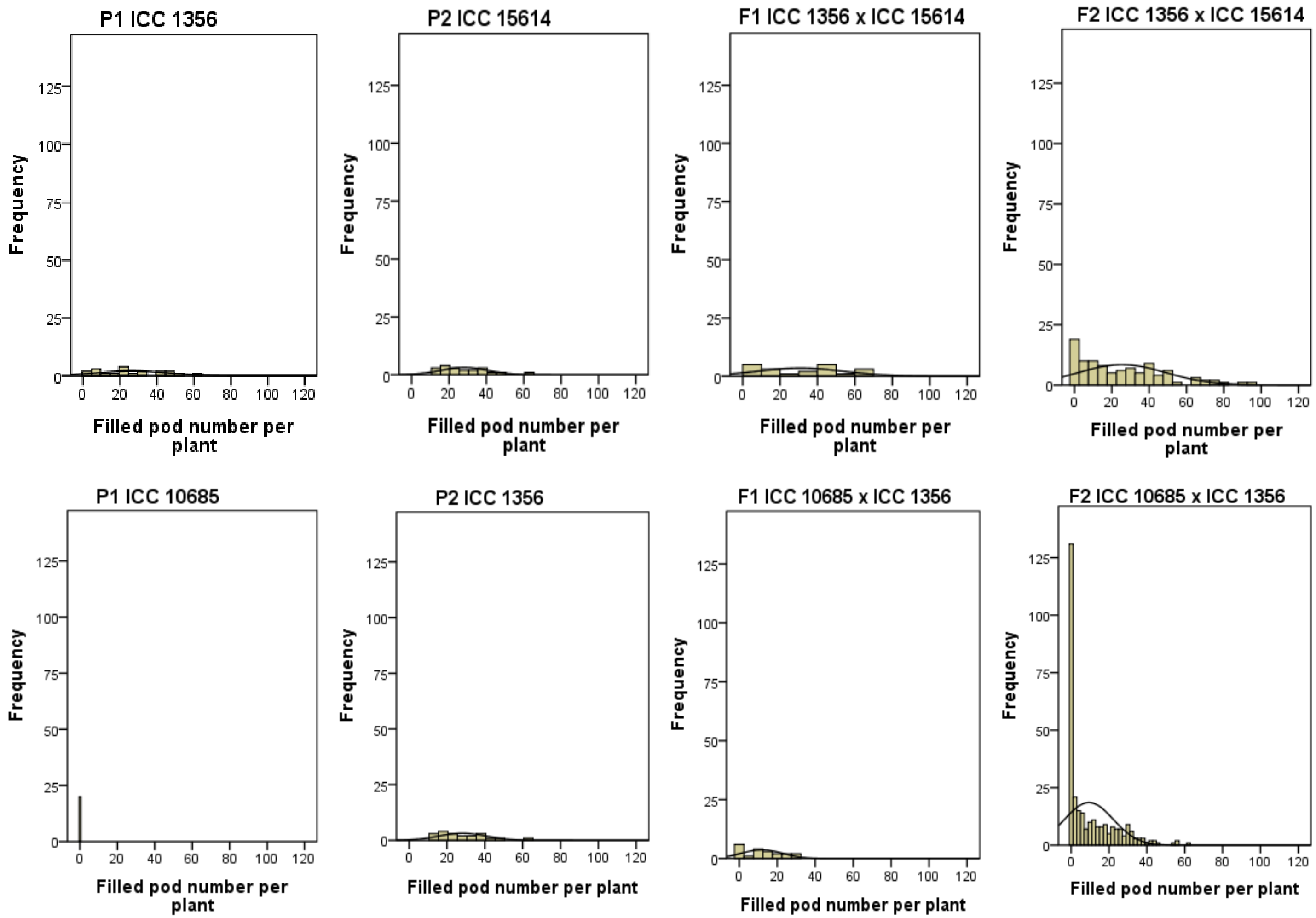
Appendix 14. Frequency distribution of pod number per plant at high temperature

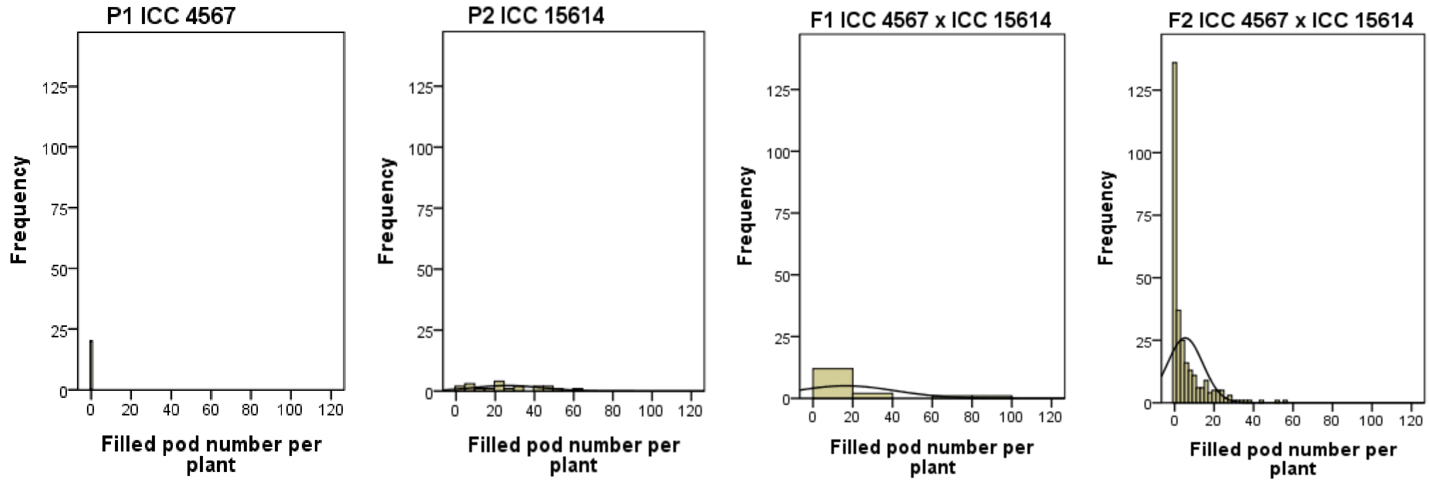




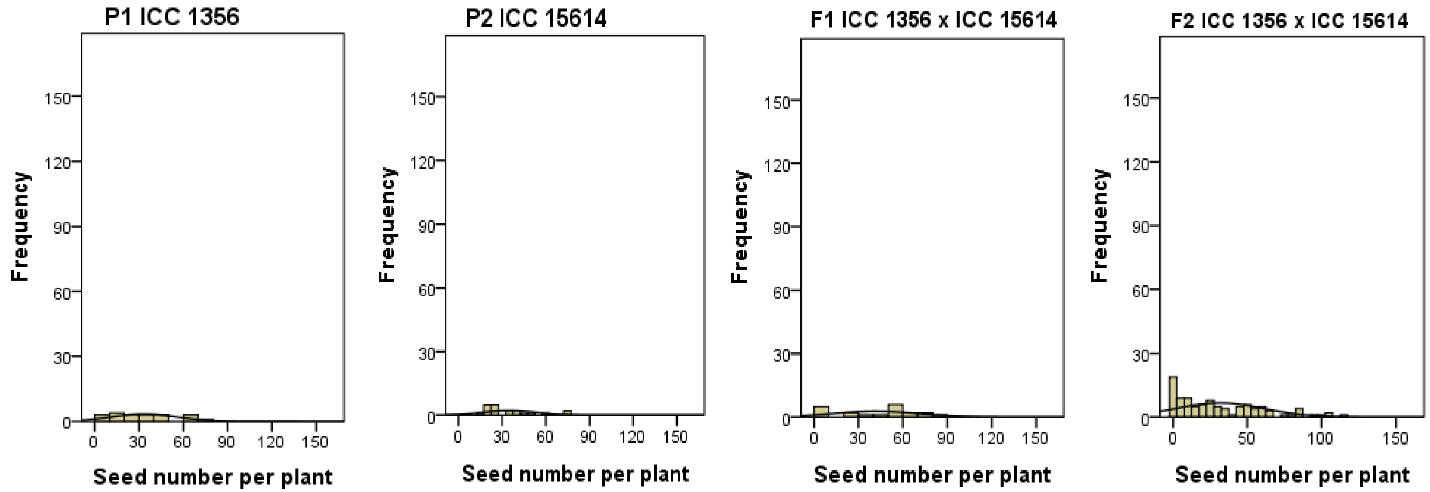
P1 ICC10685
P2 ICC 15614
F1 ICC 10685 x ICC 15614
F2 ICC 10685 x ICC 15614
P1 ICC 4567
P2 ICC 1356
F1 ICC 4567 x ICC 1356
F2 ICC 4567 x ICC 1356
Frequency
Pod number per plant
0
25
50
75
100
125
0
20
40
60
80
100
120

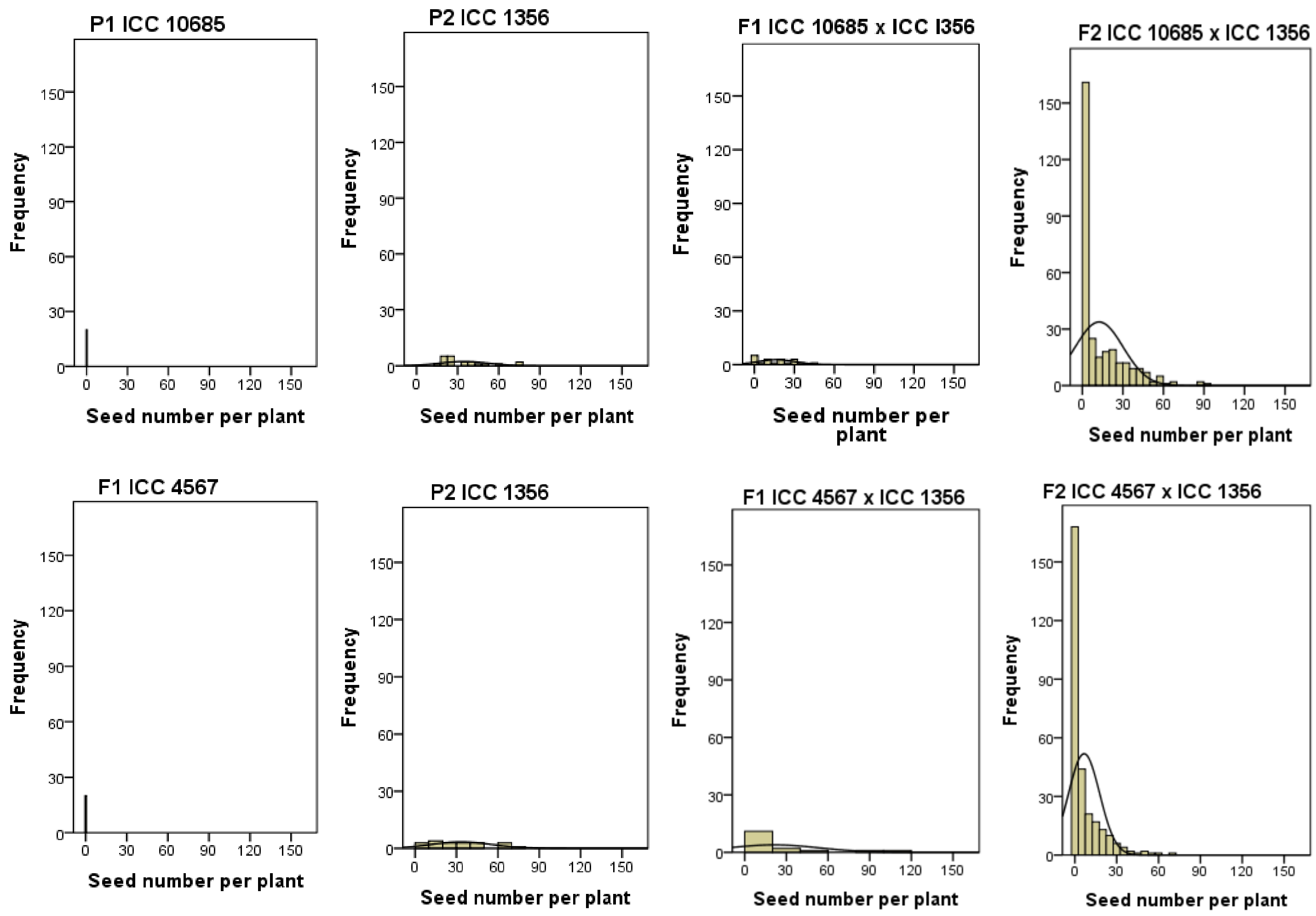
Appendix 15. Frequency distribution of filled pod number per plant at high temperature



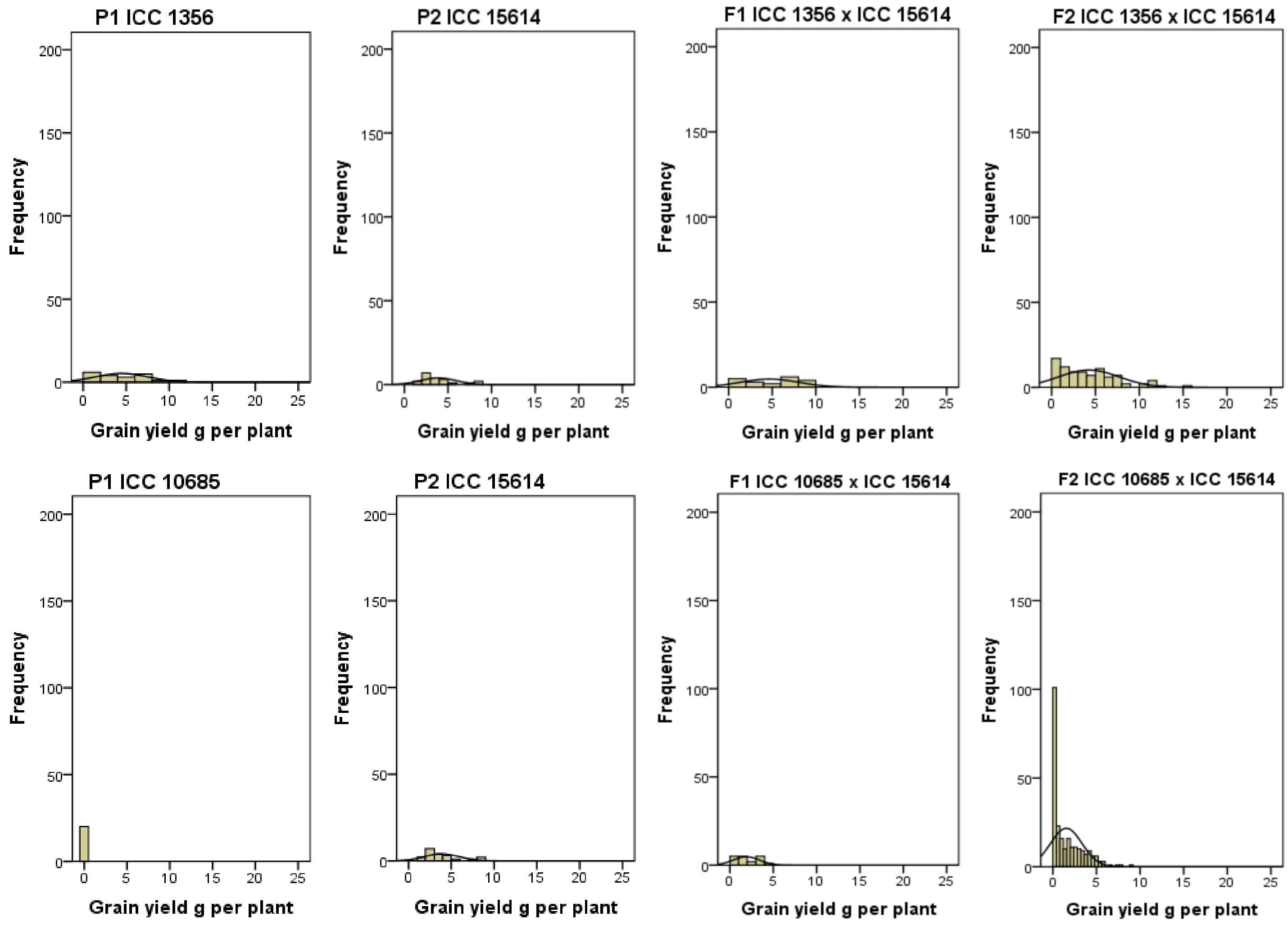


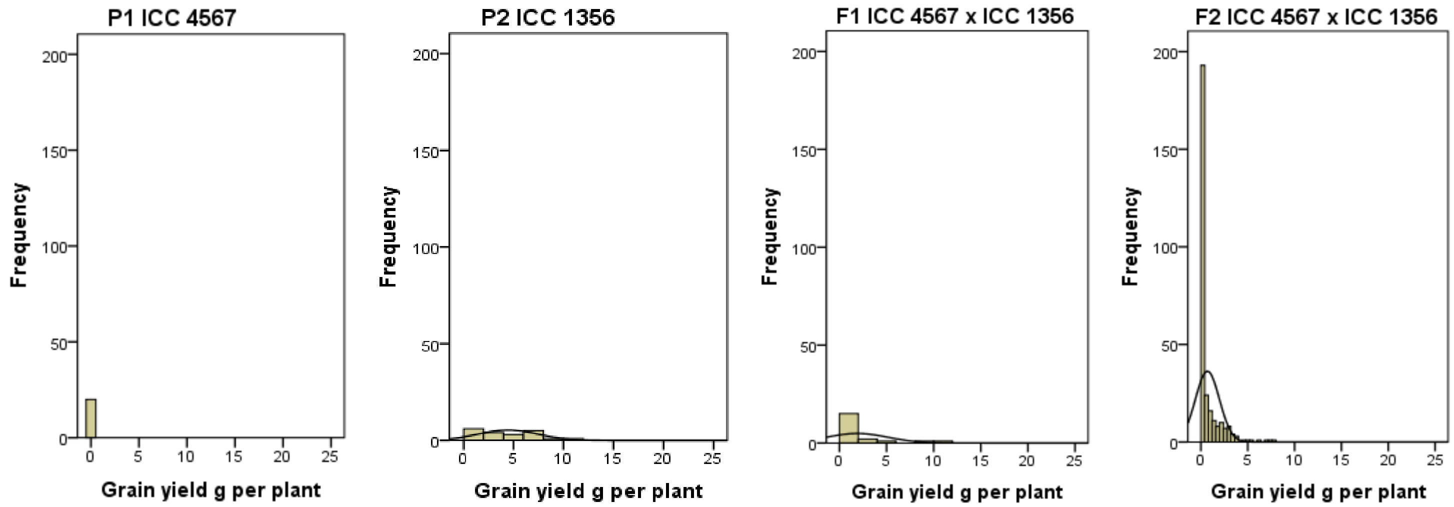
Appendix 16. Frequency distribution of seed number per plant at high temperature




P1 ICC 10685
P2 ICC 1356
F1 ICC 10685 x ICC I356
F2 ICC 10685 x ICC 1356
F1 ICC 4567
P2 ICC 1356
F1 ICC 4567 x ICC 1356
F2 ICC 4567 x ICC 1356
Frequency
Seed number per plant
0
30
60
90
120
150

Appendix 17. Frequency distribution of grain yield per plant at high temperature




P1 ICC 4567
P2 ICC 1356
F1 ICC 4567 x ICC 1356
F2 ICC 4567 x ICC 1356
Frequency
Grain yield g per plant
0
50
100
150
200
5
10
15
20
25