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IMPACT OF N FERTILIZATION AND CULTIVAR ON AMARANTH NUTRIENTS AND SOIL HEALTH

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ABSTRACT

The purpose of this study was to assess the effects of nitrogen (N) fertilization and cultivar on vitamin C content, total antioxidant capacity (TAC), and catalase (CAT) activity in amaranth leaves, as well as in the rhizosphere and non-rhizosphere soil. For this purpose, a 3-year split-plot field experiment was conducted, which included the following factors: N fertilization (kg · ha^{-1}: N0 – control, N1 – 60, N2 – 90, N3 – 120, N4 – 150), amaranth cultivars ('Rawa' and 'Aztek') and developmental stages (BBCH 13, BBCH 16, BBCH 19). The factor that most significantly differentiated the vitamin C content in the leaves was the cultivar, followed by the development stage, N fertilization, and weather conditions. The cultivar 'Aztek', stage BBCH 13, N3 fertilization, and weather conditions during the last year of the experiment gave the best results in this regard. CAT activity in the leaves significantly depended on N fertilization and developmental stage. It increased with the higher N dose and decreased with plant development. The TAC of the leaves depended only on the developmental stage and weather conditions. The highest TAC was observed at BBCH 13 and the third year of the study. CAT in the rhizosphere significantly depended on N fertilization, cultivar, and developmental stage, while in the non-rhizosphere zone, it depended on N fertilization, developmental stage, and weather conditions. This study is an essential addition to the knowledge on the use of amaranth seed forms as a vegetable with high nutritional value and antioxidant properties, as well as the effect of this plant on soil biological properties.

Key words: leaves, N fertilization, vitamin C, total antioxidant capacity (TAC), catalase (CAT) activity, soil, rhizosphere zone

INTRODUCTION

Amaranth is one of the world's oldest plants, cultivated as early as over 4,000 BC. Its history is closely linked to pre-Columbian civilizations of the New World, such as the Aztecs and the Incas. There is evidence that it was the main crop of the Aztecs, utilized in various ways, although primarily for food purposes [Weerasekara et al. 2019]. Amaranth served mainly as a staple food source for the local population, with its seeds used to produce flour and alcoholic beverages (such as chicha and beer) [Bodroža Solarov et al. 2022]. The green parts of this plant were also consumed, particularly the young shoots and leaves [Ogwu 2019]. The ornamental form of amaranth was first introduced to Europe around the 17th century.

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Interest in the seed form of this plant arose after a thorough examination of the chemical composition of its seeds, which took place only in the 1970s [Nalborczyk et al. 1994]. Amaranth seeds are a source of valuable nutrients, minerals, and vitamins in the human diet. Numerous scientific studies have provided insights into various amaranth species' nutritional, ecological, agricultural, and health benefits [Skwaryło-Bednarz et al. 2020]. Among the species of amaranth belonging to the genus *Amaranthus*, the seeds of *A. cruentus*, *A. hypochondriacus*, and *A. caudatus* have the highest nutritional value [Skwaryło-Bednarz et al. 2020]. Among others, these seeds contain a high amount of protein, averaging 13.6%, with a complementary amino acid profile, fiber, gluten-free starch with a small grain diameter, and fats that include valuable antioxidants [Venskutonis and Kraujalis 2013]. The aerial parts of vegetable and seed forms of amaranth species, recommended for consumption as vegetables similar to spinach or lettuce, are also valuable in terms of chemical composition. Currently, grain-form cultivars' tender leaves are often utilized the same way as leafy amaranth [Aderibigbe et al. 2020]. In East and West Africa, especially in Nigeria, amaranth is a common vegetable [Ruth et al. 2021]. *A. cruentus, A. dubius, A. blitum,* and *A. tricolor* are among the most popular leafy amaranths cultivated worldwide. According to market demand, *A. cruentus* is cultivated for vegetables, fodder, and grain [Aderibigbe et al. 2020]. Amaranth leaves contain varying amounts of polyphenols, betacyanins, saponins, hemagglutinins, phytin, as well as nitrate (V) and oxalate compounds [Baraniak and Kania-Dobrowolska 2022]. They serve as a source of various minerals such as Ca, P, K, Mg, Fe, and vitamins including A, B_1, B_2, C, and E [Skwaryło-Bednarz and Nalborczyk 2006, Skwaryło-Bednarz et al. 2020].

Amaranth responds very well to nitrogen (N) fertilization, both its seed and vegetable forms [Ayodele 2002, Skwaryło-Bednarz et al. 2011, Pelech 2021]. However, it is essential to fertilize carefully with nitrogen to avoid over-fertilization, which leads to excessive growth and development of the vegetative parts, a considerable prolongation of seed maturity, and an accumulation of harmful nitrates in the aerial parts of the plants [Skwaryło-Bednarz and Nalborczyk 2006, Gimponger et al. 2007]. In conditions of high nitrogen availability, amaranth tends to accumulate nitrates mainly in tissues rather than seeds [Alegbejo 2013]. Studies have demonstrated that frequently in leafy vegetables, including amaranth, sold in the eastern and northeastern parts of India, the nitrate content was higher than the acceptable daily intake (ADI) for an average person (approx. 60 kg) [Jana and Moktan 2012]. High levels of nitrates are hazardous for livestock fed a poorly diversified feed with nitrogen-overfertilized plants [O'Brien and Price 2008]. In the human digestive tract, 5–20% of nitrates involving anaerobic bacteria are converted to toxic nitrites, which are transformed into carcinogenic nitrosamines [Karwowska and Kononiuk 2020]. Excessive nitrogen fertilization also leads to plantation lodging and increased susceptibility to disease pathogens, which affects the final yield and quality [Skwaryło-Bednarz and Nalborczyk 2006]. Hence, the selection of an appropriate nitrogen dose is crucial in the cultivation of different amaranth species. In the 1990s, pre-sowing application of nitrogen (N) in the form of ammonium nitrate or urea in the amount of 80–120 kg · ha^{-1} was recommended in the cultivation of amaranth in Poland (after the introduction and breeding of domestic cultivars) [Skwaryło-Bednarz and Nalborczyk 2006]. It should be noted that the current plant fertilization strategy (e.g., in integrated production, IP) is based on systematic soil analysis, allowing for monitoring changes in nutrient levels and adjusting appropriate fertilizer doses. This approach has economic justification and helps reduce excessive fertilization while maintaining optimal nutrient content, leading to good-quality yields. Determining nitrogen fertilization doses is also crucial from the perspective of the vitamin C content in the leaves of vegetable plants [Acikgoz et al. 2014]. Excessive use of nitrogen fertilizers, in the case of certain vegetables, can lead to a reduction in their leaf vitamin C content [Lee and Kader 2000]. It should be noted that in addition to nitrogen fertilization, the vitamin C content in plants is influenced by various biotic and abiotic factors, such as plant genotype, light, temperature, fertilization with other macro- and micronutrients or irrigation [Lee and Kader 2000, Hancock and Viola 2005]. The aforementioned factors can also affect changes in the dynamics of enzyme activity such as catalase in the plant [Gurgul and Herman 1994, Telesiński et al. 2009]. Changes in catalase activity, as well as other antioxidant enzymes, can be induced by various envi-

ronmental stressors, such as high temperature, salinity, UV radiation, heavy metals, xenobiotics, or pathogens, leading to increased production of reactive oxygen species in plant cells [Telesiński et al. 2009]. Hence, the activity of the cellular antioxidant system can be assessed by measuring the catalase activity in the plant and even in different plant organs. Decomposition of toxic hydrogen peroxide in the soil is caused by organic compounds that exhibit antioxidant and mineral activities, such as heavy metal oxides, transition-metal ions – Fe^{2+}, $Cu^{1+,}$ and microorganisms containing the enzyme catalase [Bartosz 2003].

The study aimed to determine the effect of increasing N doses and amaranth cultivars on:

– vitamin C content and catalase activity in leaves,

– total antioxidant capacity of leaves in three initial developmental stages, BBCH 13, BBCH 16, and BBCH 19, as well as

– catalase activity in the rhizosphere and non-rhizosphere zones of the soil under amaranth cultivation.

MATERIAL AND METHODS

Field trial. The basis of the study was a field experiment conducted between 2015 and 2017 on an individual farm located in Bodaczów (50°71'N, 23°04'E) near Zamość, in the Lublin Voivodeship, Poland. The experiment was set up using a split-plot method in a randomized block design with three replications. The plot size was 3 m² (2m×1.5m). The experiment was established on brown soil derived from loess. Each year, before setting up the experiment, soil samples were collected for chemical analysis, determining the content of phosphorus (P), potassium (K), magnesium (Mg), organic matter, and soil pH. The abundance values for these minerals were within the high or medium content range. Each year, the humus content was typical for brown soil, and the pH was neutral (Tab. 1). The forecrop of amaranth in 2015 was winter wheat; in 2016, spring wheat with spring barley intercrop, and in 2017 – spring wheat with white mustard intercrop.

The following variable factors were adopted in the experiment – N fertilization (n = 5), cultivars (n = 2), repetitions (n = 3) (total n = 30):

– different variants of N fertilization (kg · ha^{-1}): N0 – control, N1 – 60, N2 – 90, N3 – 120, N4 – 150, with a constant value of P_2O_5 and K_2O fertilization (60 kg · ha^{-1} each),

– two cultivars:'Rawa' and 'Aztek'.

Additionally, the variable factor was the developmental stage of amaranth: 1-BBCH 13, 2-BBCH 16, and 3-BBCH 19, which did not affect the number of plots per experiment.

All tillage and cultivation practices followed generally accepted principles of good agronomy. Fertilization with phosphorus in the form of granulated single superphosphate $Ca(H_2PO_4)_2$ (fertilizer composition – 19% P_2O_5, 28% SO_3, 10% CaO, Fosfan S.A.) and potassium in the form of potassium salt KCl (fertilizer composition – 60% K_2O, Luvena S.A.) was applied in the autumn. Nitrogen in ammonium nitrate NH_4NO_3 (32% N, Grupa Azoty, Puławy) was applied once to the soil in the spring before sowing. Seeds of two amaranth cultivars ('Rawa' and 'Aztek') of full utility value were sown at a rate of 2.0 kg · ha^{-1} using a plot seeder (Tool Carrier 2700) with row spacing of 50 cm. Sowing was performed each year in the third decade of May. In each year of the field experiment, certified seed material was provided free of charge by "Szarłat" M. and W. Lenkiewicz Sp. J., Cibory Galeckie 46 near Łomża (Poland).

Before the experiment was completed, manual and mechanical field works (using a 4-blade hoeing machine with a width of 24 cm) were carried out only once to remove excessive weeds in the plantation. Among the monocotyledonous weeds, *Echinochloa crus-galli* L. was the most abundant, and of the dicotyledons – *Chenopodium album* L. and *Thlaspi arvense* L. Each year, the experiment did not require protection from pathogens and pests, which could be challenging due to the lack of plant protection products designed for amaranth cultivation in Poland.

Vitamin C content in leaves. The vitamin C content in amaranth leaves at the studied developmental stages was determined using Tillman's method according to the PN-90/A-75101/11 standard [1994]. It is a titration method involving a color reaction between ascorbic acid and a solution of 2,6-dichloroindophenol (Tillman's reagent) – a titrant solution (2,6-dichloroindophenol) of approx. 3.5×10^{-4} mol · dm^{-3} was titrated directly before the analysis to ascorbic acid standard solution. The homogenized biological material was weighed (average weight of the sample – 1 g), and

Table 1. Chemical parameters of the soil

Parameters	Years of study		
	2015	2016	2017
P (mg · kg^{-1})	155	165	160
K (mg · kg^{-1})	217	212	210
Mg (mg · kg^{-1})	118	125	110
pH in l mol · dm^{-3} KCl	6.9	6.8	6.8
Humus content (g · kg^{-1})	21.9	22.3	22.2

25.0 cm^3 of a 2.0% hydrochloric acid solution was added. It was titrated until a light pink color appeared, which persisted for 10 seconds. Each determination was performed in triplicate.

Sample extraction for determination of total antioxidant capacity (TAC) in leaves. At the specified developmental stages of amaranth, the youngest leaves were collected from 5 random plants from each microplot. Extracts were prepared according to the method of Proestos et al. [2013] with modifications. To extract 1 g of ground, dried leaves from each cultivar, 50 ml of 90% aqueous methanol was used in a tightly sealed bottle (100 ml). The samples were extracted for 1 hour using a shaken water bath (single-chamber W110E or W415, Laboplay, Poland). The extracts were filtered to determine total antioxidant capacity.

Determination of total antioxidant capacity (TAC) in leaves. To assess the antioxidant activity of amaranth extracts, the method of radical degradation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was employed following the procedure described by Alvarez-Jubete et al. [2010] with modifications. The prepared mixture consisted of 500 µl of leaf extract solution, 500 µl of a freshly made DPPH solution (0.05 mg · ml^{-1}) and 2 ml of water. The mixture was then placed in the dark for 30 minutes at room temperature. The absorbance was measured against methanol at a wavelength of λ = 515 nm using a UV-VIS spectrophotometer (Metash, Shanghai). The antioxidant capacity of the sample was then expressed as Trolox equivalent antioxidant capacity (TEAC) values (TEAC µg · g^{-1} DW).

Catalase (CAT) activity in leaves. The youngest leaves were sampled from 5 randomly selected plants in each plot in the examined developmental stages. CAT activity in leaves was determined using a modified method of Beck [Brauner and Bukatsch 1987]. Freshly collected leaves were finely chopped, 1 g was weighed, the sample was homogenized and transferred to an Eijkman tube (Labor Szkło, Lublin), and 25 ml of 3.0% H_2O_2 was added. Every minute, the height of the oxygen column released from the solution was measured in centimeters, and the results were averaged. The unit of catalase activity (U) is the volume of oxygen in milliliters released per 1 gram of air-dried plant material per minute (adjusted for moisture content). Each assay was conducted in triplicate.

Catalase (CAT) activity in rhizosphere and non-rhizosphere soil zones. Soil samples were taken from the humus horizon by shaking five randomly selected plants from each microplot at BBCH 13, BBCH 16, and BBCH 19 stages. The rhizosphere zone included soil less than 4 mm from the roots [Bielińska 2007]. Soil without roots was also sampled from the humus horizon and considered non-rhizosphere soil. The soil samples were averaged for each microplot. Sample preparation for the analysis involved air-drying, grinding in a porcelain mortar, and sieving through a 1 mm mesh. Catalase activity was measured by the volumetric method using an Eijkman tube in freshly collected soil samples and converted to air-dried weight after accounting for moisture content. The catalase activity (U) unit is the volume of oxygen in milliliters released per 1 gram of air-dried plant material per minute. Each assay was conducted in triplicate.

Weather conditions. In 2015, amaranth sowing coincided with a wet and generally warm May (total precipitation – 91.1 mm, average monthly temperature – 13.6°C) – Fig. 1, 2. In the subsequent months of am-

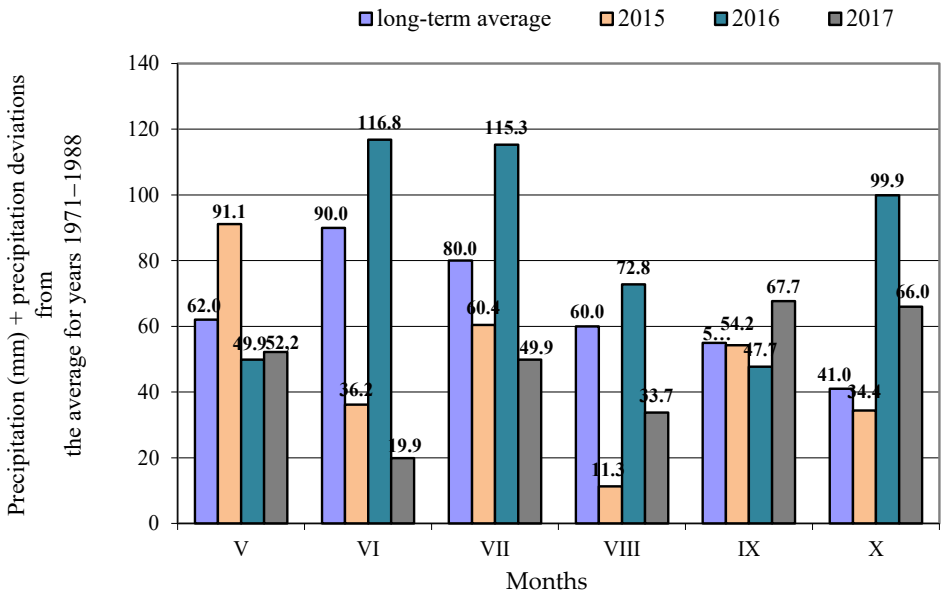


Fig. 1. Total precipitation (mm) for the years 2015–2017 and the long-term average for 1971–1988 (Testing Station in Zamość)

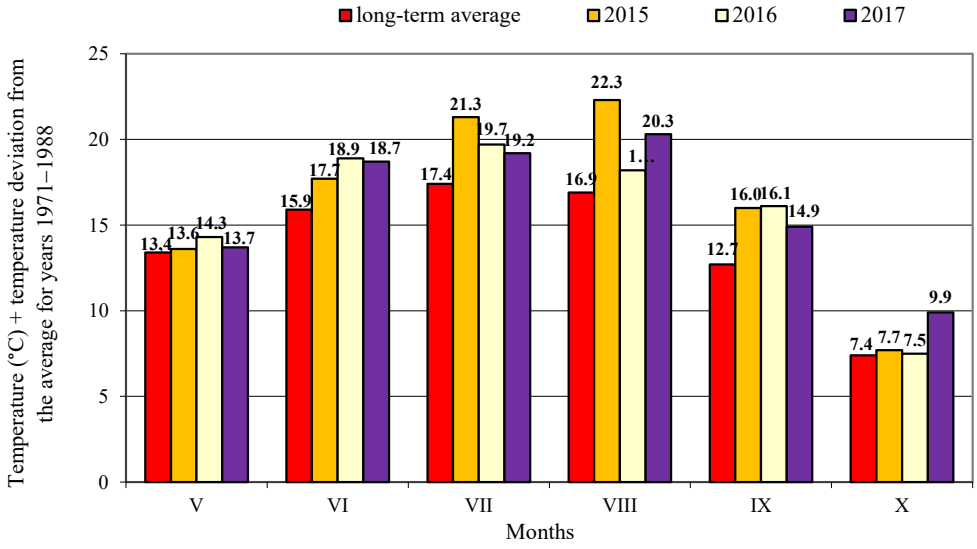
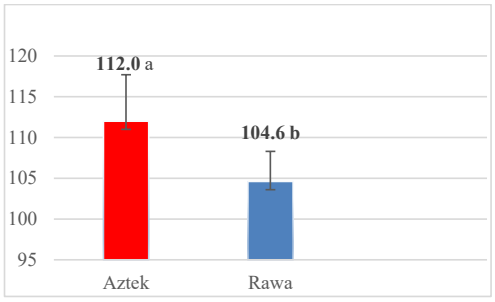


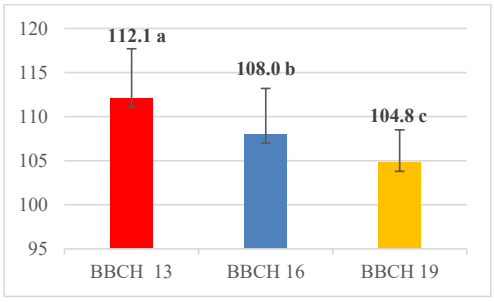
Fig. 2. Average monthly air temperatures (°C) for the years 2015–2017 and the long-term average for 1971–1988 (Testing Station in Zamość)

aranth growth, the average air temperature was higher compared to the long-term average (Fig. 2). From June to the end of amaranth vegetation, total precipitation in the study area was lower than the long-term average (Fig. 1). The shortage of precipitation, especially during the early developmental stages, contributed to soil dryness. In 2016, amaranth sowing coincided with a warm spring and rainfall deficits (total precipitation – 49.9 mm, average monthly temperature – 14.3°C) – Fig. 1, 2. Temperatures above the long-term average persisted throughout the growing season and were lower than in the first year of the study except for July



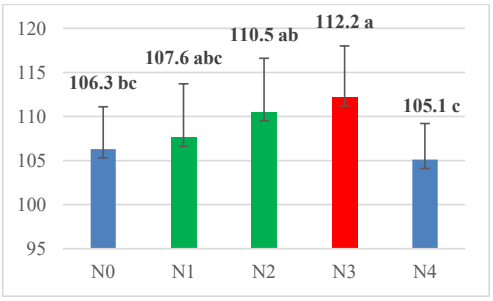
F = 52.4969, p = 1.56 · 10^{-10}, LSD = 3.3

(a)



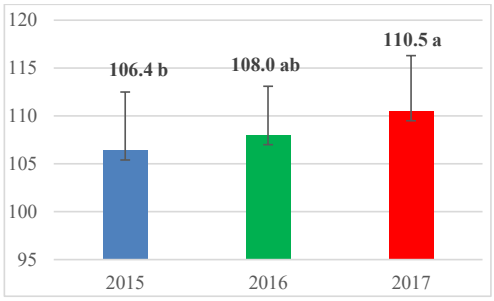
F = 13.9314, p = 5.64 · 10^{-6}, LSD = 3.2

(b)



F = 4.9748, p = 0.001, LSD = 5.2

(c)



F = 3.6606, p = 0.03, LSD = 3.7

(d)

Fig. 3. The vitamin C content in amaranth leaves (mg · 100 g–1 FW) (means for factors and years, the arrangement of consecutive plots depends on the level of significance) – (a) cultivar, (b) developmental stage, (c) N doses, (d) study years (Tukey's mean separation test, $p \leq 0.05$)

and August. Excess precipitation was observed from June to October, except for September, significantly above the long-term average (Fig. 1).

In 2017, from sowing through the growing season until harvest, temperatures recorded were higher than the long-term average, and a significant shortage of precipitation was noted until September (Fig. 1, 2). The hydrological situation improved only in September and October, during the final stages of development, but it did not hinder the harvest of amaranth (Fig. 1).

Statistical analysis. The data collected were presented as means from individual study years ± standard deviation (SD). Statistical analysis was conducted using analysis of variance (ANOVA) with Snedecor's F-test, and the probability of the F distribution was calculated. The significance of differences was assessed using Tukey's test ($\alpha = 0.05$), which was subsequently subjected to post-hoc analysis. Correlation and linear regression analyses were performed to determine the relationship and association between TAC and vitamin C content. Statistical analysis was conducted using Excel 7.0 and Statistica (StatSoft Polska, 2013). Excel 7.0 was also used to prepare the figures. The results were presented as means ± standard deviation (SD).

RESULTS

Vitamin C content in leaves. All examined factors of the experiment influenced the vitamin C content in amaranth leaves, with the cultivar and developmental stage having the most significant impact (Fig. 3a-d). The cultivar 'Aztek' accumulated 112.0 mg · 100 g^{-1}

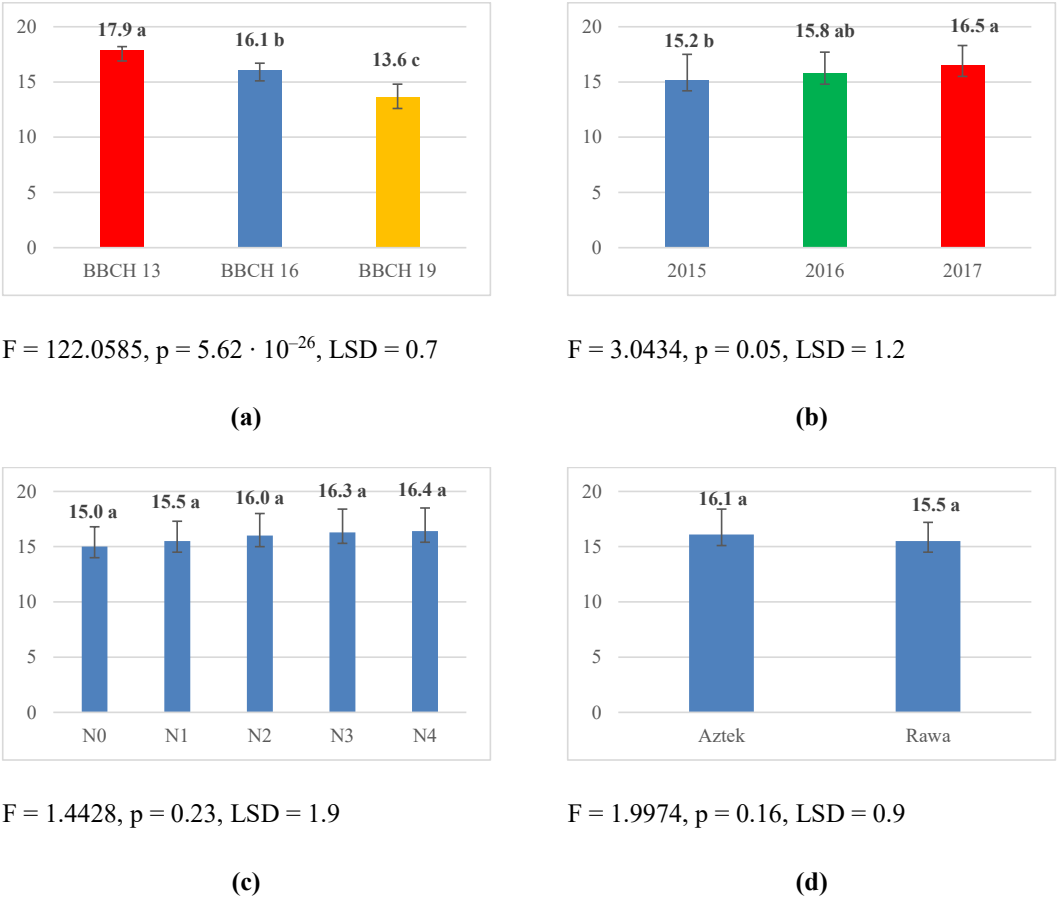


Fig. 4. TAC of amaranth leaves (TEAC µg · g–1 DW) (means for factors and years, the arrangement of consecutive plots depends on the level of significance) – (a) developmental stage, (b) study years, (c) N doses, (d) cultivar (Tukey's mean separation test, $p \leq 0.05$)

of vitamin C, which is 7.1% more than the cultivar 'Rawa' (104.6 mg · 100 g^{-1}) (Fig. 3a). Significantly, the highest amount of vitamin C was found in amaranth leaves in the developmental stage BBCH 13 (112.1 mg · 100g^{-1}). In the subsequent developmental stages, BBCH 16 and BBCH 19, the vitamin C content significantly decreased (108.0→104.8 mg · 100 g^{-1}) (Fig. 3b). Fertilization with increasing doses of nitrogen and weather conditions during the years of the study had a slightly smaller impact on the accumulation of vitamin C in amaranth leaves. Fertilization with increasing doses of N promoted the accumulation of this vitamin up to N3, where the highest amount was observed, reaching 112.2 mg · 100 g^{-1}. The highest dose, N4, led to a drastic reduction in the vitamin C content in the leaves, even lower than that of the non-fertilized control (N0) – Fig. 3c. The weather conditions during the study years had the least significant effect on the vitamin C content in amaranth leaves. In the last year of the study (2017), a vitamin C content of 110.5 mg · 100 g^{-1} was recorded in the leaves, i.e., 3.9% more than in the first year, 2015 (106.4 mg · 100 g^{-1}). The vitamin C content in the leaves in 2016 was 108.0 mg · 100 $g^{-1}$and did not significantly differ from the first and last year of the study (Fig. 3d).

TAC value in leaves. The TAC value was significantly dependent only on the developmental stage of amaranth and the course of hydrothermal conditions during the years of the study. The highest TAC value was recorded at the BBCH 13 developmental stage

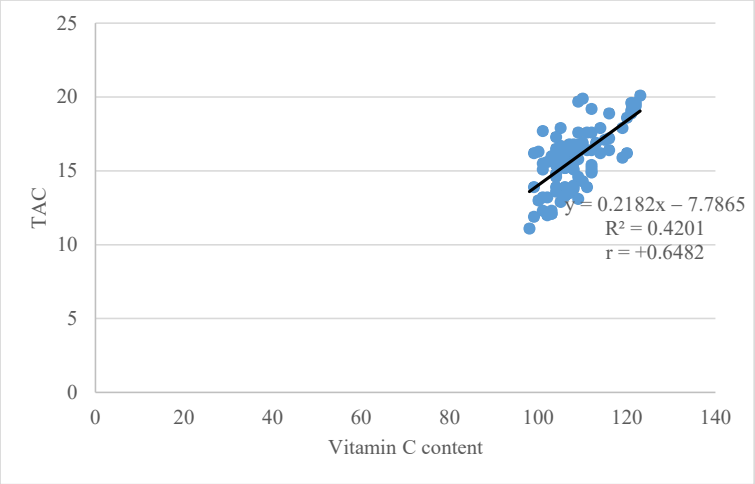
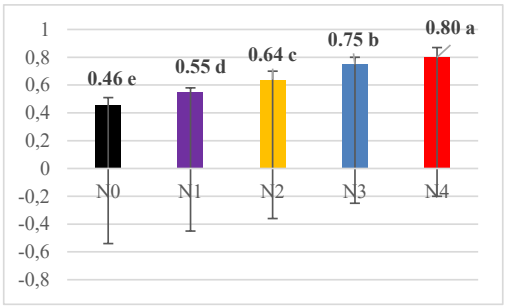


Fig. 5. Correlation between TAC and leaf vitamin C content

(17.9 TEAC µg · g^{-1} DW); it was 11.2% higher than at BBCH 16 (16.1 TEAC µg · g^{-1} DW) and 31.6% higher than at BBCH 19 (13.6 TEAC µg · g^{-1} DW) – Fig. 4a. The weather conditions during the study years also influenced the TAC value in the plant material collected from the trial plots. The plant material collected in 2017 (16.5 TEAC µg · g^{-1} DW) had a significantly higher TAC value compared to that of 2015 (15.2 TEAC µg · g^{-1} DW), with a difference of 8.6%. The TAC value in 2016 did not differ from the first and last year of the field experiment (Fig. 4b). No significant effect of cultivar and nitrogen fertilization on the differences in TAC values in amaranth leaves was observed (Fig. 4c, d). In our study, the TAC of amaranth leaves was significantly positively correlated with the vitamin C content in the leaves (r = +0.6482) – Fig. 5.

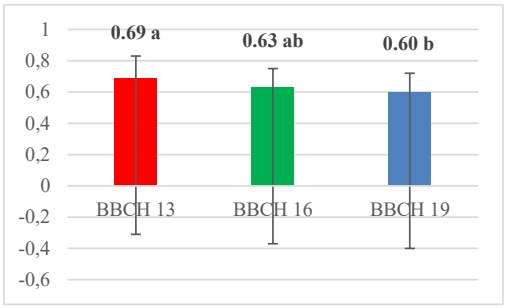
CAT activity in leaves. Of the experimental factors tested, only fertilization with increasing N doses and the developmental stage of amaranth significantly affected CAT activity in the fresh weight of leaves (Fig. 6a, b). The highest CAT activity was observed after applying the highest N dose, i.e., 0.80 U. This activity significantly decreased in the following order: N4→N3→N2→N1→N0, with a difference of 73.9% between the extreme treatments (Fig. 6a). Higher CAT activity was observed at the BBCH 13 stage (0.69 U) compared to BBCH 19 (0.60 U), with a difference of 15.0% (Fig. 6b). CAT activity at BBCH 16 (0.63 U) did not differ from the other two stages investigated (Fig . 6c). There was no significant effect of study years and cultivar on CAT in amaranth leaves (Fig. 6c, d).

CAT activity in the rhizosphere zone. The activity of CAT in the rhizosphere was influenced by fertilization with increasing N doses, genetic factors, and developmental stage of amaranth plants (Tab. 2). The highest CAT activity in the soil sampled from the rhizosphere was observed after the application of the highest N dose, i.e., N4, reaching 1.77 U. This value decreased with the introduction of lower N doses, down to 1.44 U observed in plots without N fertilization. Significant differences in CAT activity in the rhizosphere were also recorded between cultivars (Tab. 2). The cultivar 'Aztek' exhibited significantly higher CAT activity in the rhizosphere (1.71 U) compared to 'Rawa' (1.54 U), with a difference of approximately 11.0%. Significant differences in CAT activity in the rhizosphere were also observed between developmental stages of amaranth. This activity increased along with the plant development. Plants at the BBCH 19 developmental stage exhibited significantly higher CAT activity in the rhizosphere, approximately 9.0% higher than those at the BBCH 13 stage. Catalase activity in the rhizosphere at the BBCH 16 developmental stage did not differ from the other developmental stages (Tab. 2).



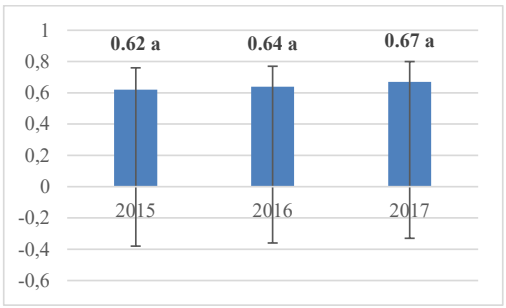
F = 114.2871, p = 2.33 · 10^{-33}, LSD = 0.05

(a)



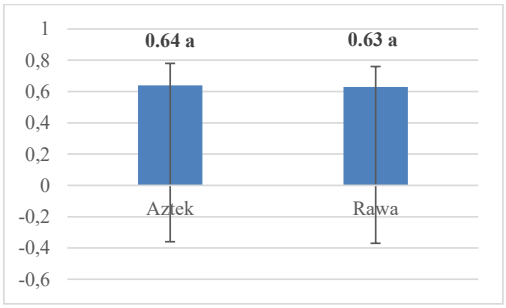
F = 3.5683, p = 0.03, LSD = 0.08

(b)



F = 1.0615, p = 0.35, LSD = 0.08

(c)



F = 0.0352, p = 0.85, LSD = 0.06

(d)

Fig. 4. TAC of amaranth leaves (TEAC µg · g–1 DW) (means for factors and years, the arrangement of consecutive plots depends on the level of significance) – (a) developmental stage, (b) study years, (c) N doses, (d) cultivar (Tukey's mean separation test, $p \leq 0.05$)

CAT activity in the non-rhizosphere zone. Catalase activity in the non-rhizosphere zone significantly depended on the cultivar, fertilization with increasing N doses, developmental stage, and weather conditions during the years of the study (Tab. 2). The cultivar 'Aztek' (1.47 U) was characterized by 16.7% higher CAT activity in the non-rhizosphere zone than the cultivar 'Rawa' (1.26 U). The influence of increasing nitrogen fertilization doses on CAT activity in the non-rhizosphere zone was similar to that in the rhizosphere, although lower values of this activity were recorded in this case. The highest activity of the tested enzyme in the non-rhizosphere zone was observed in the N4 plots (1.50 U) and the lowest in N0 (1.22 U), a difference of approximately 23.0%. Catalase activity in the rhizosphere also depended on the developmental stage, with significantly higher activity of this enzyme (1.44 U) observed in the latest examined stage – BBCH 19, compared to 1.30 U at the earliest BBCH 13 stage (Tab. 2). Catalase activity in the non-rhizosphere zone at BBCH 16 stage did not differ from the two other developmental stages. The weather conditions in the years of the trial exerted the least significant impact on the CAT activity in the non-rhizosphere zone. In the last year of the study, catalase activity in the non-rhizosphere zone was significantly higher (1.42 U, 8.4% higher) compared to the first year of the study (1.31 U). Catalase activity in the non-rhizosphere soil in the second year of the study did not differ from the first and last study years of the field experiment (Tab. 2).

Table 2. CAT in rhizosphere and non-rhizosphere (means for factors and years) (Tukey's mean separation test, $p \leq 0.05$)

Factors	CA in rhizosphere (U)	CA in non-rhizosphere (U)
N0	1.44 ±0.10 d	1.22 ±0.11 c
N1	1.57 ±0.15 c	1.30 ±0.11 c
N2	1.64 ±0.14 bc	1.34 ±0.11 bc
N3	1.70 ±0.11 ab	1.46 ±0.18 ab
N4	1.77 ±0.11 a	1.50 ±0.19 a
F	18.0368	10.9446
p	$9.32 \cdot 10^{-11}$	$3.32 \cdot 10^{-7}$
LSD	0.12	0.14
'Aztek'	1.71 ±0.13 a	1.47 ±0.17 a
'Rawa'	1.54 ±0.15 b	1.26 ±0.10 b
F	36.7599	53.2173
p	$3.26 \cdot 10^{-8}$	$1.24 \cdot 10^{-10}$
LSD	0.06	0.06
BBCH 13	1.55 ±0.17 b	1.30 ±0.19 b
BBCH 16	1.63 ±0.15 ab	1.35 ±0.16 ab
BBCH 19	1.69 ±0.14 a	1.44 ±0.15 a
F	6.5056	5.3225
p	0.002	0.007
LSD	0.10	0.10
2015	1.58 ±0.16 a	1.31 ±0.18 b
2016	1.62 ±0.16 a	1.36 ±0.17 ab
2017	1.67 ±0.16 a	1.42 ±0.16 a
F	2.2459	3.0042
p	0.11	0.05
LSD	0.10	0.11

DISCUSSION

Vitamin C content in leaves. Worldwide, amaranth seed producers often promote a production system in which the seeds and leaves are harvested. When amaranth is used extensively in the human diet, growers should prudently manage macronutrient fertilization, especially N, to prevent excessive accumulation of nitrates in the leaves [Jana and Moktan 2012]. It is also important, if necessary, to use the appropriate pesticides and recommended doses to avoid exceeding the allowed levels of these compound residues in plants. When growing amaranth in Poland, it is possible to cultivate this crop using non-chemical protection methods against pests and weeds. It reduces the likelihood of crop contamination with chemical plant protection agents. In Europe (Denmark), research is also being conducted on using low-intensity defoliation to cultivate amaranth species characterized by high aerial biomass, aiming to maintain good seed quality [Hoidal et al. 2019]. In such cases, particular caution should be exercised if leaves are planned to be used in the human diet. Amaranth leaves have a high nutritional value, containing more than 29.46% protein, 7.89% fat, 19.29% carbohydrates, 8.98% fiber, 24.3% ash, 330.3 mg · 100 g^{-1} vitamin C, more than 399.4 IU · 100 g^{-1} vitamin A, as well as other valuable vitamins and minerals [Kambabazi et al. 2021]. Currently, they can serve as a significant source of nutritional supplementation for the population in poor African countries, including Rwanda, provided there is a further increase in local awareness in this regard [Kambabazi et al. 2021]. Amaranth leaves are particularly rich in vitamin C. The daily serving of vegetables with a valuable chemical composition, such as spinach, in the USA is 30 g FW [USDA 2015]. It can be assumed that it is very similar to amaranth. According to the U.S. Food and Drug Administration, an adult's daily dose of vitamin C is 60 mg/day [U.S. FDA 2013]. Jiménez-Aguilar and Grusak [2017] reported that a serving of amaranth leaves contains 21 to 41 mg of L-ascorbic acid, representing 32.0 to 68.0% of the daily requirement for this vitamin. A serving of *A. blitum*, *A. dubius*, *A. palmeri*, *A. tricolor*, *A. viridis*, or *A. thunbergii* provides 63.0 to 68.0% of this vitamin. Additionally, a serving of *A. hybridus* or *A. spinosus* provides 32 to 35% of the daily value (DV) for vitamin C. Therefore, serving any amaranth species is considered an excellent source of vitamin C. This present study demonstrates that amaranth cultivars bred and grown for seeds in Poland – 'Rawa' (*A. cruentus* L.) and 'Aztek' (*A. hypochondriacus* × *A. hybridus* L.) – can also be an excellent source of vitamin C. Considering the recommendations of the U.S. Food and Drug Administration for the daily dose of vitamin C for adults, which is 60 mg/day, the cultivar 'Aztek' covers 56.0% of the daily requirement, and 'Rawa' covers 52.3%. It should be noted that the recommended daily allowance for vitamin C in Poland is higher, as it amounts to 80 mg/day for adults [Dz.U. nr 196 poz. 1425 2007]. For these reasons, a 30 g serving of cultivar 'Aztek' leaves would cover 42.0% of the daily vitamin C requirement, while the cultivar 'Rawa' would cover 39.2%. However, it should be emphasized that vitamin C in leaves (similarly to phenols and flavonoids) can act as a reducing agent or chelator for metals such as Fe [Andjelković et al. 2006, Etcheverry et al. 2012]. Ensuring these metals are supplemented naturally in the daily diet is crucial. The content of vitamin C in amaranth leaves is dependent on the genotype. In an experiment conducted using a randomized complete block design (RCBD) with three replicates involving 12 genotypes (GRA3, GRA4, GRA7, GRA9, GRA11, GRA12, GRA15, GRA17, GRA21, GRA23, GRA26, GRA27) of green morph amaranth grown at Bangabandhu Sheikh Mujibur Rahman Agricultural University in Bangladesh, the application of compost at a rate of 10 t · ha^{-1}, along with recommended doses of fertilizers such as urea, triple superphosphate, muriate of potash, and gypsum at doses of 200, 100, 150, and 30 kg · ha^{-1}, respectively, was found to exert significant effects. Among the different genotypes of green morph amaranth studied, GRA3 exhibited the highest vitamin C content, exceeding 100 mg · 100 g^{-1} fresh weight (FW), specifically, 101.65 mg · 100 g^{-1} FW [Sarker et al. 2020]. In the present study, both tested cultivars accumulated more than 100 mg · 100 g^{-1} FW, with the cultivar 'Aztek' showing better performance than 'Rawa.'

TAC value in leaves. Plants of the genus *Amaranthus* spp. exhibit antioxidant activity [Park et al. 2020]. However, their properties vary between individual species. It has been demonstrated that among the five species of amaranth – *A. caudatus*, *A. cruentus*, *A. hybrid*, *A. hipochondriacus*, and *A. hybridus*, significant-

ly higher TAC was observed in *A. hypochondriacus* (91.4%) and *A. cruentus* (90.2%) [Akin-Idowu et al. 2017]. It should be noted that extracts from various parts of the aerial biomass (leaves, seeds, flowers, sprouts, and stems) of different amaranth species, such as *A. cruentus*, *A. caudatus*, or *A. hypochondriacus*, exhibit antioxidant activity, although its level varies. The antioxidant activity of *A. hypochondriacus* was found to be higher in leaves (451.4 µmol Trolox equivalent (TE · g^{-1} DW)) than in seeds (<51 µmol TE · g^{-1} DW) [Li et al. 2015]. In *in vivo* studies, it was observed that the consumption of amaranth leaf extract by *Drosophila melanogaster* was associated with increased survival after exposure to H_2O_2. This survival rate depended on the extract's dose, confirming that the ethanolic extract of amaranth leaves protects against H_2O_2-induced oxidative stress [Johnmark and Kinyi 2021]. Such beneficial antioxidant properties of vegetable plants, including amaranth, can be further enhanced through appropriate agronomic practices [Skwaryło-Bednarz and Krzepiłko 2008, Skwaryło-Bednarz and Krzepiłko 2009b, Biesiada and Tomczak 2012]. The impact of mineral fertilization, including N, on the TAC of leaves in various crops has been extensively investigated.-The research conducted in this study spans three years, which would imply that it is more comprehensive. The present results are partially consistent with the one-year study by Skwaryło-Bednarz and Krzepiłko [2008], especially concerning the influence of NPK fertilization on the TAC value of plant material derived from the cultivar 'Aztek'. It can be presumed that the parameter assessing the antioxidant activity of leaves may depend not only on macronutrient fertilization and cultivar but also on weather conditions. These findings were confirmed by another one-year study by Skwaryło-Bednarz and Krzepiłko [2009b], indicating that in 2007, the TAC value of leaves increased with the increasing level of NPK fertilization. The highest NPK dose (kg · ha^{-1}: 130–70–70) reached 14.55 µM Trolox $cm^3 \cdot g^{-1}$ leaf extract for the cultivar 'Rawa'. For the cultivar 'Aztek', this value was highest after applying the lowest NPK dose (kg · ha^{-1}: 50–40–40), reaching 12.50 µM Trolox $cm^3 \cdot g^{-1}$ leaf extract, and gradually decreased with increasing fertilization doses. A study by Sarker et al. [2020] has indicated that various genotypes of amaranth (12) differ in the antioxidant activity of leaves at a specific fertilization level and that these differences are significant. TAC (DPPH) in the latter study ranged from 8.90 TEAC µg · g^{-1} DW (GRA26) to 26.56 TEAC µg · g^{-1} DW (GRA4); genotype GRA29 was characterized by high TAC (DPPH), GRA26 had the lowest TAC (DPPH), averaging 13.74 TEAC µg · g^{-1} DW; five genotypes had higher TAC values than the average value. In the present 3-year study, 'Aztek' exhibited a higher TAC (DPPH) value than 'Rawa', despite these cultivars' lack of significant differences. Significant variations, however, were found between developmental stages. The earlier the developmental stage, the richer it was in accumulating antioxidant compounds. In their study, Łata et al. [2022] also pointed to the significant influence of the genetic factor on increasing concentrations of biologically active compounds in plants. In addition, no statistical relationship was found between TAC (DPPH) values determined for leaves grown under varied N fertilization. In this case, only a slight gradual increase was observed with increasing levels of N fertilization.

CAT activity in leaves. Amaranth belongs to the group of crops with the C_4 photosynthetic type [Nalborczyk et al. 1994]. This photosynthetic pathway promotes efficient water use and effective CO_2 assimilation. As a result, amaranth exhibits drought resistance and rapid growth in height even under water stress [Rastogi and Schukla 2013]. A study by Sakrer et al. [2018] has demonstrated that drought stress increases the activity of enzyme antioxidants such as SOD (superoxide dismutase) and CAT in amaranth cultivars VA13 and VA15. The activity of SOD and CAT was significantly higher in the tolerant cultivar VA13 compared to the sensitive cultivar VA15. It suggests that SOD and CAT play a crucial role in the tolerance of *A. tricolor* to drought by catalyzing the dismutation of superoxide radicals to H_2O_2 through SOD and subsequently facilitating the disproportionation of H_2O_2 to water and oxygen through CAT. Drought tolerance of amaranth could also stem from a higher level of regulation of the toxic action of H_2O_2 by glutathione reductase (GR), ascorbate peroxidase (APX), and superoxide dismutase (SOD) [Slabbert and Krüger 2014]. Other studies have confirmed greater drought resistance of *A. tricolor* than *A. hypochondriacus* or *A. hybridus*, which results from a higher level of regulation of H_2O_2 toxicity by glutathione reductase (GR),

ascorbate peroxidase (APX) and superoxide dismutase [Slabbert and Krüger 2014]. In our study, no significant differences were found between the cultivated cultivars, which may suggest a very similar activity of this enzyme in the leaves of amaranth grown under the same weather conditions. Although the temperature and precipitation distribution during the study period did not significantly impact the CAT activity in leaves, there was a noticeable increase in the activity of this enzyme in leaves in the third year of the study, which was characterized by precipitation deficits during the research period. Dynamic changes in enzymatic activity in plant leaves can be beneficial in diagnosing plant macromineral nutrition under specific environmental conditions. Mineral nutrition of plants can affect the activity of enzymes or the regulatory system of their synthesis [Gurgul et al. 1979]. Particular physiological significance in this regard is attributed, i.a., to catalase. Biological plant control at the enzymatic level is critical, especially for vegetables. Increasing doses of mineral fertilizers most often leads to a rise in oxidative stress parameters [Gurgul and Herman 1994], while the levels of non-enzymatic antioxidants in the plant exhibit varied responses [Telesiński et al. 2008]. Another one-year study evaluated the effect of NPK fertilization on the activity of catalase enzyme in the fresh leaf weight of two amaranth cultivars – 'Rawa' and 'Aztec'– at different developmental stages (seedling stage – BBCH 12, five-leaf stage – BBCH 15, full flowering stage – BBCH 65 and full seed maturity stage – BBCH 89) [Skwaryło-Bednarz and Krzepiłko 2013]. It was shown that increasing doses of NPK fertilizer (kg · ha^{-1}: 1. 50–40–40; 2. 70–50–50; 3. 90–60–60; 4. 130–70–70) stimulated higher CAT activity. The cultivar 'Rawa' leaves exhibited higher catalase activity than the cultivar 'Aztek', irrespective of the developmental stage. These findings were consistent with our research, where N fertilization exerted the most significant positive impact on CAT activity in leaves; however, no influence of the cultivar on the activity of this enzyme was detected. Catalase plays a physiological role in the process of plant growth and development, especially auxin metabolism; thus, it is important to determine the activity of this enzyme at different developmental stages, including early periods of growth. A study by Skwaryło-Bednarz and Krzepiłko [2013] demonstrated that CAT activity in amaranth leaves exhibited a decreasing trend during the amaranth growing season. It was shown that the highest catalase activity was recorded in fresh leaf weight at the seedling stage (BBCH 12) compared to later developmental stages, consistent with the results of the present work. The level of CAT activity is also affected by the amaranth cultivar. A study in southeastern Poland found that the cultivar 'Rawa' showed a higher CAT activity than the 'Aztek' (by 6.7%) [Skwaryło-Bednarz and Krzepiłko 2013]. In the present comprehensive 3-year study, no statistical differences were recorded between catalase activity in the leaves of 'Aztek' and 'Rawa' cultivars.

CAT activity in rhizosphere and non-rhizosphere soil zones. Catalase activity in the soil primarily depends on the content of organic matter, biomass, oxygen consumption, and carbon dioxide release, as well as the activity of dehydrogenases, glucosidase amidase, and phosphodiesterase [Riffaldi et al. 2002, Brzezińska 2006]. Catalase activity indicates soil salinity, contamination with heavy metals, or chemical plant protection agents [Romanowicz and Krzepiłko 2013]. The activity of this enzyme in soil indirectly provides information about the state of oxygenation, which is crucial for the growth and development of plants and microorganisms [Brzezińska 2006]. Microbial activity is one of the most important factors responsible for soil fertility. Its level is influenced by plant roots, which can modify the habitat conditions in which they are located [Frączek 2010]. The rhizosphere is a specific environment of mutual interaction between plants, soil, and microorganisms. It is characterized by increased biological activity, especially in microbial abundance and enzyme activities, compared to the non-rhizosphere zone. It influences the overall condition of plants and their resistance to pathogens. The rhizosphere zone of plants contains a significantly greater amount of primary substrates secreted by plants than the surrounding soil. These substances serve as nutrients for soil microorganisms, and their abundance and composition vary depending on the plant species and environmental conditions. Therefore, particularly valuable are cultivated plant species, and within their cultivars, those that provide optimal, high-quality yields and have a beneficial impact on the activity of soil microorganisms, even beyond the rhizosphere. Their roots stimulate microbiological activity by pro-

viding water-soluble compounds, including organic acids, sugars, and amino acids. The activity of microorganisms in the rhizosphere exhibits the highest dynamics [Galus-Barchan and Chmiel 2019]. Many scientific works have described the interaction of microorganisms and plants in the rhizosphere, yet there are few studies specifically addressing amaranth as a test plant. One of the research topics in this series of studies was the assessment of the impact of increasing NPK doses (kg · ha^{-1}: 1. 50–40–40, 2. 70–50–50, 3. 90–60–60, 4. 130–70–70) on soil enzymatic activity in the rhizosphere and non-rhizosphere zones of two amaranth cultivars, 'Rawa' and 'Aztek' [Skwaryło-Bednarz and Krzepiłko 2009a]. A controlled field experiment cultivated amaranth in a wide-row spacing on a soil classified as a good wheat complex. It was demonstrated that the enzymatic activity in the rhizosphere zone of amaranth was significantly higher than in the non-rhizosphere soil, regardless of the cultivar. The introduction of increasing doses of macronutrients resulted in a successive increase in the activity of dehydrogenases and catalase in the rhizosphere and non-rhizosphere soil compared to the control. The best results were achieved with fertilization using the highest dose of NPK (kg · ha^{-1}: 130–70–70), and in this regard, the cultivar 'Aztek' outperformed 'Rawa' (increase in dehydrogenase activity: rhizosphere zone by 21.8%, non-rhizosphere zone by 31.0%; increase in catalase activity: rhizosphere zone by 9.4%, non-rhizosphere zone by 15.4%). It was most likely because the composition of amaranth root exudates positively impacted the abundance and activity of soil microorganisms, thereby enhancing soil fertility. The cultivar 'Aztek' exhibited particularly favorable predispositions in this aspect. In the present study, a higher CAT activity was observed in the rhizosphere zone than in the non-rhizosphere soil. Nitrogen fertilization also significantly affected CAT activity in both the rhizosphere and non-rhizosphere soil. Additionally, the cultivar 'Aztek' showed a more favorable effect on CAT activity in both the rhizosphere and non-rhizosphere zones compared to the cultivar 'Rawa.' An increase in CAT activity was observed in both zones, along with the growth of plants. Furthermore, a significant influence of weather conditions on CAT activity in the non-rhizosphere zone was observed, as drought stress in the last year of the study caused an increase in the activity of this enzyme. The results obtained are supported by another study, which evaluated the effect of mycorrhizal fungi (MF) and irrigation on CAT activity in the rhizosphere of peppers in organic field cultivation [Jamiołkowska et al. 2020]. For this purpose, MF was applied to the plants in the form of a mycorrhizal vaccine (composition: *Rhizophagus aggregatus*, *R. intraradices, Claroideoglomus etunicatum*, *Endogone mosseae*, *Funneliformis caledonium*, and *Gigaspora margarita*) and irrigated in the following treatments: mycorrhized plants (PM), mycorrhized plants with irrigation (PMI), and non-mycorrhized plants with irrigation (PI). Plants without mycorrhizal fungi and irrigation were the absolute control (P). The study showed that soil sampled from the rhizosphere from the control plants (P) and mycorrhized plants (MF) demonstrated higher CAT activity, while soil from irrigated (PI) and mycorrhized and irrigated plants (PMI) had significantly lower activity. Other studies have evaluated the effects of factors such as habitat, cultivar, and developmental stage of amaranth on CAT activity in soil (without division into zones) [Skwaryło-Bednarz et al. 2022]. The results indicated that the factors most significantly influencing the activity of this enzyme in the soil were the combinations of fertilization, cultivar ('Aztek', 'Rawa'), developmental stage of amaranth, and weather conditions. The highest CAT activity was observed in the plots treated with the highest dose of NPK fertilizers (kg · ha^{-1}: 120–70–70). Higher activity of this enzyme was found in the soil under the cultivar 'Aztek' compared to the cultivar 'Rawa'. Catalase activity gradually increased from the 5-leaf to full seed maturity stage, with its highest value recorded. In the present study, the highest CAT activity was found during the wet period of amaranth growth and development and the lowest during dry summers, consistent with the study of Brzezińska [2001]. According to the latter author, irrigating peat-bog soil with pure water did not significantly impact CAT activity

CONCLUSIONS

Cultivating plants introduced from other climate zones into regions where they are not native always raises particular concerns. As it appears, such concerns were and continue to be unfounded in the case of amaranth. Numerous amaranth cultivars have been

developed in many countries, including Poland, and are well-adapted and consistently produce high-quality seeds. These comprehensive analyses confirm the capacity for versatile use of amaranth seed cultivars. They can potentially be very useful not only as seed forms but also as vegetables with high vitamin C content and other antioxidant compounds in the leaves during the initial stages of development. The cultivar 'Aztek' can be especially recommended for cultivation in Poland, as it accumulates significantly more vitamin C in the leaves at the BBCH 13 stage when fertilized with 120 kg N · ha–1 in warm and less wet early stages of the growing season. Such developmental phases and hydrothermal conditions are optimal for accumulating antioxidant compounds; however, the producers have no control over the weather conditions. Understanding the factors affecting CAT activity in the leaves is also essential. It appears that its activity is highest in the early stages of development and increases with nitrogen dose increments. Catalase activity in the soil in both the rhizosphere and non-rhizosphere zones depends on nitrogen fertilization, cultivar, developmental stage, and weather conditions (only for the non-rhizosphere zone). The current study is a significant addition to the knowledge regarding utilizing amaranth seed forms as a vegetable with high nutritional value. It also helps optimize nitrogen fertilization doses and determine developmental stages where the highest accumulation of antioxidants occurs. Additionally, the work sheds light on this plant's influence on soil's biological properties.

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