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ABIOTIC STRESS MANAGEMENT IN POTATO CROP: EVALUATION OF ANTHOCYANIN ACCUMULATION FOR SALAD BLUE CULTIVAR

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Abstract

The present work follows the reactions of ``Salad Blue`` variety to abiotic stress factors (high temperatures and precipitation deficit in the area of the experimental fields) through the amount of anthocyanins accumulated in tubers. The experimental fields were located on the territory of Mandra in Brasov county and Rusciori in Sibiu county, both localities in Romania. The experiments took place over a period of two years, 2023 and 2024. The two years had a high climatic variability. The results show an inversely proportional relationship between the accumulation of anthocyanins in potato tubers and the abiotic stress present in the experimental plots. The highest accumulation of anthocyanins was identified in the experimental field of Rusciori locality of 2023, under less stressful conditions and the lowest amount of anthocyanins was identified in Mandra locality of 2024, where the most stressful climatic conditions were present. The compound Cyanidin-3-caffeoylsophosphorosid-5-glucoside had the highest weight in all the analyses performed regardless of environmental conditions. These results show the impact of abiotic stresses on the nutritional quality of ``Salad Blue`` potato variety and emphasize the need for increased attention to adaptive agricultural practices to mitigate the effects of climate change.

Key words: *Salad Blue potato, anthocyanins, abiotic stress, climatic conditions, nutritional quality.*

INTRODUCTION

Purple-fleshed potato varieties may be a very good choice for introduction into the human diet because they are nutritionally valuable compared to other potato varieties due to their high anthocyanin content [24]. Anthocyanins are natural pigments found in fruits and vegetables. They are responsible for their intense color, as in the case of ``Salad Blue`` potato tubers [26]. Interest in potato varieties with colored flesh has increased in recent years due to their benefits in human nutrition. Consumption of these anthocyanin-rich vegetables actually means a higher intake of antioxidants introduced into the body with anti-inflammatory effects, which reduce chronic inflammation and oxidative stress at the cellular level [38]. These compounds support the health of the cardiovascular system by improving endothelial function and reducing the risk of cardiovascular disease. Anthocyanins are stimulants of the immune system and help the body to fight infections, supporting good health [22], [29]. The relatively recent presence of these varieties in potato cultivation may bring challenges in terms of the level of public knowledge about the significant nutritional value of these colored potatoes. Even if there is a well-documented scientific basis, consumer information remains an important tool to increase interest and demand for these and also other food by-products [7], [4]. Studies suggest that the promotion of potato varieties high in anthocyanins may have a positive impact on public health [23]. Finally, the cultivation and promotion of purple-fleshed potatoes offers an agronomic advantage by diversifying supply and improving consumer health by accessing a natural source of essential bioactive compounds. Another theme of global value is climate change which presents a challenge for areas such as agriculture and for their develeopment under sustainability and circular economy concepts [14], [4]. These climate changes were directly affecting agriculture by increasing abiotic stress in established and ideal cropping areas decades ago. Increased abiotic stress can be manifested by the intensification and prolongation of periods of extreme events such as droughts and above-average temperatures, or at the other extreme by short but intense rainfall or floods. In addition to direct actions on crops through the intensification of extreme events, climate change also indirectly influences agriculture by requiring the adoption of global sustainable policies that have as main objective strategies to reduce the carbon footprint [17], [30], [13]. Abiotic stress in agriculture can be a major risk factor for food security. This phenomenon can reduce the quantity and quality of agricultural products. Moreover, agricultural areas are vulnerable to degradation, being exposed to different types of erosion. Consequently, the land can be placed in categories such as infertile or degraded, which makes it difficult to cultivate them with agricultural crops valuable for human food [34], [1], [21]. The environmental conditions, such as altitude, temperature, humidity and soil composition, are decisive factors in the accumulation of anthocyanins in plants.

The literature emphasizes that at higher altitudes anthocyanin levels increase because plants are exposed to more intense UV radiation.

As a result, plants react by producing more protective pigments to reduce radiation stress. In the same defense scenario, plants can produce more pigments under high temperatures and water stress.

The role of these pigments in plants, in the above mentioned scenarios, is to protect cells against damage caused by reactive oxygen species [20].

In the literature, researches have been identified showing variability in terms of anthocyanin accumulation in plants due to abiotic stress experienced. This variability in fact means that some varieties may accumulate more or less anthocyanins. Depending on the variety, anthocyanin biosynthesis may change. Some varieties may accumulate a higher amount of anthocyanins, while other varieties, under the same stress conditions, may decrease the amount of anthocyanins accumulated in the control. These findings show the importance of research, utilization and adaptation of specific varieties for specific cropping areas [16], [28]. For potato crops where colored-fleshed varieties are used, it is necessary to identify and develop stress resistant varieties to maintain a high quality and quantity of production while maintaining their outstanding nutritional benefits. Therefore, in this context, the present work highlights the interaction between ``Salad Blue`` genotype, environmental conditions and anthocyanin accumulation, being a part of a wider research started in the two localities of Mandra [11] and Rusciori [10].

The aim of this study is to determine and evaluate the reaction of ``Salad Blue`` variety to abiotic stress factors by measuring the amount of anthocyanins in potato tubers.

The objectives of this study are:

i. To analyze the reaction of ``Slad Blue`` variety to hydric and thermal stress by evaluating the amount of anthocyanins;

ii. To identify the trends of increase or decrease in accumulation according to the intensity of abiotic stress;

iii. Comparison of anthocyanin levels as a function of environmental conditions at the two locations to highlight differences in stress response of ``Salad Blue`` variety.

MATERIALS AND METHODS

Description of the experimental site

The present study was conducted in two experimental fields, in two different localities. Mandra locality, in Brasov county and Rusciori locality in Sibiu locality, both localities being on the territory of Romania.

Climatic conditions

The climatic conditions (temperature and precipitation) for both experimental years are shown in Table 1.

Tabel 1. Climatic conditions in the two locations of the experimental fields

	Sum of degree (°C)		Rainfall (mm)	
	2023	2024	2023	2024
Rusciori	3,211	3,728	251	272
Mandra	3,140	3,311	381	217

Source: original.

Description of biological material

Tubers of the ``Salad Blue`` variety were analyzed in the experiment (Figure 1). The planting material was obtained from the Agricultural Research and Development Station Targu Secuiesc in Romania. The variety `Salad Blue` is native to Scotland and is suitable for both organic and conventional cultivation. The tuber shape is elongated oval, the rind is purple and the flesh is purple. The inflorescence consists of simple buds with blue-purple flowers. The variety belongs to the early maturity group. It is resistant to the pathogenic golden nematode RO1. The culinary quality is good, its utilization group is A/B.

Anthocyanin analyses were performed on tubers harvested from the two experimental camps in which these seed tubers were used.

The experimental procedure

During the growing season the potato plants received no fertilizer and precipitation was not supplemented by irrigation. A detailed description of the experimental design and experimental procedure can be found in previous papers [11], [10].

Data collection and analyzing

After harvesting the potato tubers, six medium-sized tubers were randomly selected, washed (Fig. 1) and sliced.

Fig. 1. Salad Blue tubers ready for extraction
Source: original.

Then the slices were dried in a lyophilizer (Ilshin Lab Co. Ltd., South Korea). After drying the slices, the potatoes were mashed in a Grindomix GM 200 knife mill (Retsch, Germany) to powder.

Anthocyanins extraction

Anthocyanin extraction was done from one gram of sample extracted with 5 ml of methanol acidified with 1% HCl at 37% concentration by vortexing for 1 min at Heidolph Reax top vortex for 1 min, followed by a sonication bath for 15 min. In Elmasonic E 15 H sonication bath, followed by centrifugation at 10,000 rpm for 10 min and a temperature of 240°C on Eppendorf AG 5804 centrifuge. The supernatant was collected, and the above operations were repeated until complete decolorization of the sample. The extract was concentrated by vacuum evaporation at 400°C on the Heidolph Hei-VAP Expert Heidolph rotaevaporator to a final volume of 1 ml, then filtered through a 0.45 µm Chromafil Xtra nylon 0.45 µm Chromafil Xtra nylon filter and 20 µl was injected into the HPLC system.

Chromatographic conditions

The Agilent 1200 HPLC system equipped with quaternary pump, solvent degasser, autosampler, UV-Vis photodiode array detector (DAD) coupled with Agilent model 6110 single quadrupole mass detector (MS) (Agilent Techologies, CA, USA) was used for chromatography of the samples. The separation of the compounds was performed on a Kinetex XB C18 column, size 4.6 x 150 mm, with 5 µm particles (Phenomenex, USA), using the mobile phases (A) water + 0.1% acetic acid and (B) acetonitrile + 0.1% acetic acid in the gradient below, for 30 min, at a temperature of 250 °C, at a flow rate of 0.5 ml/min. Gradient (expressed in % B): 0 min, 5% B; 0-2 min, 5% B; 2-18 min, 5%-40% B; 18-20 min, 40%-90% B; 20-24 min, 90% B; 24-25 min, 90%-5% B; 25-30 min, 5% B. Spectral values were recorded in the 200-600 nm range for all peaks. Chromatograms were recorded at wavelengths λ = 520 nm. For the MS, full scan ionization positive ESI mode was used under the following working conditions: capillary voltage 3,000 V, temperature 3,000°C, nitrogen flow rate 7 l/min and m/z 120-1,200. Data acquisition and interpretation of the results was done using Agilent ChemStation software, version B.02.01 SR2.

Chemical reagents and materials

Acetonitrile, of HPLC purity, was purchased from Merck (Germany) and ultrapure water was purified with the Direct-Q UV system from Millipore (USA). Cyanidin standard was purchased from Sigma-Aldrich (USA).

Identification and quantification of anthocyanins

Anthocyanins were identified by comparing retention time, UV-Vis absorption and mass spectra with those of standard compounds and literature data.

For the quantification of anthocyanins, a calibration curve was performed by injecting 5 different concentrations of standard cyanidin dissolved in methanol (Fig. 2).

The equation of the curve was used for the quantitative calculation for each anthocyanin, and the result was expressed as cyanidin equivalent.

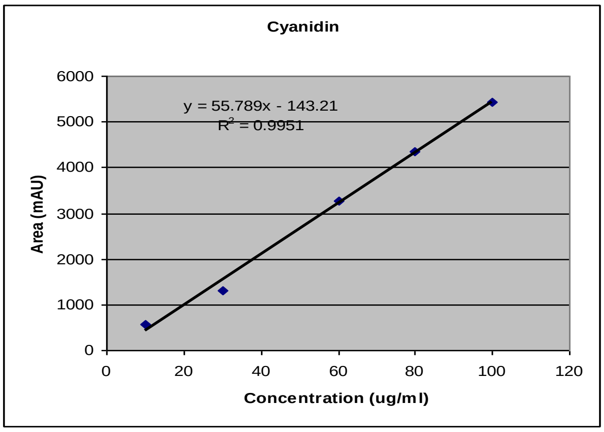

Fig. 2. Curba de calibrare pentru diferite concentratii de cyanidina
Source: original.

Data analysis

All data collected from the laboratory analyses were interpreted by IBM SPSS program, applying Tukey's test, using a one-way analysis of variance with two variables. The variables being represented by each experiment (year+location) and the anthocyanin levels in the tubers.

RESULTS AND DISCUSSIONS

Climatic conditions

According to the literature, the sum of degrees in terms of potato crop is in the range of 1,500 - 3,000°C [36], [5], [18], [8]. In the present experiment, the sum of degrees in both years exceeded the theoretical maximum threshold of 3,000°C, even exceeding the threshold of 3,700°C in 2024, in the locality of Rusciori (Figure 2 (up)), which means that the potato plants in that experiment suffered severe heat stress [35], [32] [6], [19].

(a)

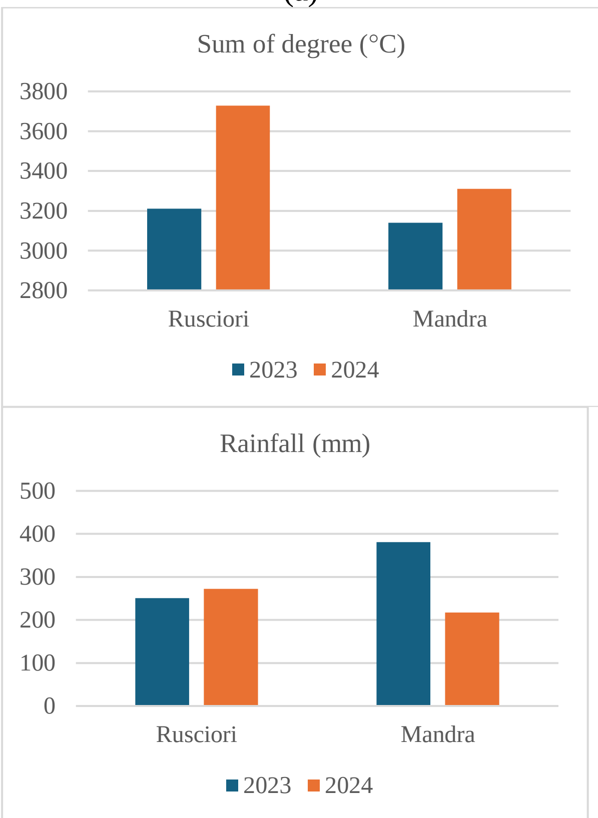

Fig. 3. Sum of degrees plotted in the four experimental years (Up) and Sum of precipitation plotted in the four experimental years (Down).
Source: Original.

In terms of precipitation, the water stress of potato plants was accentuated, because during the growing season, potato crop requires precipitation between 500 and 650 mm [25], [9], [27]. In the experimental fields, the highest amount of precipitation was recorded in 2023, in the locality of Mandra, reaching just over 380 mm, and the lowest value was observed in 2024, also in the locality of Mandra, when precipitation was below 220 mm (Figure 2(b)). These conditions resulted in severe water stress for the experiment [15], [31], [2].

Identification and quantification of anthocyanins

The results on the average amount of anthocyanins for each experimental field and each year were centralized in Table 2.

Table 2. Average anthocyanin compounds in potato tubers from experimental fields (mg/100g)

Experimental site	Mandra 2023	Mandra 2024	Rusciori 2023	Rusciori 2024
Means	352.643[b]	428.544[c]	413.139[a]	291.196[d]

Note: *p<0.5*
Source: original.

At the same time, the types of anthocyanins identified in potato tubers of ``Salad Blue`` variety in the four experimental years were centralized and classified, the means of the results being centralized in Table 3.

Table 3. Average of each anthocyanin compound in potato tubers from experimental fields (mg/100g)

Compound	Cyanidin-3-caffeoylsophoroside-5-glucoside	Peonidin-3-caffeoylsophoroside-5-glucoside	Peonidin-3-dicaffeoylsophoroside-5-glucoside
Means	244.518[a]	161.696[c]	201.546[b]

Note: *p<0.5*
Source: original.

Stress reaction

Analyzing the total results obtained from the two years of experience with ``Salad Blue`` in the two localities, an inversely proportional accumulation of anthocyanin compounds with the climatic stress experienced by the plants can be observed.

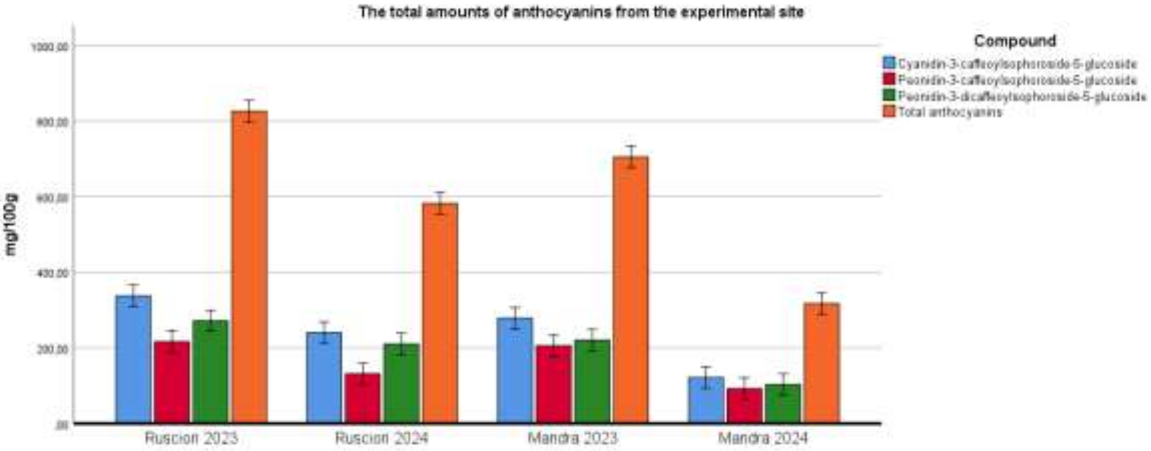

Fig. 4. Graphic representation of anthocyanin compounds within the experimental fields
Source: original.

According to Figure 4 the highest amount of accumulated anthocyanins was identified in the locality of Rusciori, in the year 2023 with a total mass over 800 mg of anthocyanins per 100g. The experiment in Rusciori locality of year 2023 coincides according to Figure 2 (Up) and (Down) with less stressful climatic conditions compared to other years. At the opposite pole are the biochemical analyses of anthocyanins for the experiment in the locality of Mandra, year 2024, which has the lowest accumulated anthocyanin mass, below 400 mg/100g (Figure 3) and coincides with the most stressful conditions from the hydric point of view, accumulating the least precipitation (Figure 2 (Down)). In the literature it is specified that the accumulation of anthocyanins under abiotic stress conditions may lead to an increase in their accumulation in potato plants or to their reduction, depending on the traits [37], [12], [33], [3]. In our case the total amount of anthocyanins decreased due to climatic stress.

Observing each anthocyanin compound studied, according to Figure 3 and Table 3, regardless of the year and location studied, there is a consistency in the size of the accumulated masses. The highest accumulated mass in all 4 experiments was Cyanidin-3-caffeoylsophoroside-5-glucoside, and the lowest mass was Peonidin-3-caffeoylsophoroside-5-glucoside.

CONCLUSIONS

In conclusion, the present study confirms and demonstrates that the accumulation of anthocyanin compounds is closely related to environmental conditions, as is the case in the literature.

For the ``Salad Blue`` variety, the accumulation of anthocyanins is inversely proportional to the intensity of heat and water stress.

The 2023 experiment, in the Rusciori locality, had a lower intensity of abiotic stress, and the accumulation of anthocyanins was the highest within the experiment.

At the opposite pole, the 2024 experiment was identified, in the Mandra locality, where the intensity of abiotic stress was the most pronounced and the accumulation of anthocyanins was the lowest.

Regarding the distribution of accumulated anthocyanin compounds, we identified a consistency in the mass stability as follows: Cyanidin-3-caffeoylsophoroside-5-glucoside had the highest mass, followed by Peonidin-3-dicaffeoylsophoroside-5-glucoside and Peonidin-3-caffeoylsophoroside-5-glucoside, with the lowest mass.

This ranking, in terms of the accumulation of the three anthocyanin compounds, was not influenced by the abiotic stress during the field experiments.

Regardless of the environmental conditions, the proportion of the three compounds remained the same.

These findings highlight the impact of abiotic stress on the nutritional quality of “Salad Blue” potato soils and emphasize the need for adaptive agricultural practices to mitigate the effects of climate change.

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