SOIL & CROP SCIENCES | RESEARCH ARTICLE

The role of root zone temperature on physiological and phytochemical compositions of some pigmented potato (Solanum tuberosum L.) cultivars

Hildegard Witbooi¹, Callistus Bvenura¹, Oluwafemi Omoniyi Oguntibeju² and Learnmore Kambizi²³

Abstract: A greenhouse study was conducted to evaluate the effects of three root zone temperatures (20, 24 and 28°C) on growth and chemical compositions in seed mini tubers of four pigmented Solanum tuberosum cultivars (Non-pigmented control (BP1), Salad Blue (SB), Pink Fir Apple (PFA) and Highland Burgundy Red (HBR)). The results indicate that RZT 24°C significantly increased plant height and tuber weight. RZT 28°C increased polyphenols (mg GA/g) in cv. BP1 (1.47) and PFA (2.09). Cultivars BP1, SB and PFA recorded 84.37, 83.08 and 124.86 µg/g ascorbic acid, respectively. The highest caffeic acid content was reported in cv. HBR (1380.74 µg/g) under the control RZT and lowest in the non-pigmented BP1 (40.48 µg/g) at 20°C RZT. In similar manner, the highest chlorogenic acid (µg/g) value was reported in cv. HBR (426.20 µg/g) under the control RZT and lowest in the non-pigmented BP1 (6.79 µg/g) at 20°C RZT. DPPH activity was highest in cv. SB (26.43 µmol TE/g) under the control RZT. Although these results indicate a variable response of various parameters to different root zone temperatures, the high values recorded under the

ABOUT THE AUTHOR

Hildegard Witbooi holds a Master of Horticulture degree while at the tail-end of her Doctoral studies at Cape Peninsula University of Technology (CPUT) in South Africa. Her interests are in Crop Production, Organic and Sustainable Horticulture, Phytomedicine and Food Security. She focuses on sustainable and organic crop production systems of socio-economic relevance not only to South Africa, but other regions of the world. She is particularly interested in rare, pigmented potatoes which are nutrient dense and how these varieties can be cultivated particularly in South Africa as an alternative nutrient dense food source. The present study forms part of a larger PhD study that assessed “Phenological and physiological responses to abiotic parameters of rare Solanum tuberosum L. cultivars”. This work was financed by National Research Foundation (NRF) of South Africa.

PUBLIC INTEREST STATEMENT

Pigmented potatoes are an important nutrient dense vegetable, with possible positive socio-economic effects and overall positive contributions to South Africa. We assessed the effects of different root zone temperatures (RZT) on growth and chemical compositions in seed mini tubers of three pigmented cultivars namely Salad Blue, Pink Fir Apple, and Highland Burgundy Red. This study was inspired by the general absence of research in South Africa on possible cultivation of these cultivars under local climatic conditions as well as their nutrient density and possible health benefits. Plant physiological growth and tuber bearing capacity were positively affected by an increase in RZT which is directly related to nutrient uptake. Polyphenols and vitamin C were subject to cultivar and RZT and higher in pigmented cultivars, indicating their potential health benefits. These cultivars could potentially produce preferable yields, nutritional, and health benefits in regions of temperatures in the range of 24 °C.
control RZT presumably show the natural concentrations of the phytochemicals at room temperature without heat application. The RZT recommendations would therefore be based on specific needs. Furthermore, the secondary metabolites reported in the pigmented cultivars SB, PFA and HBR and their associated potential health benefits offer a substantial basis for their inclusion in the diet, regardless of their low yielding capacity.

Subjects: Crop Science; Agriculture and Food; Soil Sciences

Keywords: Antioxidants; ascorbic acid; phenolic acids; root zone temperature; seed tuber potatoes

1. Introduction

The potato (Solanum tuberosum L.) is a tuber bearing food crop that belongs to the genus Solanum and the family Solanaceae. Although potatoes are now globally cultivated, the wild species is thought to have originated in the Andes region of South America (Levy & Veilleux, 2007). Potatoes are found in various skin and flesh colors such as white, yellow, purple and red. This is mainly due to the presence of anthocyanins and other pigments. Globally, there is an increasing demand for food sources high in polyphenolic content, and vitamins, which are often found in pigmented potatoes among other crops. In most African countries, potatoes are one of the most important food crops. In fact, China and India, the world’s largest potato producers accounted for a third of over 370 million metric tons produced in 2019 (STATISTA, 2021). But South Africa produced about 2.5 million tons in 2017 and has an annual per-capita consumption of 34 kg (POTATOPRO, 2021). According to Wang et al. (2008), the potato tuberization stage is a distinctive process controlled by many factors such as genotype. These factors determine tuber size, tuber number and yield potential, whereas yield performance is influenced by seed tuber physiological status. Furthermore, the assured maximum quantity and quality of a seed tuber is controlled by external tuber physiology and initiation factors such as root zone temperature, nitrogen supply, pH and water stress. Food and nutrition security are a major problem in South Africa where more than half of the children live under the poverty datum line, and 3 in 10 are stunted, resulting in chronic malnutrition as an underlying cause of death in children (UNICEF/SA, 2020). Furthermore, 30% of the children live in households with little or no access to daily healthy and balanced diets. Potatoes, therefore, provide a good source of dietary energy, fiber, carbohydrates, vitamin B1 and B6, niacin as well as minerals such as potassium, phosphorous, magnesium and ascorbic acid. In addition to the basic nutrients found in potatoes, they are a rich source of phenolic compounds. For example, chlorogenic acid constitutes between 49.3 and 90% of the total phenolic content (Friedman, 1997; Riciputi et al., 2018). Epidemiological studies have shown a positive correlation between ingesting phenolic compounds and improved health (Baker et al., 2002; Dragsted et al., 1997; Hertog et al., 1996; Knekt et al., 2002). Phenolic compounds have been shown to possess antioxidant activity and other characteristics that have the potential to promote health. Despite the known phenomena of promoted root growth in the presence of elevated temperature, little information is available of these effects on potatoes, particularly the low yielding pigmented cultivars. Therefore, we investigated the effect of various root zone temperatures on potato seed mini tubers in some pigmented cultivars Salad Blue (SB), Pink Fir Apple (PFA) and Highland Burgundy Red (HBR) in a greenhouse between the South African winter and spring (July to September 2018). Furthermore, the antioxidant potential in the ethanol aqueous extracts was also investigated as this could be a contributing factor to the potato’s health benefits. A literature search showed that no study has been performed to evaluate the effect of root zone temperature on potato growth and chemical characteristics. Therefore, the present study is the first comprehensive work to focus on the effect of root zone temperature that would provide the potential root zone temperature for potato cultivation and growth. Pigmented cultivars Salad Blue (SB), Pink Fir
Apple (PFA) and Highland Burgundy Red (HBR) which are currently not extensively cultivated and exploited by South African farmers and the market were chosen. These cultivars are a gourmet treat and are in high demand especially in high-end restaurants with high tourist turnovers. But farmers are reluctant to cultivate them due to their low yields and small, specialized market. The commercial control (BP1) is actively grown and adapted to the Western Cape Province of South where the current study was conducted and is readily available on the market.

2. Materials and methods

2.1. Plant material and site description

In this study, four pigmented potato cultivars; Salad Blue (SB), Pink Fir Apple (PFA), Highland Burgundy Red (HBR) and a non-pigmented control (BP1) were used in this experiment. The tissue culture plantlets were generated and purchased from Ruvalabs PTY (Ltd), Western Cape, South Africa. Sterile nodal explants (0.5 cm) were sub-cultured on solid full-strength MS media supplemented with 30 g L⁻¹ sucrose. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl before solidification with 8 g L⁻¹ agar bacteriological. Cultures were maintained at 25 ± 2°C in a room with 24-h light conditions and a 40–50 μmol m⁻² s⁻¹ photosynthetic flux (PPF) provided by cool white fluorescent lamps. Sub-culturing was done at 4-week intervals until enough material was produced for the experiments. Afterwards, the 6-week-old regenerates from all four varieties were transplanted for cultivation in an automatically controlled research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS coordinates—33° 55′4.1″S 18°38′35.5″E. The plants were transplanted into 175 + 150 x 350 x 125 Mic black planting bags of 10 L volume filled with pine wood sawdust and shavings. The bags were kept moist with municipal water for 7 days in the greenhouse before receiving any nutrients. Individual plantlets were carefully planted in the middle of ¾ filled bags. Wood shavings were steam sterilized a week prior to transplanting in a steam sterilizer controller by a Delta DTD 48B4 at 80°C for 1 hour. The heat treatments began in the stolon initiation growth stage 48 days after transplant (DAT) and were maintained for 25 days. The trial was conducted from July to September 2018.

3. Experimental materials and design

3.1. Nutrient solution

The plants were supplemented with a nutrient solution by means of a precision dripper system (1 dripper per plant) at a rate of 8 L h⁻¹ controlled by a precision Delta timer. Two nutrient solution reservoirs were used during this experiment. One nutrient solution included macro elements Calcium Nitrate and the second reservoir included microelements including, N = 65 g kg⁻¹; P = 45 g kg⁻¹; K = 240 g kg⁻¹; Mg = 30 g kg⁻¹; S = 60 g kg⁻¹; Fe = 1680 mg kg⁻¹; Mn = 400 mg kg⁻¹; Cu = 30 mg kg⁻¹; Zn = 200 mg kg⁻¹; Mo = 50 mg kg⁻¹; B = 500 mg kg⁻¹. The nutrient solutions were prepared by adding 1 kg of fertilizer to a 1000 L reservoir filled with municipal tap water and adjusted to pH 5.8. Each plant received 200 ml solution in the morning (06h00) and 200 ml nutrient solution in the evening (18h00). A new solution was brought to level once a week with the same pH reading. The electric conductivity (EC) of the solution was monitored and it remained at the required range of 2.0–2.5 mS/cm.

4. Root zone temperature (RZT)

All treatments were simultaneously initiated to non-controlled temperatures between 19 and 25°C (control) before they were subjected to warm RZT. Three heated tables were specifically designed with heating cables to transmit heat to the root zone and maintained at a temperature of 20, 24, 28 ± 1°C and controlled automatically by temperature sensors inside the chambers. For the control, bags containing the plants were placed on a galvanized steel table with no source of heat.

5. Data collection

Temperature and relative humidity (RH) were controlled and measured by a fully automated Envirowatch system. The greenhouse was fitted with full light and retractable (40%) ALUNET cover, extraction fan, as well as wet walls. Within the greenhouse DELTA Programmable Logic
Controllers were installed close to the experiment to collect relative environmental data. Minimum and maximum air temperature, RH, and the heated bed chamber temperature were recorded every hour, day and night.

6. Plant growth measurement after 25 days of the heat treatments

Plant height, number of leaves and shoots were recorded 48 DAT on a subset of five plants for each treatment in three replications. Data were collected weekly every seven days thereafter. Each self-standing heating table unit housed 52 plant bags in total and the bags were tightly packed to avoid the loss of heat. The four cultivars were randomly distributed on each table and received the same treatment at the same time.

7. Experiment termination/harvest

The experiment was terminated exactly 73 DAT. The whole plant above ground level was harvested and weighed individually to obtain fresh weight of the leaves and stems. Tubers were harvested and weighed. The fresh leaf weight was recorded and grouped in bags for the leaf tissue analysis at Bemlab (Bemlab Pty Ltd), Somerset West, South Africa. Tubers were recorded and grouped for total experimental parameter weight and bagged for storage at −80°C till further analysis.

8. Leaf tissue analysis

Leaf samples were taken to compare the mineral content of the plant with its growth rate, physical appearance, yield, and tuber quality. Samples were analysed for macro- and microelements using an inductively coupled mass spectrometry (ICP-MS) and a LECO nitrogen analyzer with suitable standards. Leaves were washed with Teepol solution, rinsed with de-ionised water and dried to a constant temperature at 70°C overnight in an oven. The dried leaves were then milled and 0.5 g was ashed at 480°C in a muffle furnace and later shaken up in a 50:50 HCl (50%) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). The Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn) and Boron (B) content of the extracts were then determined on an Inductively Coupled Plasma—Mass Spectrometry ICP-MS. Total Nitrogen (N) content of the leaves was determined through total combustion in a Leco N-analyser. About 120 mg of the dried sample was encapsulated and placed in a tared tin foil container and the N content was then determined following the instrument specifications. The amounts of N, P, K, Ca and Mg were converted from percentage (%) to mg/kg by a factor of 10 000.

9. Tuber sample preparation

After harvest, tuber samples were stored at −80°C prior to lyophilizing for 24 hours (VirTis genesis wizard 2.0, United Kingdom). The material was then powdered (40–60 mesh) and stored in a refrigerator at 4°C until further use. The freeze dried and powdered tubers (200 g) were extracted with 60% ethanol. After 2 hours, the extracts were filtered and used for the assays.

10. Determination of polyphenol and flavonol contents

The total phenolic content of the lyophilized extracts was determined using the Folin Ciocalteau's phenol reagent according to the method described by Singleton et al. (1999) with modifications. Using a 96-well microplate, 25 μL of the sample was mixed with 125 μL Folin–Ciocalteau reagent and diluted 1:10 with distilled water. After 5 min, 100 μL (7.5%) aqueous sodium carbonate (Na₂ CO₃) was then added to each well. The total phenolics were then determined spectrophotometrically and expressed as mg gallic acid standard equivalents (mg/GAE) per gram sample. The flavonol contents were determined using quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol as standard using a protocol described by Mazza et al. (1999). In the sample wells, 12.5 μL of the crude sample extracts were mixed with 12.5 μL 0.1% hydrochloric acid (HCl) in 95% ethanol and incubated for 30 min at room temperature. The flavonol contents of the plant extracts were determined spectrophotometrically at 360 nm and expressed as mg quercetin standard equivalents (mg/QE) per gram sample. The flavonol contents of the aqueous plant extracts were
determined colorimetrically at 640 nm using aldehyde DMACA and expressed as mg catechin standard equivalents (mg/CE) per gram sample (Delcour et al., 1985; Treutter, 1989). All determinations were done in triplicates.

11. Antioxidant capacity

11.1. Oxygen Radical Absorbance Capacity (ORAC)
The ORAC assay was conducted to kinetically measure the peroxyl radical scavenging activity in potato samples with trolox as the antioxidant standard. Using the method of Prior et al. (2003), 20 μl of blank, Trolox standard, or the sample extracts in 75 mM potassium phosphate (KH2PO4) buffer at pH 7.4 were added to wells in a 96-well microplate that was clear-bottom and black. The samples were distributed throughout the microplate and were not placed side by side. About 200 μl of 0.96 μM fluorescein in working buffer was added and incubated at 37°C for 20 min in each well, while shaking intermittently, prior to adding 20 μl of freshly prepared 119 mM ABAP, after which the microplate was inserted instantly into a plate reader at 37°C. The decay of fluorescence at 538 nm was measured with excitation at 485 nm every 5 min for 2.5 hr. Areas under the fluorescence in comparison with the time curve for the samples minus the area under the curve for the blank were calculated and compared to a standard curve of the areas under the curve for 6.25, 12.5, 25, and 50 μM Trolox standards minus the area under the curve for blank. All determinations were conducted in triplicates. The ORAC value was expressed in micromoles of trolox equivalents per gram of tissue (μmol TE/g).

12. Ferric Reducing Antioxidant Power (FRAP) assay
The FRAP assay was performed using the method described by Benzie and Strain (1996). Ferric ion-Reducing Antioxidant Power (FRAP) reagent that had been freshly prepared contained a mixture of 20 mL glacial acetic acid (300 mM, pH 3.6), 2 mL 2,4,6-Tripyridyl-s-triazine (TPTZ) solution, and 2 mL FeCl3.6H2O. The solution was kept in a water bath at 37°C for 10 min prior to use. About 5 μl of the samples was mixed with 45 μl of deionized water in a 96-well plate followed by the addition of 100 μl of freshly prepared FRAP reagent. Sample blanks were also prepared. The 96-well plate was incubated in the dark at room temperature for 30 min and the absorbance was measured at 593 nm. Different concentrations of FeSO4.7H2O dissolved in deionized water at 50, 100, 200, 400, 800, and 1000 μM (R2 = 0.9798) were used for the standard curve and the results were expressed as μmol AAE/g sample. All mentioned determinations were conducted in triplicates.

13. DPPH free radical scavenging activity
The DPPH free radical scavenging activity of the plant extracts was carried out according to a method described by Zheleva-Dimitrova (2013). DPPH solution (0.0 ml, 0.11 mM) in methanol was separately mixed with 0.01, 0.02, 0.05, and 0.1 mg/ml of extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 60 min at 10 min intervals. Catechin and butylated hydroxytoluene (BHT) were used as the reference antioxidant compounds. The absorbance of the remaining DPPH radicals was read using a suitable spectrophotometer at 519 nm. Free radical scavenging activity of the samples was expressed according to the equation below:

\[
\text{Percentage ( %) inhibition of DPPH activity} = \frac{A_{\text{sample}} - A}{A_0} \times 100
\]

where \(A_0\) is the absorbance of DPPH in solution without an antioxidant and \(A\) is the absorbance of DPPH in the presence of an antioxidant. IC50 value (concentration of sample where absorbance of DPPH decreases 50% with respect to absorbance of blank) of the sample was determined. All mentioned determinations were done in triplicates.

14. Caffeic acid and chlorogenic acid
Caffeic and chlorogenic acid were determined on a Dionex HPLC (Dionex Softron, Germering, Germany) equipped with a binary solvent manager and auto-sampler coupled to a Bruker ESI Q-TOF mass spectrometer (Bruker Daltonik GmbH, Germany). Constituents of the plant extracts
were separated by reversed chromatography on a Thermo Fischer Scientific C18 column 5 μm, 4.6 μm 150 mm (Bellefonte, USA), using a linear gradient of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as solvent at a flow rate of 0.8 mL min⁻¹, an injection volume of 10 μL, and 30°C oven temperature. Electrospray voltage was set to ±3500 V. MS spectra was acquired in negative mode. Dry gas set to 9 L min⁻¹ at a temperature of 300°C and nebulizer gas pressure was set to 35 psi.

15. Vitamin C content
This extraction was performed according to the procedure of Amin and Cheah (2003). In triplicates, 10 g of the sample was extracted with meta-phosphoric acid (0.3 M) and acetic acid (1.4 M) in a ratio of extract to sample of 1:1. This mixture was agitated in an orbital shaker for 15 min at room temperature and at 100 rpm. Vitamin C content was then determined on a High-Performance Liquid Chromatography (HP LC).

16. Statistical analysis
Where applicable, data were subjected to statistical analysis using the STATISTICA program. A one-way analysis of variance was used to compare the means of the growth parameters and chemical contents among the treatments and a two-way analysis of variance to compare the interaction between plant age and root zone temperature on various growth parameters or chemical contents. Means were segregated using Fisher’s least significant difference (LSD) test and treated as significantly different at p < 0.05.

17. Results and discussion
17.1. Plant growth parameters
The height response of different potato cultivars subjected to three R2T treatments was variable as shown in (Table 2). An interesting trend was obvious. An R2T of 24°C significantly (p < 0.05) increased height in almost all the cultivars (except for 28°C, 73 DAT) regardless of the duration after transplanting. However, the highest increases were noted in the pink fleshed PFA cultivar throughout the trial. Specifically, 48 DAT, the values ranged between 40–85 cm, 41–63 cm, 92–165 cm and 36–75 cm in cv. BP1, SB, PFA and HBR, respectively. At 55 DAT, the values increased from 55–95 cm, 46–97.8 cm, 115.8–172 cm and 34–83.6 cm in cv. BP1, SB, PFA and HBR, respectively. Sixty-three DAT, the control significantly (p < 0.05) lowered plant height in all the cultivars and ranged between 70–104 cm, 46–78 cm, 1466–175 cm and 29–87.2 cm in cv. BP1, SB, PFA and HBR, respectively. The same trend was noted 73 DAT where the control significantly slowed the growth of the plants. The values ranged between 74.6–112.6 cm, 42.4–118.8 cm, 130.6–176 cm and 33–89 cm in cv. BP1, SB, PFA and HBR, respectively. In general, 24°C significantly increased plant height and 20°C significantly slowed down plant growth. Cultivar PFA responded best to 24°C. The response of the potato cultivar leaf number to R2T was variable as shown in (Table 3). The control significantly slowed down leaf production 48 DAT in all the cultivars except in the Blue fleshed CV. SB where 20°C had this effect. The values ranged between 5 and 7 and 7–10 in BP1 and PFA, under R2T 24°C for the higher values and the control for the lower values. The control also resulted in lower leaf numbers in cv. PFA and HBR, 55 DAT while 28°C significantly improved leaf numbers in the same cultivars. However, 20°C and 24°C, respectively, decreased and increased the number of leaves in cv. BP1 and SB. At 63 DAT, 24°C significantly lowered leaf number in all cultivars except in cv. HBR (28°C). However, contrary to other treatments, the control significantly

| Table 1. Temperature and relative humidity in the greenhouse throughout the growing season |
|---------------------------------------------|------------------|-----------------|
| Temperature (0 °C) | Relative Humidity (%) |
| Min | 18.9 | 88 |
| Max | 34.3 | 62 |
| Mean | 24.6 | 62.4 |
Table 2. The combined effect of RZT and duration after transplanting on plant height in some potato cultivars

|       | 48 DAT |       |       | 55 DAT |       |       | 63 DAT |       |       | 73 DAT |       |       |
|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|--------|-------|-------|
|       | Control | 20°C  | 24°C  | 28°C  | Control | 20°C  | 24°C  | 28°C  | Control | 20°C  | 24°C  | 28°C  | Control | 20°C  | 24°C  | 28°C |
| BP1   |        |       |       |        |        |       |       |        |        |       |       |        |        |       |       |       |
|       | 40 ± 0  | 57.6 ± 2.51 | 85.5 ± 4.27 | 52.4 ± 4.51 | 55.6 ± 2.61 | 83.4 ± 2.88 | 95.4 ± 1.52 | 73.4 ± 2.88 | 70 ± 5.57 | 90.4 ± 4.93 | 104 ± 2.24 | 95.6 ± 3.78 | 74.6 ± 4.45 | 94.8 ± 3.56 | 112.6 ± 5.13 | 108 ± 2.58 |
| SB    |        | 49 ± 4.18 | 41.2 ± 1.64 | 63.6 ± 3.42 | 60.8 ± 3.92 | 46.8 ± 2.49 | 97.8 ± 4.55 | 53.2 ± 0.49 | 46 ± 3.87 | 46 ± 2.74 | 78 ± 6.04 | 55.6 ± 4.34 | 49.4 ± 5.77 | 42.4 ± 2.51 | 118.8 ± 6.42 | 73.8 ± 3.27 |
| PFA   |        | 92.6 ± 5.13 | 129 ± 10.0 | 149 ± 3.27 | 151 ± 3.27 | 172 ± 3.27 | 153 ± 3.13 | 129 ± 3.42 | 146 ± 3.39 | 175 ± 3.24 | 152 ± 5.73 | 130.6 ± 2.61 | 135 ± 4.80 | 166.6 ± 6.50 | 176 ± 3.17 |
| HBR   |        | 36 ± 4.18 | 49.6 ± 6.40 | 75 ± 0.79 | 62.2 ± 3.61 | 57.6 ± 1.67 | 83.6 ± 1.52 | 70.2 ± 2.17 | 29 ± 6.28 | 56.2 ± 4.97 | 87.2 ± 3.56 | 74.2 ± 3.19 | 33 ± 4.42 | 53.4 ± 2.70 | 89 ± 6.96 | 87.4 ± 3.44 |

Values represent Mean ± SD

Different letters along the row per DAT block represent significant differences at p < 0.05

No heat was applied to the control
Table 3. The combined effect of RZT and duration after transplanting on number of leaves in some potato cultivars

|       | 48 DAT | 55 DAT | 63 DAT | 73 DAT |
|-------|--------|--------|--------|--------|
|       | Control | 20°C    | 24°C    | 28°C    | Control | 20°C | 24°C | 28°C | Control | 20°C | 24°C | 28°C | Control | 20°C | 24°C | 28°C |
| BP1   | 4.6 ± 0.55 | 6.6 ± 0.59 | 7.4 ± 0.89 | 6.2 ± 0.45 | 8.6 ± 0.89 | 9.2 ± 1.34 | 9 ± 1.79 | 9.6 ± 1.41 | 8.8 ± 1.10 | 9 ± 1.14 | 7.4 ± 0.89 | 9 ± 1.18 | 7.6 ± 1.34 | 10 ± 1.68 |
| SB    | 5.6 ± 0.55 | 4.8 ± 0.84 | 6.2 ± 0.71 | 6.2 ± 0.45 | 6.8 ± 0.84 | 6.4 ± 1.14 | 7.4 ± 0.55 | 5.6 ± 0.89 | 6.6 ± 1.34 | 6.2 ± 0.84 | 6 ± 0.71 | 6.2 ± 0.84 | 5.8 ± 2.05 | 6.6 ± 0.55 | 5.4 ± 3.05 |
| PFA   | 6.8 ± 1.64 | 10 ± 1.22 | 10 ± 1.22 | 9.4 ± 0.89 | 10.4 ± 3.70 | 12.8 ± 1.64 | 11.6 ± 0.89 | 10.4 ± 1.14 | 10.2 ± 1.3 | 9.8 ± 2.17 | 10.4 ± 1.14 | 10 ± 1.22 | 7.8 ± 0.71 | 10.2 ± 0.71 | 6.8 ± 7.85 |
| HBR   | 4 ± 0.71 | 6.4 ± 0.59 | 6.6 ± 0.89 | 8.4 ± 0.55 | 5 ± 0.71 | 7.6 ± 1.14 | 7.4 ± 0.55 | 8.4 ± 0.84 | 7.4 ± 1.87 | 7 ± 1.48 | 6.8 ± 1.48 | 3.2 ± 0.84 | 4.4 ± 0.84 | 4.4 ± 1.52 | 3.8 ± 1.44 |

Values represent Mean ± SD

Different letters along the row per DAT block represent significant differences at p < 0.05

No heat was applied to the control
improved the number of leaves in all cultivars. At 73 DAT, the number of leaves ranged between 8–10, 4–7, 7–10 and 4–4 in cv. BP1, SB, PFA and HBR, respectively. RZT 28°C significantly lowered leaf numbers while variable outcomes involving all the treatments were noted for increasing leaf number. Looking at the leaf numbers, it appears that they were within the same range regardless of DAT.

According to Van Loon (1981), high temperatures as well as water stress are factors that negatively affect potato quality and yield. In our findings, 24°C RZT proved to be advantageous for prolific plant height in potato cultivars. A study by Sakamoto and Suzuki (2015), lettuce (Lactuca sativa L) shoot size decreased at low RZT (10°C) at seven days after treatment when compared to with RZT (20, 25 and 30°C). In cucumber (Cucumis sativus L) RZT 12°C significantly reduced total fresh weights when compared with higher temperature (20°C), due to plant growth restriction by membrane lipid peroxidation, cell root viability and water stress (Yan et al., 2013). South Africa has a variety of different climates which range from a continental climate with rainy summers and dry winters, to Mediterranean climate with rainy winters and warm summers in its south western coastal areas (Taljaard, 1986). Potatoes are grown in most of these climatic regions with dry and wet winters and summers (Haverkort et al., 2013). For this reason, it would be difficult to obtain accurate and supported data to grow the cultivars SB, PFA and HBR which are low yielding but very nutritious compared to our control cultivar BP1. As cold and heat stress can cause stunted plants (Bharti et al., 1997; Nozolillo et al., 1990), the lowest aerial biomass production was observed in control and 20°C for cv. BP1, PFA and HBR.

The results of the current study also indicate that an increase in RZT significantly (p < 0.05) increased the number of tubers in all the cultivars (Table 4). The lowest tuber number value was reported in cv. HBR (1.08 cm) in the control and the highest value was reported in cv. HBR and PFA at 28°C. These cultivars reported the same value of 2.31 cm. As shown in Table 2.3, RZT also significantly increased tuber weight in all the cultivars; however, 24°C had the most significant impact on weight and in the commercial cv. BP1. More specifically, the values ranged between 0.43–35.55 g, 0.3–30.3 g, 0.27–19.17 g and 0.15–11.92 g in cv. BP1, SB, PFA and HBR, respectively. Interestingly, the control and 24 °C, respectively, lowered and increased tuber weight in cv. BP1 and SB while 20 and 28°C had a similar effect on cv. PFA and HBR. The control temperature was not constant in the present study as there was no heat supplied to the tables and varied between 19 and 25°C according to the temperature of the greenhouse and irrigation regimes. This conceivably led to the low aerial biomass production reported in both the control and 20°C RZT. In the field, RZT is expected to vary in accordance with day and night temperatures, humidity, and irrigation among other factors.

High root zone temperatures combined with high air temperature have the potential to cause severe stress through the stimulation of shoot production and can delay tuber initiation and formation (Chang et al., 2006; Struik, 2007). The opposite was noted in cv. HBR as it responded best to RZT 28°C. Chang et al. (2006) further reported that RZT in particular is critical to the root and tuber initiation, development and growth. In general, root growth occurs when root zone temperature is between 15 and 30°C. This is a wide spectrum and we can confirm that the best yield was obtained between 24 and 28°C for the cultivars of this study. The control and 20°C expressed significantly (p < 0.05) low tuber weight as well as tuber numbers. Furthermore, we can confirm that when growing generation 0 of all four potato cultivars, the yield expectation is not significantly different from each other and comparing that of the white fleshed commercial potato type, especially when exposed to RZT 24°C. There was a significant difference in the response of the cultivars; this confirms that the response is genetic. Wang et al. (2008) reported that the tuberization stage of potatoes is a distinctive process controlled by many factors such as genotypes which determines tuber size, number, and yield potential. Root zone temperature (28°C) was effective in producing more tubers for all four cultivars but the development in size was smaller. This was expected, the tubers needed more time to develop which would have affected the tuber weight positively. Furthermore, we can confirm that this RZT can be mimicked in a controlled greenhouse for optimum production for large-scale farming throughout the year. It is widely
Table 4. The effect of RZT on number of potato tubers and weight

|        | Number of tubers | Tuber weight (g) |
|--------|-----------------|-----------------|
|        | Control | 20°C | 24°C | 28°C | Control | 20°C | 24°C | 28°C |
| BP1    | 1.23 ± 0.60<sup>b</sup> | 1.46 ± 0.66<sup>b</sup> | 1.77 ± 0.93<sup>b</sup> | 1.92 ± 0.64<sup>b</sup> | 0.43 ± 0.35<sup>a</sup> | 0.75 ± 0.54<sup>a</sup> | 35.55 ± 12.41<sup>a</sup> | 26.43 ± 11.56<sup>a</sup> |
| SB     | 1.15 ± 0.38<sup>c</sup> | 1.31 ± 0.49<sup>c</sup> | 1.69 ± 0.85<sup>c</sup> | 2.08 ± 1.04<sup>b</sup> | 0.30 ± 0.12<sup>b</sup> | 0.31 ± 0.18<sup>b</sup> | 30.30 ± 14.44<sup>b</sup> | 20.23 ± 5.03<sup>b</sup> |
| PFA    | 2.23 ± 1.36<sup>c</sup> | 1.85 ± 0.69<sup>b</sup> | 1.77 ± 0.83<sup>c</sup> | 2.31 ± 1.18<sup>c</sup> | 0.38 ± 0.22<sup>a</sup> | 0.27 ± 0.12<sup>b</sup> | 15.29 ± 11.73<sup>c</sup> | 19.17 ± 6.99<sup>b</sup> |
| HBR    | 1.08 ± 0.28<sup>c</sup> | 1.46 ± 0.52<sup>b</sup> | 1.85 ± 1.21<sup>b</sup> | 2.31 ± 1.03<sup>c</sup> | 0.19 ± 0.08<sup>c</sup> | 0.15 ± 0.07<sup>c</sup> | 0.31 ± 0.08<sup>d</sup> | 11.92 ± 3.69<sup>c</sup> |

Values represent mean±SD
Different letters down a column per block represent significant differences at p < 0.05
No heat was applied to the control
reported that cool seasons are ideal tuber production, while the most active root development occurs around 20°C (Sattelmacher et al., 1990a). But, results of the present study differ. However, the greenhouse and field planting times can be accomplished by ensuring that the root zone or soil temperature in the field reaches 24°C.

18. Leaf tissue analysis

18.1. Effects of root zone temperature on elemental composition

The uptake of macro- and micronutrients on exposure to different RZT was variable as shown in Table 5. Temperature and cultivars significantly affected the level of greenness of leaves, which is an indicator of leaf nitrogen content. Specifically, nitrogen ranged between 21 and 34.9 mg/kg in cv. BP1 at 28°C and cv. SB in the control, respectively. Phosphorus followed the same trend in terms of cultivar and RZT but ranged between 2.1 and 6.1 mg/kg.

Potassium ranged from 44.1 to 68.8 mg/kg in cv. SB and HBR, respectively, Ca between 24.2 and 36 mg/kg in HBR and PFA, respectively; Mg from 4.5 to 7.8 mg/kg in BP1 and HBR, respectively; Na between 1096 and 7210 mg/kg in PFA and HBR, respectively; Mn between 0.09 and 0.25 mg/kg in HB1 and BP, respectively; Fe from 0.33 to 0.59 mg/kg, respectively, in SB and PF; Cu between 0.30 and 1.8 mg/kg in HB and PF, respectively; Zn from 0.06 to 0.68 mg/kg in SB and PF, respectively, and B ranged from 0.03 to 0.09 mg/kg in cv. BP. These results indicate that the control played a significant (p < 0.05) role in increasing (mg/kg) Ca (24.20), N (31.90), P (3.40) and Na (2620) in comparison to other treatments while 20°C significantly increased K (48.10 mg/kg) and Cu (0.98 mg/kg). Also, 24°C significantly increased Mn (0.13 mg/kg), Fe (0.26 mg/kg) and Zn (0.07 mg/kg), while 28°C increased Mg (4.80 mg/kg) and B (0.03 mg/kg).

One would also expect warmer RZT to reduce micro-elements uptake. But a two-way ANOVA showed that there was a strong interaction between the RZT and cultivar on mineral absorption in the leaves of the potatoes. Therefore, the response is both cultivar and temperature related. Generally, micronutrients displayed higher absorbance values in RZTs of the control and at 24°C. The best result for each cultivar was observed at 24°C and HBR displayed higher values than the other cultivars.

High nutrient solution temperature under hydroponic conditions is said to increase water absorption by influencing root structure changes (Al-Harbi & Burrage, 1992). Lower solution temperatures have also been reported to reduce nitrogen uptake, levels of hormones in the roots as well as translocation; and which in turn induce physiological changes and presumably enhance tuberization induction and substantially increases tuber yield (Chang et al., 2006). The present results are at variance with those of the previous authors. The higher the RZT, the lower the nitrogen uptake. The lower nitrogen availability enhanced tuberization. From the results, it could be noticed that the root zone temperature of 28°C resulted in a significantly higher tuber weight compared to the control and RZT 20°C. This could be due to drought stress in the potato root zone that reduces the amount of water readily available for the plant as well as restrict the absorption of specific nutrients such as NO3, N, K and Ca. These are required for optimal growth rate and leaf area as described by Chang et al. (2008). Struik et al. (1989a) also reported the negative effect of high root temperature on the haulm longevity especially when RZT is in the range of 28–30°C, a range known to be supra-optimal for potatoes (Sattelmacher et al., 1990b). Also, one expects that warmer RZT will reduce micro-elements uptake (Chang et al., 2006), decrease photosynthesis and carbon net assimilation (Burton, 1972) which cause haulm senescence as expressed by lighter green leaves. Farren and Mingo-Castel (2006) further reported that an unlimited nitrogen supply causes the delay of tuberization in aeroponics due to the plants extended vegetative growth. Furthermore, the tuberization developmental stage is stimulated by nitrogen deficiency or the inhibition of nitrogen uptake as a result of low temperatures (Goins et al., 2004). Goins et al. (2004) therefore reported that a controlled environment with optimized nitrogen concentrations in solutions can improve N use efficiency and tuber yield by suppressing
Table 5. The effects of RZT on mineral compositions in potato cultivar BP1 leaves 73 DAT

|       | N (mg/kg)  | P (mg/kg)  | K (mg/kg)  | Ca (mg/kg) | Mg (mg/kg) | Na (mg/kg)  | Mn (mg/kg) | Fe (mg/kg) | Cu (mg/kg) | Zn (mg/kg) | B (mg/kg) |
|-------|------------|------------|------------|------------|------------|-------------|------------|------------|------------|------------|-----------|
| Control | 31.90 ± 1.53<sup>a</sup> | 3.40 ± 1.00<sup>a</sup> | 59.60 ± 3.21<sup>a</sup> | 24.20 ± 2.65<sup>c</sup> | 7.30 ± 1.53<sup>c</sup> | 2620.00 ± 0.58<sup>c</sup> | 0.09 ± 2.08<sup>a</sup> | 0.23 ± 1.15<sup>a</sup> | 0.30 ± 2.00<sup>a</sup> | 0.06 ± 2.52<sup>a</sup> | 0.04 ± 2.62 |
| 20°C    | 24.80 ± 3.06<sup>b</sup> | 3.20 ± 2.08<sup>a</sup> | 53.50 ± 3.06<sup>d</sup> | 27.60 ± 1.00<sup>d</sup> | 6.10 ± 1.53<sup>d</sup> | 2910.00 ± 0.58<sup>d</sup> | 0.13 ± 1.15<sup>d</sup> | 0.26 ± 2.52<sup>a</sup> | 0.78 ± 2.00<sup>d</sup> | 0.07 ± 2.52<sup>d</sup> | 0.03 ± 2.65 |
| 24°C    | 22.90 ± 1.53<sup>c</sup> | 2.30 ± 2.52<sup>d</sup> | 48.10 ± 1.53<sup>c</sup> | 30.10 ± 1.53<sup>c</sup> | 4.50 ± 2.08<sup>c</sup> | 3130.00 ± 0.58<sup>c</sup> | 0.21 ± 2.08<sup>c</sup> | 0.27 ± 2.08<sup>c</sup> | 0.98 ± 1.53<sup>c</sup> | 0.09 ± 2.00<sup>c</sup> | 0.03 ± 2.08 |
| 28°C    | 21.00 ± 1.15<sup>c</sup> | 2.10 ± 1.53<sup>d</sup> | 48.40 ± 1.00<sup>c</sup> | 25.70 ± 2.65<sup>c</sup> | 4.80 ± 2.52<sup>c</sup> | 3530.00 ± 1.73<sup>c</sup> | 0.13 ± 2.65<sup>c</sup> | 0.28 ± 2.00<sup>c</sup> | 0.65 ± 3.00<sup>c</sup> | 0.08 ± 1.73<sup>c</sup> | 0.03 ± 2.52 |

Values represent mean±SD
Different letters down the column represent significant differences at p < 0.05
No heat was applied to the control
Table 6. The effects of RZT on mineral compositions in some potato cultivar Salad blue leaves 73 DAT

|       | N (mg/kg)   | P (mg/kg)   | K (mg/kg)   | Ca (mg/kg)   | Mg (mg/kg)   | Na (mg/kg)   | Mn (mg/kg)   | Fe (mg/kg)   | Cu (mg/kg)   | Zn (mg/kg)   | B (mg/kg)   |
|-------|-------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|
| 0°C   | 34.9 ± 1.00 | 6.101 ± 2.08 | 65.4 ± 1.53 | 27.001 ± 1.53 | 7.801 ± 1.00 | 6050 ± 0.5   | 0.09 ± 1.00  | 0.20 ± 3.06  | 0.64 ± 2.00  | 0.07 ± 3.79  | 0.04 ± 2.65 |
| 20°C  | 29.40 ± 1.00 | 4.80 ± 1.00  | 53.50 ± 1.00 | 35.00 ± 0.58  | 6.01 ± 1.00  | 7210 ± 0.5   | 0.17 ± 2.52  | 0.32 ± 3.06  | 1.19 ± 2.52  | 0.11 ± 3.21  | 0.05 ± 2.00 |
| 24°C  | 28.30 ± 1.00 | 3.60 ± 1.53  | 58.40 ± 0.58 | 34.60 ± 1.00  | 5.20 ± 1.53  | 5740.00 ± 0.5| 0.18 ± 2.52  | 0.34 ± 3.06  | 1.80 ± 3.61  | 0.13 ± 0.58  | 0.05 ± 0.58 |
| 28°C  | 28.80 ± 1.73 | 4.90 ± 2.65  | 60.40 ± 2.00 | 31.00 ± 2.31  | 5.20 ± 1.53  | 6140.00 ± 1.5| 0.16 ± 2.52  | 0.37 ± 1.53  | 1.10 ± 2.08  | 0.12 ± 1.00  | 0.05 ± 0.58 |

Values represent mean±SD
Different letters down the column represent significant differences at p < 0.05
0°C represents the control
Table 7. The effects of RZT on mineral compositions in some potato cultivar pink fir apple leaves 73 DAT

|       | N (mg/kg)  | P (mg/kg)  | K (mg/kg)  | Ca (mg/kg) | Mg (mg/kg) | Na (mg/kg) | Mn (mg/kg) | Fe (mg/kg) | Cu (mg/kg) | Zn (mg/kg) | B (mg/kg) |
|-------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----------|
| 0°C   | 30.30 ± 1.00a | 3.30 ± 2.00a | 68.80 ± 2.52a | 27.30 ± 2.00a | 6.10 ± 2.00a | 4600.00 ± 1.77c | 0.12 ± 4.16c | 0.26 ± 2.52c | 0.60 ± 1.00c | 0.06 ± 1.00c | 0.04 ± 2.08c |
| 20°C  | 25.60 ± 4.04b | 2.70 ± 1.53b | 62.90 ± 2.08b | 35.10 ± 3.21b | 4.80 ± 2.65bc | 4800.00 ± 1.67bc | 0.17 ± 3.00bc | 0.33 ± 1.53bc | 1.41 ± 2.08bc | 0.11 ± 2.00bc | 0.05 ± 1.53bc |
| 24°C  | 25.70 ± 2.08a | 2.80 ± 1.53a | 63.30 ± 1.00a | 32.00 ± 2.52a | 4.60 ± 0.58a | 5190.00 ± 2.00ab | 0.18 ± 3.06ab | 0.33 ± 2.08ab | 1.29 ± 1.53ab | 0.09 ± 1.73ab | 0.06 ± 1.15ab |
| 28°C  | 22.90 ± 2.08a | 3.10 ± 3.21a | 61.20 ± 2.69a | 33.50 ± 1.53ab | 5.20 ± 2.08ab | 4150.00 ± 1.56a | 0.25 ± 3.06a | 0.30 ± 1.53a | 1.26 ± 2.08a | 0.11 ± 2.00a | 0.05 ± 1.73a |

Values represent mean±SD
Different letters down the column represent significant differences at p < 0.05
0°C represents the control
Table 8. The effect of RZT on mineral compositions in some potato cultivar highland burgundy red leaves 73 DAT

| Temperature | N (mg/kg)    | P (mg/kg)    | K (mg/kg)    | Ca (mg/kg)   | Mg (mg/kg)   | Na (mg/kg)  | Mn (mg/kg)  | Fe (mg/kg)  | Cu (mg/kg) | Zn (mg/kg) | B (mg/kg) |
|-------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|-------------|------------|------------|-----------|
| 0°C         | 28.30 ± 2.65 | 4.10 ± 2.08  | 63.40 ± 2.65 | 30.50 ± 2.00 | 7.40 ± 3.06  | 1096.00 ± 1 | 0.15 ± 2.52  | 0.59 ± 0.58  | 0.62 ± 2.08 | 0.68 ± 1.00 | 0.07 ± 1.15 |
| 20°C        | 27.90 ± 1.53 | 2.30 ± 1.00  | 44.10 ± 3.21 | 28.50 ± 2.08 | 6.30 ± 2.00  | 4330.00 ± 2 | 0.10 ± 2.08  | 0.16 ± 1.15  | 0.53 ± 3.06 | 0.06 ± 1.53 | 0.05 ± 1.00 |
| 24°C        | 27.90 ± 1.53 | 2.70 ± 1.00  | 45.00 ± 2.08 | 36.00 ± 0.58 | 6.20 ± 1.00  | 4740.00 ± 1 | 0.17 ± 2.00  | 0.31 ± 2.31  | 0.74 ± 2.31 | 0.10 ± 2.08 | 0.07 ± 1.15 |
| 28°C        | 24.50 ± 2.00 | 2.50 ± 1.53  | 47.00 ± 3.06 | 34.20 ± 2.52 | 5.90 ± 2.65  | 4910.00 ± 1 | 0.17 ± 3.00  | 0.17 ± 1.53  | 0.43 ± 1.15 | 0.11 ± 2.00 | 0.09 ± 2.08 |

Values represent mean±SD
Different letters down the column represent significant differences at p < 0.05
0°C represents the control
Table 9. The effect of RZT on polyphenolic compounds in some potato cultivars

|        | FLAVONOLS (mg QE/g) | POLYPHENOLS (mg GA/g) |
|--------|---------------------|-----------------------|
|        | Control 20°C 24°C 28°C | Control 20°C 24°C 28°C |
| BP1    | 0.35 ± 0.02a 0.21 ± 0.03c 0.33 ± 0.05bd | 0.37 ± 0.07a 1.18 ± 0.13b 0.69 ± 0.16c |
| SB     | 0.64 ± 0.08a 0.43 ± 0.08d 0.48 ± 0.03c | 0.55 ± 0.08b 3.09 ± 0.26a 2.11 ± 0.14c |
| PFA    | 0.35 ± 0.01b 0.25 ± 0.01c 0.31 ± 0.03c | 0.45 ± 0.02a 1.51 ± 0.11bc 1.37 ± 0.14c |
| HBR    | 0.76 ± 0.07a 0.39 ± 0.02b 0.33 ± 0.03c | 0.35 ± 0.01bc 4.08 ± 0.26a 2.21 ± 0.17b |

Values represent mean±SD
Different letters along the row per block represent significant differences at p < 0.05
No heat was applied to the control
shoot growth and enhancing assimilate partitioning into tubers. Previous studies have shown enhanced tuber initiation and the development of stolons and tubers under these conditions (Struik & Van Voorst, 1986). Moreover, others have reported that even shorter periods of water deficit during tuberization and stolonization caused a significant reduction in tuber number and weight and consequently yield (Lahliou & Ledent, 2005).

19. Tuber analysis

19.1. The effect of RZT on polyphenol and flavonol content

The effect of RZT on content of polyphenols was variable as shown in (Table 9). All three pigmented cultivars SB, PFA and HBR showed higher polyphenol activity in all treatments compared to the nonpigmented cultivar BP1 that was subjected to the control temperature. RZT 20°C significantly (p < 0.05) decreased polyphenols (mg GA/g) in the white fleshed commercial cv. BP1 (0.69), purple fleshed cv. SB (2.11) and pink fleshed cv. PFA (1.37). However, the highest temperature treatment significantly increased the polyphenols (mg GA/g) in cv. BP1 (1.47) and PFA (2.09). In contrast, the control significantly increased the values in cv. SB (2.09 mg GA/g) and HBR (4.08 mg GA/g). Cultivar HBR recorded the highest values although the lowest value was 1.98 mg GA/g under 24°C RZT. Similarly, RZT 20°C significantly (p < 0.05) lowered flavonols (mg QE/g) in cv. BP1 (0.21), SB (0.43) and PFA (0.25) while 24°C had a lowering effect on cv. HBR (0.33 mg QE/g). But, a RZT of 28°C significantly increased flavonols (mg QE/g) in cv. BP1 (0.37) and PFA (0.45) while the control had the same increasing effect on cv. SB (0.64) and HBR (0.76). Cultivar HBR undoubtedly presented itself with the highest content in both polyphenols and flavonols. No flavonol content was detected in the ethanol extract during DMACA. A two-way analysis of variance showed that there was a very strong interaction between the cultivar and temperature on both polyphenols and flavonols. This means that the production of flavonols in seed tuber potatoes that were subjected to various RZT is dependent on both the temperature and cultivar.

The results of the present study suggest that the pigmented potato cultivars SB, PFA and HBR had a significantly higher antioxidant activity compared to the more commonly consumed white or yellow fleshed cultivars. The Folin assay showed that the polyphenolic activity of the tuber extracts from cultivars BP1 and PFA both which are white and yellow fleshed was stimulated by the RZT of 28°C in comparison to cv. SB and cv. HBR which both had significantly higher polyphenolic activity when exposed to control temperature. The control temperature variations as determined by greenhouse temperatures, irrigation and humidity conceivably led to elevated compound synthesis observed as the RZT mimicked the actual variations that are experienced by the plants in a non-controlled environment or in the field. This can be ascribed to the novel source of its natural colorants and antioxidants, which are both associated with its phenolic compounds (Reyes et al., 2003). This increase in polyphenolic content refers to the stress resistance of the cultivars as it is forming oxidation compounds that are more toxic to pathogens, thus assisting in the healing process (Shahidi & Naczk, 1995). Thus, we can conclude from this experiment that the cultivars SB and HBR are ultrasensitive and would struggle in the traditional potato growing areas in South Africa, even more so during the seed growing phase under controlled conditions. If polyphenols can be induced by RZT, then there is a potential to use this abiotic stress as a tool to increase the health-related properties together with increased yield. Most plants suffer from biochemical and physiological damage by exposure to temperatures which are either too cold or too hot. These temperatures are not optimal for crop growth (Grace et al., 1998; Lyons, 1973). Phenolic compounds have been previously reported to defend plants against microorganisms and herbivores (Hada et al., 2001).

20. Varietal differences in antioxidant activity (ORAC, FRAP, DPPH)

The present results show mean ORAC values for ethanol extracts of tubers to be significantly (p < 0.05) higher in cv. HBR (107.27 μmol TE/g) and SB (91.47 μmol TE/g) as shown in Table 10 and when exposed to the control temperature. It is evident that the pigmented tubers have a two-fold or more presence of antioxidant activity compared to the white fleshed tuber cv. BP1. A RZT of 20°C
Table 10. The effect of RZT on antioxidant reducing capacity in some potato cultivars

|         | ORAC (μmol TE/g) | DPPH (μmol TE/g) | FRAP (μmol AAE/g) |
|---------|------------------|------------------|-------------------|
|         | Control 20°C 24°C 28°C | Control 20°C 24°C 28°C | Control 20°C 24°C 28°C |
| BP1     | 39.95 ± 1.40b 41.36 ± 1.75b 44.12 ± 1.74b | 16.06 ± 1.48b 9.23 ± 3.23 c 13.33 ± 0.95c | 16.00 ± 0.97b 3.01 ± 0.78ab 1.52 ± 0.19c |
| SB      | 91.47 ± 0.67a 68.59 ± 4.48c 72.96 ± 2.91b | 26.43 ± 0.62d 17.90 ± 0.40b 16.64 ± 2.03c | 21.66 ± 1.68b 13.10 ± 0.58c 7.09 ± 0.59c |
| HBR     | 49.81 ± 3.20c 44.06 ± 0.97c 58.38 ± 2.28c | 15.07 ± 1.26b 6.79 ± 4.71c 9.84 ± 0.81b | 16.21 ± 0.87b 5.24 ± 0.31c 2.98 ± 0.63c |

Values represent mean±SD

Different letters along the row per block represent significant differences at p < 0.05

No heat was applied to the control
resulted in significantly lower ORAC values in all cultivars except in cv. HBR (64.74 µmol TE/g) where 24°C had this lowering effect. The ORAC (µmol TE/g) values under 20°C decreased in the order SB (68) > PFA (44.06) > BP1 (30). It appears that a difference of 8°C in RZT brought about an increase of antioxidant activity. The results of the ORAC assay showed the potency of the ethanol tuber extracts to protect against oxidative damage. Although cv. HBR and SB do not need to be exposed to higher root zone temperature for more pronounced antioxidant activity, the present results have shown that the increase in yield through RZTs of pigmented seed tuber potatoes SB, PFA and HBR is still favourable compared to the naturally high yielding white fleshed potatoes.

The magnitude at which the ethanol extracts of tubers could reduce ferric ions was achieved by the FRAP assay. The ethanol extract of the tubers from cv. HBR showed a significantly higher FRAP (20 µmol AAE/g) followed by SB (13.1 µmol AAE/g) when the two were exposed to the control temperature. The root zone temperature 28°C appeared to be favored by cv. BP1 (4.68 µmol AAE/g) and PFA (7.09 µmol AAE/g). A two-way ANOVA showed a very strong interaction between RZT and cultivar on all antioxidant activities.

In the present study, all the ethanol tuber extracts showed free radical scavenging abilities. It was observed that the control significantly increased the DPPH activity and was highest in cv. SB (26.43 µmol TE/g). A RZT of 20°C significantly increased DPPH values in cv. HBR (17.09 µmol TE/g). Also, 28°C significantly increased antioxidant activities in cv. PFA (16.21 µmol TE/g) although this was not significantly higher than the 16.06 reported in BP1 (16.06 µmol TE/g) control treatment. In a cucumber study, low RZT (10°C) was shown to increase DPPH activity while a high RZT (30°C) was shown to significantly decrease these values (Sakamoto & Suzuki, 2015). The study of Haghighi and Abdolalihiour (2020) showed that the antioxidant activity was highest when the RZT was 25°C. Lee (2009) found high antioxidant activities in lentils at lower RZT of 14°C. Studies by these previous authors are variable, and it is conceivable that the variations in the RZT encountered in the control led to variable but higher antioxidant activities in the present trial. The strong interaction shown between RZT and cultivar shows the dependence of the two variables on each other for antioxidant activities.

21. Caffeic acid and chlorogenic acid content

HPLC analysis was carried out on the ethanol extract of the potato tubers. In the present study, two prominent compounds were detected viz, caffeic acid and chlorogenic acid. The chromatogram results in the peak profile showed that the mean concentrations of the compounds detected varied among the four treatments and cultivars as shown in (Table 11). Subjecting cv. BP1 and PFA to 20°C significantly (p < 0.05) lowered caffeic acid in the present trial by 40.48 and 95.47 (µg/g), respectively, as shown in Table 2.4. Although 28°C significantly lowered caffeic acid in cv. SB (596.09 µg/g), the same temperature significantly (p < 0.05) increased this phenolic acid in cv. BP1 (183.78 µg/g) and PFA (431.45 µg/g). Interestingly, the control significantly increased caffeic acid in SB (916.75 µg/g) and HBR (1380.74 µg/g). Also, as shown in Table 2.4, 20°C significantly lowered chlorogenic acid in cv. BP1 (6.79 µg/g) and SB (107.8 µg/g) while 24 and 28°C had a lowering effect on this phenolic acid in cv. HBR (79.42 µg/g) and PFA (41.46 µg/g). The control significantly increased chlorogenic acid in all the cultivars and they increased in the order: BP1 (17.06 µg/g) > PFA (247.94 µg/g) > SB (338.23 µg/g) > HBR (426.20 µg/g). A two-way ANOVA showed a very strong interaction between temperature and cultivar on caffeic and chlorogenic acid production in potatoes.

Lewis et al. (1998) reported that the chlorogenic acid is significantly higher in colored than in yellow-fleshed potatoes. Our results confirm this. Furthermore, elevated RZT did not improve the content of chlorogenic acid. It is interesting to note that caffeic acid content was promoted when PFA and BP1 was exposed to higher root zone temperature. The opposite was true for SB and HBR and therefore do not need elevated RZT for caffeic acid production. Ezekiel et al. (2013), reported chlorogenic acid in red- or purple-fleshed cultivars to have 2.2 to 3.5 times higher by comparison with yellow- and white-fleshed cultivars. Akyol et al. (2016), Furrer et al. (2017), Külen et al. (2013), Lachman et al. (2013), and Stushnoff et al. (2008) also reached similar findings.
Table 11. The effect of RZT on phenolic acids in some potato cultivars

|       | CAFFEIC ACID (μg/g) | CHLOROGENIC ACID (μg/g) |
|-------|---------------------|------------------------|
|       | Control  20°C  24°C  28°C | Control  20°C  24°C  28°C |
| BP1   | 143.32 ± 1.92 c  40.48 ± 0.82d  154.55 ± 4.87b  183.78 ± 9.04a | 17.06 ± 0.66a  6.79 ± 0.38 c  14.95 ± 0.67b  14.95 ± 0.63b |
| SB    | 916.75 ± 9.20a  534.65 ± 17.18A  604.94 ± 13.44B  596.09 ± 13.45 C | 338.23 ± 8.93a  107.80 ± 1.21 d  136.26 ± 2.42 C  184.00 ± 2.38b |
| PFA   | 302.21 ± 2.93b  95.47 ± 2.38c  282.82 ± 2.73 C  431.45 ± 10.27a | 247.94 ± 13.06a  57.92 ± 1.19c  48.8 ± 1.61 c  41.46 ± 0.91d |
| HBR   | 1380.74 ± 50.12a  227.70 ± 3.49b  98.29 ± 1.31d  127.68 ± 3.42 c | 426.20 ± 20.16a  180.72 ± 2.60b  79.42 ± 1.45d  140.54 ± 1.37 C |

Values represent mean±SD

Different letters along the row per block represent significant differences at p < 0.05

No heat was applied to the control
Table 12. The effect of RZT on Ascorbic acid (µg/g) content in some potato cultivars

|        | Control  | 20°C     | 24°C     | 28°C     |
|--------|----------|----------|----------|----------|
| BP1    | 25.46 ± 0.31 c | 84.37 ± 0.42 a | 23.36 ± 0.80 c | 36.23 ± 0.84 b |
| SB     | 31.48 ± 0.88 b | 83.08 ± 2.83 c | 14.23 ± 0.60 c | 10.63 ± 0.42 b |
| PFA    | 124.86 ± 0.88 a | 83.73 ± 0.10 b | 51.21 ± 1.02 c | 13.66 ± 0.65 c |
| HBR    | 44.03 ± 1.31 c | 83.73 ± 0.65 b | 86.04 ± 1.40 b | 160.82 ± 3.68 a |

Values represent mean±SD Different letters along the row per block represent significant differences at p < 0.05 No heat was applied to the control

22. Ascorbic acid (AA) content

The results of the current trial indicate that the response of AA to different cultivars subjected to different RZTs was variable (Table 12). A RZT of 28°C significantly (p < 0.05) lowered AA (µg/g) content in cv. SB (10.63) and PFA (13.66). In contrast, 28°C significantly increased AA (µg/g) content in the red skinned cv. HBR and this was significantly higher than in other cultivars. White fleshed commercial cv. BP1, purple skinned cv. SB and pink fleshed cv. PFA recorded 84.37, 83.08 and 124.86 (µg/g) respectively. Also, the control significantly decreased AA in cv. HBR (44.03) but contrastingly increased this vitamin in cv. PFA. A two-way ANOVA also showed a strong interaction between RZT and cultivar on AA production.

Water deficit has been closely associated with high AA content in tomato fruit (Dumas et al., 2003). In the current study, there is a trend either the highest RZT or the treatments that resulted in exceptional growth and yield, which would have been subjected to water deficit, higher AA content was noted. Furthermore, it is interesting to note that the AA was higher in the yellow fleshed, PFA when subjected to control RZT. Hejtmánková et al. (2009) obtained similar results when they reported some yellow-fleshed cultivars were tested to be about 1.4 times higher than purple-fleshed potatoes. Our results have shown that purple fleshed (SB, 20°C) and red fleshed (HBR, 28°C) seed tuber potatoes can reach significant ascorbic acid values when it is subjected to elevated root zone temperature. Nevertheless, the AA content in HBR convincingly outweighed all the other cultivars in all treatments. Our results further confirm a significant effect of cultivar on AA content, which was reported by Pawelzik et al. (1999), Weber and Putz (1999), Zgórska and Frydecka- Mazurczyk (2000) and Hamouz et al. (2009).

23. Conclusion

In conclusion, plant physiological growth and tuber bearing capacity were positively affected by an increase in root zone temperature which has a direct correlation on the macro and micronutrient uptake. The strong interaction observed between the cultivar and the root zone temperature on various variables present some interesting findings; showing the dependence of the two factors on each other for some important variable outcomes. The total activity of polyphenols and vitamin C is subject to the cultivar and treatment. As expected, secondary metabolites were more elevated in pigmented cultivars SB, PFA and HBR indicating their potential health benefits in comparison with non-pigmented potatoes. The cultivation practice of our findings are key for both growth and higher yield in a controlled environment. We provide evidence that these cultivars could produce preferable yields in regions of temperatures in the range of 24°C. Furthermore, the secondary metabolites reported in the pigmented cultivars SB, PFA and HBR of the current study and their potential health benefits offer substantial proof that these cultivars should be regarded as important when consulting dietary needs. Further field studies on the potential commercial value and the potential of exposing the plants to a RZT of 28°C to stimulate tuber formation and then reduce the temperature to 24°C with the aim to improve yield need to be conducted. Further research is necessary to investigate the potential of using a combination of 28°C and 24°C RZTs at different plant growing stages.
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Author details
Hildegard Witbooi1
Callistus Bvenura1
Oluwafemi Omoniyi Ogunbubu2
Learnmore Kambizi2
E-mail: kambizi@acu.za
1 Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, 7535, South Africa.
2 Phytochemistry Group, Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.

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