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AMARANTHUS SPECIES ASSESSMENT FOR MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS

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SUMMARY

Amaranth (*Amaranthus* spp.) is an important food crop source of nutrients and bioactive compounds. Different species of amaranth showed considerable phenotypic variations and could possess diverse nutritional properties. The presented study strove to determine the diversity of amaranth collection for morphological and biochemical traits and their antioxidant potential for developing new cultivars adapted to the environmental conditions of Central Russia. The selected 16 amaranth accessions had high variability in morphological and biochemical traits. With red-colored leaves, amaranthine content ranged from 0.319[a] to 2.031[f] mg/g fresh weight (FW). The water-soluble antioxidant content ranged from 0.425[a] to 1.439[h] mg of gallic acid equivalent (GAE)/g FW. In amaranth accessions, the phenolic compound content in leaves varied from 2.700[a] to 4.825[g] mg GAE/g FW. In the amaranth collection, the total chlorophyll content ranged from 0.9946[a] to 3.5467[j] mg/g, and carotenoids from 0.2196[a] to 0.8289[i] mg/g. A strong positive correlation ($P \leq 0.05$) was evident between total chlorophyll and carotenoid contents (r≈0.90), plant height and inflorescence length (r≈0.79), and inflorescence length and seed weight harvested per plant (r≈0.76).

In summary, when evaluating the antioxidant levels in various samples, the Valentina cultivar, characterized by red leaves, appeared to have the highest amaranthine concentration. Both Valentina and Fakel cultivars exhibited the maximum overall antioxidant capacity. Additionally, the Valentina and Duimovochka cultivars emerged as the top accumulators of polyphenols. These findings highlight the significant antioxidant potential present in these specific cultivars.

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Keywords: Amaranth (*Amaranthus* spp.), agricultural traits, antioxidant content, amaranthine, phenolic compounds, photosynthetic pigments

Key findings: In the latest study, the 16 Amaranth accessions belonging to eight species succeeded in evaluation for morphological and biochemical parameters. Based on the investigations, the different accessions identified are recommendable for breeding and developing new, improved amaranth cultivars and preferable sources of phytopigments and antioxidants at the seedling stage.

INTRODUCTION

With population pressure and climate change, a prediction arose that food supplies must double by 2050 to cope with the global food systems (Massawe *et al*., 2016). With pressures of salinity and drought conditions, reduced crop yields in most regions worldwide adversely affect food supplies (Mayes *et al*., 2011). In addition, only a few crops are beneficial, leading to a loss of agrobiodiversity and potentially threatening global food security (Khoury *et al*., 2014).

Several major staple crops (rice, wheat, and maize) cannot provide a balanced diet composition based on the essential minerals, vitamins, and bioactive substances, resulting in malnourished two billion people (Cheng *et al*., 2017). Including crops with an expanded range of nutrients required by the human body can further improve diet quality by increasing biodiversity, and Amaranth is one such crop (Joshi *et al*., 2018).

Amaranth (*Amaranthus* spp.) is a C_4 dicotyledonous plant belonging to the genus *Amaranthus* (Cai *et al*., 2004). The genus *Amaranthus* consists of 50–70 species grouped into three categories, i.e., vegetable amaranth (including *A. tricolor* L., *A. blitum* L., *A. dubius* L., *A. viridis* L., *A. graecizans* L., and *A. gangeticus* L.), grain amaranth (*A. hypochondriacus* L., *A. caudatus* L., and *A. cruentus* L.), and weedy amaranth (*A. hybridus L.* and *A. spinosus L.)* (Das, 2016). Amaranth is an ancient food crop, rich in nutrients and resistant to climate change, with a high degree of genetic variability, phenotypic plasticity, and environmental adaptability (Jamalluddin *et al*., 2022). Amaranth widely adapts and can grow in temperate, tropical climates (Joshi *et al*., 2018). It can also occur year-round with short crop rotation and low inputs. As a C_4 crop, amaranth can reduce water losses during the growing season and act as a drought and salt-tolerant crop to unfavorable environmental conditions (Lin *et al*., 2022).

Amaranth seeds and leaves are rich in protein with a balanced content of gluten-free, essential amino acids, providing a nutritional option for consumers with intolerance to this protein (Lin *et al*., 2022). Leafy species of amaranth are a rich source of micronutrients, including Ca, Fe, Zn, dietary fibers, and biologically active compounds, such as pigments (amaranthine, chlorophylls, carotenoids), phenolic acids, and flavonoids, with high antioxidant capacity and ascorbic acid (Gins *et al*., 2021; Sarker and Oba, 2021). These natural antioxidants protect against several diseases, viz., cardiovascular diseases, cancer, cataracts, atherosclerosis, retinopathy, arthritis, emphysema, and neurodegenerative ailments (Sarker and Oba, 2021). The bioactive components of the amaranth plant are also vital in detoxifying reactive oxygen species and reducing stress on the human body, including supplementation as a source of antioxidants and vitamins in functional foods.

Young seedlings of the amaranth species are edible, becoming a prevalent leafy vegetable rich in nutrients (protein, fiber, and micronutrients) and antioxidants (Benincasa *et al*., 2015; Sarker and Oba, 2021). Amaranth is a nutritious, gluten-free plant that can enhance human overall well-being. In amaranth leaves, breeding for the increased amount of bioactive compounds with antioxidant properties, based on more detailed biochemical studies, could be cost-effective in improving public health (Grubben and Denton, 2004). Unfortunately, morpho-biological and biochemical characteristics of leafy amaranths have limited studies. Therefore, the pertinent research provides a more detailed characterization of the nutrients, including the amaranth species.

The proposed collection of the amaranth vegetable and cereal species will help understand the genetic variability within and between the species for morphological and biochemical traits, quantifying how variable the traits under study may be. Since the measured traits are under ecological and genetic control, by growing accessions under the same field conditions, a conclusion could be that the measured traits were due to heritable differences in specific environmental conditions. The significant study assessed the genetic diversity in 16 amaranth accessions for morphological and biochemical characteristics. It also evaluated their antioxidant potential for new amaranth cultivar development adapted to Central Russian climatic settings.

MATERIALS AND METHODS

Plant materials

The study's object comprised 16 amaranth accessions belonging to eight different species, i.e., *A. caudatus* (2 accessions), *A. tricolor* (1), *A. cruentus* (3), *A. hypochondriacus* (3), *A. paniculatus* (2), *A. blitum* (1), *A. gangeticus* (1), and *A. graecizans* (1), and two accessions of unknown species (Tables 1 and 2). Nine of these accessions were cultivars from the Genetic Collection of Plant Resources, Federal State Budgetary Scientific Institution, Federal Scientific Vegetable Center (FSBSI, FSVC), Moscow, Russia. Seven accessions came from the collection of the Federal Research Center, N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), Saint Petersburg, Russia.

The leaves of red and green amaranth plants served as the research material. The experiment commenced in the laboratory of Plant Physiology and Biochemistry, FSBSI FSVC, Moscow region, Russia (55°39.51′ N, 37°12.23′ E). The field for growing plants contained sod-podzolic clay loam soil, with the leaves collected during the bud-formation period. The experiment had a randomized complete block design with three replicates. Before planting the amaranth seedlings, the agrochemical characteristics of the tilth-top (0–20 cm) soil layer were as follows: the content of humus (1.65%), the reaction of the medium pH_{Kcl} (6.0), the hydrolytic acidity (1.30 mg-eq/100 g of soil), the amount of absorbed substrates (19.8 mg-eq/100 g of soil), the degree of substrate saturation (92.5%), and the average content of P, K, and N were 480, 161 and 10 mg/kg, respectively.

Table 1. Characteristics of the amaranth cultivars and accessions.

Accessions	Species	Breeding center	Country of origin	Application
Bulava	*A. caudatus L.*	FSBSI FSVC	Russia	Decorative
Valentina	*A. tricolor L.*	FSBSI FSVC	Russia	Vegetable, seed
Zelenaya Sosulka	*A. caudatus L.*	FSBSI FSVC	Russia	Decorative
Duimovochka	*A. cruentus L.*	FSBSI FSVC	Russia	Vegetable
Kizlyarets (green form)	*A. hypochondriacus L*	FSBSI FSVC	Russia	Seed, fodder
Kizlyarets (red form)	*A. hypochondriacus L*	FSBSI FSVC	Russia	Seed, fodder
Krepysh	*A. hypochondriacus L.*	FSBSI FSVC	Russia	Seed, fodder
Pamyati Kovasa	*A. cruentus L.*	FSBSI FSVC	Russia	Vegetable
Fakel	*A. paniculatus L.*	FSBSI FSVC	Russia	Fodder, decorative
Green thumb	*A. cruentus L.*	VIR	-	Vegetable
K-11	-	VIR	India	Vegetable
K-21	*A. paniculatus L.*	VIR	Russia	Fodder, decorative
K-26	-	VIR	China	Vegetable
K-103	*A. gangeticus L.*	VIR	Bangladesh	Vegetable
K-269	*A. graecizans L.*	VIR	-	Vegetable
K-471	*A. blitum L.*	VIR	-	Vegetable

Table 2. Qualitative traits of the amaranth cultivars and accessions.

Accessions	Growth habit	Stem color	Leaf color	Inflorescence color	Inflorescence shape	Seed color
Bulava	Erect	Pink	Green	Pink	Drooping, club-shaped	Pink
Valentina	Erect	Purple	Intense red-purple	Intense red-purple	Erect, paniculate	Black
Zelenaya Sosulka	Erect	Green	Green	Green	Drooping, paniculate	Pink
Duimovochka	Erect	Green	Light green	Green	Erect, Spike (Dense)	Black
Kizlyarets (green form)	Erect	Green	Green	Green	Erect, paniculate	White
Kizlyarets (red form)	Erect	Pale-pink	Green-red	Red	Erect, paniculate	White
Krepysh	Erect	Green	Green	Green	Erect, paniculate	White
Pamyati Kovasa	Erect	Green	Green	Red	Erect, paniculate	White
Fakel	Erect	Pink	Red-purple	Red-purple	Erect, club-shaped	Black
Green thumb	Erect	Green	Light green	Green	Erect, Spike (Dense)	Black
K-11	Erect	Red	Green	Green	Inflorescence is erect and club-shaped in leaf axils	Black
K-21	Erect	Pink	Red-purple	Red-purple	Erect, club-shaped	Black
K-26	Erect	Green	Purple center, green edges	Green	Inflorescence is erect and club-shaped in leaf axils	Black
K-103	Erect	Red	Brown-purple	Red and green	Inflorescence is erect and club-shaped in leaf axils	Black
K-269	Erect	Pale-pink	Green	Pale-pink	Inflorescences in the axils of lateral stems and leaves	Black
K-471	Erect	Green	Purple-green	Purple	Erect	Black

Morphometric assessment of plants

Qualitative traits of amaranth plants, such as plant habitus, the color of stems, seeds, leaf, inflorescence, and leaf and inflorescence shapes, attained visual scrutiny. However, measuring the quantitative traits used a ruler for plant height (cm), inflorescence length (cm), and leaf area (cm^2), and an analytical scale for seed weight per plant (g) and 1000-seed weight (g). Plant height measuring began from the soil surface to the top of the inflorescence. All the quantitative traits are in the mean measurements of 15 amaranth plants.

Biochemical parameters

Total antioxidant content

Using the amperometric method (Gins *et al*., 2020) determined total antioxidant content (TAC), with the results expressed in gallic acid equivalents (mg GAE/g FW). The mortar and pestle helped grind the accession samples (0.1–0.2 g FW), adding 10 mL extractant at the gradual temperature increase (20 °C...25 °C). The homogenate centrifugation at 6000 g took 15 min. The resulting aliquot of the supernatant served to determine the antioxidant content, diluting as necessary. The measurements proceeded on the device 'Tsvet-Yauza 01-AA' in constant-current mode (Mamedov *et al*., 2017).

Photosynthetic pigments and amaranthine

The spectrophotometric method determined the content of photosynthetic pigments. Calculating the content of chlorophyll a, b, and the total chlorophyll content of carotenoids followed the formulas below (Lichtenthaler, 1987).

$$Chl\ a\left[\frac{mg}{g}\right] = \frac{\left(13{,}36A_{664{,}2} - 5{,}19A_{648{,}6}\right) * V}{1000 * m}$$

$$Chl\ b\left[\frac{mg}{g}\right] = \frac{\left(27{,}43A_{648{,}6} - 8{,}12A_{664{,}2}\right) * V}{1000 * m}$$

$$Car^{\Sigma}\left[\frac{mg}{g}\right] = \frac{\left(4{,}785A_{470} + 3{,}657A_{664{,}2} - 12{,}76A_{648{,}6}\right) * V}{1000 * m}$$

Where:
A_{470}, $A_{648.6}$, and $A_{664.2}$: the absorption at 470, 648.6, and 664.2 nm, respectively, and the thickness of the cuvette was 1 cm.
V = the volume of the extract (ethanol 96%) in ml
m = the weight of the sample attachment in g.

The amaranthine content calculation used the following formula:

$$\text{Amaranthine} = A_{536}(MW)V_a(DF)/\varepsilon LWa$$

Where:
A_{536} is the absorbance at 536 nm (λ_{max}), V_a is the total extract volume, DF is the dilution factor, L is the path length of the cuvette, and W_a is the fresh weight of the sample. The molecular weight (MW) and molar extinction coefficient (ε) of amaranthine were 726.6 and 5.66×10^4 $M^{-1}cm^{-1}$, respectively (Gins *et al*., 2002).

Phenolic compounds

The total phenolic content determination utilized the Folin-Ciocalteu assay (Ainsworth and Gillespie, 2007). Gallic acid served as the standard, with the TPC expressed as mg gallic acid equivalents (mg GAE/g FW). Fresh samples of 1 g sustained thorough crushing and homogenizing in 10 ml of 80% ethanol. The homogenate, placed in capped test tubes, received heat at 60 °C in a water bath for 1 h. The extract centrifugation was at 6000 g for 15 min, with the collected resulting supernatant used for the estimation of total phenolics. Each sample of 20 µl received 480 µl bidistilled water and Folin-Ciocalteu reagent (1 mL), and then incubated at room temperature for 3 min. Then, each mixture acquired 1 mL of 7% Na_2CO_3. The absorbance measurement was at 765 nm against a blank after 30 min of incubation at room temperature using the Solar UV-VIS PB 2201 spectrophotometer.

Statistical analysis

The study used three biological replicates in determining biological parameters, carrying out 3–5 analytical determinations within each biological replicate. The data analysis ran in R-Studio (calculation of mean, standard deviations, correlations, and analysis of variance) using tidyverse, emmeans, multcomp, multcomp View, and ggcorrplot packages. Evaluating normal distribution and equality of the variances employed Shapiro-Wilk's and Levene's tests, respectively, at $p > 0.05$. Tabular data presentation was mean ± standard error. The standard errors (S.E.) stayed constant within a table within each set of results. It was a consequence indicating a balanced design (equal numbers of observations in each cell) and equality of the variances (as proven by Levene's test). Mean values with no-matching letters were significantly different according to the Bonferroni test at a significant level of 5%. The Cheddock scale helped assess the strength of the correlation coefficient.

RESULTS AND DISCUSSION

In the presented study, the morphological and biochemical traits of 16 amaranth accessions gained scrutiny to quantify the level of intra- and interspecific variability and to estimate their adaptability to the environmental conditions of Russia's Central Region.

Morphometric characterization of the amaranth cultivars

Different species of amaranth (*Amaranthus* spp.) reached characterization by a high level of genetic variability and differences in phenotypic features, especially for inflorescence type, leaf color, growth mode, and pest and disease resistance (Gerrano *et al*., 2015). Plant height and flower coloration also varied among the amaranth species. In their accessions, leaf areas also generally contrasted due to different leaf shapes and plant sizes. The wide genotypic variability of amaranth species may refer to frequent interspecific and inter-varietal hybridization (Suresh e*t al*., 2014).

In the latest study, five quantitative morphological traits bore analysis, i.e., plant height (cm), inflorescence length (cm), leaf

Table 3. Morphometric characteristics of the amaranth cultivars and accessions.

Accessions	Plant height (cm)	Terminal inflorescence length (cm)	Leaf area (cm^2)	Seed weight per plant (g)	1000-seed weight (g)
Bulava	130.82^{g}	60.67^{g}	84.62^{efg}	3.910^{h}	0.7040^{ab}
Valentina	117.45^{ef}	31.64^{e}	81.25^{ef}	4.631^{i}	0.7030^{ab}
Duimovochka	61.17^{a}	25.04^{cd}	25.64^{b}	1.331^{bc}	0.6764^{a}
Zelenaya Sosulka	180.25^{h}	65.75^{h}	76.56^{e}	4.774^{i}	0.6780^{ab}
Kizlyarets (green form)	82.48^{b}	26.41^{cd}	47.11^{c}	3.512^{g}	1.0134^{e}
Kizlyarets (red form)	103.43^{d}	27.25^{cde}	63.03^{d}	3.630^{gh}	0.9728^{e}
Krepysh	97.75^{cd}	26.75^{cde}	58.25^{d}	3.501^{g}	0.9712^{e}
Pamyati Kovasa	125.03^{fg}	44.10^{f}	103.75^{h}	5.780^{j}	0.7658^{c}
Fakel	92.82^{c}	24.26^{bcd}	78.43^{e}	2.343^{e}	0.7892^{cd}
Green thumb	62.67^{a}	23.60^{bc}	24.66^{b}	1.418^{cd}	0.6850^{ab}
K-471	129.22^{g}	19.63^{b}	88.12^{fg}	1.013^{b}	0.7200^{b}
K-11	77.25^{b}	8.51^{a}	21.81^{b}	1.741^{d}	1.2680^{f}
K-21	113.43^{e}	28.61^{de}	80.52^{ef}	2.819^{f}	0.7694^{c}
K-26	67.75^{a}	6.25^{a}	89.94^{g}	0.490^{a}	0.8220^{d}
K-103	76.41^{b}	9.22^{a}	25.76^{b}	1.342^{bc}	1.2768^{f}
K-269	64.24^{a}	NA	8.15^{a}	0.501^{a}	0.9878^{e}
S.E.	1.67	0.92	1.82	0.066	0.0081

area (cm^2), seed weight per plant (g), and 1000-seed weight (g). As a result, the differences among the amaranth accessions emerged for all the measured morphological characteristics (Table 3). The maximum plant height was visible in amaranth cultivars Zelenaya Sosulka (180.25^{h} cm) and Bulava (130.82^{g} cm), belonging to the *A. caudatus* L. species. They also have a drooping type of inflorescence. Maximum inflorescence length also occurred in the cultivars Zelenaya Sosulka and Bulava (65.75^{h} and 60.67^{g} cm, respectively).

However, the observed minimum plant height showed in the amaranth cultivar Duimovochka (61.17^{a} cm), while the minimum inflorescence length appeared in the accession from China – K-26 (6.25^{a} cm). Notably, in amaranth accession K-269, the main inflorescence was also absent, with the seeds forming in the axils of leaves and shoots. The highest leaf area (103.75^{h} cm^2) manifested in the cultivar Pamyati Kovasa, while the minimum value was apparent in the accession K-269 (8.15^{a} cm^2).

The amaranth seeds collected from each plant also underwent assessment. The highest seed weight per plant resulted in the cultivar Pamyati Kovasa (5.780^{j} g), while the lowest seed weight occurred in the accession K-26 (0.490^{a} g). The maximum 1000-seed weight came from accessions K-103 and K-11 (1.2768^{f} and 1.2680^{f} g, respectively), with the minimum in cultivars Zelenaya Sosulka and Duimovochka (0.6780^{ab} and 0.6764^{a} g, respectively). Overall, the studied 16 amaranth accessions revealed the highest genetic variability for morphological traits, indicating the prospects of improving the potential of amaranth species for these traits.

Biochemical index characterization of amaranth accessions

The study also revealed considerable differences in the amaranth accessions belonging to different species for the content of pigments, antioxidant activity, and phenolic compounds in leaves (Table 4). The total antioxidant content index includes water-soluble compounds, as the extraction continued with bidistillate, including amaranthine, ascorbic acid, and phenolic compounds. The maximum water-soluble antioxidant content was available in amaranth plants of cultivars Valentina and Fakel (1.439^{h} and 1.306^{g} mg GAE/g FW, respectively). However, the minimum content of water-

Table 4. Biochemical index characterization of the amaranth cultivars and accessions.

Accessions	AO H_2O (mg GAE/g FW)	Chl a+b (mg/g FW)	Car (mg/g FW)	Am (mg/g FW)	TPC (mg GAE/g FW)
Bulava	0.614^{c}	1.8929^{e}	0.3395^{c}	NA	4.110^{d}
Valentina	1.439^{h}	2.0930^{f}	0.3995^{e}	2.031^{f}	4.808^{g}
Duimovochka	0.843^{e}	0.9946^{a}	0.2224^{a}	NA	4.825^{g}
Zelenaya Sosulka	0.598^{c}	2.1030^{f}	0.2653^{b}	NA	4.448^{ef}
Kizlyarets (green form)	0.595^{c}	1.6531^{d}	0.3995^{e}	NA	4.290^{de}
Kizlyarets (red form)	0.700^{d}	1.6731^{d}	0.3881^{de}	0.482^{b}	3.803^{c}
Krepysh	0.573^{c}	2.6236^{h}	0.6370^{g}	NA	4.327^{e}
Pamyati Kovasa	0.605^{c}	1.2089^{b}	0.2196^{a}	NA	4.603^{f}
Fakel	1.306^{g}	1.6431^{d}	0.3710^{d}	0.631^{cd}	4.315^{e}
Green thumb	0.825^{e}	1.1590^{b}	0.2453^{ab}	NA	4.600f
K-471	0.990^{f}	1.4733^{c}	0.3424^{c}	0.319^{a}	3.655^{c}
K-11	0.493^{b}	2.2381^{g}	0.5654^{f}	NA	2.753^{a}
K-21	0.935^{f}	3.5467^{j}	0.7314^{h}	0.724^{d}	4.288^{de}
K-26	0.476^{ab}	1.1890^{b}	0.3309^{c}	0.533^{bc}	3.032^{b}
K-103	0.677^{d}	3.2756^{i}	0.8289^{i}	1.154e	3.622^{c}
K-269	0.425^{a}	1.8979^{e}	0.5454^{f}	NA	2.700^{a}
S.E.	0.013	0.0248	0.0054	0.028	0.039

soluble antioxidants was evident in the green-colored leaves of amaranth accessions K-269 and K-11 (0.425^{a} and 0.493^{b} mg GAE/g FW, respectively).

In amaranth, compared with green-colored leaves, the red-colored leaf plants seemed to contain more pigments, including amaranthine and carotenoids (Sarker and Oba, 2021). The maximum content of amaranthine pigment surfaced in the amaranth cultivar Valentina (2.031^{f} mg/g FW), showing intense coloration of stems, leaves, and inflorescence. However, amaranth accessions with green-colored leaves indicated an undetected Amaranthine pigment.

Amaranthine is an influential class of water-soluble red-violet pigments belonging to the group of betalain pigments characteristic of plants of the order Amaranthus and Caryophyllales (Chen *et al.*, 2013). The low molecular weight antioxidant pigment amaranthine exhibits high reactivity, as its molecular structure contains two aromatic rings and hydroxyl groups, readily engaging in free-radical reactions, neutralizing the superoxide radical and inhibiting lipid radicals formation (Gins et al., 1998). In addition, amaranthine also exhibits antioxidant properties characterized by ascorbate, lycopene, and SOD, actively chelating variable valence ions (Ptushenko *et al.*, 2002).

Phenolic compounds are vital phytochemicals widely distributed in plants. The total amount of phenolic compounds in plants depends on their genetic makeup and environmental conditions (Motyleva *et al*., 2021; Muflihah *et al*., 2021). The maximum content of phenolic compounds was prevalent in the green-leaved cultivar Duimovochka and the red-leaved cultivar Valentina (4.825^{g} and 4.808^{g} mg GAE/g FW, respectively), and the minimum was evident in green-leaved accession K-269 (2.700^{a} mg GAE/g FW). Past research studied the effects of leaf, seed coat, and flower colors on the content of phenolic compounds in 289 amaranth samples (Li *et al.,* 2022). Their findings revealed no significant differences among the genotypes with different leaf and seed coat colors for phenolic compound content. However, the amaranth genotypes with diverse flower colors varied significantly in the phenolic compound content.

Chlorophylls a and b are the predominant forms of chlorophyll in higher plants that are crucial in the primary processes of photosynthesis, being part of the antenna complexes that allow plants to absorb light energy. In addition to chlorophylls, carotenoids are essential for photosynthesis, protecting chlorophylls from photooxidative damage (Niroula *et al*., 2019). The highest total chlorophyll was prominent in red-colored leaves of amaranth accession K-21 (3.5467^{j} mg/g), while the lowest was in green-colored leaves of the accession Duimovochka (0.9946^{a} mg/g). The maximum content of carotenoids emerged in the accession K-103 (0.8289^{i} mg/g), with the minimum in the accession Pamyati Kovasa (0.2196^{a} mg/g). Amaranth accession K-103 belonged to the species *A. gangeticus*, red spinach. It is typical for *A. gangeticus* to contain the optimum content of β-carotene and has hepatoprotective properties against chemical carcinogenesis (Sarker and Oba, 2021; Tetyannikov *et al*., 2022).

Correlation coefficients

Correlation among the morphometric traits

The correlation coefficients (r) appear in Figure 1. Several studies have proceeded on amaranth genotypes' biochemical parameters and antioxidant activities (Ishtiaq *et al*., 2014; Gins *et al*., 2021; Jahan *et al*., 2022). However, very few have investigated the relationship among agricultural traits, phytochemicals, and antioxidant activities (Gerrano *et al*., 2015; Nyonje *et al*., 2021; Li *et al*., 2022).

By examining the association among the morphological indices, a strong positive correlation (r≈0.79) occurred between the plant height and inflorescence length. It was interesting to report that amaranth accession K-471 had a rather large plant height (129.22^{g} cm); however, the percentage of its inflorescence length to plant height was only 15.19%. Inversely, the cultivars Duimovochka and Green thumb were the lowest growing accessions, but the percentage of inflorescence length to plant height was 40.94% and 37.66%, respectively in these accessions. A significant positive correlation (r≈0.76) also emerged between the inflorescence length and seed weight per plant.

Considerable positive correlations were evident between plant height and seed weight per plant (r≈0.69), plant height and leaf area (r≈0.66), and leaf area and seed weight per plant (r≈0.54). Gerrano *et al*. (2015) also reported moderate correlation between the leaf area and inflorescence length (r≈0.44), and leaf area and seed yield per plant (r≈0.49) in 32 amaranth genotypes grown in South Africa. The said findings were almost in agreement with the study results, with correlation values of r≈0.43 and r≈0.54, respectively (Gerrano *et al*., 2015). The findings of Yue *et al*. (2006) enunciated that a broader leaf area, length, and width favored the panicle development in rice.

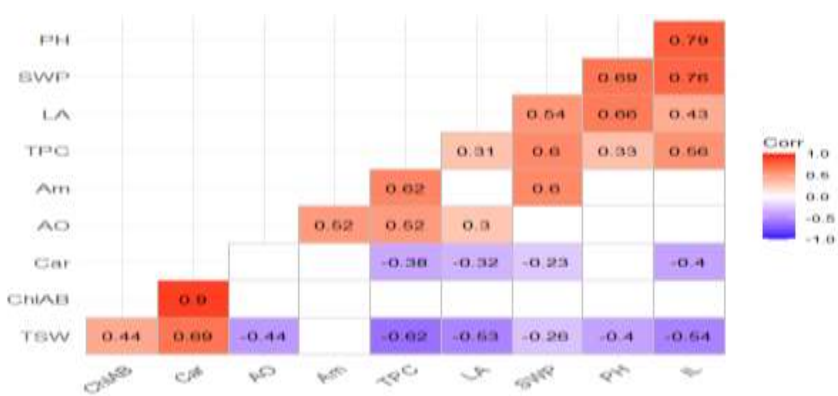

Figure 1. Pearson correlation coefficients for morphometric and biochemical parameters significantly different from zero at p ≤ 0.05. PH – Plant height (cm), IL – Inflorescence length (cm), LA – Leaf area (cm^{2}), SWP – Seed weight per plant (g), TSW – Thousand seed weight (g), AO – Water-soluble antioxidants (mg GAE/g FW), ChlAB – Total content of chlorophylls a and b (mg/g FW), Car – Carotenoid content (mg/g FW), Am – Amaranthine content (mg/g FW), and TPC – Total phenolic compounds (mg GAE/g FW).

However, considerable negative correlations (r≈-0.54 and r≈-0.53) were notable between 1000-seed weight and inflorescence length and leaf area, respectively. Indeed, four amaranth accessions, i.e., K-11, K-103, K-269, and K-26, produced large seeds (Figure 2); however, they showed the shortest inflorescence length, and even its absence, compared with the other 12 accessions. Additionally, three of these four accessions were descriptive by the smallest leaf area, except for accession K-26, displaying a broadleaf plate (Figure 3).

Correlation among the biochemical indices

By studying the relationship among the biochemical parameters, a significant positive correlation (r≈0.90) surfaced between the total chlorophyll content and carotenoids. Kopsell *et al.* (2005) also reported a positive correlation between chlorophyll and carotenoid contents in eight cultivars of sweet basil grown indoors and outdoors. Ndukwe *et al.* (2016) reported a significant correlation between chlorophyll a and b and total chlorophyll and carotenoid contents in 10 maize cultivars. The remarkable correlation relates to given growing conditions and may not be valid when light levels and temperatures differ during the growth period (Niroula *et al*., 2019).

Phenolic compounds are usually chief contributors to the amaranth's antioxidant activity. However, the high amount of betacyanin-amaranthine in the red-leaved amaranth, giving it a dark red color, also enhances the antioxidant activity (Khandaker *et al*., 2008). In the existing study, a considerable positive correlation (r≈0.62) materialized between the phenolic compounds and amaranthine and the phenolic compounds and water-soluble antioxidants (r≈0.52), and between the amaranthine and water-soluble antioxidants (r≈0.52). It indicates that phenolics are not the only source of significant contribution to the sum of antioxidants. Past studies reported a substantial positive correlation between the antioxidant activity and the phenolic compounds, as well as, the pigment and amaranthine in amaranth samples (Bang *et al*., 2021).

Correlation among the morphometric and biochemical traits

A considerable positive correlation (r≈0.69) occurred between the 1000-seed weight and carotenoid content. However, a sizable negative correlation (r≈-0.62) was evident between the 1000-seed weight and total phenolic compound content. Therefore, the 1000-seed weight may be an influential parameter for developing amaranth genotypes with a high content of carotenoids and phenolic compounds.

A considerable positive correlation was apparent between the seed weight per plant and amaranthine content with the total phenolic compound content (r≈0.60) and between phenolic compound content and inflorescence length (r≈0.56). According to the obtained correlation data, the functional interactions between these traits are conclusive. Consequently, the positive correlation between the above features may indicate that the selection and improvement of primary attributes of interest will positively influence the secondary traits in the amaranth breeding program.

Principal component and hierarchical cluster analyses

Evaluating the relationship among the studied traits of amaranth, morphometric, and biochemical parameters ran the principal component analysis (PCA) for 16 accessions, resulting in 10 principal components. Principal components 1–3 had the Eigenvalues >1 (4.13, 2.14, and 1.52, respectively) and explained 77.9% of the total variance (41.3%, 21.4%, and 15.2%, respectively) (Figure 3). The TSW (15.59%), IL (14.87%), TPC (13.84%), SWP (13.79%), PH (13.32%), and LA (12.18%) contributed most to the variance explained by PC1. However, the ChlAB (37.99%) and Car (24.80%) were the prime contributors to the variance explained by PC2. The associations between the contributions observed in PCA were consistent with the correlation obtained by the Pearson correlation analysis (Figure 4).

Figure 2. Seeds of the amaranth cultivar Valentina (left) and sample K-11 (right).

Figure 3. Amaranth cultivars and accessions studied.

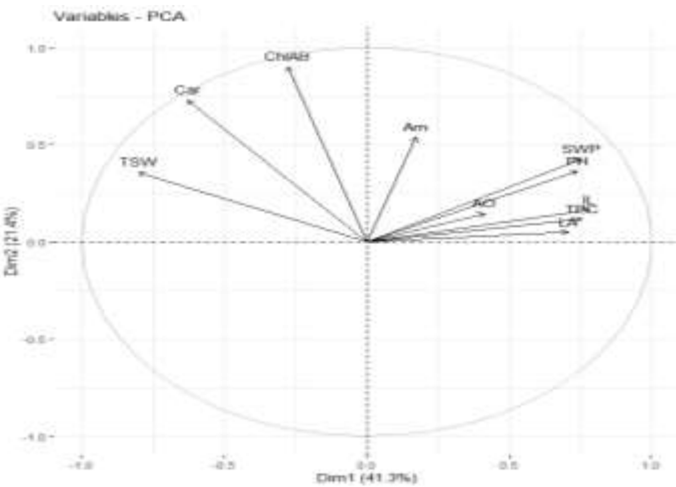

Figure 4. Principal component plot for 16 amaranth cultivars and accessions belonging to different eight species of the genus *Amaranthus* based on morphometric and biochemical parameters. PH – Plant height (cm), IL – Inflorescence length (cm), LA – Leaf area (cm^2), SWP – Seed weight per plant (g), TSW – Thousand seed weight (g), AO – Water-soluble antioxidants (mg GAE/g FW), ChlAB – Total content of chlorophylls a and b (mg/g FW), Car – Carotenoid content (mg/g FW), Am – Amaranthine content (mg/g FW), and TPC – Total phenolic compounds (mg GAE/g FW).

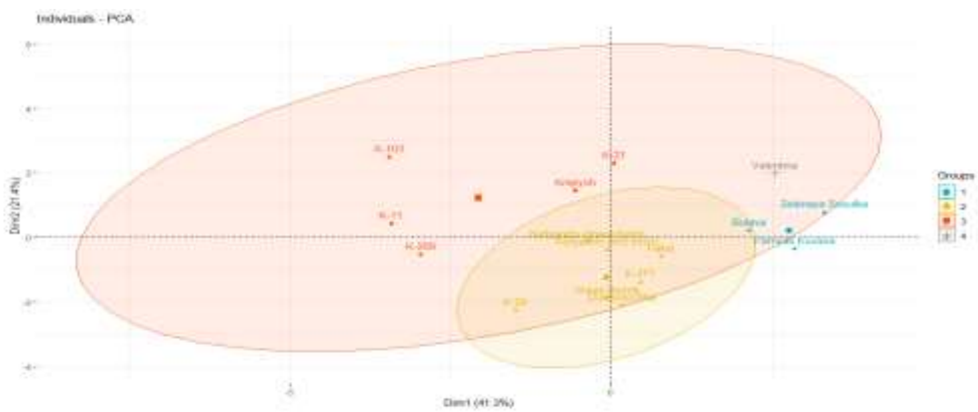

Figure 5. PCA plot showing clustering of 16 amaranth cultivars and accessions based on morphometric and biochemical parameters using the entire dataset.

Hierarchical clustering principal component (HCPC) analysis proceeded based on morphometric and biochemical parameters (Figure 5). Cluster 1 contained the three most related amaranth accessions based on plant height, inflorescence length, leaf area, and seed weight per plant. These morphometric parameters were the highest in amaranth accessions from Cluster 1; however, these accessions also showed the lowest carotenoid content. Cluster 2 consisted of seven amaranth accessions and showed the shortest plant height, minimum seed weight per plant, and less content of photosynthetic pigments (chlorophyll a, b). Cluster 3 included five amaranth accessions with high values of 1000-seed weight, total chlorophyll, and carotenoids; however, they varied by the lowest content of phenolic compounds, short inflorescence, and even absence of inflorescence. Amaranth cultivar Valentina was distinct as a separate Cluster 4 because it had the highest content of amaranthine, water-soluble antioxidants, and phenolic compounds compared with all the studied accessions.

CONCLUSIONS

The results demonstrated the genotypic diversity in 16 amaranth accessions belonging to eight species based on their morphometric and biochemical traits. The highest plant height and inflorescence length prevailed in the cultivar Zelenaya Sosulka and the leaf area in the cultivar Pamyati Kovasa. Amaranth accessions K-21, K-103, and Krepysh were distinctive by the highest amount of photosynthetic pigments. The red-leaved cultivar Valentina showed the maximum antioxidant activity and the optimum content of amaranthine and phenolic compounds. These genetic advantages could apply in breeding and developing new, improved amaranth cultivars. These promising amaranth accessions could be better options at the seedling stage as a source of phytopigments and antioxidants.

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