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AN ALTERNATIVE HEALTH CROP FOR SOUTH AFRICA: PURPLE POTATO MINI TUBER PRODUCTION AS AFFECTED BY WATER AND NUTRIENT STRESS

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ABSTRACT

Food security in South Africa ranks as one of the top ten priorities in the country. Potato is a fundamental staple food crop in South Africa, providing essential nutrition. While there are several cultivars currently in production for the potato market, there is a need to explore cultivars that are available, but not utilised within the country. Pigmented potatoes are not regarded as high value on the South African market; however, yield prospects as well as health-promoting benefits could have a positive contribution on the South African Gross Domestic Product (GDP) and on the population's health. Potato cultivar (cv.) Salad blue (SB) seems to be a drought-tolerant crop with the ability to produce reasonable yields under severe environmental conditions. In order to promote cv. SB as a possible food security option for South Africa, there is a critical need for empirical information, describing some basic horticultural as well as biochemical information and vitamin C presence. This study investigated the potential of pigmented potato SB tubers as an alternative to high yielding white potato for the South African market. Tubers of *Solanum tuberosum* cv. BP1 and SB, were used for this research. The high amounts in phenolic compounds in SB can be considered to be health-promoting phytochemicals. Anticarcinogenic, antibacterial, antiviral properties have been reported. A greenhouse, bag trial with virus-free plantlets of BP1 and SB cultivars was conducted using three water and nutrient levels and favourable root zone temperature (100% without heat, 100% heated, 50% heated, 25% heated) all grown in coco peat. Cultivar SB showed nearly two-fold yield compared to the control BP1. Methanol extracts of the tubers were assessed for their total polyphenolic, flavanol, and flavonol contents as well as 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability, ferric reducing antioxidant power (FRAP), Trolox equivalence antioxidant capacity (TEAC), anthocyanin and $_L$-ascorbic acid assays. The aqueous extract of the SB tubers was found to contain higher level of total polyphenols (320 mg GAE/g), and flavonol (85 mg QE/g) than the extract of the BP1 tubers with values of 173 mg GAE/g (total polyphenol), and 67 mg QE/g (flavonol). Similarly, the methanol extract of the tuber skins also exhibited higher DPPH (818,86 IC_{50} mg/mL), FRAP (18,19 μmol AAE/g), and TEAC (911,12 μmol TE/g) than the extract of the BP1 with DPPH (595,99 IC_{50} mg/mL), FRAP (10,86 μmol AAE/g) and TEAC (435,44 μmol TE/g). The present study provides useful information for farmers and health professionals in respect to increased yield and health-promoting benefits of an underutilized potato variety.

Key words: Drought tolerant, Food security, Potato, Root Zone Temperature, water, nutrient

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INTRODUCTION

Food security for South Africa is one of its top ten priorities [1]. In recent years, pigmented vegetables have gained commercial importance as a result of increased awareness of their health and nutritional benefits. Tuber crops together with other root crops occupy a significant role in food security, agriculture, and incomes for over 2.2 billion people in rural areas of developing countries of Africa, Asia, and the Caribbean [2,3]. Water is essential for plant growth; therefore, its deficiency is one of the most important factors that limits crop yield [4,5]. While many of the abiotic factors can be controlled by farmers, water and nitrogen are the main factors that control plant growth [6]. Drought tolerant tuber crops, capable of producing good yield under water scarce conditions, would be an important attribute given that South Africa is a water scarce country with much of the country being classified as semi-arid [7]. This study aims to evaluate the effects of water and nutrient stress in the presence of elevated root zone temperature on the growth as well as total phenolics (TP), ascorbic acid (AA) and total antioxidant (TA) of two pigmented potatoes. Potato crop yield is largely regulated by two key factors, water and nutrients in horticultural production management.

MATERIALS AND METHODS

Plant material and site description

Cultivars Salad blue (SB) and a control BP1 (*Solanum tuberosum* L.) were used in this experiment. The tissue culture plantlets were generated and purchased from Ruvalabs PTY (Ltd), Western Cape, South Africa. Sterile nodal explants (0.5cm) were subcultured 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 photosynthetic flux (PPF) 40–50 µmol m^{-2} s^{-1} provided by cool white fluorescent lamps. Subculturing was done at 4-week intervals until enough material was produced for the experiment. Afterwards, the uniform regenerates of 6-week-old plantlets from both varieties were transplanted into cultivation in an automatically controlled greenhouse research facility at the Cape Peninsula University of Technology, Bellville, South Africa; GPS co-ordinates – 33° 55′ 45.53S, 18° 38′ 31.16E. The plants were transplanted into 175 + 150 x 350 x 125 Mic black planting bags of 10L in volume and kept moist with municipal water for 7 days in the greenhouse before receiving any nutrient and planted in coco peat. Individual plantlets were carefully planted in the middle of a bag with only ¼ filled with the medium. Coco peat was steam sterilized a week prior to transplanting in a sterilizer controller by a Delta DTD 4B4B at 80°C for 1 hour. The heat treatments began 48 days after transplant and was maintained for 25 days. The control received no heat treatment. The experiments were conducted from July to October 2018.

Nutrient solution

Throughout the experiment, the plants were supplemented with a nutrient solution by means of a precision dripper system (1 dripper per plant) at a rate of 8L/h controlled by a precision Delta timer. Two nutrient solution reservoirs were used during this

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experiment. One nutrient solution reservoir included calcium nitrate (N = 157 g/kg, Ca = 192 g/kg) and the second reservoir included elements: N = 65 g/kg; P = 45 g/kg; K = 240 g/kg; Mg = 30 g/kg; S = 60 g/kg; Fe = 1680 mg/kg; Mn = 400 mg/kg; Cu = 30 mg/kg; Zn = 200 mg/kg; Mo = 50 mg/kg; B= 500 mg/kg. The nutrient solutions were prepared by adding 1kg of fertilizer to municipal tap water with the total solution volume in each reservoir at 1000L and adjusted to a pH 5.8. A new solution was brought to level once a week with the same pH reading. The electric conductivity of the solution was monitored and adjusted when it varied. The nutrient solution from the same reservoir was supplied at the same time to all four cultivars within the treatment to exclude the difference of nutrient uptake by the various cultivars. The trial consisted of 3 water levels and 3 nutrient levels. The 3 treatments exposed to 24 °C root zone temperature were based on our preliminary experiment, which was sufficient for maximum yield.

Growth measurement of plant/shoot growth, leaves and tuber differentiation after 36 days

Plant height, number of leaves, and number of shoots were recorded at 37 DAT on a subset of four plants for each treatment in three replications and then weekly every seven days thereafter. Each self-standing heating table unit housed 52 plant bags in total and was tightly packed to avoid heat loss. The four cultivars were randomly distributed in the units and received the same treatment at the same time. Commercial white fleshed [control (BP1)] cultivar and heritage cultivars, purplish-blue (SB), were used with each experimental unit. All recorded data were subjected to ANOVA and treatment means were compared using multiple comparison tests at 5% level of probability (Duncan's LSD).

Experiment termination at harvest

The experiment was terminated at 73 DAT. The whole plant above ground was harvested and weighed individually to obtain the fresh weight of the leaves and stems. All watering was stopped at this stage and the tubers were harvested. The fresh leaf weight was recorded and grouped in bags. Tubers were recorded and grouped for total experimental parameter weight and bagged for storage at -80 °C.

Sample preparation

After harvest, all the potato samples were placed in paper bags and frozen at -80 °C prior to being freeze dried for 24 hours (VirTis genesis wizard 2.0, United Kingdom). The tuber material was separated into skins and flesh, and then powdered (40-60 mesh) and stored under refrigeration until further use.

Preparation of plant extracts

The freeze-dried and powdered tubers (200g) were extracted with 80% methanol (MeOH). After 2 hours, filtration of the extracts took place and was used for the assays.

Total polyphenol, flavonol, and flavanol content analysis

The total phenolic content of the lyophilized extracts was determined using the Folin-Ciocalteu phenol reagent [8] and was determined spectrophotometrically using a microplate reader and expressed as mg gallic acid standard equivalents (GAE) per gram sample. Flavonol content of the plant extracts was determined spectrophotometrically at

360 nm and expressed as mg quercetin standard equivalents (QE) per gram sample [9]. The flavanol content of the aqueous plant extracts was determined colorimetrically at 640 nm using aldehyde DMACA and expressed as mg catechin standard equivalents (CE) per gram sample [10,11]. All determinations were done in triplicates.

Ascorbic acid

Ascorbic acid extraction was performed according to a specific procedure [12]. The 200 mg of the four tuber cultivars was added to 25 ml of 5% metaphosphoric (MPA) solution. The combined mixture was homogenized and centrifuged for 15 min at 4 °C. The supernatant was vacuum filtered through Whatman No. 1 filter paper. Following this step, 10 ml of the vacuum filtered sample was passed through a Millipore 0.45 µm membrane and thus ready to be injected in the HPLC system.

Antioxidant Capacity

DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the plant extracts was carried out according to a specific method [13]. 10 µl of the different concentrations of the plant extracts was reacted with 190 µL DPPH solution (0.00625g DPPH in 50mL methanol) and the absorbance of the samples was determined after 30 min using a Multiskan Spectrum plate reader (Thermo Fischer Scientific, USA) at 517nm. Free radical scavenging activity of the samples was expressed according to the equation below:

Percentage (%) inhibition of DPPH activity

$$= \frac{A^0 - A}{A^0} \times \mathbf{100} \qquad [14]$$

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. IC_{50} value (concentration of sample where absorbance of DPPH decreases 50% with respect to absorbance of blank) of the sample was determined. All measurements were done in replicates.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was performed by using a specific method [15]. An amount of 10 µl of the diluted aqueous plant extracts was mixed with 300 µl FRAP reagent in a 96-well clear plate. The FRAP reagent was a mixture of the following: (10:1:1, v/v/v) acetate buffer (300 mM, pH 3.6), tripyridyl triazine (TPTZ) (10mM in 40 mM HCL), and $FeCl_3.6H_2O$ (20 mM). After the room incubation period of 30 min, the plate was read at a wavelength of 593 nm in a Multiskan Spectrum plate reader (Thermo Fischer Scientific, USA). Ascorbic acid (AA) was used as the standard and the results were expressed as µmol AAE/g sample. All measurements were done in replicates.

Trolox equivalent antioxidant capacity assay

The trolox equivalent antioxidant capacity (TEAC) assay was carried out according to specific method [16] with the principle of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity. A solution of ABTS was prepared a day before by adding ABTS salt (8mM) with potassium persulfate (3mM) and then

storing the solution in the dark until the assay could be performed. The solution was further diluted with Millipore water. The plant extract (25µl) was mixed with 300µl ABTS solution in a 96-well clear microplate. The plate was read after an incubation period of 30min at room temperature in a Multiskan Spectrum plate reader (Thermo Fischer Scientific, USA) at 734 nm. Trolox was used as a standard and the results were expressed as µmol TE/g sample. All measurements were done in replicates.

Phenolic acids- High Performance Liquid Chromatography-Mass Spectrometry (HPLC)

The HPLC-MS technique was performed on a Dionex HPLC technology (Dionex Softron, Germering, Germany). Together with a binary solvent manager and autosampler coupled to a Brucker ESI Q-TOF mass spectrometer (Bruker Daltonik GmbH, Germany). Constituents of the plant extracts were separated by reversed-phase chromatography on a Thermo Fischer Scientific C18 column 5 µm, 4.6 × 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. The 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.

Statistical Analysis

Data were collected on 52 samples (13 plants per cultivar) per treatment. The morphological data were noted every seven days for 25 days. The percentage data for the morphological data for each treatment were analyzed using JMP. The differences between means reaching a minimal confidence level of 95% were considered as being statistically significant.

RESULTS & DISCUSSION

Effect of water and nutrient stress with increased root zone temperature on tuber differentiation of tubers

The effect of water and nutrient stress in combination with a suitable root zone temperature of 24 °C for cultivars BP1 and SB were tested. In cultivar SB, the mean number of tubers was significantly higher ($P < 0.05$) in all treatments. The results further confirmed that cultivar SB has the potential to remain a high yielding tuber cultivar even under lower water and nutrient additions and higher root zone temperature. A reduced water use efficiency (WUE) was observed in potato when exposed to an early season water shortage and it ultimately resulted in poor biomass accumulation and yield [17]. The response of cv. SB compared to BP1 can be attributed to WUE. It was reported [18] that an important plant physiological regulation is WUE, which is the ratio of the dry matter accumulated to the water [19]. This improvement in the WUE of cv. SB is mainly due to the accumulation of the dry matter by consuming less water due to the closing of stomata and less rate of transpiration. In cv. SB, the no heat compared with 100% water and nutrient combination, resulted in higher tuber weight. This result can be compared to that of Al-harbi & Burrage [20] where they reported higher nutrient solution temperatures as experienced under hydroponic conditions that increased water

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absorption by influencing root structure changes. The lowest combination of water and nutrient had the least significant $P > 0.05$ result in both cultivars. This result confirms that potatoes are water sensitive.

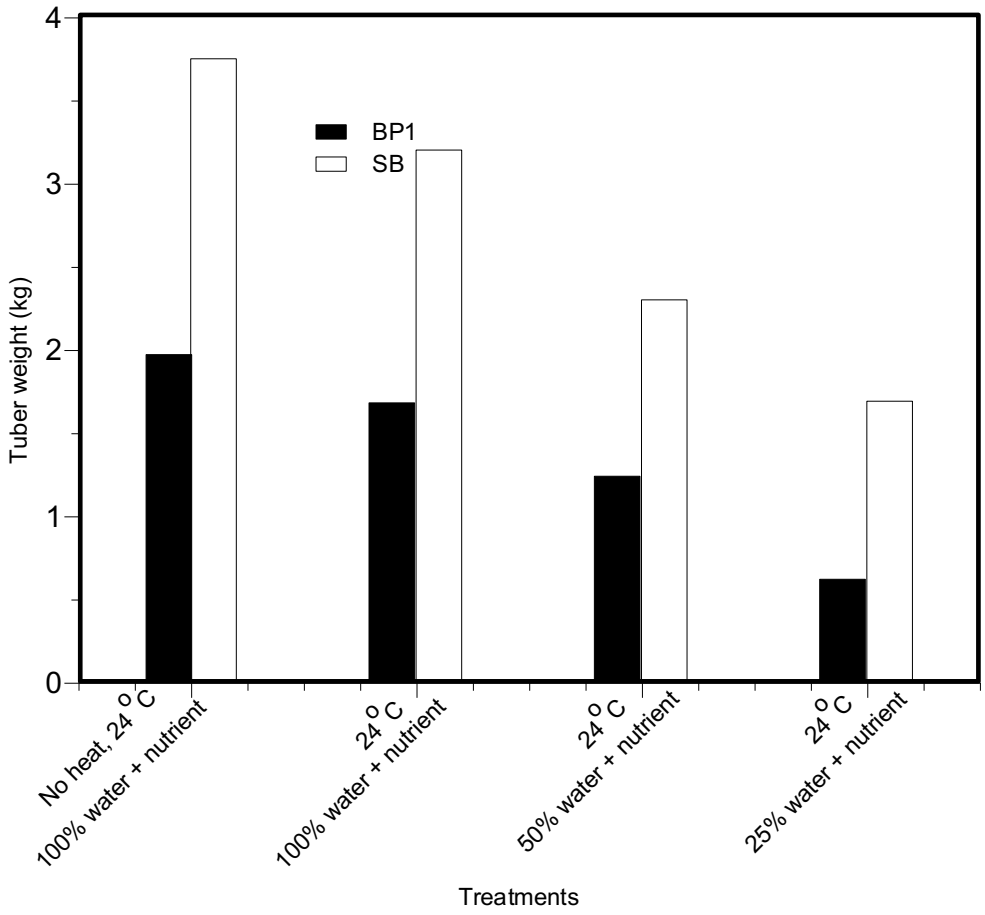

Figure 1: Effect of water and nutrient stress on cv. BP1 and SB tuber weight after 37 days under treatment

Biochemical evaluations

The results of Folin-Ciocalteu assay are presented in Table 2. Purple potato cultivar, for example, SB, is a richer source of polyphenols compared to the white potato cultivar BP1. The cultivar BP1 showed no significant results in all treatments, neither in the skins nor the tuber flesh. Among the treatments of cv. SB, the skins had significantly higher ($P < 0.05$) polyphenol presence with T0, T2 and T6 with the highest gallic acid equivalents (283 mg 100g-1, 320 mg 100g^{-1} and 230 mg 100g^{-1} DW, respectively). When 100% water and nutrients were applied to SB with or without 24 °C root zone temperature, the polyphenols were more apparent in the skins of the tubers subjected to higher root temperature by 37 mg GAE/ 100g^{-1} DW. Also, significant, but to a lesser degree, was when the tubers were subjected to 25% water and nutrients. The purple fleshed cultivar, Guincho Negra, contained similar total polyphenols, 285 mg 100g^{-1} FW compared to results herein [21]. Although this study presents phenolic content higher than Vitelotte variety 135.2 mg 100g^{-1} [22], the phenolic content differences observed

can be due to the various treatments, which have been shown to have an effect on the accumulation of phenolic acids in purple- and red-fleshed potatoes [23, 24, 25]. Total flavonols for both cultivars were clearly more present (***P*** < 0.05) in the skins of the tubers. The tuber skin extracts of the treatment T2 exudates significant presence of quercetin 85 mg QE/100mg^{-1}. This result compares to that of [26] reported flavonoids to be more than 30 mg per 100 g fresh weight in white fleshed potatoes and this level is nearly doubled in red and purple-fleshed potatoes as a result of the anthocyanins, which give the red and purple colour [27]. No anthocyanins were detected in cv. BP1, whilst all methanol extracts of SB expressed anthocyanins, which was significantly two-fold higher (408 mg/ 100g^{-1}) in T2. No flavanol content was detected in the methanol extract during DMACA.

Antioxidant activity

The DPPH radical scavenging activities (IC_{50}) of SB are shown in Figure 3. The tuber skins of cv. SB T0 had an IC_{50} value of 818,86 µmol TE/100g dry weight, whereas other values were T2 665,89, T4 592,21 and T6 572,89 µmol TE/100g, respectively. All the samples of cultivar BP1 had lower radical scavenging activity for DPPH and TEAC, except for treatment T4 with values of 725,02 and 658,14 µmol TE/100g, respectively. Cultivar SB T2, had the highest FRAP and TEAC radical scavenging activity, whereas T0 had the highest DPPH radical scavenging activity. In 2009, Ramboa *et al*. reported that the range of IC_{50} values of the sweet potato varieties was 0.7~6.4 mg/mL dried sample [28]. Further reports indicated that the range of IC_{50} values was 49~5.23 mg/mL methanol extract from sweet potato flours [23]. The FRAP radical scavenging activities of BP1 and SB are shown in Figure 3. SB had the highest activity of mg AAE/100 g dry weight. The SB tuber skin samples had the highest radical scavenging activity of T2 (18,19 µmol) AAE/100g dried samples, T0 (16,28 µmol) followed by T6 (13,52 µmol) respectively. Lachman *et al*. 2009 reported antioxidant activity in red/purple- fleshed potatoes was 72.51 – 144 mg AAE/100 g fresh weight. The present results showed that cv. SB had significantly (P < 0.05) higher radical scavenging activities compared to cv. BP1.

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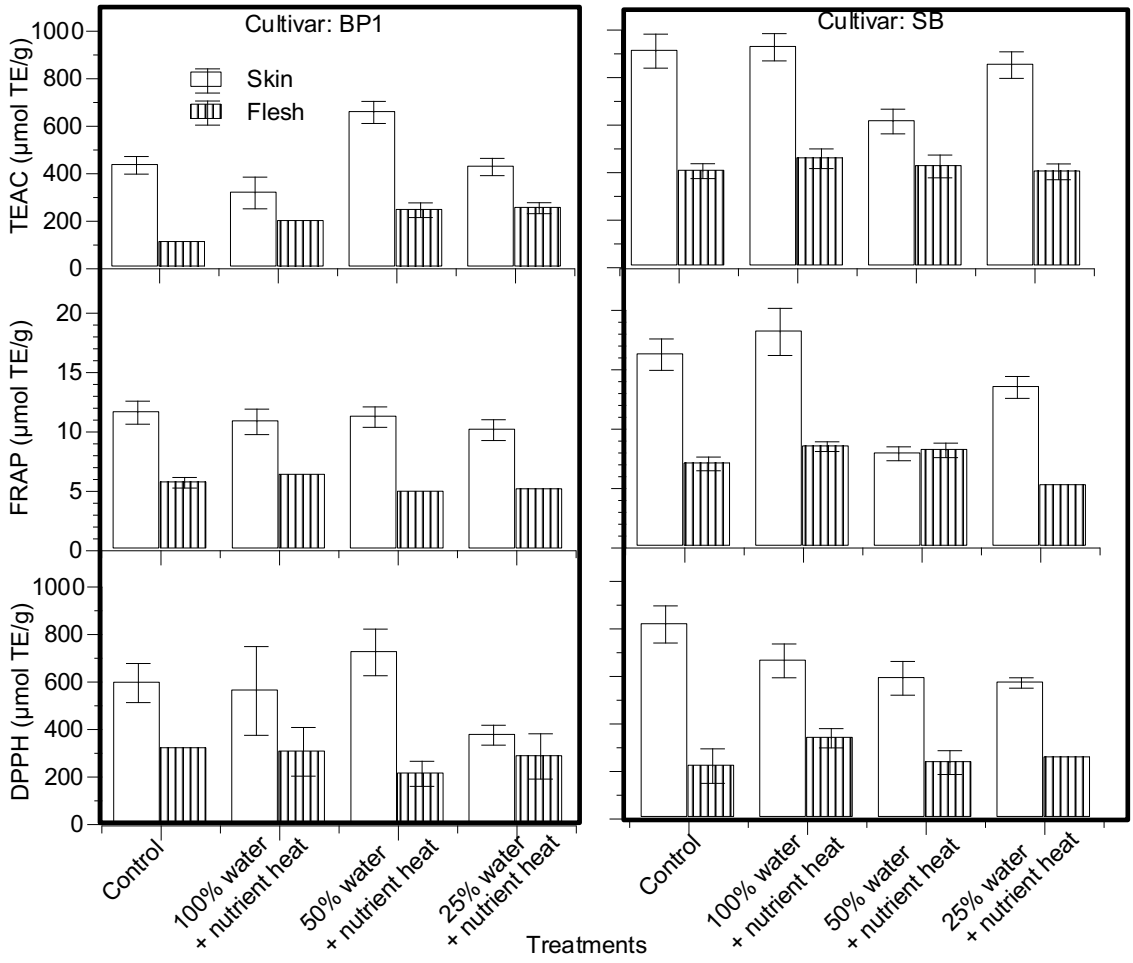

Figure 3: Antioxidant activity in skins and flesh of white fleshed (BP1) and purple fleshed (SB) potatoes represented as means (n = 3) ± standard deviation

Ascorbic acid

The contents of L-AA showed considerable variation, as shown in Figure 4. Cultivar SB resulted in significantly higher ($P < 0.05$) content of AA. It is evident that the potato flesh in all treatments contained significantly more AA, especially when it was exposed to water and nutrient stress ($P < 0.05$). The abiotic stresses that the samples were exposed to resulted in the development of reactive oxygen species (free radicals), which may be responsible to trigger an increase in vitamin C synthesis. Further reports indicated that the exposure of environmental stresses such as atmospheric ozone, UV-B radiation and sulfur dioxide, triggers the up-regulation of dehydroascorbate reductase, resulting in more rapid recycling of dehydroascorbate to vitamin C [29, 30, 31]. It has been reported that any improvement in the vitamin C content of potato products would have a beneficial impact on human nutrition [32].

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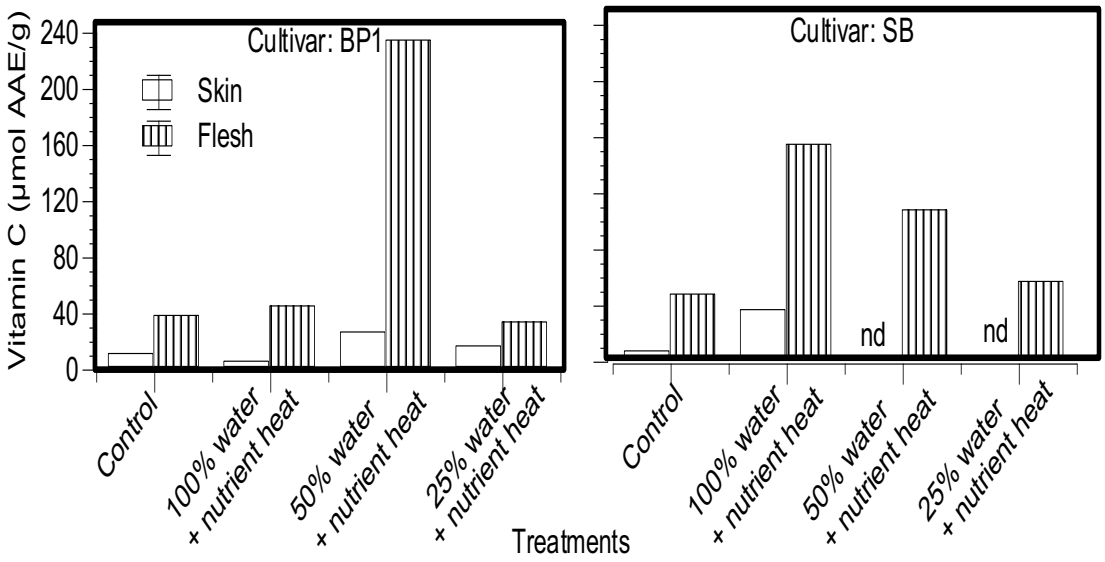

Figure 4: Concentration of ascorbic acid in skins and flesh of a white fleshed (BP1) and purple fleshed (SB) potatoes represented as means (n = 3) ± standard deviation

CONCLUSION

Cultivar SB clearly demonstrated a high content of polyphenolic compounds as well as high antioxidant capacity. The results of the study provide useful information that can inform agricultural planning and influence research for improving the productivity, food security and resilience of the under-utilized pigmented potato cultivars in drought-prone regions of South Africa.

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Table 1: Water and Nutrient level in different treatments

Rootzone Temperature	Water	Nutrient
No heat	Control (100% water)	Excess Nutrient (100%)
24 °C	Well-watered (100% water)	Excess Nutrient (100%)
24 °C	Moderate-watered (50% watered)	Sufficient Nutrient (50%)
24 °C	Water stressed (25% water)	Deficient Nutrient (25%)

Table 2: Concentrations of Total Polyphenol, Flavanol, Anthocyanin contents in methanol extracts of tuber skins and flesh

	Phenolic acids (mg GAE/ 100g DW)		Flavonols (mg QE/ 100g DW)		Flavanols (mg TE/ 100g DW)	Anthocyanidins (mg /100g DW)	
	BP1	SB	BP1	SB	BP1 | SB	BP1	SB
T0 S	189± 0,19	283± 0,27	65± 0,05	65± 0,08	*N.D.*	*N.D.*	298 ± 0,39
T1 F	71± 0,09	98± 0,12	39± 0,02	32± 0,01	*N.D.*	*N.D.*	170 ± 0,18
T2 S	173± 0,22	320± 0,40	67± 0,15	85± 0,11	*N.D.*	*N.D.*	408 ± 0,18
T3 F	87± 0,07	125± 0,08	48± 0,05	55± 0,09	*N.D.*	*N.D.*	154 ± 0,28
T4 S	183± 0,17	118± 0,12	52± 0,07	39± 0,04	*N.D.*	*N.D.*	134 ± 0,15
T5 F	55± 0,04	120± 0,12	22± 0,02	36± 0,01	*N.D.*	*N.D.*	197 ± 0,35
T6 S	161± 0,18	230± 0,18	59± 0,07	55± 0,09	*N.D.*	*N.D.*	241 ± 0,17
T7 F	63± 0,06	61± 0,06	47± 0,06	35± 0,06	*N.D.*	*N.D.*	71 ± 0,13

Values are means (n = 3) ± SD of eight determinations
Mean values that are not significantly different from each other ($P < 0.05$) are represented by the same letter

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