



Research article

Chlorophyll fluorescence and carbohydrate concentration as field selection traits for heat tolerant chickpea genotypes

Givemore M. Makonya^a, John B.O. Ogola^b, A. Muthama Muasya^a, Olivier Crespo^c, Siphon Maseko^d, Alex J. Valentine^e, Carl-Otto Ottosen^f, Eva Rosenqvist^g, Samson B.M. Chimphango^{a,*}

^a University of Cape Town, Department of Biological Sciences, Private Bag X3 Rondebosch 7701, South Africa

^b Department of Plant Production, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa

^c University of Cape Town, Climate System Analysis Group, Environmental and Geographical Science Department, Rondebosch, Cape Town 7701, South Africa

^d Department of Crop Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

^e Botany and Zoology Department, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

^f Department of Food Science, Aarhus University, Kirstinebjergvej 10, 5792 Aarslev, Denmark

^g Department of Plant and Environmental Sciences, Section for Crop Science, University of Copenhagen, Højbakkegaard Allé 9, 2630 Taastrup, Denmark

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ABSTRACT

Chickpea (*Cicer arietinum* L.), a cool season crop is severely affected by heat stress, predicted to increase due to warming climates. Research for identifying heat tolerance markers for potential chickpea genotype selection is imperative. The study assessed the response of four chickpea genotypes to a natural temperature gradient in the field using chlorophyll fluorescence, non-structural carbohydrate, chlorophyll concentrations, gas exchange and grain yield. Field experiments were carried out in two winter seasons at three locations with known differences in temperature in NE South Africa. Results showed two genotypes were tolerant to heat stress with an F_v/F_m of 0.83–0.85 at the warmer site, while the two sensitive genotypes showed lower F_v/F_m of 0.78–0.80. Both dark-adapted F_v/F_m and F_q'/F_m' (where $F_q' = F_m' - F$) measured at comparable high light levels correlated positively with grain yield. The two tolerant genotypes also showed higher photosynthetic rates, starch, sucrose and grain yield than the sensitive genotypes at the warmer site. However, these parameters were consistently higher at the cooler sites than at the warmer. These results were further validated by a climate chamber experiment, where higher F_v/F_m decline in the sensitive compared to tolerant genotypes was observed when they were exposed to short-term heat treatments of 30/25 °C and 35/30 °C. Tolerant genotypes had higher F_v/F_m (0.78–0.81) and grain yield plant^{-1} (1.12–2.37g) compared to sensitive genotypes (0.74–0.75) and (0.32–0.89g plant^{-1}) respectively in the 35/30 °C. It is concluded that chlorophyll fluorescence and leaf carbohydrates are suitable tools for selection of heat tolerant chickpea genotypes under field conditions, while the coolest site showed favourable conditions for chickpea production.

1. Introduction

Chickpea (*Cicer arietinum* L. Fabaceae) is the third most important legume crop, globally, after common bean (*Phaseolus vulgaris*) and field pea (*Pisum sativum*) (Jain et al., 2013). The crop is a relatively cheap source of protein (23%), carbohydrates (40%), oil (6%) (Gil et al., 1996), and minerals (Mg, K, P, Fe, Zn, and Mn) (Ibriki et al., 2003). Presently, chickpea is cultivated in over 40 countries across all continents (Wubneh, 2016) on about 12 million hectares globally with 65% and 8% share belonging to India and Pakistan, respectively (FAO, 2018; Muehlbauer and Sarker, 2017). Average global annual production of

chickpea is about 12.1 million tonnes with 95% production and consumption occurring in developing countries (FAO, 2018). Ethiopia (average temperatures of 18–29 °C, about 800 mm rainfall and on predominantly vertisols), Tanzania (average temperatures 19–29 °C, 1100 mm rainfall and grown on black clay soils) and Kenya (average temperatures 11–29 °C, 500 mm rainfall and grown on sandy loam soils) account for about 69% of chickpea production in Sub-Saharan Africa (SSA), and the whole African continent contributes about 4% of global chickpea production (Monyo and Laxmipathi, 2014; Muehlbauer and Sarker, 2017). Efforts in extending chickpea cultivation to regions of N.E. South Africa, where it has previously not been grown have been

* Corresponding author. Department of Biological Sciences, University of Cape Town, Rondebosch, South Africa.

E-mail address: samson.chimphango@uct.ac.za (S.B.M. Chimphango).

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initiated by the Mpumalanga Department of Agriculture and the University of Venda. The results to date show a great potential for crops in this region (Thangwana and Ogola, 2012).

Chickpea production in the SSA region is constrained by management factors such as appropriate varieties, spacing, and nutrient requirements, and abiotic stresses such as frost, terminal drought, nutrient deficiencies and heat stress (Monyo and Laxmipathi, 2014). Being a cool season crop, high temperatures during critical growth stages, like the reproductive period, can limit chickpea grain yield to a greater extent than warm season legumes (Devasirvatham et al., 2012a). Climate change is recognized as inevitable and one of the most complex challenges that human kind faces now and, in the future, with global simulation models predicting a 4–5 °C increase in atmospheric temperatures by the year 2100 (Harris and Roach, 2016). Effects of climate change are expected to be more severe in the tropics where temperatures are already quite high (Martin, 2015), hampering crop production. This necessitates the development of crop genotypes that have tolerance to heat stress as an adaptation strategy.

Even though chickpea is grown worldwide, it has a relatively narrow genetic base making development of heat stress tolerant cultivars a major challenge (Abbo et al., 2003). Reduced photosynthetic rates and carbon assimilation, as well as high transpiration rates tend to occur during high temperature stress, leading to reduced chickpea establishment and reduced carbon reserves (Singh et al., 1987; Mathur et al., 2011). Reduction in the photosynthesis is due to thermal instability of Rubisco and inhibition of the electron transport chain and Photosystem II (PSII) (Mathur et al., 2011; Brestic et al., 2012), primarily limiting photochemistry (Baker and Rosenqvist, 2004). This has been attributed to the heat induced increase in thylakoid membrane fluidity and electron transport-dependent integrity of PSII (Prasad et al., 2008). Also, the heat stress induced damage and disruption of the integrity of thylakoid membranes causes photophosphorylation process to cease (Dias and Lidon, 2009). The inactivation of the PSII reaction centres after heat stress due to the damaged thylakoid membranes (composed of different types of lipids together with a significant amount of protein) has also been associated with the phase changes and ultimately the separation that their lipid components go through (Sharkey and Zhang, 2010). Moreover, inhibition of PSII activity after exposure to heat stress usually results in reduced chlorophyll biosynthesis due to the deactivation of various enzymes (Dutta et al., 2009).

Plants acclimate to elevated temperatures by developing appropriate morphological, physiological and biochemical characteristics (Wahid et al., 2007). For example, although heat stress leads to misfolding of newly synthesised proteins and the denaturation of existing ones, it induces accumulation of heat shock proteins (HSPs) that prevent protein degradation (Wahid et al., 2007). Furthermore, increased carbohydrate (e.g. sucrose and glucose) availability during heat stress exposure represents a vital physiological trait associated with heat stress tolerance (Liu et al., 2011). Indeed, there is evidence that high cell wall and vacuolar invertases activities as well as increased sucrose import into young tomato fruit contributed to heat tolerance, through an elevated sink strength and sugar signalling activities (Li et al., 2012).

Heat stress is known to disrupt sexual reproductive success, with the pollen being most sensitive, in a variety of legume species (chickpea, *Cicer arietinum* L. – Devasirvatham et al., 2012a; common bean, *Phaseolus vulgaris* L. – Monterroso and Wien, 1990). Exposure to heat stress during flowering leads to yield losses due to the reduction of pollen viability, pollen production per flower and pod set in chickpea, hence their continued use as potential heat tolerance selection candidates in chickpea (Devasirvatham et al., 2012a).

Measurement of chlorophyll fluorescence has been used successfully as a quantitative assessment of inhibition or damage to the electron transport system (Baker and Horton, 1988) in several crops including maize (Sinsawat et al., 2004). The chlorophyll fluorescence parameter (F_v/F_m) reflects the maximum quantum efficiency of PSII

photochemistry in dark adapted leaves (Baker and Rosenqvist, 2004), with a decrease in F_v/F_m resulting in lowering of maximum quantum yield of photosynthesis (Ögren, 1988). The relationships between primary photosynthetic reactions and chlorophyll fluorescence (F_v/F_m) are important as they provide information on the plant's photosynthetic capability as well as its acclimation capacity under stressful environmental conditions (Lichtenthaler, 1987; Brestic et al., 2018). The use of chlorophyll fluorescence is becoming a common tool in plant heat stress response studies with emphasis on PSII photochemistry since the technique is relatively rapid, sensitive, non-destructive and can show damage before visible stress symptoms appear (Wilson and Greaves, 1990). A group of other fluorescence parameters called the JIP-test that quantify the stepwise flow of energy through PSII using input data from fluorescent transient have, in some studies, shown a greater sensitivity to plant heat stress (Jiang et al., 2006; Brestic et al., 2012; Brestic and Zivcak, 2013). We, however, opted to use F_v/F_m test in this study because in a previous experiment (Sharma et al., 2012), it was noted that the F_v/F_m test had no genetic component of the variation in control conditions in climate chamber experiment, while the JIP-test parameters showed an increase in the genetic component in both the heat stress and the control plants.

Therefore, the validation of the relationships between measured chlorophyll fluorescence (F_v/F_m) and carbohydrate accumulation with plant agronomic performance will strengthen the use of these markers as phenotyping tools during germplasm screening under field conditions.

The objectives of this study were to determine (i) response of chlorophyll fluorescence of chickpea to a temperature gradient under field and controlled climate chamber conditions, and (ii) the effect of heat stress on non-structural carbohydrates and gas exchange in four chickpea genotypes. With this study, we intend to establish the use of chlorophyll fluorescence and leaf carbohydrate concentrations for identifying thermotolerant genotypes with desirable agronomic traits under field conditions in southern Africa for the maintenance of higher grain yields under warming climates and as genetic resources for plant breeding.

2. Materials and methods

2.1. Field experiment (experiment 1)

2.1.1. Study sites

Field experiments were conducted in north eastern South Africa at three sites during the winter seasons of 2016 and 2017. The winter growing season traditionally falls between April and August (Thangwana and Ogola, 2012). The three sites included the University of Venda experimental farm in Thohoyandou (22°35'14.0" S and 30°15'50.3" E and 595 m asl), Vhugela River Queen farm in Louis Trichardt (23°02'37" S 29°54'11" E and 495 m asl) and the University of Limpopo experimental farm in Polokwane (23°49' S; 29°41' E and 389 m asl). The straight-line distance between the two furthest sites (Venda and Polokwane) is 149 km. Automatic weather stations located approximately 100 m from the experimental plots recorded rainfall (mm), maximum and minimum air temperatures (°C), and relative humidity (%) each day during the experiments. The three sites are along a temperature gradient with average minimum and maximum winter temperatures of 12/24 °C (Venda), 7/22 °C (Louis Trichardt) and 4/20 °C (Polokwane) characterised by different soils (Table 1), cumulative monthly rainfall and average maximum air temperatures for 2016 (Fig. 1a) and 2017 (Fig. 1b). The University of Venda and University of Limpopo experimental farms generally had the highest and lowest minimum and maximum air temperatures of the three sites throughout 2016 and 2017 respectively. The rainfall predominantly falls in the summer season and little to no rainfall in the winter season, with Venda receiving the highest cumulative rainfall and Polokwane the least rainfall of the three sites. Global radiation was measured daily

Table 1
Soil physicochemical analyses for the three experimental sites for 2016 cropping season. The sites are as in Fig. 1. Data is mean values \pm se (n = 8).

Site	Venda	Louis	Polokwane
Soil Type	Clay	Sandy Loam	Sandy loam
Carbon (%)	2.77 ^a	1.42 ^a	0.32 ^c
pH	5.10 ^b	5.88 ^a	5.18 ^b
Total P (mg/kg)	209.3 ^a	154.2 ^b	74.3 ^c
Bray II P (mg/kg)	10.00 ^c	51.00 ^a	27.75 ^b
N (%)	0.08 ^b	0.12 ^a	0.05 ^c
K (mg/kg)	311.00 ^{ab}	368.50 ^a	146.75 ^b
Ca (cmol/kg)	7.34 ^a	4.93 ^b	2.48 ^c
Mg (cmol/kg)	2.46 ^b	3.34 ^a	3.65 ^a
Fe (mg/kg)	126.70 ^b	327.00 ^a	131.02 ^b

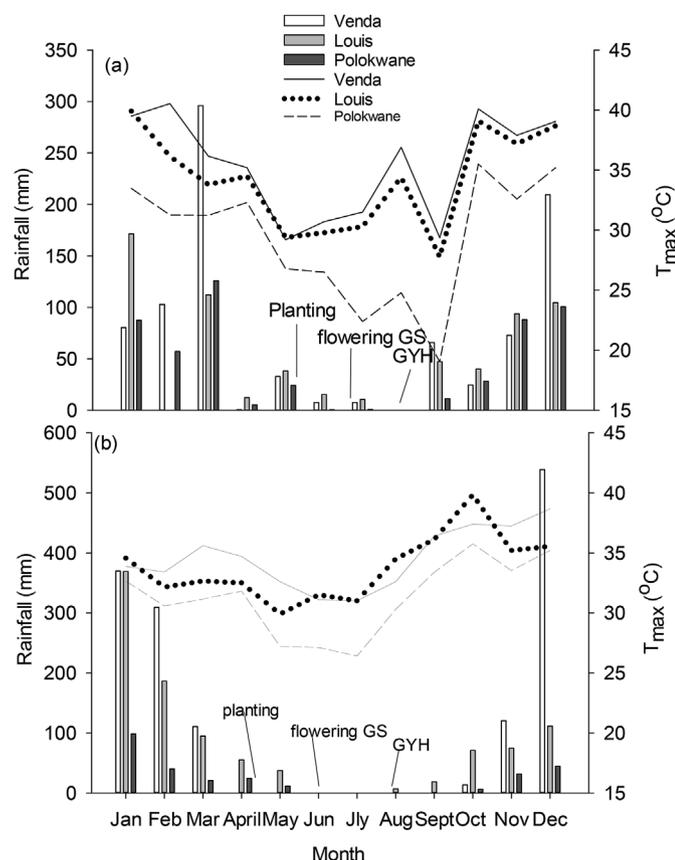


Fig. 1. Summary of total rainfall and average minimum and maximum monthly temperatures at the three experimental sites for (a) 2016 and (b) 2017 cropping seasons. The experimental sites are Venda (University of Venda experimental farm, Thohoyandou), Louis (Vhugela River Queen Farm, Louis Trichardt) and Polokwane (University of Limpopo experimental farm, Polokwane). Flowering GS is flowering growth stage and GYH is grain yield harvesting. Rainfall is the monthly accumulated precipitation and T_{max} is the monthly mean daily maximum air temperature.

and recalculated to daily light integral (DLI) across the environments. Pre-sowing analyses of soil physical and chemical properties were carried out in all the three sites at the start of the study in 2016 (Table 1).

2.1.2. Plant material, management and experimental design

The experiments consisted of a factorial treatment combination of the three sites and four desi type chickpea (*Cicer arietinum* L.) genotypes (Acc#RR-2, Acc#RR-3, Acc#7 and Acc#8). Genotypes were selected based on their superior grain yield potentials from experiments previously carried out in N. E South Africa. At each site, the treatments

were arranged in randomized complete block design and replicated four times. Each treatment consisted of a plot measuring 3.2 m \times 1.2 m with nine rows of chickpea. Spacing between plots and blocks was 0.5 m and 1 m, respectively. The plots were fertilized at planting by superphosphate fertilizer (20.3% P with 60 kg P ha⁻¹) and nitrogen (N) as limestone ammonium nitrate (LAN 28% N with 20 kg N ha⁻¹) (NTK, South Africa). The winter 2016 experiments were sown between the 7th to the 12th of May and the 2017 experiments were planted between the 14th and 19th of April. Field sowing was done manually at a spacing of 0.4 m inter-row and 0.1 m intra-row spacing. All the plots were watered uniformly after sowing to promote even germination, emergence and crop establishment. Supplemental irrigation was applied in all three experiments when necessary. Experimental plots were weeded throughout the growing seasons in all the three sites. A net was used to cover the plants at physiological maturity to deter monkeys and birds herbivory.

2.2. Controlled climate chamber experiment (experiment 2)

The experiment was conducted once in September 2018 at the Department of Food Science, Aarhus University, Aarslev, Denmark (55.30N, 10.44E). The four Desi chickpea genotypes earlier used in the field experiment 1 were sown under greenhouse conditions. Genotypes were sown with a 12 h photoperiod, combination of natural and supplementary light, 65 \pm 15% air relative humidity (RH%) and average temperature of 25 \pm 1.5 $^{\circ}$ C. Three seeds were placed one cm deep into 0.6L truncated cone plastic pots (9 cm height, 11 cm in diameter across the top and 7.5 cm in diameter across the bottom) filled with a commercial peat based potting substrate (Pindstrup Færdigblanding 2, Pindstrup Mosebrug A/S, Ryomgaard, Denmark). Seedlings were thinned to one plant per pot, when plants reached 4 fully developed leaf stage (approximately 14 days after sowing (DAS)). When the first flower appeared, a total of 12 plants per genotype were initially moved to the control climate chamber (MB teknik, Brøndby, Denmark), with 11 h photoperiod, photon flux density (PPFD) of 300 μ mol m⁻² s⁻¹ (LED FL300 Sunlight, Fionia Lighting, Sønderlø, Denmark), relative air humidity (RH%) set at 65%, and a CO₂ concentration of 400 ppm for acclimatisation. Three temperature regimes including the control were established in three separate chambers: a control chamber with day/night temperatures of 25/20 $^{\circ}$ C, moderate heat stress of 30/25 $^{\circ}$ C and high heat stress chamber with day/night temperatures of 35/30 $^{\circ}$ C. The three chambers were set at relative humidities of 65%, 70% and 80% respectively to maintain a constant VpdL across treatments. At 50% flowering, four replicates from each genotype were subjected to moderate heat stress at 30/25 $^{\circ}$ C and high heat stress at 35/30 $^{\circ}$ C for 3 days, with four replicates per genotype remaining in the control chamber. Plants in the 25/20 $^{\circ}$ C and 30/25 $^{\circ}$ C chambers were watered with a full nutrient solution twice a day with the plants in the 35/30 $^{\circ}$ C watered thrice daily to avoid moisture limitation. The day length was set from 0800 to 1900 h in all chambers. Plants were returned to the control chamber after data collection for recovery until final grain yield determination. Data on F_v/F_m , grain weight and pod numbers plant⁻¹ from Experiment 2 is presented.

2.3. Chlorophyll fluorescence

In Experiment 1 and following the fluorescence nomenclature proposed by Baker and Rosenqvist (2004), leaf chlorophyll fluorescence values, including minimal fluorescence F_o , Maximum Fluorescence F_m , variable fluorescence F_v , and the maximum photochemical efficiency of photosystem II F_v/F_m ($F_v = F_m - F_o$), were taken at the early reproductive stage on the youngest, fully expanded leaf using a PAM-2100 portable chlorophyll fluorometer (Walz, Eifeltrich, Germany). Chlorophyll fluorescence readings were taken at night from 1900 h onwards to enable prior dark adaptation (sun sets between 1730 h and 1800 h in winters of N.E. South Africa). Five plants from each of the

sixteen plots were randomly selected from the four inner-most rows, clamped on using light-exclusion clips (Walz, Eifeltrich, Germany) and readings recorded. The operating efficiency of PSII (F_q'/F_m') (where $F_q' = F_m' - F$) and leaf temperature readings were taken and recorded during the day from 0800 h on five randomly selected plants from each of the sixteen plots from the four inner most rows with the fiber optics attached to leaf clip holder 2030-B (Walz, Eifeltrich, Germany). Corresponding time and photosynthetic photon flux density (PPFD) values were recorded simultaneously.

F_v/F_m measurements in Experiment 2 were taken on four plants per genotype using a Plant Efficiency Analyser, Handy PEA (Hansatech Instrument, King's Lynn, UK) with excitation light energy of 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Data was collected on the youngest fully expanded leaf after three days of exposure to heat stress in the 35/30 °C and 30/25 °C treatments, as well as the control treatment. Leaves were prior dark adapted for 30 min using leaf dark clips (Hansatech Instrument). Measurement were taken in the afternoon after 11 h of light exposure to maximise on the stressful conditions on the adaxial leaf surface.

2.4. Gas exchange

Gas exchange variables including net photosynthetic rate (P_n), rate of transpiration (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and night respiration (R_n) were measured at CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$ using a LI-6400 portable photosynthesis system infrared gas analyser (LiCor, Lincoln, NE, USA) with an automatic cuvette of up to 6 cm^2 leaf area. Measurements were taken when the crop had reached 50% flowering growth stage. The gas exchange measurements were taken on well-watered plants to avoid moisture stress between 0800 h and 12 noon on a sunny day (average sunrise and sunset times in NE South Africa in winter are 0530 h–1800 h respectively). Five plants from each of the 16 plots were randomly selected from four inner most rows. Readings were taken from the youngest, fully expanded leaves that were allowed to equilibrate to 20 °C cuvette conditions and at PPFD of ca 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 3 min. The same procedure at PPFD of 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was repeated at night from 19 00 h until 2300 h for respiration measurements.

2.5. Non-structural carbohydrates

During the flowering stage, leaf samples were collected, and oven dried for 48 h at 70 °C. Dried samples were finely ground using a Hammer Mill (United Scientific Pty Ltd, Pretoria, South Africa) for analysis of the non-structural carbohydrates. A glucose stock solution (1.0 mg ml^{-1}) packed with the GAHK-20 kit was used to make standard solutions containing 0–5 mg ml^{-1} glucose by diluting the stock solution with deionized water prior to analysis. Concentrations of fructose, glucose and starch were determined using an enzymatic method as described by Zhao et al. (2010). In this method, 70 mg of ground tissue was mixed with 2 ml 80% ethanol and heated at 80 °C in a water bath for 15 min. The same sample was further extracted 2 more times and centrifuged for 10 min at 1811 $\times g$ in an Eppendorf 5810 R Centrifuge (Hamburg, Germany). Three supernatants from the same sample were then combined and brought to 6 ml final volume with 80% ethanol. Finely ground 60 mg of activated charcoal was added into each tube and briefly shaken to mix contents. After being left to stand for 5 min, the tubes were centrifuged at 1811 $\times g$ for 15 min to obtain clear extracts. Clear aliquots from these samples were transferred into fresh tubes and used for glucose, sucrose and fructose analyses. Glucose concentrations were determined using a glucose hexokinase (HK) assay reagent kit Sigma-Aldrich, Inc. (St Louis, MO, USA) on a microplate reader. Fructose, the second of the three assays was initiated by addition of phosphoglucose isomerase to each well with the glucose aliquots and resultant absorbance obtained as the sum of glucose and fructose. Addition of the invertase enzyme is the initial step in the determination of the final sucrose assay by obtaining the overall sum of glucose,

fructose and sucrose equivalent concentrations as glucose. Subsequently, sucrose concentration was determined using the following equation:

$$\text{Sucrose} = [\text{overall sum of glucose equivalents} - (\text{glucose} + \text{fructose})] \times 0.96$$

where 0.96 accounts for a water molecule added during sucrose hydrolysis. Starch concentration was determined by measuring glucose in the aliquot of the supernatant after hydrolysis of starch in the sample residue remaining after extraction of the non-structural carbohydrates. Hydrolysis was done using amyloglucosidase and the glucose released was measured as described above. All absorbances were obtained spectrophotometrically at 340 nm on a Thermo Multiskan Plate reader (Thermo Scientific, USA). Starch concentration was then calculated according to glucose concentrations in the tissue residue multiplied by 0.9 to account for water loss when glucose units are linked in starch formation (Zhao et al., 2010).

2.6. Total biomass, grain yield and yield components

In Experiment 1, whole plant biomass was quantified by harvesting three adjacent plants within randomly selected rows in each plot at the flowering stage (9–13 leaf stage). Plant samples were dried at 70 °C for 48 h and biomass weight recorded. Grain yield was determined at harvest maturity from 20 plants in the four inner most rows (five successive plants within a row) of the total nine rows in a plot. Pods were removed from the plants, threshed and seed air dried to 12% seed moisture and weighed to obtain total grain yield in kg ha^{-1} . Total pod number and 100 seed weight were also determined.

Number of pods plant^{-1} for Experiment 2 were determined at harvest maturity from four harvested plants per genotype and treatment, which were then dried to 12% moisture content and seed weight in grams plant^{-1} determined. A table showing which data were collected from experiment 1 in 2016 and 2017 cropping seasons is presented (Appendix 1).

2.7. Statistical analysis

Data was analysed by two-way analysis of variance (ANOVA) to test for the significance of the different environments and temperature treatments (Experiment 1 and Experiment 2 respectively) and the four genotypes on each measurable variable. The Tukey's Honestly Significant Difference (HSD) test was used to separate means that were significantly different ($P < 0.05$).

3. Results

3.1. Chlorophyll fluorescence parameters

The daily light integral (DLI), which is the number of photons in the photosynthetic range integrated over the day (Poorter et al., 2016) were measured across all sites in Experiment 1 during the cropping seasons and were relatively comparable, ranging from 26.6 to 47.3 $\text{mol m}^{-2} \text{d}^{-1}$ and 31.4–54.7 $\text{mol m}^{-2} \text{d}^{-1}$ in 2016 and 2017 seasons respectively (data not shown). The interaction of the genotypes and environment for F_v/F_m was significant ($p < 0.05$) for the plants grown in the 2017 season. At the cooler site of Polokwane, Acc#7 had higher ($p < 0.05$) F_v/F_m (0.86) compared to the other three genotypes with no significant decline at Venda (Fig. 2a). The F_v/F_m values for the other three genotypes were similar except that there was a significant decline from Polokwane to Venda for Acc#RR-2 whereas there was no decline in F_v/F_m for Acc#RR-3 and Acc#8. Noteworthy is that Acc#8 recorded lower F_v/F_m values than Acc#7 at all sites whereas the values for Acc#RR-3 were lower than Acc#7 only at Polokwane sites (Fig. 2a). The values of F_q'/F_m' at the cooler site in Polokwane were greater ($p < 0.05$) than

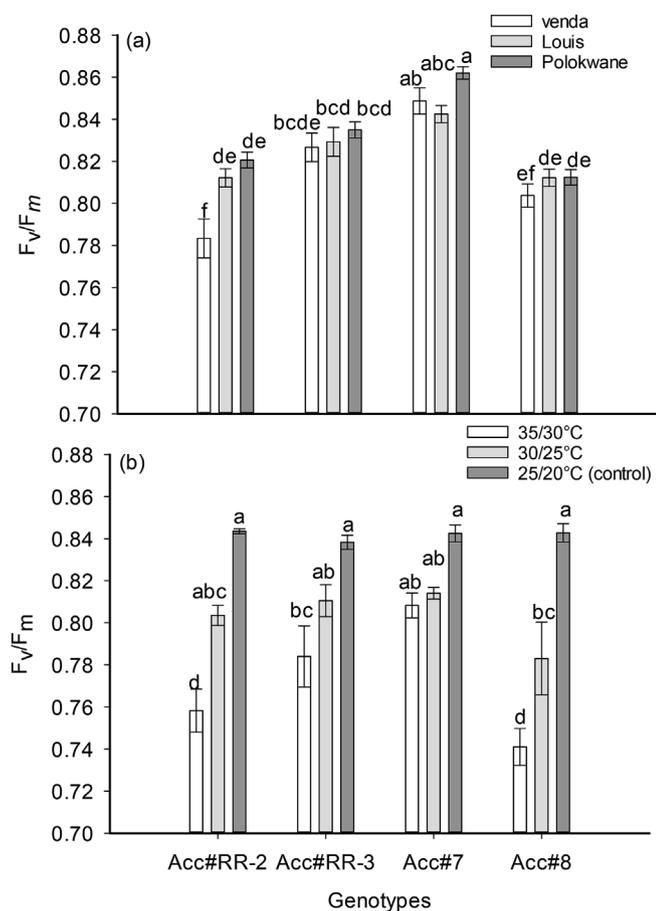


Fig. 2. Interaction of (a) genotype and environment on maximum quantum yield of PSII (F_v/F_m) at 50% flowering stage of chickpea in 2017 cropping season and (b) genotype and temperature treatment on maximum quantum yield of PSII (F_v/F_m) at 50% flowering stage of chickpea treatment in a growth climate chamber. Data is mean values \pm se ($n = 4$). Different letters indicate significant differences between genotypes and sites by Tukey's honest significant difference *post hoc* test ($p < 0.001$).

those in Venda both in 2016 and 2017 seasons (Table 2). However, the F_q'/F_m' values at Louis Trichardt were similar to those in Venda in 2016 and to those in Polokwane in 2017 season. The PPFD at the time of data collection in 2016 were significantly higher ($p < 0.001$) in Venda than in Polokwane which was similar to Louis Trichardt (Table 2). In 2017, however, the PPFD values on the day of data collections were similar across all environments. Genotypic differences in F_q'/F_m' were observed

Table 2

Effect of environment (growth site) and genotypes on operating efficiency of PSII (F_q'/F_m') and average PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at time of data collection of chickpea in 2016 and 2017 cropping seasons. The data is mean values \pm se ($n = 16$ for environment and $n = 12$ for genotype) with different letters showing significant difference as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns = not significant. The sites are as in Fig. 1.

Treatment	F_q'/F_m'		PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$	
Site	2016	2017	2016	2017
Venda	0.66 \pm 0.003 ^b	0.53 \pm 0.007 ^b	1156.2 \pm 25.9 ^a	1147.6 \pm 28.0 ^{ns}
Louis	0.65 \pm 0.006 ^b	0.57 \pm 0.009 ^a	978.5 \pm 45.3 ^b	1121.0 \pm 35.9 ^{ns}
Polokwane	0.69 \pm 0.005 ^a	0.59 \pm 0.008 ^a	887.0 \pm 17.8 ^b	1062.2 \pm 20.5 ^{ns}
Genotypes				
Acc#RR-2	0.66 \pm 0.007 ^b	0.53 \pm 0.008 ^b	995.2 \pm 55.2 ^{ns}	1041.3 \pm 24.4 ^{ns}
Acc#RR-3	0.67 \pm 0.007 ^a	0.59 \pm 0.008 ^a	988.3 \pm 56.1 ^{ns}	1136.3 \pm 37.7 ^{ns}
Acc#7	0.67 \pm 0.008 ^a	0.64 \pm 0.009 ^a	1047.1 \pm 42.6 ^{ns}	1160.1 \pm 35.3 ^{ns}
Acc#8	0.65 \pm 0.008 ^b	0.52 \pm 0.008 ^b	998.4 \pm 59.3 ^{ns}	1105.4 \pm 30.1 ^{ns}
<i>F</i> probability				
Site	29.7***	16.0***	15.3***	2.5 ^{ns}
Genotype	9.1***	25.9***	0.4 ^{ns}	2.3 ^{ns}
Site*Genotype	2.2 ^{ns}	1.7 ^{ns}	0.3 ^{ns}	1.0 ^{ns}

in both years where Acc#7 and Acc#RR-3 showed similar but significantly ($p < 0.001$) higher values than Acc#RR-2 and Acc#8 which were also similar.

In Experiment 2, genotypes and temperature treatment interaction for F_v/F_m was significant ($p < 0.05$) (Fig. 2b). Acc#7 had significantly higher F_v/F_m compared to Acc#RR-2 and Acc#8 in the high heat stress, though similar to Acc#RR-3. There was no significant decline in F_v/F_m of Acc#7 in the moderate heat stress (0.81) and high heat stress (0.81) relative to the control (0.84). A significant decline was however observed in F_v/F_m of Acc#8 in the moderate heat stress (0.78) and high heat stress (0.74) relative to the control treatment (0.84), with a decline observed only in the high heat stress for Acc#RR-2 (0.75) and Acc#RR-3 (0.78) (Fig. 2b).

3.2. Gas exchange parameters

There was no genotype by environment interactions for all the measured gas exchange parameters in neither 2016 nor 2017 (Appendix 2). There were environmental differences ($p < 0.05$) on net photosynthesis in 2016 with the cooler Polokwane site recording highest ($p < 0.05$) P_n ($8.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and Venda the lowest P_n ($6.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Table 1S). In 2017, the warmer site of Venda showed higher ($p < 0.001$) stomatal conductance g_s ($0.39 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), internal carbon dioxide concentration ($C_i = 309 \mu\text{mol CO}_2 \text{ mol}^{-1}$) and leaf transpiration rate ($E = 2.8 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than the other two sites. Results for leaf temperatures varied between the years being higher ($p < 0.05$) at the warmer Venda site than at Polokwane in 2016, while the highest leaf temperatures (24°C) were observed at Louis Trichardt site in 2017. The genotypic differences were significant ($p < 0.01$) on P_n (Appendix 2) 2017 where genotype Acc#7 was similar to Acc#RR-3, but higher ($p < 0.01$) than the two similar Acc#RR-2 and Acc#8 genotypes.

The warmer Venda site showed higher ($p < 0.01$) R_n values than the cooler Polokwane site in both years with Louis Trichardt values similar to Polokwane in 2016 and similar to Venda in 2017 (Fig. 3a and b). There were no genotypic differences in R_n in 2016 while in 2017, R_n in Acc#7 were similar to Acc#8 and Acc#RR-2, but higher ($p < 0.01$) than Acc#RR-3.

3.3. Non-structural carbohydrates

Genotype and environment did not have interactive effect on concentration of non-structural carbohydrate in leaves of plants grown in 2017. The concentration of starch in plants at the cooler Polokwane site was lower ($p < 0.05$; Fig. 4a) than that at the warmer Venda site while that of sucrose ($p < 0.001$; Fig. 4b) and glucose ($p < 0.05$; Fig. 4c) were similar in the two sites. However, leaves of plants at Louis

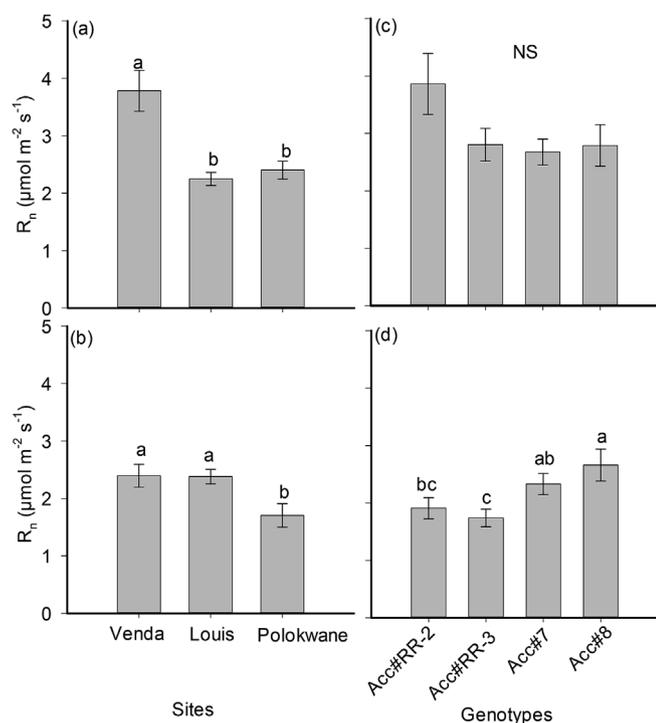


Fig. 3. Effect of environment (a and b) and genotype (c and d) on respiration at 50% flowering stage of chickpea in 2016 (a and c) and 2017 (b and d) cropping season. Data is mean values of pooled sites or genotypes \pm se ($n = 16$ for environment and $n = 12$ for genotype). Different letters indicate significant differences between genotypes or site by Tukey's honest significant difference *post hoc* test ($p < 0.001$) in respective figure; ns = not significant.

Trichardt recorded the highest ($p < 0.05$) concentrations of starch and sucrose while that of glucose was the least relative to the other two sites (Fig. 4a, b, and c respectively). The concentration of starch also differed with genotypes where Acc#7 showed similar values to Acc#8, but higher ($p < 0.05$; Fig. 4d) than Acc#RR-2. For the concentration of sucrose, Acc#7 showed similar values to Acc#RR-3 and Acc#8 but higher ($p < 0.05$; Fig. 4e) than Acc#RR-2. The concentration of glucose in leaves of Acc#7 was similar to that in Acc#8 and Acc#RR-2 but significantly higher ($p < 0.01$; Fig. 4f) than that of Acc#RR-3.

3.4. Plant harvest and yield components

In Experiment 1, there was no interaction between genotypes and environment on yield and yield components of plants grown in neither 2016 nor 2017. The environmental effects showed that the cooler site of Polokwane recorded higher ($P < 0.001$) grain yield than both Venda and Louis Trichardt 2017 (Table 3). However, grain yield in Polokwane was similar to that of Venda in 2016. Pod numbers per plant were significantly higher in Polokwane than in Venda and Louis Trichardt and in both years. The 100-seed weight was highest ($P < 0.01$) at Polokwane relative to the other sites only in 2016. However, total biomass was significantly higher ($p < 0.01$) at Venda than at Polokwane and Louis Trichardt with the two sites showing no differences. Genotypic differences were observed for grain yield and pod number in 2017 where Acc#7 recorded similar values with Acc#RR-3 and Acc#RR-2, but higher than Acc#8 (Table 3). Although Acc#7 recorded similar total biomass to all genotypes, total biomass for Acc#RR-3 was significantly higher ($p < 0.05$) than Acc#8 (Table 3).

It was interesting to see that there were significant and positive correlation of F_q'/F_m' (Fig. 5a) and F_v/F_m (Fig. 5b) with grain yield in 2017. Also, for leaf starch concentration, there was a significant negative correlation to the grain yield within each site, which was however negative

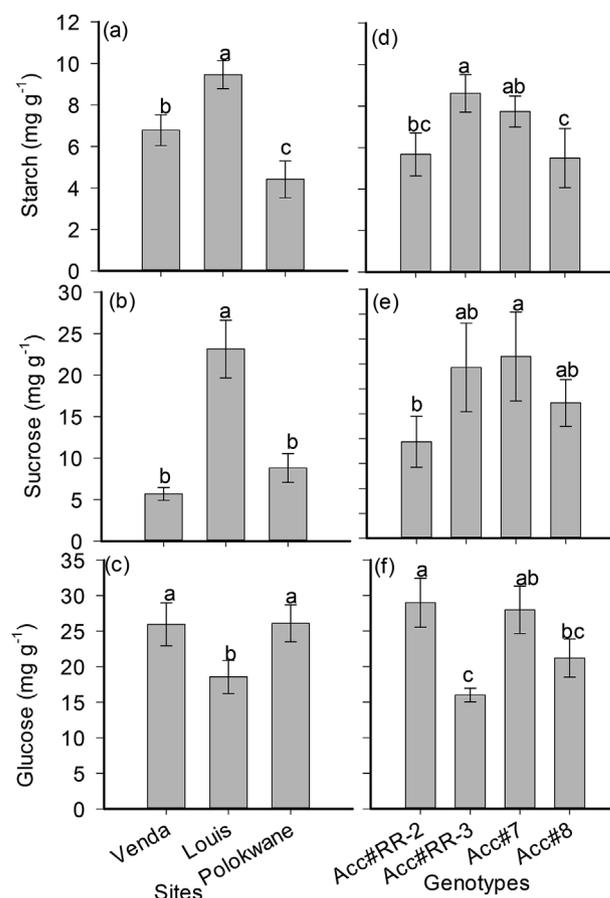


Fig. 4. Effect of environment on starch (a), sucrose (b), glucose (c), and genotype on starch (d), sucrose (e), glucose (f) leaf concentrations at 50% flowering stage of chickpea in 2017 cropping season. Data is mean values of pooled genotypes (a, c and d) or sites (b, e and f) \pm se ($n = 16$ for environment and $n = 12$ for genotype) Different letters indicate significant differences between genotypes and sites by Tukey's honest significant difference *post hoc* test ($p < 0.001$).

at the warmer site of Venda (Fig. 5c).

In experiment 2, grain yield plant⁻¹ was significantly affected by the interaction of the genotypes and temperature treatments ($p < 0.05$) (Table 4). There was a significant decline in the grain yield of all genotypes for both the moderate and high temperature chambers relative to their controls. However, Acc#8 and Acc#7 had the most and least severe decline in grain yield plant⁻¹ (94% and 65% respectively) in the high heat stress relative to their controls. Acc#7 had significantly higher grain yield plant⁻¹ compared to the other 3 genotypes exposed to the moderate heat stress. Though not different from Acc#RR-2 and Acc#RR-3, Acc#7 also had significantly higher grain yield plant⁻¹ compared to Acc#8 in the high heat stress (Table 4). Genotype and temperature treatment interaction was not significant ($P > 0.05$) for number of pods plant⁻¹ that were decreased ($P < 0.001$) by both heat stresses relative to the control. Genotypic differences ($P < 0.001$) were also observed, with Acc#7 recording higher pod numbers plant⁻¹ compared to the other three genotypes which were similar (Table 4).

4. Discussion

The maximum photochemical efficiency of PSII (F_v/F_m) measured on dark adapted leaves (Kumar et al., 2013) in combination with the operating efficiency of PS11 (F_q'/F_m') can be used as indicators of some environmental stresses and as screening tools for heat tolerance as shown in this study. The genotype Acc#7 was regarded as the most heat tolerant partly due to its high F_v/F_m at the cooler site of Polokwane with

Table 3
Effect environment and genotype on grain yield (plant^{-1} and ha^{-1}), pod yield (plant^{-1} and ha^{-1}), 100 seed weight and biomass yield (plant^{-1} and ha^{-1}) of chickpea in 2016 and 2017 winter cropping seasons. Mean \pm se with different letters are significantly different as at: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns = not significant. Venda-University of Venda experimental farm, Thohoyandou, Louis-Vhugela River Queen Farm, Louis Trichardt and Polokwane- University of Limpopo experimental farm, Polokwane.

Treatment Site	Grain yield (g plant^{-1})					Pod number plant^{-1}					100 seed weight (g)					Total Biomass (g plant^{-1})		Total Biomass (kg ha^{-1})	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	
Venda	10.9 \pm 0.68 ^a	9.7 \pm 1.37 ^c	1948 \pm 122 ^a	1729 \pm 245 ^c	41 \pm 2.63 ^b	31 \pm 4.62 ^c	23.8 \pm 1.06 ^b	25.5 \pm 1.58	12.2 \pm 1.45 ^a	1796 \pm 124 ^{ab}	2170 \pm 258 ^a								
Louis	4.0 \pm 0.61 ^{bs}	16.5 \pm 1.41 ^b	717 \pm 109 ^{bs}	2942 \pm 252 ^b	18 \pm 3.14 ^{cs}	47 \pm 4.57 ^b	20.7 \pm 1.37 ^c	25.8 \pm 0.94	8.4 \pm 0.71 ^b	2072 \pm 350 ^a	1500 \pm 128 ^b								
Polokwane	12.3 \pm 2.17 ^a	23.3 \pm 1.94 ^a	2204 \pm 388 ^a	4155 \pm 347 ^a	57 \pm 9.94 ^a	67 \pm 5.44 ^a	25.8 \pm 1.46 ^b	25.9 \pm 1.11	7.6 \pm 0.49 ^b	1599 \pm 195 ^{ab}	1369 \pm 88 ^b								
Genotypes																			
Acc#RR-2	8.1 \pm 2.20	16.1 \pm 2.07 ^{ab}	1438 \pm 391	2878 \pm 369 ^b	28 \pm 7.23	47 \pm 6.34 ^b	19.7 \pm 2.13	25.9 \pm 1.01	10.1 \pm 0.70 ^{ab}	1796 \pm 124 ^{ab}	2170 \pm 258 ^a								
Acc#RR-3	8.7 \pm 1.72	18.6 \pm 2.22 ^a	1550 \pm 302	3313 \pm 396 ^a	37 \pm 5.87	62 \pm 7.44 ^a	21.0 \pm 1.38	22.6 \pm 1.48	11.6 \pm 1.96 ^a	2072 \pm 350 ^a	1500 \pm 128 ^b								
Acc#7	9.2 \pm 1.77	19.5 \pm 3.18 ^a	1636 \pm 315	3482 \pm 569 ^a	41 \pm 7.67	52 \pm 7.02 ^{ab}	23.7 \pm 1.39	23.9 \pm 1.55	9.0 \pm 1.10 ^{ab}	1599 \pm 195 ^{ab}	1369 \pm 88 ^b								
Acc#8	7.8 \pm 1.59	11.8 \pm 1.58 ^b	1399 \pm 285	2096 \pm 281 ^b	31 \pm 7.58	31 \pm 4.65 ^c	25.7 \pm 1.09	27.7 \pm 1.34	7.0 \pm 0.43 ^b	1246 \pm 77 ^b	1500 \pm 128 ^b								
F probability	27.6***	21.8***	27.6***	21.8***	15***	20.5***	8.62***	1.5 ^{ns}	7.5**	7.5**	7.5**								
Site	0.8 ^{ns}	4.2*	0.8 ^{ns}	4.2*	2.2 ^{ns}	7.6***	2.7 ^{ns}	2.7 ^{ns}	3.6*	3.6*	3.6*								
Genotype	0.4 ^{ns}	0.9 ^{ns}	0.4 ^{ns}	0.9 ^{ns}	0.1 ^{ns}	1.3 ^{ns}	2.07 ^{ns}	1.1 ^{ns}	0.8 ^{ns}	0.8 ^{ns}	0.8 ^{ns}								
Site * Genotype																			

§ - Infestation by pod borer in 2016 affected the pod number and grain yield.

no significant decline in the warmer Venda site, a result validated by the climate chamber experiment. On the other hand, genotype Acc#2 is regarded as the most heat sensitive as it was the only one to show a significant decline in F_v/F_m at the warmer site while Acc#3 is intermediate between Acc#7 and Acc#8 for F_v/F_m at both Polokwane and Venda and also did not decline with warmer temperature. The F_v/F_m for the sensitive Acc#RR-2 in Venda is lower than the published value of 0.832 (Demmig and Björkman, 1987) for non-stressed plants, hence a lower adaptive response to heat stress compared to the heat tolerant Acc#7 genotype. These results were also consistent with the climate chamber results, where Acc#RR-2 as well as the other sensitive genotype Acc#8 had low F_v/F_m below the published value of 0.832 when exposed to the high heat stress. At the cooler Polokwane site with maximum temperatures of 27 °C at flowering stage, the tolerant genotype Acc#7 clearly had the highest F_v/F_m compared to the other three genotypes, despite none of the genotypes being stressed, suggesting a more ideal site and temperatures for chickpea production. However, when grown at the warmer Venda site, characterised by maximum temperatures around 32 °C at the flowering stage, Acc#7 and Acc#RR-3 were able to maintain a higher F_v/F_m than the heat sensitive genotype Acc#RR-2. In the climate chamber experiment, the two tolerant genotypes also registered a lower decline in F_v/F_m in comparison to the sensitive ones in 35/30 °C, relative to their controls. Similarly, heat tolerant bean genotypes were found to maintain high F_v/F_m when exposed to heat stressful conditions (Petkova et al., 2007). In a study on 30 field grown chickpea genotypes, genotype Pusa 240 maintained a high F_v/F_m when exposed to temperatures above 30 °C (Kumar et al., 2013). Contrasting reports have however reported that PSII inhibition does not occur until leaf temperatures are as high as 35 °C–42 °C (Wise et al., 2004) and around 40 °C (Al Khatib and Paulsen, 1999). Rubisco has been shown to deactivate at temperatures causing no harm to PSII (Feller et al., 1998), with this deactivation being proposed as the primary constraint to photosynthesis in this temperature range (Crafts-Brandner and Salvucci, 2000). However, it would be difficult to make similar conclusions from short term heat treatments on the chronic heat stress applied in the current study.

Genotypes Acc#7 and Acc#RR-3 were also able to maintain higher operating efficiencies (F_q'/F_m') in both cropping seasons compared to the other two genotypes. The higher values of F_q'/F_m' for Acc#7 and Acc#RR-3 (between 0.58 and 0.67) show their superior operating efficiencies under heat stress probably due to maintenance of the PSII quinone electron acceptors partially more oxidised (Rosenqvist, 2001) than the heat sensitive Acc#RR-2 and Acc#8 genotypes. Similar observations were made in a study of cotton, where 17 of the 40 selected genotypes with operating efficiencies between 0.56 and 0.67 were concluded to be the most tolerant to heat stress (Wu, 2013). A decrease in the operating efficiency of PSII from the cooler site of Polokwane to the warmer site of Venda was observed in 2016, a result attributed to the higher PPFD at Venda during the time of measurements as a natural consequence of the shape of the light dependency of photosynthesis. Fluctuations of light irradiance in the field may occur over short time scales as well as from year to year, leading to varied photosynthetic assimilation (Petridis et al., 2018), similarly making field F_q'/F_m' data collection and use a challenge. We therefore recommend using F_q'/F_m' measurements in conjunction with F_v/F_m data. Noteworthy is that the observed values of up to 0.66 for F_q'/F_m' at about 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ are higher than those reported by Bilger et al. (1995) for pumpkin plants (about 0.45 at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) grown in summer in Temperate region in the Northern Hemisphere. There are several factors than can be attributed to the differences including species and location differences with the current study using chickpea genotypes that are acclimated to semi-arid conditions in Southern Africa.

Putting together results from the F_v/F_m and F_q'/F_m' measurements, genotypes Acc#7 and Acc#RR-3 are showing heat tolerating characteristics in all sites. In a study of heat stress response by wheat, genotypic differences were also supported by superior photosynthetic

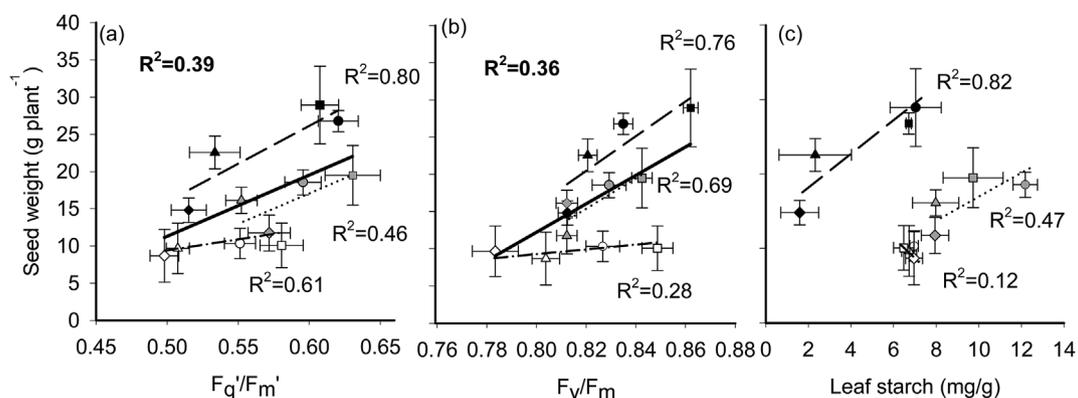


Fig. 5. Correlation of chickpea grain yield (g plant^{-1}) and operating efficiency (F_q'/F_m') (a), F_v/F_m (b) and leaf starch concentrations (mg/g) (c) in 2017 cropping season. 20 g plant^{-1} corresponds to 3333 kg ha^{-1} . The data represents mean values \pm se ($n = 4$) where the symbols are triangles (Acc#RR-2), circles (Acc#RR-3), squares (Acc#7), and diamonds (Acc#8) and the closed symbols are from Polokwane, grey from Louis Trichardt and open symbols from Venda. Bold regression lines and R^2 values represent combined regression across sites and accessions while the non-bold R^2 relate to respective sites. The flat lines for F_q'/F_m' and F_v/F_m for the Venda site are due to the scale for the y-axis (seed weight) and the non-presentation of the combined regression for leaf starch is due to the negative correlation for the Venda site.

Table 4

Effect temperature, genotype and their interactive effects on grain yield (plant^{-1}) and pod number plant^{-1} of chickpea in a climate chamber experiment. Mean \pm se with different letters are significantly different as at: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns = not significant.

Treatment	Grain yield (g plant^{-1})	pod number plant^{-1}
Temperature		
25/20 °C (control)	6.8 ± 0.29^a	19.1 ± 0.98^a
30/25 °C	2.3 ± 0.33^b	10.8 ± 1.10^b
35/30 °C	1.2 ± 0.22^c	4.1 ± 0.64^c
Genotype		
Acc#RR-2	3.3 ± 0.92^b	10.3 ± 2.03^b
Acc#RR-3	3.3 ± 0.79^b	10.1 ± 1.60^b
Acc#7	4.6 ± 0.62^a	15.2 ± 2.33^a
Acc#8	2.6 ± 0.75^b	9.7 ± 2.08^b
Temperature*Genotype		
25/20 °C (control)		
Acc#RR-2	7.4 ± 0.75^a	18.3 ± 1.4
Acc#RR-3	6.9 ± 0.63^a	16.3 ± 1.5
Acc#7	6.9 ± 0.57^a	24.0 ± 1.5
Acc#8	6.0 ± 0.36^{ab}	17.8 ± 1.1
30/25 °C		
Acc#RR-2	1.6 ± 0.26^{cd}	9.8 ± 1.9
Acc#RR-3	1.9 ± 0.14^{cd}	9.0 ± 0.9
Acc#7	4.4 ± 0.34^b	14.8 ± 1.7
Acc#8	1.4 ± 0.22^{cd}	9.8 ± 1.5
35/30 °C		
Acc#RR-2	0.9 ± 0.16^{cd}	3.0 ± 0.8
Acc#RR-3	1.1 ± 0.17^{cd}	5.0 ± 1.2
Acc#7	2.4 ± 0.44^c	6.8 ± 0.6
Acc#8	0.3 ± 0.09^d	1.5 ± 0.4
F probability		
Temperature	12.7***	8.9***
Genotype	219.3***	100.3***
Temperature*Genotype	3.0*	ns

performance of the genotypes (Sharma et al., 2015) as highlighted by the high operating efficiencies in the current study. The significant correlation of maximum photochemical efficiency and PSII operating efficiencies with grain yield seems valid because tolerant genotypes Acc#7 and Acc#RR-3 which both had higher grain yield also had higher operating efficiencies of PSII than the heat sensitive genotypes. The correlation of the two fluorescence parameters to grain yield was valid both within the sites and for the whole data set. Therefore, use of F_v/F_m as a tool for selection of field grown chickpeas continues to show potential, especially with the incorporation of F_q'/F_m' measured under

high light conditions, which to the best of our knowledge has very few records in literature, if any, on chickpea before our study for the use of a modulated chlorophyll fluorimeter in the field. This is a vital finding of our study for it signifies the potential use of both quick and non-destructive parameters (Sharma et al., 2012) as selection tools for heat tolerant chickpea genotypes under field conditions.

In this study we found a significant difference in F_v/F_m in Acc#7 compared to the other three genotypes also in the coolest location in Polokwane. This raises the question if the found differences in heat tolerance amongst the four genotypes arises from a difference in 'base line' F_v/F_m in non-stressed plants or in the decline in F_v/F_m after heat stress, or in both. In previous studies of 41 wheat genotypes of as diverse origin as Sweden and Pakistan, no genetic component was found in the variation of the control values (Sharma et al., 2012). In 28 genotypes of tomato that are used as well performing cultivars in the field in Nepal, no significant difference was found in F_v/F_m in control conditions, while they showed pronounced differences after heat stress when screened in climate chambers (Poudyal et al., 2018). Two heat tolerant and two heat susceptible cultivars were subsequently grown in a well irrigated field experiment in Nepal, where they by coincidence were exposed to a natural heat wave. The heat tolerant group had considerably smaller loss of harvest yield and stayed greener than the heat susceptible group from the climate chamber screening (Poudyal et al., 2018). In both these investigations no significant difference was found in F_v/F_m in control conditions. In our study only one out of four genotypes had higher F_v/F_m in the 'control' conditions, while two genotypes were considered more heat tolerant. For that reason, we cannot challenge the previous conclusions that it is the decrease in F_v/F_m that distinguish the heat susceptible from the heat tolerant genotypes, rather than an intrinsic difference in F_v/F_m in un-stressed plants.

Genotypic and site differences on F_v/F_m and F_q'/F_m' were further supported by photosynthetic measurements, night respiration and carbohydrates in the leaf and grain yield. For example, similar to genotypic differences in F_v/F_m and F_q'/F_m' , the tolerant genotypes Acc#7 and Acc#RR-3 had higher P_n than Acc#RR-2 and Acc#8. The ability of plants to sustain leaf gas exchange and CO_2 assimilation rates under heat stress is directly correlated with heat tolerance in snap bean (Kumar et al., 2005) and wheat (Yang et al., 2006). However, the correlation between P_n and grain yield was not universal across sites (data not shown). This is logical since growth is not directly dependent on photosynthesis but rather on the balance between photosynthesis, maintenance and growth respiration (Dewar et al., 1994). Since F_v/F_m reflects the activity in only parts of the photosynthetic apparatus, it is rather surprising to find a general correlation to the grain yield. Only

further investigation will reveal if this relationship is universal. Plant respiration at night also varied with genotypes with Acc#8 having the highest respiration rate compared to the other three genotypes. Respiration has been noted to consume between 30% and 80% of the CO₂ taken up by photosynthesis per day (Atkin et al., 2005), increasing with increase in temperature (McCullough and Hunt, 1993). High R_n in peanut was associated with a potential increase in reactive oxygen species, leading to cell damage and decrease in pollen viability (Prasad et al., 1999). Low respiration has been associated with higher biomass accumulation in a *Lolium spp.* breeding programme in a temperate environment (Wilson and Jones, 1982). Therefore, the high respiration rates, lower operating efficiency and lower photosynthesis in our study may have ultimately led to significantly lower grain yield of Acc#8.

The higher leaf starch concentrations in genotype Acc#7 and Acc#RR-3 compared to Acc#8 also suggest tolerance to heat stress (Subrahmanyam and Rathore, 1995). Reduction in the starch accumulation in heat sensitive genotypes may be partly attributed to the limited activity of starch synthesising enzymes which ultimately leads to reduced sucrose availability to developing seeds (Snider et al., 2011). In a study where heat stress induced reproductive failure, a significant reduction in sucrose concentrations in leaves of heat intolerant chickpea genotypes was observed, with the tolerant genotypes having higher sucrose concentration which correlated with higher sucrose phosphate synthase and sucrose synthase (Kaushal et al., 2013). Availability of higher sucrose concentrations for the reproductive organs (Snider et al., 2011) may have been critical in their sustained function of Acc#7. Increased sucrose availability in genotype Acc#7 may have been due to increased Rubisco activity, as noted by the high photosynthetic rates, possibly resulting in reduced flower and pod abortions, which may have contributed to its superior pod numbers and grain yield compared to Acc#8. Despite the relatively lower sucrose and starch concentrations, Acc#RR-2 was able to maintain a higher leaf glucose concentration. Concentrations of starch, fructose and sucrose decreased with glucose remaining relatively higher in some heat intolerant varieties of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) and redtop (*Agrostis alba* L.) (Chatterton et al., 1987). The correlation of leaf starch to grain yield was valid both within the sites and for the whole data set, also making it a potential selection marker for heat tolerant chickpea genotypes in the field.

In our study, photosynthesis, grain yields and yield components followed a similar pattern being significantly higher at the cooler site of Polokwane compared to the warmer site of Venda. In soybean, heat stress (38/20 °C) significantly reduced F_v/F_m (5%), photosynthesis (20%) and sucrose concentrations, 20% (Hasanuzzaman et al., 2013). A 2.2 and 3.1 °C increase in atmospheric temperatures from the ambient caused a respective 18.8 and 37.5% reduction in the P_n of chickpea (Chakrabarti et al., 2013). However, in 2017 g_s , leaf transpiration, and C_i were highest at Venda and least at Polokwane, with leaf temperatures lower at Venda. These results contrasted with 2016 observations in this study and we attribute this to the relatively lower temperatures (22.6 °C) on the day of data collection at the normally warmer site of Venda compared to the other two sites, which might have led to a higher stomatal opening, hence more internal CO₂ concentrations (Greer and Weedon, 2012).

Interestingly, there were no differences in the leaf glucose and sucrose concentrations of plants grown in Venda and those grown in Polokwane, with a higher starch concentration in plants grown in Venda that lead to higher biomass accumulation at flowering. The lower grain yield of plants at Venda compared to Polokwane site, similar to the warmer treatments in the chamber experiment, might partly be due decreased carbohydrate export from leaves to reproductive organs of plants grown in warmer conditions (Plaut et al., 2004). Starch synthase has been identified as a major gene and protein that is reduced by heat stress and this consequently leads to reduced utilization of incoming carbohydrate, followed by a reduction in sugar transport to the developing grain (Keeling et al., 1993). In heat stress

studies on potato, raised temperatures during tuber growth resulted in redirection of photosynthates to vegetative tissues at the expense of starch accumulation in growing tubers (Wolf et al., 1990). Furthermore, elevated temperatures during grain filling stages of chickpea have previously been reported to reduce grain yield as well as seed sizes, which may also lower grain yield (Ong, 1983). Reactive oxygen species (ROS) accumulate in male reproductive organs during prolonged heat stressful conditions, particularly in microspores/pollen grains, evidenced by protein and membrane degradation, potentially leading to male reproductive abortion (Sage et al., 2015). Heat stress has also been noted to result in severely reduced flower bud initiation, decreased flower number and size, leading to loss of flowers and young pods, ultimately lowering grain yields (Morrison and Stewart, 2002). The impact of heat stress on pod characteristics like pod numbers ultimately result in reduction of overall seed yield (Krishnamurthy et al., 2011). Average maximum temperatures at grain filling stages of chickpea in Polokwane in 2016 and 2017 were 27.1 °C and 27 °C respectively while in Venda they were 31.5 °C and 31.2 °C respectively. Chickpea grain yields have been reported to reduce by 53–330 kg ha⁻¹ for every 1 °C increase in mean seasonal temperatures in India (Kalra et al., 2008). The higher pod numbers per plant and grain yield in Polokwane reflect the site's association with higher chickpea reproductive efficiency. Chickpea grain yields in similarly cooler South African environments as the Polokwane site, with mean winter maximum temperatures ranging between 15 and 24 °C have average yield between 3000 and 4000 kg ha⁻¹ (Thangwana and Ogola, 2012). This is consistent to prior research on chickpea showing that elevated temperatures above 30 °C, like the Venda site at the critical growth stages between flowering and pod formation, adversely affected pod set (Devasirvatham et al., 2012a). Consequently, any potential increase in temperatures associated with climate change in cooler sites like Polokwane may adversely affect chickpea production of the area. We note that the low grain and pod number per plant at Louis Trichardt in 2016 were partly due to 50% of the grain yield being destroyed by pod borer infestation.

In the climate chamber study, the tolerant genotype Acc#7 had higher grain yield plant⁻¹ than the sensitive genotype Acc#8 when exposed to the high heat stress as well as higher than all other three genotypes in the moderate heat stress. Therefore, the observed genotype and temperature interaction on grain yield plant⁻¹ signifies that the tolerant Acc#7 could perform well relative to the other genotypes under elevated temperatures. Genotypic differences were also reported by Devasirvatham et al. (2012b) where lack of fertile pollen and pod set at 35/20 °C in the sensitive chickpea genotype ICC5912 indicated a temperature threshold for reduced fertility, resulting in lower number of pods plant⁻¹, filled pods plant⁻¹, seeds plant⁻¹ and plant biomass plant⁻¹. Wang et al., (2006) in their study also highlighted that seed yield plant⁻¹ was reduced in sensitive chickpea genotypes at 35/16 °C.

5. Conclusion

Overall, our results show that chlorophyll fluorescence parameters (F_v/F_m and F_q'/F_m') and carbohydrate concentrations for Acc#7 and Acc#RR-3 were unaffected by exposure to heat stress, showing their potential use as heat tolerance markers and genotype Acc#7 and Acc#RR-3 as heat tolerant genotypes. Heat stress in the field generally leads to reduced photosynthesis, F_q'/F_m' , F_v/F_m as well as carbohydrate concentrations in leaves of heat sensitive genotypes. However, the heat tolerant genotype Acc#7 and Acc#RR-3 maintained unaltered physiological response at flowering stage as well as their grain yields after exposure to heat stressful conditions both in the field and under controlled chamber environments. The observed relationships between measured chlorophyll fluorescence (both F_v/F_m and F_q'/F_m'), a relatively rapid and non-destructive method, with plant agronomic performance supports its use as a phenotyping tool during germplasm screening under field conditions. The site of Polokwane showed temperature range that is conducive to chickpea production.

Author contribution

GMM contributed to the design of the study, experimentation, acquisition of data, analysis and interpretation of data and drafting the article. SBMC conceptualized the project and obtained funding. SBMC, JBOO and C-OO administered the project. SBMC, JBOO, AMM, OC, SM, AJV, C-OO, ER contributed on supervision, methodology, discussion of results, critical revision of article, editing and approval of submission of the article.

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Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2019.05.031>.

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