PHENOLOGICAL AND PHYSIOLOGICAL RESPONSES TO ABIOTIC PARAMETERS IN RARE POTATO CULTIVARS (*Solanum tuberosum* L.)

By

HILDEGARD WITBOOI

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Supervisor: Prof L Kambizi

Co-supervisor: Prof OO Oguntibeju

Bellville

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DECLARATION

I, Hildegard Witbooi, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

December 2020

Signed **Date**

PREFACE

This thesis has been written in an article format based on the guidelines of the journal where it has been published or submitted for review and consists of five chapters. Repetition between chapters that may occur was thus unavoidable. **Chapter one** encompasses a detailed literature review that includes horticultural information, biochemical properties and benefits of the potato tuber to human health. This chapter provides a review of all previously reported scientific data on polyphenols in pigmented potatoes. Cultivars Pink Fir Apple, Salad Blue and Highland Burgundy Red are all unusual to find on the market in South Africa. The *in vitro* plant material is available, but due to disease risks and low yield, farmers have not taken the step to become active growers nor did research to find the most efficient protocol to grow it. These cultivars are known to have complex polyphenolic and antioxidant activity and higher availability of vitamin C. The aims of the study are included at the tail end of Chapter one. This review article has been submitted for publication in Biological Reviews. **Chapter two** is the research article entitled “The role of increased root temperatures on the physiological and biochemical responses of four pigmented potatoes (*Solanum tuberosum* L.) which has been published in the Cogent Food and Agriculture. It discusses the evaluation of the effects of three root zone temperatures on growth, leaf macro- and micro-nutrients as well as phytochemical and antioxidant capacity on seed mini tubers of four pigmented potato cultivars [*Solanum tuberosum* cv. BP1, Salad blue (SB), Pink Fir Apple (PFA), Highland Burgundy Red (HBR)]. This chapter provides information to protect these potato cultivars in South Africa and promote a new food niche to the market that supports health consciousness. It also summarises the association between potatoes, oxidative stress and health benefits. Due to the

limitation of the current information available for South African potato farmers, the need to research options for specific growing conditions as well as health alternatives for the niche market is crucial. **Chapter three** is the research article entitled “An alternative health crop for South Africa: purple potato mini tuber production as affected by water and nutrient stress”, and have been published in African Journal of Food, Agriculture, Nutrition and Development. This study provides useful information on drought tolerance for farmers, food security and health professionals in respect to increased yield and health-promoting benefits of a single potato variety. **Chapter four** is the research article “The potential of purple and white fleshed potato tuber extract (Solanum tuberosum L.): Antioxidant, antimycobacterial and anti-cancer investigation” and have been submitted to African Journal of Pharmacy and Pharmacology. This research will provide information necessary as a reference in the pharmacology industry. **Chapter five** is the last chapter of the thesis and is a general discussion and conclusion of the entire study.

ABSTRACT

This research study was conducted to measure the impact of abiotic factors including various root zone temperatures on the phenology and physiology of four potato cultivars grown under controlled greenhouse conditions. Despite the known phenomena of promoted root growth in the presence of elevated temperature, little information is available on that of *Solanum tuberosum* L. particularly the low yielding cultivars. Further studies were conducted on these cultivars to explore the contributing factors to their health beneficial effects. *In vitro* cultured plantlets were acclimatized and after growing for 48 days in individual planting bags, no heat and warm root zone temperatures (20°C, 24°C, 28°C) were applied to potato plants cv. BP1, Salad blue, Pink Fir Apple and Highland Burgundy Red for a duration of 25 days. The plantlets received the same macro and micro elements as well as a prominent amount of water. Data was collected on a weekly basis. Further experiments were conducted to establish the effect of 100%, 50% and 25% water and nutrient in combination with the root zone temperature (RZT) 24°C. Ethanol extracts of the potato tubers were assessed for their total polyphenolic, flavanol and flavonol content as well as Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), 1-diphenyl-2-picrylhyddrazyl (DPPH) scavenging ability, ferric reducing antioxidant power (FRAP) and L-Ascorbic acid assays. High Performance Liquid Chromatography (HPLC) was performed for the individual polyphenols. All increased root zone temperatures had a positive effect on tuberization and enlargement compared to the control. A significant increase in tuber weight was observed when exposed to root zone temperature of 24°C. Cultivar Salad blue had the highest content L-ascorbic acid when exposed to 20°C root zone temperature. No flavanols were detected. The two

main peaks with the HPLC method resulted in chlorogenic acid and caffeic acid, present in all samples but significant in cultivar Highland Burgundy Red. The outcome of this study demonstrated that judicious use of root zone temperature can effectively encourage tuberization. Furthermore, it provides useful information on the levels of L-ascorbic acid and its potential health promoting phytochemicals in the selection of potato varieties. To date, pigmented potatoes are not regarded as a high value crop on the South African market, however its yield prospects as well as health-promoting benefits could have a positive impact on the South African Gross Domestic Product (GDP) and in the health of the population. Potato cultivar (cv.) Salad blue (SB) seem 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 oxidative response and vitamin C presence. The results of this study further indicated that there is no activity against *M. smegmatis* at the highest test concentration of 1000 μg/ml. Antiproliferative activity against liver hepatocellular carcinoma (HepG2) cells for their and was found to have IC50 values ranging from between 267.7 ± 36.17 μg/mL and > 400 μg/mL after 72 h of incubation suggesting that it is non-cytotoxic to the specific cell line tested. The present study provides useful information for farmers and health professionals in respect of increased yield and health-promoting benefits of a single potato variety.

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BIOGRAPHICAL SKETCH

Hildegard Witbooi was born in Paarl, South Africa, on the 22nd May 1990. She attended William Lloyd Primary School and matriculated at Klein Nederburg Secondary School in 2007. She enrolled at the Cape Peninsula University of Technology in 2008 and obtained the ND Horticulture in 2010, Btech Horticulture in 2011 and Mtech Horticulture in 2013. She has been employed at Springfontein Wine Estate for the past 9 years as Chief of Agriculture.

DEDICATION

This thesis is dedicated to my:

Parents	Hendriena Witbooi
	& Willem Witbooi
Husband	Tariro Masayiti

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GLOSSARY

Abbreviation	Definition
AA	Ascorbic acid
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
ABTS	2,2’-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid
ANOVA	Analysis of variance
NH4	Ammonium
B	Boron
Ca	Calcium
CE	Catechin standard equivalents
CaN	Calcium Nitrate
Cu	Copper
Cv	Cultivar
°C	Degrees Celsius
DMACA	p-dimethylaminocinnamaldehyde
DPPH	1-diphenyl-2-picrylhydrazyl
DW	dry weight

Fe	iron
FW	fresh weight
FRAP	Ferric reducing power
g	Gram
GAE	Gallic acid equivalents
GDP	Gross Domestic Product
HBR	Highland Burgundy Red
HPLC	High performance liquid chromatography
IC_{50}	Inhibitory concentration
In vitro	“In glass”
Kg	Kilo gram
L	Litre
LSD	Least significant difference
MeOH	Methanol
Mg	Magnesium
Ml	Millilitre
mm	Millimeter
mM	Millimolar
Mn	Manganese
Mo	Molybdenum

MPA	Metaphosphoric
N	Nitrogen
Nm	Nano meter
NO3	Nitrate
Na	Natrium
ORAC	Oxygen radical absorbance capacity
P	Phosphorous
PFA	Pink Fir Apple
PPF	Photosynthetic Photon Flush
K	Potassium
QE	Quercetin standard equivalents
ROS	Reactive oxygen species
S	Sulphur
SB	Salad blue
TA	Total antioxidant
TEAC	Trolox equivalence antioxidant capacity
TP	Total phenolics
TPTZ	tripyridyl triazine
UV-B	Ultra Violet
μ	micro

WUE	water use efficiency
Zn	Zinc

CHAPTER ONE

LITERATURE REVIEW AND INTRODUCTION

Review Paper

Horticultural and nutritional description of rare pigmented potatoes- an alternative health crop for South Africa: A review

Hildegard Witbooi [1*], Learnmore Kambizi [1], Oluwafemi Oguntibeju [2]

[1]Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa.

[2]Phytomedicine & Phytochemistry Group, Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.

Keywords: polyphenols, superfood, phytonutrients, anthocyanins, pigmented potatoes, nutrient dense food, plant-based diet, potato tubers.

1. Abstract

This review article illustrates the key factors of phenolic compounds and their sources from potatoes, its cultivation aspects, possible beneficial uses as well as potential health benefits. There is currently a great interest in phytochemicals as bioactive compounds of food. Potatoes of bright pigmented color contains polyphenols that represent a group of chemical substances commonly found in plants. Due to the lack of horticultural information on pigmented potato farming in South Africa, it is necessary to effectively contribute information including its potential health benefits described. Polyphenols have been associated with health benefits and are the most abundant antioxidants in human diets and the best studied class of polyphenols is flavonoids which include several thousand compounds. Phenolic acids, tannins and flavonoids (anthocyanins) are the three major classification groups in which naturally occurring polyphenols are found. Potatoes are known as a good dietary source for health promotion through energy, fiber, carbohydrates, vitamins, niacin and minerals such as potassium, phosphorous, magnesium and ascorbic acid. Epidemiological studies correlate flavonoid intake with a reduced incidence of chronic diseases such as, reduction of the risk of coronary heart disease, prevention of some types of cancer, inhibition of cholesterol and the retardation of macular degeneration among others. Acquiring a growing protocol that would yield substantial crop may be a challenge for South African farmers. However, the benefits of its nutritional value confirm its significant importance.

2. Introduction

The potato (*Solanum tuberosum* L.) can be considered as one of the most important food crops in most African countries. In addition to being the largest vegetative propagated crop worldwide, the potato tuber has become an important staple in parts of the world where there is a limited but increasing purchasing power, an increasing pressure on scarce land and an increasing demand for food (Struik and Wiersema, 1999). With the increased popularity of heirloom vegetables and its higher source of polyphenols, the lack of pigmented potatoes in South Africa became more apparent. Local potato farmers in South Africa do not want to grow these pigmented potatoes due to its low yielding capacity as well as susceptibility to diseases during seed multiplication. Epidemiological studies have shown a correlation between ingesting phenolic compounds and improved health (Boker, *et al*. 2002. Dragsted, *et al*. 1997. Hertog, *et al*. 1996. Knekt, *et al.* 2002). Phenolic compounds have shown in many studies to have antioxidant activity and other characteristics that could promote health. Among phenolics, of special interest are anthocyanins. This is important not only because of its health benefits but its possible utilization as natural food pigments. The worldwide trend for health and health promoting crops known as superfoods is growing exponentially. Superfoods are nutrient dense food that are mainly plant based.

The production of seed tubers is mostly vegetative and based on the use of micro tubers or *in vitro* plantlets (Ranalli 1997). The rate at which potatoes multiply is very low if compared to other crops. For this reason, a large portion of crop production area is devoted to the production of seed tubers and it does take a considerable amount of time to build up seed tubers for its commercial growth. With the increase in field

generation, there is also an increase in the amount of pathogens present and this leads to the degeneration of seeds. Agronomists are faced with the high-water requirements as well as the low fertilizer use efficiencies of this crop (Struik *et al*. 2006) while other studies present the high use efficiency of other resources such as energy and land (Linnemann *et al*. 1999). Due to the ever-increasing role that the potato tuber has as a major food crop of the world and the enormous potential that is prevalent for its exploitation, new techniques must be promoted in seed production. For this reason, it is important to investigate the methods of increasing the production of minitubers (G0) from disease free *in vitro* plantlets.

As the fifth largest produced crop in the world after sugar cane (*Saccharum officinarum*), maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*), the popularity of potatoes is still growing, both in terms of areas cultivated and its total production (Douches *et al.* 1996). The potato (*Solanum tuberosum* L.) can be considered as one of the most important food crops in most African countries. Phenolic compounds were shown to have antioxidant activity and other characteristics that could promote health. Among phenolics, of special interest are anthocyanins. This is important not only because of its health benefits but its possible utilization as natural food pigments. The worldwide trend for health and health promoting crops known as superfoods is growing exponentially.

3. The potato as a horticultural crop

3.1 Botanical description

The potato is an annual herbaceous crop which originated in the Andes from areas of high altitude and tropical conditions and it generally grows up to 100cm tall. The potato

is a tuber bearing food crop and belongs to the genus *Solanum* and the family *Solanaecae.* This family is divided into two cultivar groups: Andigenum, adapted to short day conditions and Chilotanum which is adapted to long day conditions (FAO 2008). This genus *Solanum* also hosts two other economically important food crops namely the tomato (*Solanum lycopersicum*) and the eggplant (*Solanum melongena*). A productive potato plant may have two or more main stems with its leaves. The tuber of the plant is an enlarged portion of an underground stolon or stem. Tuber eyes are buds from which the next season's growth will arise. The apical end of the tuber develops the most eyes, with only a few found near the stolon or the basal end. The number of eyes and its distribution are characteristic of the potato variety. The tuber skin is composed of two cell layers. The external layer of single cells, called the epidermis is underlain by several layers of corky cells called the periderm. The cells in the periderm layer may contain a pigment that produces potatoes with colour. Below the periderm is the cortex, followed by the vascular ring, which contains the cells that transport food products to the tuber from the above ground stems. The core to the vascular ring is the medulla. The medulla represents the main storage area for the potato tuber. Excess food produced by the potato plant is transported to the medulla via the vascular tissue. The size of the tuber increases as the cells in the medulla expands with the uptake of food.

Potatoes are an upright or sprawling herb with an approximate height of 30-100 cm. Branched and robustly angular, winged stem glabrous or sporadically pubescent with simple and glandular hairs. The underground stolons may be white, brown, pink, red, purple, or purplish blue in color. The flesh can be various shapes ranging from oblate, elliptic or globose and can range in size with axillary buds and numerous lenticels.

The interruptedly odd-pinnate leaves alternate with 6-8 pairs of leaflets and unequal smaller interstitial leaflets. The petioles are 2.5- 5cm long and the leaflet blade 2 -10cm by 1- 6cm. Inflorescence emerging terminal, with the leafs opposed or axillary many-flowered with sparingly branched panicles. Pedicels are 1-2 cm and expressive near the middle of the stolon. Its calyx campanulate with 5 lanceolate lobes which are sparely pubescent accompanied by the corolla which may be pink, white or purple blue, sometimes all on one plant, 2.5-3 cm in diameter, with 5 deltate lobes. Filaments are thick with five free, erect yellow anthers of 5-6mm. Ovary glabrous. Style 8 mm with capitate stigma, yellowish green or berry green, often striped, smooth, globose and 1.5-2 cm in diameter. Approximately 300 seeds are found in one potato berry. Seeds are flat, small, suborbicular to ovate and embedded in yellowish brown mucilaginous pulp. Ranalli (1997) reported that seed potato production is mostly vegetative and based on the use of micro tubers or *in vitro* grown plant material. A large number of crop area is devoted to the production of seed tubers, mainly because the multiplication rate of potatoes is extremely low compared to other crops. When commercial seed ought to be produced, it requires a lot of time for sufficient material to be built up. Every generation of field grown potatoes and continuous multiplication results in the increase of pathogen transfer eventually leading to seed degeneration. It is imperative to investigate the best method to develop these rare potato types as minitubers (G0) from disease free *in vitro* raised plant material.

3.2 Potato Cultivars

Potatoes (*Solanum tuberosum L.*) are found in various colours, including the skin and flesh including white, yellow, purple and red. This is mainly due to the presence of anthocyanins and other pigments. Wild potatoes are known to have originated from

the Andes, South America. These potatoes were cultivated at an altitude between 2000 and 4000 m above sea level in a particular region which is known by its high light intensity cool temperatures, short day lengths and relatively high humidity (Levy and Veilleux 2007). Solar radiation during the developmental growth stages affects nutrient availability as well as the ability of the plant to take up and utilize the nutrients, which directly influences final yields (Arkin and Taylor 1981). Fageria 1992, stated that light intensity affects photosynthesis, which has a direct effect on the plants demand for nutrient. Levy and Veilleux (2007) found in their trial that at low light intensity in winter, maximum tuber weight was obtained between 12 and 14°C, and in the contrary with high light intensity in summer, maximum tuber weight was obtained at 18 to 20°C.

PSA, 2019 reported that the main cultivars that are being grown in South Africa are Mondial at 47%, Sifra at 41% and Lanorma third at 4%. According to Farmers Weekly (2015), the cultivar Mondial is popular due to its excellent scab resistance and high yield. There are currently no known pigmented cultivars grown commercially in South Africa.

Table 1 Main cultivars planted in South Africa during 2017 (PSA, 2020)

			Main cultivars planted in 2017			Total
Region	Crop		1	2	3	ha
Sandveld	Summer	Table/Process	Sifra, 27%	Mondial, 25%	FL2108, 7%	2 495
		Seed				
	Winter	Table/Process	Mondial, 39%	Sifra, 26%	FL2108, 7%	3 797
		Seed	Markies, 29%	Avalanche, 20%	Valor, 19%	319
Ceres		Table/Process	Sifra, 21%	FL2108, 17%	Fianna, 13%	1 011
		Seed	Avalanche, 78%	Valor, 12%	VDP, 8%	65
South Western Cape		Table/Process				
Northern Cape		Table/Process	Sifra, 51%	Mondial, 24%	Lanorma, 10%	468
		Seed	Lanorma, 73%	FL2108, 9%	UTD, 6%	1 764
Eastern Cape	Summer	Table/Process				
	Winter	Table/Process	Mondial, 72%	Sifra, 24%	Almera, 1%	696
Southern Cape	Summer	Table/Process				
		Seed				
	Winter	Table/Process				
		Seed				
North Eastern Cape	Summer	Table/Process	Mondial, 31%	Sifra, 17%	Lanorma, 15%	1 188
		Seed	Mondial, 60%	Sifra, 37%	Fabula, 4%	520
Western Free State		Table/Process	Mondial, 59%	Sifra, 16%	UTD, 14%	2 347
		Seed	Mondial, 42%	Sifra, 36%	Markies, 4%	4 912
South Western Free State	Early	Table/Process	Sifra, 75%	Mondial, 15%	Lanorma, 6%	1 124
	Late	Table/Process	Mondial, 45	Sifra, 15%	Lanorma, 13%	135
Eastern Free State		Table/Process	Mondial, 61%	Lanorma, 9%	Sifra, 8%	10 313
		Seed	Mondial, 41%	Markies, 26%	Lanorma, 17%	357
KwaZulu-Natal	Winter	Table/Process	Mondial, 58%	Valor, 30%	Sifra, 9%	916
		Seed	FL2108, 100%			65
	Summer	Table/Process	Valor, 24%	Mondial, 21%	UTD, 9%	844
		Seed	Mondial, 29%	FL2108, 20%	Valor, 15%	1 172
Mpumalanga		Table/Process	Mondial, 48%	Lanorma, 13%	FL2108, 11%	1 109
		Seed	Mondial, 40%	Valor, 25%	BP1, 7%	437
Loskop Valley		Table/Process	FL2108, 38%	Hertha, 20%	UTD, 12%	1 067
		Seed	FL2108, 100%			30
Limpopo	Main	Table/Process	Mondial, 58%	Sifra, 13%	Valor, 10%	7 565
		Seed	Mondial, 61%	Sifra, 26%	Valor, 6%	696
	Early	Table/Process	Mondial, 41%	Sifra, 27%	Valor, 10%%	2 832
		Seed	BP1, 75%	Markies, 14%	Lanorma, 11%	36
North West	Late	Table/Process	Sifra, 64%	Mondial, 25%	Almera, 6%	135
		Seed	Mondial, 31%	Lanorma, 22%	Sifra, 18%	548
	Early	Table/Process	Sifra, 46%	P/Dell, 24%	Innovator, 11%	1 577
		Seed	Lanorma, 54%	Almera, 18%	Markies, 7%	84
Gauteng	Early	Table/Process	Mondial, 38%	Sifra, 32%	Innovator, 13%	1 098
Total						51 722

3.2.1 Pink Fir Apple

According to the European Cultivated Potato Database, Pink Fir Apple dates back to 1850 from France, and was first imported to UK. The plants are strong and grows up to 1,2m high. Its flower colours are blue-violet, mauve taupe as well as light pastel purple. When it reached full maturity, the single flowers with normally 5 petals are 2,5cm in diameter. The knobby shaped tubers are hard to peel, but can be enjoyed with the peel on. This potato is known for its creaminess in texture and is popular for boiling, baking and frying.

Figure 1 Potato cultivar Pink Fir Apple

3.2.2 Salad blue

This potato dates back to the 1900 in Scotland. David *et al.* (2016) indicated that potato cultivar salad blue prefers acid soil (districambosoil) and areas that are colder and placed at a higher altitude compared to ordinary potato varieties. The consumer properties on the eating quality is described to be a good, firm cooking potato type. It is known for its floury consistency, especially if harvested old. It is enjoyed boiled, cold in salads. It can also be cooked in stews or fried.

Figure 2 Potato cultivar Salad blue

3.2.3 Highland Burgundy Red

This main crop variety produces moderate to low yields with high numbers of tubers per plant. Red skin and red flesh with a white ring under the skin. The consumer properties on the eating quality are described to be moderate to high dry matter, suitable for fresh market and crisping.

Figure 3 Potato cultivar Highland Burgundy Red

3.2.4 BP1

This cultivar was selected as it is actively grown and adapted to Western Cape as well as readily available on the market. The cultivar BP1 is a medium maturing cultivar which grows 90 to 110 days before maturing with a short dormancy period of approximately 50 to 90 days (Kempen, 2007). The plants are medium to tall and the stems erect. This cultivar develops rapidly and foliage good and excessive. When in flower is produces many blue flowers. The tubers are oval and very smooth with shallow eyes. The yielding potential is high. The skin and the flesh of the tubers are white. BP1 is sensitive to Metribuzin. Its needs lower levels on nitrogen. The consumer properties on the eating quality are described to be a good, firm cooking potato type. It is a good all-rounder and is preferred by many cooks. The processing quality is poor,

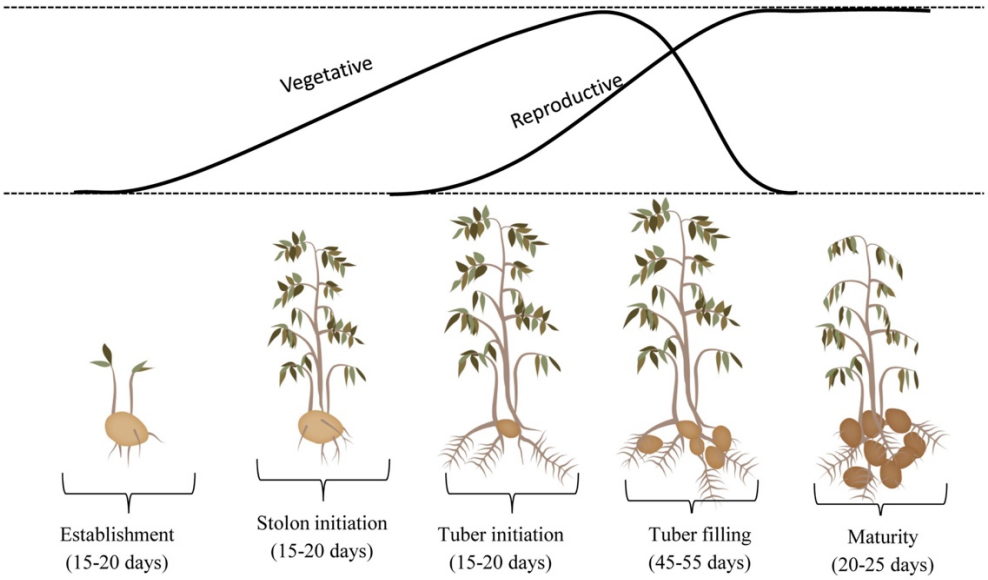
due to the poor dry matter. It is susceptible to both late and early blight, but it can be controlled by a spray programme. It is moderately susceptible to potato leaf roll virus as well as PVY, but found to be very susceptible to common scab, but immune to wart disease.

Figure 4 Potato cultivar BP1

3.3 Growth Requirements of the potato

Quality and growth of potatoes are influenced by environmental factors such as soil type, nutrient, light, temperature and moisture. Several factors that influence potato growth are largely uncontrollable such as the air and soil temperatures, length of growing season, light intensity and its duration, humidity and wind. Other controlling factors for the grower includes: the potato type, size of mother seed tubers, seed-piece type, seed-piece cutting, cut-seed size, planter operation, plant stand, stem population, moisture, nutrition, pest management, planting date and harvest date. Only when latter are at optimum levels can the best quality and most profitable yields be attained. The potato is a cool season crop that only grows in specific areas. It prefers day temperatures between 20- 25 °C and night temperatures below 20 °C. The development rate of sprouts depends on soil temperature. Sprout elongation is maximized at 18°C, elongation is slow at 9°C and very little elongation occurs at 6 °C.

For initiating tubers, the optimum soil temperature is 16-19 °C. Haulm growth needs higher temperature of about 27 °C (Bodlaender *et al.* 1962). The rate at which respiration and photosynthesis takes place is affected by temperature. According to Winkler (1971), respiration rates of the potato plant leaves in the dark roughly doubled for every 10 °C increase in temperature. High temperatures are inhibitory for tuberization in both long and short photoperiods. According to Ewing and Struik (1992), it affects the partitioning of assimilates by decreasing the partitioning to the tubers and increasing the partitioning to other parts of the plant. When the soil temperature rises above 20 °C, the tuber development declines and practically stops at 30 °C. Levy and Veilleux (2007), stated that low temperature and short-day lengths, especially at night, enhances tuber initiation, increase the number of tubers formed, allow longer periods of photosynthesis, enhance efficient translocation of assimilates from haulm to its tubers and lowers respiration rates during the cool nights.



Impact of water deficit	Delayed emergence Restrict root establishment Restrict plant development Presence of fewer stalks/stem	Restrict plant development Limited stolon initiation Limited number of stolon Restrict root growth Restricts uptake and response to nutrients	Limited foliage and plant development Limited tuber initiation Limited number of tubers	Limited plant and tuber development Restricts tuber size Promotes distorted tuber shape Faster senescence	Limited relative tuber density Limited tuber size

Figure 5 Impact of water deficit during the potato growth cycle (Obidiegwu *et al.* 2015)

In the tropics, it is usually grown in the highlands above 800 m where the temperatures are cooler. In PNG, they are grown in altituded between 1500 m and 2200 m and the high light intensities are required for optimum dry matter production. The tubers are susceptible to frost and freezing. Potato requires a well distributed rainfall of 500–750 mm in a growing period of 3– 4.5 months. Potato grows on a wide range of soils; however waterlogged soils are not suitable. It grows best in loose, friable soil with well-drained mineral or organic soils with medium loam or light or medium silty textures. Deep soils with good aeration and permeability give good growth and high tuber yields. Potato tolerates a wide range of pH from 4.8–7.

4. DEVELOPMENTAL STAGES OF POTATO GROWTH

The growth of a potato plant has been classified into four distinct growth phases, namely: sprout development, tuber initiation, tuber bulking and lastly tuber maturation (Struik and Wietsema 1999). Each of these growth phases are relying on external environmental and management factors. Furthermore, van Loon 1981 reported categories into early, mid and late maturing which is measured according to physiological criteria. Moreover, this classification is universal and can be used to compare different growing areas and different growing seasons.

1.5.1 Sprout Development and Plant Establishment

This development starts from the eyes and ends at emergence from the soil. The energy source for growth during this stage is purely from the seed piece. According to Oosterhaven (1995), potato growers in the Andean region have traditionally been using natural occurring compounds to control sprout growth. Physiological age of the seed tuber, nitrogen and water supply, air temperature and inter and intra plant competition all have a strong effect on the sprout development of the potato plant.

1.5.2 Vegetative Growth

This stage refers to the vegetative development of the potato leaves, branches, stolons and roots. This stage begins at emergence and lasts until tubers start to develop. The two initial stages of growth last from 30- 70 days depending on the planting date, physiological age of the seed tubers, soil temperature and the characteristics of the cultivar that is planted.

1.5.3 Tuber Set/ Initiation

Tubers are initiated at the stolon tips. This stage lasts approximately two weeks without any enlarging during this stage. Under Dutch conditions, stolon formation occurred by 29 DAP (days after planting) and stolon tip swelling started from 29 to 36 DAP for seven cultivars of different maturity Celis-Gamboa *et al*., 2003.

1.5.4 Tuber Bulking

This growth stage has the largest duration. The cells of the tubers expand with the accumulation of nutrients, carbohydrates and water. Tantowijoyo (2006) stated that the plant itself has stopped growing at this stage and only the tubers grow larger. Planting date and cultivar dependent, the bulking stage may last up to three months. Cells in the tuber can increase up to 18 times their normal size due to the accumulation of starch and water (Steyn 1999).

1.5.5 Maturation

Photosynthesis gradually decreases, vines turn yellow and lose their leaves and as the growth response slows the vine eventually dies. According to Tantowijoyo (2006), the skins of the tuber will gradually harden due to their increased starch content and it is a cultivar dependent cycle.

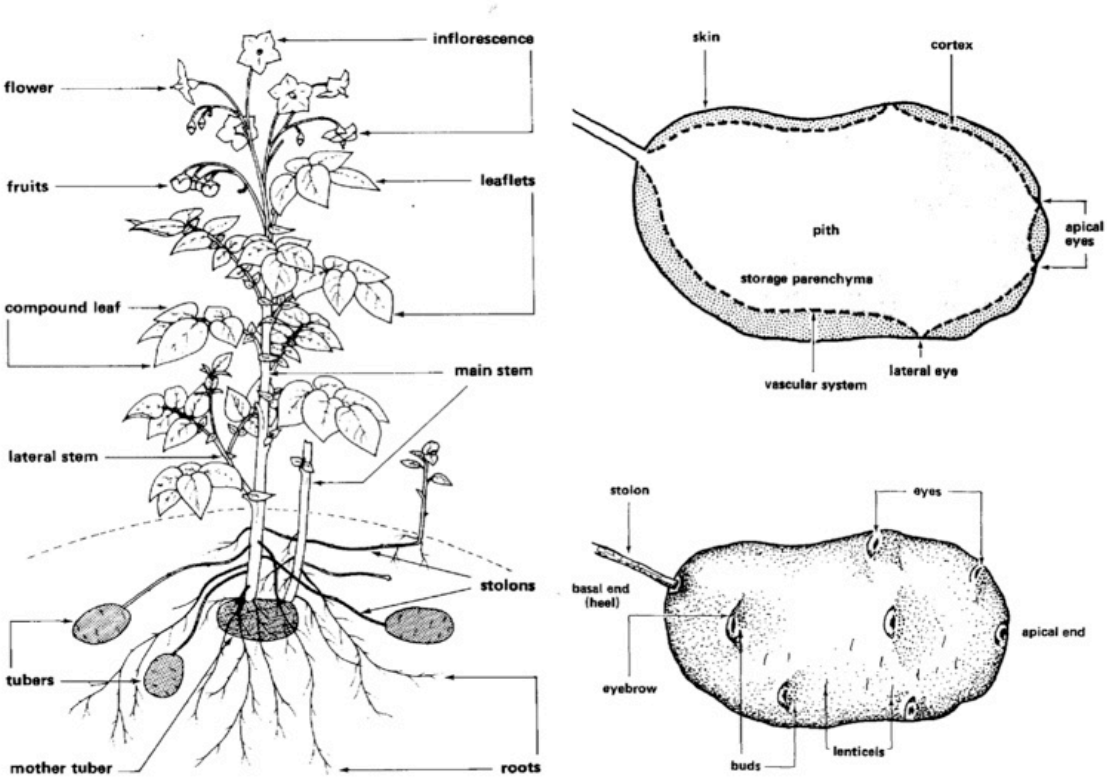


Figure 6 Morphology of the potato plant and potato tubers. An original from Huaman, 1986.

1.6.1 Nutritional Benefits

Potatoes are wholesome and nutritious and is an important food source to the diet. They contain approximately 78% water, 22% dry matter and less than 1% fat. About 82% of dry matter is carbohydrate, mainly starch, with minimal amounts of simple sugars and some dietary fiber. Potatoes are a big source of vitamin C, vitamin B3, vitamin B6, carbohydrates, high quality proteins, thiamine, iron and folic acid and contain 12 essential vitamins and minerals such as phosphorous, potassium and magnesium (Andre *et al*. 2007). Besides these basic nutrients, potatoes also provide a good source of phenolic compounds. Epidemiological studies have shown a correlation between ingesting phenolic compounds and improved health (Boker et al., 2002. Dragsted et al., 1997. Hertog et al., 1996., Knekt et al., 2002.)

1.6 Phenolic compounds

Phenolic compounds also known as polyphenols, constitute one of the most abundant and widely distributed group of substances in the plant kingdom (Harbone and Williams, 2000). Santos-Buelga and Sacalbert (2000) stated that phenolic compounds differ structurally from simple molecules such as phenolic acids, and from highly polymerized compounds such as proanthocynanidins (tannins), which occur in plants and are most common in many foods (vegetables, fruit and cereal grains) and beverages (teas, wine and beer). The most common phenolics in human diet are phenolic acids, flavonoids and tannins (King and Young, 1999). Food normally comprises of complex polyphenols which are predominantly found in the outer layers of the plants (Pandey and Rizvi, 2009). Phenolic compounds have at least one aromatic ring with one or more hydroxyl groups and may be classified as flavonoids and non-flavonoids (Del Rio *et al*. 2012). During the last years, the interest in polyphenols and other molecules with antioxidant activity has increased among agricultural and food scientists, food industry professionals, consumers and nutritionists (Harbone and Williams, 2000). Polyphenols provide health benefits by several mechanisms, including the elimination of free radicals, the protection and regeneration of other dietary antioxidants (e.g., vitamin E) and the chelation of pro-oxidant metals. According to André *et al*. 2009, antioxidative phenolic compounds are secondary metabolites found in potatoes that were shown to be health promoting phytochemicals with many beneficial antioxidant, anticancerogenic and anticholesterol properties. A wide variation both in individual and total phenolic content and antioxidant activity of commercial potatoes and varieties was reported (Rumbaboa *et al*. 2009). Potatoes is worldwide one of the most important foods of the human diet

after wheat and rice (Woolfe and Poats 1987). In this review, the significantly different phenolic compound availability in pigmented potatoes compared to commercially available white fleshed potatoes is presented. Moreover, some studies that confront the health benefits of consuming pigmented skin and flesh potatoes are mentioned.

1. Role of Phenolic Compounds in Plants and Humans

Within the living plant, phenolic compounds protect against pathogens, UV radiation, harsh climatic conditions and oxidative stress (Pandey and Rizvi, 2009). In the human body, phenolic compounds are antioxidants and have diverse biological properties (Fig 2) such as anti-cancer (Odongo *et al.* 2017 and Luo *et al.* 2017), anti-diabetic (Omodanisi *et al*. 2017 and Venkata *et al.* 2017), anti-inflammatory (Franceschelli *et al*. 2017 and Sajid *et al.* 2017), osteoprotective (Leotoing *et al*. 2016 and An *et al.* 2016), cardioprotective (Rodriguez *et al*. 2017), neuroprotective (Ben Mansour *et al.* 2017 and Sarrias *et al.* 2017), anti-asthmatic (Shaw *et al.* 2016), antihypertensive (Gomez *et al.* 2016), antiaging (Nobile *et al.* 2016), antiseptic (Le Sage *et al.* 2017), cholesterol lowering (Tenore *et al*. 2017), cerebrovascular protection (Forte *et al.* 2016), antifungal (Ayub *et al.* 2017), hepatoprotective (Jia *et al*. 2017), antibacterial (Miyamoto *et al*. 2017) and antiviral properties (Alam *et al*. 2017).

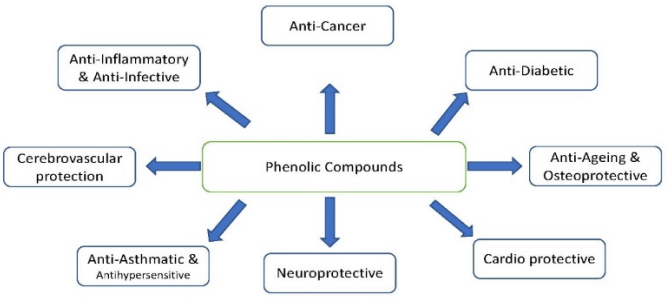


Figure 7 Role of Phenolic Compounds in Humans

2. **Abiotic Environmental Factors affecting development of Phenolic Compounds in Pigmented Potatoes**

Most polyphenols, in particular phenolic acids, have a direct implication in the response of plants to various types of stresses, such as temperature, biotic stress and injuries, as well as ultraviolet ray and ozone exposure Ngadze *et al*. (2014). These phytochemicals show antimicrobial properties through an increase in concentration after pathogen incidence and this contributes to healing by lignification of damaged zones Wang *et al*. (2015).

1.6.3.1 Chlorogenic acid and Caffeic acid

Chlorogenic acid (CA) is a phenolic compound found widely in fruit and vegetables and is the major compounds found in potatoes. It has been reported that there is insufficient knowledge in the literature about the contents of chlorogenic acid in potatoes and the factors that influence such content (Orsák *et al.* 2019). A wide range of potential health benefits of CA have been reported, including anti-inflammatory, anti-

obesity, anti-diabetic, anti-carcinogenic and may provide a non-invasive and non-pharmacological approach for treatment of some chronic diseases. Greenberg *et al*. (2006) reported that the intake of CA reduces food cravings, induces body fat loss by thermogenesis, and reduces daily calorie intake. Caffeic acid (3,4-dihydroxycinnamic) is one of the hydroxycinnamate and phenylpropanoid metabolites that is more widely distributed in plant tissues.

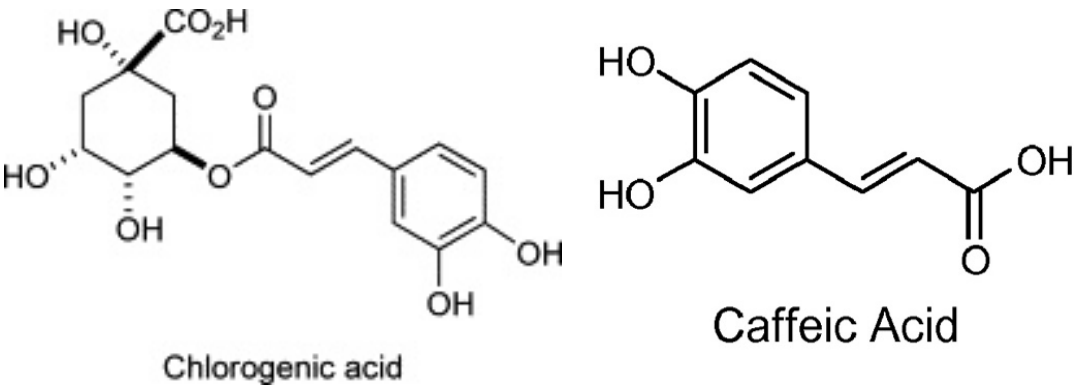


Figure 8 Chemical structure of chlorogenic acid and caffeic acid

1.6.2 Ascorbic acid

Vitamin C, i.e., ascorbic acid is a critically important nutrient in the human diet. The discovery of the vitamin was as a result of efforts to understand and to cure scurvy, a disease tightly linked to vitamin C deficiency. Vegetables and fruit are the major contributors to vitamin C in the human diet. In the countries where potatoes are produced and consumed, it is the most important source of vitamin C. Mosure (2004), cited the Dietary Guidelines Advisory Committee; among vegetables and fruits, the concentration of vitamin C in potatoes is moderate to low.

1.7 Potato Market in South Africa

Potatoes are undoubtedly an important vegetable crop in South Africa. In 2011, the potato industry contributed approximately 61% to the total gross value of vegetable production, 13% of horticultural products and 3% of total agricultural products. The potato processing industry has grown at a rapid rate over the last decade. According to Potato South Africa (PSA), the processing industry represented 17% of the total potato crop during 2011. A rapid increase of the potato processing industry may be attributed to consumer need for ready to eat, convenience foods. The primary domestic uses for potatoes are for crisps, French fries and frozen products.

Table 2 Commercial farming units in South Africa (PSA, 2020)

Number of commercial potato producers (farming units)

Regions	1993	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Limpopo	245	87	85	92	91	88	82	93	97	99	108
Sandveld	222	116	89	104	99	87	80	84	83	82	69
Eastern Free State	206	77	69	72	76	79	77	75	76	76	80
KwaZulu-Natal	226	86	83	78	72	57	72	63	67	48	56
Eastern Cape	105	38	35	46	40	39	35	38	28	21	19
Western Free State	333	59	52	50	61	52	45	43	48	40	40
Mpumalanga	143	45	38	45	48	39	31	29	29	32	26
South Western Free State	see Western FS	17	25	35	40	33	26	27	31	33	28
North West	117	19	22	45	30	29	25	21	21	21	20
North Eastern Cape	42	25	17	21	20	18	20	18	16	21	24
Northern Cape	121	26	29	22	25	23	19	17	17	16	21
Gauteng	85	15	20	19	25	23	18	17	15	19	20
Loskop Valley	see Mpumalanga	16	13	21	15	17	17	14	16	16	11
Ceres	47	21	16	17	13	12	11	13	12	7	10
South Western Cape	96	28	29	16	10	5	5	5	4	5	0
Southern Cape	43	6	7	7	14	8	6	4	6	4	0
	2 031	681	629	690	679	609	569	561	566	540	532

1.8 Aim of research

There is an increasing demand for food sources that are high in polyphenolic content as well as vitamins. Although the health benefits of potatoes have been demonstrated especially in pigmented skin and fleshed tubers, South African farmers have not explored its cultivation requirements under the diverse South African conditions.

Therefore, this study was an initiative to unravel the status quo of non-cultivation in South Africa of pigmented cultivars. Furthermore, to promote a new food niche to the market that supports health consciousness. The following studies were therefore undertaken:

1.7.1 Despite the well-known phenomena of promoted root growth in the presence of elevated temperature, little information is available on that of potatoes, particularly the low yielding pigmented cultivars. It is therefore hypothesized that increased root zone temperatures will have a positive impact on the phenology and physiology of four potato cultivars grown under controlled greenhouse conditions. Evaluating the effect of root zone temperatures (control, 20°C, 24°C, 28°C) on *S. tuberosum* cv. Pink Fir Apple, Salad blue, Highland Burgundy Red and the non-pigmented control BP1 on the growth, leaf macro- and micro-nutrients of seed mini tubers.

1.7.2 It is said that the tuberization stage of potatoes is a distinctive process controlled by various factors such as genotype, as well as external factors nitrogen supply, pH, water and stress. Therefore, it is hypothesized that controlled amounts of water and nutrient applied during critical stages of the potato plant cycle will provide favored results. Furthermore, the availability of water and nutrient in combination with elevated root zone temperature was tested. Evaluating the effects of 100%, 50% and 25% water and nutrient application on the growth cv. BP1 and Salad blue when exposed to root zone temperature 24°C.

1.7.3 With the increase in market demand for food sources that are high in polyphenolic content and vitamins, potatoes are a highly sought-after health-conscious food crop. It is hypothesized that pigmented potatoes cultivated in stress conditions will produce higher yielding polyphenolic content, antioxidant capacity, vitamins and therefore elicit anti-cancer activity. Analyzing plant and tuber (skins and flesh) extract to determine their total polyphenolic, flavanol and flavonol content as well as Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), 1-diphenyl-2-picrylhyddrazyl (DPPH) scavenging ability, ferric reducing antioxidant power (FRAP) and L-Ascorbic acid assays. High Performance Liquid Chromatography (HPLC) will be performed for the individual polyphenols. Vitamin C was also assessed.

Conclusion

The results of studies outlined in this review paper confirm and provide a current understanding on the overall phenolic compound availability in pigmented potatoes and its remarkably higher occurrence than that of commercially available white fleshed potatoes. Furthermore, its relevance to human health provides the opportunity of polyphenol rich diets which may provide significant protection against the development and progression of many chronic pathological conditions.

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CHAPTER TWO

THE ROLE OF ROOT TEMPERATURE ON THE PHYSIOLOGICAL AND PHYTOCHEMICAL COMPOSITIONS OF SOME PIGMENTED POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS

Full Length Research Paper

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

Hildegard Witbooi[1], Learnmore Kambizi[1], Oluwafemi Oguntibeju[2], Callistus Bvenura[1]

[1]Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa.

[2]Phytomedicine & Phytochemistry Group, Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.

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 (BP1, Salad Blue (SB), Pink Fir Apple (PFA), Highland Burgundy Red (HBR)). The results indicate that RZT 24^{o}C significantly increased plant height and tuber weight. RZT 28^{o}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. Subjecting cultivar BP1 and PFA to the control increased caffeic acid in cv. SB (916.75 μg/ g) and HBR (1380.74 μg/g). The control increased chlorogenic acid in the order: BP1 (17.06 μg/g) > PFA (247.94 μg/ g) > SB (338.23 μg/g) > HBR (426.20 μg/g. DPPH activity was highest in cv. SB (26.43 µmol TE/g) under control temperature conditions. These results indicate a variable response of various parameters to different RZTs. The RZT recommendations would therefore be based on specific needs. Furthermore, the secondary metabolites reported in the pigmented cultivars and their associated potential health benefits offer a substantial basis for their inclusion in the diet; regardless of their low yielding capacity.

Keywords: Antioxidant capacity, caffeic acid, chlorogenic acid, phytochemical content, vitamin C

Introduction

The potato (*Solanum tuberosum* L.) is a tuber bearing food crop that belongs to the genus *Solanum* and the family *Solanaecae.* 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. According to Wang (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. Potatoes are 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 (Riciputi *et al.* 2018; Friedman, 1997). Epidemiological studies have shown a positive correlation between ingesting phenolic compounds and improved health (Boker, *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 temperature on the cultivation of potato seed mini tubers of four cultivars in a greenhouse between July and September 2018. Furthermore, the

antioxidant potential in the ethanol aqueous extract was investigated as this could be a contributing factor to its health benefits. A literature search has shown that no scientific 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 finding the root zone temperature that would provide the best potential root zone temperature for potato cultivation and growth. Pigmented cultivars which are currently not grown by South African farmers were chosen, due to yield concerns.

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. Subculturing was done at 4-week intervals until enough material was produced for the experiments. Afterwards, the 6-week-old uniform 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 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 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 ¼ filled bags. Wood shavings were steam sterilized a week prior to transplanting in a steam sterilizer controller by a Delta DTD 4B4B 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.

2.2 Experimental materials and design

2.2.1 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 m kg^{-1}; B = 500 mg kg^{-1}. The nutrient solutions were prepared by adding 1kg of fertilizer to a 1000L reservoir filled with municipal tap water and adjusted to pH 5.8. 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.

2.2.2 Root zone temperature (RZT)

All treatments were simultaneously initiated under cool temperature (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.

2.2.3 Data collected

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 in order to collect relative environmental data. Minimum and maximum air temperature, RH, and the heated bed chamber temperature were recorded every hour, day and night.

2.3 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.

2.4 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.

2.5 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 at Bemlab. 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 ashed at 480 °C shaken up in a 50:50 HCl (50%) solution for extraction through filter paper (Campell & Plank, 1998; Miller, 1998). The Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Z) and Boron (B) content of the extracts were analysed using the ash method. Total Nitrogen (N) content of the leaves was determined through total combustion in a Leco N-analyser. The amounts of N, P, K, Ca and Mg were converted from percentage (%) to mg/kg by a factor of 10 000.

2.6 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 (200g) were extracted with 60% ethanol. After 2 hours, the extracts were filtered and used for the assays.

2.7 Determination of Total Polyphenol, Flavonol, and Flavanol 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.* (1998) and was determined spectrophotometrically using a microplate reader and expressed as mg gallic acid standard equivalents (GAE) per gram sample. The flavonol contents of the plant extracts were determined spectrophotometrically at 360 nm and expressed as mg quercetin standard equivalents (QE) per gram sample (Mazza *et al.* 1999). The flavanol contents of the aqueous plant extracts were determined colorimetrically at 640 nm using aldehyde DMACA and expressed as mg catechin standard equivalents (CE) per gram sample (Delcour *et al*. 1985 and Treutter *et al*. 1989). All determinations were done in triplicates.

2.8 Antioxidant Capacity

2.8.1 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 according to the method of Ou *et al.* (2001). All mentioned determinations were done in triplicates. The ORAC value was expressed in micromoles of trolox equivalents per gram of tissue (μmol TE/g).

2.8.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed using the method described by Benzie and Strain (1996). Ascorbic acid (AA) was used as the standard and the results were expressed as μmol AAE/g sample. All mentioned determinations were done in triplicates.

2.8.3 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). Free radical scavenging activity of the samples was expressed according to the equation below:

Percentage (%) inhibition of DPPH activity

$$= \frac{\boldsymbol{A^0 - A}}{\boldsymbol{A^0}} \times \mathbf{100}$$

(Krovánková *et al*., 2012)

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 mentioned determinations were done in triplicates.

2.9 Caffeic acid and chlorogenic acid

Caffeic and chlorogenic acid were determined on a Dionex HPLC technology (Dionex Softron, Germering, Germany) equipped 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 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. 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.

2.10 Vitamin C content

This extraction was performed according to the procedure of Brubacher, Muller-Mulot and Southgate (1985). The DHAA was calculated according to Sanchez-Mata *et al*. (2000) as the difference between the vitamin C (after reduction) and ascorbic acid (without reduction).

2.11 Statistical Analysis

Data were collected on fifty-two samples (thirteen plants per cultivar) per treatment. The morphological data were recorded every seven days for 25 days. Statistically significant differences among treatments means were determined by two-way analysis

of variance (ANOVA) at $p < 0.05$. Fisher's least significant difference (LSD) test was used to segregate means that were significantly different using a computer software program called STATISTICA.

3. Results and discussion

3.1 Plant Growth Parameters

The height response of different potato cultivars subjected to three RZT treatments was variable as shown in Table 2.1. An interesting trend was obvious. A RZT of 24^{o}C significantly ($p<0.05$) increased height in almost all the cultivars (except for 28^{o}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^{o}C significantly increased plant height and 20^{o}C significantly slowed down plant growth. Cultivar PFA responded best to 24^{o}C. The response of the potato cultivar leaf number to RZT was variable as shown in Table 2.2. The control significantly slowed down leaf production 48 DAT in all the cultivars except in the Blue fleshed CV. SB where 20^{o}C had this effect. The values ranged between 5 and 7 and 7-10 in BP1 and PFA, under RZT 24^{o}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^{o}C significantly improved leaf numbers in the same cultivars. However, 20^{o}C and 24^{o}C respectively decreased and increased the number of leaves in cv. BP1 and SB. At 63 DAT, 24^{o}C significantly lowered leaf number in all cultivars except in cv. HBR (28^{o}C). However, contrary to other treatments, the control significantly 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^{o}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^{o}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

(Nozolillo *et al.,* 1990 and Bharti *et al.*, 1997), 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 2.3). 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^{o}C. These cultivars reported the same value of 2.31 cm. as shown in Table 2c, RZT also significantly increased tuber weight in all the cultivars; however, 24^{o}C had the most significant impact on weight and in the commercial cv. BP1. More specifically, the values ranged between 0.43-35.55g, 0.3-30.3g, 0.27-19.17g and 0.15-11.92g in cv. BP1, SB, PFA and HBR respectively. Interestingly, the control and 24^{o}C respectively lowered and increased tuber weight in cv. BP1 and SB while 20^{o}C and 28^{o}C had a similar effect on cv. PFA and HBR.

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 °C 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 (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 reported that cool seasons are ideal for the production of tubers, 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.

3.3 Leaf Tissue Analysis

3.3.1 Effects of Root zone temperature (RZT) on Elemental Composition

The uptake of macro- and micronutrients on exposure to different RZT was variable as shown on Tables 3.1-3.4. 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^{o}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 – 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 – 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 to0.59 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 Ca, N, P and Na in comparison to other treatments while 20^{o}C significantly increased K and Cu. Also, 24^{o}C significantly increased Mn, Fe and Zn, while 28^{o}C increased Mg and B.

One would also expect warmer RZT to reduce micro-elements uptake. 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 and 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 NO_3-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 supraoptimal for potatoes (Sattelmacher *et al.* 1990b). Also, one expects that warmer RZT will reduce micro-elements uptake (Chang *et al.* 2006), decrease photosynthesis (Ewing 1981) and carbon net assimilation (Burton 1972) which cause haulm senescence as expressed by lighter green leaves. Farran 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 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 and 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 (Lahlou and Ledent, 2005).

3.4 Tuber Analysis

3.4.1 The effect of RZT on polyphenol and flavonol content

The effect of RZT on content of polyphenols was variable as shown in Table 4. All three pigmented cultivars showed higher polyphenol activity in all treatments compared to the non-pigmented cultivar BP1 that was subjected to the control

temperature. RZT 20^{o}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^{o}C RZT. Similarly, RZT 20^{o}C significantly ($p<0.05$) lowered flavonols (mg QE/g) in cv. BP1 (0.21), SB (0.43) and PFA (0.25) while 24^{o}C had a lowering effect on cv. HBR (0.33 mg QE/g). But, a RZT of 28^{o}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 as well as 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 potatoes that were subjected to carious RZT is dependent on both the temperature and cultivar.

The results of the present study suggest that the pigmented potato cultivars 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. 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*.,

2001). 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 and 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 (Lyons, 1973 and Grace *et al.,* 1998). Phenolic compounds have been previously reported to defend plants against microorganisms and herbivores (Hada *et al*., 2001).

3.4.2 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 5 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 resulted in significantly lower ORAC values in all cultivars except in cv. HBR (64.74 µmol TE/g) where 24^{o}C had this lowering effect. The ORAC (µmol TE/g) values under 20^{o}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 potatoes 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 favoured by cv. BP1 (4.68 µmol AAE/g) and PFA (7.09 µmol AAE/g).

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^{o}C significantly increased DPPH values in cv. HBR (17.09 µmol TE/g). Also, 28^{o}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 addition, a two-way ANOVA showed a very strong interaction between RZT and cultivar on all antioxidant activities.

3.4.5 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 6. Subjecting cv. BP1 and PFA to 20^{o}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^{o}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^{o}C significantly lowered chlorogenic acid in cv. BP1 (6.79 µg/g) and SB (107.8 µg/g) while 24 and 28^{o}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 coloured 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 a*l. (2016), Furrer *et al*. (2017), Külen *et al.* (2013), Lachman *et al.* (2013) and Stushnoff *et al*. (2008) also reached similar conclusions.

3.4.6 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 7). A RZT of 28^{o}C significantly ($p<0.05$) lowered AA (µg/g) content in cv. SB (10.63) and PFA (13.66). In contrast, 28^{o}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) 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).

4. **Conclusion**

In conclusion, plant physiological growth and tuber bearing capacity were positively affected by an increase in soil root temperature which has a direct correlation on the macro and micro nutrient uptake. The total activity of polyphenols and vitamin C is subject to the cultivar and treatment. As expected, secondary metabolites were more elevated in pigmented potatoes indicating their potential health benefits than non-pigmented potatoes. The cultivation practice of our findings is key for both growth and higher yield in a controlled environment. We provide evidence that these cultivars could have preferable yield in regions of field soil temperatures that reach 24 °C in temperature. Furthermore, the secondary metabolites reported in the pigmented cultivars 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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Disclosure Statement

No potential conflict of interest was reported by the authors.

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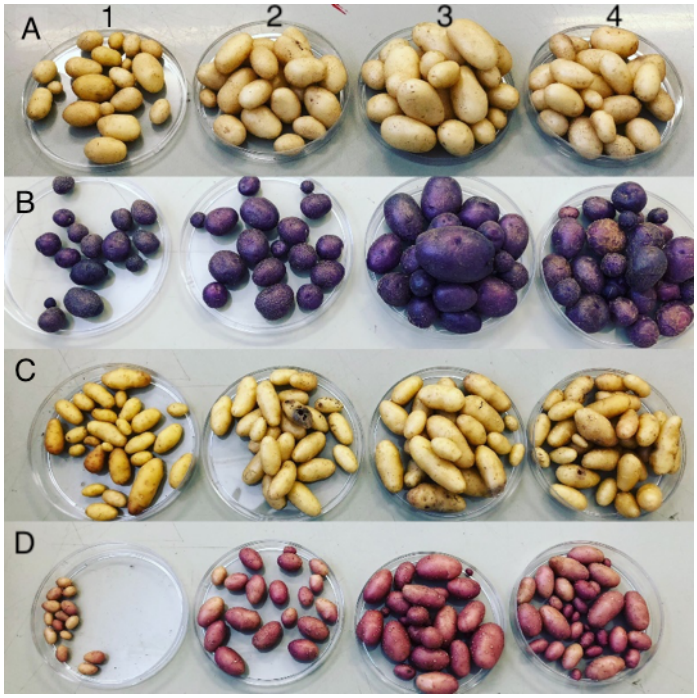
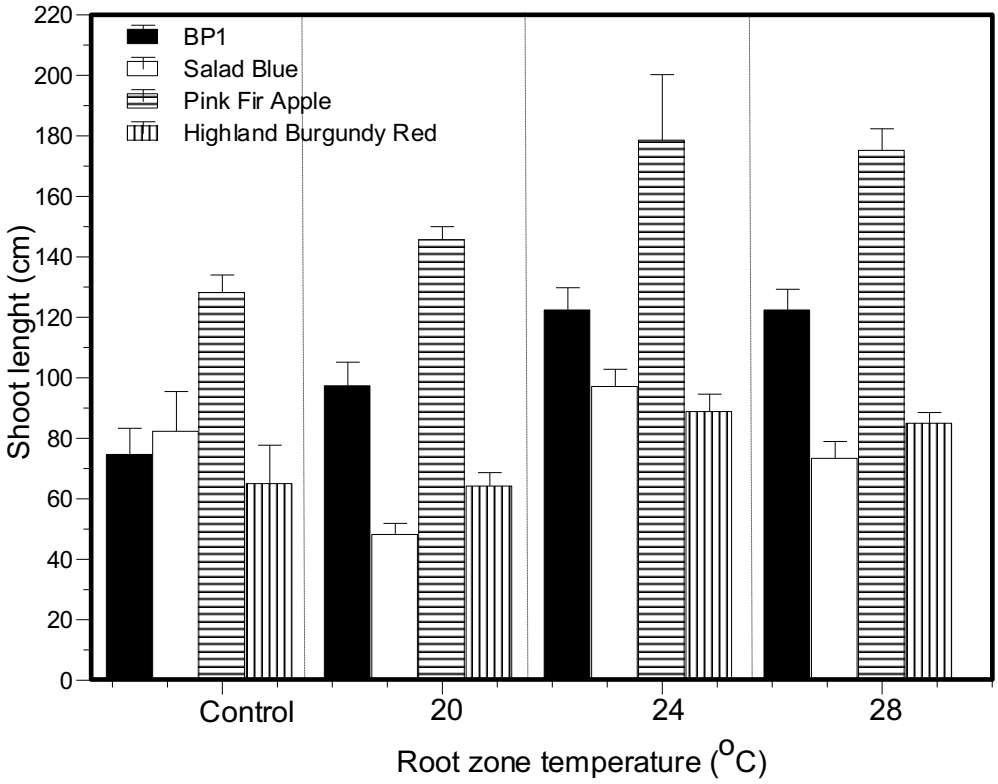
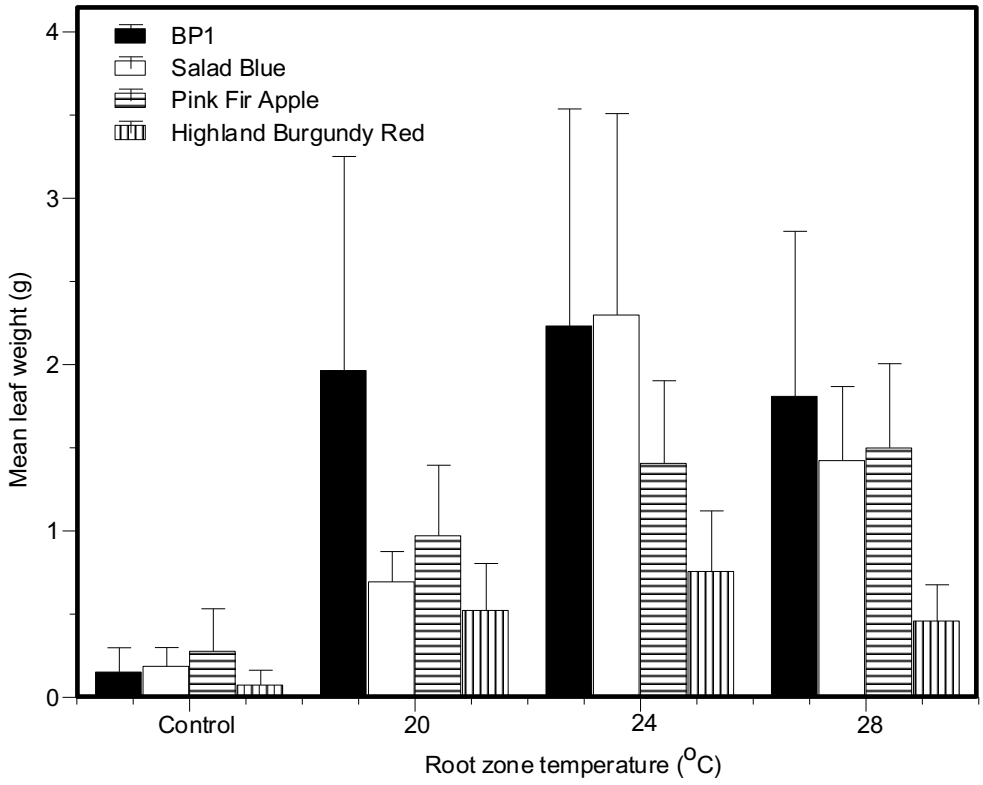


Figure 1. BP1 (A), Salad blue (B), Pink Fir Apple (C), Highland Burgundy Red (D) submitted to variable root zone temperatures T0 (control), T1 (20°C), T2 (24°C), T3 (28°C) after 73 DAT. Each point is the mean of thirteen replicates.


BP1
Salad Blue
Pink Fir Apple
Highland Burgundy Red
Shoot lenght (cm)
0
20
40
60
80
100
120
140
160
180
200
220
Control
20
24
28
Root zone temperature (°C)


BP1
Salad Blue
Pink Fir Apple
Highland Burgundy Red
Mean leaf weight (g)
0
1
2
3
4
Control
20
24
28
Root zone temperature (°C)

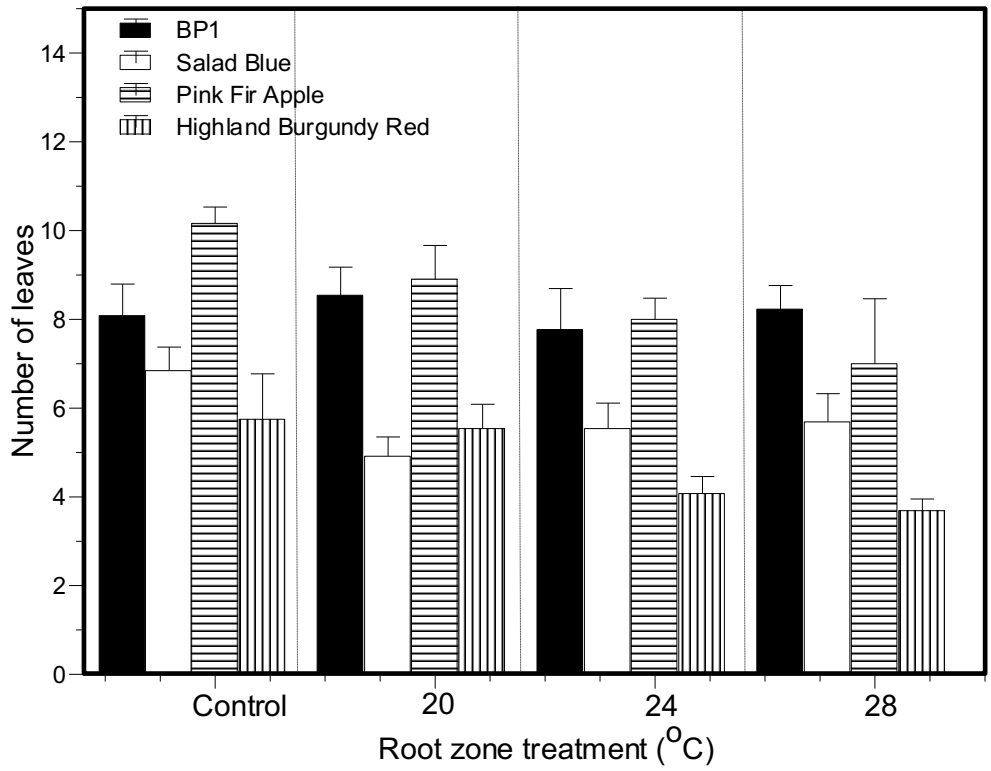


Figure 2. Relative plant height (A) total leaf weight (B) and number of leaves (C) of potato cultivars (BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red) submitted to variable root zone temperatures, 73 DAT. Each point is the mean of thirteen replicates. T0 (control), T1 (20°C), T2 (24°C), T3 (28°C)

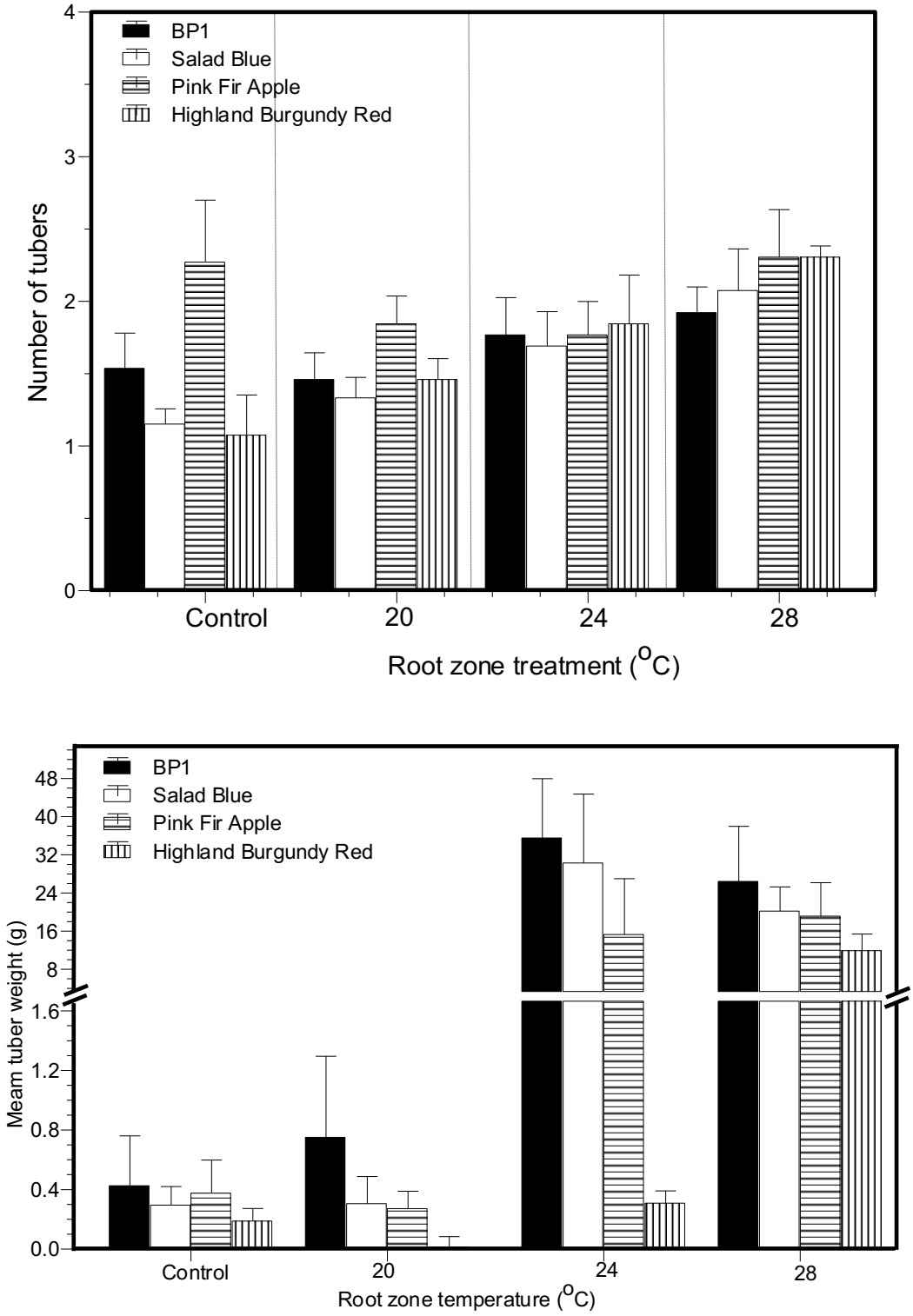


Figure 3. Number of tubers (A) and total tuber weight (B) of potato cultivars (BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red) submitted to variable root zone temperatures. Each point is the mean of thirteen replicates. T0 (control), T1 (20°C), T2 (24°C), T3 (28°C).

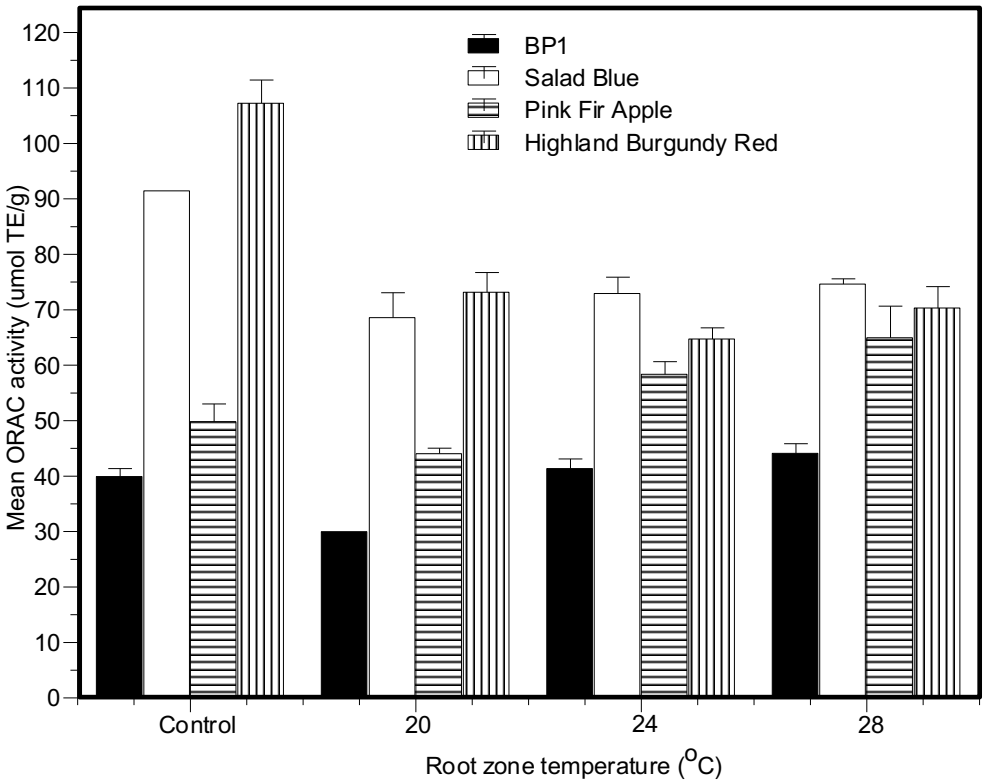


Figure 4. Antioxidant activity (ORAC) in the ethanol extracts of the tubers of *S. tuberosum* cv. BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red

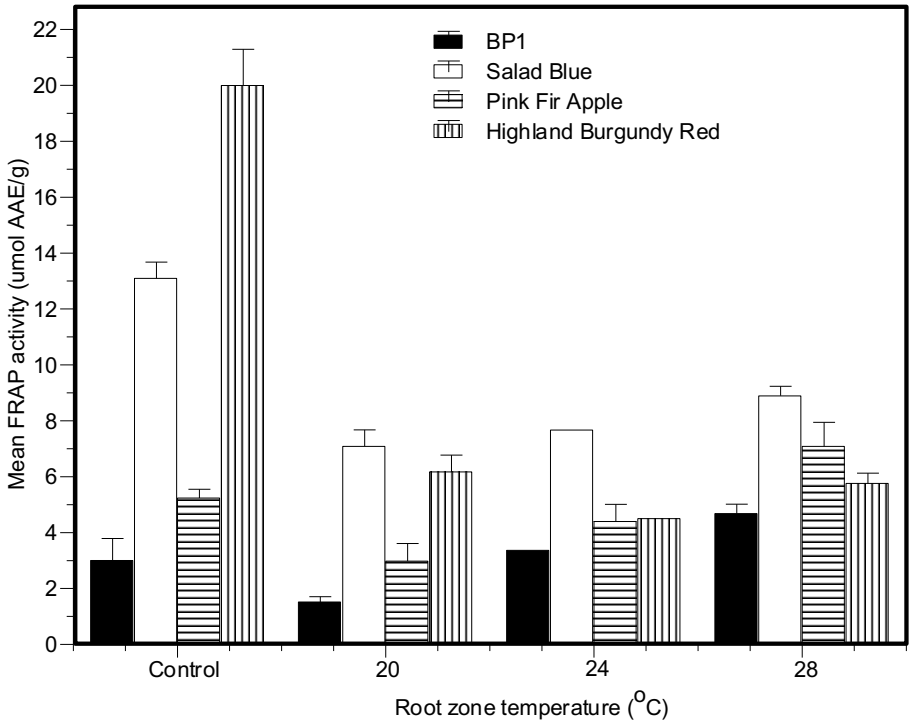


Figure 5. Antioxidant activity (FRAP) in the ethanol extracts of the tubers of *S. tuberosum* cv. BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red

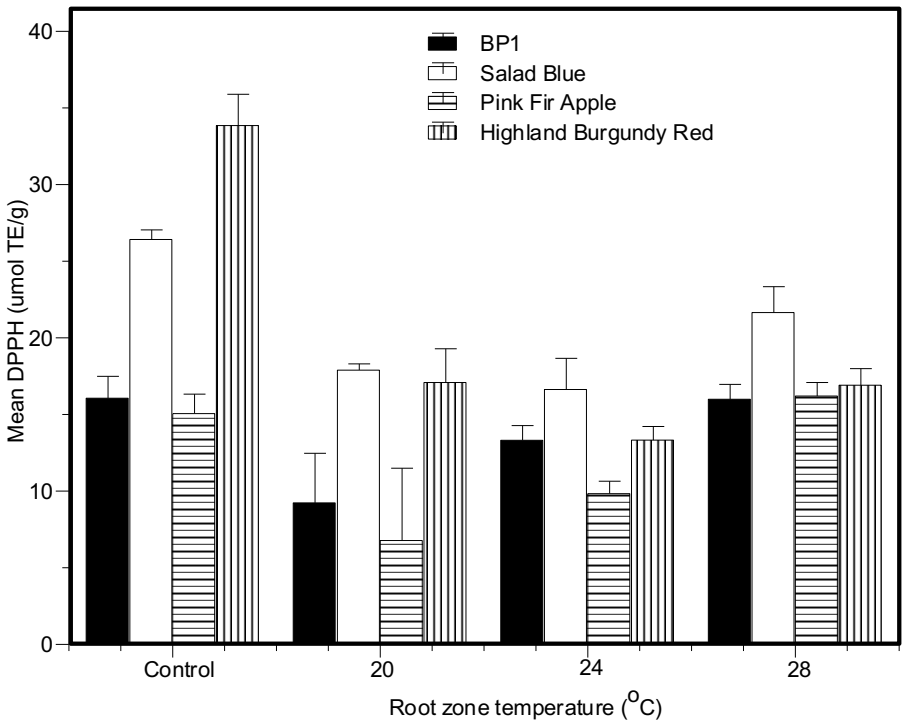


Figure 6. DPPH activity in the ethanol extracts of the tubers of *S. tuberosum* cv. BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red

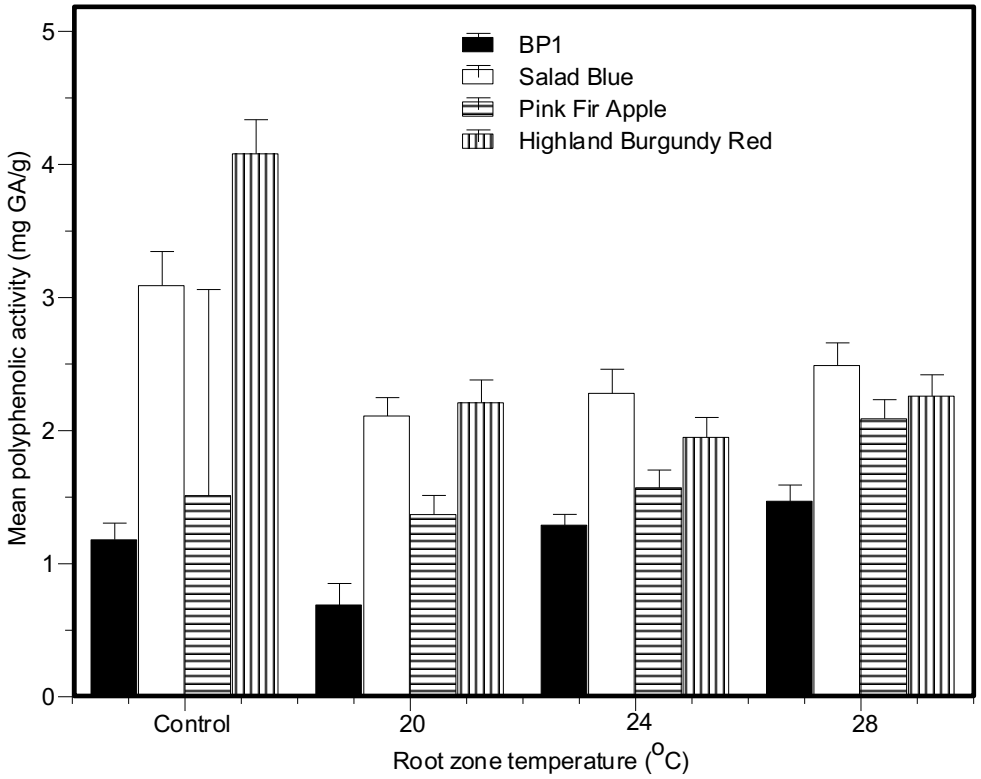
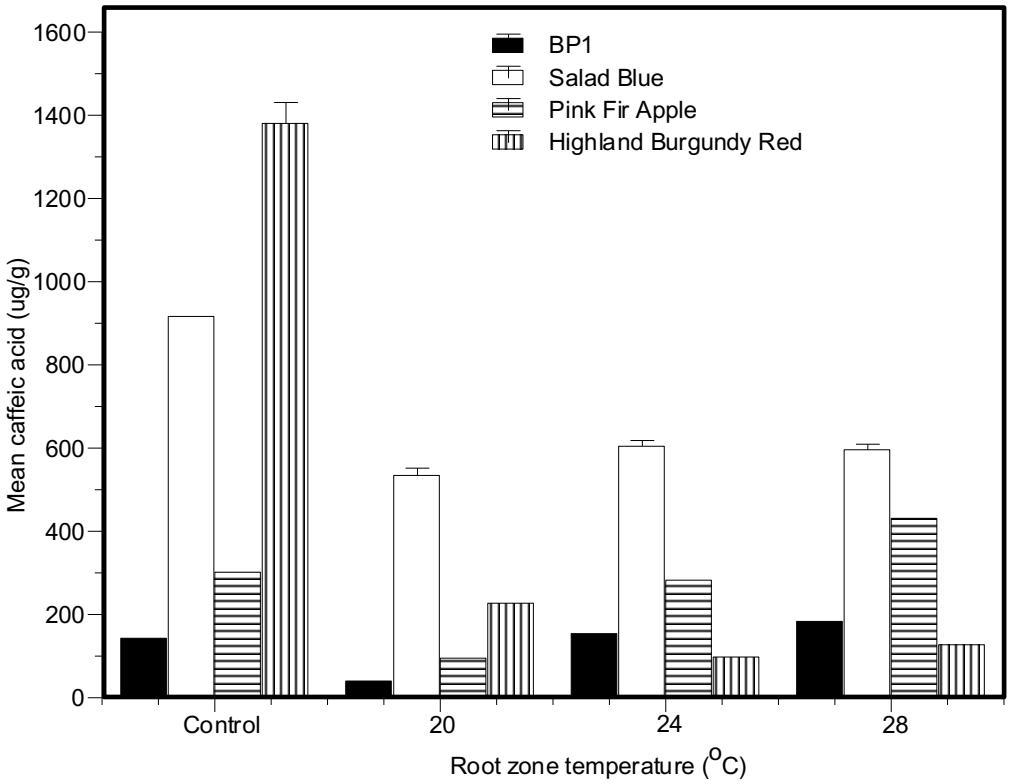


Figure 7. Polyphenol activity in the ethanol extracts of the tubers of *S. tuberosum* cv. BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red


BP1
Salad Blue
Pink Fir Apple
Highland Burgundy Red
Mean caffeic acid (ug/g)
1600
1400
1200
1000
800
600
400
200
0
Control
20
24
28
Root zone temperature (°C)

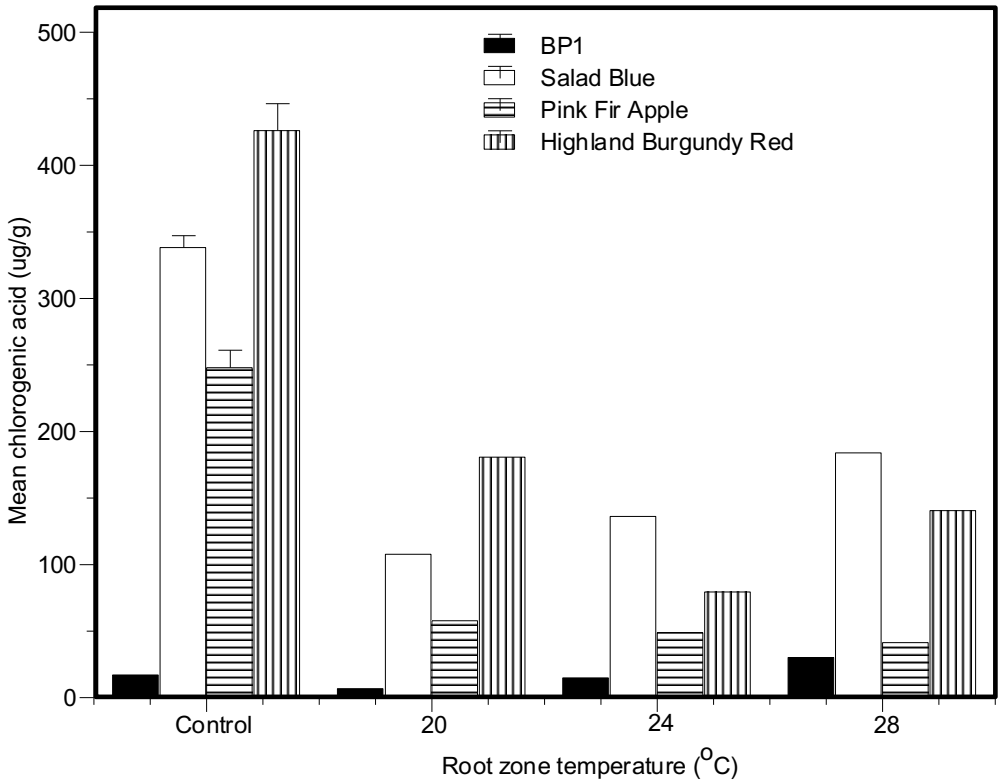

BP1
Salad Blue
Pink Fir Apple
Highland Burgundy Red
Mean chlorogenic acid (ug/g)
500
400
300
200
100
0
Control
20
24
28
Root zone temperature (°C)

Figure 8. Caffeic acid (A) and chlorogenic acid (B) activity in the ethanol extracts of the tubers of *S. tuberosum* cv. BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red

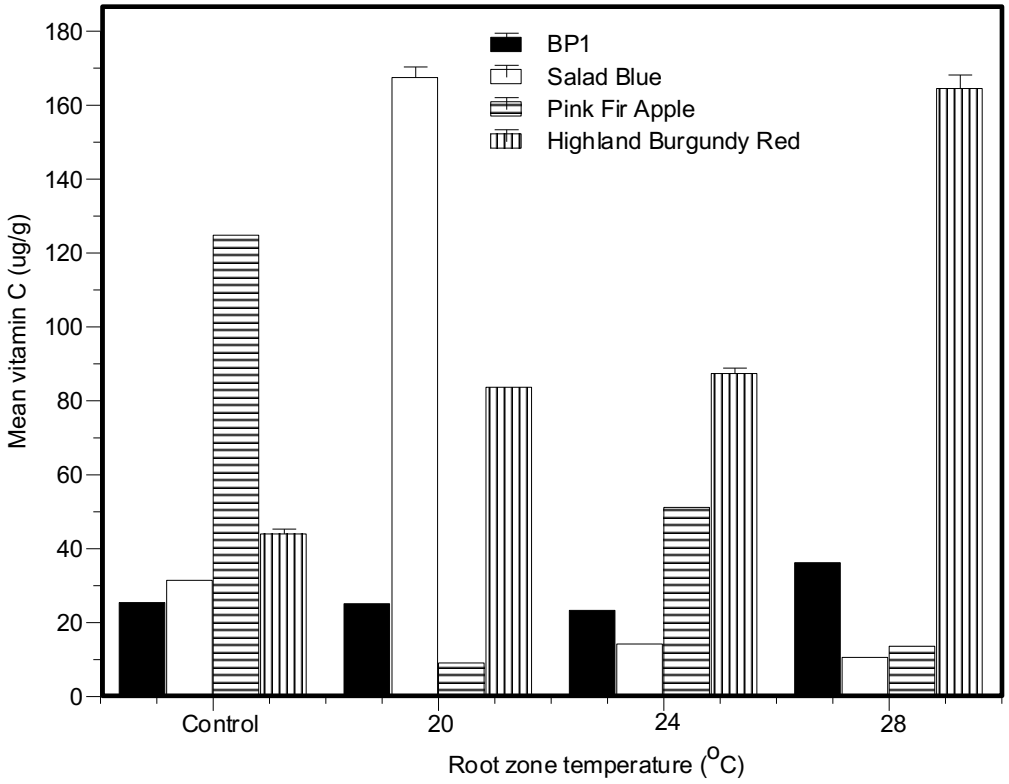


Figure 9. Ascorbic acid activity in the ethanol extracts of the tubers of *S. tuberosum* cv. BP1, Salad blue, Pink Fir Apple, Highland Burgundy Red

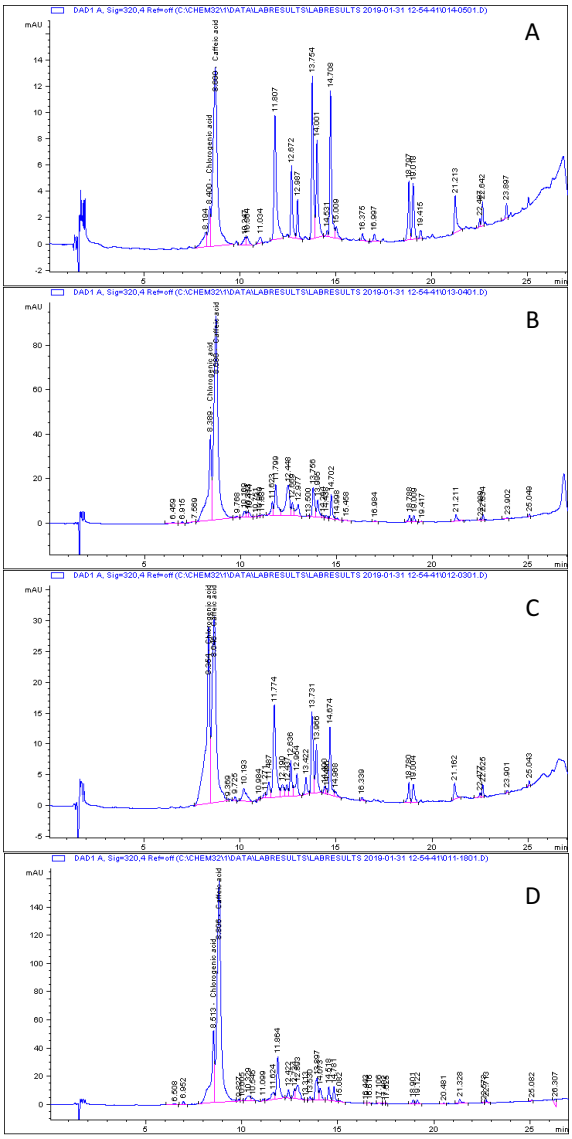


Figure 10 HPLC chromatograms activity of phenolic acids in the ethanol extracts of the tubers of the experimental control of *S. tuberosum* cv. (A) BP1, (B) Salad blue, (C) Pink Fir Apple, (D) Highland Burgundy Red

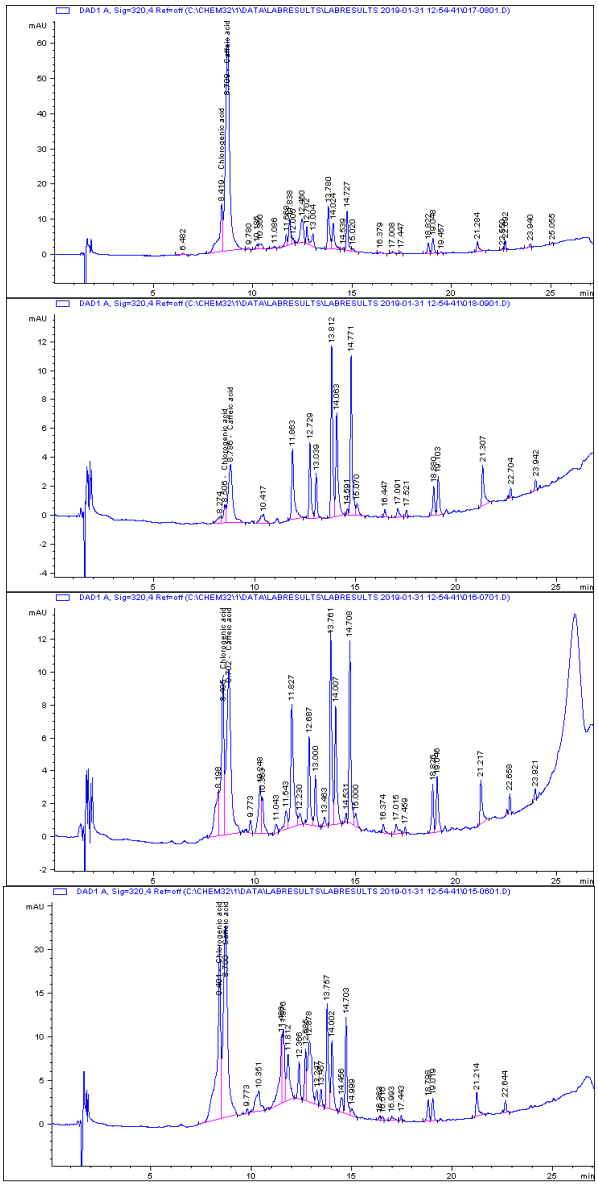


Figure 11 HPLC chromatograms activity of phenolic acids in the ethanol extracts of the tubers of the experimental RZT 20°C of *S. tuberosum* cv. (A) BP1, (B) Salad blue, (C) Pink Fir Apple, (D) Highland Burgundy Red

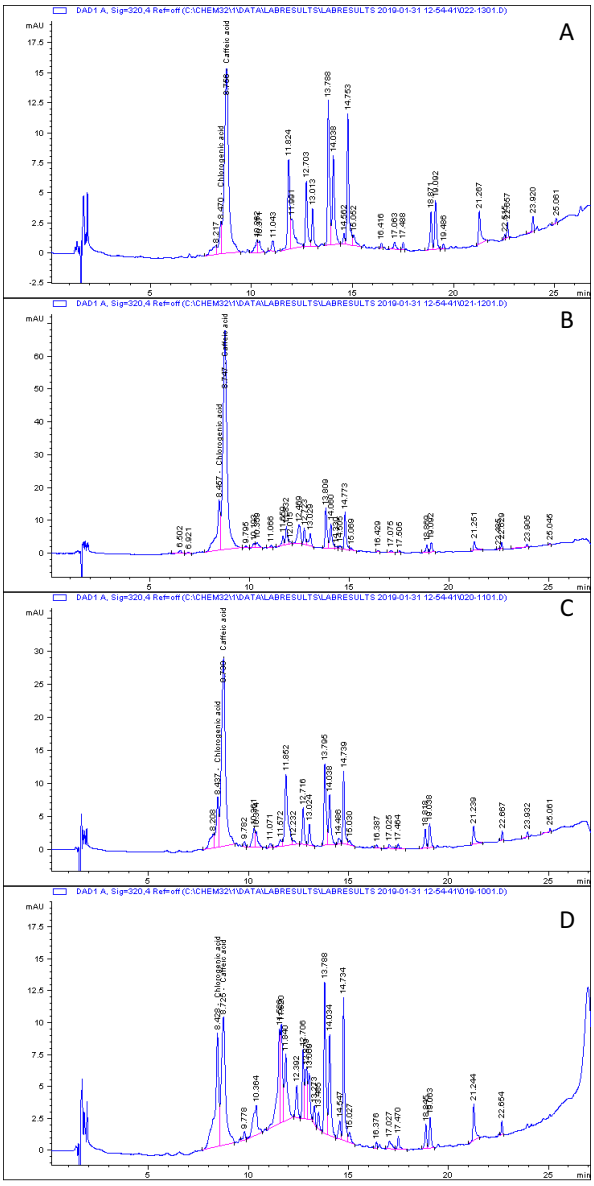


Figure 12 HPLC chromatograms activity of phenolic acids in the ethanol extracts of the tubers of the experimental RZT 24°C of *S. tuberosum* cv. (A) BP1, (B) Salad blue, (C) Pink Fir Apple, (D) Highland Burgundy Red

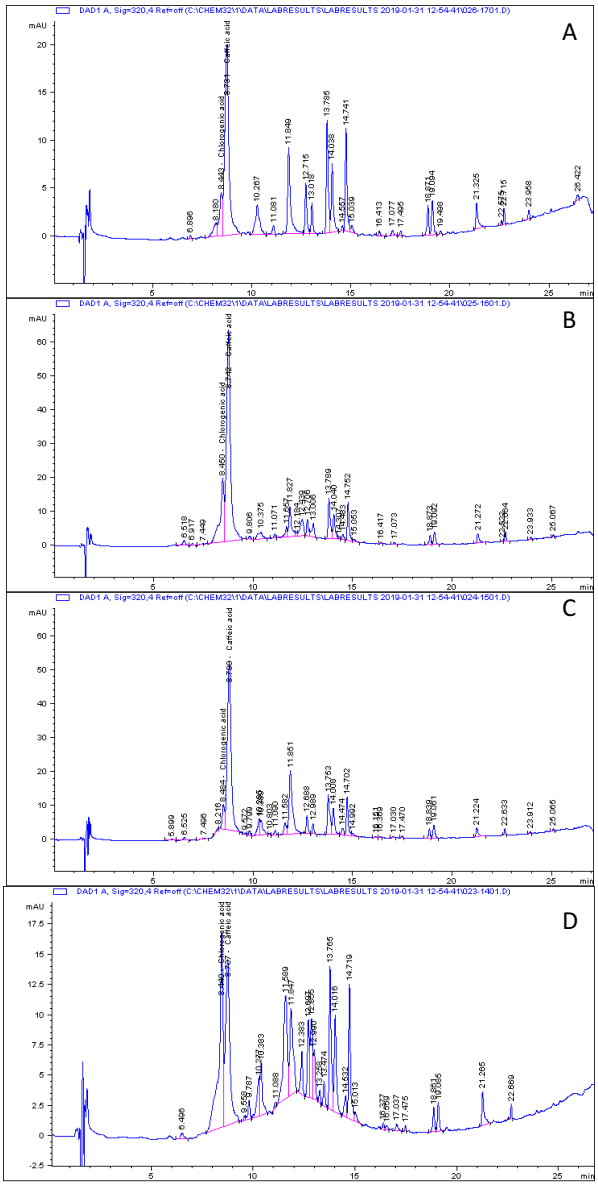


Figure 13 HPLC chromatograms activity of phenolic acids in the ethanol extracts of the tubers of the experimental RZT 28°C of *S. tuberosum* cv. (A) BP1, (B) Salad blue, (C) Pink Fir Apple, (D) Highland Burgundy Red

Figure 14 The greenhouse experiment (A) tissue culture plantlets prior to greenhouse acclimatization, (B) 30 DAT (C) 44 DAT (D) 44 DAT on heatbed

Table 1: Temperature and relative humidity in the greenhouse throughout the growing season

	Temperature (°C)	**Relative humidity (RH)**
Minimum Reading	18.9	88%
Maximum Reading	34.3%	62%
Average Reading	24.6%	62.4%

Table 2.1: The effects of RZT on plant height (cm) in potato cultivars BP1, SB, PFA and HBR

	48 DAT				55 DAT				63 DAT				73 DAT			
	0°C	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**
BP1	40±0^{c}	57.6±2.51^{b}	85.5±4.27^{a}	52.4±4.51^{b}	55.6±2.61^{d}	83.4±2.88^{b}	95.4±1.52^{a}	73.4±2.88^{c}	70±5.57^{c}	90.4±4.93^{b}	104±2.24^{a}	95.6±3.78^{b}	74.6±4.45^{c}	94.8±3.56^{b}	112.6±5.13^{a}	108±2.58^{a}
SB	49±4.18^{b}	41.2±1.64^{c}	63.6±4.16^{a}	60.8±3.42^{a}	51.6±3.97^{b}	46.8±2.49^{c}	97.8±4.55^{a}	53.2±0.45^{b}	46±3.87^{c}	46±2.74^{c}	78±6.04^{a}	55.6±4.34^{b}	49.4±5.77^{c}	42.4±2.51^{d}	118.8±6.42^{a}	73.8±3.27^{b}
PFA	92.6±5.13^{d}	129±2.24^{c}	165±10^{a}	149±4.18^{b}	115.8±3.27^{c}	151±4.24^{b}	172±4.06^{a}	153.4±3.13^{b}	129.8±3.42^{d}	146±3.39^{c}	175±3.24^{a}	152.4±5.73^{b}	130.6±2.61^{c}	135±4.80^{c}	166.6±6.5^{b}	176±2.45^{a}
HBR	36±4.18^{d}	49.6±6.40^{c}	75±0^{a}	62.2±1.79^{b}	34±3.61^{d}	57.6±1.67^{c}	83.6±1.52^{a}	70.2±2.17^{b}	29±6.28^{d}	56.2±4.97^{c}	87.2±3.56^{a}	74.2±3.19^{b}	33±4.42^{c}	53.4±2.70^{b}	89±6.96^{a}	87.4±3.44^{a}

Values represent Mean ± SD

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

0°C represents the control

Table 2.2: The effects of RZT on number of leaves in potato cultivars BP1, SB, PFA and HBR

	48 DAT				55 DAT				63 DAT				73 DAT			
	0°C	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**
BP1	4.6±0.55^{c}	6.6±0.55^{b}	7.4±0.89^{a}	6.2±0.45^{b}	8.6±0.89^{b}	8.4±1.34^{b}	9.2±1.79^{a}	9±1.41^{ab}	9.6±1.52^{a}	8.8±1.10^{b}	9±1.14^{a}	7.4±0.89^{c}	9±1.87^{b}	7.8±1.48^{c}	7.6±1.95^{c}	10±1.71^{a}
SB	5.6±0.55^{ab}	4.8±0.84^{bc}	6±0.71^{a}	6.2±0.45^{a}	6.8±0.84^{ab}	6.4±1.14^{b}	7.4±0.55^{a}	5.6±0.89^{c}	6.6±1.34	6.2±0.84	6±0.71	6.2±0.45	5.8±2.05^{b}	4.2±1.64^{c}	6.6±0.55^{a}	5.4±3.05^{b}
PFA	6.8±1.64^{b}	10±1.22^{a}	10±1.22^{a}	9.4±0.89^{a}	10.4±0.89^{c}	12.8±3.70^{a}	10.8±1.64^{c}	11.6±0.89^{b}	10.4±1.14	10.2±1.3	9.8±2.17	10.4±1.14	10±1.22^{a}	7.8±1.10^{b}	7±0.71^{b}	6.8±7.85^{bc}
HBR	4±0.71^{c}	6.4±0.55^{b}	6.6±0.89^{b}	8.4±0.55^{a}	5±0.71^{c}	7.6±0.55^{b}	7.4±1.14^{b}	8.4±0.55^{a}	3.8±0.84^{b}	7.4±0.89^{a}	7±1.87^{a}	6.8±1.48^{ab}	3.2±0.84^{b}	4.4±0.89^{a}	4.4±1.52^{a}	3.8±1.10^{b}

Values represent Mean ± SD

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

0°C represents the control

Table 2.3: The effect of RZT on number of tubers and weight (g)

	Number of tubers				Tuber weight (g)			
	0°C	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**
BP1	1.23±0.60^{b}	1.46±0.66^{b}	1.77±0.93^{b}	1.92±0.64^{bc}	0.43±0.35^{a}	0.75±0.54^{a}	35.55±12.41^{a}	26.43±11.56^{a}
SB	1.15±0.38^{bc}	1.31±0.49^{bc}	1.69±0.85^{bc}	2.08±1.04^{b}	0.30±0.12^{b}	0.31±0.18^{b}	30.30±14.44^{b}	20.23±5.03^{b}
PFA	2.23±1.36^{a}	1.85±0.69^{a}	1.77±0.83^{b}	2.31±1.18^{a}	0.38±0.22^{a}	0.27±0.12^{b}	15.29±11.73^{c}	19.17±6.99^{b}
HBR	1.08±0.28^{c}	1.46±0.52^{b}	1.85±1.21^{a}	2.31±1.03^{a}	0.19±0.08^{c}	0.15±0.07^{c}	0.31±0.08^{d}	11.92±3.49^{c}

Values represent mean±SD
Different letters down a column per block represent significant differences at $p < 0.05$
0°C represents the control

Table 3.1: The effects of root zone temperature on mineral compositions in potato cultivar BP1 leaves at 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)
$0^{o}C$	31.90±1.53^{a}	3.40±1.00^{a}	59.60±3.21^{a}	24.20±2.65^{c}	7.30±1.53^{a}	2620.00±0.58^{c}	0.09±2.08^{d}	0.23±1.15^{b}	0.30±2.00^{d}	0.06±2.52^{b}	0.04±2.62
$20^{o}C$	24.80±3.06^{b}	3.20±2.08^{a}	53.50±3.06^{b}	27.60±1.00^{b}	6.10±1.53^{b}	2910.00±0.58^{b}	0.13±1.15^{b}	0.26±2.52^{a}	0.78±2.00^{b}	0.07±2.52^{ab}	0.03±2.65
$24^{o}C$	22.90±1.53^{c}	2.30±2.52^{b}	48.10±1.53^{c}	30.10±1.53^{a}	4.50±2.08^{c}	3130.00±0.58^{b}	0.21±2.08^{a}	0.27±2.08^{a}	0.98±1.53^{a}	0.09±2.00^{a}	0.03±2.08
$28^{o}C$	21.00±1.15^{c}	2.10±1.53^{b}	48.40±1.00^{c}	25.70±2.65^{c}	4.80±2.52^{c}	3530.00±1.73^{a}	0.13±2.65^{c}	0.28±2.00^{a}	0.65±3.00^{c}	0.08±1.73^{a}	0.03±2.52

Values represent mean±SD

Different letters down the column represent significant differences at $p < 0.05$

$0^{o}C$ represents the control

Table 3.2: The effects of root zone temperature on mineral compositions in some potato cultivar Salad Blue leaves at 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^oC	34.9±1.00^a	6.101±2.08^a	65.4±1.53^a	27.001±1.53^{bc}	7.801±1.00^a	6050±0.58^b	0.09±1.00^b	0.20±3.06^c	0.64±2.00^c	0.07±3.79^b	0.04±2.65
20^oC	29.40±1.00^b	4.80±1.00^b	53.50±1.00^c	35.00±0.58^a	6.01±1.00^{ab}	7210±0.58^a	0.17±2.52^a	0.32±3.06^b	1.19±2.52^b	0.11±3.21^a	0.05±2.00
24^oC	28.30±1.00^b	3.60±1.53^c	58.40±0.58^b	34.60±1.00^a	5.20±1.53^c	5740.00±0.58^{bc}	0.18±2.52^a	0.34±3.06^{ab}	1.80±3.61^a	0.13±0.58^a	0.05±0.58
28^oC	28.80±1.73^b	4.90 ± 2.65^b	60.40±2.00^b	31.00±2.31^b	5.20±1.53^c	6140.00±1.53^b	0.16±2.52^a	0.37±1.53^a	1.10±2.08^b	0.12±1.00^a	0.05±0.58

Values represent mean±SD

Different letters down the column represent significant differences at $p < 0.05$

0^oC represents the control

Table 3.3: The effects of root zone temperature on mineral compositions in some potato cultivar Pink Fir Apple leaves at 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^oC	30.30±1.00^a	3.30±2.00^a	68.80±2.52^a	27.30±2.00^c	6.10±2.00^a	4600.00±1.73^b	0.12±4.16^c	0.26±2.52^c	0.60±1.00^c	0.06±1.00^c	0.04±2.08
20^oC	25.60±4.04^b	2.70±1.53^b	62.90±2.08^b	35.10±3.21^a	4.80±2.65^{bc}	4800.00±1.00^b	0.17±3.00^b	0.33±1.53^a	1.41±2.08^a	0.11±2.00^a	0.05±1.53
24^oC	25.70±2.08^b	2.80±1.53^{ab}	63.30±1.00^b	32.00±2.52^b	4.60±0.58^c	5190.00±2.08^a	0.18±3.06^b	0.33±2.08^a	1.29±1.53^b	0.09±1.73^{ab}	0.06±1.15
28^oC	22.90±2.08^c	3.10±3.21^a	61.20±2.65^b	33.50±1.53^{ab}	5.20±2.08^{ab}	4150.00±1.53^c	0.25±3.06^a	0.30±1.53^b	1.26±2.08^b	0.11±2.00^a	0.05±1.73

Values represent mean±SD

Different letters down the column represent significant differences at $p < 0.05$

0^oC represents the control

Table 3.4: The effect of root zone temperature on mineral compositions in some potato cultivar Highland Burgundy Red leaves at 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^oC	28.30±2.65^a	4.10±2.08^a	63.40±2.65^a	30.50±2.00^c	7.40±3.06^a	1096.00±1.53^d	0.15±2.52^b	0.59±0.58^a	0.62±2.08^b	0.68±1.00^a	0.07±1.15^a
20^oC	27.90±1.53^a	2.30±1.00^b	44.10±3.21^c	28.50±2.08^c	6.30±2.00^b	4330.00±2.08^c	0.10±2.08^c	0.16±1.15^c	0.53±3.06^c	0.06±1.53^c	0.05±1.00^b
24^oC	27.90±1.53^a	2.70±1.00^b	45.00±2.08^{bc}	36.00±0.58^a	6.20±1.00^b	4740.00±1.68^b	0.17±2.00^a	0.31±2.31^b	0.74±2.31^a	0.10±2.08^b	0.07±1.15^a
28^oC	24.50±2.00^b	2.50±1.53^b	47.00±3.06^b	34.20±2.52^b	5.90±2.65^c	4910.00±1.00^a	0.17±3.00^a	0.17±1.53^c	0.43±1.15^d	0.11±2.00^b	0.09±2.08^a

Values represent mean±SD

Different letters down the column represent significant differences at $p < 0.05$

0^oC represents the control

Table 4: The effect of RZT on polyphenolic compounds

	FLAVONOLS (mg QE/g)				POLYPHENOLS (mg GA/g)			
	0°C	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**
BP1	0.35 ± 0.02^{a}	0.21 ± 0.03^{c}	0.33 ± 0.05^{ab}	0.37 ± 0.07^{a}	1.18 ± 0.13^{b}	0.69 ± 0.16^{c}	1.29 ± 0.08^{ab}	1.47 ± 0.12^{a}
SB	0.64 ± 0.08^{a}	0.43 ± 0.08^{d}	0.48 ± 0.03^{c}	0.55 ± 0.08^{b}	3.09 ± 0.26^{a}	2.11 ± 0.14^{c}	2.28 ± 0.18^{bc}	2.49 ± 0.17^{b}
PFA	0.35 ± 0.01^{b}	0.25 ± 0.01^{c}	0.31 ± 0.03^{b}	0.45 ± 0.02^{a}	1.51 ± 0.11^{bc}	1.37 ± 0.14^{c}	1.57 ± 0.13^{b}	2.09 ± 0.14^{a}
HBR	0.76 ± 0.07^{a}	0.39 ± 0.02^{b}	0.33 ± 0.03^{c}	0.35 ± 0.01^{bc}	4.08 ± 0.26^{a}	2.21 ± 0.17^{b}	1.95 ± 0.15^{c}	2.26 ± 0.16^{b}

Values represent mean±SD
Different letters along the row per block represent significant differences at $p < 0.05$
$0^{o}C$ represents the control

Table 5: The effect of RZT on antioxidant reducing capacity in some potato cultivars

	ORAC (µmol TE/g)				DPPH (µmol TE/g)				FRAP (µmol AAE/g)			
	0°C	20°C	24°C	28°C	0°C	20°C	24°C	28°C	0°C	20°C	24°C	28°C
BP1	39.95± 1.40^{b}	30.00±0.35^{c}	41.36±1.75^{b}	44.12±1.74^{a}	16.06±1.43^{a}	9.23±3.23^{c}	13.33±0.95^{b}	16.00±0.97^{a}	3.01±0.78^{b}	1.52±0.19^{c}	3.37±0.14^{ab}	4.68±0.34^{a}
SB	91.47±0.67^{a}	68.59±4.48^{c}	72.96±2.91^{b}	74.65±0.94^{b}	26.43±0.62^{a}	17.90±0.40^{c}	16.64±2.03^{c}	21.66±1.68^{b}	13.10±0.58^{a}	7.09±0.59^{c}	7.67±0.06^{bc}	8.89±0.34^{b}
PFA	49.81±3.20^{c}	44.06±0.97^{d}	58.38±2.28^{b}	64.97±5.69^{a}	15.07±1.26^{a}	6.79±4.71^{c}	9.84±0.81^{b}	16.21±0.87^{a}	5.24±0.31^{b}	2.98±0.63^{c}	4.40±0.61^{bc}	7.09±0.85^{a}
HBR	107.27±4.16^{a}	73.90±3.54^{b}	64.74±1.99^{d}	70.33±3.84^{c}	33.86±2.03^{a}	17.09±2.19^{c}	13.33±0.90^{d}	16.92±1.07^{b}	20.00±1.29^{a}	6.17±0.60^{b}	4.50±0.15^{bc}	5.76±0.37^{b}

Values represent mean±SD
Different letters along the row per block represent significant differences at $p < 0.05$
0°C represents the control

Table 6: The effect of RZT on phenolic acids in some potato cultivars

	CAFFEIC ACID (μg/g)				CHLOROGENIC ACID (μg/g)			
	0°C	**20°C**	**24°C**	**28°C**	**0°C**	**20°C**	**24°C**	**28°C**
BP1	143.32±1.92^{c}	40.48±0.82^{d}	154.55±4.87^{b}	183.78±9.04^{a}	17.06±0.66^{a}	6.79±0.38^{c}	14.95±0.67^{b}	14.95±0.63^{b}
SB	916.75±9.20^{a}	534.65±17.18^{d}	604.94±13.44^{b}	596.09±13.45^{c}	338.23±8.93^{a}	107.80±1.21^{d}	136.26±2.42^{c}	184.00±2.38^{b}
PFA	302.21±2.93^{b}	95.47±2.38^{d}	282.82±2.73^{c}	431.45±10.27^{a}	247.94±13.06^{a}	57.92±1.19^{b}	48.8±1.61^{c}	41.46±0.91^{d}
HBR	1380.74±50.12^{a}	227.70±3.49^{b}	98.29±1.31^{d}	127.68±3.42^{c}	426.20±20.16^{a}	180.72±2.60^{b}	79.42±1.45^{d}	140.54±1.37^{c}

Values represent mean±SD
Different letters along the row per block represent significant differences at $p < 0.05$
0°C represents the control

Table 7: The effect of RZT on Ascorbic Acid (μg/g) content in some potato cultivars

	0°C	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^{a}	14.23±0.60^{c}	10.63±0.42^{d}
PFA	124.86±0.88^{a}	83.73±0.10^{b}	51.21±1.02^{c}	13.66±0.65^{d}
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 $p < 0.05$
0°C represents the control

CHAPTER THREE

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

Full Length Research Paper

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

Hildegard Witbooi [1*], Learnmore Kambizi [1], Oluwafemi Oguntibeju [2]

[1]Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa.

[2]Phytomedicine & Phytochemistry Group, Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.

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 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

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 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 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 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

$$= \frac{\boldsymbol{A^0 - A}}{\boldsymbol{A^0}} \times \mathbf{100}$$

[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]. 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 [20] where they reported higher nutrient solution temperatures as experienced under hydroponic conditions that increased water 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.

Biochemical evaluations

The results of Folin-Ciocalteu assay are presented in Table 2. Purple potato cultivar, i.e., 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 Fig 3 (A). The tuber skins of cv. SB T0 had an IC_{50} value of 818,86 μmol TE/100g dry weight, whereas other values wereT2 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 Fig 3 (B). 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.

Ascorbic acid

The contents of L-AA showed considerable variation, as shown in Fig. 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].

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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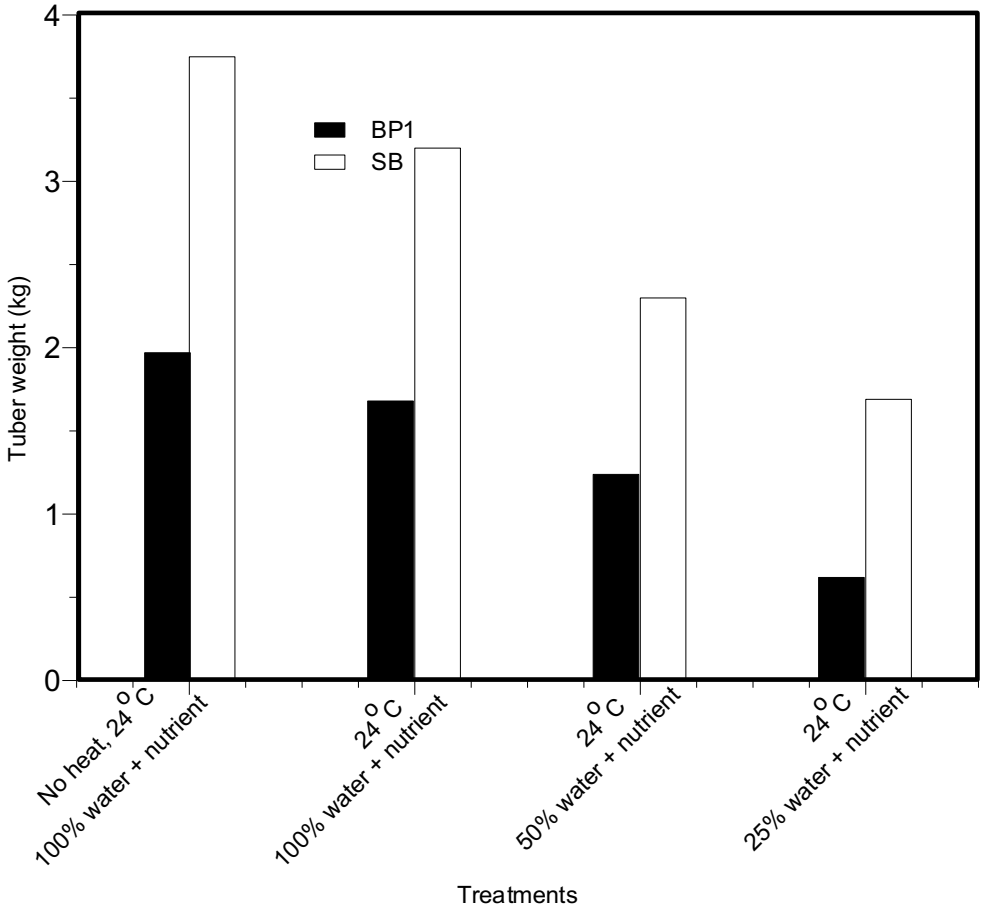

Figure 1: Effect of water and nutrient stress on cv. BP1 and SB tuber weight after 37 days under treatment

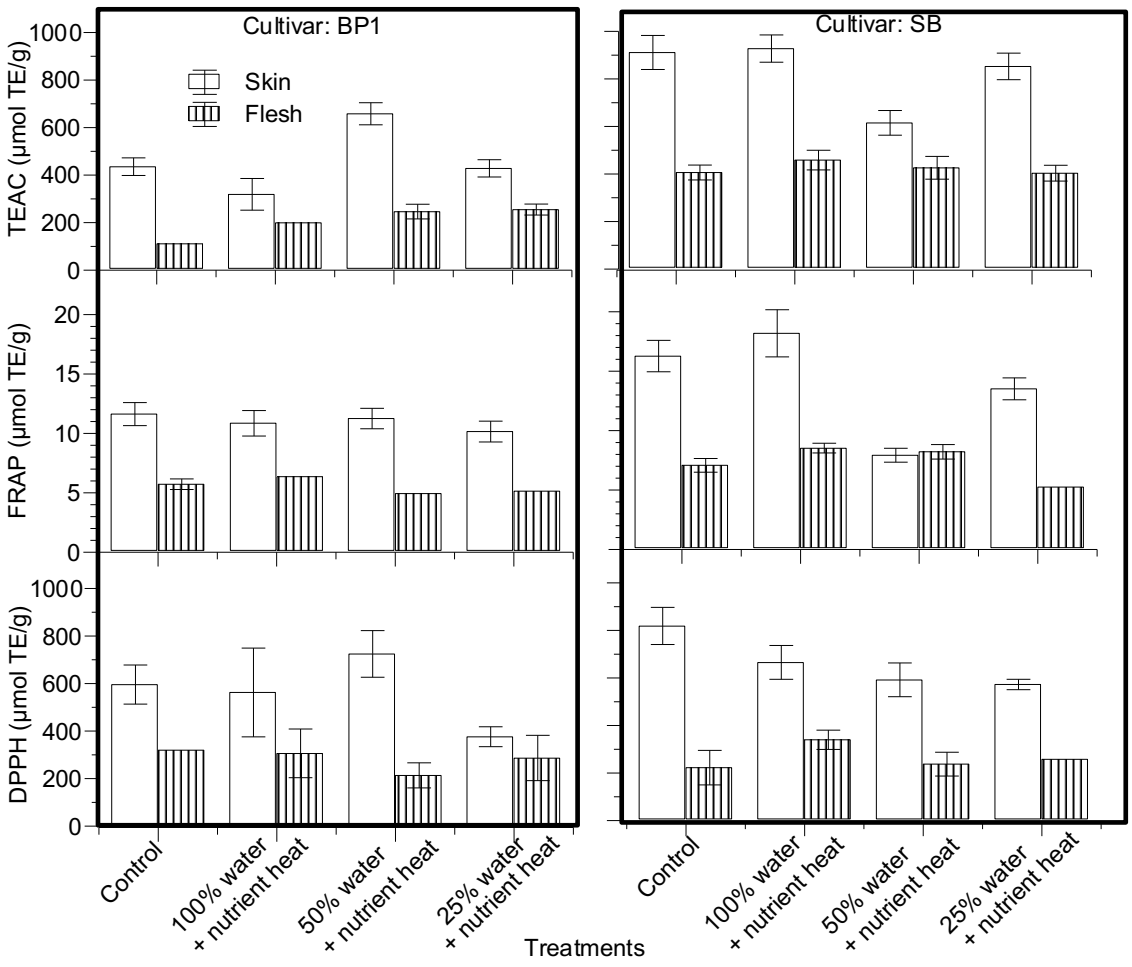

Figure 3: Antioxidant activity in skins and flesh of white fleshed (BP1) and purple fleshed (SB) potatoes represented as means (n = 3) ± standard deviation

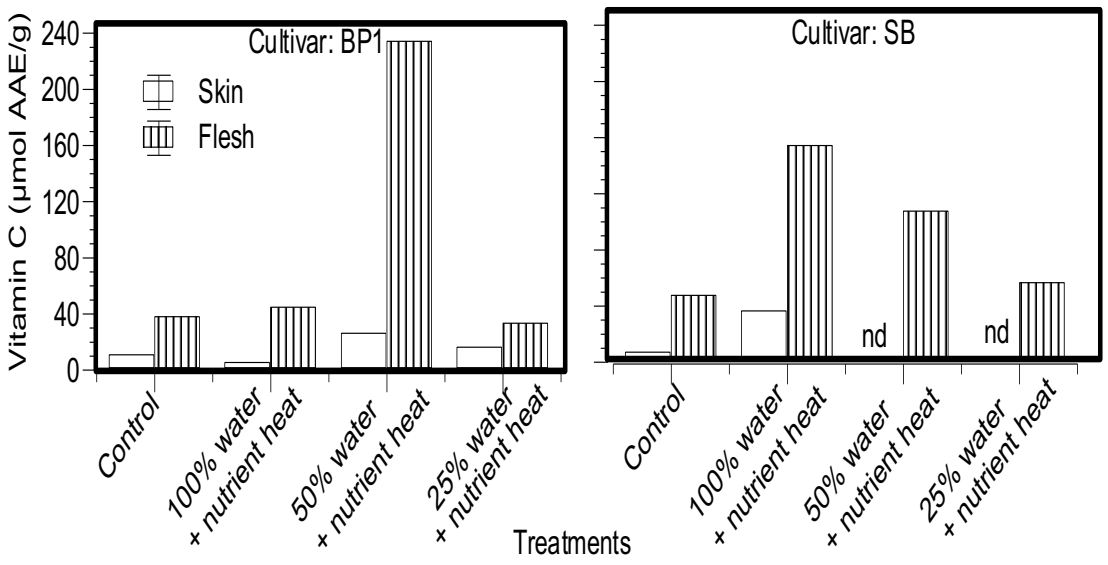

Figure 4: Concentration of ascorbic acid in skins and flesh of a white fleshed (BP1) and purple fleshed (SB) potatoes represented as means (n = 3) ± standard deviation

Figure 5: Greenhouse experiment showing non-heated and heated beds for elevated RZT

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, Flavonol, Anthocyanin contents in methanol extracts of tuber skins and							
	Phenolic acids		Flavonols		Flavanols	Anthocyanidins	
	(mg GAE/ 100g DW)		(mg QE/ 100g DW)		(mg TE/ 100g DW)	(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 represent by the same letter.							

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CHAPTER FOUR

THE POTENTIAL EFFECT OF ELEVATED ROOT ZONE TEMPERATURE ON THE CONCENTRATION OF CHLOROGENIC, CAFFEIC, AND FERULIC ACIDS AND THE BIOLOGICAL ACTIVITY OF SOME PIGMENTED *Solanum tuberosum* L. CULTIVAR EXTRACTS

Communication

The potential effect of elevated root zone temperature on the concentration of chlorogenic, caffeic, and ferulic acids and the biological activity of some pigmented Solanum tuberosum L. cultivar extracts

Hildegard Witbooi[1], Learnmore Kambizi[1], Oluwafemi Omoniyi Oguntibeju[2], Callistus Bvenura[1], Namrita Lall[3], Anna Kok[3]

[1]Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa.

[2]Phytomedicine & Phytochemistry Group, Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.

[3]Department of Plant & Soil Sciences, Medicinal Plant Sciences, University of Pretoria, Pretoria, South Africa.

ABSTRACT

Several plant extracts have been studied in particular for its ability to treat cancer, tuberculosis as well as other ailments. The present study explores the properties of *Solanum tuberosum L.* cultivars Salad Blue (SB) and BP1 investigating its antioxidant capacity, anticancer as well as antimycobacterial activity. Phenolic acids were determined through HPLC analysis. The antiproliferative activity of *S. tuberosum* against human hepatocellular carcinoma (HepG2) was investigated as well as the ability to inhibit *Mycobacterium smegmatis* with minimum inhibitory concentration range of (MIC) 10 to 0.156 μg/mL. Chlorogenic acid was the most prominent phenolic acid in both treatments as well as cultivars. Subjecting cv. BP1 and SB to 24^{o}C significantly ($p<0.05$) increased chlorogenic acid in the present trial. The ethanol extracts of all the samples showed no activity at the highest test concentration of 1000 μg/ml (ciprofloxacin MIC of 0.325 μg/mL) against *M. smegmatis*. Tuber samples tested against liver hepatocellular carcinoma (HepG2) cells for their antiproliferative activity was found to have IC50 values ranging from between 267.7 ± 36.17 μg/mL and >400 μg/mL. The present study provides useful information for farmers and health professionals in respect to health-promoting benefits of an underutilized potato variety.

Keyword: *Solanum tuberosum*, antimycobacterial, antioxidant capacity, hepatocellular carcinoma, pigmented potatoes

Introduction

Natural medicine is still one of the primary sources of health care in Southern Africa.

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 (Witbooi et al. (2020). The potato (*Solanum tuberosum* L.) is a tuber bearing food crop that belongs to the genus *Solanum* and the family *Solanaecae.* In most African countries, potatoes are one of the most important food crops. Potatoes are 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 (Riciputi *et al.* 2018; Friedman, 1997). Epidemiological studies have shown a positive correlation between ingesting phenolic compounds and improved health (Boker, *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. There is little or no information on the effects of potatoes (particularly the cultivars that were examined in this current study) on the antimycobacterial and anticancer activity. Therefore, we investigated the effect of root zone temperature 24°C on the pharmacology of cv. SB and BP1. Furthermore, the antioxidant potential in the ethanol aqueous extract was investigated as this could be a contributing factor to its health benefits. Constant reaction of the human body to oxygen through breathing, results in the cells producing

energy. Due to this activity, highly reactive molecules are produced within human and animal cells known as free radicals and oxidative stress occurs. Excessive oxidative stress may lead to inflammation and has been implicated in cancer (Mosaki, 2010). In the current study, the tuber's antioxidative compounds which can suppress oxidative stress might also have anticancer and anti-mycobacterial activity. To date, health benefits of *Solanum tuberosum* L phytochemicals and those of other pigmented vegetables and their free radical scavenging and antioxidant capacities are widely available. However, their anticancer and antimycobacterial activities have not been well studied. A literature search has shown that no scientific study has been performed to evaluate the effect of root zone temperature on potato growth and chemical characteristics. The present study explores the unknown *S. tuberosum* cv. SB, investigating its antioxidant, anticancer and antibacterial properties.

Materials and Methods

Plant growth, harvest and extraction

Potato tubers of cultivars Salad blue and BP1 were grown in a greenhouse, as described by Witbooi *et al*. (2020), for 73 days to test the effect of a controlled RZT (24°C) and non-controlled RZT. 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; skins and flesh and then powdered (40-60 mesh) and stored under refrigeration until further use. The lyophilized and powdered tubers (200g) were extracted. Samples received 20x the

volume of ethanol (etOH) (Absolute; B&M Scientific) per mass (10g > 200ml) overnight, ultra-sonicated for approximately 15 min at 40C. Samples were filtered using 0.22 µm syringe filters (25mm) and concentrated using Genevac miVac sample concentrator.

Phytochemical analysis

Caffeic, chlorogenic and ferulic acid were determined on a Dionex HPLC technology (Dionex Softron, Germering, Germany) equipped 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 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. 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.

Antimycobacterial activity of the ethanolic extract of *Solanum tuberosum* L

M. smegmatis is a non-pathogenic and fast-growing species of mycobacterium. This model is most commonly used in the physiology of mycobacteria, as it has relevance to the pathogenic species-*M. tuberculosis.* The MIC values were determined according to the method of Lall *et al.* (2013). All tuber samples were dissolved in 20% DMSO, in sterile Middlebrook 7H9 media. The outer wells of the plate received sterile distilled water (200µL) to compensate for evaporation during the incubation period for *M. tuberculosis*. Two-fold dilutions of each sample were made in sterile Middlebrooks 7H9

media to yield a final assay volume of 200μL. Ciprofloxacin served as a positive drug control for *M. smegmatis*, at a concentration range of 10 to 0.156 μg/mL. The solvent control (DMSO 2%), as well as untreated bacterial control was carried out in triplicates. The plates were sealed using parafilm, before incubation at 37 °C for seven days and 24 h for *M. smegmatis*. Prior to further incubation of 2 to 24 h, presto blue was added to each well. The result was defined by the MIC value where the lowest concentration had no color change from blue to pink.

Antiproliferative activity

The human hepatocellular carcinoma (HepG2) cell line was cultured and maintained. The hepatocellular carcinoma (HepG2) cells (100 μL) were counted and seeded in 96 well plates with a cell density of 10 000 cells and left to incubate overnight at 37°C and 5% CO_2 to allow attachment. Each of the samples were prepared to a stock solution of 2000 μg/mL. Serial dilutions of the extracts were made up to final test concentrations ranging from 400 to 12.5 μg/ml. An incubation period of 72 h at 37 °C and 5% CO_2. The concentration range of the positive control Antinomycin D was 0.5 to 0.02 μg/ml with DMSO at 2% serving as a solvent control.

Statistical analysis

Data were collected on fifty-two samples (thirteen plants per cultivar) per treatment. Statistically significant differences among treatments means were determined by two-way analysis of variance (ANOVA) at $p < 0.05$. Fisher's least significant difference (LSD) test was used to segregate means that were significantly different using a computer software program called STATISTICA**.** The mean IC50's and percentages were used to perform statistical analysis with GraphPad Prism (Version 7) using

ANOVA. The Dunnett's multiple comparison test was performed to identify significance compared to a control value. The data is expressed as the mean ± standard deviation, n=3 or more.

Results and Discussion

Caffeic, chlorogenic and ferulic acid content

HPLC analysis performed on the ethanol extract of the potato tubers. In the present study, three prominent compounds were detected *viz*, caffeic acid, chlorogenic acid, as well as ferulic acid. The chromatogram results in the peak profile showed that the mean concentrations of the compounds detected varied among the two treatments and cultivars as shown in Table 1. Chlorogenic acid was the most prominent phenolic acid in both treatments as well as cultivars. Subjecting cv. BP1 and SB to 24^{o}C significantly ($p<0.05$) increased chlorogenic acid in the present trial by 40.48 and 95.47 (µg/g) respectively as shown in Table 1. Although 24^{o}C significantly lowered caffeic acid in cv. BP1 skins and flesh (0.291 – 0.027 µg/g), the same temperature significantly ($p<0.05$) increased this phenolic acid in cv. SB skins (0.390 µg/g) and flesh (0.054 µg/ g). Interestingly, the control significantly increased in caffeic acid in BP1 skins (0.367 µg/ g) and its flesh decreased (0.051 µg/g). Also, as shown in Table 1, 24^{o}C lowered chlorogenic acid in cv. BP1 skins (0.530 µg/g) and BP1 flesh (0.531 µg/g) while 24^{o}C an increase in phenolic acid in cv. SB skins (0.779 µg/g) and flesh (0.707 µg/g). The control significantly lowered chlorogenic acid in both cultivars. Ferulic acid was present, but at low concentration with cv. SB showing the highest availability. 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 chlorogenic acid is significantly higher in coloured than in yellow-fleshed potatoes. Our results confirm this. Furthermore, elevated RZT showed minimal increase in the content of chlorogenic acid. It is interesting to note that caffeic acid content was promoted when SB and BP1 was exposed to higher root zone temperature. 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 a*l. (2016), Furrer *et al*. (2017), Külen *et al.* (2013), Lachman *et al.* (2013) and Stushnoff *et al*. (2008) also reached similar conclusions.

Antimycobacterial activity

All samples were tested against *M. smegmatis*. Antibacterial properties of *Solanum tuberosum (*ethanol extracts) of both cultivars and treatments of BP1 and Salad blue were tested. The ethanol extracts of all the samples showed no activity (Table 2) at the highest test concentration of 1000 μg/ml (ciprofloxacin MIC of 0.325 μg/mL) against *M. smegmatis*. According to Gibbons (2004), natural products that show MIC values of 1000 μg/ml and lower, are considered as noteworthy activity.

Antiproliferative assay

All tuber samples were tested against liver hepatocellular carcinoma (HepG2) cells for their antiproliferative activity and was found to have IC50 values ranging from between 267.7 ± 36.17 μg/mL and > 400 μg/mL after 72 h of incubation (Table 2). According to Geran *et al*. (1972) plant extracts with a IC50 value of more than 100 μg/mL are considered as non-cytotoxic to the specific cell line, after a 72h incubation period.

The α-solanine has demonstrated *in vitro* and *in vivo* anti-cancer, alongside protective actions in the plant and potential teratogenic effects in animals, with most studies identifying activation of apoptosis as the underlying mechanism of α-solanine anti-tumor activity. Contrary to our results, Friedman (2015) reported that apoptotic effects of α-solanine in potatoes were demonstrated in vitro in HepG2 cells, in which α-solanine caused cell cycle arrest, decreased the duration of G phase and increased the duration of S phase of the cell cycle as well as decreased the synthesis of anti-apoptotic regulatory protein Ji *et al*. (2008) Bcl-2.

Conclusions

The antioxidant capacity, anticancer as well as antimycobacterial activity in pigmented potato tubers are cultivar specific. Our insights into the effects of heat on the latter, had significant effects on the antioxidants but not on its anticancer and antimycobacterial activity. Our results may offer the opportunity to test the sample against other cancer cells. These findings are of interest to build up information of experimental investigations of these cultivars in Southern Africa.

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Table 1: The effect of RZT on phenolic acids in S. tuberosum cv. BP1 and Salad blue

	CAFFEIC ACID (μg/g)		CHLOROGENIC ACID (μg/g)		FERULIC ACID (μg/g)	
	0°C	**24°C**	**0°C**	**24°C**	**0°C**	**24°C**
BP1 SKINS	0.367±0.004^{b}	0.291±0.003^{c}	0.386±0.006^{e}	0.530±0.002^{c}	0.002±0.001^{f}	0.003±0.001^{e}
BP1 FLESH	0.051±0.001^{ef}	0.027±0.000^{g}	0.458±0.050^{d}	0.531±0.003^{c}	0.001±0.001^{c}	0.002±0.001^{a}
SB SKINS	0.254±0.003^{d}	0.390±0.005^{a}	0.416±0.005^{e}	0.779±0.014^{a}	0.007±0.001^{d}	0.009±0.001^{g}
SB FLESH	0.046±0.002^{f}	0.054±0.001^{e}	0.511±0.007^{c}	0.707±0.007^{b}	0.008±0.001^{b}	0.009±0.001^{c}

Values represent mean±SD
Different letters along the row per block represent significant differences at $p < 0.05$
0°C represents the control

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSION

5.1 LITERATURE REVIEW AND INTRODUCTION

The introductory chapter established a rationale for the study with a review of literature, to provide an understanding of the conceptual framework and theoretical underpinning of the economical and well as health importance of pigmented potatoes as a potentially new health crop for South Africa. The study strived to identify new knowledge that may be used to solve farming problems, thereby increasing the information available to farmers and well as pharmaceutical companies. This reference study showed that the underutilized potato cultivars should no longer be overlooked at should be explored by vegetable farmers. These pigmented potato cultivars have the potential to start a new niche and contribute to South Africa's GDP. The few existing unusual crops as well as heirloom and other pigmented crops available provide evidence that the development potential of these pigmented potatoes has not been fully realised.

The reference study therefore investigated current cultivation records of potatoes and found no records or field data for rare pigmented potato cultivars in South Africa. The study suggests that farmers did not fully explore all benefits of its cultivation nor the potential cultivation factors responsible for the further growth which led to the, the loss of data and the lack of food source development in the local market. Furthermore, local superfood niche market needs to be established with pigmented potatoes. It is also necessary to investigate the biochemical and health benefits of the cultivars. This

provided ample proof for the necessity of its farming

5.2 THE ROLE OF ROOT TEMPERATURE ON THE PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF FOUR PIGMENTED POTATOES (*SOLANUM TUBEROSUM* L.)

Chapter 2 investigated the effects of three root zone temperatures (20, 24 and 28 °C) on growth, leaf macro- and micro-nutrients as well as phytochemical and antioxidant capacity of seed mini tubers of four pigmented potato cultivars [*Solanum tuberosum* cv. BP1, Salad Blue (SB), Pink Fir Apple (PFA), Highland Burgundy Red (HBR)]. The study found that RZT 24^{o}C significantly increased plant height and 20^{o}C reduced plant growth. Tuber weight values ranged between 0.43-35.55g, 0.3-30.3g, 0.27-19.17g and 0.15-11.92g in cv. BP1, SB, PFA and HBR respectively and 24^{o}C recorded the most favourable tuber weight value. The control temperature also increased Ca, N, P and Na while 20^{o}C increased K and Cu. RZT 20^{o}C decreased polyphenols (mg GA/g) in cv. BP1 (0.69), SB (2.11) and PFA (1.37), but 28^{o}C increased polyphenols (mg GA/g) in cv. BP1 (1.47) and PFA (2.09). Similarly, RZT 20^{o}C lowered flavonols (mg QE/g) in cv. BP1 (0.21), SB (0.43) and PFA (0.25). RZT 28^{o}C significantly lowered AA (μg/g) content in cv. SB (10.63) and PFA (13.66). Cultivars BP1, SB and PFA recorded 84.37, 83.08 and 124.86 μg/g AA respectively. Subjecting cultivar BP1 and PFA to 20^{o}C lowered caffeic acid and recorded 40.48 and 95.47 (μg/g) respectively, but, the control increased caffeic acid in cv. SB (916.75 μg/ g) and HBR (1380.74 μg/g). The control increased chlorogenic acid in all the cultivars in the order: BP1 (17.06 μg/g) > PFA (247.94 μg/ g) > SB (338.23 μg/g) > HBR (426.20 μg/g. The ORAC values were higher

in cv. HBR (107.27 µmol TE/g) and SB (91.47 µmol TE/g) when exposed to the control temperature but decreased at 20^{o}C in the order SB (68) > PFA (44.06) > BP1 (30). DPPH activity was highest in cv. SB (26.43 µmol TE/g) under control temperature conditions although 20^{o}C also significantly increased these values in cv. HBR (17.09 µmol TE/g). These results clearly indicate a variable response of various parameters to different root zone temperatures. Root zone temperature recommendations would therefore be based on specific needs. However, a clear interaction was established between RZT and potato cultivar on physiological and chemical characteristics including the antioxidant activities of the tubers. Furthermore, the secondary metabolites reported in the pigmented cultivars from the current study; and their potential health benefits offer a substantial basis for their inclusion in the diet; regardless of their low yielding capacity.

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

Chapter 3 investigated the potential of SB potato tubers as a potential high yielding potato crop for the South African market, with added health benefits. The yield and health benefits of the purple cultivar was compared to the white fleshed cv. BP1. The bag experiment using virus-free plantlets of BP1 and SB was conducted using three water and nutrient levels and favourable root zone temperature (100% without heat, 100% heated, 50% heated and 25% heated), 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.

The study concluded provides useful information for farmers and health professionals in respect to increased yield and health-promoting benefits of an underutilized potato variety. Cultivar SB clearly demonstrated a high content of polyphenolic compounds as well as high antioxidant capacity. The results of the study provide 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. The successful production of quality potatoes for the market is largely defined by the grower who is able to use the correct horticultural information to produce quality seed potatoes which will succeed once planted for production.

5.4 THE POTENTIAL OF PURPLE AND WHITE FLESHED POTATO TUBER EXTRACTS (*SOLANUM TUBEROSUM* L.): ANTIOXIDANT, ANTIMYCOBACTERIAL AND ANTI CANCER INVESTIGATION.

Chapter 4 described a study on the potato extracts studied in particular for its ability to treat cancer and tuberculosis. This study explores the properties of *Solanum tuberosum L.* cultivars Salad Blue (SB) and BP1 investigating its antioxidant capacity, anticancer as well as antimycobacterial activity. Phenolic acids were determined through HPLC analysis. The antiproliferative activity of *S. tuberosum* against human hepatocellular carcinoma (HepG2) was investigated as well as the ability to inhibit *Mycobacterium smegmatis* with minimum inhibitory concentration range of (MIC) of 10 to 0.156 μg/mL. Chlorogenic acid was the most prominent phenolic acid in both treatments as well as cultivars. Subjecting cv. BP1 and SB to 24^{o}C significantly ($p<0.05$) increased chlorogenic acid in the present trial. The ethanol extracts of all the samples showed no activity at the highest test concentration of 1000 μg/ml (ciprofloxacin MIC of 0.325 μg/mL) against *M. smegmatis*. Tuber samples tested against liver hepatocellular carcinoma (HepG2) cells for their antiproliferative activity and was found to have IC50 values ranging from between 267.7 ± 36.17 μg/mL and >400 μg/mL. The present study provides useful information for farmers and health professionals in respect to health-promoting benefits of an underutilized potato variety.

5.5 MAJOR FINDINGS OF THE STUDY

Chapter 2 found that plant physiological growth and tuber bearing capacity were positively affected by an increase in soil root temperature which has a direct correlation on the macro and micro nutrient uptake. The total activity of polyphenols and vitamin C is subject to the cultivar and treatment. As expected, secondary metabolites were more elevated in pigmented potatoes indicating their potential health benefits than non-pigmented potatoes. The cultivation practice of our findings is key for both growth and higher yield in a controlled environment. We provide evidence that these cultivars could have preferable yield in regions of field soil temperatures that reach 24 °C in temperature. Furthermore, the secondary metabolites reported in the pigmented cultivars 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.

Chapter 3 The results showed that cultivar Salad blue 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.

Chapter 4 found that chlorogenic acid was the most prominent phenolic acid in both treatments as well as cultivars. Subjecting cv. BP1 and SB to 24^{o}C significantly ($p<0.05$) increased chlorogenic acid in the present trial. The ethanol extracts of all the samples showed no activity at the highest test concentration of 1000 μg/ml (ciprofloxacin MIC of 0.325 μg/mL) against *M. smegmatis*. Tuber samples tested against liver hepatocellular carcinoma (HepG2) cells for their antiproliferative activity and was found to have IC50 values ranging from between 267.7 ± 36.17 μg/mL and >400 μg/mL. The present study provides useful information for farmers and health professionals in respect to health-promoting benefits of an underutilized potato variety.

This final chapter provided a summary of the research activities and the findings. The ensuing recommendations culminate in farming, as well as health beneficial information for the potato cultivars in question. This study was aimed at identifying new knowledge that may be used to address the various challenges faced by farmers. It is hoped that the findings of this study will support the potato industry in propagating and cultivating potatoes for a niche market through sustainable farming.

5.6 RECOMMENDATIONS

This study recommends that the farming of pigmented crops in particular potato tubers of this study should receive greater attention from both farmers as well as health professionals. Further research studies will provide additional information in terms of other abiotic growth factors for what we consider as an alternative health crop for South Africa. Furthermore, small scale farmers should be encouraged to supply the

need for local tourism-based businesses such as restaurants to increase employment in their region. Other developments may include fresh food markets, as well refining for products such a flour. Once the immediate issues have been solved e.g., food security, these tubers may also be considered in the beverage industry for distillation of alcohol for products such as vodka or gin.

LIST OF APPENDICES

Cape Peninsula
University of Technology

Faculty of Applied Sciences
Department of Horticulture
Tel No: 021 959 6479, Address: PO Box 1906, Bellville 7535
E-mail: KambiziL@cput.ac.a

To whom it may concern:

Dear Lourens van der Merwe

Herewith I would like to request *in vitro* plantlets of *Solanum tuberosum L.* (BP1, Pink Fir Apple, Salad Blue, Highland Burgundy Red).

My study will focus on improved seed propagation of these rare potato types under Andean and organic conditions as well as the polyphenolic content in greenhouse grown tubers. For this study, I will require 300 plantlets of each type.

Please note that this material will be used solely for research purposes and the plant material produced at termination of the project will be discarded.

Kind Regards,

Hildegard Witbooi
Doctoral Candidate
+27 72 345 7243
hildegardwitbooi@gmail.com

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

Witbooi H[1]*, Kambizi L[2] and O Oguntibeju[3]

Hildegard Witbooi

*Corresponding author email: kambizil@cput.ac.za

[1]Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa

[2]Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa

[3]Phytomedicine and 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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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

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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

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

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

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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.

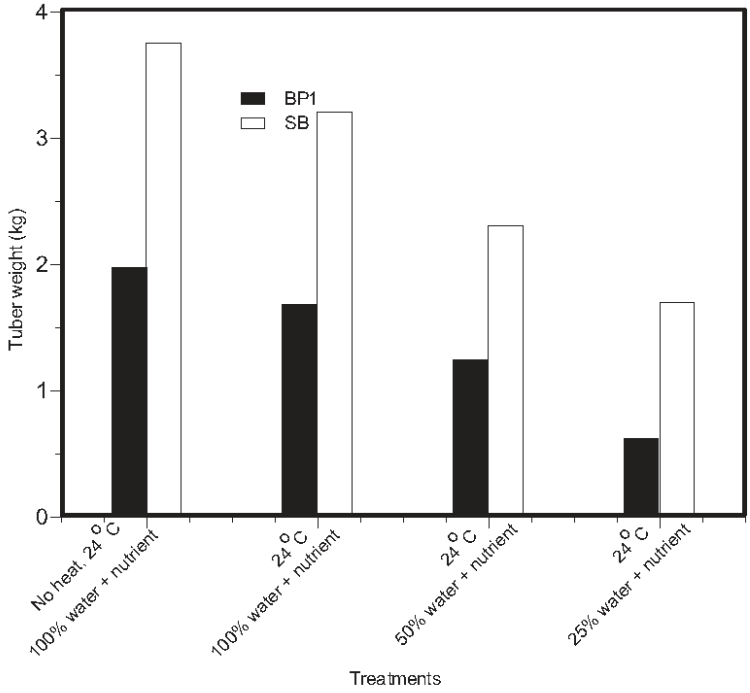

Figure 1: Effect of water and nutrient stress on cv. BP1 and SB tuber weight after 37 days under treatment

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

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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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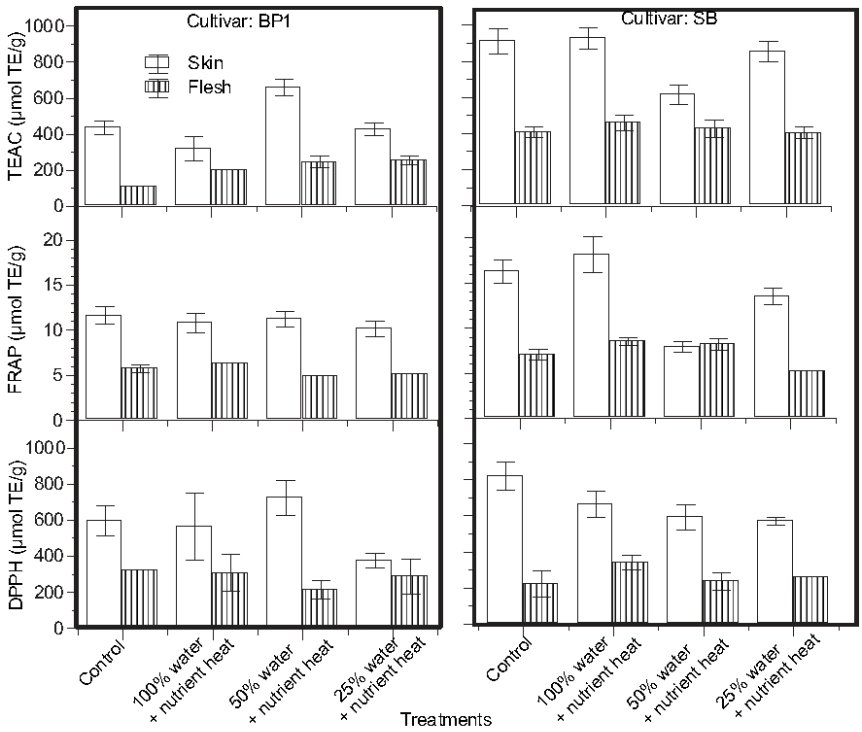

Figure 3: Antioxidant activity in skins and flesh of white fleshed (BP1) and purple fleshed (SB) potatoes represented as means (n = 3) ± standard deviation

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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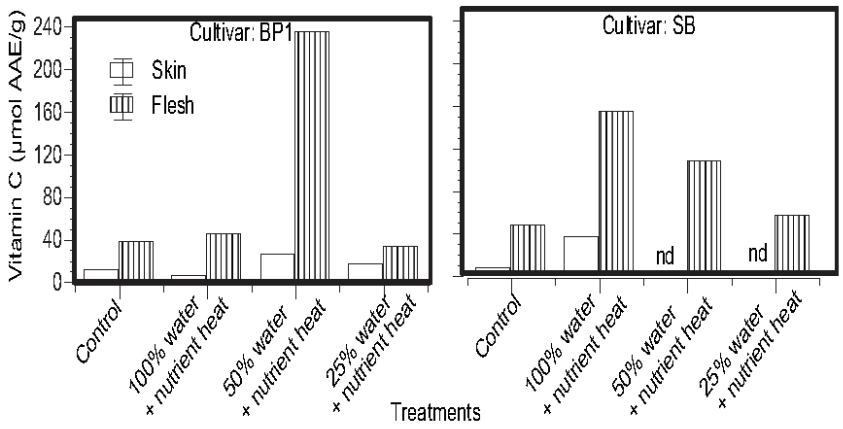

Figure 4: Concentration of ascorbic acid in skins and flesh of a white fleshed (BP1) and purple fleshed (SB) potatoes represented as means (n = 3) ± standard deviation

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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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 Witbooi[1], Callistus Bvenura[1], Oluwafemi Omoniyi Oguntibeju[2] and Learnmore Kambizi[1*]

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*Corresponding author: Learnmore Kambizi, Department of Horticulture, Faculty of Applied Sciences, Bellville Campus PO Box 1906, Bellville, 7535, South Africa.
Email: kambiziL@cput.ac.za

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Manuel Tejada Moral, University of Seville, Seville, Spain

Additional information is available at the end of the article

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

Hildegard Witbooi

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 (Boker 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

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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 uniform 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° 55'47.1"S 18°38'35.5"E. The plants were transplanted into 175 + 150 x 350 × 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 ¼ filled bags. Wood shavings were steam sterilized a week prior to transplanting in a steam sterilizer controller by a Delta DTD 4B4B 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 m 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–Ciocalteu reagent and diluted 1:10 with distilled water. After 5 min, 100 µL (7.5%) aqueous sodium carbonate (Na_2CO_3) 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 acetate buffer (300 mM, pH 3.6), 2 mL 2,4,6-Tripyridyl-s-triazine (TPTZ) solution, and 2 ml $FeCl_3.6H_2O$. 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 $FeSO_4.7H_2O$ 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:

$$\text{Percentage (\%) inhibition of DPPH activity } = \frac{A^0 - A}{A^0} \times 100 \text{ (Krovánková et al., 2012)}$$

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 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 Brucker ESI Q-TOF mass spectrometer (Bruker Daltonik GmbH, Germany). Constituents of the plant extracts

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were separated by reversed chromatography on a Thermo Fischer Scientific C18 column 5 µm, 4.6µ 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 RZT treatments was variable as shown in (Table 2). An interesting trend was obvious. An RZT 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 RZT 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 RZT 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 ^{c}	57.6 ± 2.51^{b}	85.5 ± 4.27^{a}	52.4 ± 4.51^{b}	55.6 ± 2.61^{d}	83.4 ± 2.88^{b}	95.4 ± 1.52^{a}	73.4 ± 2.88 ^{c}	70 ± 5.57 ^{c}	90.4 ± 4.93^{b}	104 ± 2.24^{a}	95.6 ± 3.78^{b}	74.6 ± 4.45 ^{c}	94.8 ± 3.56^{b}	112.6 ± 5.13^{a}	108 ± 2.58^{a}
SB	49 ± 4.18^{b}	41.2 ± 1.64 ^{c}	63.6 ± 4.16^{a}	60.8 ± 3.42^{a}	51.6 ± 3.97^{b}	46.8 ± 2.49 ^{c}	97.8 ± 4.55^{a}	53.2 ± 0.45^{b}	46 ± 3.87 ^{c}	46 ± 2.74 ^{c}	78 ± 6.04^{a}	55.6 ± 4.34^{b}	49.4 ± 5.77 ^{c}	42.4 ± 2.51^{d}	118.8 ± 6.42^{a}	73.8 ± 3.27^{b}
PFA	92.6 ± 5.13^{d}	129 ± 2.24 ^{c}	165 ± 10^{a}	149 ± 4.18^{b}	115.8 ± 3.27 ^{c}	151 ± 4.24^{b}	172 ± 4.06^{a}	153.4 ± 3.13^{b}	129.8 ± 3.42^{d}	146 ± 3.39 ^{c}	175 ± 3.24^{a}	152.4 ± 5.73^{b}	130.6 ± 2.61 ^{c}	135 ± 4.80 ^{c}	166.6 ± 6.5^{b}	176 ± 2.45^{a}
HBR	36 ± 4.18^{d}	49.6 ± 6.40 ^{c}	75 ± 0^{a}	62.2 ± 1.79^{b}	34 ± 3.61^{d}	57.6 ± 1.67 ^{c}	83.6 ± 1.52^{a}	70.2 ± 2.17^{b}	29 ± 6.28^{d}	56.2 ± 4.97 ^{c}	87.2 ± 3.56^{a}	74.2 ± 3.19^{b}	33 ± 4.42 ^{c}	53.4 ± 2.70^{b}	89 ± 6.96^{a}	87.4 ± 3.44^{a}

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^{c}	6.6 ± 0.55^{b}	7.4 ± 0.89^{a}	6.2 ± 0.45^{b}	8.6 ± 0.89^{b}	8.4 ± 1.34^{b}	9.2 ± 1.79^{a}	9 ± 1.41^{ab}	9.6 ± 1.52^{a}	8.8 ± 1.10^{b}	9 ± 1.14^{a}	7.4 ± 0.89^{c}	9 ± 1.87^{b}	7.8 ± 1.48^{c}	7.6 ± 1.95^{c}	10 ± 1.71^{a}
SB	5.6 ± 0.55^{ab}	4.8 ± 0.84^{bc}	6 ± 0.71^{a}	6.2 ± 0.45^{a}	6.8 ± 0.84^{ab}	6.4 ± 1.14^{b}	7.4 ± 0.55^{a}	5.6 ± 0.89^{c}	6.6 ± 1.34	6.2 ± 0.84	6 ± 0.71	6.2 ± 0.45	5.8 ± 2.05^{b}	4.2 ± 1.64^{c}	6.6 ± 0.55^{a}	5.4 ± 3.05^{b}
PFA	6.8 ± 1.64^{b}	10 ± 1.22^{a}	10 ± 1.22^{a}	9.4 ± 0.89^{a}	10.4 ± 0.89^{c}	12.8 ± 3.70^{a}	10.8 ± 1.64^{c}	11.6 ± 0.89^{b}	10.4 ± 1.14	10.2 ± 1.3	9.8 ± 2.17	10.4 ± 1.14	10 ± 1.22^{a}	7.8 ± 1.10^{b}	7 ± 0.71^{b}	6.8 ± 7.85^{bc}
HBR	4 ± 0.71^{c}	6.4 ± 0.55^{b}	6.6 ± 0.89^{b}	8.4 ± 0.55^{a}	5 ± 0.71^{c}	7.6 ± 0.55^{b}	7.4 ± 1.14^{b}	8.4 ± 0.55^{a}	3.8 ± 0.84^{b}	7.4 ± 0.89^{a}	7 ± 1.87^{a}	6.8 ± 1.48^{ab}	3.2 ± 0.84^{b}	4.4 ± 0.89^{a}	4.4 ± 1.52^{a}	3.8 ± 1.10^{b}

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; Nozolillio 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^{b}	1.46 ± 0.66^{b}	1.77 ± 0.93^{b}	1.92 ± 0.64^{bc}	0.43 ± 0.35^{a}	0.75 ± 0.54^{a}	35.55 ± 12.41^{a}	26.43 ± 11.56^{a}
SB	1.15 ± 0.38^{bc}	1.31 ± 0.49^{bc}	1.69 ± 0.85^{bc}	2.08 ± 1.04^{b}	0.30 ± 0.12^{b}	0.31 ± 0.18^{b}	30.30 ± 14.44^{b}	20.23 ± 5.03^{b}
PFA	2.23 ± 1.36^{a}	1.85 ± 0.69^{a}	1.77 ± 0.83^{b}	2.31 ± 1.18^{a}	0.38 ± 0.22^{a}	0.27 ± 0.12^{b}	15.29 ± 11.73^{c}	19.17 ± 6.99^{b}
HBR	1.08 ± 0.28^{c}	1.46 ± 0.52^{b}	1.85 ± 1.21^{a}	2.31 ± 1.03^{a}	0.19 ± 0.08^{c}	0.15 ± 0.07^{c}	0.31 ± 0.08^{d}	11.92 ± 3.49^{c}

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 NO_3, 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. Farran 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^{a}	3.40 ± 1.00^{a}	59.60 ± 3.21^{a}	24.20 ± 2.65 ^{c}	7.30 ± 1.53^{a}	2620.00 ± 0.58 ^{c}	0.09 ± 2.08^{d}	0.23 ± 1.15^{b}	0.30 ± 2.00^{d}	0.06 ± 2.52^{b}	0.04 ± 2.62
20°C	24.80 ± 3.06^{b}	3.20 ± 2.08^{a}	53.50 ± 3.06^{b}	27.60 ± 1.00^{b}	6.10 ± 1.53^{b}	2910.00 ± 0.58^{b}	0.13 ± 1.15^{b}	0.26 ± 2.52^{a}	0.78 ± 2.00^{b}	0.07 ± 2.52^{ab}	0.03 ± 2.65
24°C	22.90 ± 1.53 ^{c}	2.30 ± 2.52^{b}	48.10 ± 1.53 ^{c}	30.10 ± 1.53^{a}	4.50 ± 2.08 ^{c}	3130.00 ± 0.58^{b}	0.21 ± 2.08^{a}	0.27 ± 2.08^{a}	0.98 ± 1.53^{a}	0.09 ± 2.00^{a}	0.03 ± 2.08
28°C	21.00 ± 1.15 ^{c}	2.10 ± 1.53^{b}	48.40 ± 1.00 ^{c}	25.70 ± 2.65 ^{c}	4.80 ± 2.52 ^{c}	3530.00 ± 1.73^{a}	0.13 ± 2.65 ^{c}	0.28 ± 2.00^{a}	0.65 ± 3.00 ^{c}	0.08 ± 1.73^{a}	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 RZI 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^{b}	6.101 ± 2.08^{a}	65.4 ± 1.53^{a}	27.001 ± 1.53^{bc}	7.801 ± 1.00^{a}	6050 ± 0.58^{b}	0.09 ± 1.00^{b}	0.20 ± 3.06^{c}	0.64 ± 2.00^{c}	0.07 ± 3.79^{b}	0.04 ± 2.65
20°C	29.40 ± 1.00^{b}	4.80 ± 1.00^{b}	53.50 ± 1.00^{c}	35.00 ± 0.58^{a}	6.01 ± 1.00^{ab}	7210 ± 0.58^{a}	0.17 ± 2.52^{a}	0.32 ± 3.06^{b}	1.19 ± 2.52^{b}	0.11 ± 3.21^{a}	0.05 ± 2.00
24°C	28.30 ± 1.00^{b}	3.60 ± 1.53^{c}	58.40 ± 0.58^{b}	34.60 ± 1.00^{a}	5.20 ± 1.53^{c}	5740.00 ± 0.58^{b}	0.18 ± 2.52^{a}	0.34 ± 3.06^{ab}	1.80 ± 3.61^{a}	0.13 ± 0.58^{a}	0.05 ± 0.58
28°C	28.80 ± 1.73^{b}	4.90 ± 2.65^{b}	60.40 ± 2.00^{b}	31.00 ± 2.31^{b}	5.20 ± 1.53^{c}	6140.00 ± 1.53^{b}	0.16 ± 2.52^{a}	0.37 ± 1.53^{a}	1.10 ± 2.08^{b}	0.12 ± 1.00^{a}	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 ± 1.00^{a}	3.30 ± 2.00^{a}	68.80 ± 2.52^{a}	27.30 ± 2.00^{c}	6.10 ± 2.00^{a}	4600.00 ± 1.73^{b}	0.12 ± 4.16^{c}	0.26 ± 2.52^{c}	0.60 ± 1.00^{c}	0.06 ± 1.00^{c}	0.04 ± 2.08
20°C	25.60 ± 4.04^{b}	2.70 ± 1.53^{b}	62.90 ± 2.08^{b}	35.10 ± 3.21^{a}	4.80 ± 2.65^{bc}	4800.00 ± 1.00^{b}	0.17 ± 3.00^{b}	0.33 ± 1.53^{a}	1.41 ± 2.08^{a}	0.11 ± 2.00^{a}	0.05 ± 1.53
24°C	25.70 ± 2.08^{b}	2.80 ± 1.53^{ab}	63.30 ± 1.00^{b}	32.00 ± 2.52^{b}	4.60 ± 0.58^{c}	5190.00 ± 2.08^{a}	0.18 ± 3.06^{b}	0.33 ± 2.08^{a}	1.29 ± 1.53^{b}	0.09 ± 1.73^{ab}	0.06 ± 1.15
28°C	22.90 ± 2.08^{c}	3.10 ± 3.21^{a}	61.20 ± 2.65^{b}	33.50 ± 1.53^{ab}	5.20 ± 2.08^{ab}	4150.00 ± 1.53	0.25 ± 3.06^{a}	0.30 ± 1.53^{b}	1.26 ± 2.08^{b}	0.11 ± 2.00^{a}	0.05 ± 1.73

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

	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^{a}	4.10 ± 2.08^{a}	63.40 ± 2.65^{a}	30.50 ± 2.00 ^{c}	7.40 ± 3.06^{a}	1096.00 ± 1.53^{a}	0.15 ± 2.52^{b}	0.59 ± 0.58^{a}	0.62 ± 2.08^{b}	0.68 ± 1.00^{a}	0.07 ± 1.15^{a}
20°C	27.90 ± 1.53^{a}	2.30 ± 1.00^{b}	44.10 ± 3.21 ^{c}	28.50 ± 2.08 ^{c}	6.30 ± 2.00^{b}	4330.00 ± 2.08 ^{c}	0.10 ± 2.08 ^{c}	0.16 ± 1.15 ^{c}	0.53 ± 3.06 ^{c}	0.06 ± 1.53 ^{c}	0.05 ± 1.00^{b}
24°C	27.90 ± 1.53^{a}	2.70 ± 1.00^{b}	45.00 ± 2.08^{bc}	36.00 ± 0.58^{a}	6.20 ± 1.00^{b}	4740.00 ± 1.68^{b}	0.17 ± 2.00^{a}	0.31 ± 2.31^{b}	0.74 ± 2.31^{a}	0.10 ± 2.08^{b}	0.07 ± 1.15^{a}
28°C	24.50 ± 2.00^{b}	2.50 ± 1.53^{b}	47.00 ± 3.06^{b}	34.20 ± 2.52^{b}	5.90 ± 2.65 ^{c}	4910.00 ± 1.00^{a}	0.17 ± 3.00^{a}	0.17 ± 1.53 ^{c}	0.43 ± 1.15^{d}	0.11 ± 2.00^{b}	0.09 ± 2.08^{a}

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 ± 0.02^{a}	0.21 ± 0.03^{c}	0.33 ± 0.05^{ab}	0.37 ± 0.07^{a}	1.18 ± 0.13^{b}	0.69 ± 0.16^{c}	1.29 ± 0.08^{ab}	1.47 ± 0.12^{a}
SB	0.64 ± 0.08^{a}	0.43 ± 0.08^{d}	0.48 ± 0.03^{c}	0.55 ± 0.08^{b}	3.09 ± 0.26^{a}	2.11 ± 0.14^{c}	2.28 ± 0.18^{bc}	2.49 ± 0.17^{b}
PFA	0.35 ± 0.01^{b}	0.25 ± 0.01^{c}	0.31 ± 0.03^{b}	0.45 ± 0.02^{a}	1.51 ± 0.11^{bc}	1.37 ± 0.14^{c}	1.57 ± 0.13^{b}	2.09 ± 0.14^{a}
HBR	0.76 ± 0.07^{a}	0.39 ± 0.02^{b}	0.33 ± 0.03^{c}	0.35 ± 0.01^{bc}	4.08 ± 0.26^{a}	2.21 ± 0.17^{b}	1.95 ± 0.15^{c}	2.26 ± 0.16^{b}

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 (Lahlou & 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 carious 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 ± 1.40^{b}	30.00 ± 0.35^{c}	41.36 ± 1.75^{b}	44.12 ± 1.74^{a}	16.06 ± 1.43^{a}	9.23 ± 3.23^{c}	13.33 ± 0.95^{b}	16.00 ± 0.97^{a}	3.01 ± 0.78^{b}	1.52 ± 0.19^{c}	3.37 ± 0.14^{ab}	4.68 ± 0.34^{a}
SB	91.47 ± 0.67^{a}	68.59 ± 4.48^{c}	72.96 ± 2.91^{b}	74.65 ± 0.94^{b}	26.43 ± 0.62^{a}	17.90 ± 0.40^{c}	16.64 ± 2.03^{c}	21.66 ± 1.68^{b}	13.10 ± 0.58^{a}	7.09 ± 0.59	7.67 ± 0.06^{bc}	8.89 ± 0.34^{b}
PFA	49.81 ± 3.20^{c}	44.06 ± 0.97^{d}	58.38 ± 2.28^{b}	64.97 ± 5.69^{a}	15.07 ± 1.26^{a}	6.79 ± 4.71^{c}	9.84 ± 0.81^{b}	16.21 ± 0.87^{a}	5.24 ± 0.31^{b}	2.98 ± 0.63^{c}	4.40 ± 0.61^{bc}	7.09 ± 0.85^{a}
HBR	107.27 ± 4.16^{a}	73.90 ± 3.54^{b}	64.74 ± 1.99^{d}	70.33 ± 3.84^{c}	33.86 ± 2.03^{a}	17.09 ± 2.19^{c}	13.33 ± 0.90^{d}	16.92 ± 1.07^{b}	20.00 ± 1.29^{a}	6.17 ± 0.60^{b}	4.50 ± 0.15^{bc}	5.76 ± 0.37^{b}

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 24oC 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 Abdolahipour (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 ± 0.82^{d}	154.55 ± 4.87^{b}	183.78 ± 9.04^{a}	17.06 ± 0.66^{a}	6.79 ± 0.38 ^{c}	14.95 ± 0.67^{b}	14.95 ± 0.63^{b}
SB	916.75 ± 9.20^{a}	534.65 ± 17.18^{d}	604.94 ± 13.44^{b}	596.09 ± 13.45 ^{c}	338.23 ± 8.93^{a}	107.80 ± 1.21^{d}	136.26 ± 2.42 ^{c}	184.00 ± 2.38^{b}
PFA	302.21 ± 2.93^{b}	95.47 ± 2.38^{d}	282.82 ± 2.73 ^{c}	431.45 ± 10.27^{a}	247.94 ± 13.06^{a}	57.92 ± 1.19^{b}	48.8 ± 1.61 ^{c}	41.46 ± 0.91^{d}
HBR	1380.74 ± 50.12^{a}	227.70 ± 3.49^{b}	98.29 ± 1.31^{d}	127.68 ± 3.42 ^{c}	426.20 ± 20.16^{a}	180.72 ± 2.60^{b}	79.42 ± 1.45^{d}	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^{a}	14.23 ± 0.60 ^{c}	10.63 ± 0.42^{d}
PFA	124.86 ± 0.88^{a}	83.73 ± 0.10^{b}	51.21 ± 1.02 ^{c}	13.66 ± 0.65^{d}
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 Witbooi[1]
Callistus Bvenura[1]
Oluwafemi Omoniyi Oguntibeju[2]
Learnmore Kambizi[1]
E-mail: kambiziL@cput.ac.za
[1] Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, 7535, South Africa.
[2] Phytomedicine and 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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Communication

The Potential Effect of Elevated Root Zone Temperature on the Concentration of Chlorogenic, Caffeic, and Ferulic acids and the Biological Activity of some Pigmented *Solanum tuberosum* L. Cultivar Extracts

Hildegard Witbooi [1], Callistus Bvenura [1], Anna-Mari Reid [2], Namrita Lall [2,3], Oluwafemi Omoniyi Oguntibeju [4] and Learnmore Kambizi [1,*]

[1] Department of Horticulture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa; hildegardwitbooi@gmail.com (H.W.); bvenurac@cput.ac.za (C.B.)
[2] Department of Plant & Soil Sciences, Medicinal Plant Sciences, University of Pretoria, Pretoria 0002, South Africa; u29086354@tuks.co.za (A.-M.R.); namrita.lall@up.ac.za (N.L.)
[3] School of Natural Resources, University of Missouri, Columbia, MO 65211, USA
[4] Phytomedicine & Phytochemistry Group, Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville 7530, South Africa; Oguntibejuo@cput.ac.za
* Correspondence: kambizil@cput.ac.za

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Abstract: Without a doubt, potatoes play a vital food and nutrition security role in the world as more than a billion people consume this vegetable. Furthermore, the polyphenolic constituents of pigmented potato cultivars and their associated health benefits have been reported. However, the antioxidant, anticancer, and antimycobacterial activity of pigmented cultivars are scanty. Therefore, the present study explores the phenolic acids and biological activities of cv. Salad Blue (SB) and non-pigmented control (BP1) extracts. The antiproliferative activity of *S. tuberosum* L. against human hepatocellular carcinoma (HepG2) was investigated, as well as the ability to inhibit *Mycobacterium smegmatis*. Chlorogenic acid was the most prominent phenolic acid in both treatments as well as cultivars. In the current trial, 24 °C significantly increased chlorogenic acid in cv. SB and BP1. Ethanolic extracts of all the samples showed no activity at the highest test concentration of 1000 µg/mL (ciprofloxacin MIC of 0.325 µg/mL) against *M. smegmatis*. The antiproliferative activity of the tuber samples against HepG2 liver cells had IC_{50} values ranging between 267.7 ± 36.17 µg/mL and >400 µg/mL. Since the health benefits of these cultivars are highly valued, the present study provides useful information for future oncology studies, for human nutrition, as well as for how these underutilized cultivars can be fortified to improve their health benefits.

Keywords: *Solanum tuberosum*; antimycobacterial; antioxidant capacity; hepatocellular carcinoma; pigmented potatoes

1. Introduction

Of the crops that feed the world, potatoes are the third most consumed after rice and wheat, with more than a billion people relying on them for food and nutrition security [1]. This is because they are an important staple crop that is well endowed with complex carbohydrates and thus very high in energy, in addition to other nutritional benefits. This crop is easy to cultivate under a diverse range of climatic conditions except those found in Antarctica [2]. Latest statistics indicate that by 2019, over 17 million hectares were under potato cultivation. Global production also increased from 334.73 to 370.43 million metric tons (MMT) between 2009 and 2019 [2]. Current data [3] further show that global leaders in potato production by MMT are the following: China (91.92) > India (50.19) > Russia (22.07) > Ukraine (20.27) > USA (19.18) > Germany (10.60) > Bangladesh (9.65) > France (8.56) > Netherlands (6.96) > Poland (6.48).

In South Africa, potatoes also play a major food and nutrition security role, with about 677.46 km^2 under potato cultivation in the 2016/2017 agricultural season [4]. Furthermore, during the 2016/2017 agricultural season, about 2.5 MMT of potatoes were produced at a rate of approximately 3,617,200 kg/km^2 [4]. According to the same statistics, the average per capita consumption of potatoes in South Africa is 30 kg.

The usefulness of the potato peel, which is regarded as a waste product, has grown [5,6]. Due to its high antioxidant and antimicrobial effectiveness [7], it is used in food preservation. The potato peel has also been reported as a pharmaceutical ingredient in wound management [8,9], and glycoalkaloids in the leaves and other parts of the plant have been reported to offer natural protection against plant pests [10,11].

In the world today, the demand for polyphenolic and vitamin rich food sources is on the rise, and pigmented potatoes, among other crops, often possess these constituents [12].

Potatoes are rich in phenolic compounds; for example, about 49% to 90% of the total phenolics in this vegetable are in fact chlorogenic acid [13,14]. In addition, as shown in some epidemiological studies, a clear correlation has been established between consuming phenolic rich diets and better health [15–18]. Nevertheless, the antioxidant activity and some health promoting aspects of plant-derived phenolic compounds cannot be overemphasized. Anthocyanins in some pigmented potato cultivars have been reported to suppress stomach cancer in mice [19], prostate cancer cells [20], colon cancer cells [21], as well as liver cancer cells [22]. Furthermore, glycoalkaloids in these cultivars have been shown to inhibit the growth of colon, liver, stomach, and lymphoma cancer cells, among others [23,24]. However, the potential role of root zone temperature (RZT) on antioxidant, anticancer, as well as antimycobacterial activity of pigmented cultivar extracts has not been reported. We previously reported root zone temperature's role on physiological growth and polyphenolic contents in both pigmented and non-pigmented potato cultivars [25]. The results of this study showed the polyphenolic superiority of pigmented cultivars under a diverse range of root zone temperatures over the non-pigmented cultivar. Therefore, as an offshoot of this study, we tested the antiproliferative activity of the extracts of both the pigmented and non-pigmented cultivars against liver hepatocellular carcinoma (HepG2) cells and their antimycobacterial activity against *M. smegmatis*. In addition, chlorogenic and caffeic acid were significantly higher in SB under 24 °C in our previous study, and this informed our decision for the selected temperature and cultivar of the current study.

2. Materials and Methods

2.1. Plant Growth, Harvest, and Extraction

Potato tubers of cv. BP1 and SB were cultivated in a greenhouse, as described by [25], for 73 days to test the effect of controlled RZT (24 °C) and non-controlled RZT (ranging between 19 °C and 25 °C). Postharvest, the samples were separated into flesh and skin, and frozen at −80 °C in paper bags, after which they were freeze dried for 24 h in a VirTis genesis wizard 2.0 (UK). The samples were then powdered and sieved through a 40–60 mesh and stored at 4 °C until further use. About 200 g of the powdered and lyophilized tubers were extracted with 20 × the volume of 60% ethanol (EtOH) (Absolute; B&M Scientific) per mass (10 g > 200 mL) overnight and then ultra-sonicated for approximately 15 min at 40 °C. Samples were filtered using 0.22 µm syringe filters (25 mm) and concentrated using the Genevac miVac sample concentrator.

2.2. Phytochemical Analysis

A Dionex HPLC (Dionex Softron, Germering, Germany) was used to determine chlorogenic, caffeic, and ferulic acids in the samples. This HPLC has a Brucker ESI Q-TOF MS coupled autosampler and is equipped with a binary solvent manager. A reversed chromatography on a Thermo Fisher Scientific C18 column 5 µm, 4.6 × 150 mm (Bellefonte, PA, USA) was used to separate plant extract constituents via the use of a linear gradient of 0.1% formic acid in acetonitrile (solvent A) and water (solvent B) at 0.8 mL min^{-1} flow rate, an electrospray voltage of +3500 V, an oven temperature of 30 °C, and a 10 µL injection

volume. The negative mode was used to acquire the MS spectra. The nebulizer gas was set at 35 psi and the dry gas to 9 L min^{-1} at 300 °C.

2.3. Antimycobacterial Activity of the Ethanolic Extract of Solanum tuberosum L.

Mycobacterium smegmatis (*M. smegmatis*) is a non-pathogenic and fast-growing species of mycobacterium. This model is most used in the physiology of mycobacteria, as it has relevance to the pathogenic species of *Mycobacterium tuberculosis*, the causative pathogen for tuberculosis. The minimum inhibitory concentration (MIC) values were determined according to [26], with slight modifications.

A stock solution of 20% DMSO (Sigma-Aldrich, Saint Louis, MO, USA) was used to dissolve all tuber ethanolic extracts in Sterile Middlebrook 7H9 media. Furthermore, using this sterile media, two-fold dilutions were made of each sample into a final assay yield volume of 200 µL. Ciprofloxacin (Sigma-Aldrich, Saint Louis, MO, USA) served as a positive drug control at a concentration range of 0.156–10 µg/mL. The solvent control (DMSO 2%) as well as the untreated bacterial control were carried out in triplicates. The plates were sealed using parafilm before incubation at 37 °C for 24 h. After incubation of 24 h, PrestoBlue (Thermofischer, South Africa) as a viability indicator was added to each well (20 µL). The minimum inhibitory concentration (MIC) values were defined as the concentration at which no color change was visible from blue to pink.

2.4. Antiproliferative Activity

The antiproliferative activity assay was carried out according to the method of [27]. In all, 100 µL of HepG2 cells with a cell density of 10,000 cells per well was seeded in 96 well plates after careful counting and left at 5% CO_2 and 37 °C overnight to incubate in order to allow for attachment. To prepare each sample, a stock solution of 2000 µg/mL was used. A final test concentration of 12.5 to 400 µg/mL in serial dilutions of the sample extracts in the cell-containing plates was made. The plates were then incubated for 72 h at 37 °C and 5% CO_2. Actinomycin D was used as the positive control (0.02 to 0.5 µg/mL) and DMSO at 2% as the solvent control. After incubation, PrestoBlue was added (20 µL) to each well, and the plates were left to incubate for a further 2–4 h. After incubation, the absorbance values were read (490 nm wavelength, including a reference wavelength of 690 nm) using a BIO-TEK Power Wave XS multi-well reader. The mean 50% inhibitory values (IC_{50}) were calculated, and statistical analysis was performed.

2.5. Statistical Analysis

Data were collected on 52 samples (13 plants per cultivar) per treatment. Statistically significant differences among treatment means were determined by two-way analysis of variance (ANOVA) at $p < 0.05$. Fisher's least significant difference (LSD) test was used to segregate means that were significantly different using a computer software program called STATISTICA (Palo Alto, California, USA). The mean IC_{50} values (three replicates) were used to perform statistical analysis using GraphPad Prism (Version 7, San Diego, CA, USA) and two-way ANOVA. To identify significance in comparison to the control value, the Dunnett's MCT was performed. All experiments were conducted in triplicates.

3. Results

3.1. Caffeic, Chlorogenic, and Ferulic acid Content in Solanum tuberosum L. Exposed to Higher Root Zone Temperature

The results of the present study revealed the presence of chlorogenic, caffeic, and ferulic acids in the ethanolic extracts of the potato tuber cultivars, as shown in Table 1. The chromatographic peaks in the result profiles showed some variations in the mean concentrations among the two root zone temperatures and the cultivars. Chlorogenic acid was the most prominent phenolic acid in both treatments and cultivars. Cultivar BP1 flesh and skins increased chlorogenic acid by 13% and 26%, respectively, on exposure to an RZT of 24 °C. Similarly, cv. SB flesh and skins increased by 28% and 46%, respectively, on

exposure to an RZT of 24 °C. Although the set RZT of 24 °C significantly lowered caffeic acid in cv. BP1 skins and flesh (0.29–0.03 µg/g), the same RZT significantly increased this specific phenolic acid in the cv. SB skins (0.39 µg/g) and the flesh (0.05 µg/g). Interestingly, the control temperature (no heat applied) significantly increased the concentration of caffeic acid in BP1 skins (0.367 µg/g) and decreased the concentration in the flesh (0.051 µg/g). In addition, as shown in Table 1, at 24 °C the concentration of chlorogenic acid in cv. BP1 skins was lowered to 0.530 µg/g and in the BP1 flesh to 0.531 µg/g; however, a set temperature of 24 °C had the ability to increase the concentration of chlorogenic acid in cv. SB skins (0.779 µg/g) and the flesh (0.707 µg/g). The control temperature significantly lowered the concentration of the chlorogenic acid in both cultivars. Ferulic acid was present, but in very low concentrations only in cv. SB. Using a two-way analysis of variance, a strong interaction was established between the specific cultivar and RZT on chlorogenic and caffeic acid contents in the present trial.

Table 1. The effect of root zone temperature on the phenolic acid content in *S. tuberosum* cv. BP1 and Salad blue.

	Caffeic Acid (µg/g)		Chlorogenic Acid (µg/g)		Ferulic Acid (µg/g)	
	Control	24 °C	Control	24 °C	Control	24 °C
BP1 Skins	0.37 ± 0.004^{aA}	0.29 ± 0.003^{bB}	0.39 ± 0.006^{bC}	0.53 ± 0.002^{aC}	0.00 ± 0.001	0.00 ± 0.001
BP1 Flesh	0.05 ± 0.001^{C}	0.03 ± 0.000^{C}	0.46 ± 0.050^{bB}	0.53 ± 0.003^{aC}	0.00 ± 0.001	0.00 ± 0.001
SB Skins	0.25 ± 0.003^{bB}	0.39 ± 0.005^{aA}	0.42 ± 0.005^{bBC}	0.78 ± 0.014^{aA}	0.01 ± 0.001	0.01 ± 0.001
SB Flesh	0.05 ± 0.002^{C}	0.05 ± 0.001^{C}	0.51 ± 0.007^{bA}	0.71 ± 0.007^{aB}	0.01 ± 0.001	0.01 ± 0.001

Values represent mean ± SD. Different small letters along the row per block represent significant differences at $p < 0.05$ and different capital letters down the column represent significant differences at $p < 0.05$. No heat was applied to the control. BP1 = Non-pigmented control; SB = Salad Blue.

3.2. Antimycobacterial Activity

The antimycobacterial activity of *Solanum tuberosum* (ethanol extracts) of both cultivars and treatments of BP1 and SB was investigated. The ethanolic extracts of all the tested samples did not show activity at the highest test concentration of 1000 µg/mL, as shown in Table 2. The positive drug control ciprofloxacin showed an MIC value of 0.325 µg/mL.

Table 2. Antimycobacterial activity against *M. smegmatis* (MIC µg/mL).

	Antimycobacterial Activity against M. *smegmatis* (MIC µg/mL)
Control SB	NA
24 °C SB	NA
Control BP1	NA
24 °C BP1	NA
Controls	
Ciprofloxacin	0.325

NA—Not Active at the highest test concentration of 1000 µg/mL.

3.3. Antiproliferative Assay

The antiproliferative ethanolic extract activity of *S. tuberosum* L. cultivars SB and BP1 subjected to two RZTs was tested against HepG2 liver cells. The IC_{50} values of the samples ranged between 267.7 ± 36.17 µg/mL and >400 µg/mL, following 72 h of incubation as shown in Table 3. According to [28], after 72 h of incubation, plant extracts with IC_{50} values greater than 100 µg/mL are non-cytotoxic to the particular cell line. However, there is an increase in activity when SB and BP1 varieties are compared.

Table 3. Antiproliferative activity against Hepatocellular carcinoma cells (HepG2) (IC50 µg/mL).

	Antiproliferative Activity against Hepatocellular Carcinoma Cells (HepG2) (IC_{50} µg/mL)
Control SB	267.7 ± 36.17
24 °C SB	290.8 ± 39.35
Control BP1	393.0 ± 34.17
24 °C BP1	NA
Controls	
Actinomycin	0.49 ± 15.91

NA—Not Active at the highest test concentration of 1000 µg/mL.

4. Discussion

4.1. Caffeic, Chlorogenic, and Ferulic acid Content in Solanum tuberosum L. Exposed to Higher Root Zone Temperature

The results of the current study confirmed what was seen in the study conducted previously by [29], which showed that chlorogenic acid concentration was significantly lower in yellow-fleshed potatoes in comparison with the high values reported in colored potatoes. Furthermore, an elevated RZT showed minimal chlorogenic acid concentration increase. Interestingly, caffeic acid concentration increased when SB and BP1 were exposed to a higher RZT. The concentration of chlorogenic acid in red- or purple-fleshed cultivars has previously been reported to be 2.2 to 3.5 times higher than in yellow- and white-fleshed cultivars [30]. Similar results have been reported by other authors, including [31–35]. Phenolic compounds have a direct function in the type of response given by the plant when exposed to stress, such as from sun exposure or pathogen infection [36]. This is, therefore, a direct indication as to why the increase in RZT has a direct effect on the concentration of the phenolic, as seen in the current study.

4.2. Antimycobacterial Activity

Many literature studies have shown that the potato contains a variety of phenolic acids as a means of protection against microbes, viruses, and insects [37]. The mechanism of action of the antimicrobial potential of phenolic compounds has been proposed to be through the destabilization and permeation of the membrane of the microbe, which results in changes to the efflux activity and polarization; in addition, virulence factors, such as hydrophobicity, are directly affected [29]. A study conducted by [38] showed that the phenolic compound myricetin showed low antimycobacterial inhibition against *M. smegmatis* with an MIC value of 32 mg/L. Another study conducted by [39] showed that chlorogenic acid showed no inhibition against *M. smegmatis* with an MIC value of >2500 µg/mL. Moreover, during this study, a direct correlation of phenolic content to antimycobacterial activity could not be shown [39]. Due to the high levels of chlorogenic acid found in both cultivars, it could be concluded that this might be why no inhibitory activity was found against *M. smegmatis*. Further studies based on previous literature could focus on the activity of chlorogenic acid against other Gram-negative and Gram-positive bacteria and microbes.

4.3. Antiproliferative Activity

Several studies have concluded that phenolics are important sources of antioxidants and that a diet rich in antioxidants can have remarkable effects on the risk of developing cardiovascular and neurogenerative diseases including cancer and diabetes [40–43]. The anticancer activity of chlorogenic acid was investigated both in vitro and in vivo against the HepG2 cell line and HepG2 xenografts in nude mice. The study concluded that chlorogenic acid in greater concentrations had increased inhibition of HepG2 cells. The xenograft studies on nude mice achieved the same results and showed the suppression of the progression of the HepG2 xenograft [44]. Although there was no effective antiproliferative activity, as seen in the results against the HepG2 cell line by both the cultivars tested, it

should be noted that the increase in chlorogenic acid content in the SB cultivar showed increased antiproliferative activity when compared with BP1. It should also be emphasized that although chlorogenic acid is present in high amounts in both cultivars, it is not the only phenolic compound, or compound in general, that is present, and the synergistic effects of all compounds in *Solanum tuberosum* L. should be noted when looking at antiproliferative activity.

5. Conclusions

The pigmented potato tubers' antioxidant capacity (through the presence of ferulic, chlorogenic, and caffeic acids), antiproliferative activity, and antimycobacterial activity are cultivar specific. In our study, increasing the RZT had a significant effect on caffeic and chlorogenic acid in the pigmented cultivar SB. The same effect was reported in the antiproliferative study. Our results may offer the opportunity to test the same and other cultivars of *Solanum tuberosum* against other cancer cell lines. These findings are of interest because they increase the availability of information on the experimental investigations of different cultivars found within Southern Africa.

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