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# Quantification of the photosynthetic performance under high temperature in chickpea by means of non-invasive techniques

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**Abstract**
Chickpea (*Cicer arietinum* L.) is an important food crop grown in India under rainfed conditions. The crop usually encounters terminal high temperature (*HT*) during pod filling stage. The objective of the work is to determine the degree of *HT* effect on two contrast genotypes *viz.* ICCV 92944 and HC 5 differing in their tolerance detectable from net photosynthesis ($P_N$), stomatal conductance ($g_s$), *SPAD*, chlorophyll stability index (*CSI*), photochemical efficiency of *PSII* measured as chlorophyll fluorescence (*CF*) and canopy temperature depression (*CTD*) under field condition. *HT* was applied by the changing of two sowing dates *i.e*. normal and late sown. With increasing days after exposure to *HT* a close relationship in decline photosynthesis, chlorophyll content and *CTD* was observed. In comparison to HC 5, ICCV 92944 exhibited the better chlorophyll content, photosynthetic activity and yield, the variance of different parameters statistically related to yield decrease. The measured parameters of ICCV 92944 (identified as *HT* tolerant), declined relatively more slowly than those of HC 5. Results showed that *HT* induced stress is detectable from $P_N$, $g_s$, *SPAD, CSI, CF* and *CTD,* these physiological non-invasive techniques shown correlated with yield and all can be useful tools to help in screening of chickpea germplasm for *HT* tolerance.

**Keywords:** quantum yield; crop improvement; crop physiology

## Introduction
Global climate change is making *HT* a critical factor for plant growth and productivity. IPCC reported that temperature from 2.5 to 3.5°C will be raised in coming 2050 years. Chickpea is the most important legume crop of the arid and semi-arid zones grown under conserved soil moisture. The crop encounters terminal *HT* stress during grain filling stage and it is considered relatively susceptible to *HT* (>30°C). Temperature stress has devastating effects on plant growth and metabolism (Hasanuzzaman *et al*. 2013) [3]. The seriousness of *HT* stress depends on its timing, duration and intensity during the reproductive stage is a major cause of yield loss (Krishnamurthy *et al*. 2011) [5].

*HT* inhibited $P_N$ and $g_s$ significantly in many plant species (Morales *et al.* 2003) [10]. *CF* is a fast, non-invasive, non-destructive and informative tool to study physiological state of photosynthetic apparatus of any photosynthesizing material. Siebke *et al.* (1997) [13] demonstrated that *CF* parameters were related directly to the photosynthetic $CO_2$ assimilation rate of leaves. The ratio of variable to maximum fluorescence (*Fv/Fm*) of *PSII* measures the efficiency of excitation energy captured by *PSII* and it is one of the sensitive components for heat denaturation (Zhang and Sharkey 2009) [15]. *CTD* has been used to assess plant water status as it is a product of the leaf's energy balance, it is itself is a mechanism of heat escape including both environmental and physiological responses to water and *HT* stress (Leinonen *et al.* 2006, Balota *et al.* 2008) [1, 9] and it has been shown to correlated with yield in chickpea under *HT* (Kumar *et al*. 2012, 2013) [6, 7].

Now a days, with the availability of performance non-invasive digital physiological data correlates with plant growth, development and economic yield under *HT* stress. Infra red gas analyzer (*IRGA*) most widespread device is used to analyze $P_N$, $g_s$ of leaves in different crops. The relative chlorophyll content is assessed with a hand held chlorophyll meter *SPAD*. The main advantage of this method is that many leaf samples can be assessed in a short period of time. The using of *CF*, *SPAD*, *IRGA* and *CTD* measurements are useful tools in screening of two contrast genotypes of chickpea for *HT* tolerance.

## Materials and Methods
The present study was carried out to understand the tolerance mechanism to *HT* in two chickpea genotypes, namely ICCV 92944 variety of International Crops Research Institute for

the Semi-Arid Tropics (ICRISAT) and HC 5 variety from CCS Haryana Agricultural University, Hisar, 125 004, India, differing in sensitive to *HT*. The experiment was conducted in the research area of CCS HAU. Hisar is located in Indo Gangetic plains of North – West India with latitude of 29° 10´ North and longitude of 75° 46´ East. Hisar has a semi–arid, sub-tropical weather with severe cold during winter, hot and dry days with desiccating winds during reproductive phase. Mean relative humidity decrease to 40 to 50 per cent by the end of April (period coincides with reproductive phase of this crop). *HT* was given by manipulation of sowing dates *i.e.* normal sown was 11th November, 2011 and late sown 16th December, 2011 (*rabi* season) of the year 2011- 2012. Observations were recorded as the temperature rises above 30°C at reproductive phase at 4, 8 and 12 days after exposure (DAE) and the control sampling was done at the temperature below 30°C. During the course of experimentation, the values of minimum and maximum temperature, sunshine, rain fall and relative humidity are presented in Fig.1.

Gas exchange measurements were made with an *IRGA* portable gas exchange system (Model LCA-4ADC, U K) on the third fully expanded leaves. The leaf chlorophyll content was measured by *SPAD-502* chlorophyll meter (Konica Minolata Sensing). Chlorophyll stability index (*CSI*) was measured by the method described by Gunes *et al*. (2007) [2] using dimethyl sulphoxide. Photochemical efficiency was determined by using an amplitude modulated OS-30P (Model Optic science, Inc., Hudson, USA) chlorophyll fluorometer. *CTD i.e.* Transpirational cooling measured by using Infra-red thermometer (Model AG-42 Tele-temp Corp. CA) which was focused on 1 meter distance of crop canopy level. All measurements were recorded between 10:00 – 11:00 h.

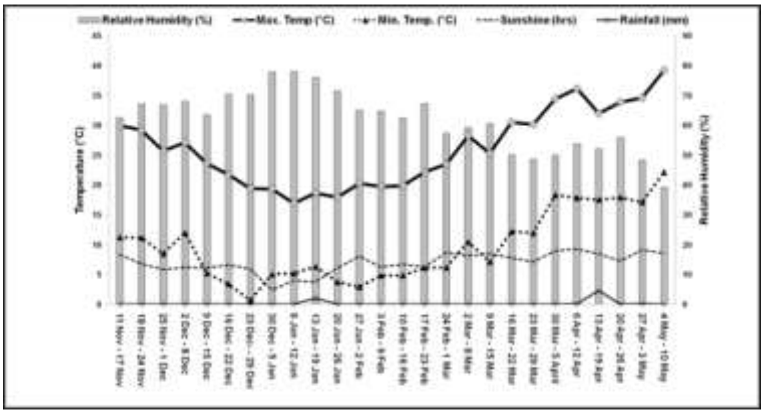


**Fig 1:** Agro meteorological data during the period of experimentation on weekly basis from November 11, 2011 to May 10, 2012

**Statistical analysis:** The reported data represent the mean ± *SE*. Statistical analysis was done using the analysis of variance (*ANOVA*) using Online Statistical Analysis Package (OPSTAT, Computer Section, CCS HAU, Hisar – 125 004, India).

## Results

Fig. 2*A* shows the effect of *HT* on $P_N$. When subjected to *HT*, $P_N$ decreased significantly in both genotypes with increased DAE to *HT*. The $P_N$ of ICCV 92944 decline relatively slower (18.65%) than that of HC 5 (21%). The decrease of $P_N$ under *HT* was associated with heat tolerance of genotypes. Stomatal conductance ($g_s$) showed different modifications with increased DAE to *HT* in each genotype (Fig. 2*B*). $g_s$ presented significant reductions in both genotypes under *HT* conditions. The maximum reduced values *i.e.* 33.47% observed in plants of HC 5 and minimum was in (29.89%) ICCV 92944. *SPAD* values showed in Fig. 3*A*, decreased from 59.11 to 51.98 with increased DAE to *HT*. The highest readings were found in ICCV 92944 (56.82) and lowest in HC 5 (54.68). *CSI* (%) also follows similar trend like *SPAD*. *CSI* decreased with increased DAE to *HT* (>30°C) in both genotypes and the decline values varied from 95.58 to 67.79. The mean values of *CSI* in leaves were higher in the genotype of ICCV 92944 (86.92) as compared with HC 5 (78.04) (Fig. 3*B*). The data on quantum yield (*Fv/Fm*) presented in Fig. 4*A*, indicated that significant differences were noticed with increased DAE to *HT* in both genotypes. The values of *Fv/Fm* significantly declined from 0.787 to 0.649 with the increasing in DAE period. The maximum value of quantum yield (0.746) was observed in ICCV 92944 and lower in HC 5 (0.69). The value of *CTD* declined significantly under *HT* condition in both genotypes over their respective control (Fig. 4*B*). With the increase in days to exposure *CTD* value decreased from -4.7°C to -2.9°C. The highest depression was noticed in HC 5 (-4.5 to -2.3) and lowest was in ICCV 92944 (-5.0 to -3.5).

Significant effect of *HT* was noticed on mean seed test mass (g) in both the dates of sowing. The highest mean test mass was observed under normal sown (17.4) than late sown (15.2) (Table 1). A significant reduction in test mass was observed in both genotypes, however more decline in test mass was observed in HC 5 (18.1%) than ICCV 92944 (9.4%). The mean seed yield/plant of late sown treatment was less than the mean seed yield of normal sown due to *HT* (>30°C), it was 23.3 g per plant in normal sown, while it was 16.1 g in late sown (Table 1). The mean seed yield was higher *i.e*. 22.6 g with lowest per cent decline 21.0% in ICCV 92944 as compared to 16.8 g and 43.0%, respectively in HC 5.

**Table 1:** Seed test mass (100 seed mass) and yield per plant of chickpea under normal sown (NS) and late sown (LS) conditions

| Genotypes | 100 seed mass [g] | | | | Yield plant$^{-1}$ [g] | | | |
|---|---|---|---|---|---|---|---|---|
| | **NS** | **LS** | **Mean** | **Per cent Decline** | **NS** | **LS** | **Mean** | **Per cent Decline** |
| ICCV 92944 | 21.1 | 19.1 | 20.1 | 9.4 | 25.3 | 20.0 | 22.6 | 21.0 |
| HC 5 | 13.8 | 11.3 | 12.5 | 18.1 | 21.4 | 12.2 | 16.8 | 43.0 |
| Mean | 17.4 | 15.2 | | | 23.3 | 16.1 | | |
| C.D. at 5% | *Genotypes (G) = 0.67; Temperature (T) = 0.67; G ×T = NS** | | | | *Genotypes (G) = 0.81;Temperature (T) = 0.81; G ×T = 1.14* | | | |

* Non Significant

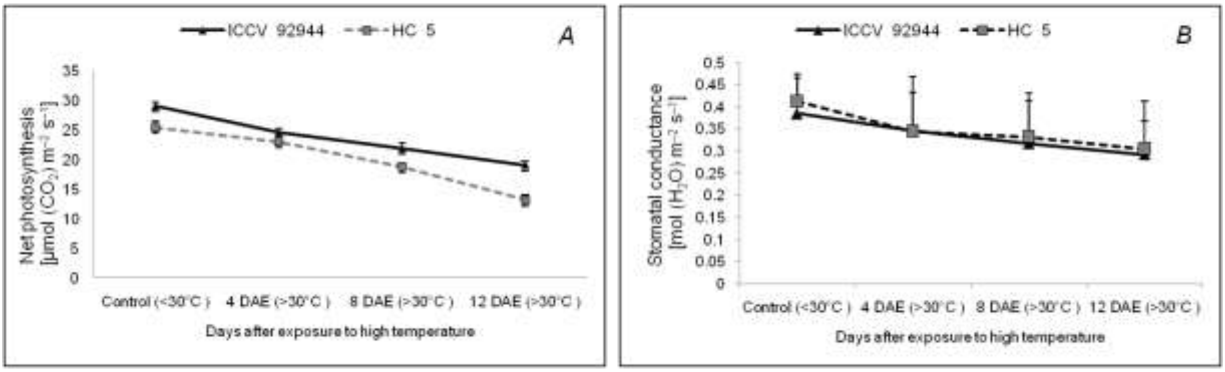


**Fig 2:** Changes in net photosynthesis (*A*) and stomatal conductance (*B*) of chickpea genotypes exposed to high temperature. *Bars represent ± SE of means of 4 replicates*

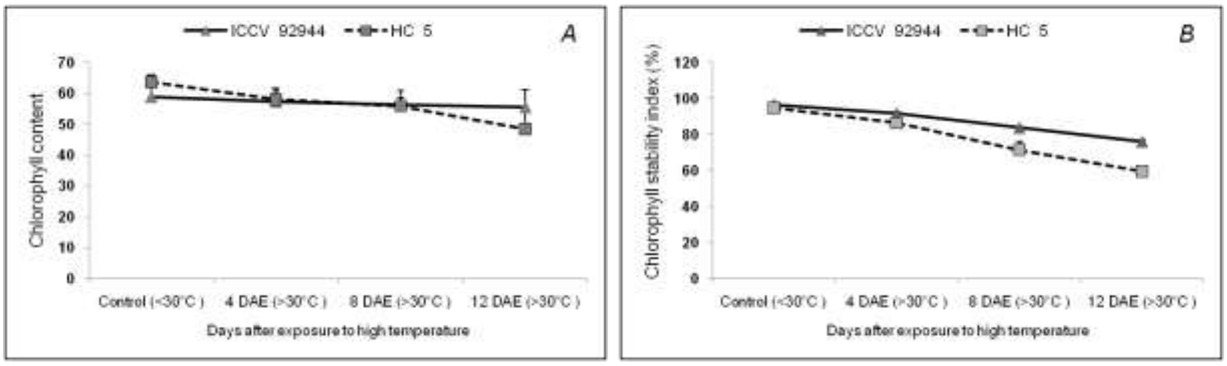


**Fig 3:** Changes in chlorophyll content (*SPAD*) (*A*) and chlorophyll stability index (%) (*B*) of leaves in chickpea genotypes as affected by high temperature. *Bars represent ± SE of means of 4 replicates*

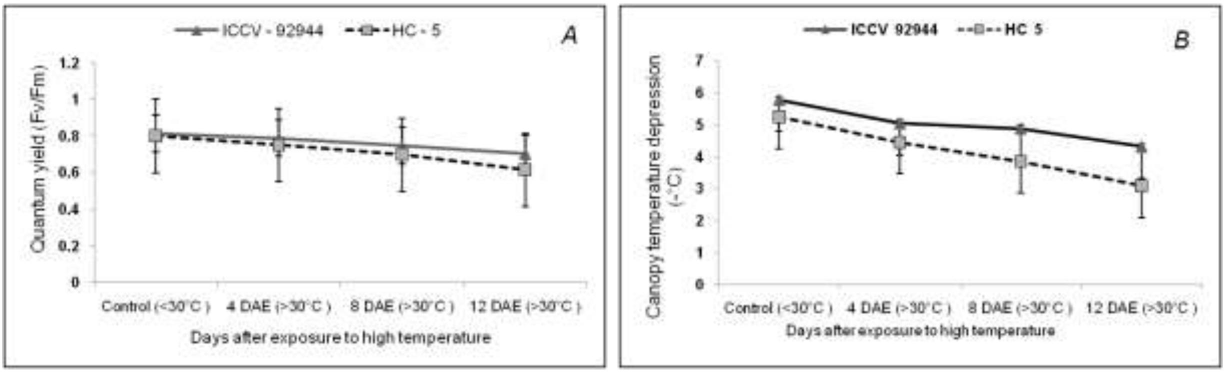


**Fig 4:** Changes in quantum yield *PSII* (*Fv/Fm*) (*A*) and canopy temperature depression (˚C) (*B*) of leaves in chickpea genotypes as affected by high temperature. *Bars represent ± SE of means of 4 replicates*

## Discussion

Photosynthesis is the most sensitive physiological process to elevated temperature in chickpea and any reduction in photosynthesis affects growth and grain yield (Krishnamurthy *et al*. 2011) [5]. *HT* reduces photosynthesis through reduction in chlorophyll content of leaves, as evidenced by the decrease in photosynthetic activity in genotypes under *HT* as compared to the controls. In fact, different DAE to *HT* have different effects on genotypes were observed in both ICCV 92944 and HC 5 genotypes. This study clearly shows that *HT* significantly decreased $P_N$, chlorophyll content and *CSI*. With increasing DAE to *HT* $P_N$ decreased by 18.65% and 20.88% in ICCV 92944 and HC 5, respectively. *SPAD* values decline very slower in ICCV 92944 *i.e.* 5.6% than HC 5 (23.85%) at 12 DAE. A strong relationship between chlorophyll content (*SPAD*) and photosynthesis was observed. ICCV 92944 had the maximum chlorophyll content and photosynthetic activity, a decrease of chlorophyll with *HT* implies a lowered capacity of light harvesting which subsequently led to lower yields. The inactivation of chloroplast enzymes, mainly induced by oxidative stress or inhibition of chlorophyll synthesis due to heat stress may also reduce the rate of leaf photosynthesis. *CSI* was linearly decreased at three investigated DAE to *HT* and follows similar trend with total chlorophyll content. Leaves of genotype HC 5 showed lowest *CSI* value than ICCV 92944 at 12 DAE. The reduced *CSI* is due to more destruction of chlorophyll under *HT* and poor translocation of photosynthates from the sink to the source in the present study could be the other reasons for poor quantum yield and *CTD*. After 4, 8 and 12 DAE to *HT* affects the $P_N$ but not $g_s$, which indicate the stomatal component did not limit $CO_2$ diffusion. Similar stomatal response to *HT* has been observed in both tested genotypes could be associated to tolerance mechanisms to avoid an excessive transpiration maintaining gas exchange values. The photochemical efficiency/ quantum yield (*Fv/Fm*) can be used to detect damage to *PSII* and possible photo inhibition reduced under *HT* indicated damage to the functional status of *PSII* in the photosynthetic apparatus. Two genotypes showed a marked decrease of the *Fv/Fm* with increase in period of DAE to *HT*. Thus, the heat tolerant ICCV 92944 genotype retained significantly greater $P_N$, $g_s$, chlorophyll content and *CSI* than the HC 5 genotype. It was apparent from these observations that the tolerant genotypes were able to minimize *HT* stress injury to the leaf tissues to a greater extent than the sensitive genotype and the decline observed in *Fv/Fm* can be rather a regulatory adjustment to limiting carbon availability under *HT* due to chlorosis of photosynthetic pigments. Thus, an inhibition in carbon assimilation may lead to a decrease in *PSII* function. Similar results were observed under *HT* by Zhang and Sharkey (2009) [15].

Leaf temperatures are depressed below air temperature when the temperature above the optimal condition with water evaporates from their surface was noticed as *CTD*. One of the factors determining evapo-transpiration is $g_s$, which itself is regulated by the rate of carbon fixation and it is itself is a mechanism of heat escape. *CTD* values were significantly changed between normal and *HT* condition in both genotypes. The depression values were lower in HC 5 as compare with ICCV 92944. It understood that ICCV 92944 genotype showed some degree of temperature depression and had cooler plant canopy than HC 5. The genotype ICCV 92944 had higher *CTD* showed higher $P_N$, $g_s$ and quantum yield as compared with HC 5. This genotype also maintained high rates of photosynthesis as well better system for transport of nutrients and assimilates lead to higher seed mass and yield. Reynolds *et al*. (1998) [11] reported that *CTD* shows high genetic correlation with yield and high values of proportion of direct response to selection, indicating that the trait is heritable and therefore amenable to early generation selection. *CTD* was used as a method of screening for heat tolerance (Rosyara *et al*. 2010, Kumar *et al*. 2012) [12, 7]. The effect of *HT* on seed mass and yield of two genotypes is related with their thermo tolerance. ICCV 92944 is relatively *HT* tolerant genotype, whose grain mass and yield less affected by *HT* compared with HC 5. Earlier reports of Krishnamurthy *et al*. (2011) [5] and Upadhyaya *et al*. (2011) [14] also confirmed that *HT* decreases the crop yield. Higher heat tolerance of ICCV 92944 is an integrated expression of thermo tolerance mechanism especially $P_N$, *Fv/Fm* and *CTD*, which decline photosynthetic parameters decline more slowly than those of HC 5 during the whole *HT* period. It is suggested that ICCV 92944 is identified a variety for chickpea breeder with the high efficiency of photosynthesis and thermo tolerance.

## Conclusion

Based on above physiological parameters *i.e*. $P_N$, $g_s$, *SPAD*, *CSI*, *Fv/Fm* and *CTD* and yield characters, it is concluded that genotype ICCV 92944 showed better performance under *HT* than HC 5 and in future these traits can be used in crop improvement programmes. We recommend that these new technologies in Plant Physiology/Crop Physiology can play a major role in screening of different biotypes in large scale with very fast, non-invasive, non-destructive and informative tools.

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