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Research Article

Variability and heritability of technological characteristics of *Amaranthus* leaves and seeds

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Abstract

This study aimed to determine the variability properties of four major types of *Amaranthus* species in protein and amino acids content in leaves and flowers. Obtained results by ion exchange chromatography in our study have shown high values for the content of essential amino acids lysine and methionine. In the leaf, the lysine content ranged from 3.9 (*A. caudatus*) to 7.0 (*A. cruentus*; *A. moleros*), and in the flowers from 4.2 (*A. caudatus*) to 6.7 (*A. molleros*). The methionine content ranged from 3.1 (A. caudatus) to 7.4 (*A. mantegazzianus*) in the leaf and in the flower from 2.9 (*A. caudatus*) to 6.7 (*A. mantegazzianus*). Besides lysine and methionine, significant values of other essential amino acids were recorded, respectively. Significant concentrations of total proteins were recorded in all examined genotypes. The heritability of the studied characters as protein and mineral content of seeds and leaves, and oil contents of seed were significantly high. The maximum values of the protein content of seeds were 16.55% (*A. cruentus*), in leaves 20.10% (*A. caudatus*), and the minerals in seeds 2.73% (*A. moleros*), and leaves 18.76% (*A. mantegazzianus*). The oil content of seeds was 6.16% (*A. moleros*). The oil content of the seed's proportion of genetic variance to total phenotypic variance was 72%, and it has a significant impact on ecological factors. Tested divergent *Amaranthus* genotypes may serve as parents for further crossing. *Amaranth* seeds is gluten-free and is important in the diet of celiac patients and contains amino acids, especially lysine, which acts against the herpes virus. Amaranth from amaranth leaves biologically active substance that prevents heart muscle damage during ischemic processes. *Amaranth* seed oil has hypolipemic, anti-atherosclerotic, hypotensive and antioxidant activity.

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Keywords: *Amaranthus*; chemical characters; protein and amino acid; leaves and seeds; use in medicine

Introduction

The *Amaranthus* genus has about 60 species, which has the potential for daily diet diversification and alternative food for Celiac disease. Despite having been neglected over many years, Amaranths is promising food crop, mainly due to their resistance to heat, drought, diseases, and pests. In addition, the nutritional value of both the seeds and leaves is excellent. Concerning their high grain protein concentration, they are more superior to corn and other major cereal foods. Amaranthus is significant in terms of protein content, and protein digestibility varies from 76 to 87%, but studies have confirmed values around 85 %. There is a unique opinion in the literature that the impact of technological processes on the quality of amaranth protein is small. The content of essential amino acids in Amaranthus is about 47.65 g/100 g of protein (Delgado-Zamarreño *et al.,* 2004). Amaranth and soybean are similar sources protein, they have amino acids that can be compared to egg white proteins, and in some cases, they are larger (Delgado-Zamarreño *et al.*, 2004; Popović *et al.*, 2013; 2016; 2019; 2020; 2021; Božović *et al.*, 2022; Zejak *et al.*, 2022; Filipović *et al.*, 2023). Amaranth is a dicotyledonous C4 species that originates from Central and South America and is rarely grown as the specialty crop in Europe. The genus Amaranthus has about 60 species which are wild forms, and only several species have been cultivated. In developing countries of Central and South America, Asia, and Africa, amaranth leaves are consumed in raw and/or processed form. Amaranths are divided into two groups: field crops and vegetable crops. As the main vegetable crops appear in *A. tricolor* L., *A. graecizans* L., and *A. cruentus* L., while *A. cruentus* L., *A. caudatus* L., *A. hybridus* L., and A. mantegazzianus L. are used as grains (Grubben and Sloten, 1981). According to Brenner *et al.* (2000), *A. caudatus* L., *A. cruentus* L., and *A. hypochondriacus* L., for their exceptional nutritional value, are cultivating worldwide for seeds and leaves, lately. Chemical grain composition varies within the *Amaranthus* species (Pešić *et al*., 1997; Muchová *et al.*, 2000). Amaranth grain contents range are: crude protein 15.2–18.6%, crude fat 5.4–8.6%, crude fiber 3.5–4.2%, ash 2.7–3.2%, and carbohydrates 66.7–72.7%. High seed water contents involved stronger microbial infection and reduced germination (Gimplinger *et al*., 2007). Amaranthus species are widespread as ornamental and forage crops (Fasuyi *et al.*, 2007; Fasuyi *et al.*, 2008), and red food colorants (Paśko *et al.*, 2011). Amaranthus species are preferred due to the colorful history of the exotic crop (Myers, 1996; Pešić *et al.*, 1997). There are just a few crops that can be used safely in gluten-free diets among as amaranth, rice, maize, millet, buckwheat, quinoa, and legumes (Erbas *et al.*, 2005). That is important for individuals who suffer from Celiac disease also known as gluten intolerance.

Due to changes in crop profiles in world, everything is more current to look for new plants with a good nutritional potential to combined with health benefits (Popović *et al.*, 2021; Kolarić *et al.*, 2021; Nožinić *et al.*, 2022; Božović *et al.*, 2022; Petrović *et al.*, 2022). Amaranth is a crop with a dual character, combining the features of food and health-promoting product. This study aimed to determine the variability properties of four major types of amaranth plants. This investigation has a contribution to planning for future activities related to Amaranth, and above all what separate divergent genotypes which could serve as parents for further crossing in breeding programs. In addition, the goal of the study was to estimate the initial selection material (varieties and lines) of *Amaranthus* and to identify donors for high content of essential amino acids. This will enable the successful continuation of the selection of these cultures.

Materials and Methods

Experimental design

The field research was carried out in Aleksinac in Serbia, on plots of an amaranth area of 10 m^2 in three replications, during the vegetation period from years of 2017 and 2018. Ten genotypes of amaranth were tested, that belong to species: *A. molleros*, *A. caudatus*, *A. mantegazzianus*, and *A. cruentus* (Stevanović, 2022) (Figure 1a-d).

Figure 1. *Amaranthus molleros, a., Amaranthus mantegazzianus, b., Amaranthus caudatus, c., Amaranthus cruentus, d.*

During the experiment, in both experimental years, standard cultivation technology was used. The plant materials were taken for analysis at technological maturity. The seeds were separated from their shells/pod having dried appropriately, milled with a blender, and stored in air-tight containers till their use for analysis. Plant materials were analyzed by standard ICC (International Association for Cereal Science and Technology) methods for crude protein content (ICC, 1996a) and ash content (ICC, 1996b). Protein content determination involved the use of routine Kjeldahl nitrogen assay (N×6.25). The oil content of the seeds (%) was determined by extracting samples in a Soxhlet apparatus using any hydrous diethyl ether as the solvent. All analyses were performed in three repetitions.

The amino acid profile of amaranth leaves was examined in 4 genotypes belonging to the following species: *A. cudatus* L., *A. cruentus* L., *A. mantegazzianus* L., *and A. molleros* L. The composition of total proteins of the leaf was determined, as well as the content of 18 essential amino acids in the protein hydrolysate (Mol%). The amino acid analyses of *Amaranthus* leave were performed by ion exchange chromatography using an automatic amino acid analyzer Biochrom 30+ (Biochrom, Cambridge, UK), according to Spackman *et al.* (1958). The technique was based on amino acid separation using strong cation exchange chromatography, followed by the ninhydrin color reaction and photometric detection at 570 nm and 440 nm (for proline). The procedure is the same as those described (Tomičić *et al.*, 2020). Samples of the oilseed by-products were previously hydrolyzed in 6M HCl (Merck, Germany) at 110 °C for 24h, and then cooled to room temperature. After hydrolysis, samples were filtered and made up to 25 mL in sodium citrate buffer (pH 2.2) (Biochrom, Cambridge, UK). The results were expressed as g/100g on the dry matter basis of a sample.

Statistical analysis

The mean value ($\bar{x}$), standard deviation (SD), and variation coefficient (CV, %) were taken as indicators of the variability of searched characteristics, calculated by equations indicated in Table 1.

Table 1. Equations and formulas used in calculations

Sample mean value $\bar{x} = \frac{\Sigma\ xi}{N}$	*Standard deviation* $S = \sqrt{\frac{\Sigma\ (x-\bar{x})^2}{N-1}}$	*Coefficient of variance* $Cv = \frac{S \times 100}{\bar{x}}$
Formula 1. *Genetic variance* $(\delta^2 g) = (MS_2 - MS_1) / b$ MS_2: Mean square of genotype; MS_1: Mean square of experimental error.		Formula 2. *Phenotypic variance* $(\delta^2 f) = \delta^2 g + \delta^2 p/b$ $\delta^2 p$: Variance of experimental error; b: Repetition
Formula 3. *A least significant difference* (LSD-test) LSD-Se "t" SE - standard error of the treatment environment; Se - $(2MSe/b)^{1/2}$ t - tabulation value of the degrees of freedom for error.		Formula 4. *The coefficient of genetic* (GCV) $GCV = \frac{(\delta^2 g)^{1/2}}{\bar{x}} \cdot 100$
Formula 5. *Phenotypic (PCV) variance for all traits analyzed* $PCV = \frac{(\delta^2 f)^{1/2}}{\bar{x}} \cdot 100$		Formula 6. *The coefficient heritability* $h^2 = \frac{\delta^2 g}{\delta^2 f} \cdot 100$

The data on the technological and chemical properties of seeds and leaves were evaluated by analysis of variance which was used for the random system (Tables 1 and 2) that was adopted by Hadživuković (1991). Variance components were calculated by Formula 1 and Formula 2 to test the significance of differences between mean values of the characteristics which were calculated by the least significant difference (LSD - test), the significance threshold of 1% and 5% for levels of error according to the Formula 3. LSD - Se "t"; SE - standard error of the treatment environment: Se - (2MSe/b) 1/2, t - tabulation value of the degrees of freedom for error. The coefficient of genetic (GCV, Formula 4) and phenotypic variance (PCV, Formula 5) of all traits was analyzed, and representing the degree of genetic relationship phenotypic variation was compared to the average value of the traits. The percentage of genetic variability in the total phenotypic variability of the analysed traits was determined based on heritability (h^2, Formula 6). The coefficient heritability can be used to determine the probability of whether a particular form can provide the same or similar offspring.

Results and Discussion

Technological properties of Amaranth at present, the commercial interest in amaranth grain is restricted to the health food market. To be economically competitive with other grain crops, amaranth requires high grain yields and high-quality levels of the seed.

The protein content of amaranth seeds, PCS

The protein content of amaranth seed varied in the range of 14.88% (*A. caudatus*) to 16.55% (*A. cruentus*). The coefficient of variation within the analyzed *Amaranthus* species ranged from 0.70% (*A. molleros*) to 1.32% (*A. mantegazzianus*). The coefficient of variation between the studied species was 5.29% (Table 3, Figure 2). Analysis of variance showed that the analyzed species are very significant differences in PCS (Table 3). The average percentage of PCS of *A. cruentus* is 17.8% and *A. caudatus* 14.9% (Hadživuković, 1991).

Table 2. Analysis of variance of the random system

Sources of variation	Degrees of freedom	Sum of squares	Middle square (MS)	
Replication	(b-1)	Qb	MS_3	Qb / (b-1)
Genotype (g)	(g-1)	Qg	MS_2	Qg / (g-1)
Error	(b-1) (g-1)	Qp	MS_1	Qp/ [G(b-1) (g-1)]
In total	btg - 1	Q		

b – repetitions; g-years; Qb – sum of squares of replications; Qg – sum of squares of genotypes; Qp- sum of squares of experimental error; MS_1 - middle square of experimental error; MS_2 - middle square of genotype; MS_3 – middle square if replications; t – tabular value for degrees of freedom of error; G - years.

Table 3. Mean values ($\bar{x}$) coefficients of variation (CV) for protein, ash, and oil (%) content in seeds and leaves of *Amaranthus* species

Species	Protein content of seeds		Protein content of leaves		Ash content of seeds		Ash content of leaves		Oil content of seed	
	$\bar{x}$	Cv (%)	$\bar{x}$	Cv (%)	$\bar{x}$	Cv (%)	$\bar{x}$	Cv (%)	$\bar{x}$	Cv (%)
A. molleros	14.89	0.26	15.94	0.37	2.73	2.56	17.63	0.62	6.16	1.45
A. caudatus	14.88	0.70	20.10	0.92	2.32	8.18	16.34	2.14	5.76	3.56
A. mantegazzianus	15.13	1.32	17.03	0.35	2.54	16.14	18.76	0.69	5.56	7.19
A. cruentus	16.55	0.68	18.06	0.47	2.39	10.46	18.26	0.82	6.05	3.38
SD	0.79		1.77		0.18		1.045		0.23	
CV (%)	5.29		9.95		7.24		7.24		4.64	

$\bar{x}$ - mean value; CV - coefficient of variation; SD - standard deviation.

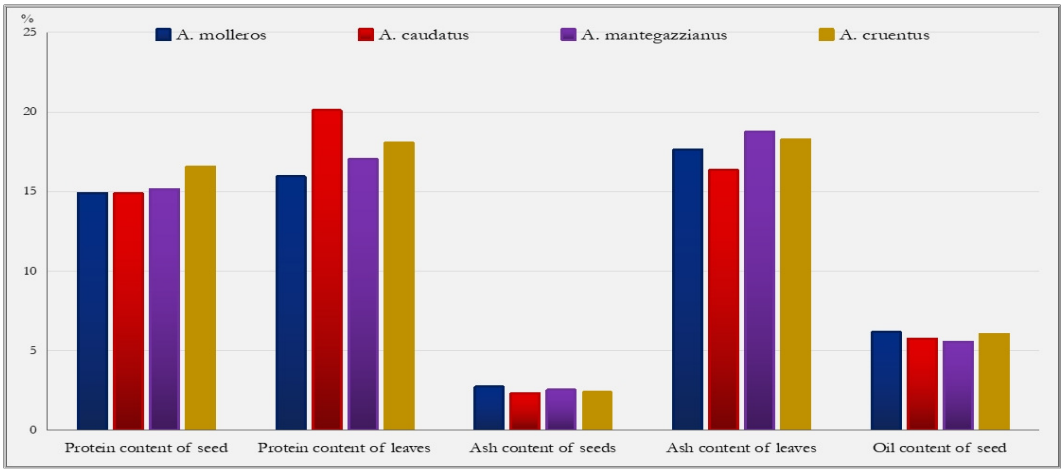

Figure 2. Protein content and ash content of seeds and leaves, and oil content of seed in Amaranthus species

The protein content of amaranth leaves, PCL.

PCL in addition to the PCS is the most important qualitative character of *Amaranthus*. PCL varied in the range from 15.94% to 20.10%. *A. molleros* had the lowest average value (15.94%), while *A. caudatus* had the biggest average value (20.10%) of these features traits (Table 3, Figure 2). Indicators of variability within the analyzed species varied in a very small range of 0.35% (*A. mantegazzianus*) to 0.95% (*A. caudatus*), while among the analyzed species coefficient of variation is 9.95% (Table 3). The influence of the type is highly significant for the PCL of amaranth (Table 3). In previous studies, protein contents of Amaranth seeds were reported as 9.2% in *A. blitoides*, and 15% in *A. muricatus* (Juan *et al*., 2007). PCL of Amaranth species s have

been reported differently such as in *Amaranthus* hybridus was 17.92% (Akubugwo *et al*., 2007) and in *Amaranthus cruentus* was 23.0% (Fasuyi *et al*., 2007).

Amino acids content of amaranth leaves, AACL

Common amino acids are present in amaranth leaf proteins. Among them, all essential amino acids are present (tryptophan and cysteine decompose during acid hydrolysis).

The results obtained for the content of lysine and methionine are very significant (Table 4). In the amaranth leaf, the lysine content ranged from 3.9 g/100 g protein (*A. caudatus*) to 7.0 g/100 g protein (*A. cruentus*) and (*A. moleros*). In amaranth flower, the content of lysine ranged from 4.2 g/100 g of protein (A. caudatus) to 6.7 g/100 g of protein (*A. molleros*). The methionine content ranged from 3.1 g/100 g of protein (*A. caudatus*) to 7.4 g/100 g of protein (*A. mantegazzianus*) in the leaf, or 2.9 g/100 g of protein (*A. caudatus*) to 6.7 g/100 g of protein (*A. mantegazzianus*) in amaranth flowers (Table 5). Amaranth leaves protein was rich in all essential amino acids compared with the FAO/WHO (1973) requirements since their contents were higher than that in the reference protein. Only the amino acid content of tryptophan is at the level of the reference protein.

Table 4. Amino acids content (g/100 g protein) in leaves of *Amaranthus* species

Amino acid	*A. caudatus*	*A. cruentus*	*A. mantegazzianus*	*A. molleros*
Glycine	9.6	9.3	9.0	9.2
Alanine	6.7	6.6	6.4	5.1
Leucine	6.3	5.6	4.4	5.5
Isoleucine	4.1	3.9	2.6	4.0
Valine	6.3	4.8	4.5	3.8
Serine	8.2	5.1	4.8	6.2
Aspartic acid	9.8	9.4	7.5	6.2
Glutamic acid	17.2	17.2	17.3	17.9
Cysteine	4.2	4.1	2.6	4.4
Methionine	3.1	4.6	7.4	4.4
Lysine	3.9	7.0	5.7	7.0
Arginine	11.5	9.7	8.3	9.2
Tyrosine	4.2	5.1	3.3	4.4
Tryptophan	0.4	1.2	0.6	0.6
Histidine	3.5	2.5	3.0	2.1
Phenylalanine	4.9	4.5	3.7	3.0
Threonine	4.0	3.9	3.5	2.4
Proline	5.2	4.1	4.6	4.1

Ash content of amaranth seeds, MCS

Among the analyzed species of amaranth were no significant differences in the MCS (Table 3, Figure 2). Ash content of seeds varied in a very narrow interval (2.32-2.73%). Type *A. caudatus* had a lower average value (2.32%) for this property, while the highest average value was recorded for the species *A. molleros* (2.73%) (Table 3). Coefficient of variation within the analyzed species varied in the range from 2.56% (*A. molleros*) to 16.14% (*A. mantegazzianus*) and between the studied species it was 7.24% (Table 3). Concerning the MCS, analysis of variance, statistically significant difference between the study's species of *Amaranthus* was not find (Table 3). Ash content of leaves, MCL. Ash content in the leaves of the analyzed amaranth species, varied from 16.34% (*A. caudatus*) to 18.76% (*A. mantegazzianus*). The variation coefficient of the analyzed amaranth

species was between 0.62% (*A. moleros*) and 2.14% (*A. caudatus*). The results of the ash content of the leaves show a significant difference between the studied species.

Akubugwo *et al.* (2008) reported that *A. hybridus* leaves contain an appreciable amount of nutrients, minerals, vitamins, amino acids, and phytochemicals and low levels of toxicants.

The oil content of seeds, OCS

The average value of the oil content in amaranth seeds varied slightly from 5.56% (*A. mantegazzianus*) to 6.16% (*A. molleros*) (Table 3). Within the analyzed species of amaranth, the highest CV was observed in *A. mantegazzianus* (7.19%) and the lowest value was in *A. molleros* (1.45%), Table 3. The standard deviation is consistent with the value of the coefficient of variation. A similar result was reported by He and Corke (2003) - that in *Amaranthus* grain of 104 genotypes of 30 species the overall average oil content was 5.0%, and ranging from 1.9 to 8.7%.

Table 5. Amino acids content in flowers of *Amaranthus* species

Amino acid, g/100 g protein	*A. caudatus*	*A. cruentus*	*A. mantegazzianus*	*A. mollersos*
Glycine	8.9	8.9	7.9	8.6
Alanine	5.8	6.2	6.7	4.8
Leucine	7.8	5.2	6.1	4.9
Isoleucine	4.3	3.5	2.9	4.5
Valine	5.9	5.0	4.7	4.5
Serine	7.8	5.3	4.9	6.7
Aspartic acid	9.1	8.0	7.9	6.5
Glutamic acid	19.1	16.7	17.5	16.8
Cysteine	4.4	3.7	3.1	4.6
Methionine	2.9	3.5	6.7	3.9
Lysine	4.2	6.6	6.1	6.7
Arginine	10.7	9.4	9.5	8.7
Tyrosine	4.4	5.4	4.2	3.8
Tryptophan	0.8	0.8	1.0	1.3
Histidine	3.8	2.3	3.2	1.9
Phenylalanine	5.1	4.7	3.2	3.4
Threonine	3.8	4.1	3.9	2.9
Proline	5.4	3.8	4.2	3.9

Analysis of variance was very significant for protein content of seed and leaves and ash content of leaves (Table 6).

Table 6. Analysis of variance of the random system for protein, ash, oil content of seeds and leaves of *Amaranthus* species

Sources of variation	DF	Protein content of seeds	Protein content of leaves	Ash content of seeds	Ash content of leaves	Oil content of seeds
		1.906**	9.392**	0.09	3.28**	0.22
Error	8	0.016	0.012	0.07	0.04	0.06

The components of variance, of coefficient of variation and heritability of amaranth.

The protein content of seeds (PCS) and leaves (PCL), and the ash content of leaves (MCL) are more controlled by genetic basis, while the influences of environmental factors are lower.

The percentage of genetic variance to total phenotypic variance for PCS, PCL, and MCL was 0.63%, 3.12%, and 1.08% respectively, and also the relation of genetic and ecological variance in the phenotypic variance (Table 7).

Heritability was high for PCS, PCL, and MCL, which confirms a pronounced influence on the variability of genotypes (Table 6). The lowest value of the genetic coefficient of variation was 5.16% (PCS), and the highest value of 9.93% (PCL). The phenotypic coefficient of variation varied in the range of 5.18% (PCS) to 9.94% (PCL). A small difference in genetic and phenotypic coefficient of variation indicates a greater impact of genetic factors on the expression of the respective properties.

The oil content of seeds and the proportion of genetic variance to total phenotypic variance was 72%. This value indicates that in addition to genetic factors, the inheritance of oil content of seeds of Amaranthus has a significant impact and ecological factors (Table 7).

Table 7. Genetic (δ^2 g), ecological (δ^2 e), phenotypic (δ^2 f) variance; coefficients of genetic (GCV) and phenotypic (PCV) variance and heritability (h^2) of technological properties of *Amaranthus* species

Traits	δ^2 g	δ^2 e	δ^2 f	h^2 (%)	GCV (%)	PCV (%)
Protein content of seeds	0.63	0.005	0.635	99	5.16	5.18
Protein content of leaves	3.12	0.004	3.124	99	9.93	9.94
Ash content of seeds	0.0096	0.023	0.0326	29	3.92	7.16
Ash content of leaves	1.08	0.014	1.094	98	5.85	5.89
Oil content of seeds	0.054	0.021	0.075	72	3.95	4.65

Within all the technological characteristics analysed, only the traits of the ash content of seeds have a low heritability (29%) indicating that the trait is controlled by a small number of genes. The phenotypic coefficient of variation (7.16%) is significantly different from the genetic coefficient of variation (3.92%), which indicates that the ecological factors have a strong influence on variability of these traits.

The average values of protein content in amaranth seeds are in *A. teunifolious* from 19% to 23%, *A. cruentus* from 15% to 18%, *A. spinosus* 11% and A. polygamous only 5% (Singhal and Kulkarni, 1988). The values of protein content in amaranth seeds were reported as 15.3% in *A. cruentus* and 16.6% in *A. caudatus* (Gorinstein *et al.,* 1991), and 14.90% in *A. viridis,* 17% in *A. spinosus*, 12.90% in *A. tricolor*, and 12.17% in *A. blitum* (Srivastava and Roy, 2012).

The size and shape of leaves can be varying significantly between different genotypes within the same species and they implicitly influence plant growth and therefore productivity (Cosmulescu *et al*., 2020). Protein contents of amaranth species leaves have been differently reported - in *Amaranthus hybridus* was 17.92% (Akubugwo *et al.*, 2007), in *Amaranthus cruentus* was 23.0% (Fasuyi *et al.*, 2007), in *Amaranthus hypochondriacus* L. was between 2.101 mg g^{-1} to 3.152 mg g^{-1} (Pandey and Singh, 2010), in *A. dubius* flour was 21.53% (Rodríguez *et al.*, 2011), and in *A. hybridus* 17.2% (Adeyeye and Omolayo, 2011). Protein content depends on genotype and growing conditions (Bošković and Isajev, 2007; Bošković *et al.*, 2016), and it was decreased by plant age and increasing growth temperatures (Modi, 2007; Villegas-Olguín *et al*., 2019). Akubugwo *et al*. (2008) reported that *A. hybridus* leaves contain an appreciable amount of nutrients, minerals, vitamins, amino acids, and phytochemicals and low levels of toxicants.

High seed yields and high-quality levels of the amaranth seed may be achieved partly by optimal crop management and partly by selection and breeding. The origin rather than the botanical species is the decisive factor for a suitable genotype (Kaul *et al*., 1996). Breeding targets are high-yielding, short, early, and uniformly maturing lines with reduced seed shattering and large seeds of high nutritional value (Brenner, 2002; Stevanović, 2022).

Using of Amaranth in medicine

Kolesnikov and Gins (1997) points out that *A. cruentus* and *A. tricolor* are close to *Mentha piperita*. Amaranth leaves are relatively rich in proteins, pectins and flavinoids, which puts amaranth among medicinal plants.Lily (1980) and Dastur (1970) confirm the medicinal properties of amaranth and state that amaranth is still used in folk medicine in Asian and American countries today as a medicinal plant. Pešić *et al.* (1997) points out that from amaranth leaves amaranth is isolated, a biologically active substance that prevents heart muscle damage during ischemic processes.

Amaranth has an important role in the diet of people suffering from celiac disease (allergy to gluten), because it is gluten free. Due to the higher content of calcium and iron, it is recommended for women because it strengthens the bones and prevents anemia and reduces the level of cholesterol in the blood. It contains valuable amino acids, among them lysine, which acts against the herpes virus. Saponins, protoalkaloids and betacyans are responsible for the pharmacological activity of amaranth (PDR, 2000). Amaranth plants have beneficial activity on the cardiovascular and nervous systems, hypoglycemic effect, antimicrobial activity, antioxidant activity. Amaranth is widely used in the pharmaceutical industry to produce medicinal products against artherosclerosis, gastric ulcers, tuberculosis, as well as antiseptic, antifungal, and anti-inflammatory preparations (Szwejkowska and Bielski, 2012). According to Khare (2004) the seeds of *Amaranthus hypochondriacu*s L.in Unani medicine are considered as a spermatogenetic drug and tonic. Amaranth is characterized by many advantages for health (Baraniak and Kania-Dobrowolska, 2022) (Figure 3). New scientific research of biological activities of amaranth preparations on human health is needed.

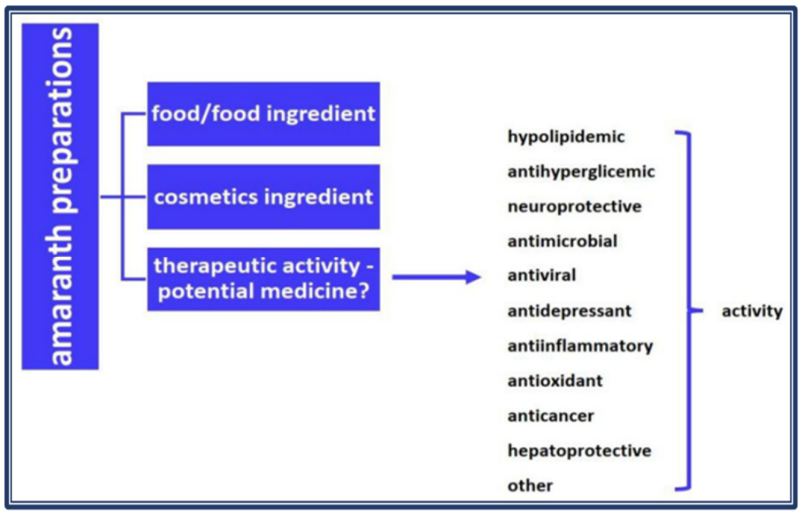

Figure 3. Benefits of amaranth for health

A. decoction is used in heavy menstrual bleeding, its flowers are as remedium for diarrhea, dysentery, cough, and hemorrhages. *Amaranthus polygamus* Willd. is used as a spasmolytic, emmenagogue, galactagogue factor (PDR, 2000). *Amaranthus spinosus* Linn. is taken to reduce heavy menstrual bleeding and in cases of excessive vaginal discharge and also as a diuretic medium. The whole plants of *Amaranthus blitum* Linn., *Amaranthus gangeticus* Linn., *Amaranthus mangostanus* Linn., and *Amaranthus tricolor* Linn. are considered as astringent, diuretic, demulcent and cooling (Khare, 2004). Amaranth seed oil exhibits hypolipemic, anti-atherosclerotic, hypotensive and antioxidant activity (Moszak *et al.*, 2018). Its consumption may lead to inhibition or delay in the development of diet-related diseases of civilization.

Conclusions

Based on the present finding it can be concluded that Amaranth is widely used in the food and in pharmaceutical industry to produce medicinal products against artherosclerosis, gastric ulcers, tuberculosis, as well as antiseptic, antifungal, and anti-inflammatory preparations. The obtained results in this investigation indicated that the variability of protein, ash and oil content is largely affected by genetic basis. The obtained results showed significant values of total proteins and essential amino acids for the examined genotypes. In the amaranth leaf, the lysine content ranged from 3.9 g/100 g to 7.0 g/100 g depending on genotype, while in the amaranth flower it ranged from 4.2 g/100 g to 6.7 g/100 g in the amaranth flower. A similar tendency was recorded for methionine amino acid, respectively. Furthermore, the heritability was high for these three parameters, which confirms a pronounced influence on the variability of genotypes. These results have shown a very good initial material, which provides excellent starting point for further works on the selection and processing of this new agricultural crop. The high seed yields and high quality of seeds may be achieved by optimal crop management, selection, and breeding. This study provides contribution to further activities on *Amaranthus* especially because of that separate divergent genotypes may serve as parents for further crossing.

Authors' Contributions

Conceptualization, A.S., J.B., Vl.P., V.P.; methodology, V.Z., V.P.; software, V.P.; validation, Lj.S.T.; formal analysis, M.Ć., M.B., V.P.; investigation, V.P.; resources, Vl.P., A.S.; data curation, V.P.; writing—original draft preparation, A.S., J.B., V.P.; writing—review and editing V.Z., M.Ć., M.B., V.P.; visualization, A.S., Lj.ST., V.Z., M.B.; supervision, M.B., A.S., J.B., Vl.P.; project administration, V.P.; funding acquisition, A.S., M.Ć. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Adeyeye EI, Omolayo FO (2011). Chemical composition and functional properties of leaf protein concentrates of *Amaranthus hybridus* and *Telfairia occidentalis*. Agriculture and Biology Journal of North America 2(3):499-511. *https://doi.org/10.5251/abjna.2011.2.3.499.511*

Akubugwo IE, Obasi NA, Chinyere GC, Ugbogu AE (2008). Mineral and phytochemical contents in leaves of *Amaranthus hybridus* L and *Solanum nigrum* L. subjected to different processing methods. African Journal of Biochemistry Research 22:40-44.

Baraniak J, Kania-Dobrowolska M (2022). The dual nature of Amaranth-functional food and potential medicine. Foods 11(4):618. *https://doi.org/10.3390/foods11040618*

Bošković J, Hojka Z, Zečević V, Prodanović R, Vukić M (2016). Significant resources of sustainable agriculture and organic food production system in Serbia. Acta Agriculturae Serbica 21(41):65-85.

Bošković J, Zečević V, Galonja Coghill T, Matković M, Trkulja N, Vukašinović D (2013). Assessing ecological aspects of biosafety of genetically modified crops to environment. Acta Agriculturae Serbica 36:143-1671.

Bošković ZJ, Isajev V (2007). Genetika. Megatrend University Belgrade. (In Serbian).

Božović D, Popović D, Popović V, Živanović T, Ljubičić N, Ćosić M, Spahić A, Simić D, Filipović V (2022). Economical productivity of maize genotypes under different herbicides application in two contrasting climatic conditions. Sustainability 14:5629. *https://doi.org/10.3390/5629*

Brenner DM (2002). Non-shattering grain amaranth populations. Chapter. In: Janick J, Whipkey A (Eds). Trends in New Crops and New Uses. Alexandria, ASHS Press, pp 104-106.

Brenner DM, Baltensperger DD, Kulakow PA, Lehmann JW, Myers RL (2000). Genetic resources and breeding of *Amaranthus*. Plant Breeding Reviews 19:227-285. *https://doi.org/10.1002/9780470650172.ch7*

Cosmulescu S, Scrieciu F, Manda M (2020). Determination of leaf characteristics in different medlar genotypes using the Image J program. Horticultural Sciences 47:117-121. *https://doi.org/10.17221/97/2019-HORTSCI*

Dastur JF (1970). Medicinal Plants of India and Pakistan. Taraporevala Sons, Bombay, India.

Delgado-Zamarreño MM, Bustamante-Rangel M, Sánchez-Pérez A, Carabias-Martínez R (2004). Pressurized liquid extraction prior to liquid chromatography with electrochemical detection for the analysis of vitamin E isomers in seeds and nuts. Journal of Chromatography A 1056:249-252. *https://doi.org/10.1016/j.chroma.2004.09.033*

Erbaş CM, Certel M, Uslu MK (2005). Microbiological and chemical properties of tarhana during fermentation and storage as wet sensorial properties of tarhana soup. LWT Food Science and Technology 38:409-416. *http://dx.doi.org/10.1016/j.lwt.2004.06.009*

FAO/WHO - Energy and Protein Requirements. (1973). Report of FAO Nutritional Meeting Series, No. 52, Rome.

Fasuyi AO, Dairo FAS, Adeniji AO (2007). Protein supplementary quality of tropical vegetable (*Amaranthus cruentus*) leaf meal in broiler starter diets: bionutritional evaluation. International Journal of Agricultural Research 2:976-986.

Fasuyi AO, Dairo FAS, Adeniji AO (2008). Tropical vegetable (*Amaranthus cruentus*) leaf meal as alternative protein supplement in broiler starter diets: bio nutritional evaluation. Journal of Central European Agriculture 9:23-34. *https://doi.org/10.3923/ijar.2007.976.986*

Filipović V, Ugrenović V, Popović V, Dimitrijević S, Popović S, Aćimović M, Dragumilo A, Pezo L (2023). Productivity and flower quality of different pot marigold (*Calendula officinalis* L.) varieties on the compost produced from medicinal plant waste. Industrial Crops and Products 192:116093. *https://doi.org/10.1016/j.indcrop.2022.116093*

Gimplinger DM, Dobos G, Schönlechner R, Kaul HP (2007). Yield and quality of grain amaranth (*Amaranthus* sp.) in Eastern Austria. Plant Soil and Environment 53(3):105-112. *https://doi.org/10.17221/2224-PSE*

Gorinstein S, Pawelczik E, Delgado-Licon E, HaruenkitR, Weisz M, Trakhtenberg S (2002). Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses. Journal of the Science of Food and Agriculture 82:886-891. *https://doi.org/10.1002/jsfa.1120*

Grubben CJH, Sloten DH (1981). Genetic resources of *Amaranthus*. AGP IBPPG 80 2 Int. Board for plant Genet. Res. Food and Agric., Org. of the U.N. Rome, pp 57-57.

Hadživuković S (1991). Statistical methods (the application in agricultural and biological research). Institute of Agricultural Economics and Rural Sociology, Novi Sad, pp 1-244.

He HP, Corke H (2003). Oil and squalene in *Amaranthus* grain and leaf. Journal of Agricultural and Food Chemistry 51(27):7913-7920. *https://doi.org/10.1021/jf030489q*

ICC (1996a). ICC Standard Method No. 105/1: Determination of Crude Protein in Cereals and Cereal Products for Food and Feed. International Association for Cereal Chemistry, Vienna, Austria.

ICC (1996b). ICC Standard Method No. 104/1: Determination of Ash in Cereals and Cereal Products. International Association for Cereal Chemistry. Wienna, Austria.

Juan R, Pastor J, Alaiz M, Vioque J (2007). Electrophoretic characterization of *Amaranthus* L. seed proteins and its systematic implication. Botanical Journal of the Linnean Society 155:57-63. *https://doi.org/10.1111/j.1095-8339.2007.00665.x*

Kaul HP, Aufhammer W, Laible B, Nalborczyk E, Pirog S, Wasiak K (1996). The suitability of amaranth genotypes for grain and fodder use in Central Europe. Die Bodenkultur 47:173-181.

Khare CP (2004). Indian herbal remedies: rational Western therapy, ayurvedic, and other traditional usage, botany. Springer science & business media.

Kolarić L, Popović V, Živanović L, Ljubičić N, Stevanović P, Šarčević Todosijević Lj, Simić D, Ikanović J (2021). Buckwheat yield traits response as influenced by row spacing, nitrogen, phosphorus, and potassium management. Agronomy 11(12):2371. *https://doi.org/10.3390/agronomy11122371*

Perry LM, Metzger J (1980). Medicinal plants of east and southeast Asia: attributed properties and uses. MIT press.

Modi AT (2007). Growth temperature and plant age influence on nutritional quality of *Amaranthus* leaves and seed germination capacity. Water SA 33:369-376. *https://doi.org/10.4314/wsa.v33i3.49118*

Moszak M, Zawada A, Grzymisławski M (2018). Właściwości oraz zastosowanie oleju rzepakowego i oleju z amarantusa w leczeniu zaburzeń metabolicznych związanych z otyłością (The properties and the use of rapeseed oil and amaranth oil in the treatment of metabolic disorders related to obesity). Forum Zaburzeń Metabolicznych 9:53-64.

Muchová Z, Čuková L, Mucha R (2000). Seed protein fractions of amaranth *(Amaranthus* sp.). Rostl. Výr. 46: 331–336.

Myers RL (1996). Amaranth: new crop opportunity. In: Janick J (Ed). Progress in New Crops. Alexandria, ASHS Press. pp 207-220.

Nožinić M, Lakić Ž, Popović V (2022). Medicinal properties and main indicators of seed and oil quality of flaxseed - *Linum usitatissimum* L. Agriculture and Forestry 68(3):57-69. *https://doi.org/10.17707/AgricultForest.68.3.04*

Pandey RM, Singh R (2010). Genetic studies for biochemical and quantitative characters in grain amaranth (*Amaranthus hypochondriacus* L.). Plant Omics 3(4):129-134.

Paśko P, Bartoń H, Zagrodzki P, Gorinstein S (2011). Effect of amaranth seeds (*Amaranthus cruentus*) in the diet on some biochemical parameters and essential trace elements in blood of high fructose-fed rats. Natural Product Research 25(8): 844-849. *https://doi.org/10.1080/14786419.2010.513976*

PDR (2000). PDR for Herbal Medicines. 2nd ed. Medical Economics Company; Montvale, NJ, USA, pp 75-76.

Pešić V, Stamenković V, Nikolić M, Berenji J, Bodroža-Solarov M, Vujačić V (1997). Genetic inbreeding in *Amaranthus* in Yugoslavia and its usage value. The Second international Symposium 'Novel and Nonconventional Plants: Prospect of Their Practical Use'. Puscino, Russia Proceeding V, pp 995-996.

Petrović B, Vukomanović P, Popović V, Šarčević-Todosijević Lj, Burić M, Nikolić M, Đorđević S (2022). Significance and efficacy of triterpene saponin herbal drugs with expectorant action in cough therapy. Agriculture and Forestry 68(3):221-239. *https://doi.org/10.17707/AgricultForest.68.3.17*

Popović V, Malešević M, Miladinović J, Marić V, Živanovic Lj (2013*).* Effect of agroecological factors on variations in yield, protein and oil contents in soybean grain. Romanian Agricultural Research 30:241-247.

Popović V, Mihailović V, Vučković S, Ikanović J, Rajičić V, Terzić D, Simić D (2019). Prospects of *Glycine max* production in the world and in the Republic of Serbia. Chapter 7. In: Janjev I (Ed). Serbia: Current Issues and Challenges in the Areas of Natural Resources, Agriculture and Environment. Agriculture and Environment. Nova Science Publishers, INC., USA, pp 171-194.

Popović V, Šarčević-Todosijević Lj, Petrović B, Ignjatov M, Popović BD, Vukomanović P, Milošević D, Filipović V (2021). Economic justification application of medicinal plants in cosmetic and pharmacy for the drugs discovery. Chapter 3. In: Emerald M (Ed). An Introduction to Medicinal Herbs. Nova Science Publishers, USA, pp 1-365. *https://doi.org/10.52305/TKAL3430*

Popović V, Vučković S, Jovović Z, Ljubičić N, Kostić M, Rakaščan N, Glamočlija-Mladenović M, Ikanović J (2020). Genotype by year interaction effects on soybean morpho-productive traits and biogas production. Genetika 52(3):1055-1073. *https://doi.org/10.2298/GENSR2003055P*

Popović V, Tatić M, Sikora V, Ikanović J, Dražić G, Đukic V, Mihailović B, Filipović V, Dozet G, Jovanović Lj, Stevanović P (2016). Variability of yield and chemical composition in soybean genotypes grown under different agroecological conditions of Serbia. Romanian Agricultural Research 33:29-39.

Rodríguez P, Pérez E, Romel G, Dufour D (2011). Characterization of the proteins fractions extracted from leaves of *Amaranthus dubius* (*Amaranthus* spp.). African Journal of Food Science 5(7):417-424.

Singhal RS, Kulkarni PR (1988). Review: Amaranths - an underutilized resource. International Journal of Food and Technology 23:125-139. *https://doi.org/10.1111/j.1365-2621.1988.tb00559.x*

Spackman DH, Stein WH, Moore S (1958): Automatic recording apparatus for use in chromatography of amino acids. Analytical Chemistry 30:1190-1206. *https://doi.org/10.1021/ac60139a006*

Srivastava R, Roy BK (2012). Analysis of genetic variation among accessions of wild and cultivated species of *Amaranthus* based on RAPD and SDS - PAGE markers. International Journal of Pharma and Bio Sciences 3(4):168-178.

Stevanović A (2022). Genetic values of production traits of introduced species of the amaranth genus (*Amaranthus* L.). Doctoral dissertation. Faculty of Biofarming, Backa Topola, Megatrend University, Belgrade. (In Serbian).

Szwejkowska B, Bielski S (2012). Wartość prozdrowotna nasion szarłatu (*Amaranthus cruentus* L.) Postepy Fitoterapi 4:240-243.

Tomičić Z, Spasevski N, Popović S, Banjac V, Đuragić O, Tomičić R (2020). By products of the oil industry as sources of amino acids in feed. Food and Feed Research 47(2):131-137. *https://doi.org/10.5937/ffr47-28435*

Villegas-Olguín MA, Cabrera De la Fuente M, Benavides-Mendoza A, Juárez-Maldonado A, Sandoval Rangel A, Fernandez CE (2020). Commercial and nutraceutical quality of grafted melon cultivated under different water tension. Horticultural Sciences 47:139-149. *https://doi.org/10.17221/139/2019-HORTSCI*