THE IMPACT OF HEAT STRESS DURING FLOWERING ON THE PHYSIOLOGICAL AND YIELD TRAITS OF VEGETABLE-TYPE SOYBEAN

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Abstract

Heat stress during the reproductive stage of legumes can have a great impact on crop production. The aim of this study was to investigate the effect of heat stress during flowering on physiological parameters and yield traits of vegetable-type soybean. Three cultivars were planted in the glasshouse using a randomized complete block design, with three replications. Control plants were kept at a constant temperature of 18°C minimum / 26°C maximum, while the heat-treated plants were exposed to 26°C minimum / 36°C maximum at flowering. Photosynthetic rate, relative chlorophyll content and chlorophyll pigments of the heat-treated plants were higher than control plants. Five of the seven physiological parameters showed on average a significant increase with heat treatment at flowering. However, for most physiological traits, the timing of data collection responded significantly with the treatment. This indicated that different results are expected early in the heat stress treatment as compared to a few days within the heat stress treatment. In addition, this is also cultivar-specific as cultivar UVE8 responded different form UVE14 and UVE17. UVE8 showed a decrease in relative chlorophyll content and chlorophyll b, over time, while the other two cultivars showed an increase. Proline content of cultivars UVE14 and UVE17 decreased over time while their photosynthesis parameters increased. Cultivar UVE8 showed the opposite response in terms of proline and photosynthesis parameters. This indicated that cultivars use different coping meganisms under heat exposure. Eight of the 13 morphological traits were significantly influenced by the heat treatment and showed an average increase in their mean values. This indicated that the brief heat treatment during flowering contributed the necessary heat units to improve growth and that yield traits were not negatively impacted by the heat treatment. In future, a more extended heat stress period over pod-filling could be tested since parameters could respond differently across different growth stages.

Resumen

El estrés por calor durante la etapa reproductiva de las leguminosas puede tener un gran impacto en la producción de cultivos. El objetivo de este estudio fue investigar el efecto del estrés por calor durante la floración sobre los parámetros fisiológicos y las características de rendimiento de la soja de tipo hortícola. Se sembraron tres variedades en invernadero utilizando un diseño de bloques completos al azar, con tres repeticiones. Las plantas de control se mantuvieron a una temperatura constante de 18°C mínimo/26°C máximo, mientras que las plantas tratadas térmicamente se expusieron a 26°C mínimo/36°C máximo durante la floración. La tasa fotosintética, el contenido relativo de clorofila y los pigmentos de clorofila de las plantas tratadas térmicamente fueron mayores que los de las plantas de control. Cinco de los siete parámetros fisiológicos mostraron en promedio un aumento significativo con el tratamiento térmico en la floración. Sin embargo, en la mayoría de

los caracteres fisiológicos, el momento de la recopilación de datos respondió significativamente con el tratamiento. Esto indicó que se esperan resultados diferentes al principio del tratamiento contra el estrés por calor en comparación con unos pocos días dentro del tratamiento contra el estrés por calor. Además, esto también es específico de la variedad, ya que la variedad UVE8 respondió de manera diferente que UVE14 y UVE17. La variedad UVE8 mostró una disminución en el contenido relativo de clorofila y de clorofila b con el tiempo, mientras que las otras dos variedades mostraron un aumento. El contenido de prolina de las variedades UVE14 y UVE17 disminuyó con el tiempo mientras que sus parámetros de fotosíntesis aumentaron. La variedad UVE8 mostró la respuesta opuesta en términos de parámetros de prolina y fotosíntesis. Esto indicó que las variedades utilizan diferentes mecanismos de afrontamiento bajo la exposición al calor. Ocho de los 13 caracteres morfológicos fueron influenciados significativamente por el tratamiento térmico y mostraron un aumento promedio en sus valores medios. Esto indicó que el breve tratamiento térmico durante la floración contribuyó con las unidades de calor necesarias para mejorar el crecimiento y que las características de rendimiento no se vieron afectadas negativamente por el tratamiento térmico. En el futuro, se podría probar un período de estrés por calor más prolongado durante el llenado de las vainas, ya que los parámetros podrían responder de manera diferente en las diferentes etapas de crecimiento.

Introduction

Plants experience heat stress when optimum temperatures are exceeded. The duration (longer exposure) and increased intensity of heat can cause permanent damage on plants (Zaidi et al. 2014). The intensity of heat stress is getting higher due to climate change, which poses a great threat to crop production areas worldwide (Fedoroff et al. 2010). This condition is more tragic in the arid and semi-arid regions like in South Africa, where weather conditions are ever fluctuating and production area is limited. Although all tissues and developmental stages of plants are sensitive to high temperatures, reproduction is the most sensitive. A slight temperature increase during flowering will lead to a great decrease in yield (Lobell et al. 2011). Photosynthetic capacity is important for biological- and grain yield, which are positively correlated with crop yield during the reproductive stage (Liang et al. 2010). Photosynthesis, the utmost important process in plants, is extremely sensitive to heat stress (Hasanuzzaman et al. 2013). In C_3 plants, like soybean, photosynthetic capacity is affected by high temperatures and leaf photosynthesis is generally restrained when leaf temperatures rise above 38°C (Wise et al. 2004).

Chlorophyll is one of the main chloroplast components for photosynthesis and has a positive association with photosynthetic rate. By upholding a higher chlorophyll content for a longer period of time during in the reproductive stages is crucial for increasing crop production (Guo et al. 2008). Chlorophyll fluorescence parameters are generally used to characterise the natural action of photosystem II (PSII), which is interconnected to the photosynthetic capacity of plants. The variable fluorescence ratio to the maximum fluorescence (Fv/Fm) is an estimation of maximum quantum efficiency of PSII photochemistry. This ratio is used to display stress and damage to the PSII reaction centres (Murchie and Lawson 2013). Plant vitality could be characterised by performance index, expressed on absorption basis (PIabs), as it reflects the functionality of both PSI and PSII, providing information on the overall photosynthetic efficiency of a plant's performance under stress conditions (Strasser et al. 2000).

The physiological and biochemical changes occur as adaptive strategies to abiotic stresses. Proline and sugars are compatible solutes that are often viewed as a basic strategy for the protection and survival of plants under abiotic stress (Chen et al. 2007). Proline is an osmolyte that contributes to osmotic adjustment, stabilisation of sub-cellular structures, foraging of free radicals and buffering cellular redox potential in stress conditions (Kishore et al. 2005). Sucrose plays a key role in osmoregulation and cryoprotection (Guy 1990). In soybean, the precise mechanisms initiating lower photosynthesis under heat stress is not yet fully understood and requires more attention

(Djanaguiraman et al. 2011). The survival of plants at high temperatures can be achieved through delayed leaf senescence, escape, avoidance and tolerance mechanisms (Hasanuzzaman et al. 2013).

Vegetable-type soybean is of the same species as the traditional grain-type soybean (*Glycine max* L. Merrill) and exhibits similar physiology. However, the crop is harvested green at full-pod (R6) stage and the beans are larger with a sweet and nutty flavour (Zhang et al. 2015). The earliest documentation of vegetable-type soybean dates back to China in the second century (Shurtleff and Aoyagi 2009). Vegetable-type soybean is most popular in East Asia, China, Taiwan and Korea (Mimura et al. 2007). However, due to its high nutritional value and being a rich source of protein, the crop has gained more interest across many countries in recent decades (Zhang et al. 2017b). The crop was introduced to South Africa in 2009 by the Edamame Development Program to be grown by small-scale farmers (Van der Merwe 2021). The crop has the potential to improve these farmers' income since no additional equipment is needed as it can be planted and harvested by hand. It will further increase job opportunities and improve nutrition in communities. However, it is evident that the crop is susceptible to abiotic stress through previous research that showed a seed yield reduction ranging between 12% to 80% in grain soybean and 25% to 85% in vegetable-type soybean when drought stress was imposed during growth cycle (Mwenye et al. 2016; Van der Merwe et al. 2018).

Currently there is no information available on the effects of heat stress on vegetable-type soybean and no breeding for heat stress is currently being performed in South Africa. By gaining knowledge of the physiological responses of vegetable-type soybean to heat stress can help improve the tolerance of this crop to South Africa's adverse weather conditions. Therefore, this research aims to establish the effect of heat stress at flowering on the physiological parameters and yield traits of vegetable-type soybean. The study was conducted to 1) measure the chlorophyll fluorescence and chlorophyll pigments to estimate photosynthesis capacity, 2) determine proline and total soluble sugars content as these osmolytes assist plants to cope with heat stress and 3) determine the impact of heat stress on the yield traits of three cultivars.

Material and Methods

Plant material and experimental design

Three vegetable-type soybean cultivars (UVE8, UVE14 and UVE17) were selected for this study. These cultivars were characterized according to their drought tolerance responses in a previous study (Van der Merwe et al. 2018). UVE8 is a top-performing cultivar when subjected to ideal conditions; however, it is highly unstable under drought stress conditions. UVE14 is stable under drought stress conditions but is not a high yield performer. UVE17 is not a stable cultivar under drought stress conditions. The seeds were sown in trays filled with Hygromix seedling growth medium (from Hygrotech) and inoculated with rhizobium. Trays with seeds were watered daily. At the first trifoliate stage (V1) the seedlings were transplanted to 20 mm pots containing 7 kg of loamy sandy soil (one plant per pot). A randomised complete block design with three replications was used. Each replication consisted of four pots. Two heat treatments, each under controlled environmental conditions in two glasshouse cubicles were applied. The control cabinet was set at a temperature of 18°C minimum, and 26°C maximum, while the cabinet for the heat treatment was set at a temperature of 26°C minimum, and 36°C maximum. For the heat treatment, plants were transferred during flowering for 12 days only from the control cabinet to the heat cabinet. After the 12 days, the plants exposed to the heat treatment were transferred back to the control cabinet. Plants were watered daily to prevent avoid drought stress.

Physiological measurements

During flowering, leaves were sampled to collect data on the various physiological parameters. At first, non-destructive measurements (chlorophyll fluorescence and relative chlorophyll content) were done, followed by destructive measurements. The same leaves used for the non-destructive measurements were frozen in liquid nitrogen, each pot per replication separate, and crushed in liquid nitrogen to form a homogenous fine powder, followed by storage at -26°C. For chlorophyll fluorescence, readings were taken every four days (i.e., 4-, 8- and 12-days post heat stress treatment, d.p.t) during the 12 days of flowering. Chlorophyll fluorescence (using a Hansatech pocket PEA chlorophyll fluorimeter) was measured by placing specialised clips on the young, fully expanded trifoliate leaves of each plant. The clips were closed to exclude all light from the tissue and left for 30 minutes. Variable fluorescence ratio to the maximum fluorescence (Fv/Fm) and performance index on chlorophyll basis (PIabs) parameters were used to represent the photosynthesis capacity of the plants. For relative chlorophyll content, three readings were taken on each leaflet of the young, fully expanded compound leaf using a Hansatech chlorophyll meter. The average of three readings per leaf was used to determine the relative chlorophyll content (RCC) of each plant.

The destructive measurements included chlorophyll a, chlorophyll b, proline content and total soluble sugar content. The chlorophyll content analysis was done according to Su et al. (2010). A 0.1 g frozen leaf sample was ground to a fine paste in 5 ml of 80% acetone on ice. Samples were centrifuged at 3000 rpm for 10 minutes. Absorbance was measured using an ultraviolet-visible spectrophotometry at 663, 645 and 480 nm. Individual pigments were calculated using formulas:

Chl. a = [(12.72 x OD 663) – (2.59 x OD 645)]……..……..……[1]
Chl. b = [(22.9 x OD 645) – (4.68 x OD 663)]…….[2]
Total chlorophyll mg/L = 20.2 OD 645 + 8.02 OD 663……..…[3]

The volume and mass of Chlorophyll a and b were interpreted by Chl. x v/w.

Free proline was extracted and determined using a ninhydrin-based method according to Gibson et al. (2000) as modified by Carillo and Gibson (2011). A crushed leaf sample (50 mg) was mixed with 70% (v/v) ethanol. The mixture was placed in 2 ml reaction tubes and centrifuged at 14000 rpm for 5 min. Supernatant (500 µl) was added to 1000 µl 1% (w/v) ninhydrin solution prepared in 60% (v/v) glacial acetic acid in 2 ml reaction tubes. Tubes were vortexed to homogenise the mixture and a hole was poked in the lid of each tube to prevent it from popping open. The tubes were heated in a 95°C warm water bath for 20 min and centrifuged again for 1 min at 10000 rpm. The supernatant was poured into disposable cuvette and the absorbance was read at 520 nm against a blank. A standard curve was prepared using proline standard solution (1 mM). Amount of proline accumulated in the extract was derived using the equation:

$$\text{Proline in } \mu mol.g^{-1}FW = (Abs_{sample} - \text{blank})/\text{slope}*Vol_{sample}/Vol_{aliquot}*1/FW \quad [4]$$

Where: Abs_{sample} is the absorbance determined with the extract, blank (expressed as absorbance) and slope (expressed as absorbance $nmol^{-1}$) are determined by linear regression, Vol_{sample} is the total volume of the extract, $Vol_{aliquot}$ is the volume used in the assay, FW (expressed in mg) is the amount of plant material extracted.

Determination of total soluble sugars (TSS) was done according to a method described by Irigoyen et al. (1992). Frozen leaf powder (0.1 g) was homogenised in 96% (v/v) ethanol, followed by incubation (80°C for 10 min) and centrifuged (4000x g) for 10 min. This procedure was done for each replication. Ethanoic extract (50 µL) was added to 1450 µL Anthrone reagent (150 mg mL^{-1}) prepared in 72% (v/v) sulphuric acid. The mixture was vortexed vigorously and incubated at 80°C for 15 min.

Absorbance was measured at 625 nm with an ultraviolet-visible spectrophotometry. The estimation TSS was calculated from glucose standard.

Yield trait measurements

At maturity (R8 growth stage), morphological data were collected. From four plants per plot, single plant measurements were taken and the average used for analysis. Plant height was measured in centimeters with a measuring tape. The following traits were determined based on counts number of branches per plant, number of pods per branch, number of pods on the mainstem, total number of pods per plant, number of nodes on the main stem, number of nodes that contains pods, number of aborted pods, total number of filled seed per plant, and the number of aborted seed per plant. Seed mass and the biomass (dry stems, empty pods and branches) of each plant were weighed using a laboratory balance. For plant biomass, the leaves were excluded since leaf abscission occurred during maturity Harvest index (HI) per plant, expressed as a percentage was determined using the formula:

HI (%) = [seed mass (g) / biomass (g)] x 100……[5]

Data analysis

The average of each plot was used to determine the analysis of variance (ANOVA) using Genstat 18th edition (VSN International 2017) software package. Physiological parameters were subjected to a repeated measures ANOVA to compare the mean score of one group (control treatment) to another group (heat treatment) on different observations. Yield trait averages were subjected to a two-way ANOVA to determine whether the variability of the outcomes is due to chance or to the factors in the analysis. Mean differences were tested for significance at 5% using the least significant test (LSD).

Results and Discussion

Increasing temperatures affects the developmental and physiological plant processes that leads to a decrease in crop yield and quality (Hatfield and Prueger 2015). Optimum temperatures for all growth stages of soybean is 25°C (DAFF 2010). Temperatures higher than 30°C adversely affects soybean yield and higher than 35°C cause high-temperature stress (Pannar 2006). Leaves of plants would exhibit chlorosis and a significant change in chlorophyll content when exposed to abiotic stresses. Therefore, chlorophyll is a good indicator that reflects a plants resistance to stress (Zhang et al. 2017a). In the ANOVA (table 1a) significant treatment effects with respect to Fv/Fm (chlorophyll fluorescence) were observed. This indicated that the heat treatment (with a mean of 0.81) resulted in a significant increase in Fv/Fm compared to the control treatment (0.78) (table 2) and that the heat treatment might contribute to an increase in photosynthetic rate. The relatively high Fv/Fm values (>0.73) for both the control and heat treatments indicated that the photosynthetic rate was high, and plants were regarded healthy. Results further indicated significant time effects and time interactions with the cultivars and treatments (table 1b). This indicated that the rate of photosynthesis was affected over time and that both treatments and cultivars rankings changed over time.

Table 1a. Analysis of variance showing mean square values of physiological parameters analysed for three cultivars subjected to a control and heat treatment.

Source of variance	Cultivar (C)	Treatment (T)	C x T
Degrees of freedom	2	1	2
Fv/Fm	0.00	0.01**	0.00
PIabs	0.21**	0.64**	0.00
RCC	11.76**	5.35*	2.45
Chlorophyll a	0.00**	0.01**	0.00*
Chlorophyll b	0.03*	0.19**	0.01*
Proline	0.25**	0.03	0.09
Total soluble sugar	0.00	0.00	0.00

* Significant at p<0.05, ** Significant at p<0.01, Fv/Fm = chlorophyll fluorescence, PIabs = chlorophyll performance index, RCC = relative chlorophyll content

Table 1b. Analysis of variance showing mean square values of physiological parameters analysed for three cultivars subjected to a control and heat treatment.

Source of variance	Time (Ti)	Ti x C	Ti x T	Ti x C x T
Degrees of freedom	2	4	2	4
Fv/Fm	0.00**	0.00**	0.00**	0.00**
PIabs	0.11**	0.17**	0.03*	0.02
RCC	1.10	11.13**	2.84**	2.82**
Chlorophyll a	0.00**	0.00	0.00*	0.00**
Chlorophyll b	0.03*	0.06**	0.01	0.01
Proline	0.02	0.08**	0.05*	0.01
Total soluble sugar	0.00	0.00	0.00	0.00

* Significant at p<0.05, ** Significant at p<0.01, C = cultivar, T = treatment, Fv/Fm = chlorophyll fluorescence, PIabs = chlorophyll performance index, RCC = relative chlorophyll content

Table 2. Mean values of cultivars and treatments for physiological parameters.

	Fv/Fm	PIabs	RCC ($mg.L^{-1}$)	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Proline ($\mu mol.g^{-1}$)	TSS ($mg.g^{-1}$)
Time							
1	0.78	2.36	5.30	0.25	0.12	0.90	0.10
2	0.80	3.03	5.19	0.24	0.12	0.90	0.10
3	0.81	3.46	5.66	0.23	0.11	0.90	0.10
$LSD_{0.05}$	0.01	0.421	ns	0.01	0.02	0.07	ns
Cultivar							
UVE8	0.80	2.18	4.52	0.24	0.11	0.80	0.10
UVE14	0.80	3.07	5.50	0.24	0.11	0.90	0.10
UVE17	0.80	3.61	6.12	0.25	0.13	1.00	0.10
$LSD_{0.05}$	ns	0.437	0.66	0.01	0.01	0.06	ns
Treatment							
Control	0.78	2.26	5.07	0.23	0.10	0.90	0.10
Heat	0.81	3.65	5.70	0.25	0.13	0.90	0.10
$LSD_{0.05}$	0.01	0.357	0.54	0.004	0.01	ns	ns
Grand mean	0.80	2.95	5.38	0.24	0.12	0.90	0.10

Fv/Fm = chlorophyll fluorescence, PIabs = chlorophyll performance index, RCC = relative chlorophyll content, TSS = total soluble sugars, LSD = least significant difference, ns = not significant

The control and heat stress treatments of cultivar UVE8 showed similar decreasing and increasing responses in chlorophyll fluorescence (figure 1). The Fv/Fm values of the heat-treated plants remained higher than the control plants during the 12 days of exposure. For UVE14, the heat-treated plants showed dramatic increases in Fv/Fm values from 0.78 to 0.83 during 4 and 8 d.p.t. followed by a slight decrease to 0.82 on 12 d.p.t. The control plants Fv/Fm values were lower than the heat-treated plants. Cultivar UVE17 heat-treated plants Fv/Fm values also remained higher than the control plants during the days of exposure. The control treatment values ranged from 0.73 to 0.81 and the heat treatment from 0.77 to 0.83. UVE14 and UVE17 ended up with higher Fv/Fm values and the chlorophyll content increased in both treatments indicating that the plants were more tolerant. UVE8 had a decrease, followed by a slight increase in Fv/Fm with both treatments. This indicates that the plants were having healthy photosynthetic rates even though plants showed less tolerance to heat stress.

PIabs (chlorophyll performance index) is appropriate to distinguish genotypes with different performances under the same range of heat stress in an effective, quick, and non-destructive approach that can be employed by both researchers and breeders to identify cultivars adapted to heat stress indices. For PIabs, significant cultivar and treatment effects were observed in the ANOVA (table 1a). Cultivar UVE17 had the highest mean PIabs (3.61) and UVE8 the smallest (2.18) (table 2). The significant treatment effects indicated that the heat treatment contributed to an increased PIabs in plants. However, cultivars responded the same to the treatments. Results further indicated significant time effects and time interactions with the cultivars and treatments (table 1b). This indicated that PIabs was affected over time and that both treatments and cultivars rankings changed over time.

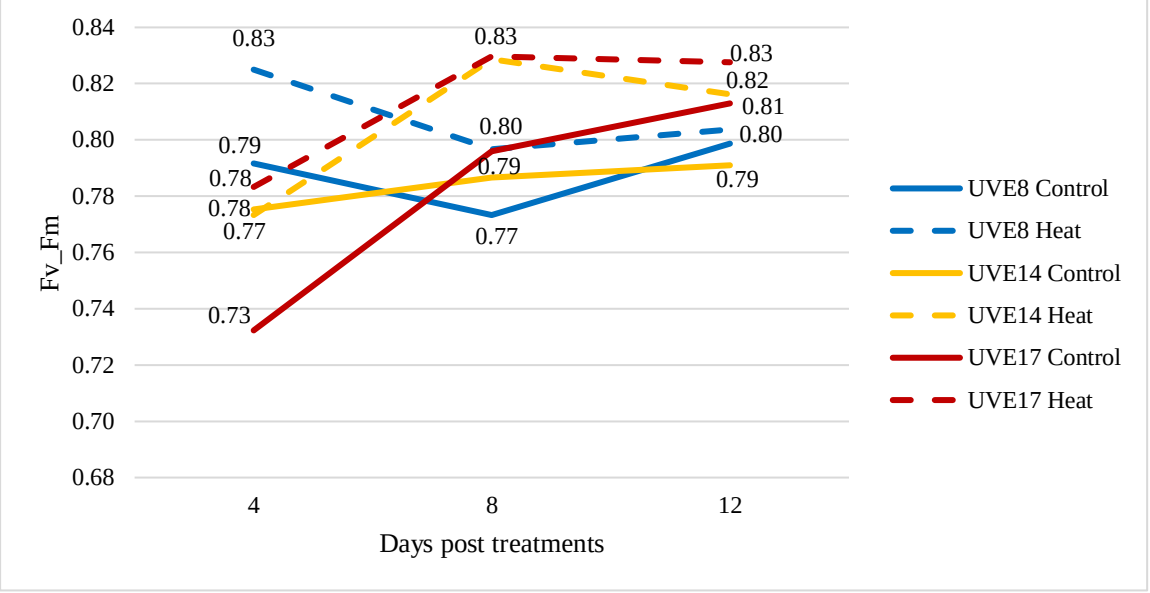

Figure 1. Chlorophyll fluorescent of three cultivars each subjected to control and heat treatments and Fv/Fm readings taken 4, 8- and 12-days post treatment.

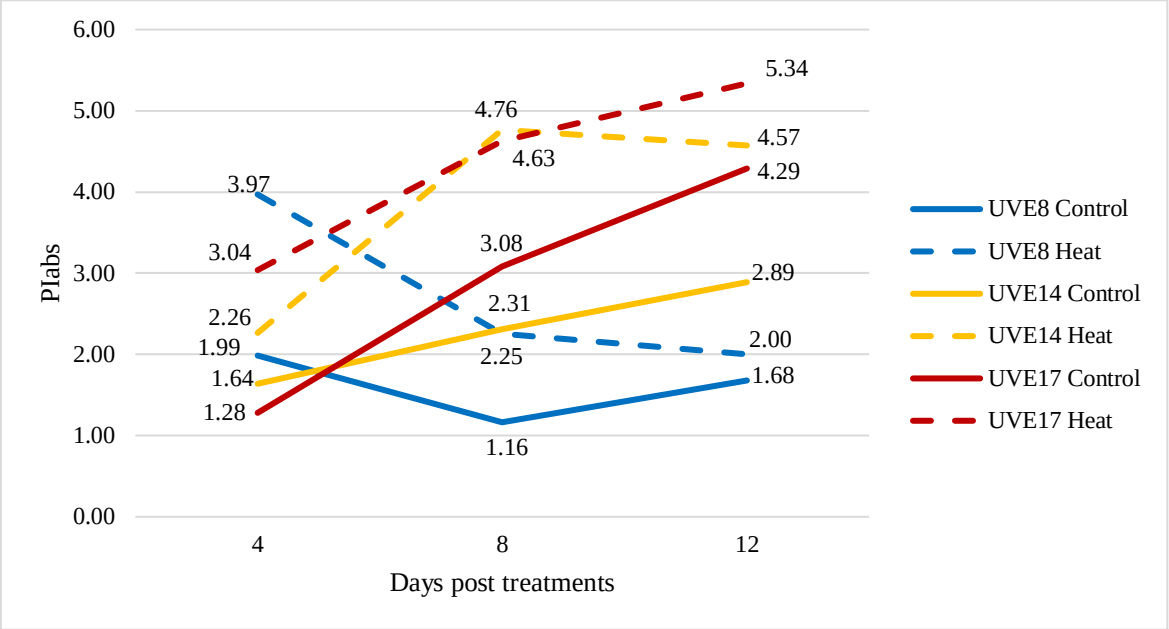

Figure 2. Performance index absorbance (PIabs) of three cultivars subjected to control and heat treatments and readings taken 4, 8- and 12- days post treatment.

For both the control and heat treatment of UVE8, PIabs decreased from 4 to 8 d.p.t. (1.99 to 1.16 and 3.97 to 2.25, respectively) (figure 2). Thereafter, heat treatment kept decreasing whereas the control increased to 1.68 on 12 d.p.t. UVE14 control treatment increased from 1.64 to 2.89 during the 12 d.p.t. and the heat treatment also increased on 8 d.p.t. but decreased slightly. UVE17 treatments showed both the same increasing response until 12 d.p.t. The PIabs values of the heat treatments for all cultivars were higher than the controls during the days of exposure.

In the ANOVA, significant cultivar and treatment effects were observed for RCC (Relative chlorophyll content) (table 1a). UVE17 had the highest mean RCC (6.12 $mg.L^{-1}$) and UVE8 the smallest (4.52 $mg.L^{-1}$) (table 2). The significant differences between treatments indicated that the heat treatment might contribute to an increased RCC in plants. However, cultivars responded the same to the treatments. On the other hand, significant time interaction effects with cultivars and treatments were observed (table 1b). This indicated that both treatments and cultivars rankings changed over time. In both the control and heat treatment for UVE8, a decrease in RCC from 4 to 12 d.p.t. (4.09 $mg.L^{-1}$ to 3.32 $mg.L^{-1}$, and 7.96 $mg.L^{-1}$ to 3.32 $mg.L^{-1}$, respectively) was observed (figure 3). The heat treatment values remained higher than the control value. However, on 12 d.p.t. both were the same value of 3.32 $mg.L^{-1}$. RCC for the heat-treated plants of UVE14 ended up higher on 12 d.p.t (7.29 $mg.L^{-1}$) than the control (6.35 $mg.L^{-1}$). The opposite occurred for UVE14 and UVE17, where 4 d.p.t values were lower than 12 d.p.t in both control and heat treatments.

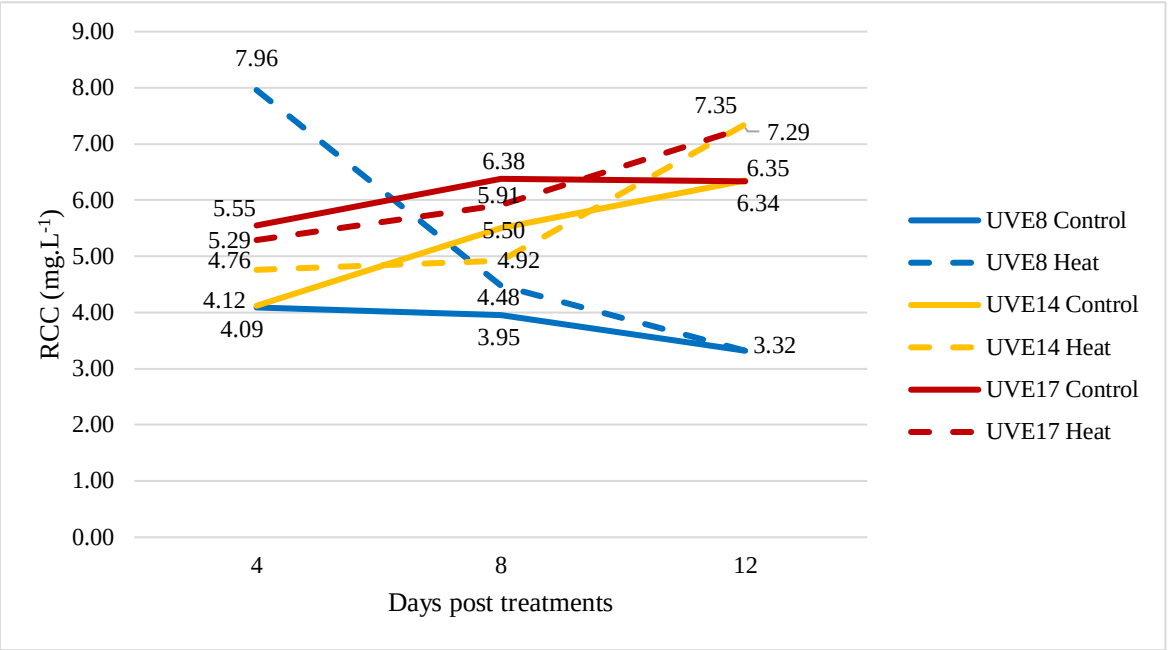

Figure 3. Relative chlorophyll content (RCC) of three cultivars subjected to control and heat treatments and readings taken 4, 8- and 12- days post treatment.

In terms of the chlorophyll pigments (chlorophyll a and b), significant cultivar, treatment and C x T effects were observed (table 1a). This indicated that the cultivars differed significantly in terms of the pigments and that they responded differently across the two treatments as well. UVE17 had the highest chlorophyll a and chlorophyll b contents (table 2). The heat treatment (with a mean of 0.25) had a significantly higher chlorophyll a value compared to the control treatment and the grand mean. This indicated that the heat treatment might contribute an increase in chlorophyll a content. Also, the heat treatment (with a mean of 0.13) had a higher chlorophyll b value compared to the control treatment and the grand mean. This indicated that the heat treatment might contribute an increase in chlorophyll b content. Significant time effects (Table 1b), Ti x C and Ti x T effects indicated that cultivar and treatment rankings changed over time and thus differences in responses as a result of time effect.

For UVE8, chlorophyll a content for the control and heat-treatment decreased (0.24 $mg.g^{-1}$ to 0.21 $mg.g^{-1}$ and 0.25 $mg.g^{-1}$ to 0.23 $mg.g^{-1}$ respectively) over the 12 days (figure 4a). Chlorophyll b content also decreased over the 12 days for the control and heat treatment (0.13 $mg.g^{-1}$ to 0.07 $mg.g^{-1}$ and 0.17 $mg.g^{-1}$ to 0.08 $mg.g^{-1}$ respectively) (figure 4b). For UVE8 the heat-treatment remained higher than the control. Chlorophyll a in UVE14 for the heat treatment showed an increase from 0.24 $mg.g^{-1}$ (8 d.p.t.) to 0.25 $mg.g^{-1}$ (12 d.p.t.) whereas control treatment decreased from 0.23 $mg.g^{-1}$ (8 d.p.t.) to 0.20 $mg.g^{-1}$ (12 d.p.t. (figure 4a). The same occurred for UVE14 chlorophyll b content. Control treatment decreased from 0.10 $mg.g^{-1}$ to 0.07 $mg.g^{-1}$ over the 12 days and heat treatment increased from 0.11 $mg.g^{-1}$ (8 d.p.t.) to 0.17 $mg.g^{-1}$ (12 d.p.t.) (figure 4b).

Chlorophyll a in UVE 17 for the control treatment increased from 0.23 $mg.g^{-1}$ (4 d.p.t.) to 0.25 $mg.g^{-1}$ (8 d.p.t.) while for the heat treated plants, this pigment decreased from 0.29 $mg.g^{-1}$ (4 d.p.t.) to 0.25 $mg.g^{-1}$ (8 d.p.t.). Both treatments remained constant at 0.25 $mg.g^{-1}$ until 12 d.p.t. The chlorophyll b content in UVE17 for the heat treatment remained higher than the control treatment (Figure 3a). The control treatment remained constant at 0.12 $mg.g^{-1}$ from 8 to 12 d.p.t.

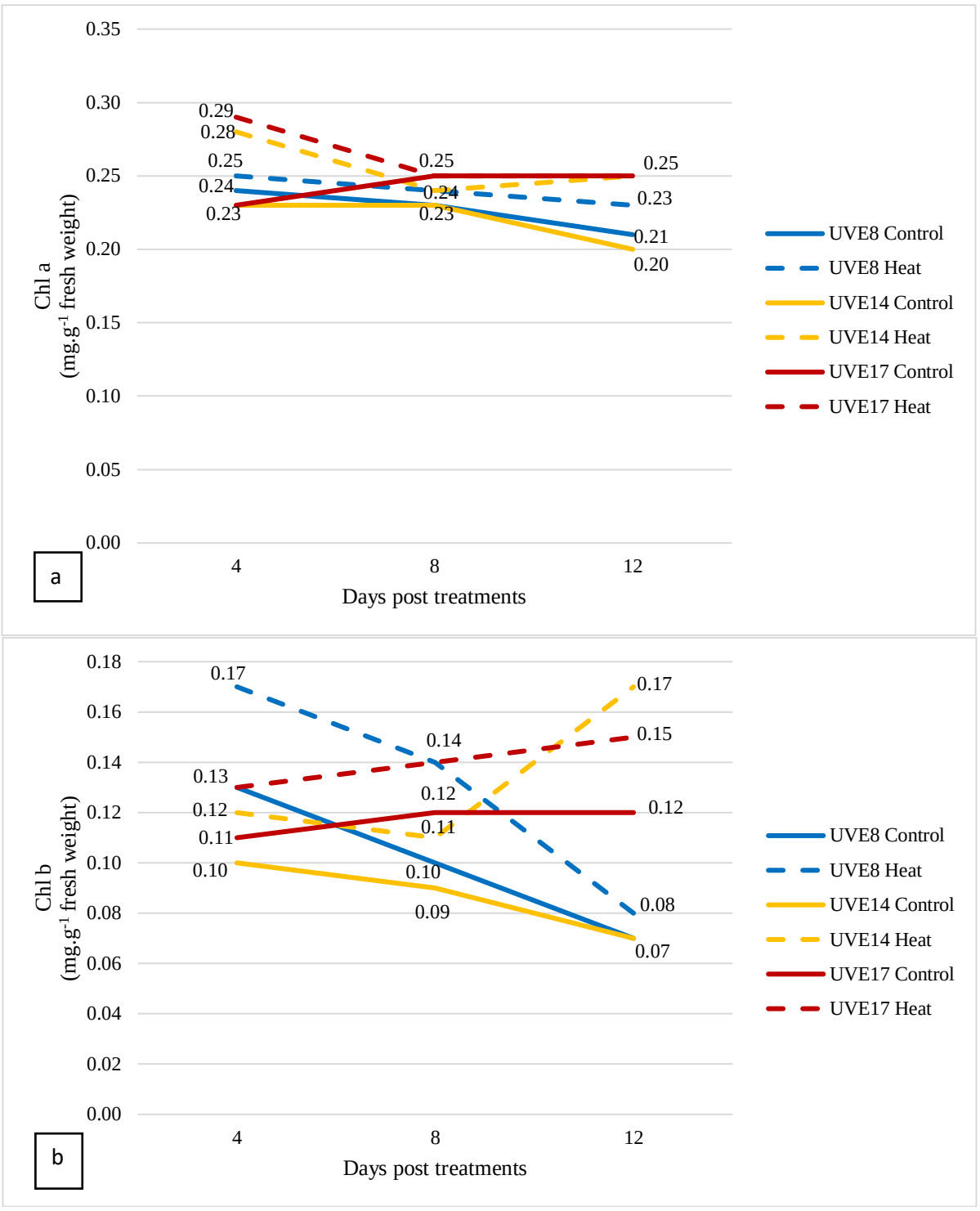

Figure 4. Chlorophyll a and b content of three cultivars subjected to control and heat treatments and readings taken 4, 8- and 12-days post treatment.

The accumulation of proline and total soluble sugars is often regarded as a basic strategy for the protection and survival of plants under abiotic stress (Chen et al. 2007). In the ANOVA, significant differences were observed between cultivars (Table 1a) where UVE17 had the highest proline content (table 2). Non-significant C x T and T effects indicated that this parameter was less responsive to the heat treatment. However, significant Ti x C and Ti x T interaction effects were observed (table 1b). These indicated that the cultivar rankings and changed over time as well as the treatment means.

The proline content of UVE8 was the lowest at 8 d.p.t for both the control and heat treatment with 0.70 µmol.g^{-1}and increased at 12 d.p.t. to 0.80 µmol.g^{-1} (figure 5). For UVE14, the proline content was constant on 4 to 8 d.p.t. and decreased on 12 d.p.t. for the control treatment from 1.00 µmol.g^{-1}to 0.90 µmol.g^{-1}. For the heat treatment, proline content decreased from 4 to 12 d.p.t (0.90 µmol.g^{-1}to 0.60 µmol.g^{-1}). For UVE17, the proline content decreased (4 to 12 d.p.t.) from 1.20 µmol.g^{-1} to 0.80 µmol.g^{-1} in the heat treatment. However, in the control treatment a slight increase on 8 d.p.t. from 1.00 µmol.g^{-1} to 1.10 µmol.g^{-1} was observed, followed by a decrease again to 1.00 µmol.g^{-1} on 12 d.p.t.

For TSS (total soluble sugar content), no significant effects on cultivar, treatment, time and all their interactions were observed (tables 1a and 1b). This indicated that the cultivars did not differ genetically, their responses to the heat treatment did not change and that the heat treatment itself did not have an impact on this parameter. Although non-significant effects were observed, higher TSS contents were observed for UVE8 with the control treatments on 4 and 12 d.p.t. at 0.098 and 0.100 respectively (figure 6). Heat-treated plants content was only higher than the control on 8 d.p.t. with 0.102. For UVE14, the heat-treated plants were higher than the control on 4 and 12 d.p.t. but on 8 d.p.t. they showed the same value of 0.094. On 4 d.p.t., the heat-treated plants of UVE17 showed a TSS content higher than the control (0.101 and 0.098 respectively). TSS content for the heat-treated plants decreased gradually from 0.101 to 0.100 on 8 and 12 d.p.t. However, the TSS content of the control plants was higher than the heat-treated plants on 8 and 12 d.p.t. with 0.104 and 0.102. the TTS content for UVE8 and UVE17 was higher than UVE14 for both treatments.

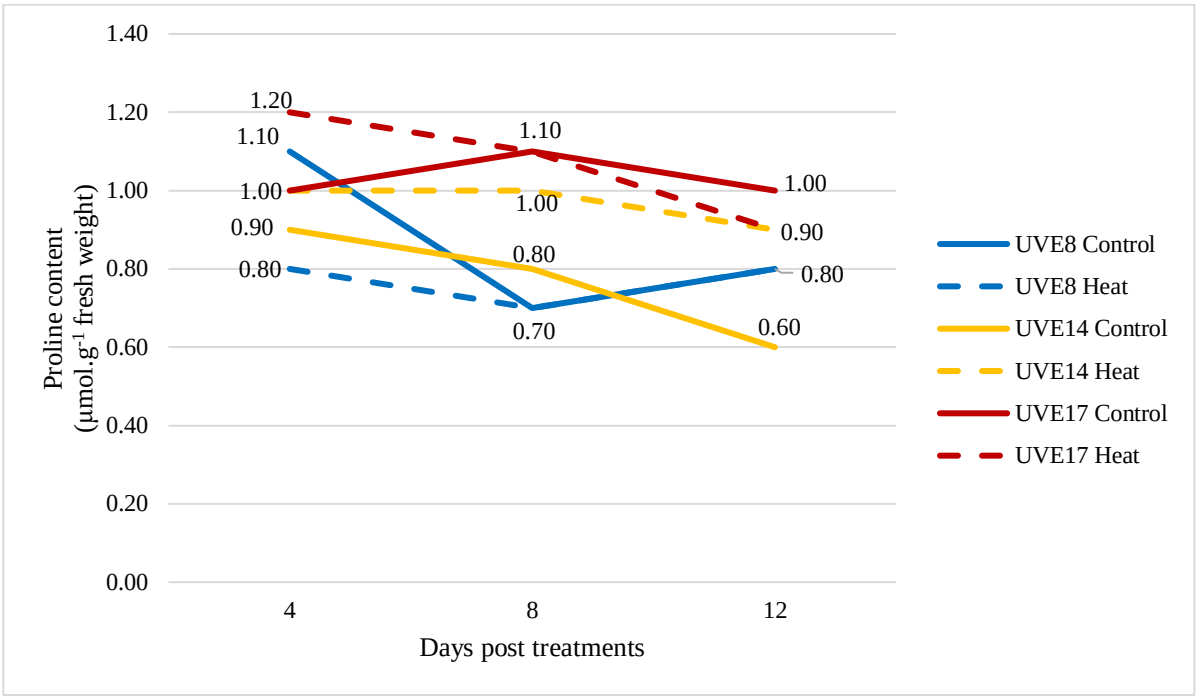

Figure 5. Proline content of three vegetable-type soybean cultivars subjected to a control and heat treatments and readings taken 4, 8- and 12-days post treatment.

In general, proline and TSS content in UVE8 showed an increase for both treatments while for UVE14, proline and TSS content decreased. In a previous study it was shown that the TSS increased under osmotic stress for osmotic adjustment in many legumes (Sassi-Aydi et al. 2014). The fact that UVE14 and UVE17 showed a decrease in proline during the heat treatment suggests the plants did not have this type of protection mechanism for heat stress.

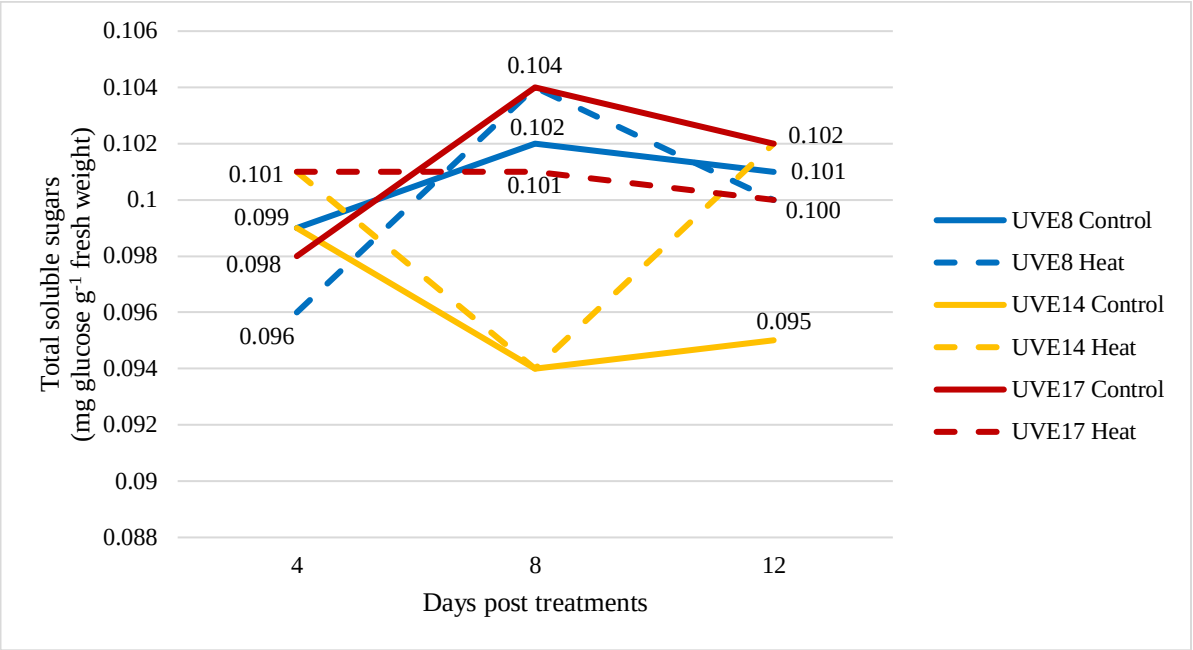

Figure 6. Total soluble sugars (TSS) content for three vegetable-type soybean cultivars subjected to a control and heat treatments and readings taken 4, 8- and 12-days post treatment.

Legumes exposed to heat stress during reproduction results in substantial seed yield loss due to a reduction in the number of seeds, decrease in pod numbers and seeds per pods (No et al. 2021). Therefore, the effect of a brief heat stress during flowering on morphological traits was of interest. In the ANOVA (Ttble 3) significant differences were observed between cultivars for plant height (PH). UVE8 was the tallest of the three cultivars at 33.57 cm and was higher than the grand mean of 29.31 (Table 4a). UVE14 was second with a height of 27.85 cm and UVE17 in third of 26.5 cm. No significant differences were observed between treatments as well as for the C x T interaction. This indicated that plant height was not affected by the heat treatment and cultivars responded similarly.

Table 3. Analysis of variance showing mean square values of yield traits analysed for three vegetable-type soybean cultivars subjected to a control and heat treatment at flowering stage.

Source of variance	Cultivar (C)	Treatment (T)	C x T
Degrees of freedom	2	1	2
Plant height	84.67**	0.52	1.14
Number of branches	6.72**	9.40**	1.06
Number of pods per branch	189.50**	133.39**	24.06*
Number of pods on main stem	3.10*	0.01	0.26
Total number of pods per plant	134.01**	120.13**	12.79*
Number of nodes on main stem	0.50	0.06	0.06
Number of nodes with pods	2.06*	2.72*	0.72
Number of aborted pods	6.43*	0.35	10.10*
Total number of filled seeds per plant	242.89**	566.72**	22.89
Number of aborted seeds	0.08	0.24*	0.23**
Seed mass per plant	9.19**	37.48**	2.78
Plant biomass	0.09**	0.03*	0.01
Harvest index	319.48**	97.13**	19.57

***** Significant at p<0.05, *** Significant at p<0.01

Significant cultivar and treatment effects with respect to the number of branches per plant (table 3) were observed. UVE8 had the highest number of branches (5.8 branches), with UVE14 and UVE17 having both 4.0 branches per plant (table 4a). The heat treatment (5.3 branches) had significantly more branches per plant compared to the control (3.9 branches). This indicated that the heat treatment resulted in an increase in the number of branches per plant. No significant C x T interaction effects were observed, indicating similar cultivar responses to heat treatment. Significant differences were observed between cultivars and treatments with respect to the number of pods per branch (PPB). UVE8 had the highest mean of 19.7, which was higher than the grand mean of 13.5. The heat treatment resulted in an increase in PPB. Significant C x T interaction indicated differences in cultivars responses to the heat treatment.

Table 4a. Mean values of cultivars and treatments for yield traits.

	PH (cm)	Branches	PPB	PMS	TPP	NMS	NWP
Cultivar							
UVE8	33.57	5.83	19.67	6.25	25.58	10.00	4.33
UVE14	27.85	4.00	12.17	7.67	20.17	10.00	5.50
UVE17	26.50	4.00	8.67	7.17	16.17	10.50	4.33
$LSD_{0.05}$	3.38	0.73	2.36	0.99	2.25	NS	0.80
Treatment							
Control	29.48	3.89	10.78	7.00	18.06	10.11	5.33
Heat	29.14	5.33	16.22	7.06	23.22	10.22	4.56
$LSD_{0.05}$	2.76	0.60	1.93	0.81	1.84	0.51	0.66
Grand mean	29.31	4.61	13.50	7.03	20.64	10.17	4.94

PH = Plant height, PPB = pods per branch, PMS = pods on main stem, TPP = total pods per plant, NMS = nodes on main stem, NWP = nodes with pods, LSD = least significant difference

Significant cultivar effects were observed for the number of pods on the mainstem (PMS) (table 3). UVE14 (7.7) and UVE 17 (7.2) had the highest mean PMS (table 4a). Both cultivars were above the grand mean of 7.0. No significant treatment as well as C x T interaction effects were observed. This indicated that PMS was not affected by the heat treatment and cultivars responded similarly. Significant differences were observed between cultivars and treatments for the total number of pods per plant (TPP). UVE8 had the highest mean of 25.9 which was also above the grand mean of 20.6. The heat treatment (23.2) had significantly more pods per plant compared to the control. Significant C x T interaction effects indicated differences in cultivars responses to the heat treatment.

No significant cultivar, treatment and C x T interaction effects were observed for number of nodes on main stem NMS (table 3). All cultivars had more less the same NMS with means close to the grand mean of 10.2 (table 4a). Results indicated that NMS was not responsive to genotype, environment or their interaction for the material used in the study and should not be considered as a selection criterium under heat stress breeding. However, significant differences were observed between cultivars for the number of nodes with pods (NWP). UVE14 had the highest NWP (5.5). Significant differences were also observed between treatments for NWP, with the control treatment (5.3) having significantly more NWP compared to the heat treatment. On the other hand, cultivars showed similar responses to the heat treatment.

Significant cultivar effects were observed for number of aborted pods per plant (AbP) (table 3). UVE8 had the highest mean of aborted pods (7.3) whereas UVE14 had the lowest AbP (5.5) (table 4b). Non-significant treatment effects indicated that AbP were not affected by the heat treatment. However,

significant C x T interaction indicated different cultivar responses to heat treatment. Highly significant cultivar and treatment effects were observed for total number of filled seeds per plant (FSP). UVE8 had the highest mean of 33.8 total filled seeds per plant, closely followed by with UVE14 (31.5). The heat treatment (34.7) significantly increased FSP compared to the control. However, cultivars responded the same to the heat treatment. For number of aborted seeds per plant (AbS), no significant cultivar effects were observed although significant treatment and C x T interaction effects were observed. The heat treatment showed a significant lower AbS compared to the control (4.4) (table 4b). Although cultivars were not significantly different, UVE8 had the lowest (2.2) while UVE17 had the highest (4.7) AbS.

Table 4b. Mean values of cultivars and treatments for yield traits.

	AbP	FSP	AbS	SM (g)	Biomass (g)	HI (%)
Cultivar						
UVE8	7.33	33.83	2.17	8.78	14.6	37.33
UVE14	5.50	31.50	3.50	9.77	9.05	51.89
UVE17	5.58	21.83	4.67	7.31	8.63	45.43
$LSD_{0.05}$	1.56	3.76	NS	1.15	1.19	2.94
Treatment						
Control	6.00	23.44	4.44	7.18	9.72	42.56
Heat	6.28	34.67	2.44	10.06	11.80	47.20
$LSD_{0.05}$	NS	3.07	1.15	0.94	0.97	2.40
Grand mean	6.14	29.06	3.44	8.62	10.76	44.88

AbP = aborted pods per plant, FSP = filled seeds per plant, AbS = aborted seeds per plant, SM = seed mass, HI = harvest index, LSD = least significant difference

Significant cultivar and treatment effects were observed for seed mass per plant (SM) (table 3). UVE14 had the highest SM of 9.8 g, followed by UVE8 with 8.8 g (table 4b). The heat treatment (10.1 g) significantly increased SM compared to the control. However, cultivars responded similarly to heat treatment. Significant cultivar and treatment effects were observed for plant biomass. UVE8 (14.6 g) had a significantly higher plant biomass compared to the other two cultivars. In addition, this trait was highly responsive to the heat treatment showing an increase (mean of 11.8 g) compared to the control (9.7 g). No significant C x T effects indicated similar responses of cultivars to the heat treatment. For harvest index (HI), significant differences were observed between cultivars and treatments. UVE14 had the highest HI (51.9) of the three cultivars. In addition, the heat treatment (47.2%) resulted in a significant increase in HI compared to the control (42.6%). However, the cultivars did not respond differently to the two heat treatments.

The significant differences between cultivars for most of the yield traits indicated that there is enough genetic variation to identify parents for the breeding programme. In general, UVE8 performed the best and was the top-ranking cultivar for branches, pods per branch, total pods per plant and filled seeds per pod. It also had the lowest number of aborted seeds. UVE14, was the top-ranking cultivar for pods on the main stem, number of nodes with pods, seed mass per plant and harvest index. This cultivar came second for most of the yield traits showing an overall generally good performance. Significant differences between the control and heat treatments indicated that a higher temperature increased the mean values of most yield traits. Therefore, the increase in temperature during flowering did not have negative influence on vegetable-type soybean as expected.

The high minimum temperature (26°C), which simulates the night temperature might be the reason for the improvement in yield traits under heat treatment. Blignaut and Taute (2010) suggested that in warmer areas in South Africa, high soybean yields can be expected under higher night-time temperatures and not higher day-time temperatures. Also, the accumulation of a sufficient number of heat units is required for good production levels.

Conclusion

The brief heat treatment during flowering caused an increase in Fv/Fm, which indicated that plants were still healthy under the elevated temperature conditions. PIabs showed that UVE14 and UVE17 were able to maintain their vitality. The accumulation in proline in UVE8 might have contributed to the high mean values observed for some yield traits in this cultivar, even though photosynthesis parameters decreased. On the other hand, cultivars UVE14 and UVE17, showed lower proline contents, with increased photosynthesis parameters, which might have assisted with yield-trait maintenance in these cultivars. Results indicated that cultivars use different mechanisms to cope with a brief heat stress. In this study, proline accumulation and total soluble sugars were not significantly impacted by the heat treatment, although photosynthesis-related parameters proved to be the better selection criteria during flowering. In future, additional studies can be done to examine the impact of extended heat stress periods during pod-filling, and not only flowering. The intensity of the heat stress treatment could also be increased.

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