

# Timing of sampling for the canopy temperature depression can be critical for the best differentiation of drought tolerance in chickpea

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## Abstract

Maintenance of water supply during grain filling is critical for the drought adaptation of chickpea and therefore measurements of canopy temperature depression (CTD) could be used as a proxy for water extraction. However the conditions and timing of such measurements are not yet clear. CTD has been recognized as a highly dependable trait for estimating the drought tolerance in chickpea. This study was aimed at identifying an optimal CTD sampling time under drought stress and an optimally irrigated condition as well. In addition, it also involved using 12 selected germplasm accessions to understand the association of CTD, with the previously established drought response and early rooting characteristics. The growth stage involved identifying a sample which included (i) combination of high genetic variation, (ii) best differentiation of accessions by CTD, (iii) large number of accessions exhibiting highly negative CTDs, (iv) high heritability of CTD and (v) the best association of CTD with grain yield. Also, the growth stage was decided to be the critical time of sampling for canopy temperature measurements. Under drought stress, the critical timing was 66 days after sowing (DAS) in 2009-10 and 70 DAS in 2010-11. Under optimally irrigated condition, the critical timing of sampling was 70 DAS in 2009-10 and 63 DAS in 2010-11. But the changes that occurred in the indicative parameters were marginal till 82 DAS, denoting that, in an optimally irrigated environment, a longer window of growth stages was required to be permitted for sampling. The drought tolerance and local adaptation of chickpea accessions were explained by the CTD under drought stress as well as under optimally irrigated condition.

## Introduction

Currently, breeding for drought tolerance lack traits for selection that offer a combination of robustness, close association with the grain yield, rapidity in estimation and high throughput affordability matching the extent of the breeding populations or germplasm accessions. CTD has been recognized as one such trait and is used for assessing the plant response to environmental stress, making genotypic comparison for water use and heat, and estimating the drought tolerance (Balota et al. 2007). CTD is expected to be influenced by a number of factors like the capacity of the crop plant to extract water, the transpirational difference in response to the vapor pressure deficits, and the phenology. Also CTD is a manifestation of the transpiration status of a crop plant, showcasing the extent of physiological and metabolic activity. Among other things, high CTD was used to distinguish between the stress-avoidant and the stress-sensitive chickpea genotypes and was suggested as a selection criterion for improving the tolerance to drought (Kashiwagi et al. 2008).

The suitability of CTD as an indicator of yield and stress tolerance, however, must be determined for the individual environments (Balota et al. 2007). For instance, CTD in winter wheat has been recognized as a poor indicator of drought tolerance as in this case the yield is highly dependent on the limited soil-stored water (Winter et al., 1988; Royo et al., 2002). The terminal drought environments are characterized by the receding soil water conditions, where the available soil water constantly declines with the increasing demand for water by the exponentially growing canopy. Under such conditions, CTD can be expected to become critical at a specific

point of time during the peak reproductive growth, with a wide genetic differentiation for water extraction. Typically, CTD is sampled at noon when maximum genotypic differentiation is expected (Amani et al. 1996). However, sampling in night and predawn was also found to be useful for the best CTD-based yield prediction in case of wheat (Balota et al. 2007). However, the issues relating to determining the optimal CTD sampling time and obtaining the maximum genetic discrimination were seldom addressed. More importantly, the information on the optimum time of sampling in order to obtain the largest probability of detecting genotypic differences is an immediate need.

Therefore, the objectives of this study were (i) to determine the optimal CTD sampling time in terms of growth stage, and (ii) to understand the CTD response of diverse germplasm which were already known for their drought response and early rooting characteristics, as compared to the well adapted genotypes of the region.

## Materials and methods

### *Crop management*

Ten chickpea germplasm accessions and two well-adapted varieties of the region (namely Annigeri and ICCV 10) with good contrasts for rooting ability, grain yield-based drought tolerance, and canopy temperature (Table 1) were grown in a Vertisol field under terminal drought stress and optimally irrigated conditions during the post-rainy seasons in the years 2009-10 and 2010-11, at ICRISAT, Patancheru. These germplasm accessions (except ICC 4958) were close in growth duration and matured between 87 to 97 days in 2009-10 and between 86 to 95 days in 2010-11 under terminal drought conditions (Table 2). The crop was raised on broad bed and furrows with 1.2 m wide beds flanked by 0.3 m furrows. Surface application and incorporation of di-ammonium phosphate on 18 kg N ha<sup>-1</sup> and 20 kg P ha<sup>-1</sup> were carried out. The experiment was conducted in a randomized complete block design (RCBD) with three replications. Seeds were treated with 0.5% Benlate® (E.I. DuPont India Ltd., Gurgaon, India) plus Thiram® (Sudhama Chemicals Pvt. Ltd., Gujarat, India) mixture for both the seasons. The seeds were hand sown manually on 31 October, 2009 and 20 November, 2010, at a depth of 2 to 3 cm, with 10 cm between plant and 30 cm between rows, and with a row length of 4 m. About 61 seeds were used for each 4 m row and at 15 DAS, the plants were thinned maintaining a plant-to-plant spacing of 10 cm. A sprinkler irrigation, 20 mm, was applied immediately after sowing to ensure uniform emergence. Subsequently, the plants in one treatment were grown under rainfed conditions while the other

plants were treated with additional four (in 2009-10) or three 50mm furrow irrigations. The plots were kept weed free by hand weeding and intensive protection measures were taken against pod borer (*Helicoverpa armigera*).

### *Canopy temperature*

The thermal images of the plant canopies were recorded on 66, 70, 76, and 81 DAS in 2009-10, and 63, 70, 72 and 82 DAS in 2010-11, respectively, using an infrared camera, IR FLEXCAM (Infrared Solutions, Inc, MN, USA) with a sensor size of 160 × 120 pixels, sensitivity of 0.09°C, and an accuracy of ±2%. The target area of the image obtained was about 30 cm × 20 cm at one of the central row of each plot, and the images were captured from north, in order to avoid shading of the target area (Kashiwagi et al., 2008). The software SmartView 2.1.0.10 (Fluke Thermography), was used for the image analysis and the estimation of canopy temperatures after removing the soil (background) emissions (Zaman-Allah et al., 2011). The camera was strapped on shoulder at a height of 1.0 m and the observations were recorded between 1400 and 1530 hrs.

The CTD of each genotype was estimated as Ambient air temperature (°C) - canopy temperature (°C).

The CTD mostly remained as a negative value when the canopies were warmer than the air.

### *Final harvest*

At physiological maturity, plant aerial parts were harvested at ground level from an area of 5.4 m<sup>2</sup>, with care to eliminate the border effects and to dry the plant components to constant weight in hot air dryers at 45°C, and the total shoot dry weights were recorded. Grain weights were recorded after threshing.

## Results and discussion

### *The extent of variation in CTD and grain yield*

Large range of variation among the accessions for CTD was found during all the times of observations (Table 2). Also, the genotypic variation among the accessions was significantly different at the highest level of probability ( $P < 0.001$ ), in all the sampling times, across irrigations and years. The range and the heritability of the CTD under drought stress, with the sampling time at 81 DAS in 2009-10 and 82 DAS in 2010-11, was relatively high. However, it was recommended that this observation be considered cautiously, as some of the accessions like ICC 4958 and Annigeri had already matured and had a warmer canopy whereas others were still cooler. Consequently, this final sample recorded the highest values and range in canopy temperature.

The second year crop had matured two days earlier, as a consequence of the late sowing in 2010-11, particularly under drought stress leading to an early development of warmer canopy (Table 3). Under the drought stress conditions, the indicators like (i) high range of genetic variation, (ii) best differentiation (widest range) in CTD, (iii) prevalence of challenging stress opportunity (as indicated by the highly negative overall mean CTD at the sampling time), (iv) high heritability (Tables 2 and 3) and (v) the best association of CTD with grain yield (Fig. 1) had occurred at both 66 and 70 DAS in 2009-10 and at 70 DAS in 2010-11. Most of these indicators reduced to 76 DAS in 2009-10 and 72 DAS in 2010-11, respectively.

Under the optimally irrigated condition, the range of genetic variation, differentiation in CTD, heritability (Table 2), and the association of CTD with grain yield (Table 3; Fig. 1) indicated no clear change pattern, as was seen under the drought stress condition. However, the high range of genetic variation, heritability, and the fitness of association with grain yield had indicated, 70 DAS in 2009-10 and 63 DAS in 2010-11 to be the most suitable time for these observations. Since the maturity was delayed by 15 to 20 days, optimally irrigated environment seemed to provide extended periods of time for sampling CTD, when the periods proximal (before and after) to the irrigation were avoided. The heritability of CTD under drought stress was found to be as good as the heritability of CTD under optimal irrigation, contrary to the previous observations reordered with the winter wheat growing environment (Winter et al., 1988; Royo et al., 2002).

### ***The relationship between CTD and grain yield***

Irrigations can reduce the canopy temperatures to a large extent and these differences can narrow down with time after irrigation. A two days prior-irrigation in 2009-10 and six days prior-irrigation in 2010-11 had brought down the canopy temperature by 10.5°C at 81 DAS in 2009-10 and 8.1°C at 82 DAS in 2010-11. On the other hand, an 12 days prior application of irrigation treatment in 2009-10 and 17 days prior application of irrigation treatment in 2010-11 had only brought down the canopy temperature by 3.7°C at 76 DAS in 2009-10 and 3.5°C at 72 DAS in 2010-11, thus indicating that the level of canopy cooling can vary to a great extent depending upon the time of canopy temperature sampling in relation to the irrigation time. Thus, CTD seemed to be an ideal tool for devising the right water management strategies and maximizing the grain yield by optimizing vegetative growth and harvest indices.

Under drought stressed condition, the best adapted varieties Annigeri and ICCV 10 maintained a CTD close to the mean at all the stages of samplings, except for an insignificant increase at 82 DAS in 2010-11 (Table 3).

The grain yield of Annigeri was greater than the mean in 2010-11 and that of ICCV 10 was the best in both the years. It was apparent that these varieties were able to maintain the extraction of water even at the later stages. It is likely that these plants had a balanced root to shoot ratio, deeper root system, and less VPD sensitivity. The CTD of ICC 4958 was clearly lower than the mean which was at 70 DAS in 2009-10 and 72 DAS in 2010-11, respectively. This early large rooting accession, ICC 4958, was the earliest in duration and matured by 79 DAS in 2009-10 and 83 DAS in 2010-11, escaping the major part of the terminal drought. This relatively brought faster development and the likely root senescence at the approach of maturity (Table 3) which led to the lower CTD of canopy or warmer canopy, while maintaining a higher grain yield. The grain yield of ICC 4958 was greater than the mean in 2010-11 notwithstanding the low CTD effect that was a result of the early maturity. Another large rooting accession, ICC 8261, was kabuli and relatively late in duration. The CTD of this accession was close to the mean or marginally lower at the later stages of observation. In spite of the fact that the shoot biomass production was above average, this accession produced lower grain yield. The highly drought tolerant accessions, ICC 867 with an above mean CTD, and ICC 14778 with an occasionally high CTD, yielded greater than the site mean. The drought tolerant accessions, ICC 3325 and ICC 14799 maintained a higher CTD than that of the lower CTD accessions, ICC 7184 and ICC 3776. Their grain yields were close to the mean. The drought tolerant accessions, ICC 1882 and ICC 283 maintained a higher CTD occasionally in the early stages of observation but edged closer to the mean CTD at later stages. These accessions also maintained grain yields similar to the mean. The drought sensitive accession, ICC 3776, maintained a CTD closer to the mean but produced grain yield lower than the site mean. One more drought sensitive accession, ICC 7184, maintained a lowest CTD during all the sampling time over years and also produced the least grain yield.

Under optimally irrigated condition, the CTD and grain yield recorded were substantially higher than the observations recorded under drought stress. On the other hand, the relationship of CTD with grain yield and the relative ranking of the accessions remained closely similar to the reaction under drought stress condition (Table 3). The best adapted varieties, Annigeri and ICCV 10, not only maintained a CTD close to the mean at all the stages of samplings but also maintained a greater CTD than the mean grain yield with ICCV 10 out-yielding Annigeri. The CTD of ICC 4958 was close to the mean in both the years and ICC 4958 also produced an average grain yield. The higher CTD expression under irrigation compared to the drought stressed environment was due to maintenance of active

transpiration linked to the delayed phenology of about 30 days in 2009-10 and 20 days in 2010-11, respectively. Accessions, ICC 8261 also maintained a CTD similar to the mean or marginally lower than ICC 4958 at all the stages. This genotype produced a lower grain yield. The highly drought tolerant accession, ICC 867 often maintained a high CTD as compared to the mean produced by the next highest yield after ICCV 10. The next highly drought tolerant, ICC 14778 maintained CTDs close to mean and yielded more than the site mean in the year 2010-11. Both the drought tolerant accessions, ICC 3325 and ICC 14799, had higher CTD but the yields were moderate and were close to the mean grain yield. The drought tolerant accessions, ICC 1882 and ICC 283, maintained CTDs and grain yield similar to the mean. The drought sensitive accessions namely ICC 3776(at the early sampling) and ICC 7184, always maintained the least CTD and produced the lowest grain yields. This study confirmed that the previously reported drought tolerance reaction of the accessions, was more due to the maintenance of better transpirational status and cooling off for a longer growth period.

## Conclusion

In conclusion, CTD was related with the grain yield both under drought stressed and optimally irrigated environments. The relative ranking of accessions for CTD remained the same at both the soil water environments, designating CTD as a constitutive trait. The large variation in CTD and cooler canopies leading to greater grain yields provided enough indication to believe that, continuous supply of water for stomatal conductance and transpiration through enhanced soil water absorption was necessary for enhanced drought tolerance. Under both the terminal drought stress and optimal irrigation conditions, best genotypic differentiation through CTD was obtained by sampling within 66 to 70 DAS but the optimally irrigated condition provided an opportunity to extend this window of sampling time for another 10 days with minimum reduction in quality. The superior CTD and drought tolerance of ICCV 10 highlights the success achieved in combining both drought tolerance and high yield through conscious breeding efforts of the past.

**Table 1. The root, drought and canopy temperature reactions of the germplasm accessions and the checks (locally adapted varieties) used for assessing the best stage of measurement of canopy temperature depression in chickpea.**

S. No	Germplasm accession	Root strength at 35 days age	Drought reaction (4)	Canopy temperature (3)
1	ICC 4958	Large (2)	Moderately tolerant	Cool
2	ICC 8261	Large (2)	Moderately tolerant	
3	ICC 867		Highly tolerant	Cool
4	ICC 3325		Tolerant	Cool
5	ICC 14778		Highly tolerant	Cool
6	ICC 14799		Tolerant	Cool
7	ICC 1882	Small (2)	Tolerant	
8	ICC 283	Small (2)	Tolerant	
9	ICC 3776		Highly sensitive	Warm
10	ICC 7184		Highly sensitive	Warm
11	Annigeri		Tolerant, adapted variety	
12	ICCV 10	Large (1)	Wider adapted variety	

<sup>(1)</sup> Ali et al. 2002., <sup>(2)</sup> Kashiwagi et al. 2005., <sup>(3)</sup> Kashiwagi et al. 2008., <sup>(4)</sup> Krishnamurthy et al. 2010.

**Table 2. Mean canopy temperature depression (CTD) measured at different days after sowing (DAS) and grain yield for the 12 chickpea accessions during the post-rainy season of 2009-10 and 2010-11 under optimally irrigated and drought stressed environment.**

Season/ environment	Trial mean	Range of means	S.Ed	$\sigma^2g$ (F pr.)	Heritability ( $h^2$ )
<b>2009-10, Drought stressed</b>					
CTD_66DAS	-0.02	-2.45 – 1.03	0.533	6.21 (<0.001)	0.63
CTD_70DAS	-0.69	-2.70 – 0.59	0.475	7.45 (<0.001)	0.68
CTD_76DAS	-2.61	-3.82 – -1.69	0.421	4.94 (<0.001)	0.57
CTD_81DAS	-5.77	-8.21 – -3.94	0.476	10.7 (<0.001)	0.76
Grain yield (kg ha <sup>-1</sup> )	1795	1093 – 2078	102.4	13.6 (<0.001)	0.81
<b>2009-10, Optimally irrigated</b>					
CTD_66DAS	4.99	4.06 – 5.61	0.323	3.54 (0.006)	0.46
CTD_70DAS	3.51	1.04 – 4.31	0.333	18.4 (<0.001)	0.85
CTD_76DAS	1.08	-0.84 – 2.23	0.487	7.24 (<0.001)	0.68
CTD_81DAS	4.76	1.84 – 5.85	0.33	23.1 (<0.001)	0.88
Grain yield (kg ha <sup>-1</sup> )	1871	1308 – 2362	149.6	9.59 (<0.001)	0.74
<b>2010-11, Drought stressed</b>					
CTD_63DAS	-1.86	-4.25 – -1.08	0.465	7.29 (<0.001)	0.68
CTD_70DAS	-2.17	-4.40 – -0.95	0.498	8.59 (<0.001)	0.72
CTD_72DAS	-1.41	-2.62 – -0.49	0.389	5.79 (<0.001)	0.61
CTD_82DAS	-4.78	-7.98 – -3.04	0.733	6.54 (<0.001)	0.65
Grain yield (kg ha <sup>-1</sup> )	1681	1078 – 2118	71.1	44.0 (<0.001)	0.93
<b>2010-11, Optimally irrigated</b>					
CTD_63DAS	2.93	-0.12 – 4.20	0.610	7.39 (<0.001)	0.68
CTD_70DAS	3.06	-0.56 – 5.31	0.809	7.09 (<0.001)	0.67
CTD_72DAS	2.07	-0.14 – 3.53	0.603	5.37 (<0.001)	0.59
CTD_82DAS	3.35	0.42 – 5.19	0.626	9.11 (<0.001)	0.73
Grain yield (kg ha <sup>-1</sup> )	3037	1876 – 4202	89.9	93.7 (<0.001)	0.97

**Table 3. Mean canopy temperature depression (CTD) measured at different days after sowing (DAS), days to 50% flowering, days to maturity and grain yield for the 12 chickpea accessions during the post-rainy season of 2009-10 and 2010-11 under drought stressed and optimally irrigated environment.**

Year Irrigation/Accession	CTD at 66 DAS	CTD at 70 DAS	CTD at 76 DAS	CTD at 81 DAS	Days to flowering	Days to maturity	Grain yield (kg ha <sup>-1</sup> )
<b>2009-10, Drought stressed</b>							
ICC 4958	-0.31	-1.54	-3.42	-8.21	38	79	1916
ICC 8261	0.12	-1.44	-3.18	-6.36	48	97	1674
ICC 867	0.47	-0.72	-2.31	-5.52	48	90	2078
ICC 3325	-0.49	-0.12	-1.90	-5.44	48	93	1752
ICC 14778	-0.08	-0.99	-1.69	-5.17	52	96	2016
ICC 14799	0.39	-0.39	-2.44	-3.94	50	94	1734
ICC 1882	0.72	0.42	-1.96	-4.96	45	89	1871
ICC 283	1.03	-0.38	-2.84	-6.02	45	87	1789
ICC 3776	-0.81	-0.92	-3.10	-4.91	49	98	1628
ICC 7184	-2.45	-2.70	-3.82	-7.04	50	100	1093
Annigeri	0.51	-0.04	-2.28	-5.77	41	82	1923
ICCV 10	0.69	0.59	-2.32	-5.83	47	93	2069
Mean	-0.02	-0.69	-2.61	-5.77	47	92	1795
S.Ed (±)	0.53	0.48	0.42	0.48	0.8	2.2	102.4
<b>2009-10, Optimally irrigated</b>							
ICC 4958	5.12	3.32	0.30	4.62	49	111	1894
ICC 8261	4.66	2.92	0.29	4.22	53	115	1308
ICC 867	5.61	4.05	1.18	5.35	51	111	2158
ICC 3325	4.95	4.22	1.61	5.52	51	113	2086
ICC 14778	5.04	3.71	1.76	5.01	54	112	2035
ICC 14799	5.46	4.35	2.23	5.85	53	113	1842
ICC 1882	4.83	4.16	1.81	5.46	51	114	1949
ICC 283	4.56	4.01	1.57	5.31	51	113	1982
ICC 3776	4.82	2.15	0.05	3.45	53	110	1529
ICC 7184	4.06	1.04	-0.84	1.84	53	112	1309
Annigeri	5.29	3.94	1.85	5.24	50	114	1993
ICCV 10	5.46	4.31	1.12	5.31	50	115	2362
Mean	4.99	3.51	1.08	4.76	52	113	1871
S.Ed (±)	0.32	0.33	0.49	0.33	1.0	0.9	270.8

(cont'd)

**Table 3. Mean canopy temperature depression (CTD) measured at different days after sowing (DAS), days to 50% flowering, days to maturity and grain yield for the 12 chickpea accessions during the post-rainy season of 2009-10 and 2010-11 under drought stressed and optimally irrigated environment. (cont'd)**

Year Irrigation/Accession	CTD at 66 DAS	CTD at 70 DAS	CTD at 76 DAS	CTD at 81 DAS	Days to flowering	Days to maturity	Grain yield (kg ha <sup>-1</sup> )
<b>2010-11, Drought stressed</b>							
ICC 4958	-2.11	-2.87	-2.22	-7.98	33	83	1905
ICC 8261	-1.68	-3.14	-1.98	-5.46	52	95	1113
ICC 867	-1.32	-1.76	-1.11	-3.83	47	90	1878
ICC 3325	-2.20	-1.16	-0.70	-4.21	49	92	1894
ICC 14778	-1.88	-2.02	-0.49	-3.56	52	93	1911
ICC 14799	-1.41	-1.76	-1.24	-3.04	51	92	1694
ICC 1882	-1.08	-0.95	-0.76	-4.06	43	93	1797
ICC 283	-1.44	-1.49	-1.64	-4.64	41	86	1535
ICC 3776	-2.61	-3.29	-1.90	-4.01	47	94	1355
ICC 7184	-4.25	-4.40	-2.62	-5.48	44	91	1078
Annigeri	-1.29	-1.74	-1.08	-5.54	35	87	1873
ICCV 10	-1.11	-1.44	-1.12	-5.60	44	90	2118
Mean	-1.86	-2.17	-1.41	-4.78	45	91	1681
S.Ed (±)	0.47	0.50	0.39	0.73	0.5	0.8	71.1
<b>2010-11, Optimally irrigated</b>							
ICC 4958	3.32	2.72	1.66	3.57	47	103	3141
ICC 8261	2.46	2.35	1.69	2.51	55	107	2183
ICC 867	3.81	3.51	1.88	3.75	51	103	3205
ICC 3325	3.49	4.19	2.90	4.89	53	104	3174
ICC 14778	3.39	3.68	2.46	4.34	54	103	3134
ICC 14799	4.20	5.31	3.53	5.19	54	105	3161
ICC 1882	3.48	4.16	3.18	3.23	49	95	3194
ICC 283	2.76	3.31	2.27	2.11	49	104	3094
ICC 3776	1.62	1.24	1.08	2.46	53	106	2485
ICC 7184	-0.12	-0.56	-0.14	0.42	53	106	1877
Annigeri	3.57	3.59	2.55	4.37	50	103	3597
ICCV 10	3.23	3.21	1.82	3.38	50	103	4202
Mean	2.93	3.06	2.07	3.35	51	104	3037
S.Ed (±)	0.61	0.81	0.60	0.63	0.5	1.9	89.9

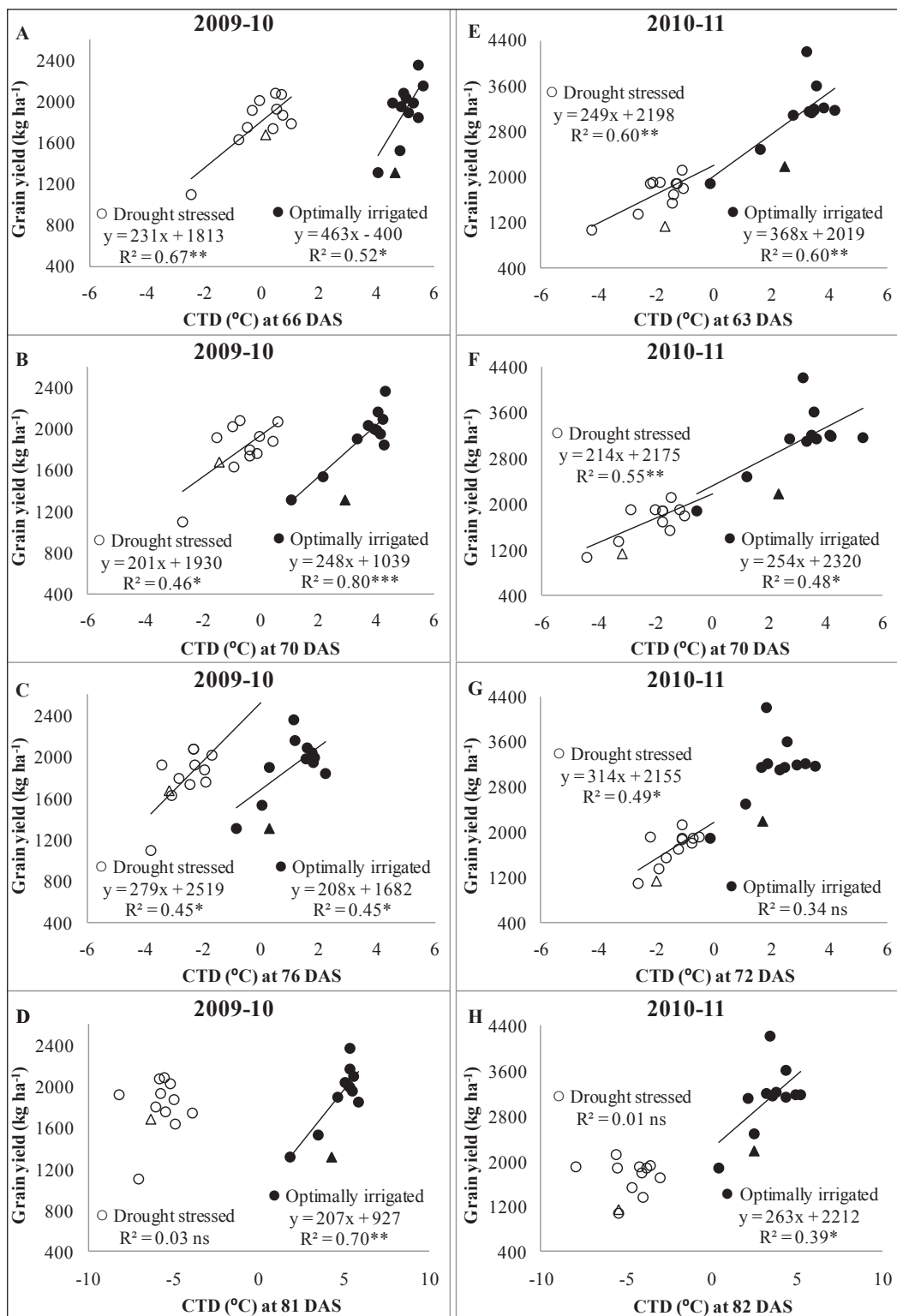


Figure 1. The relationship between canopy temperature depression (CTD) at different days after sowing (DAS) during crop reproductive stage and the grain yield for the 12 chickpea accessions during the post-rainy season of 2009-10 and 2010-11, under optimally irrigated and drought stressed environment. The kabuli accession ICC 8261 is marked as  $\Delta$  (drought stressed) and  $\Delta$  (optimally irrigated) were not considered for the regression line.



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