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Association of canopy temperature depression and membrane relative injury with seed and biological yield in chickpea (*Cicer arietinum* L.) genotypes under field conditions

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Abstract
Twenty chickpea genotypes were screened for canopy temperature depression (CTD) and membrane relative injury (RI) at flowering, poding and grain-filling stages under natural field conditions at the Nuclear Institute for Food & Agriculture (NIFA), Peshawar, Pakistan in 2020-21 and 2021-22 crop growth seasons. Eight genotypes showed highest averaged CTD values of 4.2-5.5 °C, 3.9-4.8 °C and 4.3-4.8 °C across two seasons at flowering, poding and grain-filling stages, respectively. The same eight genotypes showed lowest average RI values ranging from 24.9-45.5%, 41.2-45.5% and 40.9-48.5% across two seasons at flowering, poding and grain-filling stages, respectively. These genotypes also produced average higher biological yield plant^{-1} (BYPP) ranging from 59.5 g to 70.4 g and highest seed yield plant^{-1} (SYPP) of 16.3 g to 20.2 g across 2020-21 and 2021-22. The CTD was significantly ($P \leq 0.01$) and positively correlated with BYPP and SYPP with a strong correlation with SYPP (r = 0.9888). The RI had a negative correlation with BYPP and SYPP, being strongly negatively correlated (r = -0.9743) with SYPP. Regression analysis showed positive and negative linear relationship of CTD and RI, respectively, with BYPP and SYPP. A positive association of CTD was found with BYPP and SYPP with a strong association between CTD and SYPP (R^2 = 0.977), whereas a strong negative association was found between RI and SYPP (R^2 = -0.9489). Based on these results, CTD and RI may be utilized as positive indirect indicators to breed chickpea genotypes for better yield performance under natural environments with variable temperatures.

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Introduction

Chickpea, commonly called as gram, is an important food legume crop being cultivated across the world including Australia, Europe, North America, South America, Asia and Africa (Gaur et al., 2012; Phiri et al., 2023). It is traditionally used in a variety of ways in Pakistan, India and Bangladesh. Being a minor crop, chickpea is grown on lands with marginal or no application of external inputs. As a result, its production is affected by various natural factors particularly heat (Jha et al., 2014; Garg et al., 2015;

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Kadiyala et al., 2016; Devi et al., 2023) under field conditions.

Being a cool season's crop, chickpea is affected by high temperature (above 30 ºC) during the course of growth in the field affecting seed yield and flower pollen related functions (Kaushal et al., 2013; Kumar et al., 2013; Devasirvatham et al., 2015; Devi et al., 2023). Canci and Toker (2009) reported 100% increase in yield losses in chickpea genotypes with increasing temperature. For example, poding in chickpea is severely affected by increased temperatures due to impaired source and sink relations leading to mortality of pollen grains (Aswathi et al., 2014; Karalija et al., 2022).

Direct selection for seed yield in chickpea under natural field conditions has severe limitations due to unpredictable environmental conditions, particularly rising temperature often leads to erroneous selection and wastage of both time and financial resources. In order to come up with a reliable end product, non-conventional indirect selection criteria under abnormal temperature conditions are needed to be adopted. Cell membrane structure and function is negatively affected by high temperature which is resulted in the form of loss in membrane integrity and ion leakage from the cell (Salvucci and Crafts-Brandner, 2004). Investigation of cell membrane function through recording ion leakage under increased temperature is therefore a positive strategy, and has been studied in chickpea and other crops (Ibrahim, 2011; Awasthi et al., 2014; Ray et al., 2020).

Abiotic stresses tend to disturb transpiration which in turn creates a reason for changes in leaf temperature (Ibrahim, 2011). Transpiration from open stomata sustains canopy temperature resulting in cool canopy, and is an important trait conferring tolerance to high temperature in cereals (Munjal and Rana, 2003). Additionally, canopy temperature depression (CTD) indicates difference between canopy of the plant and air temperature. Under stress, plant closes its stomata for a certain period of time resulting in change in canopy temperature of the plant. In chickpea, CTD had usefully been applied in screening large sets of chickpea genotypes for heat tolerance under natural field conditions (Devasirvatham et al., 2015; Huang et al., 2023). Investigation of CTD in chickpea can be a useful tool to screen for tolerance against heat under natural field conditions. Thus, the primary objective of the current manuscript was to carry out the screening of advanced chickpea genotypes for CTD and RI under field conditions and to draw their relationship with biological and seed yield.

Materials and Methods

Plant material and sowing

Twenty (20) advanced chickpea recombinant genotypes (Table 1) developed at the Nuclear Institute for Food & Agriculture (NIFA), Peshawar were used in the current study. The experimental material was planted on the research farm of NIFA in Rabi (Winter) 2020-21 and 2021-22 seasons. The experiment was laid-out in a Randomized Complete Block Design. Each genotype was planted in four rows, each of 4 m in length and plant-to-plant and row-to-row distance of 10 cm and 30 cm, respectively. Each genotype was replicated three times. The experimental material was planted under natural field conditions and no supplemental irrigation was applied after sowing.

Table 1. List of chickpea genotypes used in the study; all genotypes were developed at NIFA

Genotypes	Parentage/Pedigree	Genotypes	Parentage/Pedigree
NDC-18-20-2	NIFA-2005 x NDC-6-I-7-	NDC-18-39-3	NIFA-2005 x NDC-6-I-7-
NDC-18-20-3	-do-	NDC-18-40-4	-do-
NDC-18-20-4	-do-	NDC-18-41-3	-do-
NDC-18-20-7	-do-	NDC-18-42-1	-do-
NDC-18-21-3	-do-	NDC-18-44-1	-do-
NDC-18-22-2	-do-	NDC-18-46-1	-do-
NDC-18-34-1	-do-	NDC-18-47-3	-do-
NDC-18-36-2	-do-	NDC-18-66-2	-do-
NDC-18-37-2	-do-	NDC-18-104-2	-do-
NDC-18-39-1	-do-	NDC-18-152-2	Dasht x NIFA-2005

Phenotypic evaluation

Canopy temperature depression (CTD)

Canopy temperature depression (CTD) was recorded in the field using infrared thermometer (DT-8811) at flowering, poding, and grain-filling stages. Data were recorded at noon under full sunlight and windless conditions. The device was pointed at the canopy at an angle covering whole canopy of each genotype in each replication. CTD was calculated using the following formula:

$$CTD = Ta - Ct$$

Where Ta = air temperature, and Ct = canopy temperature

Relative injury (RI)

Relative injury was recorded in the field at NIFA at flowering, poding and grain-filling stages using an electrical conductivity meter (CON 510). The leaf samples were collected from five representative plants in each genotype in each replication, and later on pooled together genotype-wise for analysis. Relative injury was determined using the following formula:

$$RI = \{1-[1-(T1/T2)]/[1-C1/C2)]\} \times 100$$

Biological and seed yield

Biological yield was recorded at physiological maturity. The above-ground parts of 10 representative plants from each genotype in each replication were randomly harvested and subsequently weighed. Average data was used to determine per plant biological yield of each genotype in grams. The same plants used to determine the biological yield were also used for the determination of seed yield. The plants were individually threshed, weighed on an electronic balance and average data was used to determine per plant seed yield in grams in each genotype.

Data analysis

Analysis of variance (ANOVA) was carried-out for CTD and RI at flowering, poding, and grain-filling stages according to Steel and Torrie (1980). ANOVA for the final biological yield per plant (BYPP) and seed yield per plant (GYPP) was conducted after harvest. Correlation coefficients between CTD and RI at the three stages with the measured traits at the respective stages were determined using the Pearson Product Moment Correlation test. All statistical analyses were carried out using the SPSS statistical software version 19.0 (IBM SPSS Statistics, USA). Regression analyses were carried out through the Microsoft Excel program. Average data was used for correlation and regression analyses.

Results

Canopy temperature depression (CTD)

Analysis of variance (ANOVA) of data revealed significant ($P \leq 0.05$) differences among genotypes for CTD at all three stages (flowering, poding, and grain-filling) in the Rabi 2020-21 and 2021-22 crop growth seasons (Table 2 and3). At flowering, the highest CTD values were recorded for 8 genotypes (NDC-18-404, NDC-18-20-7, NDC-18-22-2, NDC-18-21-3, NDC-18-44-1, NDC-18-20-3, NDC-18-152-2 and NDC-18-37-2) ranging from 4.3 °C to 5.6 °C compared with those of other genotypes (0.7 °C to 2.1 °C) (Table 2).

Table 2. Canopy temperature depression (CTD) and relative injury (RI) of 20 chickpea genotypes evaluated in Rabi 2020-21.

Genotypes	CTD (°C)			RI (%)		
	Flowering	Poding	Grain-filling	Flowering	Poding	Grain-filling
NDC-18-40-4	5.6^{A}	5.4^{A}	5.2^{A}	24.1^{R}	46.5^{G}	49.2^{K}
NDC-18-20-7	5.5^{AB}	5.3^{AB}	5.1^{AB}	24.7^{Q}	46.9^{G}	49.2^{K}
NDC-18-22-2	5.4^{B}	5.2^{ABC}	4.9^{AB}	35.0^{P}	46.6^{G}	49.1^{K}
NDC-18-21-3	5.2^{C}	4.9^{ABC}	4.9^{AB}	39.3^{O}	44.8^{G}	48.4^{L}
NDC-18-44-1	5.1^{C}	4.8^{BC}	4.8^{AB}	41.2^{N}	44.7^{G}	48.4^{L}
NDC-18-20-3	5.0^{C}	4.7^{C}	4.8^{AB}	41.5^{N}	43.3^{G}	40.2^{M}
NDC-18-152-2	4.5^{D}	4.2^{D}	4.7^{AB}	42.1^{M}	35.9^{H}	39.1^{N}
NDC-18-37-2	4.3^{E}	3.9^{D}	4.6^{B}	45.4^{L}	35.0^{HI}	32.3^{O}
NDC-18-36-2	2.1^{F}	1.5^{E}	1.8^{C}	54.5^{K}	66.5^{BC}	81.4^{A}
NDC-18-39-3	1.9^{G}	1.3^{EF}	1.5^{CD}	55.7^{J}	78.8^{A}	75.3^{B}
NDC-18-46-1	1.8^{G}	1.2^{EF}	1.4^{CD}	57.2^{I}	70.6^{B}	74.2^{C}
NDC-18-39-1	1.6^{H}	0.9^{FG}	1.1^{D}	58.3^{H}	70.2^{B}	70.6^{D}
NDC-18-42-1	1.6^{H}	0.9^{FG}	1.1^{D}	59.4^{G}	68.6^{B}	70.4^{D}
NDC-18-20-2	1.2^{I}	0.5^{GH}	0.6^{E}	61.3^{F}	66.6^{BC}	70.3^{D}
NDC-18-34-1	1.2I	0.5^{GH}	0.6^{E}	61.4^{F}	66.3^{BC}	69.5^{E}
NDC-18-47-3	1.0^{IJ}	0.3^{H}	0.3^{E}	62.1^{E}	65.9^{BCD}	66.3^{F}
NDC-18-41-3	1.0^{IJ}	0.3^{H}	0.3^{E}	65.2^{D}	62.4C^{DE}	65.2^{G}
NDC-18-66-2	0.9^{JK}	0.2^{H}	0.2^{E}	66.3^{C}	61.1^{CDE}	63.5^{H}
NDC-18-20-4	0.8^{KL}	0.2^{H}	0.2^{E}	67.4^{B}	60.1^{DEF}	62.1^{I}
NDC-18-104-2	0.7^{L}	0.2^{H}	0.1^{E}	67.9^{A}	57.2^{EF}	59.3^{J}

Means carrying different letters differ significantly from other mean values at $P \leq 0.05$ within each attribute.

At poding stage, the same 8 genotypes showed statistically significant higher values for CTD which ranged from 3.9 °C to 5.4 °C against CTD values of 0.2 °C to 1.5 °C of the remaining 12 genotypes. At grain-filling stage, the earlier-mentioned genotypes also significantly out-performed the other genotypes with CTD values ranging from 4.6 °C to 5.2 °C (Table 2) as compared with those of the other genotypes (0.1 °C to 4.6 °C). Significant differences were also noticed in the genotypes evaluated for CTD at three stages during the Rabi, 2021-22 crop growth season (Table 3). The same genotypes out-performed all other genotypes with CTD values of 4.1-5.3 °C, 3.8-4.1 °C, and 3.9-4.4 °C at the flowering, poding and grain-filling stages, respectively, compared with the other test genotypes (Table 3).

Relative injury (RI)

The results of ANOVA for percent RI at the flowering, poding and grain-filling stages during the 2021-21 and 2021-22 crop growth seasons are shown in Table 2 and 3. The genotypes differed significantly ($P \leq 0.05$) for the RI trait at all three stages. In season 2020-21 at flowering stage, the genotypes NDC-18-404, NDC-18-20-7, NDC-18-22-2, NDC-18-21-3, NDC-18-44-1, NDC-18-20-3, NDC-18-152-2 and NDC-18-37-2 showed the lowest percent relative injuries ranging from 24.1% to 42.1% compared with those of the other genotypes which ranged from 54.5% to 67.9% (Table 2). At the poding and grain-filling stages, the same genotypes significantly out-performed the other genotypes with values ranging from 35% to 46.5% and 32.3% to 49.2%, respectively compared with the values ranging from 57.2% to 78.8% and 59.3% to 81.4%, respectively, of the other genotypes (Table 2). During 2021-22, the same genotypes significantly out-performed the other test genotypes for RI with values ranging from 25.6% to 45.6%, 36.2% to 47.4% and 35.2% to 49.4% at the flowering, poding and grain-filling stages, respectively (Table 3).

Table 3. Canopy temperature depression (CTD) and relative injury (RI) of 20 chickpea genotypes evaluated in Rabi 2021-22 at NIFA, Peshawar

Genotypes	CTD (°C)			RI (%)		
	Flowering	Poding	Grain-filling	Flowering	Poding	Grain-filling
NDC-18-40-4	5.3^{A}	4.1^{AB}	4.4^{AB}	25.6^{J}	36.2^{I}	35.2^{M}
NDC-18-20-7	5.2^{B}	4.2^{AB}	4.5^{AB}	25.9^{J}	36.4^{I}	41.1^{L}
NDC-18-22-2	5.1^{C}	3.8^{B}	4.3^{AB}	37.0^{I}	44.3^{H}	42.3^{L}
NDC-18-21-3	4.9^{D}	3.9^{B}	4.4^{AB}	39.9^{H}	45.1H	47.2^{K}
NDC-18-44-1	4.9^{D}	3.8^{B}	4.1^{AB}	40.9^{GH}	45.4^{H}	48.6^{JK}
NDC-18-20-3	4.8^{E}	4.7^{A}	4.9^{A}	42.0^{GH}	45.6^{H}	49.2^{J}
NDC-18-152-2	4.8^{E}	3.5^{B}	3.8^{B}	43.0^{FG}	47.3^{H}	49.3^{J}
NDC-18-37-2	4.1^{F}	3.8^{B}	3.9^{B}	45.6^{F}	47.4^{H}	49.4^{J}
NDC-18-36-2	2.2^{G}	0.8^{CDE}	1.2^{CD}	57.0^{E}	55.3^{G}	62.1^{I}
NDC-18-39-3	2.1^{H}	1.4^{CD}	1.1^{CDE}	57.4^{DE}	62.2^{F}	63.3^{HI}
NDC-18-46-1	1.8^{I}	1.4^{CD}	0.8^{CDE}	58.8^{CDE}	62.4^{F}	64.4^{H}
NDC-18-39-1	1.6^{J}	1.5^{C}	1.3^{C}	59.9^{BCD}	63.1^{EF}	66.5^{G}
NDC-18-42-1	1.5^{K}	0.8^{CDE}	0.5^{CDE}	60.1^{BCD}	66.2^{DEF}	68.3^{FG}
NDC-18-20-2	1.4^{L}	0.7^{DE}	0.7^{CDE}	60.4^{BC}	67.3^{CDE}	69.8^{EF}
NDC-18-34-1	1.3^{M}	1.3^{CD}	0.7^{CDE}	60.5^{BC}	67.3^{CDE}	71.2E
NDC-18-47-3	1.1^{N}	0.4^{E}	0.4^{DE}	60.9^{BC}	67.5^{BCDE}	71.2^{DE}
NDC-18-41-3	1.1^{N}	0.4^{E}	0.3^{DE}	62.3^{B}	69.3^{BCD}	73.1^{D}
NDC-18-66-2	0.8^{O}	0.4^{E}	0.4^{DE}	67.4^{A}	71.5^{BC}	75.3^{C}
NDC-18-20-4	0.7^{P}	0.3^{E}	0.3^{DE}	68.1^{A}	72.2^{B}	77.6^{B}
NDC-18-104-2	0.5^{Q}	0.4^{E}	0.2^{E}	68.5^{A}	79.1^{A}	79.8^{A}

Means carrying different letters differ significantly from other mean values at $P \leq 0.05$ within each attribute.

Biological yield plant^{-1} (BYPP)

Significant ($P \leq 0.05$) differences among the genotypes were observed by ANOVA for BYPP in 2020-21 and 2021-22 (Table 4 and 5). In 2020-21, the highest per plant biological yield was produced by the genotypes NDC-18-404, NDC-18-20-7, NDC-18-22-2, NDC-18-21-3, NDC-18-44-1, NDC-18-20-3, NDC-18-152-2 and NDC-18-37-2 with values ranging from 60.3 to 69.3 g plant^{-1} compared with lower values of 22.3 to 47.3 g plant^{-1} recorded for the rest of the genotypes (Table 4). In 2021-22, the highest BYPP was recorded for the same genotypes with values ranging from 58.6 to 71.4 g plant^{-1} compared with those of the remaining genotypes evaluated (Table 5).

Table 4. Biological (BY) and seed yield (SY) of 20 chickpea genotypes evaluated in Rabi 2020-21

Genotypes	BY (g plant^{-1})	SY (g plant^{-1})	Genotypes	BY (g plant^{-1})	SY (g plant^{-1})
NDC-18-40-4	69.3^{A}	20.3^{A}	NDC-18-46-1	34.0^{F}	11.7^{DEF}
NDC-18-20-7	69.3^{A}	19.3^{AB}	NDC-18-39-1	32.3^{FG}	11.3^{DEF}
NDC-18-22-2	68.7^{A}	18.7^{ABC}	NDC-18-42-1	32.3^{FG}	11.3^{DEF}
NDC-18-21-3	68.3^{A}	18.7^{ABC}	NDC-18-20-2	32.0^{FG}	11.3^{DEF}
NDC-18-44-1	65.7^{B}	18.3^{ABC}	NDC-18-34-1	30.3^{GH}	11.3^{DEF}
NDC-18-20-3	61.3^{C}	18.3^{ABC}	NDC-18-47-3	30.3^{GH}	11.0^{EF}
NDC-18-152-2	60.3^{C}	18.0^{BC}	NDC-18-41-3	29.7^{H}	10.3^{FG}
NDC-18-37-2	60.3^{C}	16.7^{C}	NDC-18-66-2	29.7^{H}	10.0^{FG}
NDC-18-36-2	47.3^{D}	13.3^{D}	NDC-18-20-4	24.3^{I}	9.7^{FG}
NDC-18-39-3	37.0^{E}	12.2^{DE}	NDC-18-104-2	22.3^{I}	8.3^{G}

Means carrying different letters differ significantly from other mean values at $P \leq 0.05$ within each attribute.

Seed yield plant^{-1} (SYPP)

The genotypes also differed significantly ($P \leq 0.05$) for per plant seed yield during both seasons (Table 4 and 5). In 2020-21, 8 genotypes, i.e., NDC-18-404, NDC-18-20-7, NDC-18-22-2, NDC-18-21-3, NDC-18-44-1, NDC-18-20-3, NDC-18-152-2 and NDC-18-37-2 out-yielded the other genotypes with values ranging from 16.7 to 20.3 g plant^{-1} compared with those of the other genotypes that ranged from 8.3 to 13.3 g plant^{-1} (Table 4). In 2021-22, the same genotypes also out-yielded the rest of the genotypes with per plant seed yield ranging from 15.8 to 18.2 g plant^{-1} (Table 5).

Table 5. Biological yield (BY) and seed yield (SY) of 20 chickpea genotypes evaluated in Rabi 2021-22 at NIFA, Peshawar

Genotypes	BY (g plant^{-1})	SY (g plant^{-1})	Genotypes	BY (g plant^{-1})	SY (g plant^{-1})
NDC-18-40-4	66.3^{AB}	18.2^{BC}	NDC-18-46-1	33.5^{FGH}	11.5^{DEF}
NDC-18-20-7	71.4^{A}	21.1^{A}	NDC-18-39-1	31.9^{GH}	10.4^{EF}
NDC-18-22-2	66.5^{AB}	17.8^{BC}	NDC-18-42-1	31.7^{GH}	10.2^{F}
NDC-18-21-3	65.9^{ABC}	17.9^{BC}	NDC-18-20-2	30.8^{GHI}	9.40^{F}
NDC-18-44-1	67.4^{AB}	19.4^{AB}	NDC-18-34-1	29.9^{GHI}	11.5^{DEF}
NDC-18-20-3	59.7^{CD}	18.9^{AB}	NDC-18-47-3	34.3^{FG}	10.7^{DEF}
NDC-18-152-2	62.5^{BCD}	17.4^{BC}	NDC-18-41-3	27.8^{HIJ}	10.6^{DEF}
NDC-18-37-2	58.6^{D}	15.8^{C}	NDC-18-66-2	27.2^{HIJ}	9.8^{F}
NDC-18-36-2	44.2^{E}	13.1^{D}	NDC-18-20-4	25.1^{IJ}	9.3^{F}
NDC-18-39-3	39.4^{EF}	12.9^{DE}	NDC-18-104-2	23.4^{J}	9.2^{F}

Means carrying different letters differ significantly from other mean values at $P \leq 0.05$ within each attribute.

Associations of CTD and RI with BYPP and GYPP

Highly significant ($P \leq 0.01$) positive and negative correlations of CTD and RI with SYPP and BYPP, respectively, were observed (Table 6). Highest positive correlation (r = 0.9888) of CTD was noticed with SYPP, whereas highest negative correlation (r = -0.9743) was observed between RI and SYPP. Regression analysis also showed that CTD and RI were significantly ($P \leq 0.01$) positively and negatively associated with SYPP and BYPP, respectively (Fig. 1). The positive association was stronger between CTD and SYPP with a regression coefficient (R^2) of 0.977 compared with the association of CTD and BYPP with a regression coefficient value of 0.971 (Fig. 1). RI was strongly negatively associated with SYPP (R^2 = 0.9489) as compared with its association with BYPP having a regression coefficient value of 0.9466 (Figure. 1).

Table 6: Correlation coefficients between CTD (°C) and RI (%) with SYPP and BYPP under field conditions

	BYPP (g)	CTD	RI
CTD	0.9859**		
RI	-0.9727**	-0.9853**	
SYPP (g)	0.9908**	0.9888**	-0.9743**

**. Correlation is significant at the 0.01 level. SYPP (g): Seed yield per plant (g); BYPP: Biological yield per plant (g)

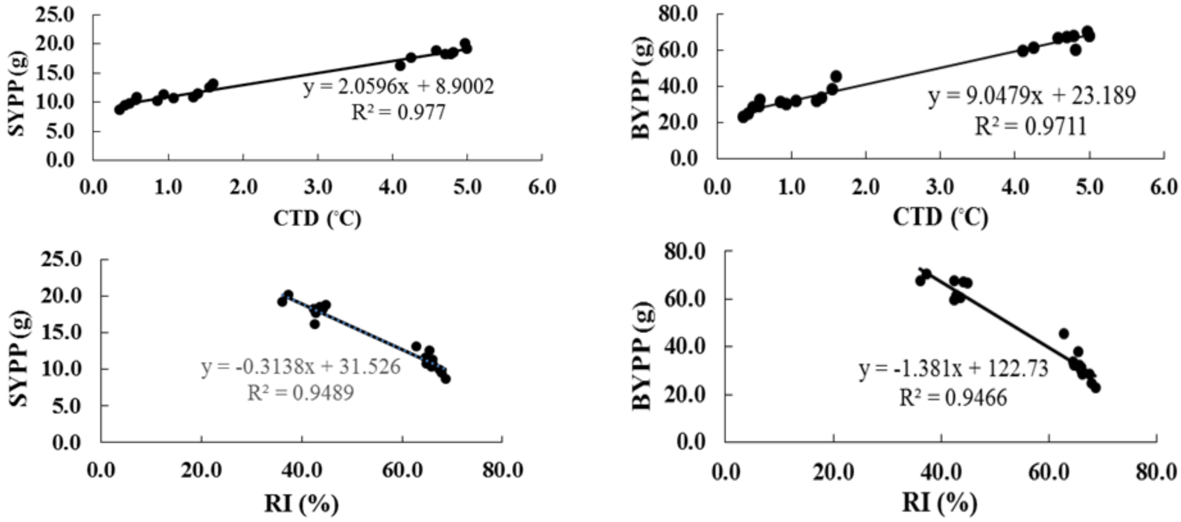

Figure 1. Regression analysis between CTD (°C) and RI (%) with SYPP and BYPP under field conditions
SYPP: Seed yield plant-; BYPP: Biological yield per plant; CTD: Canopy temperature depression; RI: Relative injury

Discussion

Chickpea is a crop adapted to cool season and gives good performance at temperatures below 30 °C, whereas the climate change is posing a serious threat to the chickpea growing area due to increasing temperature (Berger and Turner, 2007; Berger et al., 2011; Upadhaya et al., 2011; Devi et al., 2023). Heat shocks of different intensities and durations affect chickpea and other grain crops at different stages (Khattak et al., 2006; Devasirvatham et al., 2012b; Huang et al., 2023). Canopy temperature depression under field conditions is a useful indicator of plant respiratory health, particularly for maintaining cooler plant canopies. This trait has a positive and significant correlation with yield traits in chickpea and other field crops (Huang et al., 2023). In our case, the genotypes with cooler canopies produced higher per plant biological yield and seed yield (Tables 2 to 5). CTD also had a highly significant positive correlation with per plant biological yield and seed yield (Table 6). Regression analysis also revealed a positive relationship of CTD with biological yield and seed yield. Global chickpea R&D endeavors also reported similar results where CTD showed positive associations with yield traits in chickpea and other crops (Karla et al., 2008; Devasirvatham et al., 2012a; Mason and Singh, 2014; Devasirvatham et al., 2015; Purushothaman et al., 2015; Priya et al., 2018; Narayan et al., 2022; Huang et al., 2023). These findings emphasize that CTD can confidently be employed as an indirect selection criterion under natural field as well as controlled conditions for chickpea genotypes with predictions to perform better under heat-stressed conditions.

Global food security is being negatively affected by the increasing temperatures, and therefore, overall agricultural production has substantially dropped (Jha et al., 2014; Janni et al., 2020; Choukri et al., 2022). Cell membrane performs dual job of protecting the cell from its surrounding as well as controlling solutes and water movement into and out of the cell. Damage to cell membrane in leaf is a valid measure of tolerance/susceptibility to heat stress in legumes (Ibrahim, 2011; Alsamir et al., 2020). In susceptible genotypes, heat stress disintegrates cell membrane ultrastructure or produces reactive oxygen species (ROS) that attack cell membrane. Moreover, the damaged cell membrane due to increased temperature can decrease chlorophyll content (Rossi et al., 2017), tissue death (Omae et al., 2012), reduction in chlorophyll fluorescence (Sita et al., 2018; Van der Westhuizen et al., 2020) and inhibition of RuBisCo activity and photosynthesis (Kaushal et al., 2011; Bindra et al., 2021). In the present study, the genotypes showing lower relative injury (RI) percentages, performed better in terms of seed yield and biological yield (Table 2-5), meaning that these genotypes had fully integrated cell membranes which protected vital cell organelles, thereby ensuring normal physiological functioning of the cell.

These results were also confirmed by the negative association of RI with per plant seed yield and biological yield (Table 6, Fig. 1). Based on two years' results, it was found that 8 genotypes performed better for the physiological traits studied under field conditions. These genotypes also showed better performance for per plant biological yield and seed yield. It is therefore concluded that these physiological traits can confidently be used as indirect selection criteria for heat tolerance in chickpea under field conditions.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceptualized and designed the study: IS, GSSK, AJ, SA, AW. Conducted field experiments: IS. Analyzed the data: IS, GSSK, AJ, SA. Reviewed and edited the manuscript: IS, GSSK, AJ, SA, AW. All authors have read, reviewed and agreed to publish the current version of the manuscript in IJAaEB.

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This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

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