

## TERMINAL HEAT STRESS ADVERSELY AFFECTS CHICKPEA PRODUCTIVITY IN NORTHERN INDIA— STRATEGIES TO IMPROVE THERMOTOLERANCE IN THE CROP UNDER CLIMATE CHANGE

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### ABSTRACT:

Chickpea (*Cicer arietinum* L.) is a cool-season legume well adapted within temperature range of 30/15<sup>0</sup>C (day maximum and night minimum) for optimum growth and pod filling. The northern plains of India once represented a potential production zone for chickpea due to long winter favouring high biomass production and pod filling. However, the crop in this region is now adversely affected by climatic change, showing a trend of increasing minimum night temperature more than that of maximum day temperature. The asymmetric pattern of temperature rise resulted in a warmer winter, less dew precipitation and heavy evapo-transpirational water loss. The crop often experiences abnormally high temperature (>35<sup>0</sup> C) and atmospheric drought during reproductive stage. The chickpea varieties are now gradually replaced by newly bred short duration varieties escaping terminal heat, or breeding for heat tolerance has been initiated to enhance productivity. A large number of germplasm were physiologically characterized for thermo tolerance and screening techniques developed based on membrane stability, photosynthetic efficiency (quantum yield, ratio of variable to maximal chlorophyll fluorescence Fv/Fm) and pollen germinability. The foliar resistance was much higher (above 40<sup>0</sup> C) than reproductive component like pollen germination (usually occurs below 35<sup>0</sup>C). The fluorescence inductions kinetics showed a large differences in fluorescence peaks and quenching pattern when leaves pretreated at 20, 30 40 and 46<sup>0</sup>C with an irreversible damage of photosynthetic systems at 46<sup>0</sup>C. Membrane stability was significantly correlated (R<sup>2</sup>= 0.7) with quantum yield (Fv/Fm) and proved to be viable screening technique for thermo tolerance combined with pollen germinability at high temperatures.

### 1. INTRODUCTION

South Asia and particularly Indo-gangetic plains of India is most vulnerable to climate change. Latest projections indicate that after 2050, temperatures would rise by 3-4 degrees over current levels. Major impacts of climate change will be on rain fed crops including pulses which account for nearly 60% of cropland area. Almost all cool-season winter legumes under northern plains are gradually shifting towards “Warm winter”. This is primarily because of - asymmetric pattern of warming that is night-time minimums increasing more rapidly than daytime maximums.

Among pulses, chickpea (*Cicer arietinum* L.) is an important protein rich cool-season food legume grown under rainfed conditions in northern plains of India during winter. This regions under rainfed are now severely threatened by climatic changes responsible for recurrent incidence of foggy weather and abnormally less dew precipitation. This dew water is highly beneficial for chickpea for biomass production before onset of reproductive phase. The crop often experiences abnormally high temperature (>35<sup>0</sup>C) during reproductive phase.

High temperature adversely affects seed germination, photosynthesis, respiration, membrane stability, fertilization, fruit maturation, quality of seeds, nutrient absorption, protoplasmic

movement, transport of materials and also modulated level of hormones and primary and secondary metabolites (Chen et al, 1982, Fowden et al., 1993 and Wahid et al., 2007). Summerfield et al (1984) observed lower grain yields with greater exposure to hot days (30-35<sup>0</sup>C), during the reproductive period.

Heat stress at reproductive stage is thus increasingly becoming a serious constraint to chickpea production in northern India due to climate change. Two major objectives were set in order to address the impact of climate change on chickpea productivity i.e. assessment of high temperature on possible yield loss, development of screening techniques and physiological characterization for heat tolerance.

### 2. MATERIALS AND METHODS

A large set of chickpea germplasm including 211 Minicore collections from International Crops Research Institute for semi-arid tropics (ICRISAT), Patancheru, India, advanced breeding lines and released varieties were assessed for heat tolerance. Genotypes were sown under normal cool (15<sup>th</sup> November, winter) and late high (15<sup>th</sup> January, Spring) temperature condition under irrigated conditions in order to create two different temperature regimes.

Visual observation on forced maturity were recorded when temperature exceeded beyond 35<sup>0</sup> C'. The above ground biomass

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and seed size were compared in each genotype grown under normal and late sown condition. Under late sown conditions, at least 10 flowers were tagged when daytime maximum temperature exceeded beyond 35°C and after ten days pods were removed from the plants to ensure pod setting.

### 2.1 Membrane Injury Test

The fourth leaf from top was sampled for membrane injury test through electrolyte leakage. The certain amount of leaf materials were washed with distill water, surface dried between the fold of filter paper and dipped into deionized water for 20°C for 1 h. Measured the electrical conductivity (EC) of tissue leachets by using Systronic conductivity meter model 302. The same water was repeatedly used with same leaf dipped for 1 hour for 40, 50, 60, 70°C followed by 100°C and EC measured. The relative membrane stability or injury index at each temperature was calculated by the formula given by Blum and Ebercon (1981).

Membrane Stability or injury index =  $C_1/C_2$

$C_1$  = Electrical conductivity (EC  $\mu$ S) at test temperature for 1h.

$C_2$  = Electrical conductivity (EC  $\mu$ S) at 100°C for 1 h

### 2.2 Chlorophyll Fluorescence Measurement

Chlorophyll fluorescence of the leaves was measured by using Pulse amplified modulated fluorometer (*FMS-2 model of Hansatech, U.K*) according to Schreiber *et al.* (1986). Intact leaf dipped in water in a test tube maintained at different temperatures e.g. 20, 30, 40, and 46°C in water bath for 30 min prior to fluorescence measurements. Minimal fluorescence  $F_o$  of the dark adapted leaves was measured by exciting the leaf with weak modulated radiation (LED<sub>655</sub>) of 0.15  $\mu$ mol  $m^{-2}s^{-1}$  at frequency of 0.6 kHz. Thereafter, saturation pulse of 4000  $\mu$ mol  $m^{-2}s^{-1}$  was applied through fibre optic cable for 400 milliseconds to obtain maximal fluorescence,  $F_m$ . Quantum yield ( $F_v/F_m$ ) was calculated automatically by using the formula  $(F_m - F_o)/F_m$ , where  $F_v$  is the difference between  $F_m$  and  $F_o$  i.e.  $F_m - F_o$ . Soon after the  $F_v/F_m$  measurement in dark-adapted leaf, samples were irradiated by actinic radiation (8 V/20 W halogen lamp). The leaves were gradually exposed to higher irradiance from 55, 134, 260, and 440  $\mu$ mol  $m^{-2}s^{-1}$  and fluorescence induction kinetics were monitored for each level of light and temperature. Saturation pulse was triggered after every 1 min light acclimation in correspondence to each irradiance to obtain quantum yield  $F_v'/F_m'$  (e'PS2) in light.

Relative electron transport rate (*ETR*) of the leaf sample was determined by using formula as given below:

$ETR = \text{Quantum yield} \times \text{PAR} \times 0.5 \times \text{ETR FACTOR}$ , Where ETR factor = 0.84. This factor corresponds to the fraction of incident radiation absorbed by various leaf species.

### 2.3 Fluorescence Induction Kinetics

PAM was connected with PC to monitor fast fluorescence induction kinetics using FMS 2.0 software and data were analyzed for graphical presentation using MS Excel.

### 2.4 Pollen Viability Experiment

The Mercado *et al* (1994) growth medium for pollen viability having 10% sucrose, 0.1 mmol boric acid and one mmol calcium chloride was used. For gelling of the medium, 1% agar was used. Pollen grains obtained from heat treated and check groups of flowers were grown in Petri dishes at 25°C. Each treatment was replicated three times and ANOVA was performed.

### 2.5 Observation of Pollen Tubes Inside the Style

Specimens of style and ovary tissues were passed through the following solutions (10% sucrose, 0.1 mmol boric acid and one mmol calcium chloride). Fixation for 24 hours in a fixative containing formalin, 80% ethanol and glacial acetic acid (1:8:1). Rinsing for 24 h with tap water. Transferred to 8N NaOH solution for 8-24 h. Rinsing for 24 h with tap water. Staining with aniline blue dye solution for 4 h (Martin, 1986)

## 3. RESULTS AND DISCUSSION

Nearly two decades of temperature data at Kanpur on maximum/minimum temperature (January-February) showed that minimum temperature during winter night increases more than the day-time maximum (Figure 1). During reproductive stage, the day temperature maximum in the month of March 2008, abruptly increased to 41°C which was detrimental to pod setting and abortion (Figure 2). The maximum day temperature normally during grain filling stage in March rarely exceeds above 35°C. However, for the past 2-3 years the maximum day time temperature during March increased beyond 41°C which is highly detrimental for chickpea causing forced abortion of the flower and pods.

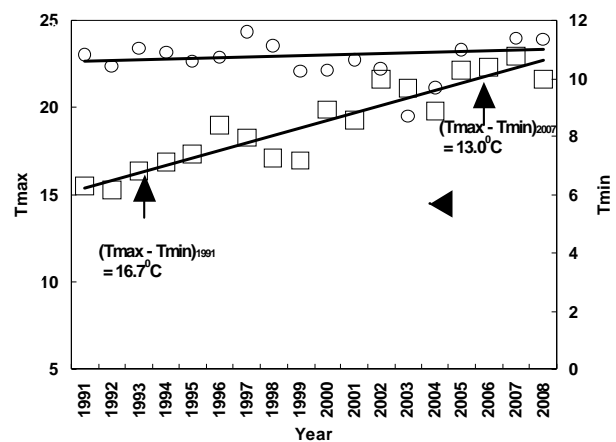


Figure 1. Mean Temperature Data (Tmax/Tmin) of January-February of Kanpur, India for the Last 18 Year

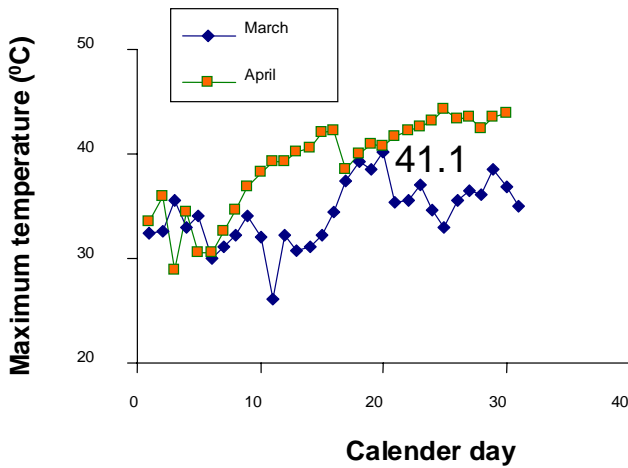


Figure 2. Maximum Day Temperature During March-April, 2008

Most of the genotypes could not set pods and pollen viability reduced when temperature touches to 35°C. The pollen viability and germination was extremely sensitive to high temperature (>35°C), though a wide genotypic variation in the pollen germinability was observed (Figure 3).

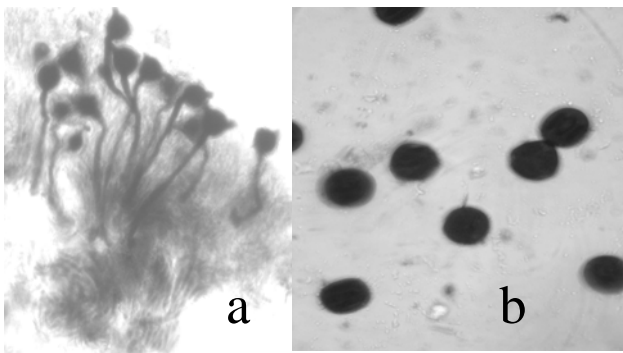


Figure 3. Pollen Germination Inside Stigma at 37°C of Heat Tolerant (a) and Sensitive (b) Genotype

More than 90% of the chickpea population failed to set pods when temperature suddenly increased to 41°C during March under late sown conditions. Few of them like JG 74 could able to set pods

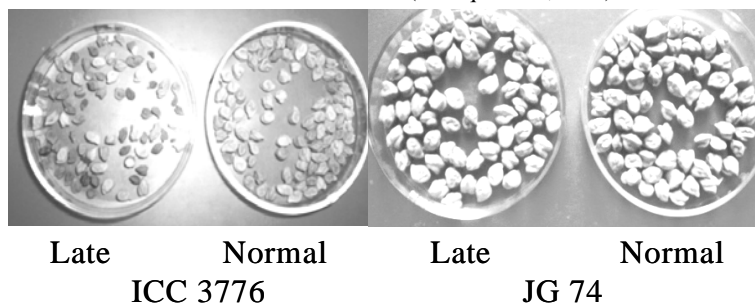


Figure 5. Deformed and Reduced Seed Size in ICC 3776 Under Late Sown High Temperature Conditions while Seeds of JG 74 Remain Unchanged

with normal seed size (Figure 5), however, majority of genotypes like ICC 3776 had reduced, shriveled or deformed grains at high temperatures exceeding 35°C (Figure 4). The critical temperature range for damage of reproductive organs was found somewhere in between 35-40°C, however, sensitivity varied among genotypes. Pollen germination in other crops has been reported to be higher vulnerability at high temperatures, pollen germination and the degree of pollen tube growth is reduced significantly (Kakani et al., 2005). Pollen germination in pepper is drastically reduced when plants are grown in 38°C as compared to 25°C (Kafizadeh et al., 2008).



Figure 4. Variations in the Seed Size and Morphology among Chickpea Genotypes Exposed to High Temperature >35°C

Earlier reports suggest that brief exposure of plants to high temperatures during seed filling accelerate senescence, diminish seed set and seed weight and reduce yield (Siddique *et al.*, 1999). Pulse legumes are particularly sensitive to heat stress at the bloom stage; only a few days of exposure to high temperatures (30-35°C) can cause heavy yield losses through flower drop or pod abortion (Siddique et al., 1999).

The results showed that foliar resistance to heat stress in chickpea was much higher than the tolerance of reproductive parts. The electrical conductivity (EC) increased as a result of rise in the temperature (Figure 6), indicating the membrane injury due to heat shock, however sensitivity of genotypes differed.

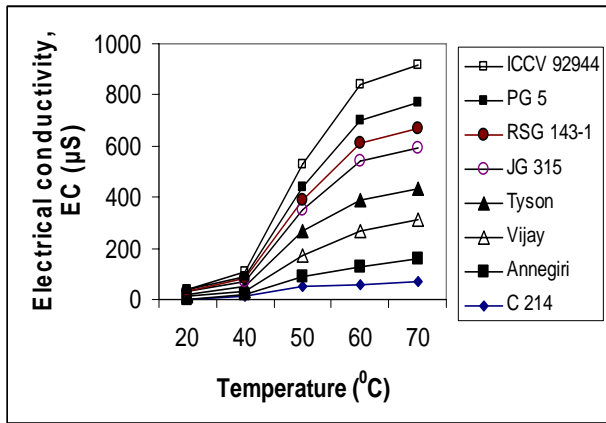


Figure 6. Electrolyte Leakage (EC) in Relation to Temperatures among Different Chickpea Genotypes

The relative membrane stability among genotypes and their tolerance to heat could be worked out on the basis of membrane injury index as described by Blum and Ebercon (1981) (data not presented). Sullivan and Ross (1979) suggested that electrolyte leakage from leaf following a heat shock can serve as a simple, rapid and reliable technique for measuring heat tolerance. The membrane thermo sensitivity test has been used most frequently as a technique to screen for heat tolerance in cool-season food legumes.

The quantum yield (Fv/Fm) measured by modulated chlorophyll fluorescence system did not show any reduction at this temperature (35°C) (Figure 7). The reduction in the quantum yield (e'PS2) in light-adapted leaf of chickpea was noticed when treatment temperature reached to 46°C (Figure 7).

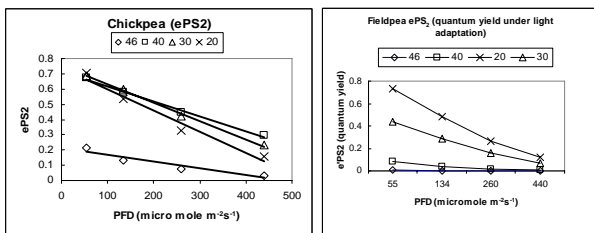


Figure 7. Light-adapted Quantum Yield (ePS2) at Different Temperatures

On the other hand, quantum yield drastically declined in fieldpea even when test temperature exceeded beyond 30°C (Figure 7) indicating that fieldpea is more sensitive to high temperature compared to chickpea. The response of ETR (electron transport rate) with respect to increasing photon flux density (PPFD) is typically similar to the response of rate of photosynthetic oxygen evolution (O<sub>2</sub>) or CO<sub>2</sub> fixation in respect to light. No reduction in

ETR was observed in chickpea up to 40°C except at very high temperature of 46°C, however in fieldpea it drastically declined beyond 20°C (Figure 8). This again suggested that heat sensitivity of fieldpea is more than chickpea. Chlorophyll fluorescence, the ratio of variable to maximum fluorescence (Fv/Fm), and the base fluorescence (F<sub>0</sub>) have been shown to correlate with heat tolerance (Yamada et al., 1996) and are related with PSII (photosystem II) and carbon fixation. PSII is highly thermolabile, and its activity is greatly reduced or even partially stopped under high temperatures (Carnejo et al., 2005). The fluorescence inductions kinetics studies showed large difference in the fluorescence peaks and quenching pattern when leaves were pretreated at 20, 30, 40 and 46°C (Figure 9). In general, no fluorescence peaks were observed either in chickpea and fieldpea at 46°C indicating the threshold temperature beyond which photosynthesis may completely stop (Figure 9). The pretreatment at 46°C showed irreversible damage of photosynthetic systems and quantum yield declined below <0.1 (Figure 9).

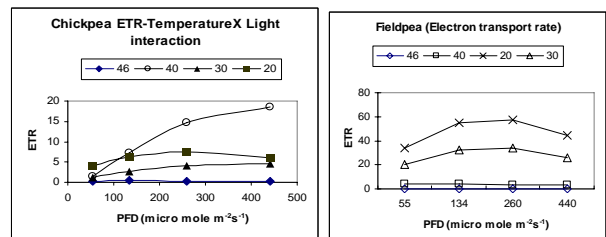


Figure 8. Electron Transport Rate in Pulses

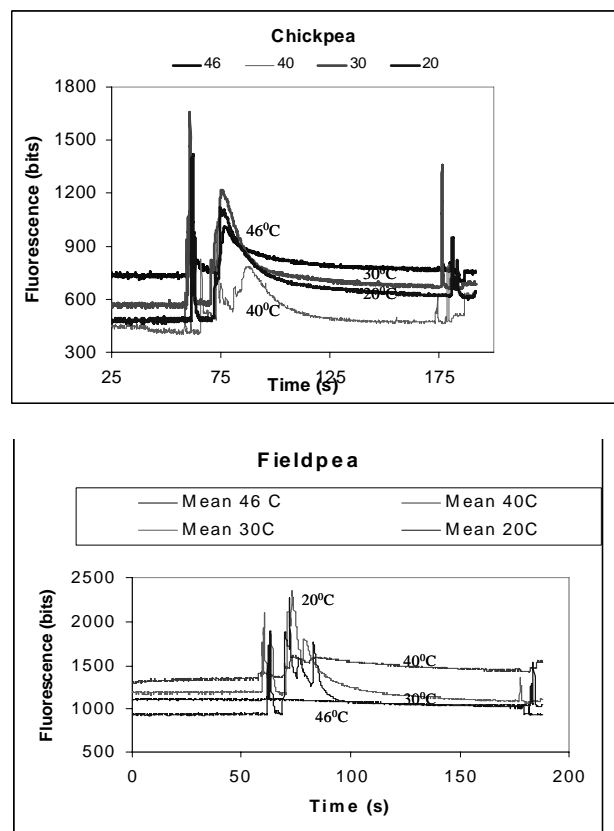


Figure 9. Fluorescence Induction at Different Temperatures

Foliar membrane stability and quantum yield (Fv/Fm) was significantly correlated ( $R^2 = 0.7$ ) (Figure 10), indicating that quantum yield and membrane stability parameters could be used as viable screening technique for identifying thermo tolerance.

The field experiment in two seasons under normal and late planting allowed identifying some of the putative heat tolerant lines such as ICCV 92944, ICCV 37, ICC 67, JKG 1, GCP 101, PG 12 based on the above screening techniques.

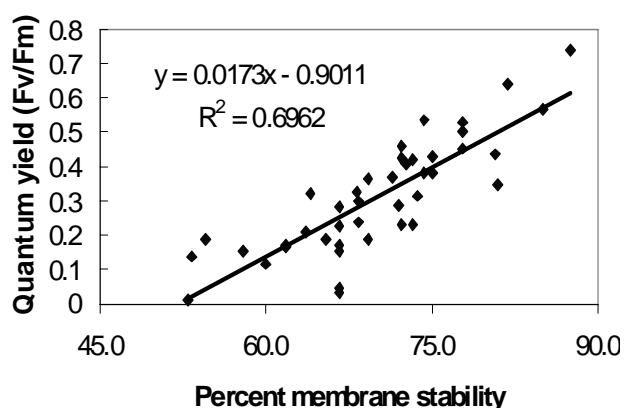


Figure 10. Relationship Between Quantum Yield and Membrane Stability

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