Research Project

Improving Heat Tolerance in Chickpea for Enhancing its Productivity in Warm Growing Conditions and Mitigating Impacts of Climate Change

Technical Report - Year 1

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ICRISAT
Science with a human face

International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Hyderabad 502 324, AP

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CONTENT

1. Project Profile

1.1 Project Title: Improving heat tolerance in chickpea for enhancing its productivity in warm growing conditions and mitigating impact of climate change

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1.6 Project duration: Four years

1.7 Date of start of the project 1 October 2009

1.8 Budget outlay of the project: Rs. 329.65 Lacs

2. Executive Summary and Project Brief

2.1 Executive Summary

Chickpea (*Cicer arietinum* L.) is a cool season food legume and incurs heavy yield losses when exposed to high temperatures (≥35^{0}C) at reproductive stage. Heat stress is increasingly becoming a major constraint to chickpea production in India because of continuing shift in its area from cooler long-season environments (northern India) to warmer short-season environments (southern India), increase in area under late sown conditions due to increasing cropping intensity or late maturity of rainy season crop, and expected increase in overall temperatures due to climate change. Heat stress during flowering and pod development causes severe yield losses. Heat tolerant varieties are needed for improving chickpea yields in warm season environments and late sown conditions, expansion of its cultivation to new niches and improving its resilience to the impacts of climate change.

This project is aimed at refining screening techniques for reproductive stage heat tolerance in chickpea, identifying heat tolerant genotypes, understanding mechanisms and genetics of heat tolerance, identifying molecular markers for gene(s) controlling heat tolerance, identification/development of heat tolerant varieties with desired agronomic background and evaluating heat tolerant varieties/elite lines at farmers' fields.

A late planting technique was standardized for effective screening of chickpea genotypes for reproductive stage heat tolerance. It involves shifting of sowing date from October/November to January/February. The late-sown crop generally experiences >35^{0}C at flowering and pod development stages. Additionally, efforts are underway to standardize a microscopy based pollen viability analysis to screen for heat tolerance. If successful, this could be an additional lab-based tool for heat tolerance screening.

Efforts were made to identify heat tolerant genotypes by screening diverse germplasm/breeding lines for heat tolerance. The reference collection of chickpea germplasm (280 lines) was screened for high temperature tolerance at two locations (Patancheru and Kanpur) by delayed sowing and synchronizing the reproductive phase of the crop with higher temperatures (≥35°C). The results were very exciting as we observed large variations for heat tolerance. Many heat tolerant lines were identified and some of these lines showed no reduction in yield under heat stress conditions. The reference set showed significant variations for heat tolerance index (HTI), phenology, yield and yield components at both the locations. A cluster analysis of the HTI of the two locations yielded five cluster groups: (i) stable tolerant [18 genotypes] (ii) tolerant only at Patancheru [34] (iii) tolerant only at Kanpur [23] (iv) moderately tolerant [120] and (v) stable sensitive [82]. Among the eighteen stable and heat tolerant genotypes identified, six genotypes (ICC 637, ICC 1205, ICC 3362, ICC 14402, ICC 14815 and ICC 15618) yielded higher under stress conditions and also had higher HTI values. Some of the genotypes (e.g. ICC 14778 and ICC 4958) identified as heat tolerant in this study were earlier identified as drought tolerant. This suggests that it is possible to develop cultivars with combined tolerance to terminal drought and heat stresses. The pod number per plant and the harvest index were identified as key traits that can be used in selections for heat tolerance.

Another set of 60 germplasm/breeding lines, including both desi and kabuli types, was evaluated at four locations (ICRISAT, Kanpur, Jabalpur and Nandyal) under normal and late-sown conditions to identify stable heat tolerant genotypes. Observations were recorded on phenological characters, yield components and yield. Based on grain yield under heat stress conditions, several heat tolerant genotypes were identified. The top ten heat tolerant genotypes included 8 breeding lines/released varieties [ICCV 2, ICCV 06302, ICCV 07118, ICCV 07109, ICCV 96970 (JG 16), ICCV 93952 (JAKI 9218), ICCV 92311 (KAK 2) and ICCV 87207 (Vishal)] and two germplasm lines (ICC 8474, ICC 9942). The heat tolerant varieties/breeding lines can be evaluated under late-sown conditions on farmers' fields and used as donors in heat tolerance breeding programs.

Physiological studies on reproductive biology of earlier identified heat tolerant (ICCV 07110 and ICCV 92944) and sensitive (ICC 14183 and ICC 5912) genotypes were carried out at IIPR-Kanpur, PU-Chandigarh and ICRISAT-Patancheru. Flowering time of sensitive genotypes had a higher decrease compared to the tolerant genotypes. Pollen viability, germination and tube growth were all affected by increasing temperatures especially beyond 35^0C. However, pollen viability and pollen germination were fairly high in the two tolerant genotypes (ICCV 07110 and ICCV 92944). A significant drop in pollen tube growth beyond 38^0C was observed, it was ~ 35 µm in sensitive genotypes (ICC 14183 and ICC 5912) compared to ~ 52 µm in the tolerant ones.

Pollen load, viability, germination and stigma receptivity were also analyzed under field conditions for the four genotypes under normal (22^0C, day /10^0C, night) and heat stress conditions (38/25^0C). Among the four genotypes analyzed only the tolerant genotype, ICCV 92944 had higher values and also performed almost identically at the normal and high temperature conditions. Further, yield parameters (biomass, pod number, filled pods, seed weight and harvest index) invariably decreased under stress conditions, but, the two heat tolerant genotypes were characterized by less reduction in yield parameters. Further, they showed lesser membrane damage and better membrane injury index compared to the sensitive genotypes at 38^0C and above. The heat tolerant genotype ICCV 92944 maintained higher chlorophyll content and lower cellular respiration at higher temperatures compared to the remaining three genotypes. The analysis of photosynthesis and fluorescence parameters indicates 20-35^0C to be the best temperature range for photosynthesis in chickpea. Sucrose synthase levels (controls seed size) were higher in the two tolerant chickpea genotypes (ICCV 92944/ICCV 07110) in leaves; and ICCV 07110 had higher in its seed compared to the sensitive genotypes at >35^0C. Three genotypes (tolerant- ICCV 07110/92944; sensitive- ICC 14183) had large seeds, higher sucrose synthase and invertase activity.

Pollen studies conducted at ICRISAT indicated that hot day (≥35º C) and warm night (≥20º C) temperature can potentially reduce the pollen viability and subsequent pod set in heat sensitive chickpea genotypes. Pollen grains were completely and partially fertile at 35/20ºC and 40/20ºC respectively in heat tolerant genotype ICCV 92944. Additionally, pollen germination and pollen tube growth were noticed in tolerant type at 35/20ºC whereas, the sensitive types did not show any. Further, sensitive genotype (ICC 5912) showed reduced pod set at 35/20ºC compared to the tolerant one (ICCV 92944).

Eleven crosses were made at ICRISAT to understand the genetics of heat tolerance. These included sensitive x tolerant, tolerant x tolerant and sensitive x sensitive crosses. At least one sensitive x tolerant cross will be used for development of recombinant inbred lines (RILs) and molecular mapping of heat tolerance gene(s). We will also use the approach of association mapping for identifying molecular markers for heat tolerance. Genotypic data is already available on the reference set of chickpea screened for heat tolerance under this project. These data sets are being used for association mapping.

Two heat tolerant genotypes (ICCV 92944 and ICCV 07110) were crossed with leading cultivars to introgress the heat tolerant trait. The crosses were performed at ICRISAT-Patancheru, IIPR-Kanpur, JNKVV-Jabalpur and RARS-Nandyal. Further ICCV 92944 (JG 14), an early maturing heat tolerant desi chickpea variety was evaluated at farmers' fields at four locations in Fatehpur and Ramabai Nagar districts of Uttar Pradesh. The average grain yield of 1720 kg/ha and 1672 kg/ha was obtained in Fatehpur and Ramabai Nagar districts, respectively. These results indicate that heat tolerant chickpea varieties like JG 14 will be very useful for late-sown chickpea and provide an opportunity of bringing chickpea in cereal dominated (mainly rice-wheat) cropping system, which is very much desired for improving soil fertility and long term sustainability of the cropping system.

2.2 Project goal, objectives and major activities

2.2.1 Project goal: The overall goal of the project is to enhance chickpea production in warm growing conditions by developing heat tolerant and climate-resilient cultivars.

2.3.1 Key objectives:

- Refine techniques for effective screening of heat tolerance at reproductive stage.
- Identify chickpea genotypes with reproductive stage heat tolerance.
- Understand mechanisms and genetics of heat tolerance.
- Identify molecular markers for gene(s) controlling heat tolerance.
- Introgress heat tolerance in selected cultivars/elite breeding lines.
- Evaluate selected heat tolerant lines at farmers fields.

2.3.2 Major activities

- Standardization of techniques for large scale screening of chickpea germplasm for heat stress at reproductive stage. This may involve study of pollen viability and pollen tube growth, pod filling, cell membrane thermo-stability, chlorophyll fluorescence and canopy thermal imagery.

- Screening of a set of genotypes (germplasm, cultivars and breeding lines) at multiple locations for their ability to flower and set pods at high temperatures (March-May).
- Crossing of heat tolerant germplasm with improved cultivars/elite lines for incorporating heat tolerance trait in suitable agronomic background.
- Study genetics of heat tolerance.
- Development of recombinant inbred lines (RILs) from a cross involving the most contrasting heat tolerant and heat sensitive genotypes for mapping heat tolerance gene(s).
- Rapid advancement of RILs and breeding materials by taking 2-3 generations per year.
- Identification of molecular markers for heat tolerance gene(s).
- Screening of segregating material under late sown conditions for establishing lines with enhanced heat tolerance and improved agronomic traits.
- Evaluation of selected heat tolerant lines at farmers fields.

3. Objective-wise Technical Report

(1 October 2001 to 30 September 2010)

3.1 Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop globally, with a production of 9.8 m t from an area of 11.1 m ha (FAO STAT, 2009). It is even more important for India as the country's production accounts for 67% of the global chickpea production and chickpea constitutes about 40% of India's total pulse production. In spite of India being the largest chickpea producing country, a deficit exists in domestic production and demand which is met through imports.

Chickpea is a winter-season crop and often experiences increasing high temperature stress with advancing stages of crop growth. During the past three decades, there has been a significant shift in the growing environment of chickpea in India from the cooler, long-season environments of northern India to the warmer, short-season environments of central and southern India (Gaur et al., 2008; Gowda et al., 2009). Terminal drought and heat stresses are major constraints to chickpea production in warmer short-season environments. Also, the chickpea area under late-sown conditions is increasing, particularly in northern and central India, due to inclusion of chickpea in new cropping systems and intense sequential cropping practices. Heat stress during the reproductive period is a major limitation in this situation too. It is also estimated that about 11.7 million ha of rice area in India, currently remains fallow after late harvest of rice during the winter season in the central and north-eastern India (Subbarao et al. 2001). These lands potentially offer expansion in chickpea cultivation provided genotypes capable of standing heat stress are made available. Finally, heat stress is expected to be an increasingly important constraint in near future due to climate change and global warming. By 2050, a rise in temperature by at least 2^0C, particularly the night temperatures, is being predicted with higher levels of warming in northern parts of India. It can be envisaged that the increases in temperature will have more adverse effects on cool-season crops (e.g. chickpea) than the rainy-season crops (Kumar, 2006). So, there is an urgent need to search the gene bank for diverse sources of heat tolerance. However, no such systematic search had been taken up in chickpea except for a limited effort with 25 diverse genotypes leading to the identification of two genotypes, ICCV 88512 and ICCV 88513, to have heat tolerance at reproductive stage (Dua, 2001).

Flowering and podding in chickpea is known to be very sensitive to changes in external environment, and exposure to heat stress at this stage leads to reduction in seed yield (Summerfield et al., 1984). Drastic reductions in chickpea seed yields were observed when plants at flowering and pod development stages were exposed to high (35^0C) temperatures (Summerfield et al., 1984, Wang et al., 2006). Heat stress is known to adversely affect pollen viability, fertilization and seed development leading to a reduced harvest index. Yet, it is still not clear how heat affects the growth and development of chickpea and whether that can explain part of the differences in seed yield under heat stress. So, a pre-requisite, before undertaking a more thorough physiological analysis of the traits involved in heat stress tolerance, is the identification of heat tolerant genotypes.

Also there is an urgent need to develop simple and effective screening techniques for screening germplasm and breeding materials for reproductive stage heat tolerance in chickpea.

3.2 Objective 1: Refine techniques for effective screening of heat tolerance at reproductive stage.

ICRISAT recently developed a rapid generation advancement method for chickpea in which two generations are taken in field, the first crop sown in Sep/Oct and the second crop in Jan/Feb immediately after the harvest of the first crop. As the second crop faces relatively high temperatures at the reproductive stage, it was proposed that Jan/Feb sowing can be used for screening of chickpea genotypes for high temperature tolerance (Gaur et al. 2007).

We further refined this delayed-sowing method for screening reproductive stage heat tolerance in chickpea. We studied long-term weather data from all locations and decided suitable sowing dates for each location to coincide high temperatures ($\geq 35^0$C) at the reproductive stage. This delayed-sowing method was used at four locations (ICRISAT-Patancheru, RARS-Nandyal, JNKVV-Jabalpur and IIPR-Kanpur) and was found very effective in screening for reproductive stage heat tolerance in chickpea. The Fig. 1 shows the range of temperatures the crop encounters during the regular (winter) and the summer season at Patancheru. It is very apparent from Fig.1 that during summer planting the crop faces high temperatures especially during its reproductive phase allowing the breeders to effectively screen for heat tolerance under field conditions.

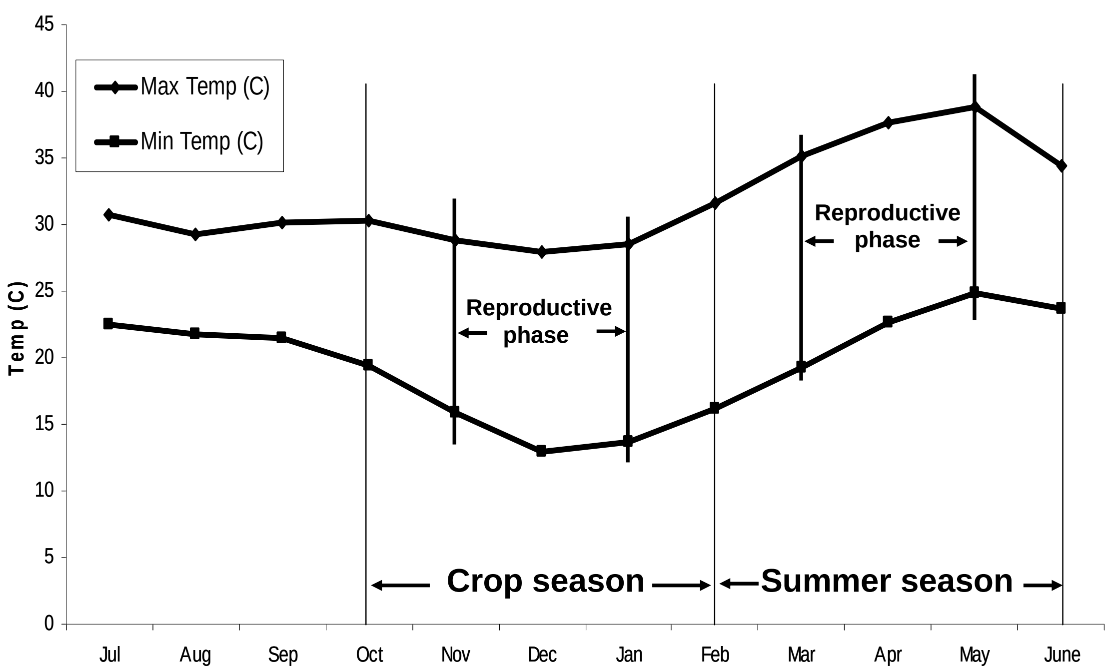


Fig. 3.2.1: Average minimum and maximum temperatures at ICRISAT, Patancheru for the past thirty four years.

3.3 Objective 2: Identify chickpea genotypes with reproductive stage heat tolerance.

3.3.1 Screening of chickpea reference collection for heat tolerance

Field evaluation of the reference collection of chickpea germplasm devoid of the wild accessions and very long duration accessions (n=280) was conducted during the post-rainy and summer season of 2009-10 in two sowing dates (normal and late sowing) on a vertisol at ICRISAT-Patancheru (17° 30' N; 78° 16' E; altitude 549 m) and in an inceptisol (sandy loam) at the New Research Farm, IIPR-Kanpur. At ICRISAT, the field used was solarized using polythene mulch during the preceding summer to sanitize the field, particularly to eradicate wilt causing fungus *Fusarium oxysporum f. sp. ciceri*. After the soil solarization in summer, the field was kept fallow. At Kanpur, the soil was deep ploughed twice and kept fallow after harvest of greengram (mungbean) in the end of September, for another one and half months, before sowing of chickpea.

At ICRISAT, the field was prepared into broad bed and furrows with 1.2 m wide beds flanked by 0.3 m furrows for the normal time sowing, while it was 60 cm ridges and furrows for the late sowing. Surface application and incorporation of 18 kg N ha^{-1} and 20 kg P ha^{-1} as di-ammonium phosphate was carried out before sowing. The plot size was 4 m × 0.75 m with a 30 × 10 cm spacing for the normal sowing and 2m x 0.6m (one row) with a 60 × 10 cm spacing for the late sowing. The design was a 14 x 20 alpha design (280 accessions) with three replications in normal and two in late sowings. The normal time sown crop was grown under receding soil moisture condition without any irrigation (apart from a post-sowing irrigation) while it was optimally irrigated in late sown condition. 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 experiment was planted on 31st Oct 2009 and the late sown on 2nd Feb 2010. During both the plantings, the fields were inoculated with Rhizobium strain IC 59 using liquid inoculation method (Brockwell 1982). Need-based insecticide sprays against pod borer (*Helicoverpa armigera*) were provided and the plots were kept weed free by manual weeding.

At Kanpur, both the normal and late sowings were sown on a flat bed with a plant spacing of 60 x 10 cm and a plot size of 3 x 0.6 m on 13 Nov 2009 and 13 Jan 2010, respectively. The experiments were planted in an 8 x 35 alpha design (280 accessions) with three replications. The seeds were treated with Bavistin (BASF India Ltd, Panoli, Bharuch, Gujarat) containing carbendazim 50% W/P @ 1g per 100 g seeds and was hand planted. After presowing irrigation, a 50 mm irrigation through surface irrigation was applied on 2 Feb 2010 (80 days after sowing) for the normal sowing but three such irrigations on 2 Feb, 12 Mar and 26 Mar 2010 (19, 37 and 50 days after sowing) were applied for the late-planted crop. Although pod borer (*Helicoverpa armigera*) is not a major pest in Kanpur, Endosulfan, EC 35%, (Excel Crop Care, Limited, Mumbai) (at 2 ml L^{-1} of water) was sprayed when 1 to 2 larvae $plot^{-1}$ were noticed, more as a prophylactic pest control measure. Pre-emergence weedicide Pendimethalin @ 3ml $litre^{-1}$ was applied immediately after sowing the crop. Manual weeding was followed thereafter at regular intervals.

Phenology

By regular observation, the date when 50% or more of the plants in a plot flowered was recorded as 50% flowering time of the plot and when 80% of the pods in a plot were mature was recorded as the time of maturity for each plot.

Final harvest

At physiological maturity, plant aerial parts were harvested from an area of 4 m x 0.75 m under normal and 4m x 0.6 m under late sown condition in Patancheru. Similarly, plant material was harvested from an area of 3 m x 0.6 m under both normal and late-sown conditions in Kanpur in each plot. The material was dried to constant weight in hot air driers at 80°C, and total shoot dry weights were recorded. Grain weights were recorded after threshing. Harvest index (%) was calculated as 100 × (Seed yield/total shoot biomass at maturity).

Heat tolerance index (HTI) estimation

Differences in crop duration and yield potential (Saxena et al 1987) are known to contribute to the seed yield under both drought and salinity stress and the removal of these effects from seed yield under stress provides a reliable measure of stress tolerance *per se* (Vadez et al 2007). Similar escape mechanism is also expected with heat. Since the temperature increased linearly during the late planting period and all the short-duration genotypes could start flowering and filling seeds even before the temperatures increased to critical levels (Saxena et al 1987). Previous work related to drought has shown that the residual yield remaining unexplained after removal of effects due to drought escape (early flowering) and yield potential (optimally irrigated yield) of a genotype gave a good indication of the true drought tolerance of that genotype (Bidinger et al 1987; Saxena et al 1987; Saxena 2003; Vadez et al 2007; Krishnamurthy et al 2011). These residuals were calculated using the multiple regression approach of Bidinger et al (1987). This approach considers grain yield under drought stress condition (Y_s) as a function of yield potential (Y_p), time to 50% flowering (F), and a drought tolerance index (DTI) such that the yield of a genotype can be expressed as follows:

$$Y_{si} = a + bY_p + cF_i + DTI_i + E,$$

where E is random error with zero mean and variance σ. The Drought Tolerance Index (DTI) was calculated as the difference between the actual and estimated yields under stress upon the standard error of the estimated yield (σ). For this multiple regression, 50% flowering (F_i) under stress for every individual plot, and for yield potential (Y_p) arithmetic mean across the three replications were considered. Similar approach was adopted for estimating HTI, as flowering time and yield potential are expected to determine the yields of genotypes that are limited by heat stress.

Statistical analysis

The replication-wise values of HTI along with other traits were used for statistical analysis of each environment using ReML considering genotypes as random. Variance components due to genotypes (σ^2_g) and error (σ^2_e) and their standard errors were determined. Environment wise best linear unbiased predictors (BLUPs) for the mini core accessions and others were calculated for the different environments. The significance of genetic variability among accessions was assessed from the standard error of the estimate of genetic variance σ^2_g, assuming the ratio σ^2_g/SE (σ^2_g) to follow normal distribution asymptotically.

While pooling the data over two sites, Bartlett (Snedecor and Cochran 1983) test indicated heterogeneity in error variances. Appropriate transformation was applied and data was tested for presence of G×E interaction. Upon detection of significant G×E interaction, data from each site was analyzed individually and significance of genotypes and their relative ranks were obtained. Spearman's rank correlation coefficient was calculated to have an idea of difference in genotype ranking over sites. Cluster analysis using Ward's incremental sum of squares method was employed to group the genotypes over sites for HTI.

Variation in weather

The late sown crop, subjected to heat stress, was sown on 2 Feb 2010 at Patancheru, and on 13 Jan 2010 at Kanpur. A 20-day early sowing date for Kanpur was chosen as the crop duration in general is longer (by 20 to 30 days) at Kanpur compared to Patancheru; and thus the heat stress imposition is applied at the same phenological stage across locations. The maximum temperature reached the threshold level of 35°C at 27 DAS in Patancheru while at 60 DAS at Kanpur (Fig. 3.3.1.1). Also the minimum temperatures were higher than 17°C after this stage at both locations. At the mean flowering time (52 DAS) in Patancheru the maximum air temperature had reached to 39°C while it was much less (31°C) at mean flowering time (56DAS) at Kanpur.

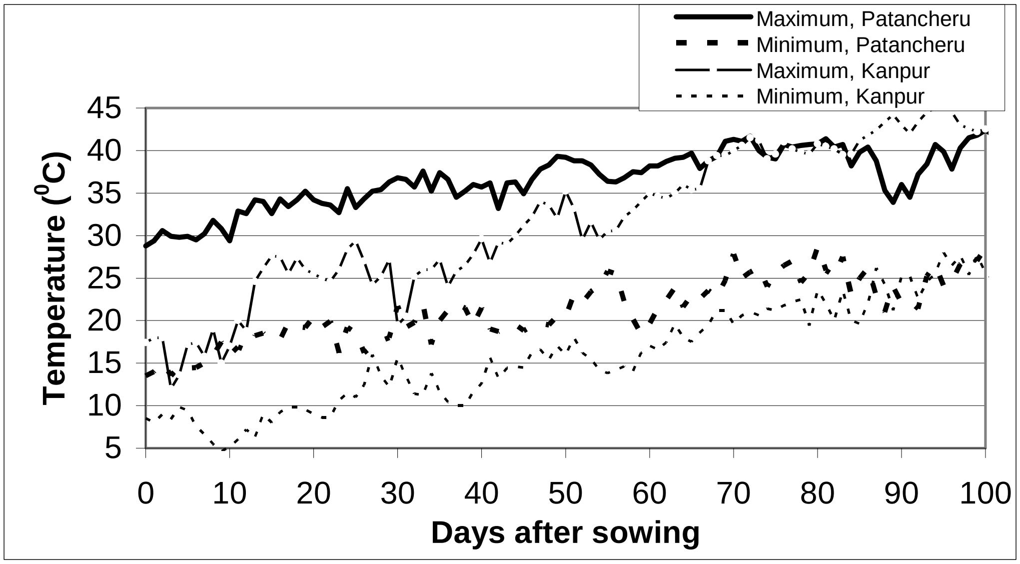


Fig. 3.3.1.1 Daily maximum and minimum temperatures (^{0}C) during the late sown crop growing period both at Patancheru and Kanpur in 2010. The 0 day or the sowing date was 2 Feb 2010 at Patancheru and 13 Jan 2010 at Kanpur.

Variation in phenology in the reference collection accessions

There were large and highly significant differences in flowering time of the accessions in both the sowing times and locations. All the genotypes tend to mature more or less close to each other, irrespective of their differences in flowering time at Kanpur. The overall means for each sowing time had shown that late sowing delayed the days to 50% flowering while the days to maturity was hastened in Patancheru. However, both these stages were reached earlier with late sowing in Kanpur (Table 3.3.1.1). In terms of thermal time (growing degree days, ^{0}Cd) taken to reach mean flowering it was 1094 ^{0}Cd under normal sowing, while it was 1377^{0}Cd under late sown condition at Patancheru. Such increase in requirement of thermal time to attain any developmental stage by the higher soil moisture grown crop is well documented (Desclaux and Roumet 1996; Krishnamurthy et al 1999). However this requirement was mainly to negate the irrigation-led cooling of the microclimate around the plants which is shown to be about 10^{0}C cooling of the soil temperature (Reddy et al 1989). At Kanpur, the late sown crop took 1032 ^{0}Cd and the normal sown 1486^{0}Cd. Providing optimum irrigation is known to extend the growth duration substantially in chickpea. The late sown crop at Patancheru received irrigations at 8-12 day intervals during the whole growing period while the normal sowing conditions was grown under residual moisture stress. Similarly, the crop at Kanpur received only 3 irrigations during the vegetative growth period. There was large range of variation for flowering time under late sown conditions in Patancheru (37 to 73 d) as well as in Kanpur (49 to 67 d) leading to an increased level temperature exposure with the delay in flowering time leading to partial disadvantages of the later genotypes.

Table 3.3.1.1 Trial means, range of best linear unbiased predicted means (BLUPs) and analysis of variance of the 280 accessions of the reference collection of chickpea germplasm for days to 50% flowering and days to maturity in the field experiments during 2009-10 both at Patancheru and Kanpur post rainy (normal) and summer (heat stress) seasons.

Location/Sowing time	Trial mean	Range of predicted means	S.Ed	σ^2_g (SE)
		Days to 50% flowering		
Patancheru				
Heat stress	51.8	37.2 – 73.2	3.64	40.1 (4.13)
Normal	48.4	34.8 – 65.7	2.00	37.8 (3.38)
Kanpur				
Heat stress	55.7	49.3 – 66.8	1.81	8.98 (0.93)
Normal	89.4	81.6 – 102.9	2.79	22.72 (2.34)
		Days to maturity		
Patancheru				
Heat stress	88.8	76.0 – 107.4	3.21	47.2 (4.51)
Normal	95.2	78.7 – 114.7	3.18	82.0 (7.41)
Kanpur				
Heat stress	NA	NA	NA	NA
Normal	NA	NA	NA	NA

Influence of flowering time and normal sown yield on late sown yield

At Patancheru seed yield under heat stress was negatively associated to the time to flowering ($r^2 = 0.51^{***}$; significant above 0.001 level), while it was positively associated with the normal sown yields, considered here as potential normal yield ($r^2 = 0.50^{***}$). Similar significant negative association with 50% flowering time ($r^2 = 0.18^{***}$) and yield under normal sowing ($r^2 = 0.09^{**}$) was also seen at Kanpur. Therefore, categorization of the accessions in terms of seed yield under heat stress for heat response would partly lead to a categorization for escape from heat and yield potential. Therefore heat tolerance indices were computed to characterize the heat tolerance *per se* in this study, i.e. the proportion of the genetic variation for seed yield under heat that was not accounted for differences in time to flowering and yield potential.

Variation in yield and yield components

Between the two locations the shoot biomass and yield produced in Kanpur was manifolds less than that at Patancheru. This was due to a combination of effects that did not promote a normal crop growth such as broader spacing practiced, sandy and poor water holding nature of the soil, recently developed marginal land and receding soil moisture conditions during major reproductive growth with only three supplementary irrigations after sowing (Table 3.3.1.2). Under heat stress conditions in Patancheru, the shoot biomass produced was higher than the normal sown crop as the heat stressed crop was optimally irrigated, while the normal sown one was on receding soil moisture condition. However mean seed yield of all the accessions were reduced to two-thirds. In Kanpur the shoot biomass was reduced by half under heat stress and the seed yield by one-fourth (Table 3.3.1.2). The overall harvest indices were lower under heat stress compared to the normal sown conditions and it was higher in Patancheru in any of the sowing conditions.

Table 3.3.1.2 Trial means, range of best linear unbiased predicted means (BLUPs) and analysis of variance for chickpea reference collection for different during 2009-10.

Season/Environment	Trial mean	Range of predicted means	S.Ed	σ^2_g (SE)
		Shoot biomass (g m^{-2})		
Patancheru				
Heat stress	473.3	356.6 – 615.6	65.8	4261 (824)
Normal	412.0	282.2 – 549.9	43.1	3031 (379)
Kanpur				
Heat stress	74.1	38.1 – 120.8	19.4	436.7 (68.7)
Normal	146.4	83.8 – 237.1	33.2	1115 (187)
		Seed yield (g m^{-2})		
Patancheru				
Heat stress	97.9	8.0 – 265.4	28.6	4150 (384)
Normal	152	44.2 – 231.4	20.9	1343 (137)
Kanpur				
Heat stress	10.4	3.2 – 34.7	5.1	48.2 (5.7)
Normal	41.1	16.8 – 87.4	12.8	215.2 (29.0)
		Harvest index		
Patancheru				
Heat stress	22.0	0.7 – 53.3	4.28	242.9 (21.2)
Normal	37.7	11.3 – 57.0	2.77	133.0 (11.6)
Kanpur				
Heat stress	13.8	5.5 – 30.5	5.15	37.5 (5.06)
Normal	27.3	14.8 – 40.2	4.90	30.8 (4.35)

There were highly significant variations for the shoot biomass as well as seed yield across the accessions and these variations were about two-fold for the shoot biomass at maturity at both heat stressed as well as normal sown crop and many-fold for seed yield among the accessions again at both the sowing times tested (Table 3.3.1.2). There was a highly significant, large range of variation in harvest index in both the sowing times and locations. At Patancheru, the variance component for the HTI of accessions (0.585, SE 0.070) was highly significant and the means ranged from -2.5 to 2.7. Similarly at Kanpur, the variance component (0.298, SE 0.041) was highly significant for HTI and the means ranged from -0.7 to 1.9. The pooled analysis of data from both the locations had revealed that there were highly significant genotype effects and also equally significant genotype × location (G×E) interactions for all the characteristics that were studied except for the yield component, seeds per pod. In spite of this interaction the rank correlation of the accession means between the location had indicated that 50% flowering ($r = 0.51^{***}$), seed yield g m^2 ($r = 0.60^{***}$), harvest index ($r = 0.57^{***}$) and HTI ($r = 0.27^{***}$) were closely related except for the shoot biomass production ($r = 0.06^{NS}$).

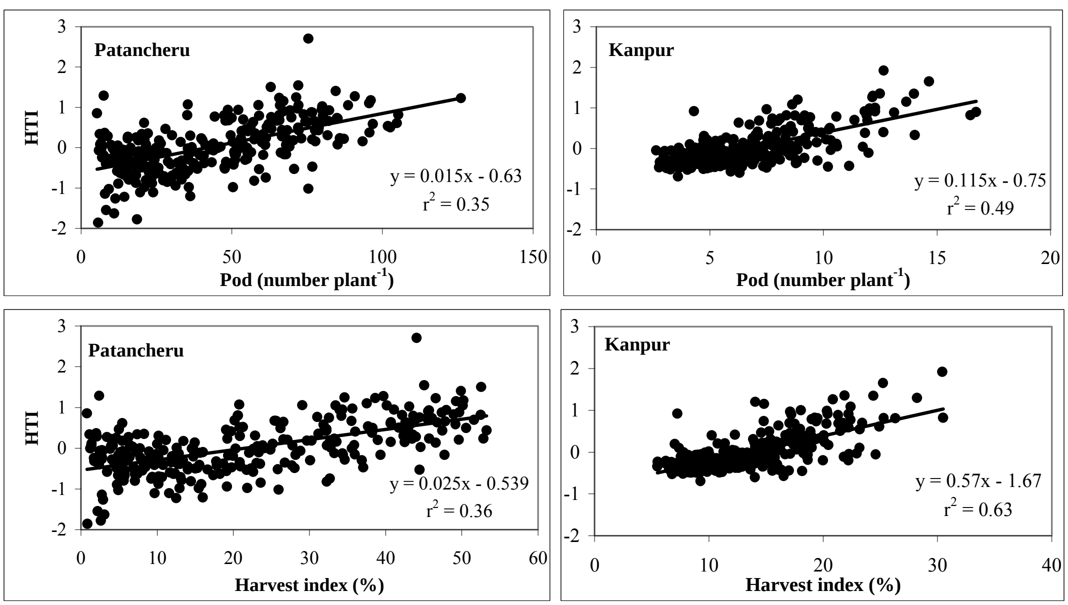


Fig. 3.3.1.2 Relationship of pod number per plant with the HTI and the harvest index (%) with the HTI both at Patancheru and Kanpur.

Contribution of yield components

Among the yield components pods $plant^{-1}$ was most affected by heat stress in Kanpur but this effect could not be seen in Patancheru due to the compensation caused by irrigation (Table 3.3.1.3). The range in pod number $plant^{-1}$ was large. Time to 50% flowering (representing earliness), shoot biomass at maturity, harvest index, pods borne on a plant and seed size were related either negatively or positively to the seed yield or the HTI to various degree, depending on the sowing time and the location (data not shown). However, the pod numbers per plant (r^2 = 0.81 at Patancheru and 0.64 at Kanpur) and harvest index (r^2 = 0.92 at Patancheru and 0.63 at Kanpur) were the two parameters that were very closely associated with the seed yield (figures not shown) and as a consequence with the HTI (Fig 3.3.1.2) and other related characteristics. These relationships were very close in Patancheru than at Kanpur.

Heat response categorization

As there was a significant interaction between accessions and years, the HTI of the accessions were grouped into representative groups using the BLUPs for HTI by a hierarchical cluster analysis (using Ward's incremental sum of squares method) and this analysis yielded five clusters that differed significantly. Based on the extent of cluster group means of the HTI these were identified as: 1. stable tolerant (with HTI means 0.81 in Patancheru and 1.01 in Kanpur), 2. tolerant only at Patancheru (1.04 and 0.09), 3. tolerant only at Kanpur (-0.15 and 0.71), 4. moderately tolerant (0.10 and -0.18) and 5. stable sensitive (-0.71 and -0.15). The stable tolerant group comprised of 18 accessions (Table 3.3.1.4), while the stable sensitive group comprised of 82 accessions out of the 277 used for clustering. For the sake of brevity, the data of 5 genotypes that were the most sensitive and made a sub-cluster with in the sensitive cluster is being presented (Table 3.3.1.4). The tolerant only at Patancheru group comprised of 34 accessions (Table 3.3.1.5). The tolerant only at Kanpur group comprised of 23 accessions (Table 3.3.1.6). The moderately tolerant group comprised 120 entries, respectively. ICC 14778, a stable drought tolerant entry and ICC 4958, a well known drought tolerant genotype with high root mass (Krishnamurthy et al., 2010) have also ranked as stable heat tolerant entries in this study (Table 3.3.1.4). Ten other entries that ranked as the next order drought tolerant accessions in the previous work also appeared as stable heat tolerant ones. Similarly 13 stable sensitive entries appeared also in a previous drought tolerance assessment and 11 of them were ranked to be moderately tolerant (Data not shown).

Table 3.3.1.4 Time to flowering, shoot and seed yield at maturity and heat tolerance indices of the consistently heat tolerant (stable in both locations) and five out of 82 consistently most heat sensitive cluster group members of chickpea reference collection at Patancheru and Kanpur under heat stress during 2010 summer. Flower, shoot, yield and HTI denotes to days to 50% flowering, shoot biomass g m^2, seed yield g m^2 and heat tolerance index, respectively and the characters P and K for Patancheru and Kanpur, respectively.

S.No	Accession	P-Flower	P-shoot	P-Yield	P-HTI	K-Flower	K-shoot	K-Yield	K-HTI
Stable heat tolerant									
1	ICC 456	50.3	412.2	145.7	0.64	54.9	81.7	19.9	0.55
2	ICC 637	53.6	514.1	139.2	0.68	54.6	102.8	26.9	1.08
3	ICC 1205	48.6	461.8	190.8	0.79	55.9	114.0	23.0	0.96
4	ICC 3362	47.4	491.3	196.3	1.28	55.7	104.3	28.9	1.34
5	ICC 3761	50.3	565.7	130.7	0.68	51.5	85.9	22.1	0.70
6	ICC 4495	49.1	503.1	199.2	1.23	55.7	73.6	21.4	0.81
7	ICC 4958	42.4	485.2	231.4	0.93	51.5	91.4	20.0	0.81
8	ICC 4991	49.9	460.4	180.6	0.50	53.7	90.6	19.7	0.81
9	ICC 6279	42.0	453.3	220.1	0.82	52.4	81.4	27.0	1.29
10	ICC 6874	49.1	503.9	195.2	1.10	55.4	92.7	21.2	0.99
11	ICC 7441	49.5	464.0	199.1	0.74	53.7	97.4	20.8	0.74
12	ICC 8950	45.7	452.4	177.3	0.81	55.1	96.7	22.7	0.90
13	ICC 11944	48.6	515.3	173.1	0.58	54.3	86.6	24.4	0.91
14	ICC 12155	44.1	433.5	191.1	1.05	51.8	97.3	34.7	1.92
15	ICC 14402	42.0	457.2	184.9	0.59	53.2	112.4	28.4	1.26
16	ICC 14778	47.8	441.8	153.5	0.50	55.4	91.7	21.1	0.89
17	ICC 14815	49.9	506.6	178.8	0.93	54.6	110.5	25.6	1.15
18	ICC 15618	48.6	431.5	187.4	0.59	52.3	112.7	23.8	1.00
	Mean	47.7	475.6	182.7	0.81	54.0	95.8	23.7	1.00
Stable heat sensitive									
1	ICC 4567	50.7	535.2	18.8	-1.80	57.9	86.5	6.5	-0.37
2	ICC 10685	47.4	520.7	8.6	-1.88	55.9	85.4	4.3	-0.27
3	ICC 10755	48.6	474.9	14.8	-1.57	55.4	61.6	5.4	-0.37
4	ICC 16374	37.8	356.6	30.4	-2.53	50.9	63.6	5.0	-0.49
5	IG 7087	49.1	505.8	20.1	-1.65	59.0	61.2	4.8	-0.07
	Mean	46.7	478.6	18.6	-1.89	55.8	71.6	5.2	-0.31

Table 3.3.1.5 Time to flowering, shoot and seed yield at maturity and heat tolerance indices of the heat tolerant only at Patancheru cluster group members of chickpea reference collection at Patancheru and Kanpur under heat stress during 2010 summer. Flower, shoot, yield and HTI denotes to days to 50% flowering, shoot biomass g m^2, seed yield g m^2 and heat tolerance index, respectively and the characters P and K stand for Patancheru and Kanpur, respectively.

S.No	Accession	P-Flower	P-shoot	P-Yield	P-HTI	K-Flower	K-shoot	K-Yield	K-HTI
1	ICC 67	48.2	547.6	202.3	1.07	53.2	86.5	12.8	0.12
2	ICC 283	44.1	482.2	217.7	0.94	55.9	82.0	9.7	-0.09
3	ICC 506	46.1	448.8	183.5	0.77	55.1	75.9	10.8	0.07
4	ICC 708	50.7	513.1	186.6	1.26	55.1	86.8	9.0	-0.44
5	ICC 1164	51.6	470.8	164.8	0.99	60.7	72.2	8.2	-0.33
6	ICC 1356	44.9	464.5	203.1	0.75	52.6	90.8	15.7	0.22
7	ICC 2072	49.5	466.5	153.1	0.80	54.6	79.2	15.4	0.07
8	ICC 2263	52.4	496.1	176.7	0.68	58.2	82.8	10.9	0.11
9	ICC 2629	57.4	460.3	93.7	0.81	59.6	88.2	10.8	0.04
10	ICC 2969	50.3	487.3	219.7	1.57	57.9	120.8	19.2	1.20
11	ICC 3325	44.1	480.2	206.8	0.67	54.3	97.5	16.0	0.17
12	ICC 4657	57.0	457.6	127.3	1.07	56.5	75.9	9.4	-0.12
13	ICC 5434	48.6	404.3	162.8	0.89	55.7	41.4	5.9	-0.05
14	ICC 5613	44.5	441.5	180.6	0.92	54.6	68.1	12.2	0.25
15	ICC 5878	45.3	454.9	199.1	1.24	57.9	54.5	6.7	-0.04
16	ICC 6816	43.2	489.6	201.8	0.65	56.2	71.2	15.4	0.26
17	ICC 8318	40.7	434.6	198.7	0.70	51.3	73.3	14.0	0.12
18	ICC 8522	68.2	420.0	8.0	0.86	51.8	56.1	9.7	-0.05
19	ICC 10018	45.3	447.6	205.7	1.19	56.3	56.6	6.9	-0.18
20	ICC 10393	42.8	442.3	195.6	0.84	54.5	79.2	15.3	0.22
21	ICC 10945	46.6	475.6	191.7	0.96	55.1	79.9	14.4	0.41
22	ICC 11279	50.3	442.2	176.3	2.74	54.3	77.3	10.9	-0.05
23	ICC 12492	54.5	521.2	116.0	0.82	54.8	53.2	9.4	-0.08
24	ICC 12654	51.6	488.8	168.4	0.94	49.8	64.0	12.7	0.11
25	ICC 13124	44.1	497.5	265.4	1.52	52.9	64.7	9.2	-0.25
26	ICC 13892	49.1	416.6	157.1	0.73	52.3	72.0	14.5	0.33
27	ICC 14595	44.9	460.2	213.2	1.17	52.6	72.5	12.3	-0.30
28	ICC 14799	44.5	486.6	195.6	1.06	58.5	101.6	12.9	0.21
29	ICC 15612	49.9	436.4	183.6	0.89	53.7	82.9	13.8	0.20
30	ICC 15614	47.4	464.6	220.9	1.42	54.0	78.8	12.8	0.25
31	ICC 15868	49.9	476.0	164.0	0.96	54.6	89.9	18.1	0.47
32	ICC 16915	44.1	486.2	209.8	1.04	53.2	79.3	16.5	0.40
33	IG 5909	52.0	596.5	147.1	1.08	55.9	62.0	5.1	-0.35
34	IG 6154	73.2	599.7	20.6	1.30	63.2	56.6	5.9	0.21
	Mean	49.3	475.2	174.0	1.04	55.2	75.7	11.8	0.09

Table 3.3.1.6 Time to flowering, shoot and seed yield at maturity and heat tolerance indices of the heat tolerant only at Kanpur cluster group members of chickpea reference collection at Patancheru and Kanpur under heat stress during 2010 summer. Flower, shoot, yield and HTI denotes to days to 50% flowering, shoot biomass g m^2, seed yield g m^2 and heat tolerance index, respectively and the characters P and K stand for Patancheru and Kanpur, respectively.

S.No	Accession	P-Flower	P-shoot	P-Yield	P-HTI	K-Flower	K-shoot	K-Yield	K-HTI
Heat tolerant only at Kanpur									
1	ICC 1083	41.6	405.1	174.0	0.24	54.0	92.0	20.3	0.82
2	ICC 1882	42.8	439.3	187.8	0.44	54.1	100.4	18.8	0.46
3	ICC 2507	47.8	526.1	89.9	-0.42	52.1	84.2	19.1	0.68
4	ICC 2884	50.7	429.2	88.5	-0.04	51.8	82.5	18.3	0.50
5	ICC 3631	47.0	466.4	61.3	-1.00	52.9	100.3	19.5	0.69
6	ICC 4182	49.5	451.2	67.1	-0.50	52.1	90.7	22.7	0.77
7	ICC 4363	42.4	462.5	128.6	-0.07	52.3	75.9	17.6	0.69
8	ICC 4418	49.9	508.5	115.8	-0.12	52.9	75.3	20.9	0.82
9	ICC 4814	47.8	479.1	109.1	-0.51	51.5	105.5	30.6	1.35
10	ICC 5383	45.3	477.0	157.7	0.23	54.6	109.0	21.9	0.39
11	ICC 6293	57.0	444.8	28.2	-0.69	52.4	110.1	19.0	0.74
12	ICC 6537	52.8	480.2	127.3	0.21	58.4	81.1	17.3	0.64
13	ICC 6579	51.1	400.2	114.8	0.00	54.0	79.8	16.6	0.50
14	ICC 9002	49.1	443.2	147.6	0.08	56.2	91.9	20.0	0.79
15	ICC 9895	50.7	465.0	112.4	0.11	55.4	67.9	16.0	0.59
16	ICC 11121	51.1	396.9	104.1	-0.75	54.6	98.3	19.5	0.56
17	ICC 11198	52.4	436.0	122.1	0.36	57.3	109.8	16.5	0.41
18	ICC 12028	55.3	546.7	60.4	-0.08	54.6	82.1	15.7	0.67
19	ICC 13524	54.5	506.4	59.6	-0.72	51.8	71.5	17.8	0.61
20	ICC 14669	42.0	425.8	176.7	-0.17	53.2	102.9	30.6	1.65
21	ICC 14831	45.3	601.4	163.1	0.22	54.6	99.6	22.4	0.92
22	ICC 15510	52.0	457.4	96.1	-0.59	54.3	66.8	16.7	0.69
23	ICC 15606	43.2	499.3	196.5	0.21	56.2	94.9	15.4	0.47
	Mean	48.7	467.3	116.9	-0.15	54.0	90.1	19.7	0.71

Large genotypic variation was available among the reference collection of chickpea germplasm for heat tolerance that underlines the utility of the reference collection for applied breeding programme. These new sources of heat tolerance can be used for physiological and genetic studies and in heat tolerance breeding. Harvest index and pod number per plant are the two key traits that can be used in selections. The heritability of yield under heat stress environment was even better than the normal growing condition offering opportunity for direct selection of yield under optimally irrigated Vertisols. The HTI represented a selection index devoid of the yield potential and phenology effects and this index potentially offers a selection criterion for adaptation to higher temperatures valid across wider agro-ecological zones.

3.3.2 Multilocation evaluation of 60 heat tolerant lines

Sixty chickpea genotypes were evaluated for their tolerance to high temperature stress at ICRISAT-Patancheru, IIPR-Kanpur, JNKVV-Jabalpur and RARS-Nandyal. The normal planting was done on 31st Oct, 2009, 17th Nov, 2009, 23th Nov 2009 and 20th Nov 2009 at Patancheru, Kanpur, Jabalpur and Nandyal respectively. The late/summer planting was done on 1st Feb 2010, 14th Jan 2010, 15th Jan 2010 and 2nd Feb 2010 at Patancheru, Kanpur, Nandyal and Jabalpur respectively. Late planting exposed the chickpea genotypes to reproductive stage heat stress. The experimental design used was randomized block design (RBD) with two replications. Each plot included two 4 m rows with 60 cm spacing between the rows and 10 cm between the plants. The standard recommended package of practices like application of fertilizers, insecticides/fungicides and weeding were followed across both the locations to have a good crop stand. Data was recorded on phenological characters (days to flowering [DF], days to fifty percent flowering [DFF], days to first pod [DFP} and days to plant maturity [DPM]), yield components (total number of pods per plant, filled pod number per plant [FPDN], seed number per plant [SDN] and test weight) and grain yield per plant.

The mean and range values of phenological, yield and yield components are shown in Tables 3.3.2.1 and 3.3.2.2. Wide variation was observed for all the characters including yield at the four locations. Both the mean and range values under high temperature stress have declined for all the phenological characters (DF, DFF, DFP, and DPM) analyzed at all the four locations (Tables 3.3.2.1/2). As it can be observed from Table 3.3.2.1 and 3.3.2.2, the effect of heat stress on yield and yield components is very significant at all the locations. There was an overall average decrease in yield and yield components (filled pod number and seed number) by ~ 67% across all the four locations. Genotypes at Kanpur location were the worst affected by high temperatures, showing almost >90% decrease in yield and its related components (Tables 3.3.2.1/2).

Table 3.3.2.1 Mean and range values of phenological characters of the sixty genotypes grown under normal and summer planting

Location	DF (N)	DF (S)	DFF (N)	DFF (S)	DFP (N)	DFP (S)	DPM	DPM
Nandyal	44.1 (32.5-57.0)	37.4 (29.0-51.0)	48.6 (38.0-66.5)	41.6 (33.5-57.0)	50.9 (40.5-68.0)	44.9 (37.5-61.0)	96.9 (83.5-107.5)	78.8 (67.0-95.5)
Jabalpur	60.9 (39.5-71.0)	44.6 (36.0-56.5)	65.7 (45.0-76.0)	49.2 (39.5-61.5)	82.4 (74.0-89.0)	55.6 (46.0-85.0)	111.4 (106.0-118.5)	82.0 (68.5-89.0)
Kanpur	76.6 (59.0-85.5)	78.3 (41.5-56.0)	84.6 (72.5-95.5)	52.2 (46.0-58.5)	92.9 (84.5-98.0)	56.9 (48.5-64.0)	126.8 (124.0-129.0)	81.6 (78.0-88.5)
ICRISAT	39.8 (28.0-51.5)	38.9 (29.0-55.0)	42.9 (31.0-56.0)	42.2 (32.5-59)	47.1 (36.0-59.5)	45.3 (35.0-59.5)	101.3 (85.5-112.0)	81.3 (69.5-91.0)

DF-days to flowering; DFF-days to fifty % flowering; DFP-days to first podding; DPM-days to plant maturity; N-normal; S-summer; values in brackets are ranges

Table 3.3.2.2 Mean and range values of yield and yield components of the sixty genotypes grown under normal and summer planting

Location	FPDN (N)	FPDN (S)	SDN (N)	SDN (S)	Yield (g)/pt (N)	Yield (g)/pt (S)	Test Wt (N)	Test Wt (S)
Nandyal	93.8 (46.5-143.5)	36.8 (0.0-208.5)	96.5 (46.5-146.5)	43.3 (0.0-261)	20.8 (9.7-38.3)	6.3 (0.0-33.3)	21.8 (12.0-35.1)	12.8 (0.0-31.5)
Jabalpur	63.3 (21.3-147.2)	23.6 (5.6-57.6)	81.7 (28.5-199.5)	27.7 (6.1-88.5)	15.9 (8.7-30.5)	4.9 (1.0-16.5)	21.4 (11.3-39.2)	19.3 (9.4-34.2)
Kanpur	211.3 (55.5-408.5)	12.8 (4.1-23.6)	239.1 (62.5-481.5)	15.6 (6.3-28.5)	49.1 (26.4-111.8)	2.7 (1.2-5.2)	22.8 (10.4-42.3)	17.8 (9.6-29.8)
ICRISAT	58.3 (24.6-89.7)	31.0 (13.1-62.5)	68.5 (28.3-104.0)	38.1 (14.2-79.6)	15.1 (8.9-25.7)	7.1 (1.9-13.1)	23.9 (11.8-44.5)	19.8 (9.4-34.7)

FPDN-filled pod number; SDN-seed number/pt; N-normal; S-summer; values in the brackets are ranges

The grain yield of sixty genotypes is given in Table 3. The yield range of the mean values for the sixty genotypes was 18.9-38.7 and 1.7-12.0 gm per plant under normal and summer/late planting at the four locations. Top ten heat tolerant genotypes were identified and ranked (Table 3.3.2.3) based on their mean yield performance under stress conditions. The top ten identified genotypes identified from this study could be used in breeding programs aimed at developing heat tolerant and high yielding genotypes.

Table 3.3.2.3 Total and mean yield of all the sixty genotypes at four locations in normal and summer planting

Entry	I-N	I-S	J-N	J-S	K-N	K-S	N-N	N-S	Mean (N)	Mean (S)	R
1	18.9	8.0	16.7	7.2	52.2	2.3	23.8	2.0	27.9	4.9	
2	13.6	8.6	18.0	5.1	62.9	2.7	19.4	5.8	28.5	5.5	
3	18.6	3.1	18.8	4.1	52.7	2.3	20.4	0.3	27.6	2.5	
4	13.1	6.9	14.2	4.7	47.4	1.2	14.9	4.2	22.4	4.3	
5	19.6	13.1	13.1	6.8	55.1	2.5	25.4	0.0	28.3	5.6	
6	15.4	5.1	13.0	1.8	40.7	2.4	19.0	6.8	22.0	4.0	
7	17.5	10.0	17.9	7.3	67.7	3.0	15.6	9.5	29.7	7.5	8
8	11.0	7.4	12.3	5.3	41.8	1.7	12.6	33.3	19.4	11.9	2

9	17.2	8.2	12.5	5.8	38.7	2.4	12.9	5.5	20.3	5.5	
10	15.2	3.9	18.1	4.6	56.8	3.3	15.8	6.0	26.5	4.4	
11	14.8	5.3	19.5	3.1	43.1	1.8	13.8	0.0	22.8	2.5	
12	12.9	5.3	12.4	5.3	37.1	2.8	19.6	3.0	20.5	4.1	
13	14.3	10.0	17.6	3.1	43.8	3.7	33.7	4.5	27.4	5.3	
14	11.8	8.3	25.2	3.2	28.8	3.6	9.7	5.3	18.9	5.1	
15	15.8	7.9	22.5	11.0	42.0	2.8	20.3	0.0	25.1	5.4	
16	14.3	8.2	13.9	6.1	41.8	2.8	35.3	5.3	26.3	5.6	
17	16.4	5.5	16.7	6.1	33.9	2.2	20.5	9.5	21.9	5.8	
18	11.6	4.9	14.1	1.8	27.6	2.8	24.4	21.5	19.4	7.8	6
19	18.8	5.8	8.7	7.0	51.1	3.6	25.8	1.0	26.1	4.4	
20	16.6	3.3	18.7	1.3	41.8	2.4	17.4	0.1	23.6	1.8	
21	10.9	8.2	15.7	6.1	40.8	5.2	16.8	2.0	21.0	5.4	
22	14.3	8.9	11.5	4.3	33.7	3.1	25.8	17.0	21.3	8.3	5
23	18.3	10.4	23.0	1.6	57.0	1.5	19.6	3.1	29.5	4.1	
24	15.2	5.8	14.2	4.2	56.4	2.8	32.4	1.0	29.5	3.5	
25	14.0	7.9	24.7	6.6	52.4	3.3	26.1	4.8	29.3	5.6	
26	13.9	9.2	17.0	2.9	59.5	2.6	14.9	2.3	26.3	4.3	
27	18.5	6.7	16.8	4.2	48.9	2.4	21.0	9.5	26.3	5.7	
28	11.6	8.7	25.2	2.2	51.4	3.4	27.4	1.0	28.9	3.8	
29	14.4	8.7	19.1	10.1	56.7	2.7	19.5	13.8	27.4	8.8	4
30	25.7	8.3	12.7	3.8	48.7	1.8	18.3	3.5	26.3	4.4	
31	12.2	8.9	11.2	3.6	52.6	2.3	22.8	2.0	24.7	4.2	
32	11.7	9.9	10.1	3.8	74.2	2.5	21.1	2.0	29.3	4.6	
33	25.7	5.5	14.0	2.5	28.5	2.6	27.5	0.3	23.9	2.7	
34	16.7	7.1	15.5	5.4	51.9	3.2	22.7	4.5	26.7	5.0	
35	17.2	9.4	30.5	3.3	60.9	2.9	22.6	17.5	32.8	8.3	5
36	11.5	7.5	13.4	4.5	26.4	2.4	27.6	2.3	19.7	4.1	
37	12.6	7.3	17.3	3.3	43.1	2.0	22.7	4.1	23.9	4.2	
38	14.2	4.6	9.8	1.0	42.9	1.7	11.3	2.5	19.6	2.4	
39	10.9	4.7	14.3	3.8	42.1	1.7	11.9	3.3	19.8	3.4	
40	8.9	2.5	14.5	1.7	41.8	1.6	12.9	1.0	19.5	1.7	
41	10.1	6.3	10.8	3.3	44.8	3.3	13.5	7.0	19.8	5.0	
42	13.4	1.9	10.7	3.3	60.1	2.6	11.9	1.3	24.0	2.3	
43	15.8	3.7	12.5	3.9	56.0	3.1	27.0	0.1	27.8	2.7	
44	10.3	4.3	20.5	3.7	48.0	1.8	20.9	8.5	24.9	4.6	
45	16.5	4.8	11.3	8.9	81.2	3.0	15.0	0.3	31.0	4.2	
46	16.2	5.4	16.5	2.8	59.1	1.5	18.3	0.0	27.5	2.4	
47	16.8	8.2	18.3	8.4	43.3	2.0	15.2	27.6	23.4	11.5	3
48	19.2	5.9	11.7	7.7	52.8	2.8	21.0	2.3	26.2	4.6	
49	14.8	6.2	14.0	5.8	38.6	2.7	26.5	11.5	23.5	6.5	
50	17.0	7.7	17.4	5.9	55.3	2.6	38.3	19.0	32.0	8.8	4
51	13.6	8.3	13.8	4.2	52.5	1.9	19.5	2.3	24.8	4.1	
52	11.7	10.9	13.3	7.8	37.3	4.8	15.9	0.1	19.5	5.9	
53	17.7	6.1	22.1	6.9	47.0	3.7	22.5	6.1	27.3	5.7	
54	12.8	6.6	10.1	3.1	31.9	4.4	26.2	8.0	20.2	5.5	

55	10.3	10.9	12.1	16.5	49.4	4.1	34.7	16.6	26.6	12.0	1
56	21.8	4.0	13.5	1.5	34.8	1.6	22.2	16.5	23.1	5.9	
57	13.9	9.1	19.3	3.5	45.1	2.6	24.6	4.3	25.7	4.8	
58	16.6	10.9	20.1	3.5	41.3	2.4	17.1	4.8	23.8	5.4	
59	14.8	7.6	14.5	2.6	111.8	1.7	13.7	12.0	38.7	6.0	
60	15.7	7.0	16.5	13.2	76.9	2.7	21.9	7.5	32.7	7.6	7

I-N, ICRISAT normal; I-S, ICRISAT summer; J-N, Jabalpur normal; J-S, Jabalpur summer; K-N, Kanpur normal; K-S, Kanpur summer; N-N, Nandyal normal; N-S, Nandyal summer; R- Rank

Based on grain yield under heat stress conditions, several heat tolerant genotypes were identified. The top ten heat tolerant genotypes included 8 breeding lines/released varieties [ICCV 2, ICCV 06302, ICCV 07118, ICCV 07109, ICCV 96970 (JG 16), ICCV 93952 (JAKI 9218), ICCV 92311 (KAK 2) and ICCV 87207 (Vishal)] and two germplasm lines (ICC 8474, ICC 9942).

3.4 Objective 3: Understand mechanisms and genetics of heat tolerance

3.4.1 Studies on mechanisms of heat tolerance

3.4.1.1 Studies on reproductive biology of heat tolerant and sensitive lines at PU-Chandigarh and IIPR-Kanpur

Physiology experiments were conducted at IIPR, Kanpur and PU Chandigarh using two heat tolerant chickpea lines (ICCV 07110 and ICCV 92944) and two sensitive lines (ICC 14183 and ICC 5912). These four chickpea genotypes were assessed for sensitivity towards high temperatures primarily based upon reproductive biology such as pollen viability, pollen germination, pollen tube growth, stigma receptivity, seed size and percent pod setting. The degree of tolerance to temperatures was further assessed by specific physiological tests such as membrane injury, chlorophyll fluorescence, photosynthesis and carbohydrate metabolizing enzymes (sucrose synthase and invertase) in developing grains. This enables us to have a basic understanding of source-sink relationship under high temperature and other factors limiting productivity of chickpea under this stress.

Dates of sowing

Chickpea genotypes were sown on 4th November 2009 as normal and 11th February 2010 as late sown condition at PU, Chandigarh and similarly on 27th Oct, 2009 and 15th January, 2010 at Kanpur on a small plots following standard cultural practices. The late sown condition exposes the crop to high temperatures during reproductive phase.

Phenology

In general two heat tolerant lines were early flowering type while sensitive ones were invariably late under both Kanpur and Chandigarh condition. Flowering time (days to first flower) was further shortened in all the genotypes under late sown condition and the days to first flower reduced to almost half in both the sensitive lines while flowering was 10 days advanced in both the tolerant ones (Table 3.4.1.1.1). Thermal time requirement remained little affected in tolerant genotypes when grown under late planting. But, greater thermal time for flowering in sensitive genotypes subjected the plants to set pods at much higher temperature than the tolerant lines. Thus early flowering has the advantage over late flowering type in terms of avoiding terminal heat stress. In late-sown (heat stressed) condition, the heat tolerant genotypes ICCV 07110 and ICCV 92944 showed 50.3 and 68.4% pod set relative to heat sensitive genotypes ICC 14183 and ICC 5912 showing 45.2 and 47.3% pod set (Table 3.4.1.1.1).

Table 3.4.1.1.1 Phenology of chickpea genotypes under normal and late-sown conditions

Accessions	Profile	Days to flowering		Thermal time Flowering		Days to podding		Thermal time (Podding)		Pod set (%)	
		Normal	Late	Normal	Late	Normal	Late	Normal	Late	Normal	Late
ICC 14183	Heat Sensitive	89	46	1425	1031	106	51	1708	1167	70.4	45.2
ICC 5912	Heat Sensitive	78.3	47.3	1382	1031	108	52	1708	1167	73.4	47.3
ICCV 07110	Heat Tolerant	51.7	41.3	990	950	106.3	47.6	1693	1112	78.8	50.3
ICCV 92944	Heat Tolerant	50.6	42.3	978	947	103.3	47	1617	1058	80.1	68.4

Reproductive Biology

The functioning of male and female components was tested using pollen germination, pollen viability, pollen tube growth and stigma receptivity. Pollen grains collected form the plants growing under normal-sown conditions were subjected to varying temperatures of 30, 35, 38 and 40^{0}C with 25^{0}C as control. The pollen viability was tested by using TTC solution. The loss of purple stain indicated decrease in pollen viability. The viability decreased in all the genotypes with increasing incubation temperatures as indicated by loss of purple stain. The pollen remained viable up to 35^{0}C but significantly decreased beyond 35^{0}C. However genotypic differences were significant in pollen germinability at high temperature beyond 35^0 C. The tolerant genotypes ICCV 07110 and ICCV 92944 showed significantly higher pollen viability, germination and pollen tube growth than the heat sensitive genotypes (ICC 14183 and ICC 5912; Table 3.4.1.1.2).

At Kanpur, pollen were collected from excised unopened flowers and incubated in the pollen germination medium at temperature 33, 37, 38 and 39^{0}C for 2h. At least 20 microscopic fields were scanned for percent germination for each variety. Pollen tube growth decreased with increasing temperature beyond 33 ^{0}C (Fig 3.4.1.1.1), but germination was observed even at 39^{0}C. However, per cent pollen germination was fairly high (> 50%) in genotypes ICCV 92944 and ICCV 07110. On the other hand ICC 5912 was found to be very sensitive to high temperature with almost cessation of pollen germination at 39 ^{0}C (Fig 3.4.1.1.1). Genotype ICC 14183 showed medium heat tolerance. The length of pollen tube was maximum within a short period of 2h in ICCV 92944 followed by ICCV 07110, ICC 14183 and ICC 5912 at 38^{0}C (Fig 3.4.1.1.2). The results at both the locations were consistent.

Table 3.4.1.1.2 Pollen functionality in respect to increasing order of temperatures under laboratory

Genotypes	Pollen viability (%)					Pollen germination (%)					Pollen tube growth (µm)				
	25^{0}C	30^{O}C	35^{O}C	38^{O}C	40^{0}C	25^{0}C	30^{O}C	35^{O}C	38^{O}C	40^{0}C	25^{0}C	30^{O}C	35^{O}C	38^{O}C	40^{0}C
ICC 14183 (sensitive)	93.2	95.3	89.1	70.2	46.3	92.4	92.6	86.4	69.2	53.1	77.1	80.1	68.4	55.4	35.2
ICC 5912 (sensitive)	95.6	92.3	86.3	68.3	44.3	91.6	94.2	88.3	71.2	58.4	84.2	82.2	65.3	58.9	38.2
ICCV 07110 (Tolerant)	96.2	93.4	89.4	77.4	56.1	94.3	93.6	88.7	80.3	66.9	78.6	82.9	68.9	64.8	48.6
ICCV 92944 (Tolerant)	94.3	96.4	88.3	83.4	61.3	93.2	94.1	91.6	84.3	82.1	80.4	84.1	71.1	67.6	56.9

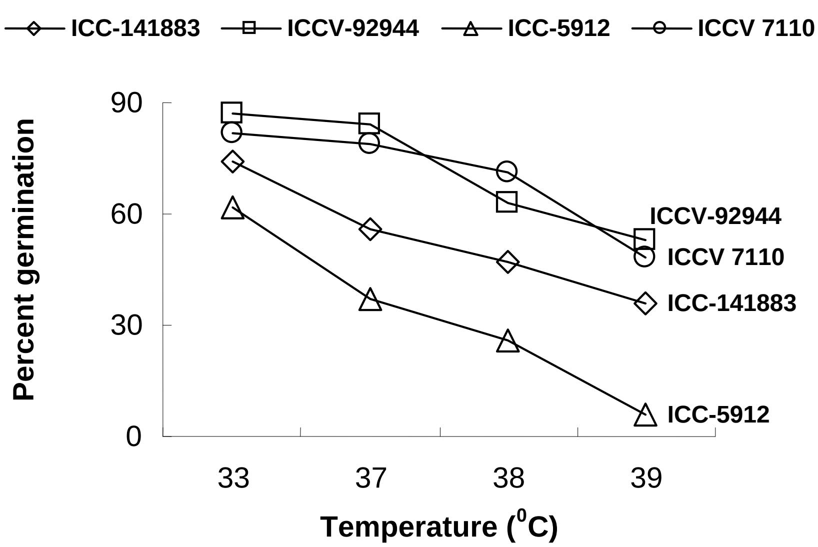
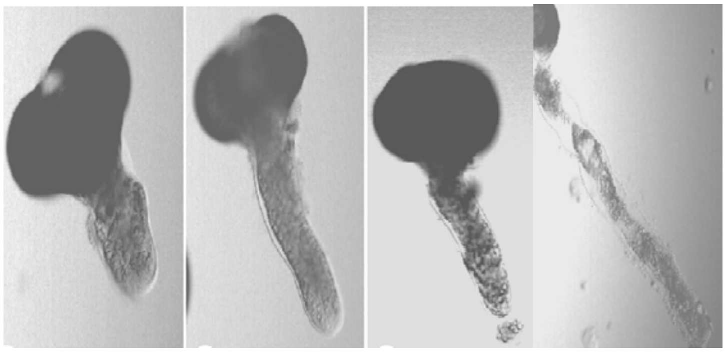


Fig. 3.4.1.1.1 Pollen germination in response to temperature



ICC 14183 (6 h) **ICCV 07110 (4 h)** **ICC 5912 (8 h)** **ICCV 92944 (2 h)**

Fig. 3.4.1.1.2 Length of pollen tubes at different incubation periods at 38^0 C

Pollen viability, pollen load and pollen germination (in vivo)

Under field condition, pollen of the flowers exposed to high temperature with a maximum/minimum, day/night temperatures approximately $38/25^0$C showed higher pollen viability (>80%), pollen load (29.3) and *in vivo* pollen germination (>1.0) in heat tolerant genotypes ICCV 07110 and ICCV 92944 compared to heat sensitive genotypes- ICC 14183 and ICC 5912 (Table 3.4.1.1.3). Under late-sown conditions, there was a decrease in stigma receptivity with maximum effect on ICC 14183 (4.3). The heat tolerant genotype ICCV 92944 showed highest receptivity (6.0) as compared to sensitive ones

Table 3.4.1.1.3 Pollen viability, pollen load and pollen germination (in vivo) in selected heat sensitive and tolerant lines

Accessions	**Pollen viability**		**Pollen load (no of pollen on stigma/pt)**		**In vivo pollen Germination (1-5 scale)**		**Stigma receptivity (1-10 scale)**	
	Approx Max/Min temperature during the period when flowers were collected							
	Normal $22/10^0$C	Late $38/25^0$C	Normal $22/10^0$C	Late $38/25^0$C	Normal $22/10^0$C	Late $38/25^0$C	Normal $22/10^0$C	Late $38/25^0$C
ICC 14183 (Sensitive)	94.2	78.6	27.66	17.7	2.3	0.7	5.3	4.3
ICC 5912 (Sensitive)	96.1	76.2	48.22	14.0	1.6	1.0	5.6	5.1
ICCV 07110 (Tolerant)	82.4	83.1	37.7	15.3	2.0	1.3	5.3	5.1
ICCV 92944 (Tolerant)	92.4	88.4	31.3	29.3	3.2	1.7	6.3	6.0

*Values are the averages of three replications. *(temperature at the time of sampling)

Yield parameters

Biomass, pod number, filled pods, seed weight and harvest index invariably decreased in all the test genotypes grown under late sown condition. Heat tolerant genotypes were characterized by less reduction in biomass, higher pod numbers, seed weight and harvest index at late sown, high day/night temperatures as compared to sensitive lines (Table 3.4.1.1.4).

Table 3.4.1.1.4 Yield parameters of chickpea genotypes under normal and late-sown conditions

Accessions	**Profile**	**Biomass (g/plant)**		**Pod No./plant**		**Filled Pods/plant**		**Seed wt./plant (g)**		**Harvest index**	
		N	L	N	L	N	L	N	L	N	L
ICC 14183	Sensitive	8.3	6.4	21	11	17	5	3.69	1.01	44.4	15
ICC 5912	Sensitive	10.2	4.3	31	9	19	3	2.89	0.39	28.2	9
ICCV 07110	Tolerant	8.4	5.8	17	10	17	10	4.92	1.90	58.6	33
ICCV 92944	Tolerant	9.7	5.1	16	15	17	14	3.47	2.37	46.0	37

Physiological and biochemical mechanisms of thermo-tolerance

Membrane damage

The damage to membranes increased (Fig. 3.4.1.1.3) with elevation of temperature. At 35 ^{0}C, the heat tolerant genotype ICCV 92944 experienced the lowest membrane damage while at 40^{0}C, another heat tolerant genotype ICCV 07110 had the least membrane damage while the heat sensitive genotypes experienced greater damage to membranes at these temperatures.

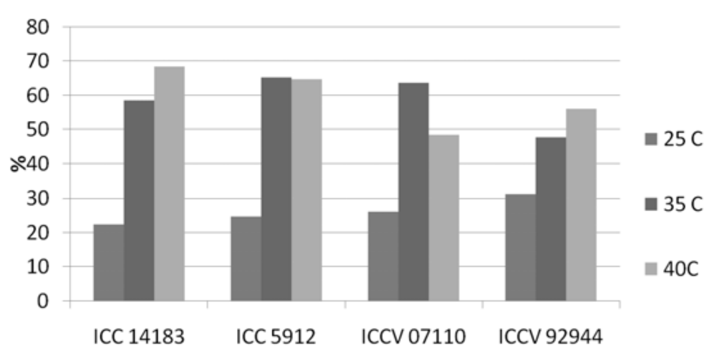


Fig. 3.4.1.1.3 Membrane damage in chickpea genotypes with respect to different temperatures

Membrane injury index

Electrolyte leakage of leaves was measured at temperature 40^{0}C (C_1) and subsequently the same at 100^{0}C (C_2). The ratio C_1/C_2 represented the membrane injury index (MII) which is shown in Table 3.4.1.1.5. The genotypic variation in the MII was evident. Out of four genotypes tested, ICCV 92944 and ICCV 07110 showed the lowest MII suggesting higher membrane stability at high temperature (Table 3.4.1.1.5)

Table 3.4.1.1.5 Membrane injury index (MII) of four chickpea genotypes

Genotypes	Membrane injury index (MII)
ICC-14183	0.123
ICCV-92944	0.084
ICC-5912	0.113
ICCV-07110	0.090

Total chlorophyll

With increase in temperature, the chlorophyll content (Fig. 3.4.1.1.4) showed reduction in all the genotypes. At 35 and 40^{0}C, heat tolerant genotype ICCV 92944 maintained greater chlorophyll content than other genotypes. The chlorophyll content in the other heat tolerant genotype (ICCV 07110) was observed to be almost similar to the heat sensitive genotypes.

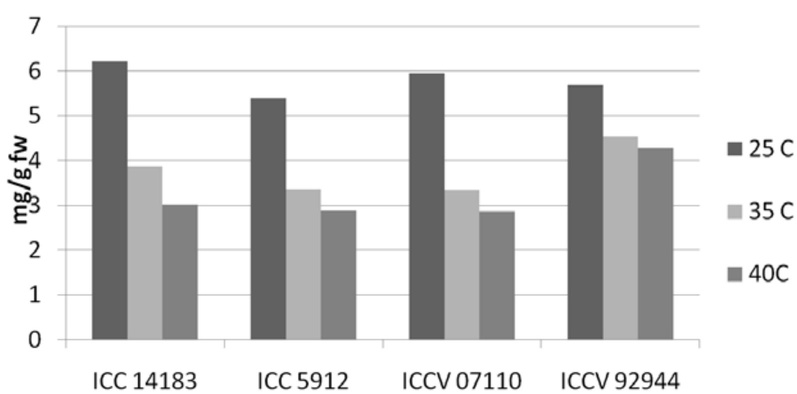


Fig. 3.4.1.1.4 Total chlorophyll content in chickpea genotypes with respect to different temperatures

Cellular respiration

The cellular respiration (Fig. 3.4.1.1.5) showed increase with elevation of temperature in all the genotypes. The heat sensitive genotypes experienced greater increase in respiration than the heat tolerant ones. At 40 ^{0}C, ICCV 07110 had the lowest respiration levels compared to other genotypes.

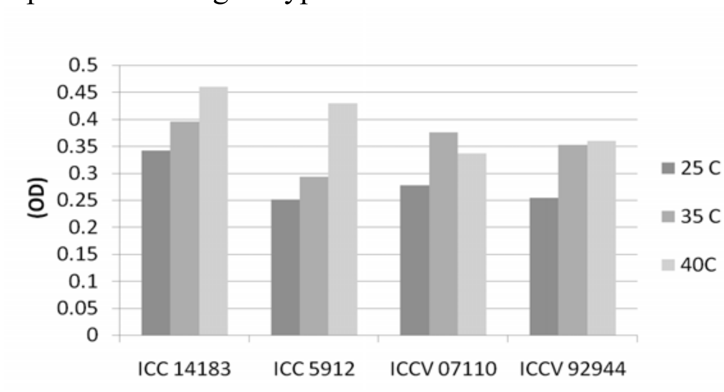


Fig. 3.4.1.1.5 Cellular respiration in chickpea genotypes with respect to different temperatures

Photosynthesis and chlorophyll fluorescence

Photosynthetic rates increased with temperature and reached to maximum level at 35^0 C and decreased above this temperature (Fig 3.4.1.1.6). The trend of fluorescence induction kinetics also confirmed that optimum photosynthesis in chickpea occurs at 35 ^{0}C (Fig 3.4.1.1.7). Leaves were incubated at temperatures 20, 25, 30, 35, 40, 45 and 50 ^{0}C for half an hour prior to the measurement of chlorophyll fluorescence. Minimal fluorescence (Fo) increased linearly with rising temperature suggesting conformational change in the membrane structural integrity or electron carriers (Fig. 3.4.1.1.8). In general, other important fluorescence parameters e.g. variable fluorescence (Fv; Fig. 3.4.1.1.9), quantum yield of PS II (Fig. 3.4.1.1.10) and ETR derived from quantum yield (Fv/Fm) (Fig. 3.4.1.1.11) decreased after a threshold temperature (35 ^{0}C). The results suggested that the optimum temperature for photosynthesis in chickpea falls within the range of 20 to 35 and beyond this the photochemical efficiency drastically declines.

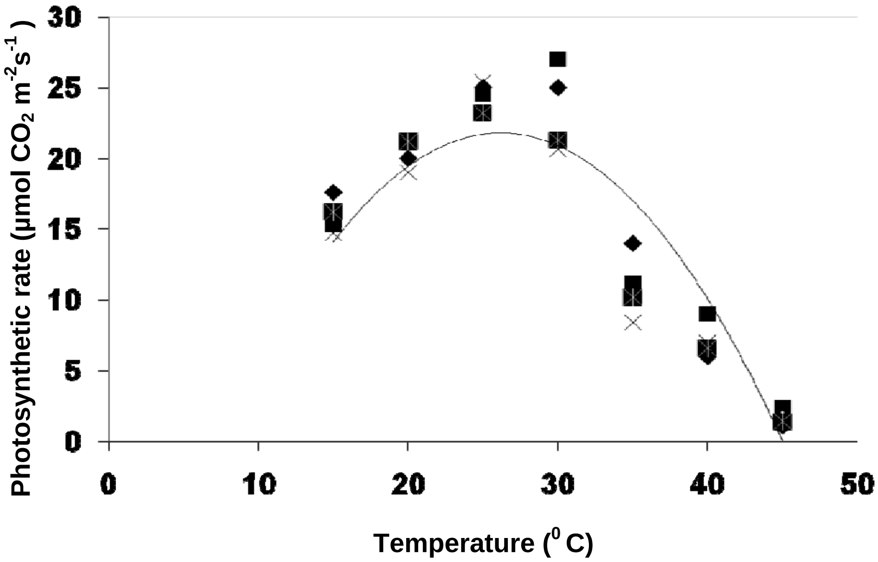


Fig. 3.4.1.1.6 Photosynthetic rates of chickpea with increasing temperatures

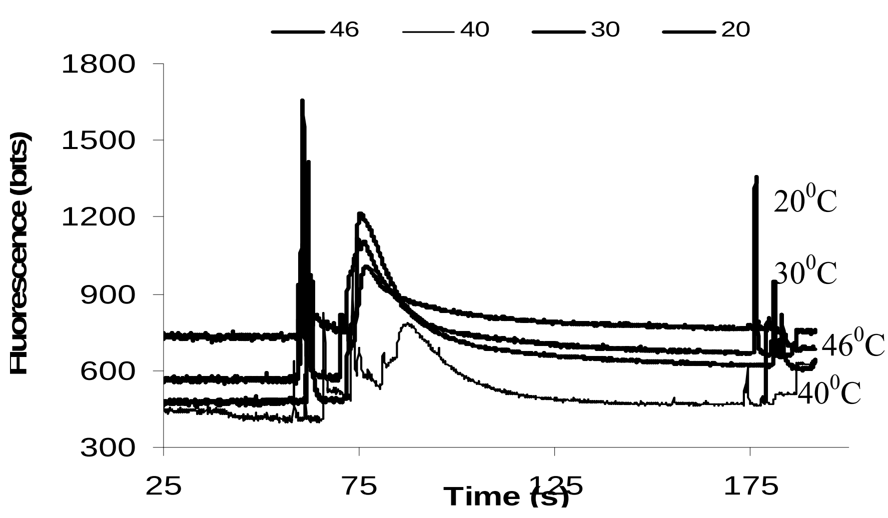


Fig. 3.4.1.1.7 Chlorophyll fluorescence induction kinetics at different temperatures

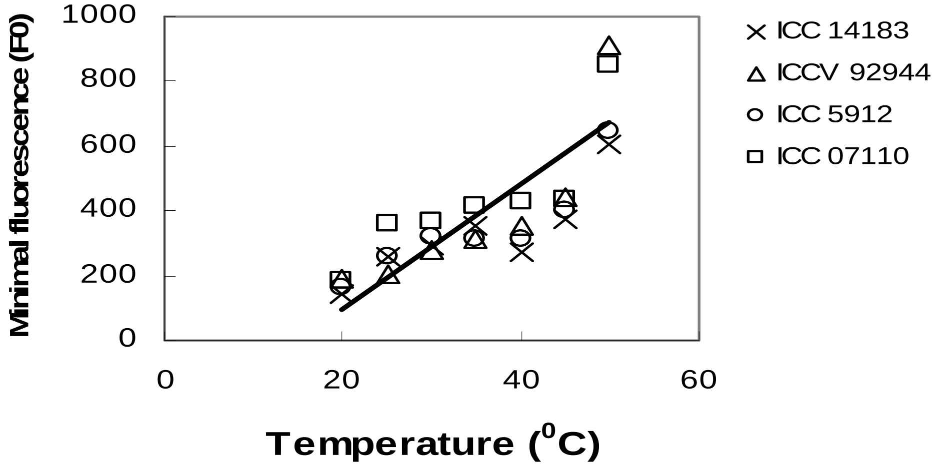


Fig. 3.4.1.1.8 Minimum fluorescence (Fo) with increasing temperatures

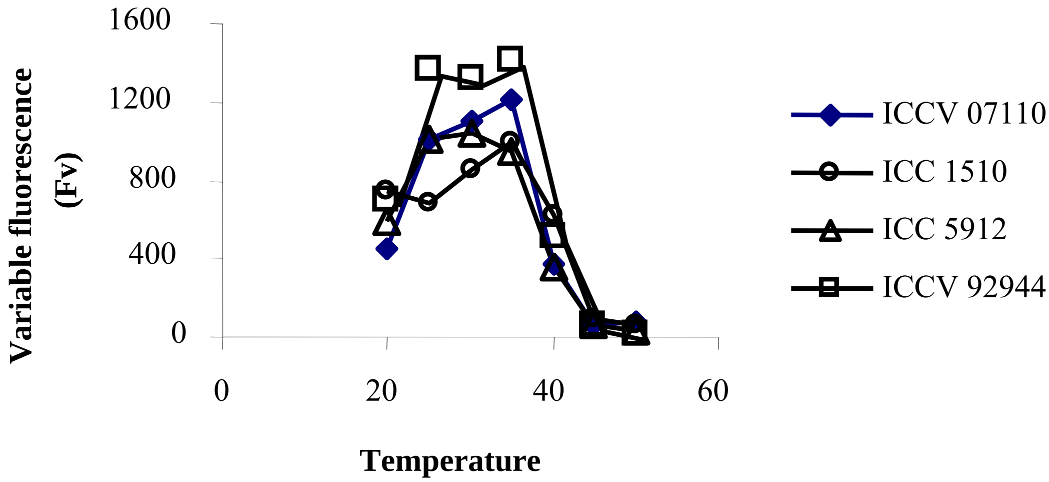


Fig. 3.4.1.1.9 Variable fluorescence (Fv) at different temperatures

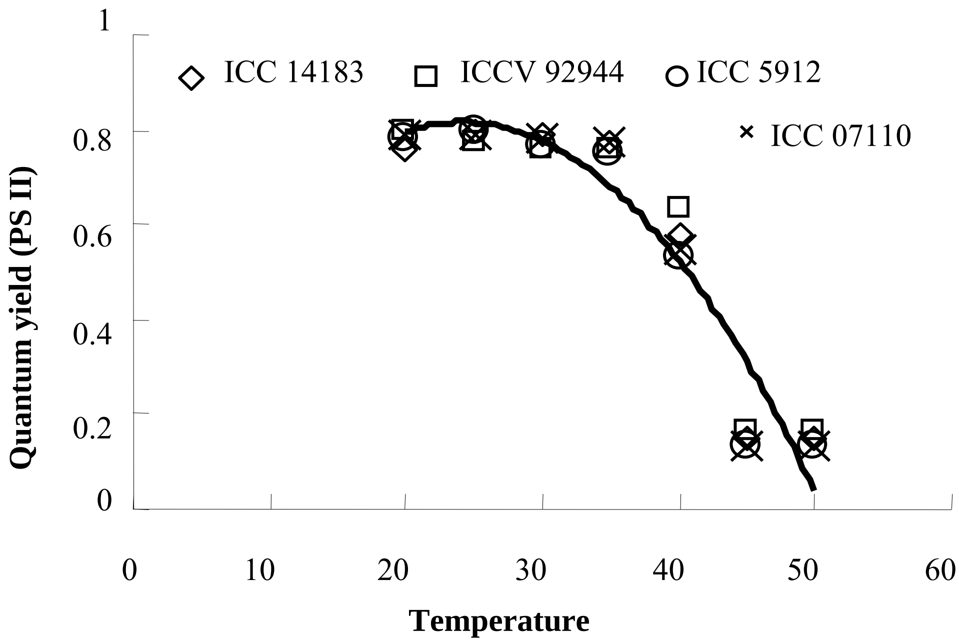


Fig. 3.4.1.1.10: Quantum yield (Fv/Fm) at different temperatures

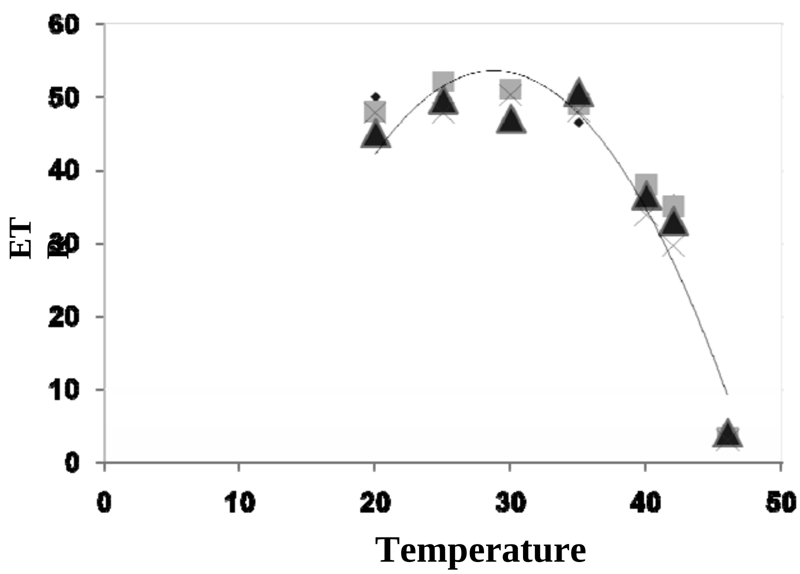


Fig. 3.4.1.1.11 Electron transport rate (ETR) at different temperatures

Table 3.4.1.1.6 Changes in the various fluorescence parameters with increasing temperatures

Temp (^{0}C)	F0	Fm	Fv	Fv/Fm	e'PS2	Qp	QNP	ETR
20	712	2697	1939	0.71	0.4	0.74	0.54	51.8
30	860	2750	1889	0.69	0.36	0.94	0.60	49.1
40	942	1867	1019	0.52	0.27	0.22	1.52	18.7
46	1215	1135	1203	0.11	0.04	0.14	2.32	4.6
CD 5%	45	275	112	0.11	0.06	0.12	0.02	3.5

Quantum yield of photosystem II has fairly good correlation with membrane stability (Fig 12) suggesting that transition changes in the photosynthesis could be due to alteration of the photosynthetic membrane, hence quantum yield (Fv/Fm) proved to be a very simple, rapid and non-destructive assessment of the leaves towards high temperature tolerance

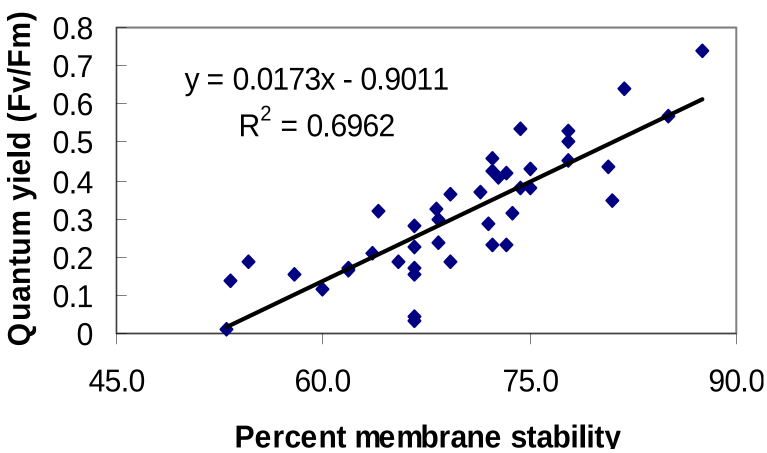


Fig. 3.4.1.1.12 Relationship with membrane stability with quantum yield (Fv/Fm)

Sucrose synthase and invertase activity

Seed size was found to be one of the worst affected components lowering the yield of chickpea beyond 35^{0}C. The size invariably decreased at high temperature (Fig. 3.4.1.1.14), although varietal differences in seed size were evident. The seed size is predominantly controlled by sucrose synthase activity in developing grains (Fig. 3.4.1.1.15).

The enzyme activity (Fig. 3.4.1.1.13) was assayed in both the leaves and seeds. Compared to the control (25^{0}C), the activity of enzymes increased at 35^{0}C but decreased at 40^{0}C in all the genotypes. At 35^{0}C, the heat tolerant genotype ICCV 07110 maintained higher activity levels in leaves and seeds while the other heat tolerant genotype ICCV 92944 possessed greater activity only in seeds. At 40^{0}C, both the heat tolerant genotypes had higher activity in leaves and seeds.

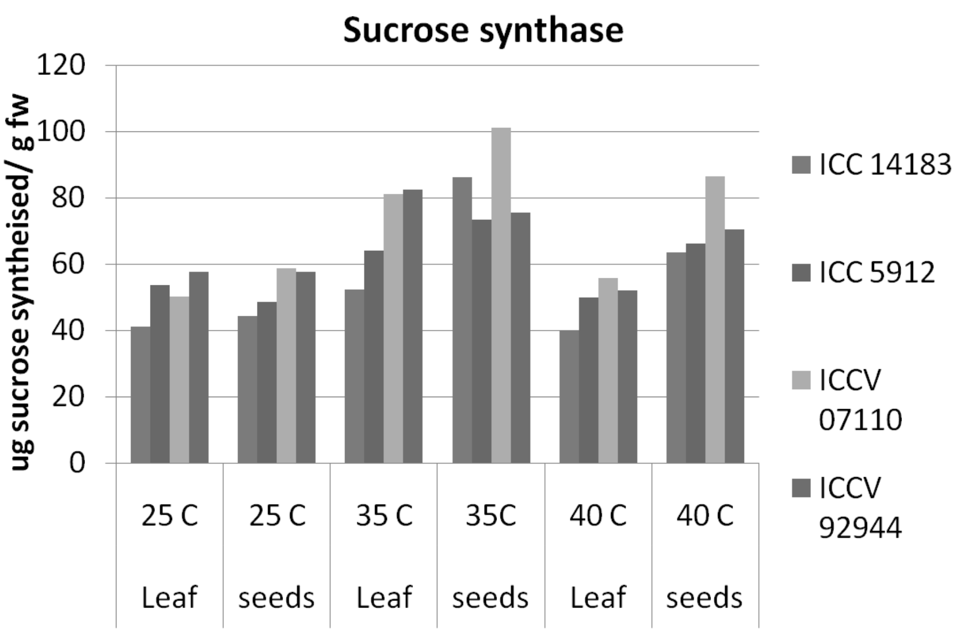
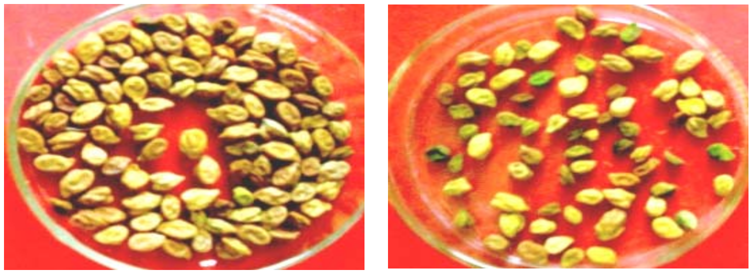


Fig. 3.4.1.1.13 Sucrose synthase activity in chickpea genotypes with respect to different temperatures

Sucrose synthase and invertase activity measured in cotyledons of all the four chickpea genotypes grown under late sown high temperature conditions. The three genotypes (ICC 14183, ICCV 92944 and ICCV 07110) having large seed size possessed higher sucrose synthase which contributed towards rapid grain development (Table 7). On the contrary, lower sucrose synthase activity was shown by ICC 5912 but had higher invertase activity. The antagonistic features i.e lower sucrose synthase and higher invertase activity retards growth of grain and consequently seeds remain smaller or underdeveloped.



Normal **Late**

Fig.3.4.1.1. 14 Reduction in seed size under late sown high temperature condition

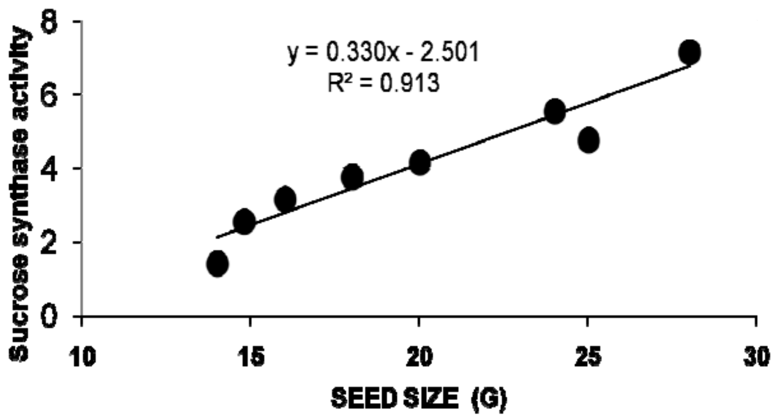


Fig. 3.4.1.1.15 Relationship with seed size and sucrose synthase activity

Table 3.4.1.1.7 Sucrose synthase and invertase activity in developing cotyledons of four chickpea genotypes

Genotype	Sucrose synthase	Invertase
	(nmol sucrose mg^{-1} protein h^{-1})	(nmole hexose mg^{-1} protein h^{-1})
ICC 14183	4.70	2.90
ICCV 92944	5.84	3.89
ICC 5912	1.40	6.45
ICCV 07110	4.40	3.95

The pollen germination in chickpea was found to be relatively stable up to 35^0C. However, two heat tolerant genotypes ICCV 92944 and ICCV 7110 showed about 60% germination even at 39^0C. While sensitive line ICC 5912 showed almost negligible germination beyond 33^0C. Based on the yield parameters at high temperatures, both heat tolerant lines ICCV 07110 and ICCV 92944 were found superior over sensitive lines. Besides inherent mechanisms of heat tolerance, less thermal time for phenological development such as flowering and pod setting was associated with the best strategy to avoid terminal stress as shown by heat tolerant lines. At cellular level, membrane injury index was found to have good correlation with heat tolerance with low value indicate higher tolerance to heat. Genotypes with large seed size appear to be more heat tolerant being attributed by higher sucrose synthase activity enabling to draw photosynthates with faster rate. Photosynthesis in chickpea was found to be stable within the temperature range of 20-35^0C. The genotypic variation in chlorophyll fluorescence was evident; however screening technique based on fluorescence parameters is yet to be developed.

3.4.1.2 Studies on reproductive biology of heat tolerant and sensitive lines at ICRISAT-Patancheru

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, (17.53°N; 78.27°E; 545m) India.

Controlled environment experiment

The two chickpea genotypes were grown in a controlled environment with five replications. 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). The phenology of the two genotypes was different. To overcome this problem, staggered sowing was conducted in the glasshouse. The plants were grown at 28/160C in a greenhouse and transferred to a growth room at the first appearance of flowers to expose them to high temperature. The plants used as a non-stressed control continued to grow in the glasshouse at 28/160C. The temperature in the growth room was increased daily by 1ºC, e.g. 29 to 40^0C during day and 16 to 25^0C during night (Table 3.4.1.2.1). Therefore, the plants were exposed to a gradual increase in temperature.

Experiment-1

The effects of a one day exposure to temperature from $31/16^0$C to $35/20^0$C during pre-anthesis were studied to determine the critical temperature. One day before anthesis (pre-anthesis), five flower buds were collected between 08:00 and 08:15 h from $31/16^0$C to $35/20^0$C to examine pollen viability. The heat tolerant genotype was examined at $40/25^0$C (extreme temperature) for pollen viability. Anthers stained with Alexander stain and examined under electron microscope are shown in Fig. 3.4.1.2.1 and Fig. 3.4.1.2.2. The fertile pollen grains inside the anthers were red in colour and the sterile pollen grains were green. Flowers at $35/20^0$C were tagged to observe the pod set. ANOVA was performed for flower data using Genstat (12^{th} Ed. VSN International Ltd).

Experiment-2

Following experiment-1, hand pollination was used in the $35/20^0$C treatment to achieve pollination (post-anthesis). Five flowers were collected 15 and 30mins after pollination to observe the pollen germination on the stigma and pollen tube growth through the style. The flowers were fixed for 24 h in 80% alcohol. The pistils (styles and ovary) were removed from the flowers, cleared with 6N NaOH for 48 h and thoroughly rinsed with water. The pistils were stained with aniline blue and observed under a fluorescence microscope.

Table 3.4.1.2.1 Details of temperature regime, pre-anthesis, and post-anthesis of chickpea flower collection under controlled environments

Days	Temp regime (day and night - ºC)	Flower buds collected one day before anthesis to check critical temperature (pollen viability)	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

Controlled environment experiment

The one day exposure of flower buds to high temperature influenced pollen viability. In ICC 5912, the pollen grains inside the anthers were fertile up to $34/19^0$C (Fig. 3.4.1.2.1a). But, at $35/20^0$C, all pollen grains inside the anther became sterile (Fig. 3.4.1.2.1b). This difference was similar in all 10 anthers positioned inside a flower. In addition, there was no pod set in ICC 5912 at $35/20^0$C. In ICCV 92944, the high temperature ($35/20^0$C) did not influence pollen fertility and the pollen grains were fertile (Fig. 3.4.1.2.2b). At $40/25^0$C, the pollen grains inside the anther were partially sterile in ICCV 92944 (Fig.3.4.1.2.2c). Therefore, the critical temperature for pod set in ICC 5912 was $35/20^0$C. The pollen viability in chickpea was affected by high temperature ($\geq 35^0$C), which concurs with Prasad et al (1999). Similarly, the pod set and yield in chickpea was reduced and associated with poor pollen viability due to exposure to high temperatures at microsporogenesis (Warrag and Hall 1984). Pollen germination and pollen tube growth was found in ICCV 92944 at 35/20^{0}C (Fig. 3a, 3b). Though the pollen tube had callus formation, the tube reached the mouth of the ovary in 30mins (Fig. 3.4.1.2.3c). However there was no pollen germination on the stigma of ICC 5912 at 35/20^{0}C. At 35/20^{0}C, ICC 5912 had reduced pod set (*P*<0.001) compared with ICCV 92944 and the control (28/16^{0}C) (Table 3.4.1.2.2). These results confirm the heat tolerance nature of ICCV 92944 and heat sensitivity of ICC 5912. The yield reduction was linked with the pollen trait. Consequently, there is potential for developing the screening techniques for heat tolerance in the field and in the controlled environments for chickpea breeding programs using the differences in pollen viability. There is also a possibility of using a pollen selection method in breeding for heat tolerance. Further research is needed to investigate more germplasm and to confirm these results.

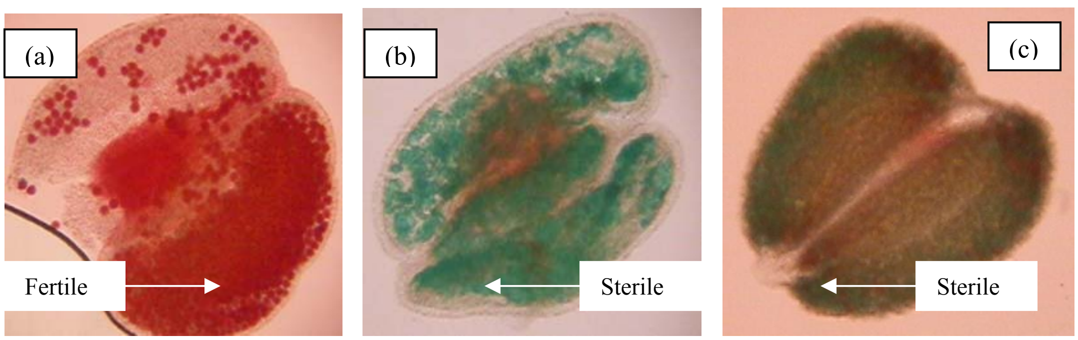


Fig. 3.4.1.2.1 ICC5912 (a) Anther-34/19ºC (pollen grains are fertile) (b) Anther-35/20ºC (pollen grains are sterile) (c) Anther-40/25ºC (pollen grains are sterile) (Fertile pollen grains are red; Sterile pollen grains are green)

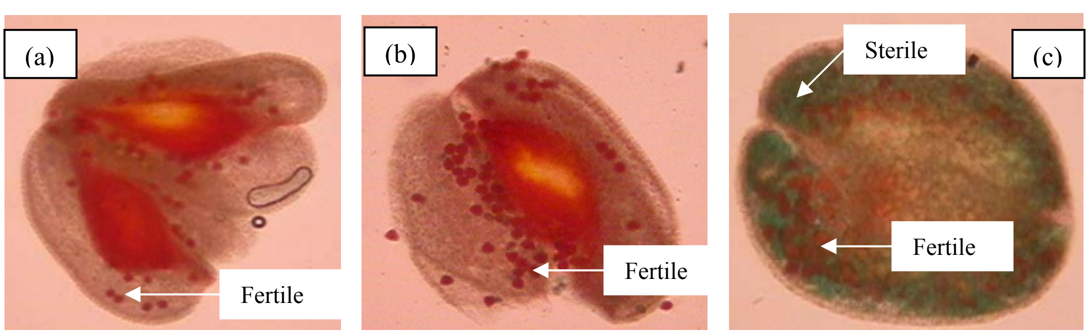


Fig. 3.4.1.2.2 ICCV92944 (a) Anther-34/19ºC (pollen grains are fertile) (b) Anther-35/20ºC (pollen grains are fertile) (c) Anther-40/25ºC (pollen grains are fertile and sterile) (Fertile pollen grains are red; Sterile pollen grains are green)

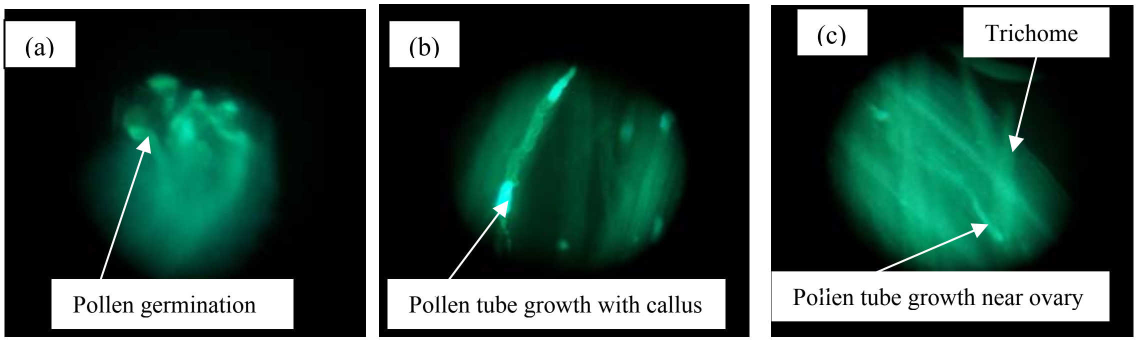


Fig. 3.4.1.2.3 ICCV92944-(a) Pollen germination on stigma 15min after pollination (b) Pollen tube growth on style with callus 15mins after pollination (c) Pollen tube growth near the mouth of the ovary 30mins after pollination (Trichome is present at the mouth of the ovary)

Table 3.4.1.2.2 Comparison of high (35/20º C) and optimum (28/16º C) temperature on flowers under controlled environments (Significant difference at ***P*<0.01; ****P*<0.001 respectively and NS-Not significant; Means followed by different letters within the same column are significantly different at *P*=0.05)

Genotypes	Temp regime (day/night) – (ºC)	Total no of flowers	No of dry flowers	% of dry flowers	Total no of pods	% of dry pods	% of pod set (retain pods)
ICCV92944	28/16	14	1	7a	13	7	99a
ICCV92944	35/20	9	2	10a	7	1	93b
ICC5912	28/16	12	1	10a	11	3	87c
ICC5912	35/20	6	6	100b	0	0	0d
Temperature effect							
28/16		13	1a	9a	10a	2	93a
35/20		7	4b	60b	6b	4	46b
Genotype effect							
ICCV92944		12	1a	13a	12	4	96a
ICC5912		9	4b	55b	4	1	44b
Temperature LSD (*P*=0.05)		NS	** (1.4)	*** (7.9)	** (4.0)	NS	*** (3.7)
Genotype LSD (*P*=0.05)		NS	** (1.4)	*** (7.9)	NS	NS	*** (3.7)
Genotype x Temperature LSD (*P*=0.05)		NS	NS	*** (11.2)	NS	NS	*** (5.3)

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

Hot days (≥35º C) and warm night (≥20º C) temperature can potentially reduce the pollen viability and subsequent pod set in heat sensitive chickpea genotype. Reduced pod set was a consequence of poor pollen viability. The critical temperature for pollen viability was ≥35º C in the heat sensitive chickpea genotype. Therefore, pollen viability at high temperatures is a possible indirect selection criterion to improve the heat stress tolerance in chickpea.

3.4.2 Genetics of Heat Tolerance

The crosses outlined in Table 3.4.2.1 were performed to understand the genetic mechanisms involved in providing heat tolerance in chickpea. The crosses were performed at ICRISAT, Patancheru. The crosses were carried out between: (i) Susceptible X Susceptible; (ii) Tolerant X Tolerant and; (iii) Susceptible X Tolerant genotypes. The F1 seeds have also been harvested for most of these crosses.

Table 3.4.2.1 Crosses performed between susceptible and tolerant genotypes at Jabalpur and ICRISAT, Patancheru locations

S.No	Location	Crosses
1	ICRISAT, Patancheru	ICC 4567 (S) X ICC1205 (T)
		ICC 4567 (S) X ICC 8950 (T)
		ICC 10685 (S) X ICC 1205 (T)
		ICC 10685 (S) X ICC 8950 (T)
		ICC 10685 (S) X ICC 15614(T)
		ICC 10685 (S) X ICC 1356 (T)
		ICC 4567 (S) X ICC 15614 (T)
		ICC 4567 (S) X ICC 1356 (T)
		ICC 1205 (T) X ICC 1356 (T)
		ICC 1356 (T) X ICC 15614 (T)
		ICC 4567 (S) X ICC 10685 (S)

3.5 Objective 4: Identify molecular markers for gene(s) controlling heat tolerance.

3.5.1 Identification of molecular markers for heat tolerance using association mapping:

The reference set of chickpea has been screened for heat tolerance (Section 3.3.1). This reference set has been genotyped using large number of SSR markers in other projects. These datasets are being used for association analysis.

3.5.2 Identification of molecular markers using linkage mapping: Of the eight sensitive x tolerant crosses made (Table 3.4.2.1), one cross will be used for development of recombinant inbred lines (RILs). These RILs will be late genotyped and phenotyped for heat tolerance for molecular mapping of heat tolerance genes(s).

3.6 Objective 5: Introgress heat tolerance in selected cultivars/elite breeding lines

Two heat tolerant genotypes, ICCV 92944 (JG 14) and ICCV 07110, genotypes were crossed with the leading cultivars/elite lines to introgress the heat tolerant trait. The center-wise crosses made are listed Table 3.6.1.

Table 3.6.1: Introgession crosses carried out different locations

Location	Crosses
ICRISAT-Patancheru	JG 11 X ICCV 07110
	JG 11 X JG 14
	ICCC 37 X ICCV 07110
	ICCC 37 X JG 14
	(ICCC 37 x JG 130) x JG 14
	(ICCV 10 x GG 2) x JG 14
	(JG 11 x JAKI 9218) x JG 14
	(JG 16 x JAKI 9218) x JG 14
IIPR-Kanpur	DCP 92-3 x JG 14
	DCP 92-3 x ICCV 07110
	KWR 108 x JG 14
	KWR 108 x ICCV 07110
JNKVV-Jabalpur	JG 63 X JG 14
	JG 16 X JG 14
	JG 130 X JG 14
	JG 11 X JG 14
	JG 12 X JG 14
	JAKI 9218 X JG 14
	JG 63 X ICCV 07110
	JG 16 X ICCV 07110
	JG 11 X ICCV 07110
	JG 12 X ICCV 07110
RARS-Nandyal	JG 11 x JG 14
	JG 11 x ICCV 07110
	JAKI 9218 x JG 14
	JAKI 9218 x ICCV 07110

3.7 Objective 6: Evaluate selected heat tolerant lines at farmers fields.

ICCV 92944e, an early maturing heat tolerant desi chickpea breeding line identified in this project, has been released as JG 14 in Madhya Pradesh during 2008 for late sown conditions. Thus, this variety was evaluated at farmers' field to assess its performance under late sown conditions and to know farmers' perception about this variety. JG 14 was grown at four locations in Fatehpur and Ramabai Nagar districts of Uttar Pradesh under late-sown conditions during 2010-11 crop season. The common cropping systems in these two districts include rice-wheat, rice-wheat-mungbean, rice-chickpea, fallow-chickpea/mustard, sesame/urdbean-chickpea/wheat and short duration pigeonpea-wheat.

In canal irrigated area, farmers usually grow long duration rice which is harvested during late November or mid-December. The farmers generally grow wheat after rice in these areas as wheat varieties suitable for late-sown conditions are available. Availability of heat tolerant varieties of chickpea, such as JG 14, can provide opportunity for rice-chickpea crop rotation. The cereal-legume crop rotation will be better than cereal-cereal (rice-wheat) cropping system for long term sustainability of soil fertility and system productivity.

The heat tolerant variety JG 14 was sown during 22-24 December 2009 at farmers' fields. The crop received rains during the first fortnight of February, therefore supplemental irrigation was not required. The grain average grain yield of 1720 kg/ha and 1672 kg/ha was obtained in Fatehpur and Ramabai Nagar districts, respectively (Table). The results indicate huge potential for popularization of heat tolerant varieties in such late-sown conditions. IIPR Kanpur has purchased 50 kg seeds from these farmers' for distribution among another group of farmers in the same districts during 2010-11.

Table 3.7.1: Performance of heat tolerant variety JG 14 under late sown conditions in Fatehpur and Ramabai Nagar districts of Uttar Pradesh

Fatehpur District					
Farmers' Name	**Village**	**Area sown (mt^2)**	**Date of sowing**	**Date of harvesting**	**Yield (kg/ha)**
Sri Onkar Nath Singh	Sai	1200	22-12-2009	29-03-2010	1760
Sri Ram Prakash Singh	Harsingpur	1200	23-12-2009	28-03-2010	1680
				Average grain yield :	**1720 kg/ha**
Ramabai Nagar District					
Farmers' Name	**Village**	**Area sown (mt^2)**	**Date of sowing**	**Date of harvesting**	**Yield (kg/ha)**
Sri Anil Mishra	Kuint Kheda	1200	24-12-2009	31-03-2010	1620
Sri Santosh Tewari	Kuint Kheda	1200	24-12-2009	31-03-2010	1725
				Average grain yield :	**1672 kg/ha**

3.8 References

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