

## EFFECTS OF NPK FERTILIZATION, CULTIVAR, AND ROW SPACING ON SEED YIELD AND FATTY ACID COMPOSITION OF AMARANTH (*Amaranthus* spp.)

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### ABSTRACT

Amaranth (*Amaranthus* spp.) is a pseudo-cereal with increasing agronomic and nutritional importance due to its high seed yield potential and valuable lipid composition. The aim of this study was to evaluate the effects of NPK fertilization level, cultivar, row spacing and their interactions on yield components, crude fat content, fat yield and fatty acid composition of amaranth seeds. A three-year field experiment (2016–2018) was conducted in southeastern Poland using two cultivars (Rawa and Aztek), four levels of NPK fertilization and two row spacings (30 and 55 cm). The results showed that cultivar was the main factor differentiating seed yield, crude fat content, fat yield and fatty acid composition. The Aztek cultivar produced significantly higher seed yield and fat yield, whereas Rawa was characterized by a higher proportion of unsaturated fatty acids. Row spacing significantly affected plant density and yield structure, while increasing NPK fertilization enhanced seed and fat yield. Significant two-factor interactions were identified. The interaction between fertilization level and cultivar significantly affected palmitic (C<sub>16:0</sub>) and stearic (C<sub>18:0</sub>) acids, whereas the interaction between row spacing and cultivar influenced linoleic acid content (C<sub>18:2</sub>, n-6) and the n-6/n-3 fatty acid ratio. These results confirm that both quantitative and qualitative traits of amaranth seeds are determined by complex interactions between genetic and agrotechnical factors.

**Keywords:** amaranth, NPK fertilization, cultivar, row spacing, yield structure, fat content

### INTRODUCTION

Amaranth (*Amaranthus* spp.) is a multifunctional crop characterized by high aboveground biomass production, considerable plant height, and well-developed inflorescences, which makes it attractive for a wide range of agricultural, horticultural, and environmental applications, including animal feeding, ornamental use, and soil protection [Schafleitner et al. 2022, Jan et al. 2023]. Field and controlled-environment studies have demonstrated that the growth performance and biomass production of amaranth are strongly influenced by cultivation system and environmental conditions, with substantial differences observed between open-field and protected cultivation; in particular, Managa et al. [2023] reported pronounced effects of production system on growth dynamics and mineral composition of amaranth biomass, highlighting the crop's high phenotypic plasticity.

In recent years, increasing attention has been paid to amaranth seeds, which are valued for their high nutritional quality, particularly their protein content, oil fraction, and abundance of bioactive compounds such as

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squalene, tocopherols, and phytosterols [Skwaryło-Bednarz et al. 2020, Jan et al. 2023, Malik et al. 2023]. Among amaranth species, *Amaranthus cruentus*, *A. hypochondriacus*, and *A. caudatus* are recognized as species with the highest nutritional value of seeds and considerable agronomic potential under diverse environmental conditions [El Gendy et al. 2018, Skwaryło-Bednarz et al. 2020]. Amaranth seed oil typically constitutes approximately 5–10% (with a mean value of about 7.0%) of seed dry matter and is characterized by a favorable fatty acid composition a dominated by unsaturated fatty acids, mainly linoleic acid C<sub>18:2</sub> (n-6) and oleic acid C<sub>18:1</sub> (n-9), which together represent the predominant fraction of seed lipids [Baraniak and Kania-Dobrowolska 2022, Azri et al. 2025].

Beyond its fatty acid profile, amaranth seed oil is distinguished by a high content of unsaponifiable bioactive compounds, particularly squalene, tocopherols, and phytosterols, which contribute to its antioxidant properties and support its utilization in food, cosmetic, and pharmaceutical applications, underlining the multifunctional nature of this crop [Sayed-Ahmad et al. 2022]. Beyond total oil content, increasing attention has been paid to the qualitative composition of fatty acids, especially the ratio of omega-6 (n-6) to omega-3 (n-3) fatty acids, which is considered a key indicator of nutritional quality and health-promoting potential of plant oils. An excessive n-6/n-3 ratio has been associated with metabolic and cardiovascular disorders, whereas lower values are regarded as more desirable from a dietary perspective [Simopoulos 2016, Calder 2017]. Although amaranth oil is rich in bioactive compounds with documented antioxidant and hypolipidemic properties, its n-6/n-3 ratio is generally high, often exceeding 30:1, which may limit its suitability as a primary dietary oil [USDA FoodData Central 2023].

Recent studies indicate that the fatty acid composition of amaranth oil is influenced not only by genetic factors but also by environmental conditions and agronomic practices, including nutrient availability and cultivation intensity [Tyrus et al. 2024]. Nevertheless, despite growing interest in amaranth as a functional crop, there is still limited information on how genotype interacts with agronomic factors such as fertilization level and row spacing to shape fatty acid profiles and their ratios, particularly under temperate European climatic conditions. This research gap is especially relevant in the context of sustainable nutrient management and breeding strategies that increasingly focus on improving oil quality and nutritional value rather than yield alone [Petkova et al. 2019, Jan et al. 2023, Azri et al. 2025].

Cultivars representing different *Amaranthus* species may differ in their physiological response to nutrient supply, lipid biosynthesis pathways, and stress tolerance, which may ultimately affect both the quantity and quality of seed oil [Schafleitner et al. 2022, Azri et al. 2025]. Recent comprehensive reviews have emphasized the high nutritional and nutraceutical potential of amaranth seeds, particularly in relation to their lipid fraction rich in unsaturated fatty acids, tocopherols, and other bioactive compounds, while simultaneously highlighting the scarcity of field-based experiments linking agronomic practices with fatty acid composition and quality parameters under realistic cultivation conditions [Jan et al. 2023].

Improving the nutritional quality of amaranth seed oil, rather than increasing oil yield alone, is increasingly considered a key objective in sustainable crop production and functional food development. In this context, fatty acid composition and their relative proportions are regarded as critical quality indicators determining the health-promoting potential of amaranth-derived products [Jan et al. 2023].

Therefore, the objective of this study was to evaluate the effects of differentiated NPK fertilization, row spacing, and cultivar on selected morphological traits, seed yield, oil content, and fatty acid composition of two amaranth cultivars: Rawa (*Amaranthus cruentus* L.) and Aztek (*Amaranthus hypochondriacus* × *A. hybridus* L.), cultivated under the soil and climatic conditions of southeastern Poland. The null hypothesis (H<sub>0</sub>) assumes that NPK fertilization and row spacing would not significantly affect morphological traits, seed yield, oil content, or the fatty acid composition of the studied cultivars. The alternative hypothesis (H<sub>1</sub>) assumes that increased fertilization intensity and wider row spacing would significantly influence these traits and that the magnitude of these effects would depend on cultivar-specific responses.

## MATERIALS AND METHODS

### Field experiment description

This study was based on a field experiment conducted from 2016 to 2018 on a private farm field located in the village of Bodaczów (50°71'N, 23°04'E), near Zamość, in the Lublin Voivodeship. The experiment was established using a split-plot design arranged in randomized blocks with three replications. The experimental factors included NPK fertilization, cultivar, and row spacing. Each elementary plot covered an area of 10 m<sup>2</sup> (2 m × 5 m). Within each replication (block), plots were arranged in a compact layout with 0.5 m wide alleys between adjacent plots and

1.0 m wide alleys between blocks to allow for machinery access and to minimize edge effects. The total experimental area, including plots and alleys, was approximately 720 m<sup>2</sup> per year. The experiment was set up on brown soil developed from loess. Each year, prior to establishing the experiment, soil samples were collected for chemical analysis to determine the levels of phosphorus (P), potassium (K), magnesium (Mg), and soil pH. The soil's nutrient content in terms of P, K, and Mg was classified as high, while the soil pH was neutral.

The preceding crops for amaranth were: spring barley in 2016, spring wheat with white mustard as an intercrop in 2017, and winter wheat in 2018.

The experiment included the following variables: NPK fertilization (n = 4), cultivars (n = 2), row spacing (n = 2), and replications (n = 3), for a total of 48 plots:

I. NPK fertilization levels (kg·ha<sup>-1</sup>):

1. NPK0 (Control) – 0 N, 0 P<sub>2</sub>O<sub>5</sub>, 0 K<sub>2</sub>O
2. NPK1 – 80 N, 50 P<sub>2</sub>O<sub>5</sub>, 50 K<sub>2</sub>O (equivalent to 80 N, 22 P, 41.5 K = 143.5 NPK)
3. NPK2 – 110 N, 70 P<sub>2</sub>O<sub>5</sub>, 70 K<sub>2</sub>O (110 N, 30.8 P, 58.1 K = 198.9 NPK)
4. NPK3 – 140 N, 90 P<sub>2</sub>O<sub>5</sub>, 90 K<sub>2</sub>O (140 N, 39.6 P, 74.7 K = 254.3 NPK)

II. Cultivars:

1. Rawa (*A. cruentus* L.)
2. Aztek (*A. hypochondriacus* × *A. hybridus* L.)

III. Row spacing:

1. 30 cm
2. 55 cm

All tillage and cultivation practices were carried out in accordance with standard agronomic recommendations for amaranth. Seeds of two amaranth cultivars (Rawa and Aztek) with certified quality were sown at a rate of 2.0 kg·ha<sup>-1</sup> using a plot seeder (Tool Carrier 2700). In narrow-row sowing (30 cm), plants were sown in four rows per 1 m<sup>2</sup>, with the first row located approximately 25 cm from the longer edge of the plot. In wide-row sowing (55 cm), rows were spaced evenly, with the first row located approximately 45 cm from the longer edge of the plot. Border rows and border plants were excluded from sampling and measurements; all observations and harvests were performed only from the central area of each plot to eliminate edge effects. Sowing was performed in the third decade of May (2016 and 2017) or the first decade of June (2018). In each year of the experiment, certified seed material was provided free of charge by "Szarłat" M. and W. Lenkiewicz sp.j.

Phosphorus (P) fertilization in the form of granular single superphosphate and potassium (K) fertilization were applied in autumn. Nitrogen (N), in the form of ammonium nitrate, was applied to the soil in spring in two equal split doses: the first before sowing and the second four weeks after plant emergence.

Each season, the experimental field underwent two rounds of weed control, combining manual and mechanical methods. No chemical protection against agrophages (pathogens and pests) was applied, due to the lack of registered plant protection products for amaranth; therefore, crop protection relied solely on mechanical and manual methods. Harvesting of seeds was carried out manually in the third decade of October (2016) and the first decade of November (2017 and 2018). Post-harvest, seed moisture content was high and required rapid drying to maintain quality and health standards. Moisture content directly after threshing was 28.9% in 2016, 21.3% in 2017, and 24.4% in 2018. Seeds were dried each year to approximately 10.0% moisture.

### Weather conditions

Meteorological data for the study period (2016–2018) were obtained from the Institute of Meteorology and Water Management – National Research Institute (IMGW) at the nearest weather station in Zamość. The following parameters were analyzed: average monthly air temperature (°C) and total monthly precipitation (mm) during the amaranth growing season (May to October).

### Assessment of plant density per 1 m<sup>2</sup> and selected morphological traits before harvest

At the BBCH 89 growth stage, the number of plants per 1 m<sup>2</sup> (plants/m<sup>2</sup>) was counted prior to harvest, and biometric measurements were performed on 30 randomly selected plants from each plot. The following traits were evaluated: plant height (cm), inflorescence length (cm), seed mass per plant (g), and thousand seed weight (g) [Martínez-Núñez et al. 2019].

After seed harvest at the BBCH 92 stage, seed yield was determined in kg per plot and converted to t·ha<sup>-1</sup>. From each plot, a 0.5 kg seed sample was collected for further analyses of chemical composition.

## Chemical analyses

Each year, the following chemical analyses were conducted on the harvested seeds of cultivated amaranth:

- Crude fat content was determined according to the Polish Standard PN-EN ISO 6492:2005 Animal feeding stuffs – Determination of fat content [PN-79R-65950];
- Fatty acid composition was determined by gas chromatography. The analysis was carried out using a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (FID). Prior to analysis, triglycerides were transesterified into fatty acid methyl esters (FAMES) using potassium methanolate. Fatty acid separation was performed on an Rtx-2330 capillary column (105 m × 0.32 mm, 0.20 μm).

## Statistical analysis

The results are presented as averages for each year of the study. The data were analyzed using analysis of variance (ANOVA) appropriate for a split-plot design in a randomized block arrangement. The experimental factors included NPK fertilization, cultivar, and row spacing, as well as their interactions. All two-factor interactions between experimental factors were included in the statistical model and evaluated accordingly. The null hypothesis ( $H_0$ ) was tested using Snedecor's F-test, and the corresponding probabilities were determined. Year was included in the analysis as an environmental factor, and treatment effects were evaluated across years. Differences between means were assessed using Tukey's honestly significant difference (HSD) test at a significance level of  $\alpha = 0.05$ . To assess data variability, the coefficient of variation (CV%) was calculated. Relationships between selected traits were examined using Pearson correlation and linear regression analyses. All statistical analyses were performed using Excel 7.0 and Statistica software (StatSoft Polska, 2013).

## RESULTS AND DISCUSSION

### Environmental conditions during the study period

Weather conditions during the study years were highly variable (Fig. 1).

In 2016, amaranth was found to be sown during a wet and generally warm May, with total precipitation amounting to 49.9 mm and an average monthly temperature of 14.3°C (Fig. 1). Throughout the growing season, mean air temperatures consistently exceeded the long-term average. Precipitation levels from June to October also remained above the climatological norm. Notably, October recorded an exceptionally high total precipitation of 99.9 mm, more than double the long-term average, which considerably hindered the seed harvest.

In 2017, sowing occurred in a warm spring characterized by a marked precipitation deficit (52.2 mm) and an average temperature of 13.7°C (Fig. 1). Despite temperatures generally remaining above the long-term mean, they were lower than in 2016, except in July and August. Cumulative rainfall throughout the growing season was substantially below average, adversely affecting seed germination, plant development, and biomass accumulation. September (67.7 mm) and October (66.0 mm) were notably wet, significantly delaying seed maturation and complicating harvest operations.

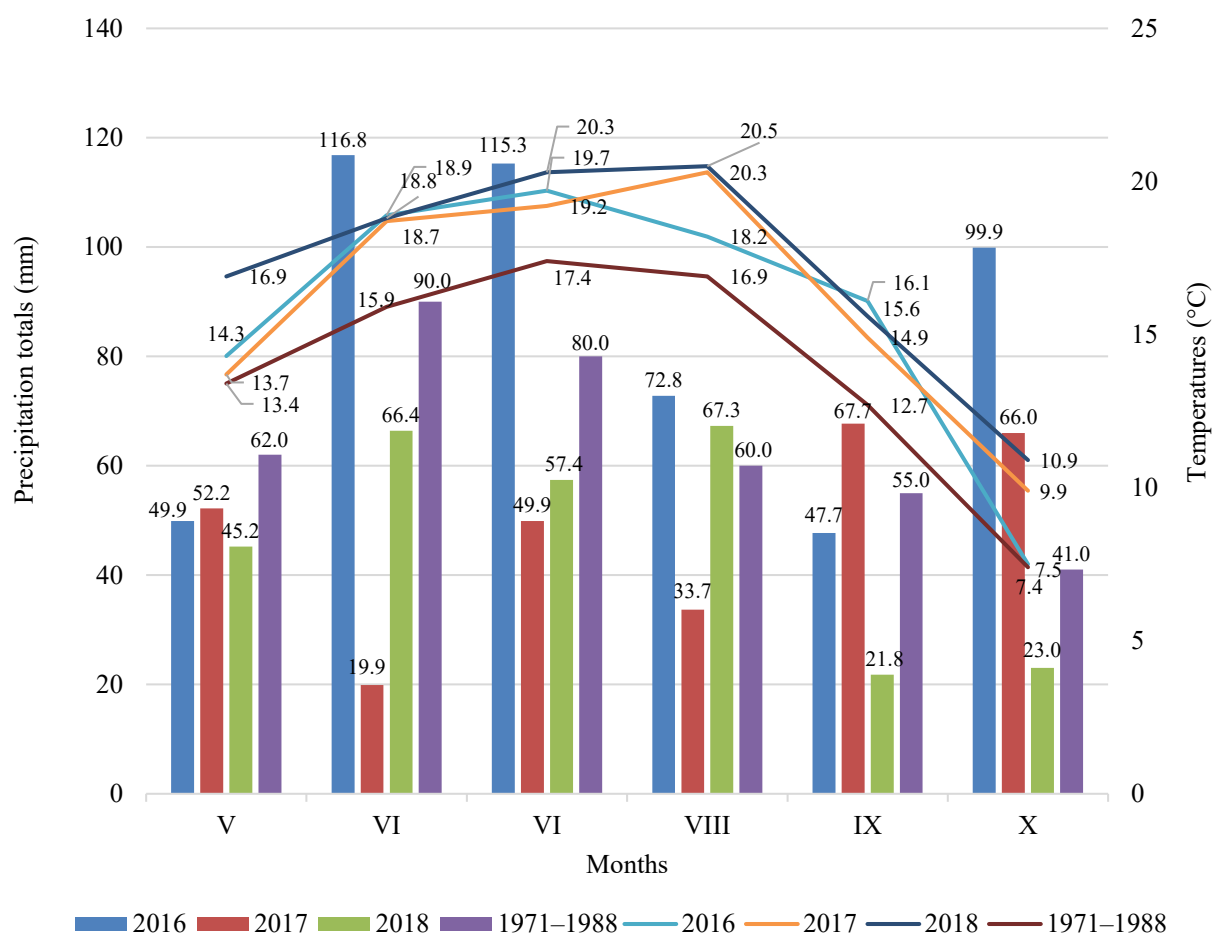
In 2018, the entire growing season – from sowing to harvest – was marked by temperatures consistently surpassing long-term norms, making it the warmest year of the study (Fig. 1). May experienced the most severe rainfall deficit of the experimental period (45.2 mm; 37.2% below the long-term average). Although July remained relatively dry, precipitation levels were higher than those observed in 2017. August precipitation slightly exceeded the long-term average by 7.3 mm. Favorably low precipitation in October facilitated efficient seed harvest.

### Plant stand structure and morphological traits

A synthesis of the three-year research results demonstrated that plant density was a key factor shaping yield structure in amaranth cultivation. In the present study, the number of plants per square meter was significantly affected only by row spacing, either 30 cm (narrow-row) or 55 cm (wide-row) – as in Table 1. This trait showed relatively high variability, as reflected by the average coefficient of variation of 26.82% (Tab. 1), indicating a strong dependence on stand structure rather than genetic control.

Proper plant density is a critical determinant of uniform plant height and appropriate development within the canopy. For amaranth, it has been suggested that maintaining a plant population per square meter that allows optimal inflorescence development is particularly important, as inflorescences may account for approximately 50% of the total plant biomass [Kozak et al. 2011]. However, achieving such stand density can be challenging under field conditions, especially for the Rawa cultivar, due to unfavorable weather during establishment and early growth stages [Jendrzyszczak and Śmigierska 2014].

**Figure 1.** Average monthly air temperatures (°C) and precipitation totals (mm) for the years 2016–2018 and the long-term averages for 1971–1988 (Meteorological Station in Zamość)



Excessive plant density per unit area is known to increase the proportion of vegetative organs, such as leaves and stems, and to promote plant lodging and stem breakage under conditions of strong wind and heavy rainfall [Skwaryło-Bednarz and Nalborczyk 2006]. Dense stands may also enhance susceptibility to fungal diseases [Pusz et al. 2015]. The results showed that narrow-row sowing with dense plant growth resulted mainly in the development of a single main stem bearing one inflorescence, whereas wider spacing favored stem branching and the formation of multiple inflorescences. Conversely, lower plant density per unit area may positively affect seed quality traits, including fat content, as previously reported for amaranth [Kozak et al. 2011].

In the present study, plant height exhibited very low variability (CV = 5.80), while inflorescence length showed moderate variability (CV = 25.25) – as in Table 1. Both morphological traits were significantly influenced only by cultivar. Averaged across the three growing seasons, the Aztek cultivar was taller by 16.9 cm and produced inflorescences that were 19.5 cm longer than those of Rawa (Tab. 1). These results are consistent with earlier findings indicating substantial cultivar-dependent differences in amaranth plant architecture [Kozak et al. 2011, Schafleitner et al. 2022].

Seed weight per plant was significantly affected only by row spacing (Tab. 1). Wide-row sowing (55 cm) enhanced growth dynamics by promoting stem branching and the formation of additional inflorescences, resulting in a higher number of seeds compared with narrow-row sowing (30 cm), with an average difference of 11.3 g per plant. The coefficient of variation for seed weight per plant reached 31.09%, indicating moderate variability of this trait (Tab. 1). Similar positive effects of reduced plant density and wider spacing on amaranth seed production have been reported under various environmental conditions [Olofintoye et al. 2015, Akamine et al. 2021, Tyrus et al. 2024]. Under favorable growing conditions, including low plant density, amaranth is capable of exceptionally high reproductive output. Gontarczyk [1996] reported that a single plant may produce more than 500 g of seeds.

In the present study, the 1000-seed weight was primarily influenced by hydrothermal conditions prevailing during the study years, as well as by cultivar and row spacing. In contrast, no significant relationships were observed between the analyzed morphological traits and increasing NPK fertilization levels (Tab. 1). This lack of response suggests that, under the conditions of the present experiment, fertilization intensity played a secondary role in shaping amaranth morphology. The very low coefficient of variation for 1000-seed weight (CV = 2.53%) indicates high stability of this trait across experimental treatments (Tab. 1). Nevertheless, previous studies have shown that the response of amaranth morphological traits to mineral fertilization may depend strongly on genotype, environmental conditions, and cultivation system [Akamine et al. 2021, Mndzebele et al. 2023, Tyrus et al. 2024]. Moreover, it has been emphasized that weather variability and stand structure frequently override the direct effects of fertilization on plant morphology, particularly under temperate and humid climatic conditions [Schafleitner et al. 2022, Azri et al. 2025].

**Table 1.** Number of plants per 1 m<sup>2</sup> and selected morphological traits before harvest (averages for factors and years)

Factor	Number of plants per 1 m <sup>2</sup> before harvest (pcs)	Plant height (cm)	Inflorescence length (cm)	Seed mass per plant (g)	Thousand seed weight (g)
NPK0	13.9 a	174.6 a	42.1 a	19.4 a	0.71 a
NPK1	14.0 a	178.3 a	45.7 a	20.0 a	0.72 a
NPK2	14.2 a	181.1 a	46.4 a	20.6 a	0.73 a
NPK3	14.2 a	183.1 a	46.8 a	21.5 a	0.71 a
LSD <sub>0.05</sub>	4.3	11.3	12.8	7.2	0.03
F value	0.01	1.6	0.4	0.2	2.6
p	n.s.	n.s.	n.s.	n.s.	n.s.
A	14.1 a	187.7 a	55.0 a	21.9 a	0.71 b
R	14.0 a	170.8 b	35.5 b	18.9 a	0.73 a
LSD <sub>0.05</sub>	2.2	3.6	3.6	3.7	0.01
F value	0.01	87.4	121.6	2.7	12.2
p	n.s.	***	***	n.s.	**
N	17.8 a	176.6 a	42.5 a	14.7 b	0.71 b
W	10.3 b	181.9 a	48.0 a	26.0 a	0.73 a
LSD <sub>0.05</sub>	0.3	6.0	6.6	1.7	0.01
F value	2008.0	3.1	2.9	185.8	9.9
p	***	n.s.	n.s.	***	**
2016	14.6 a	184.1 a	47.6 a	22.3 a	0.73 a
2017	13.9 a	176.0 a	41.6 a	17.9 a	0.70 c
2018	13.9 a	177.6 a	46.5 a	20.9 a	0.72 b
LSD <sub>0.05</sub>	3.3	8.7	9.8	5.4	0.01
F value	0.1	2.9	1.3	2.1	11.16
p	n.s.	n.s.	n.s.	n.s.	***
CV (%)	26.82	5.80	25.25	31.09	2.53

Legend: NPK0–NPK3 – fertilization levels; A – Aztek cultivar, R – Rawa cultivar; N – narrow row spacing, W – wide row spacing; 2016–2018 – study years; values followed by the same letters do not differ significantly at  $p \leq 0.05$ ; \*, \*\*, and \*\*\* indicate significance at  $p \leq 0.05$ , 0.01, and 0.001, respectively; CV – coefficient of variation

The results of our study showed that only the interaction between weather conditions during the study years and row spacing significantly affected amaranth plant density per 1 m<sup>2</sup> before harvest (Tab. 2). No significant interaction effects were detected for the other factors, and no significant interactions among the studied factors were observed for the other analyzed morphological traits. These findings indicate that amaranth plant density is shaped predominantly by environmental variability and stand structure rather than by interactions among ag-

ronomic factors, which is consistent with reports highlighting the dominant role of climatic conditions and plant density in determining amaranth morphological traits [Akamine et al. 2021, Schafleitner et al. 2022, Mndzebele et al. 2023, Tyrus et al. 2024].

**Table 2.** Significant two-factor interactions affecting yield components and fatty acid composition of amaranth

Trait	Interaction	F value	Significance
Number of plants per 1 m <sup>2</sup> before harvest (pcs)	year × row spacing	3.90	*
Seed yield (t·ha <sup>-1</sup> )	year × cultivar	4.95	*
Palmitic acid C <sub>16:0</sub> (%)	fertilization × cultivar	5.07	*
Stearic acid C <sub>18:0</sub> (%)	fertilization × cultivar	5.63	*
Linoleic acid C <sub>18:2</sub> (n-6) (%)	row spacing × cultivar	6.90	**
n-6/n-3 fatty acid ratio	row spacing × cultivar	7.46	**

Only statistically significant two-factor interactions are presented  
\*, \*\* indicate significance at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively

### Seed yield and its determinants

The seed yield of cultivated amaranth was influenced to varying degrees by the studied factors, including fertilization level, row spacing, cultivar, and weather conditions, and was characterized by relatively low variability (CV = 12.33%) – as in Table 3. In the present study, seed yield depended primarily on hydrothermal conditions during the study years rather than on cultivar or the level of NPK fertilization, whereas row spacing (30 or 55 cm) did not significantly affect this trait (Tab. 3).

The significantly highest seed yield was recorded in the first year of the experiment (2016), exceeding the yield obtained in the second year (2017) by 22.0%, and that in the third year (2018) by 8.9% (Tab. 3). These differences can be attributed mainly to variable weather conditions in the Zamość region during the study years. In the first year, the precipitation sum (402.5 mm) recorded during the amaranth growing period (from June to early September) exceeding the long-term average, and positively affected seed yield despite sowing into poorly moistened soil. In contrast, in the second and third year of the study low precipitation (223.4 mm and 258.1 mm, respectively), their unfavorable distribution, and prolonged drought periods during vegetation limited yield formation and final yield size (Fig. 1). Air temperature also influenced yield formation, as mean temperatures during all study years exceeded the long-term average, with the warmest conditions recorded in 2018, followed by 2016 and 2017.

The obtained results confirm earlier reports indicating that amaranth exhibits relatively high, although differentiated, tolerance to variable moisture conditions. This tolerance is partly related to its classification as a C<sub>4</sub> photosynthesis plant, characterized by high water-use efficiency and effective CO<sub>2</sub> fixation associated with efficient utilization of solar radiation [Gontarczyk 1996, Achremowicz et al. 2015, Schafleitner et al. 2022]. Nevertheless, amaranth is highly sensitive to soil water deficiency, particularly during the emergence and flowering stages [Olufolaji et al. 2010, Azri et al. 2025]. Insufficient soil moisture significantly delays emergence and contributes to plant thinning, while drought during inflorescence formation impairs flowering and fruit set, leading to reduced seed yield [Graham 2010, Grobelnik Mlakar et al. 2012]. Hydrothermal conditions during the growing period are therefore crucial not only for pseudo-cereal crops, which largely belong to the C<sub>4</sub> photosynthesis group, but also for cereal species predominantly classified as C<sub>3</sub> plants [Haliniarz 2013].

Cultivar was another important factor determining amaranth seed yield. Averaged across the study years, the Aztek cultivar produced a significantly higher seed yield than Rawa, with a difference of approximately 13% in favor of Aztek (Tab. 3). These results support previous findings indicating that genotype plays a decisive role in yield formation of amaranth under diverse environmental conditions. Studies conducted in Poland and other European regions, as well as in tropical and subtropical environments of East Africa (Tanzania) and East Asia (Taiwan), have consistently shown that cultivars related to *A. hypochondriacus* are characterized by higher yield potential compared with *A. cruentus* [Kozak et al. 2011, Skwaryło-Bednarz et al. 2011, Schafleitner et al. 2022, Tyrus et al. 2024]. According to these authors, the higher yield potential of the Aztek results not only from increased production of generative organs, but also from greater vegetative biomass accumulation, which may enhance assimilate supply to reproductive structures. The stronger yield response of Aztek to increased fertilization observed in this study

may therefore be associated with higher growth vigor, more efficient nitrogen utilization, and greater allocation of assimilates to seed production, traits commonly reported for genotypes derived from *A. hypochondriacus*.

**Table 3.** Seed yield, crude fat content, and fat yield in amaranth seeds (averages for years and factors)

Factor	Seed yield (t·ha <sup>-1</sup> )	Crude fat content (g·kg <sup>-1</sup> )	Fat yield (t·ha <sup>-1</sup> )
NPK0	2.48 b	65.93 b	0.16 b
NPK1	2.57 ab	67.49 ab	0.17 ab
NPK2	2.71 ab	68.98 a	0.19 a
NPK3	2.82 a	70.00 a	0.20 a
LSD <sub>0.05</sub>	0.03	2.65	0.03
F value	2.75	2.82	3.85
p	*	***	*
A	2.83 a	69.58 a	0.20 a
R	2.46 b	66.63 b	0.16 b
LSD <sub>0.05</sub>	0.16	1.41	0.01
F value	22.92	17.85	23.09
p	***	***	***
N	2.61 a	67.69 a	0.18 a
W	2.68 a	68.51 a	0.18 a
LSD <sub>0.05</sub>	0.19	1.64	0.02
F value	0.59	1.03	0.79
p	n.s.	n.s.	n.s.
2016	2.90 a	68.89 a	0.20 a
2017	2.38 c	65.99 b	0.16 c
2018	2.66 b	69.42 a	0.19 b
LSD <sub>0.05</sub>	0.22	2.08	0.02
F value	16.95	9.24	13.96
p	***	***	***
CV (%)	12.33	4.10	15.85

Legend: NPK0–NPK3 – fertilization levels; A – Aztek cultivar, R – Rawa cultivar; N – narrow row spacing, W – wide row spacing; 2016–2018 – study years; values followed by the same letters do not differ significantly at  $p \leq 0.05$ ; \*, \*\*, and \*\*\* indicate significance at  $p \leq 0.05$ , 0.01, and 0.001, respectively; CV – coefficient of variation

Compared with weather conditions and cultivar effects, NPK fertilization exerted a weaker but still significant influence on seed yield. Statistical analysis indicated that increasing macronutrient fertilization significantly increased seed yield, with the highest yield recorded at the third fertilization level (140 kg N, 90 kg P<sub>2</sub>O<sub>5</sub>, and 90 kg K<sub>2</sub>O·ha<sup>-1</sup>), which was 13.6% higher than the unfertilized control. Numerous studies have emphasized the importance of mineral nutrition, particularly nitrogen, in shaping the yield of pseudo-cereal crops, including amaranth [Skwaryło-Bednarz 2010, Kozak et al. 2011, Bielski and Szwejkowska 2015, Akamine et al. 2021, Mndzebele et al. 2023]. However, excessive nitrogen fertilization in amaranth cultivation may stimulate excessive vegetative growth, leading to lodging, delayed seed maturation, and accumulation of undesirable nitrate levels in aboveground plant parts [Skwaryło-Bednarz and Nalborczyk 2006, Gimplinger et al. 2007]. Lodging further increases susceptibility to pathogenic diseases and complicates harvesting, thereby negatively affecting final yield size and quality [Skwaryło-Bednarz and Nalborczyk 2006]. Consequently, numerous studies have aimed to identify optimal NPK fertilization strategies for amaranth cultivation. Recent research indicates that seed yield generally responds positively to increasing nitrogen application, although the optimal dose depends on environmental conditions, cultivar, and production objectives. Under diverse agro-climatic conditions, nitrogen rates in the range

of 80–120 kg·ha<sup>-1</sup> have most frequently resulted in the highest seed yields, whereas higher doses tend to enhance vegetative growth at the expense of seed quality [Kozak et al. 2011, Skwaryło-Bednarz et al. 2014, Deryło and Chudzik 2015, Akamine et al. 2021, Mndzebele et al. 2023]. More recent investigations further emphasize that excessive nitrogen fertilization can delay seed maturation and negatively affect quality traits, underscoring the need for balanced and site-specific nutrient management strategies in amaranth cultivation [Akamine et al. 2021, Mndzebele et al. 2023]. These fertilization ranges are consistent with recent findings reported for amaranth cultivated under Central and Eastern European climatic conditions [Tyrus et al. 2024]. The results of the present study indicate that only the interaction between weather conditions during the study years and cultivar significantly affected seed yield (Tab. 2). In each experimental year, the Aztek cultivar produced a significantly higher seed yield than Rawa. No significant interactions were detected among the other analyzed factors with respect to amaranth seed yield.

### Crude fat content and fat yield

One of the basic components of amaranth seeds is crude fat, which contributes significantly to their nutritional and functional value [Krasowska 2022, Malik et al. 2023]. In the present study the factor exerting the strongest influence on crude fat content was cultivar, followed by weather conditions and macronutrient fertilization. The crude fat content in seeds of the studied cultivars showed very low variability, with a coefficient of variation of 4.10% (Tab. 3). The Aztek accumulated significantly more crude fat than Rawa, with a difference of 2.95 g·kg<sup>-1</sup> (Tab. 3), which is consistent with earlier reports [Skwaryło-Bednarz 2012]. The crude fat content recorded in this study was higher than that reported by Tömösközi et al. [2009], but lower than the values reported by Soriano-García and Aguirre-Díaz [2019]. Previous studies have demonstrated that amaranth cultivars differ considerably in seed fat content [Piecyk et al. 2009, Petkova et al. 2019, Azri et al. 2025]. In particular, *A. hypochondriacus* has been shown to exhibit consistently higher fat content than *A. cruentus*, with reported values typically ranging from approximately 4.3 to 7.8% [Bozorov et al. 2018, El Gendy et al. 2018, Azri et al. 2025] which agrees well with the results of the present study. Similar conclusions were also reported by Skwaryło-Bednarz [2010], who emphasized that seed fat content in amaranth depends primarily on species, cultivar, and, to a lesser extent, fertilization level.

Differences in lipid accumulation among cultivars are mainly attributed to genetic variability affecting seed metabolism and storage compound synthesis, as confirmed in large-scale evaluations of amaranth germplasm collections [El Gendy et al. 2018]. Similar conclusions regarding cultivar-dependent variation in seed oil content were reported by Skwaryło-Bednarz [2010], who highlighted the combined role of genotype and agronomic conditions in shaping fat accumulation in amaranth seeds.

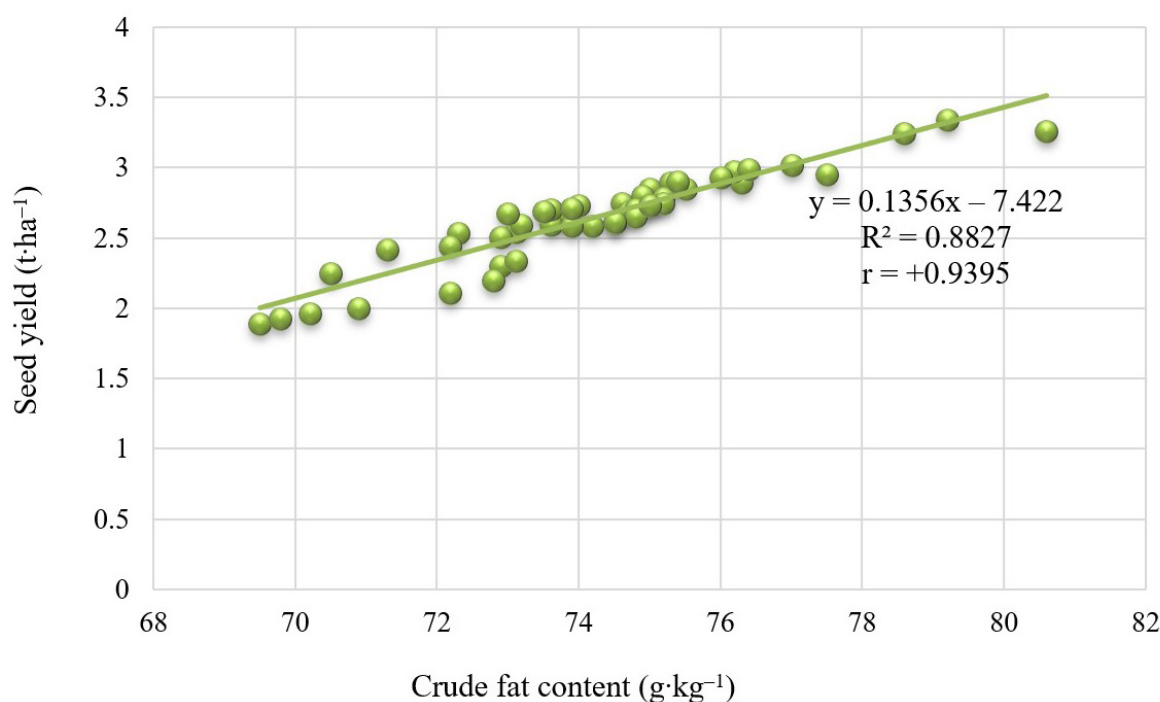
The present study also confirmed a significant influence of weather conditions on crude fat content (Tab. 3). Seeds harvested in the first and third year contained significantly more crude fat than those obtained in the second year of the study. Most likely, moisture conditions prevailing during the growing seasons favored lipid accumulation in seeds. Comparable relationships between hydrothermal conditions and oil accumulation have been widely documented in oilseed crops, where adequate water supply during seed filling enhances lipid biosynthesis. Kotecki et al. [2007] demonstrated a significant effect of weather conditions on crude fat accumulation in rapeseed. In general, years characterized by favorable rainfall distribution during seed development and maturation are particularly conducive to higher crude fat content in seeds of various crop species.

In contrast to cultivar and weather effects, the level of NPK fertilization exerted the weakest influence on crude fat content. The highest fat content was recorded in seeds from the NPK3 fertilization treatment, whereas the lowest values were observed in the unfertilized control (NPK0). No significant differences in fat content were found between the first and second fertilization levels and the control (Tab. 3). According to He and Corke [2003], oil content in *Amaranthus* species may vary depending on genetic and environmental factors, while the response to fertilization is often inconsistent, which may explain the relatively weak effect of increasing NPK doses observed in this study.

Fat yield, determined as a function of seed yield and crude fat content, exhibited low variability, with a coefficient of variation of 15.83% (Tab. 3). Fat yield was influenced more strongly by cultivar than by hydrothermal conditions or NPK fertilization level (Tab. 3). Genetic factors played a significant role, as the higher seed yield of Aztek resulted in a substantially greater crude fat yield compared with Rawa. On average, Aztek exhibited a 20.1% higher fat yield than Rawa (Tab. 2). Similar cultivar-related differences in fat yield have been reported for other oilseed and pseudo-cereal crops, emphasizing the importance of genotype selection in improving both yield and quality traits [Bartkowiak-Broda et al. 2005, Sułek et al. 2016].

Fat yield also varied significantly between years, reflecting the strong influence of weather conditions. The highest fat yield was recorded in the first year of the study, whereas the lowest occurred in the second year (Tab. 3), which corresponds well with earlier findings highlighting the decisive role of hydrothermal factors in oil yield for-

**Figure 2.** The relationship between amaranth seed yield and crude fat content



mation [Kotecki et al. 2005]. Fertilization level also significantly affected fat yield (Tab. 3). The highest values were observed under the NPK3 treatment, while the lowest occurred in the control plots, with a difference of 20.7%. No significant differences in fat yield were found between the first and second fertilization levels and the control (Tab. 3).

Correlation analysis revealed a strong and significant positive relationship between amaranth seed yield and crude fat content ( $r = +0.9395$ ) – as in Figure 2. Similar strong correlations between seed yield and oil content have been reported for oilseed crops such as rapeseed [Bartkowiak-Broda et al. 2005, Spasibionek 2007]. In the present study, neither crude fat content nor fat yield was affected by interactions among the analyzed factors.

#### Fatty acid composition of amaranth seed oil

Amaranth seeds contain a considerable amount of fat with a favorable qualitative composition. Due to its fatty acid composition, amaranth oil is classified as a functional food and is rich in biologically active compounds that positively affect human health through hypolipidemic, hypotensive, antioxidant, hepatoprotective, and anti-atherogenic actions [Ratusz and Wirkowska 2006, Moszak et al. 2008, Barrio and Añón 2010]. Similar health-promoting properties of amaranth seed oil, including its functional food potential and bioactive lipid fraction, have been highlighted in recent comprehensive reviews [Malik et al. 2023].

#### Saturated fatty acids

In amaranth seed oil, eight saturated and seven unsaturated fatty acids were identified (Tab. 4), which is consistent with recent compositional analyses of amaranth oil obtained from different genotypes and cultivation conditions [Bozorov et al. 2018].

Among saturated fatty acids, lignoceric acid C<sub>24:0</sub> exhibited moderate variability (CV = 25.29%), while pentadecanoic acid C<sub>15:0</sub> showed low variability (CV = 14.06%). The other saturated fatty acids were characterized by very low variability, including stearic acid C<sub>18:0</sub> (3.76%), palmitic acid C<sub>16:0</sub> (3.85%), behenic acid C<sub>22:0</sub> (4.79%), myristic acid C<sub>14:0</sub> (4.86%), arachidic acid C<sub>20:0</sub> (6.21%), and heptadecanoic acid C<sub>17:0</sub> (9.18%), as in Table 4.

Hydrothermal conditions significantly affected only the content of behenic acid C<sub>22:0</sub> (Tab. 4), although this factor differentiated its concentration to a lesser extent than NPK fertilization and cultivar. The highest content of behenic acid was recorded in the first year of the study and the lowest in the second year – difference of 0.02 percentage points (Tab. 4). The greatest accumulation of this acid was observed at the third fertilization level (0.31%), while the lowest occurred under NPK0 and NPK1 (0.29%). Seeds of Rawa cultivar contained 0.02 percentage points more behenic acid than Aztek (Tab. 4).

**Table 4.** The content of saturated fatty acids in amaranth seed oil (means for factors and years)

Factor	Saturated fatty acids (%)							
	C <sub>14:0</sub>	C <sub>15:0</sub>	C <sub>16:0</sub>	C <sub>17:0</sub>	C <sub>18:0</sub>	C <sub>20:0</sub>	C <sub>22:0</sub>	C <sub>24:0</sub>
NPK0	0.24 d	0.05 c	22.67 a	0.10 b	4.12 b	0.61 a	0.29 c	0.17 a
NPK1	0.25 c	0.06 b	22.55 a	0.10 b	4.16 ab	0.60 a	0.29 c	0.18 a
NPK2	0.26 b	0.06 b	23.00 a	0.11 a	4.23 a	0.60 a	0.30 b	0.20 a
NPK3	0.27 a	0.07 a	22.80 a	0.11 a	4.29 a	0.59 a	0.31 a	0.21 a
LSD <sub>0.05</sub>	0.01	0.01	0.99	0.01	0.16	0.04	0.01	0.05
F value	56.89	7.38	0.57	0.09	3.19	0.42	20.05	1.62
p	***	***	n.s.	**	*	n.s.	***	n.s.
A	0.25 a	0.06 a	23.52 a	0.11 a	4.34 a	0.64 a	0.29 b	0.15 b
R	0.26 a	0.05 b	21.99 b	0.10 b	4.06 b	0.57 b	0.31 a	0.24 a
LSD <sub>0.05</sub>	0.02	0.003	0.25	0.004	0.04	0.01	0.01	0.01
F value	0.4709	39.7794	150.9417	62.3685	162.5563	370.9221	5.7446	281.3377
p	n.s.	***	***	***	***	***	*	***
N	0.25 a	0.06 a	23.00 a	0.10 a	4.176 a	0.60 a	0.30 a	0.19 a
W	0.26 a	0.06 a	22.52 a	0.11 a	4.219 a	0.60 a	0.30 a	0.19 a
LSD <sub>0.05</sub>	0.01	0.01	0.49	0.01	0.093	0.02	0.01	0.03
F value	0.84	3.12	3.79	1.98	0.89	0.42	0.09	0.10
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2016	0.26 a	0.06 a	22.52 a	0.11 a	4.22 a	0.60 a	<b>0.31 a</b>	0.20 a
2017	0.25 a	0.06 a	23.02 a	0.11 a	4.18 a	0.60 a	<b>0.29 c</b>	0.18 a
2018	0.26 a	0.06 a	22.74 a	0.11 a	4.20 a	0.60 a	<b>0.30 b</b>	0.19 a
LSD <sub>0.05</sub>	0.01	0.01	0.75	0.01	0.14	0.03	<b>0.01</b>	0.04
F value	1.12	0.09	1.29	0.09	0.29	0.08	<b>3.26</b>	0.59
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
CV (%)	4.86	14.06	3.85	9.18	3.76	6.21	4.79	25.29

Legend: NPK0–NPK3 – fertilization levels; A – Aztek cultivar, R – Rawa cultivar; N – narrow row spacing, W – wide row spacing; 2016–2018 – study years; values followed by the same letters do not differ significantly at  $p \leq 0.05$ ; \*, \*\*, and \*\*\* indicate significance at  $p \leq 0.05$ , 0.01, and 0.001, respectively; CV – coefficient of variation

Saturated fatty acids: C<sub>14:0</sub> – myristic acid; C<sub>15:0</sub> – pentadecanoic acid; C<sub>16:0</sub> – palmitic acid; C<sub>17:0</sub> – heptadecanoic acid; C<sub>18:0</sub> – stearic acid; C<sub>20:0</sub> – arachidic acid; C<sub>22:0</sub> – behenic acid; C<sub>24:0</sub> – lignoceric acid

The content of myristic acid C<sub>14:0</sub> was significantly influenced only by fertilization level. Seeds from the control plot contained 0.24% of this acid, and its content increased with successive fertilization levels, reaching 0.27% under NPK3 (Tab. 4).

Pentadecanoic acid C<sub>15:0</sub> was more strongly affected by the genetic factor than by fertilization. Seeds of Aztek contained 0.20% more of this acid than Rawa. Although occurring at the lowest concentration among saturated fatty acids, its content was also significantly influenced by fertilization, with the lowest level observed under NPK0 and the highest under NPK3, while NPK1 and NPK2 showed identical values (Tab. 4).

Palmitic acid C<sub>16:0</sub> and arachidic acid C<sub>20:0</sub> were significantly differentiated only by cultivar (Tab. 4). The Aztek cultivar contained higher levels of both acids, with differences of 1.53% for palmitic acid and 0.07% for arachidic acid. The dominance of palmitic acid among saturated fatty acids in amaranth oil is consistent with previous reports [He et al. 2002, He and Corke 2003, Nasirpour-Tabrizi et al. 2020, Azri et al. 2025]. Genotype-dependent differences in saturated fatty acid composition have also been demonstrated in evaluations of diverse amaranth germplasm collections [El Gendy et al. 2018, Petkova et al. 2019].

The content of heptadecanoic acid C<sub>17:0</sub> was influenced more strongly by cultivar than by fertilization, with Aztek containing 0.01 percentage points more than Rawa (Tab. 4). Its concentration also increased slightly at higher fertilization levels (NPK2 and NPK3) compared with NPK0 and NPK1.

Stearic acid  $C_{18:0}$  content depended mainly on cultivar, with Aztek containing 0.28 percentage points more than Rawa (Tab. 4). The highest content was recorded under NPK3, which did not differ significantly from NPK1 and NPK2, but differed from NPK0. Different trends reported by Azri et al. [2025] under contrasting environments indicate strong genotype  $\times$  environment interactions governing stearic acid accumulation in amaranth seeds.

Lignoceric acid  $C_{24:0}$  was most strongly modified by the genetic factor, with the Rawa cultivar containing 0.09 percentage points more than Aztek (Tab. 4).

Significant interactions were observed for palmitic acid  $C_{16:0}$  and stearic acid  $C_{18:0}$ , where the interaction between fertilization level and cultivar played a decisive role, confirming the importance of genotype in modifying lipid metabolism under varying agronomic conditions [He and Corke 2003, Skwaryło-Bednarz 2010, El Gendy et al. 2018].

### Unsaturated fatty acids

Seven unsaturated fatty acids were identified in amaranth seed oil, with oleic acid  $C_{18:1}$  (n-9) and linoleic acid  $C_{18:2}$  (n-6) as the dominant components (Tab. 5). The predominance of these fatty acids is consistent with earlier and recent studies [Ratusz and Wirkowska 2006, Bozorov et al. 2018, El Gendy et al. 2018, Nasirpour-Tabrizi et al. 2020], although interspecific differences within the genus *Amaranthus* have also been reported [Jahaniaval et al. 2000].

**Table 5.** The content of unsaturated fatty acids in amaranth seed oil (means for factors and years)

Factor	Unsaturated fatty acids (%)						
	$C_{16:1}$ (n-7)	$C_{16:1}$ (n-9)	$C_{18:1}$ (n-7)	$C_{18:1}$ (n-9)	$C_{18:2}$ (n-6)	$C_{18:3}$ (n-3)	$C_{20:1}$ (n-9)
NPK0	0.41 d	0.02 b	1.15 a	29.85 a	39.03 a	1.09 a	0.20 b
NPK1	0.42 c	0.03 ab	1.21 a	29.59 a	39.32 a	1.04 a	0.20 b
NPK2	0.43 b	0.03 ab	1.20 a	29.26 a	39.06 a	1.06 a	0.20 b
NPK3	0.44 a	0.04 a	1.16 a	29.21 a	39.20 a	1.08 a	0.21 a
LSD <sub>0.05</sub>	0.01	0.02	0.07	5.72	4.57	0.24	0.008
F value	17.95	3.39	2.07	0.04	0.01	0.10	4.84
p	***	*	n.s.	n.s.	n.s.	n.s.	**
A	0.42 b	0.02 b	1.13 b	24.46 b	43.16 a	1.25 a	0.20 b
R	0.43 a	0.04 a	1.23 a	34.50 a	35.14 b	0.89 b	0.21 a
LSD <sub>0.05</sub>	0.01	0.006	0.02	0.20	0.15	0.06	0.004
F value	9.32	88.4	102.31	10628.34	12321.31	152.51	32.66
p	**	***	***	***	***	***	***
N	0.42 a	0.03 a	1.17 a	29.41 a	39.02 a	1.07 a	0.20 a
W	0.42 a	0.03 a	1.19 a	29.54 a	39.28 a	1.07 a	0.20 a
LSD <sub>0.05</sub>	0.01	0.01	0.04	2.99	2.38	0.12	0.005
F value	1.14	0.12	1.06	0.01	0.05	0.002	0.11
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2016	0.43 a	0.04 a	1.20 a	29.56 a	39.23 a	1.07 a	0.21 a
2017	0.41 c	0.03 a	1.16 a	29.42 a	39.07 a	1.04 a	0.20 b
2018	0.42 b	0.03 a	1.18 a	29.46 a	39.17 a	1.10 a	0.20 b
LSD <sub>0.05</sub>	0.01	0.02	0.05	4.45	3.56	0.18	0.007
F value	3.84	1.61	1.72	0.003	0.01	0.33	4.39
p	*	n.s.	n.s.	n.s.	n.s.	n.s.	*
CV (%)	3.49	54.09	5.19	17.07	10.26	19.52	4.19

Legend: NPK0–NPK3 – fertilization levels; A – Aztek cultivar, R – Rawa cultivar; N – narrow row spacing, W – wide row spacing; 2016–2018 – study years; values followed by the same letters do not differ significantly at  $p \leq 0.05$ ; \*, \*\*, and \*\*\* indicate significance at  $p \leq 0.05$ , 0.01, and 0.001, respectively; CV – coefficient of variation

Unsaturated fatty acids:  $C_{16:1}$  (n-7) palmitoleic acid;  $C_{16:1}$  (n-9) palmitoleic acid;  $C_{18:1}$  (n-7) elaidic acid;  $C_{18:1}$  (n-9) oleic acid;  $C_{18:2}$  (n-6) linoleic acid;  $C_{18:3}$  (n-3) linolenic acid;  $C_{20:1}$  (n-9) eicosenoic acid

**Table 6.** Fatty acid composition in amaranth seed oil (means for factors)

Factor	Fatty acid composition of cultivated amaranth oil (%)		
	Sum of saturated fatty acids (%)	Sum of unsaturated fatty acids (%)	Ratio of linoleic acid C <sub>18:2</sub> (n-6) to linolenic acid C <sub>18:3</sub> (n-3)
NPK0	28.25 a	71.75 a	37.14 a
NPK1	28.20 a	71.80 a	38.04 a
NPK2	28.77 a	71.23 a	37.24 a
NPK3	28.66 a	71.34 a	36.75 a
LSD <sub>0.05</sub>	1.10	1.10	3.74
F value	0.96	0.96	0.30
p	n.s.	n.s.	n.s.
A	29.37 a	70.63 b	34.81 b
R	27.57 b	72.43 a	39.78 a
LSD <sub>0.05</sub>	0.25	0.25	1.31
F value	203.42	203.42	58.55
p	***	***	***
N	28.68 a	71.32 a	37.59 a
W	28.26 a	71.74 a	37.00 a
LSD <sub>0.05</sub>	0.58	0.58	1.96
F value	2.10	2.10	0.36
p	n.s.	n.s.	n.s.
2016	28.27 a	71.73 a	36.94 a
2017	28.69 a	71.31 a	38.17 a
2018	28.45 a	71.55 a	36.77 a
LSD <sub>0.05</sub>	0.87	0.87	2.88
F value	0.68	0.68	0.83
p	n.s.	n.s.	n.s.
CV (%)	3.51	1.40	8.88

Legend: NPK0–NPK3 – fertilization levels; A – Aztek cultivar, R – Rawa cultivar; N – narrow row spacing, W – wide row spacing; 2016–2018 – study years; values followed by the same letters do not differ significantly at  $p \leq 0.05$ ; \*, \*\*, and \*\*\* indicate significance at  $p \leq 0.05$ , 0.01, and 0.001, respectively; CV – coefficient of variation

Palmitoleic acid C<sub>16:1</sub> (n-9) showed strong variability (CV = 54.09%), whereas linolenic acid C<sub>18:3</sub> (n-3), oleic acid C<sub>18:1</sub> (n-9), and linoleic acid C<sub>18:2</sub> (n-6) exhibited low variability (CV = 10.26–19.52%), as in Table 5. The remaining unsaturated fatty acids were characterized by very low coefficients of variation – CV < 10% (Table 5), which is consistent with previous findings [Petkova et al. 2019]. Hydrothermal conditions significantly affected only palmitoleic acid C<sub>16:1</sub> (n-9) and eicosenoic acid C<sub>20:1</sub> (n-9). The highest contents of both acids were recorded in the first year, while lower or unchanged values were observed in subsequent years (Tab. 5). Similar sensitivity of minor unsaturated fatty acids to hydrothermal variability has been reported previously [Gamel et al. 2007, Tyrus et al. 2024].

The strongest differentiation of palmitoleic acid C<sub>16:1</sub> (n-9) resulted from NPK fertilization, with the highest content under NPK3 and the lowest under NPK0 (Tab. 5). Cultivar also significantly affected this acid, with higher concentrations in Rawa. Similar fertilization effects on minor fatty acids have been reported elsewhere [Mndzebele et al. 2023, Tyrus et al. 2024].

Palmitoleic acid C<sub>16:1</sub> (n-9), elaidic acid C<sub>18:1</sub> (n-7), oleic acid C<sub>18:1</sub> (n-9), linoleic acid C<sub>18:2</sub> (n-6), linolenic acid C<sub>18:3</sub> (n-3), and eicosenoic acid C<sub>20:1</sub> (n-9) were primarily influenced by cultivar (Tab. 5). Oleic acid content was substantially higher in Rawa, exceeding values reported for several amaranth genotypes grown under different conditions [Jahaniaval et al. 2000, León-Camacho et al. 2001, Escudero et al. 2004]. In contrast, Aztek exhibited higher concentrations of linoleic and linolenic acids (Tab. 5). Such cultivar-dependent variation is attributable to genetic differences in lipid biosynthesis pathways, including  $\Delta 9$  and  $\Delta 12$  desaturase activities [Cerone et al. 2022].

A significant interaction between row spacing and cultivar was observed for linoleic acid C<sub>18:2</sub> content (Tab. 2), indicating that plant spatial arrangement may modulate seed quality traits in a genotype-dependent manner, as reported previously [Gamel et al. 2007, Petkova et al. 2019].

### **Fatty acid ratios and nutritional implications**

Coefficients of variation for the sum of saturated and unsaturated fatty acids and for the linoleic to linolenic acid ratio were very low (Tab. 6), indicating stable proportions of major fatty acid groups, consistent with earlier studies [Petkova et al. 2019, Baraniak and Kania-Dobrowolska 2022].

Cultivar was the main factor differentiating the sums of saturated and unsaturated fatty acids and the n-6/n-3 ratio (Tab. 6). The Aztek cultivar contained a higher proportion of saturated fatty acids, while Rawa showed a higher proportion of unsaturated fatty acids. The saturated-to-unsaturated fatty acid ratio in both cultivars was close to 1:3, in agreement with previous reports [Gontarczyk 1996, He et al. 2002, Januszewska-Jóźwiak and Synowiecki 2008, El Gendy et al. 2018].

The n-6/n-3 ratio was higher in Rawa (39.78:1) than in Aztek (34.81:1) – as in Table 6, confirming that amaranth oil is generally characterized by a high n-6/n-3 ratio, as also reported in food composition databases [USDA Food-Data Central 2023]. Nevertheless, genotype-dependent variability has been widely documented [El Gendy et al. 2018, Petkova et al. 2019]. From a nutritional perspective, these differences are highly relevant, as emphasized by Jan et al. [2023]. Although the observed ratios exceed recommended dietary values, cultivar-dependent variation and significant genotype × agronomic interactions indicate potential for improving fatty acid balance through targeted selection and cultivation practices [Simopoulos 2016, Calder 2017].

### **CONCLUSIONS**

The three-year field experiment demonstrated that seed yield, crude fat content, fat yield, and fatty acid composition of amaranth cultivated under temperate climatic conditions are determined by the combined effects of genetic and agrotechnical factors. Among the analyzed variables, cultivar proved to be the primary factor differentiating both yield potential and oil quality traits. The Aztek cultivar was characterized by higher seed yield and fat yield, whereas the Rawa cultivar exhibited a more favorable fatty acid composition, particularly in terms of the degree of unsaturation.

Row spacing significantly influenced plant density and yield structure. Wider row spacing (55 cm) promoted higher seed mass per plant and modified fatty acid composition in a cultivar-dependent manner, indicating the importance of adjusting stand architecture to genotype. Increasing NPK fertilization level enhanced seed yield and fat yield; however, its effect on fatty acid composition was limited and depended on cultivar. Significant interactions between fertilization level and cultivar, particularly for palmitic (C16:0) and stearic (C18:0) acids, confirm genotype-specific responses to nutrient supply. Weather conditions markedly affected yield formation and fat accumulation, but did not alter the overall direction of cultivar and agrotechnical effects.

From a practical perspective, the results indicate that effective amaranth cultivation under temperate climate conditions should combine appropriate cultivar selection with optimized fertilization level and row spacing. In particular, the integration of moderate to high mineral fertilization with wider row spacing may contribute to increased seed productivity while maintaining favorable oil quality traits. The findings also indicate that cultivar-specific responses should be taken into account when formulating agrotechnical recommendations.

Further research should focus on refining fertilization strategies and stand management under variable climatic conditions, with particular emphasis on their effects on seed yield, crude fat content, and fatty acid composition. Improved understanding of genotype × environment × management interactions may contribute to more precise optimization of agrotechnical practices aimed at maintaining high yield levels while ensuring stable and desirable oil quality traits of amaranth seeds.

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