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Bioactive compounds in potatoes: Accumulation under drought stress conditions

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ABSTRACT

Background: Potato (*Solanum tuberosum*) is a valuable source of bioactive compounds. Besides starch, crude fibre, amino acids (AAS), vitamins and minerals, the tubers contain diverse phenolic compounds. These phenolics and AAS confer anti-oxidant protection against reactive oxygen species, tissue damage, and diseases like atherosclerosis, renal failure, diabetes mellitus, and cancer. Climate change and drought stress may become a major risk for crop production worldwide, which could result in decreased access for those who depend on this staple crop and its nutritional value.

Objective: The aim of this study is to determine the effect of drought stress on water, lipid soluble antioxidants, anthocyanins (Ac), soluble phenols, proteins, free AAS, peroxidase (POD) and lipid acyl hydrolase activity (LAH) in tuber tissue.

Methods: The study was carried out on three potato genotypes comprising one yellow-fleshed cultivar and two purple breeding clones. The plants were grown in pots (from April to September) in a glasshouse with sufficient water supply and under drought stress conditions. After harvest, the tubers of both variants were analysed for antioxidants measured as ascorbic acid (ACE) and Trolox equivalent (TXE) using a photo-chemiluminescent method. Samples of anthocyanins (Ac), soluble phenols, proteins, as well as POD and LAH activities were analysed using a UV photometer. Finally, free AAS were measured by HPLC.

Results: The results revealed that drought stress significantly reduces tuber yield, but has no significant effect on antioxidants, Ac, soluble phenols and POD. Drought stress significantly increased the levels of soluble protein ($P < 0.0001$) and LAH ($P < 0.001$). Total amounts of free

AAS were also higher in the drought stressed tubers (+34.2%, on average) than in the tubers grown with a sufficient water supply. Above all, proline was elevated due to drought stress.

Key words: Anthocyanins, antioxidants, free amino acids, phenols, proteins, tuber quality

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are one of the most important staple crops in cultivation. The tubers are rich in starch, crude fibre, proteins, minerals and vitamins [1]. Potatoes contain various phenolic compounds [2], e.g. *p*-coumaric, caffeic, chlorogenic and ferulic acid, as well as anthocyanins [3] which are all secondary metabolites synthesized *via* phenylpropanoid metabolism [4]. The potato protein also has good biological value and nutritive quality [5]. Environmental stress induces antioxidant protein [6], which may be impacted by climate change. Plants can adapt to such stressful conditions by synthesizing a set of protective proteins as reported for potato [7], chickpea [8], different *Vitex* species [9] and rice [10]. Furthermore, Late Embryogenesis Abundant (LEA) proteins were discovered to be involved in protecting plants, cell membranes and other proteins from drought stress [11, 12, 13, 14]. However, such stress responses alter biochemistry and nutritional quality. Therefore, the aim of this study was to determine the effect of drought stress on antioxidants in potato tubers measured as ascorbic acid (ACE) and Trolox equivalent (TXE), anthocyanins (Ac), soluble phenols, proteins, free amino acids (AAS), peroxidase (POD) and lipid acyl hydrolase enzyme activities (LAH).

METHODS

Plant Material: One yellow-fleshed cultivar (cv.) Agave and two purple breeding clones St 89403 and St 3792 were involved in the experiments, which were carried out in two consecutive years (2010/11). The plants were grown in pots filled with a turf (95%)-sand mixture (5%) from April to September in a greenhouse. The mean temperature (°C) during the growing seasons of the first and second year was as followed: May, 9.7/12.8; June, 15.1/16.1; July 20.6/17.9; August, 17.1/17.1; September, 12.8/14.7. The standard recommended rates for commercial pesticides and fertilizer were applied throughout the growing seasons of both experimental years. Hakaphos^{R} Red (8+12+24+4; COMPO, Germany) was used as a NPK fertilizer supplemented with magnesium (MgO) and trace elements B, Cu, Fe, Mn, Mo and Zn. After harvest, the tuber yield was determined for each genotype and variant, and the tubers were stored at 5 °C until analysis.

Drought Stress: The drought stress was applied as described by Wegener et al. [15]. Control plants were grown with sufficient water supply, being watered daily. Drought stressed plants were also watered daily for seven weeks after planting. Following tuber initiation, the water supply was completely stopped for six days. After the drought period, each plant received only 50 ml of water per day, an amount which was further reduced to 30 ml daily per plant, from the middle of August until the end of experiments. The summer of the second test year was colder and darker. Consequently, a second drought period of six days was inserted 11 weeks after planting in that year. Each experimental set for the control and the drought stress variant of each

genotype was carried out with four replicates, comprising four plants per genotype and replication. The plants were grown in a randomized experimental design.

Analyses: Lyophilized tissue samples were used for the analyses of anthocyanins according to Fuleci and Francis [16] and for the assay of LAH activity according to Bohac [5], both with modifications [17]. Total amounts of Ac were measured at 545 nm on a UV spectrometer (Kontron Instruments, Germany), then calculated as malvidin-3-*p*-coumaryl-glucoside and expressed as milligrams per 100 grams freeze-dried matter (FDM). The LAH activities were assayed at 410 nm on a UV photometer, and one enzyme unit was defined as the increase in 0.1 absorbance units per minute and milligram FDM. The POD activity was assayed in tissue extracts at 470 nm on a UV photometer according to Bi *et al.* [18] with modifications [15]. One POD enzyme unit was defined as the increase in 0.1 absorbance units per minute and microliter of the tissue extract. Antioxidants present in the tissue extracts were measured by means of a photo-chemiluminescent method described by Popov & Lewin [19] on a Photochem instrument (AnalyticJena AG, Germany), using an ACW kit for water soluble antioxidants and an ACL kit for lipid soluble antioxidants according to the manufacturer's recommendations (AnalytikJenaAG). Antioxidants were expressed in microgram equivalents of the appropriate reference compound, i.e. as ascorbic acid (ACE) and Trolox equivalent (TXE) per milligram of fresh weight (FW), respectively. Soluble phenols were measured in the tissue extracts using Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) as described [20]. The assays were performed at 725 nm on a UV photometer and the amounts of phenols (coumaric acid equivalent) were expressed in grams per kilogram of tuber FW. Soluble proteins were determined in the tissue extracts by means of a Bradford assay using Roti^{R}-Quant reagent (Roth, Germany) and bovine serum albumin (Sigma-Aldrich, Germany) as a standard. The absorbance was measured at 595 nm on a UV spectrophotometer. Amounts of proteins were expressed as mg/mL of tissue extract. Total amounts of free AAS were measured by the method of Cohen & Michaud [21] adapted to a Luna C18 (2) bonded silica column (Phenomenex, Germany) according to Hernández-Orte *et al.* [22] with modifications [17]. The HPLC assay was carried out on an Agilent 1100 series liquid chromatograph system (Agilent Technologies, Germany), and amounts of AAS were expressed in milligrams per 100 g of FW.

All analyses mentioned above were performed at least in triplicate. The analyses started in November and were finished in December of each year. For the preparation of lyophilized tissue powder and extract samples used in the analyses, ten medium sized tubers were taken from each genotype and replica as an average sample.

Statistics: The data was subjected to a generalized linear model for the analysis of variance, using the SAS 9.2 statistical package (PROC GLM, Tukey-test, SAS Institute Inc., Cary, NC, USA) and $P \leq 0.05$ was regarded to be statistically significant.

RESULTS AND DISCUSSION

Yield: The tuber yield of all three genotypes was significantly ($P \leq 0.05$, all) reduced by drought stress in both years, i.e. on average by 44% in the first year and by 34% in the second year [15]. This strong yield reduction indicates that the drought stress was successfully applied, therefore enabling the study of its impact on the bioactive compounds in tuber tissue. Moreover, it should

be emphasized that the free amino acid proline as a stress-related marker was enhanced in drought stressed tubers of all genotypes [17].

Anthocyanins: Ac pigments, belonging to the widely distributed flavonoid group, are bioactive plant polyphenols with known salutary effects [23]. They are involved in response to various environmental stresses such as excessive light, UV-B radiation, drought, and pathogen attack. Moreover, these compounds possess antioxidant capacity [24]. Their radical scavenging capacity was reported to be many times higher than that of vitamins E and C [25]. Antioxidant activity is considered to be one of the major functions of anthocyanins [24]. Therefore, it is significant that in both years the purple clones exhibited relatively high amounts of Ac in their control and drought-stressed tubers, whereas no colour pigments were found in tissue of the yellow-fleshed cv. Agave [15]. The differences in Ac between the two purple clones were not significant statistically. Their Ac contents ranged on average from 235 (Year 1) to 362 mg 100 g^{-1} (Year 2) for the control tubers grown with sufficient water supply and from 206 (Year 1) to 359 mg 100 g^{-1} (Year 2) for drought-stressed tubers. The levels of colour pigments were on average higher in the second year than in the first year with its relatively warm summer. As reported, the differences between the two years were statistically significant ($P \leq 0.01$) [15]. The cooler temperature during the main growing period of the second year fostered the accumulation of Ac pigments. Moreover, the differences in Ac between the control and the drought stress variants of purple clones were statistically insignificant [15]. As a result, it appears that drought stress has no evident effect on the amount of Ac pigments in tuber tissue.

Peroxidases: The results revealed significant genotypic differences in POD enzyme activities [15]. The latter ranged on average from 0.29 (Year 2) to 0.43 Uμl^{-1} (Year 1) for the control tubers and from 0.29 (Year 2) to 0.57 Uμl^{-1} (Year 1) for the drought stressed tubers of the three genotypes involved in this study. The POD levels were significantly ($P \leq 0.05$) higher in the first year than in the second year, a tendency which was contrary to that observed for anthocyanins. However, the differences in POD between the control and the drought stress variants were not significant statistically [15]. Therefore, drought stress appears to have no evident effect on POD, which has been similarly established for Ac. The peroxidase enzymes are assumed to compliment anthocyanins in the plant antioxidant system [24]. The data, which indicated that both POD and Ac react similarly under drought stress conditions, was consistent with their overlapping function.

Antioxidants: The three genotypes varied considerably in their concentrations of antioxidants measured as ascorbic acid (ACE) and Trolox equivalent (TXE) (Table 1). In both types of antioxidants, the purple clones exceeded the yellow fleshed cv. Agave. Thus, St 89403 had several times higher antioxidant activities than the latter. Moreover, the observations revealed that the ACE and TXE values were higher on average in the first year than in the second year (Table 1), a tendency coinciding with POD values that were also higher in the first test year. In fact, the differences in antioxidants between the years were all statistically significant ($P \leq 0.05$). But in this frame, it is also interesting to note that the differences in ACE and TXE between the control and the drought stressed variants were ultimately not significant statistically (Table 1).

As a result, it appears that the effect of drought stress on both types of antioxidants is less prominent, as noticed similarly with Ac and POD.

Table 1. Contents of antioxidants measured as ascorbic acid (ACE) and Trolox equivalent (TXE) in control and drought stressed tubers (Mean ± SD).

Genotypes	ACE (µg mg^{-1})		TXE (µg mg^{-1})	
	Control	Drought	Control	Drought
1. Year				
St 89403	2.36 ± 0.36a†	2.08 ± 0.09a†	4.33 ± 0.25a†	4.46 ± 0.48a†
St 3792	1.14 ± 0.12b†	1.32 ± 0.24b	3.67 ± 0.38b†	4.30 ± 0.28a$^{\dagger *}$
Agave	0.38 ± 0.07c†	0.21 ± 0.05c*	0.34 ± 0.02c†	0.28 ± 0.03b
Average	1.29 ± 0.88†	1.20 ± 0.81†	2.78 ± 1.84†	3.01 ± 2.04†
2. Year				
St 89403	1.76 ± 0.11a†	1.61 ± 0.17a†	2.92 ± 0.28a†	2.81 ± 0.21a†
St 3792	0.94 ± 0.07b†	0.98 ± 0.15b	2.50 ± 0.18a†	2.97 ± 0.53a†
Agave	0.25 ± 0.04c†	0.24 ± 0.02c	0.27 ± 0.03b†	0.26 ± 0.03b
Average	0.99 ± 0.65†	0.94 ± 0.60†	1.90 ± 1.22†	2.01 ± 1.33†

a,b,c Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Differences between the years are significant at $^{\dagger}P \leq 0.05$. Differences between the control and drought stress variants are significant at the level $^{*}P \leq 0.05$.

Soluble Phenols: Several phenols, i.e. non-flavonoids mainly derived from cinnamic acid, are also part of the plant antioxidant system. Most common hydroxycinnamates are *p*-coumaric, caffeic, and ferulic, as well as 5-*O*-caffeoylquinic acid (referred to as chlorogenic acid) [24]. As the results have shown, there were significant differences in soluble phenols between the genotypes (Table 2). In both years, the purple clones exhibited the highest levels. The differences in the phenol content between the two years were statistically significant ($P \leq 0.05$) only in the control variant. However, it is important to note that the differences between the control and the drought stressed variants were not significant statistically (Table 2). This may imply that the effect of drought stress on soluble phenols is less evident, as noticed for Ac, POD, ACE and TXE (Table 1).

It appears that the level of antioxidants comprising phenols, Ac and POD was high enough to respond successfully to drought, which is a condition associated with oxidative stress. The finding that these components are unaffected by drought stress might be a considerable crop advantage of tubers, especially regarding antioxidants and their protective effects [26]. Besides Ac and POD, the phenols may have also contributed to the overall antioxidant potential measured in tuber tissue (Table 1).

Soluble Proteins: Potatoes are one of the most important protein sources in the world [27], and the amino acid profile may be superior to cereals and legumes [28]. Importantly, the three genotypes tested all differed in concentrations of soluble protein (Table 2), with St 89403 being

revealed as having the highest level. The statistically significant differences in protein were only within the control variant between the years (Table 2). Additionally, it should also be noted that in all three genotypes the amount of soluble protein was significantly increased due to drought stress (Table 2). This overall trend suggests that drought stress, which had no evident effect on Ac, POD, ACE, TXE and soluble phenols, has a clear effect on protein expressed in tuber tissue. Accordingly, the latter might play an important role within the whole network of stress and/or adaptive responses in tuber tissue.

With regard to the nutritional value of tubers, this enhancement can be considered an advantage, because plant protein is an important source for amino acids in human nutrition. About 20-40% of soluble protein in potato tubers comprises patatin, a plant glycoprotein exhibiting lipid acyl hydrolase (LAH) activities [29]. Lipid acyl hydrolases are lipolytic enzymes associated with changes of membrane lipids and the release of fatty acids [30]. In fact, the LAH was significantly ($P \leq 0.05$) enhanced in drought-stressed tubers, compared to control tubers grown with sufficient water supply [17]. Therefore, the LAH levels were elevated on average by 77.1% (Year 1) and 87.6% (Year 2) due to drought stress, a tendency which may support the findings obtained in the context with soluble protein.

Table 2. Contents of soluble phenols and protein in control and drought stressed tubers (Mean ± SD).

Genotypes	**Soluble phenols (g kg^{-1})**		**Soluble protein (mg ml^{-1})**	
	Control	**Drought**	**Control**	**Drought**
1. Year				
St 89403	2.93 ± 0.18a	2.92 ± 0.25a	7.60 ± 1.37a†	12.64 ± 1.22a***
St 3792	1.77 ± 0.11b†	2.00 ± 0.27b	4.54 ± 0.50b	4.58 ± 0.08c
Agave	0.62 ± 0.08c	0.65 ± 0.04c	3.46 ± 0.14c†	7.68 ± 0.30b$^{\dagger **}$
Average	1.77 ± 1.00†	1.86 ± 0.99	5.20 ± 1.99†	8.30 ± 3.53***
2. Year				
St 89403	3.12 ± 0.21a	2.62 ± 0.14a*	8.05 ± 0.10a†	10.95 ± 0.48a**
St 3792	2.26 ± 0.04b†	1.97 ± 0.10b*	4.83 ± 0.13b	6.00 ± 0.28c**
Agave	0.62 ± 0.02c	0.64 ± 0.05c	4.15 ± 0.33c†	6.45 ± 0.19b$^{\dagger **}$
Average	2.00 ± 1.09†	1.74 ± 0.87	5.68 ± 1.79†	7.80 ± 2.35***

a,b,c Genotype means followed by different letters in the same column differ significantly at $P \leq 0.05$. Differences between the years are significant at $^{\dagger}P \leq 0.01$. Differences between the control and drought stress variants are significant at $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.0001$.

Free Amino Acids: About 49% of total amino acids in tuber tissue are non-proteinogenic, free amino acids [26]. Total free AAS was elevated in drought-stressed tubers of all genotypes (+34.2%, on average) [17]. A further experiment on these three genotypes (Wegener & Jürgens,

unpublished data) underlined this tendency and confirmed that differences in total AAS between control tubers (2.51±0.82 g 100 g^{-1} FDM) and drought-stressed tubers (3.16±1.13 g 100 g^{-1} FDM) are statistically significant ($P < 0.001$). Besides proline as a stress-related signal, asparagine was especially increased under drought stress conditions. These results were not surprising, since AAS function as osmolytes which can stabilize proteins and other macromolecules under stress conditions [31]. Furthermore, the observed increase in total amounts of free AAS due to drought may confer a nutritional advantage to this crop.

In summary, these results indicate that free amino acids, together with soluble protein such as lipid acyl hydrolases are associated with the drought stress responses of tubers. Moreover, anthocyanins, peroxidases, soluble phenols, water (ACE) and lipid soluble antioxidants (TXE) seem to be minimally affected. However, it should acknowledged that the drought stress applied in this study represents only one of many scenarios that plant may experience in nature. Therefore, it is necessary for the findings presented here to be transferred to other scenarios and to other potato genotypes when grown under drought stress conditions. Further studies will focus on starch accumulated under conditions of drought stress, because the potato starch is seen as a risk factor of type 2 diabetes [32].

Conclusions: This data has clearly demonstrated that drought stress has no significant effect on anthocyanins, peroxidases, soluble phenols, water (ACE) and lipid soluble antioxidants (TXE) accumulated in potato tuber tissue. However, it is important to note that drought stress significantly increased the contents of soluble protein, lipid acyl hydrolase activities and total concentration of free amino acids. Increased amino acid content increases the nutritional value of potatoes. Furthermore, the purple clones (above all St 89403) ranked on the highest levels in ACE, TXE, phenols and proteins.

Abbreviations: amino acids (AAS), anthocyanins (Ac), ascorbic acid equivalent (ACE), cultivar (cv.), freeze-dried matter (FDM), high performance liquid chromatography (HPLC), lipid acyl hydrolase (LAH), peroxidase (POD), Trolox equivalent (TXE)

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Author's contributions: All author contributed to this work.

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