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Full Length Research Paper

Protein concentration and amino acid composition in grain amaranth (*Amaranthus cruentus* L.) as affected by sowing date and nitrogen fertilization

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A four-year field experiment was used to evaluate the effects of sowing date and nitrogen fertilization on protein content and amino acid composition of grain of amaranth (*Amaranthus cruentus* L.). Seeds of *A. cruentus* cv. 'G6' were sown at two sowing dates (May and June) and fertilized at rates to obtain three target levels of available soil mineral nitrogen (N_{min}) set to 140, 200 and 260 kg N ha^{-1}. Grain protein content and the sum of essential amino acids (EAA) were affected by growing season and date of sowing. There was a higher protein content (165 g kg^{-1}) and lower EAA in protein (39.3 g kg^{-1}) in grain of plants sown in June. Nitrogen application up to an N_{min} target value of 140 kg N ha^{-1} raised protein concentration in grain, and maintained the content of EAA in protein. EAA in grain fertilized to the target value 140 kg N ha^{-1} was higher (397 g kg^{-1}) than the standard requirement for preschool children (339 g kg^{-1}). Among essential amino acids, only valine concentration responded to nitrogen supply. Leucine was the limiting amino acid in grain protein.

Key words: Amino acid composition, grain amaranth, nitrogen fertilization, N_{min}, protein concentration, sowing date.

INTRODUCTION

Grain amaranth (*Amaranthus* spp.), an important ancient staple food in the pre-colonized South American civilizations' diet, has been identified as a protein-rich pseudo cereal. Due to its attractive chemical composition and superior nutritive value in comparison to other common grains, it may be used as an alternative food or incorporated into food as an ingredient (Grobelnik Mlakar et al., 2009a; de la Barca et al., 2010; Bodroža-Solarov et al., 2010). Outstanding nutritive value is particularly connected to a relatively high concentration of crude protein in dry matter, and well-balanced amino acid composition. The essential amino acid pattern in amaranth grain proteins has a remarkably high chemical score of 72 to 81, which is close to optimum in the human diet according to FAO/WHO (Bressani, 1994; Williams et al., 1995; Berghofer and Schoenlechner, 2002; Gorinstein et al., 2002; Bavec and Bavec, 2006; Grobelnik Mlakar et al., 2009b).

Since nitrogen is one of the dominant yield-creating factors, and nitrogen supply determines the protein accumulation in the seed, it is important to know more about amaranth's protein quantity and quality response connected to nitrogen fertilization. However, for cost-efficient nitrogen fertilization that meets the plant demand and requirements of environmental protection, the amount of plant-available soil mineral nitrogen (N_{min}) at a given growth stage should be considered (Olfs et al., 2005). The N_{min} target value concept, defined in the procedure developed by Wehrmann and Scharpf (1979), considers N_{min} at a particular time, using an average soil net nitrogen mineralization. The approach to establish the

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Table 1. Previous crops and soil physical and chemical characteristics.

Parameter	Unit	Values*	
		2001/2002	2005/2006
Previous crop		**Maize**	**Vegetables**
Organic matter	g kg^{-1}	27	26
P_2O_5	mg kg^{-1}	410	419
K_2O	mg kg^{-1}	203	107
pH		6.2	6.8
Bulk density			
0.0-0.3 m	g cm^{-3}	1.28	1.19
0.3-0.6 m	g cm^{-3}	1.35	1.35
0.6-0.9 m	g cm^{-3}	1.40	1.43
N_{min} (total 0.0-0.9 m)			
May	kg ha^{-1}	69.5/58.4	71.0/67.9
June	kg ha^{-1}	72.8/94.5	137.0/147.8

*A single set of data represents the first and the second two years of a trial.

N_{min} target values for crops, and to perform nitrogen fertilization according to defined values is a common practice in many countries (Olfs et al., 2005; Mengel et al., 2006).

Protein concentration strongly depends on amaranth species, genotype and climatic conditions, and is in the range 140 to 201 g kg^{-1} in European production conditions (Aufhammer et al., 1995; Aufhammer et al., 1999; Gimplinger et al., 2007). Although there have been some nitrogen fertilization studies on grain amaranth, information on its protein concentration (Bressani et al., 1987; Elbehri et al., 1993; Aufhammer et al., 1995; Schulte auf'm Erley et al., 2005; Pospišil et al., 2006; Olaniyi et al., 2008) and grain amino-acid composition response (Thanapornpoonpong et al., 2008) is still limited. Moreover, to date, there have been few investigations of the effect of nitrogen supply combined with other agronomic factors, such as plant sowing date and plant density on the protein and its amino acid pattern. Thus, in European growing conditions, the only factors studied have been amaranth yields, grain protein concentration and morphological trait responses (Pospišil et al., 2006; Pospišil et al., 2007a, b; Gimplinger et al., 2008).

The present study was part of a trial established to optimize amaranth production practice and promote exploitation of the crop, and was particularly conducted to determine the effects of sowing date, plant density, nitrogen fertilization (considering N_{min} at the time of sowing), and their interactions on crude protein content in *A. cruentus* cv. 'G6' grain and its amino acid composition. To the best of our knowledge, this is the first paper to report results from a field experiment.

MATERIALS AND METHODS

Locality description

The field experiment was carried out at the University Agriculture Centre Pohorski Dvor, Maribor, North-East Slovenia (46º 39' N, 15º 41' E, 282 m a.s.l) in 2001, 2002, 2005 and 2006. The site conditions for this experiment were a sandy-loam soil (Dystric Cambisol on slate metamorphic rocks) with 9.8ºC and 1047 mm of precipitation as the long-term annual averages. The field experiment was carried out on two fields which were 100 m apart. Previous crops, some physical and chemical characteristics of the soils of the trial site are reported in Table 1. The average climatic conditions (1961 to 1990) of the location and weather conditions in the course of the investigated growing seasons are presented in Figure 1. In all investigated growing seasons, mean temperatures from May to August were higher or similar to long-term average values. Mean temperatures in September 2001 and 2002 were below average. Precipitation was very variable among the growing seasons. Growing season 2001 was characterized by a considerably low precipitation in May, July and August, whereas high precipitation was recorded in June and September. In 2002, monthly precipitations were below average from May to July and above average in August. Precipitations in 2005 were below average in May and June, whereas extremely large monthly rainfall totals was recorded in July. Precipitations in 2006 were well above average in May, below average in June, July and September, and similar to long term values in August.

Plant

Mature grain samples of *A. cruentus* cv. 'G6' were obtained from a field experiment in which plant density, sowing date, and nitrogen fertilization where studied. The genotype was donated in 1999 from professor Bajuk, Mendoza, Argentina, and was the most appropriate of tested genotypes for our local production conditions.

Design of experimental fields

Field trial was assigned as a factorial experiment in a randomized block design with four replications. The experimental plot size was 8.4 m^2 (four rows 0.70 m wide and 3.5 m long). Plots were hand-sown on two dates; at mid May, and at mid June. Stands were hand-thinned to a population of 50 and 75 plants m^{-2} at the 4 to 6 leaf-stage in 2001 and 2002. Because of pronounced self-thinning for the greater density treatment, the stands in 2005 and 2006 were thinned only to a population of 50 plants m^{-2}. Plots were hand weeded and harvested (at the end of September, beginning of October), grain samples were cleaned and oven dried (70ºC) to constant mass.

Nitrogen application

Nitrogen was applied according to N_{min} and the stated N_{min} target values in three levels: control (C) = no mineral nitrogen addition, and N1 and N2 with N_{min} target values of 140 and 200 kg N ha^{-1}, respectively. Soil nitrogen content in three soil layers (0.3 m each) down to a depth of 0.9 m was determined at the time of sowing. From each replication, 12 soil cores were taken and separated by layers, mixed and cooled immediately after collection. Later, soil samples were extracted with 0.01 mol L^{-1} $CaCl_2$ solution and analyzed for nitrate-N and ammonium-N using a Cary 500 spectrophotometer (Varian, Canada). The actual N_{min} values were calculated using the analyses and measured soil bulk density (Table 1). Since N_{min} status in the treatments of June sowings in

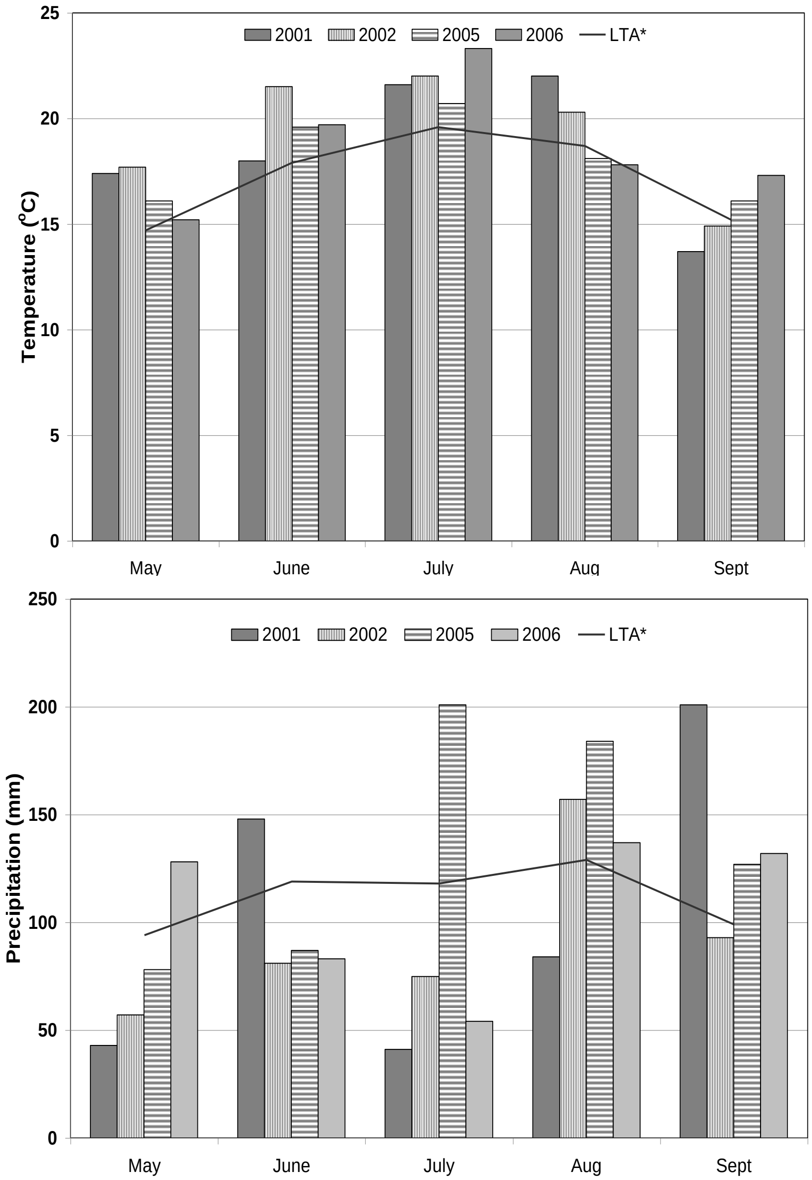

Figure 1. Total monthly precipitation, monthly air temperature and their long term average (LTA*; 19961–1990) during the experiments.

2005 and 2006 were near or higher than 140 kg ha^{-1}, the N1 and N2 N_{min} target values of these were stated at 200 and 260 kg N ha^{-1}, respectively. The difference between the N_{min} target value and the actual N_{min} content represented the amount of fertilizer required. Nitrogen (as calcium ammonium nitrate) was applied manually at the growth stage of 4 to 6 developed leaves to both fertilized treatments (N1 and N2) up to the N1 N_{min} target value (140 or 200 kg N ha^{-1}). N2 treatments were fertilized with additional nitrogen up to the N2 N_{min} target value (200 or 260 kg N ha^{-1}) at the stage of inflorescence visible.

Analytical methods

Protein analyses were conducted at the Institute of Agriculture and Forestry Murska Sobota (Slovenia) and partly at the Department of Chemistry and Biochemistry, Faculty of Animal Science, University of Kaposvar (Hungary) where amino acid composition analyses were also carried out. Seed samples were cleaned, dried and milled as needed before analyses. Crude protein concentration was calculated on a dry mass basis (original moisture content in grain was 90 to 120 g kg^{-1}) and was determined according to Kjeldahl method (ISO 5983, 2005). Nitrogen to protein conversion factor of 6.25 was used in the calculation. Grain protein values obtained by Kjeldahl method from two different laboratories were not significantly different by *t*-test. Analyses were performed for four replications in each treatment.

Grain samples for amino acid determination were hydrolyzed with hydrochloric acid: 5 mL of 6 M hydrochloric acid was added to 100 mg of sample and heating in a closed glass vessel at 110 ± 1ºC for 24 h in a nitrogen atmosphere. After hydrolysis, the samples were diluted, filtrated and kept frozen at -24ºC. The required pH of 2.2 for the solution for ion exchange column chromatography analysis was set with 4 M sodium hydroxide solution. Analyses were carried out

Table 2. Grain protein (g kg^{-1})[1], sum of essential amino acids (EAA) and individual essential amino acids concentration (g kg^{-1} protein) as affected by year of production (Y), sowing date (SD), nitrogen fertilization (N), and significant factors' maximum three-order interactions (SI).

	Protein	EAA	THR	CYS	VAL	MET	ILE	LEU	TYR	PHE	LYS	HIS	TRP
Y	**	**	**	**	**	**	NS	**	**	**	**	NS	NS
2001	157±1.4^{c}	397±1.1^{a}	38.6±0.4^{a}	23.4±0.3^{a}	42.7±0.3^{d}	23.2±0.4^{a}	36.1±0.3	58.2±0.3^{a}	34.8±0.3^{a}	42.1±0.2^{c}	58.2±0.3^{b}	27.6±0.1	1.22±0.03
2002	161±2.8^{b}	396±0.9^{a}	37.1±0.2^{b}	20.9±0.4^{b}	43.7±0.3^{c}	22.8±0.3^{a}	36.4±0.1	57.4±0.3^{b}	33.5±0.2^{bc}	44.6±0.3^{a}	58.4±0.3^{b}	27.4±0.3	1.24±0.02
2005	164±1.7^{a}	391±1.2^{b}	37.0±0.3^{b}	20.7±0.2^{b}	44.3±0.2^{b}	21.5±0.2^{b}	36.4±0.1	57.0±0.2^{b}	33.8±0.1^{b}	41.6±0.2^{d}	59.4±0.3^{a}	27.7±0.4	1.15±0.03
2006	161±1.9^{b}	396±2.4^{a}	38.6±0.3^{a}	20.5±1.0^{b}	45.3±0.6^{a}	22.0±0.3^{b}	36.2±0.3	58.1±0.4^{a}	32.3±0.2^{c}	42.8±0.4^{b}	60.4±0.5^{a}	28.6±0.4	1.17±0.01
SD	**	**	**	NS	*	NS	**	**	NS	NS	NS	NS	NS
May	156±1.2^{b}	397±0.7^{a}	38.2±0.3^{a}	21.3±0.3	43.9±0.5^{b}	22.8±0.3	36.6±0.1^{a}	58.1±0.2^{a}	33.7±0.2	42.6±0.2	59.2±0.3	27.5±0.2	1.20±0.02
June	165±1.1^{a}	393±1.2^{b}	37.3±0.2^{b}	21.6±0.5	44.2±0.3^{a}	22.1±0.2	35.9±0.1^{b}	57.0±0.2^{b}	34.1±0.2	42.9±0.4	58.6±0.3	28.0±0.2	1.19±0.02
N	**	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C	157±1.6^{b}	395±0.9^{ab}	37.8±0.4	21.7±0.5	43.9±0.5	2.2.6±0.2	36.3±0.1	57.8±0.2	33.9±0.2	42.7±0.4	59.1±0.3	27.5±0.2	1.18±0.02
N1	161±2.1^{a}	396±1.4^{a}	37.6±0.3	21.4±0.4	44.2±0.3	22.6±0.4	36.3±0.2	57.8±0.3	34.1±0.3	43.0±0.4	59.2±0.4	28.1±0.3	1.21±0.03
N2	163±1.7^{a}	393±1.3^{b}	37.8±0.3	21.4±0.5	43.9±0.3	22.1±0.3	36.3±0.2	57.3±0.2	33.7±0.2	42.6±0.4	58.5±0.3	27.6±0.2	1.21±0.03
SI		SD×N*	Y×SD*	Y×SD**	Y×SD** Y×N*	Y×SD*	-	-	-	Y×SD** SD×N*	-	-	-

[1]Nx6.25, **, *Significant at $P < 0.01$ and $P < 0.05$, respectively; NS not significant, [a–d]Mean values (± SEM) followed by different letters within a column and an individual factors are significantly different (LDS, α = 0.05).

with an amino acid analyzer (INGOS AAA 400, CZ). Amino acids were separated on a cation-exchange column (350 × 3.7 mm, 'OSTION Lg ANB' resin), postcolumn ninhydrin derivatives of amino acids were detected with an online photometer at 570 nm according to Csapo et al. (2008). For tryptophan determination, the sample was hydrolyzed with alkali (barium hydroxide), and the free tryptophan reacted with 4-dimethylaminobenzaldehyde in sulphuric acid solution. After a treatment with sodium nitrite, the blue color intensity of the formed product was measured spectrophotometrically at a wavelength of 590 nm. EAA is the sum of essential amino acids: threonine (THR), cystine (CYS), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), tyrosine (TYR), phenylalanine (PHE), lysine (LYS), histidine (HIS) and tryptophan (TRP). NEAA is the sum of non-essential amino acids: aspartic acid (ASP), serine (SER), glutamic acid (GLU), proline (PRO), glycine (GLY), alanine (ALA) and arginine (ARG). Amino acid analyses were performed for each treatment (year × sowing date × nitrogen fertilisation) in two replications.

Data analysis

Data were analyzed by analysis of variance (ANOVA) using the Statgraphics Centurion XV (Statpoint Technologies Inc., Herndon, VA, USA). Before performing the ANOVA, the homogeneity of variance of all characteristics was verified according to Cochran's and Bartlett's tests. Data were analysed according to a factorial design, where the sources of variation were year, sowing date, nitrogen fertilization, and their maximum three-order interactions. Comparison of means was done by Fisher's least significant difference (LSD) procedure (α = 0.05). The results are presented as the mean of replications ± standard error of mean (SEM). Relationships between variables were determined by Pearson product-moment correlation coefficients.

RESULTS AND DISCUSSION

Statistical analysis showed non-significant effects of plant density in 2001 and 2002 on all examined variables, except for ALA content in protein (data not presented). The density of 50 plants m^{-2} resulted in higher ALA content in grain than was achieved at density of 75 plants m^{-2} (41.5 and 40.9 g kg^{-1} of protein, respectively). Regarding the results, the factor plant population was excluded from the statistical model. The grain protein content, EAA, NEAA and the individual amino acids concentrations as affected by examined factors, and their interactions are summarized in Tables 2 and 3. Grain protein and EAA responses to available soil mineral nitrogen are presented in

Table 3. Sum of non-essential amino acids (NEAA), individual non-essential amino acids concentration and ammonia (g kg^{-1} protein) as affected by year of production (Y), sowing date (SD), nitrogen fertilization (N), and significant factors' maximum three-order interactions (SI).

	NEAA	ASP	SER	GLU	PRO	GLY	ALA	ARG	Ammonia
Y	*	**	**	**	**	**	**	*	NS
2001	581±1.2^{b}	82.9±0.3^{b}	60.2±0.5^{b}	178.4±0.6^{b}	44.0±0.9^{b}	82.9±0.4^{b}	40.8±0.2^{b}	91.2±0.8^{a}	21.9±0.5
2002	583±0.8^{ab}	81.1±0.4^{c}	61.3±0.2^{a}	185.6±1.0^{a}	40.1±0.8^{c}	84.8±0.5^{a}	41.5±0.2^{a}	88.8±0.6^{b}	20.7±0.2
2005	585±1.0^{a}	85.7±0.5^{a}	59.2±0.3^{c}	173.8±0.5^{c}	49.6±0.7^{a}	83.4±0.6^{b}	41.6±0.2^{a}	91.8±0.8^{a}	21.5±0.1
2006	584±2.6^{a}	83.6±0.7^{b}	61.0±0.4^{ab}	184.0±2.8^{a}	39.5±1.5^{c}	85.7±1.0^{a}	40.4±0.4^{b}	89.7±0.6^{ab}	20.7±0.6
SD	*	**	NS	**	**	NS	NS	*	NS
May	582±0.8^{b}	83.9±0.5^{a}	6.01±0.3	178.8±1.1^{b}	45.0±0.1^{a}	83.4±0.4	41.4±0.2	89.5±0.5^{b}	21.5±0.3
June	584±1.0^{a}	82.7±0.5^{b}	60.7±0.3	181.1±1.4^{a}	42.7±1.1^{b}	84.5±0.04	41.0±0.1	91.4±0.6^{a}	21.0±0.2
N	*	*	NS	**	NS	NS	NS	NS	NS
C	584±0.9^{a}	83.7±0.6^{a}	60.4±0.4	179.1±1.3^{b}	44.3±1.5	84.3±0.6	41.5±0.2	90.4±0.5	20.8±0.2
N1	581±1.2^{b}	83.5±0.7^{a}	60.5±0.3	178.5±1.2^{b}	43.4±1.3	84.0±0.5	41.1±0.2	90.3±0.9	21.6±0.5
N2	584±1.2^{a}	82.7±0.6^{b}	60.1±0.4	182.2±2.0^{a}	43.9±1.3	83.7±0.5	40.9±0.3	90.7±0.7	21.3±0.2
SI	Y×SD×N*	Y×SD**	Y×SD**	Y×SD* SD×N** Y×SD×N*	Y×SD×N*	Y×SD** Y×N*	Y×SD*	-	-

**, *Significant at P < 0.01 and P < 0.05, respectively; NS not significant, [a–c]Mean values (± SEM) followed by different letters within a column are significantly different (LDS, α = 0.05).

Figure 2.

Protein content and amino acids concentration

For the various treatments in the present study, grain protein content had range of 149 to 172 g kg^{-1}. In the literature, average values for *A. cruentus* and for *A. cruentus* cv. 'G6' were 147 to 229 (Grobelnik Mlakar et al., 2009b) and 165 g kg^{-1} (Pospišil et al., 2007a), respectively. The EAA average concentration obtained on control plots (395 g kg^{-1} protein) were lower than those reported by Thanapornpoonpong (2004). Although TRP was not determined in the above mentioned study, among all 17 *A. cruentus* varieties screened, only the protein of variety 'K266' comprised less EAA (389 g kg^{-1}) than *A. cruentus* cv. 'G6' in the present study.

The year of production and sowing date had both highly significant effects on grain protein and EAA concentrations (Table 2). The highest grain protein concentration of 164 g kg^{-1} was obtained in 2005, followed by 2002 and 2006 (161 g kg^{-1}). Protein concentration in 2001 (157 g kg^{-1}) was significantly lower than for other years. Beside the protein concentration, the growing season 2001 revealed the least favourable also in amaranth growth and yield performance. Compared to other years, plant height, inflorescence length and harvest index were significantly lower, the yield of above-ground biomass was lower by 41 to 53% and grain yield by 37 to 45% (data not present). The reason for the inferior amaranth performance in 2001 may be attributed to unfavourable weather conditions in this particular year (Figure 1), which resulted in the highest proportion of plants failed in the period from the thinning to harvesting (12.3%), and additionally, unfavourable conditions reflected also in less intense nitrogen mineralisation in comparison to other years (Table 1).

If sufficient nitrogen supply for plant growth and development in all examined years was assumed, then the lower amaranth aboveground and grain yields obtained in later sowings due to shortening of the growing season (data not presented) would be expected to result in the observed elevated protein concentrations in grain of June sowings. Thus, compared to plants sown in May, the protein concentration was on average 8 g kg^{-1} higher in grain of plants sown in June. Similarly, higher seed yield, but lower grain protein concentration in later sowings of *A. cruentus* cv. 'G6' was reported by Pospišil et al. (2007a).

Since the significant, but relatively weak negative linear correlation between grain protein concentration and EAA (r = -0.42) was noticed, the latter revealed to be the lowest in 2005 (391 g kg^{-1} protein) and attained lower values also in June sowings (393 g kg^{-1} protein). Particularly, the average concentrations of THR and ILE (they were not affected by year of production), and LEU and LYS in grain protein were lower; and among essential amino acids only VAL concentration was

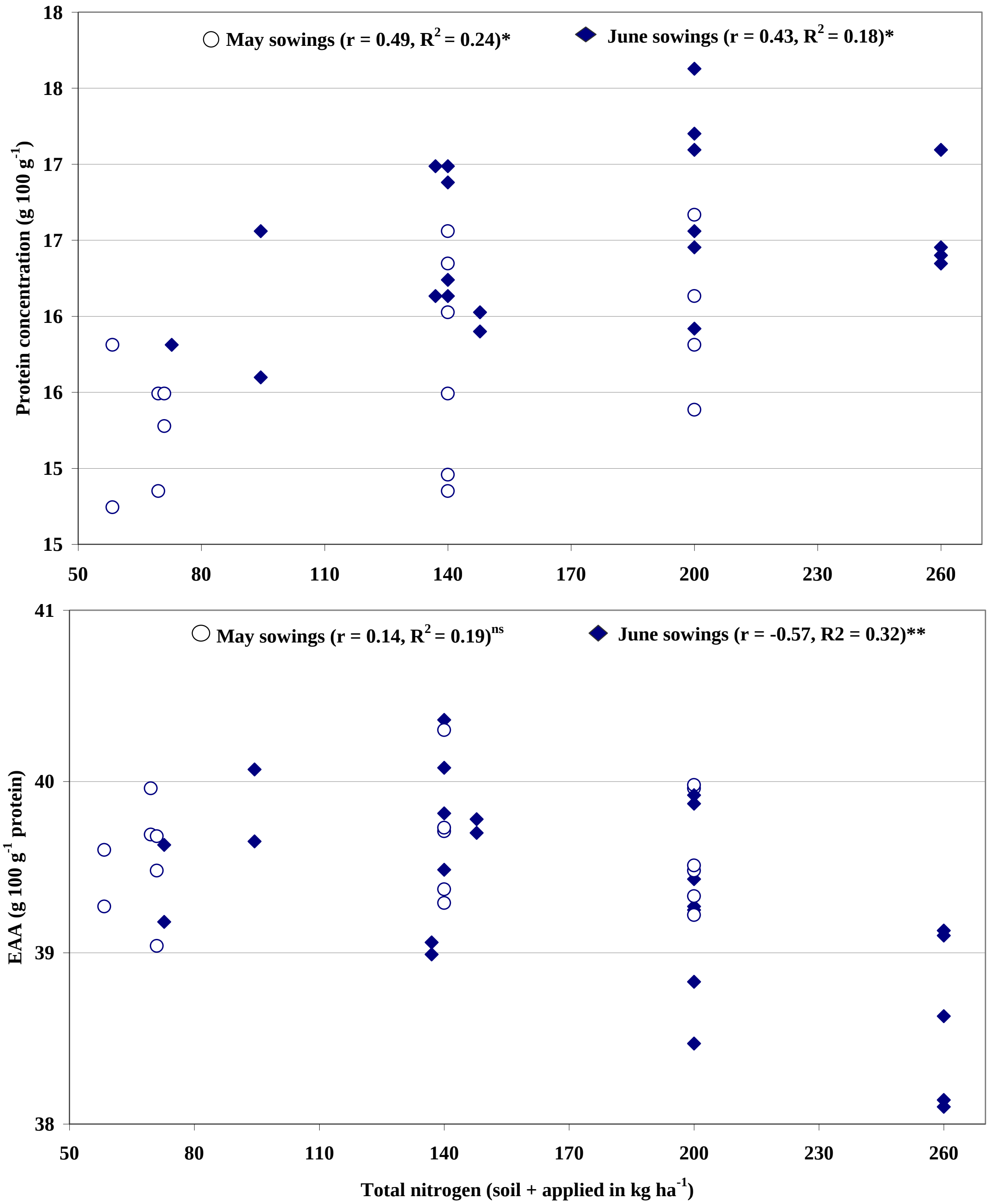

Figure 2. Grain protein (g 100 g^{-1}) and EAA (g 100 g^{-1} protein) response to available soil mineral nitrogen (kg ha^{-1}) at different amaranth sowing dates.

significantly higher in June sowings. Concentrations of CYS, MET and PHE were not influenced by the date of sowing, but their concentrations depended on the year of production. TYR, HIS and TRP accumulation in grain protein were not affected by year of production or by sowing date (Table 2).

In comparison to control, there were higher protein concentrations in amaranth grain for treatments where nitrogen was added. However, although there were different nitrogen target values in particular years and sowing dates (according to N_{min} status at sowing time), the average protein concentrations for N1 (161 g kg^{-1}) and N2 treatments (163 g kg^{-1}) were not significantly different, but the EAA concentration attained an average of 3 g kg^{-1} protein higher value for N1 than N2 treatments (Table 2), even the concentration of any individual essential amino acid was influenced by nitrogen fertilization. Moreover, the target levels of 200 and 260 kg N ha^{-1} resulted in similar values of protein concentration (164 and 166 g kg^{-1}, respectively), and the EAA concentration response to total nitrogen (N_{min} + N applied) was non-significant in May sowings, and moderately negative (r = -0.57) in June sowings (Figure 2). The results indicated that fertilization to the target value of 140 kg N ha^{-1} was sufficient, and that split application of nitrogen at the stage of inflorescence development (which is also difficult to perform in practice) is redundant for enhancing amaranth grain quality.

According to Misra et al. (1983), the protein content in grain of wild and cultivated *A. hypochondriacus* was positively correlated with LYS and MET and negatively correlated with THR concentration. In the present study, there were significant positive correlations between protein content and GLY (r = 0.31), protein and ARG (r = 0.35), and negative correlations between protein and MET (r = -0.54), and protein content and LEU concentration (r = -0.65).

Field and pot experiments measuring amaranth response to applied nitrogen have generated variable results. Likewise, rates of 80 and 120 kg N ha^{-1} (the latter was similar to our method in a split application) in southern Germany did not alter grain nitrogen concentration (Schulte auf'm Erley et al., 2005). Similarly, an inconsistent response pattern in protein concentration of different amaranth selections was found by Bressani et al. (1987) when NPK fertiliser (12-24-12) was applied at rates of 0 to 90 kg ha^{-1}. No protein response to pre-sowing nitrogen application at rates of 50 and 100 kg N ha^{-1} was also reported by Pospišil et al. (2006) who conducted field experiments with the same variety of *A. cruentus* (that is, cv. 'G6') as well as with *A. hypochondriacus* cv. '1008' in Croatia. Although tested rates influenced some other agronomic traits, the grain protein concentration was not affected and had range of 162 to 169 g kg^{-1} (averaged among the species). In contrast, Elberhri et al. (1993) reported a linear increase of protein level in grain (from 160 to 168 g kg^{-1}) when nitrogen up to 180 kg N ha^{-1} was applied. A study in Nigeria found that amaranth grain yield, as well as its nutritional quality increased with nitrogen application from 0 to 45 kg N ha^{-1} and declined thereafter (Olaniyi et al., 2008). Thanapornpoonpong et al. (2008) studied the effect of nitrogen (0.16 and 0.16 plus 0.08 g N kg^{-1} soil: additional nitrogen applied at flowering time) on seed protein fractions and amino acid composition of amaranth and quinoa (*Chenopodium quinoa* Willd.) in a pot experiment. Averaged across species and genotypes, the grain protein was 108, 137, and 151 g kg^{-1} when 0, 0.16, and 0.24 g N kg^{-1} soil was applied, respectively; but, in contrast to our findings, the EAA concentration was not affected.

The year of production and sowing date had both highly significant effects on NEAA concentrations (Table 3). The concentration of all individual non-essential amino acids was influenced by the year of production, and among them ASP, PRO, GLU and ARG were influenced by sowing date. The concentration of ASP and PRO was significantly higher in grain of May sowings (1.2 and 2.3 g kg^{-1} protein, respectively) and oppositely, the concentration of GLU and ARG was higher in June sowings (2.3 and 1.9 g 100 g^{-1}, respectively). Nitrogen fertilization influenced ASP and GLU; the N2 treatments resulted in lowest ASP, but the highest GLU concentration in comparison to C and N1 treatments. Similarly, Lošák et al. (2010) observed that increasing nitrogen rates resulted in reduced accumulation of ASP (and GLY) in protein of maize grain. Since the first product of nitrogen assimilation in plants is ammonia, further in the process is converted to glutamine (GLN) and GLU, higher GLU concentration observed in grain derived from N2 treatments, as well as in grain of June sowings, may be ascribed to higher soil N_{min} status in those treatments. Both amino acids act as amino group donors for biosynthesis of all the protein, primarily of PRO and ARG (Mengel and Kirkby, 2001). Fluctuation of ammonia (within 19.1 to 26.9 g kg $protein^{-1}$) was not influenced by any of the examined factors (Table 3).

Thanapornpoonpong et al. (2008) reported negative correlations of PRO and GLU with ARG concentration. The present study demonstrated, beside the weak negative correlation between GLU and ARG concentrations (r = -0.33), that there were moderate negative correlations (r values in range 0.5 to 0.75) between the following amino acid pairs: GLU-ASP, PHE-ASP, CYS-VAL and ARG-ILE, PRO-SER, PRO-GLU, PRO-GLY and PRO-PHE. There were moderate positive relationships for GLU-SER, GLU-PHE, and ALA-VAL, MET-LEU, PRO-ASP and LYS-ASP (data not presented).

Protein chemical score

According to mean values of particular essential amino acids obtained in treatments fertilized up to the N_{min} target value of 140 kg ha^{-1}, and compared to the FAO (1985) standard for preschool children, the limiting amino acid was LEU (chemical score 87). All other essential amino acids had high chemical scores. The average value of summed EAA evaluated in the above mentioned treatments was higher (397 g kg^{-1}) than the standard requirements for preschool children (339 g kg^{-1}). The biological value of protein in *A. cruentus* cv. 'G6' grain was well balanced (Table 4) compared with standards for adults and school children.

Table 4. Average amino acid composition (g kg^{-1} of protein) in grain of *Amaranthus cruentus* cv. 'G6' fertilized to target value 140 kg ha^{-1} in comparison to stated amino acid requirements.

Amino acid	HIS	ILE	LEU	LYS	MET	CYS	PHE	TYR	THR	TRP	VAL
Average[a]	27.5±0.54	36.4±0.15	57.6±0.17	59.7±0.20	22.8±0.18	21.5±0.28	42.7±0.22	33.9±0.15	38.1±0.19	11.9±0.15	44.7±0.22
Minimum[a]	26.2	35.9	57.0	58.9	22.1	19.9	41.8	33.0	37.1	11.3	44.1
Maximum[a]	26.9	36.8	58.3	60.7	23.7	21.0	43.9	35.1	39.0	12.5	45.5
Standard[b]											
Preschool child	19.0	28.0	66.00	58.00	25.0[c]		63.0[d]		34.0	11.00	35.0
School child	19.0	28.0	44.00	44.00	22.0		22.0		28.0	9.00	25.0
Adult	16.0	13.0	19.00	16.00	17.0		19.0		9.0	5.00	13.0
Chemical score*	145	130	87	103	177		122		112	108	128

[a]Average (± standard deviation), minimal and maximal amino acid concentration obtained in trial, [b]FAO/WHO/UNU standard (1985) taken from Table 38; requirements for preschool children (2 to 5 years), school children (10 to 12 years) and adults, [c]Methionine + cystine, [d]Phenylalanine + tyrosine, *Grain protein supplies 100% or more of the requirement. Limiting amino acid is in italics. Chemical score based on FAO (1985) pattern for preschool child.

Conclusion

According to growing season, grain protein content of *A. cruentus* cv. 'G6' had range 157 to 164 g kg^{-1}. As expected, higher protein content was obtained in grain of June compared to May sowings (165 and 156 g kg^{-1}, respectively). Nitrogen fertilization affected protein content; however, the target levels of 200 and 260 kg N ha^{-1} resulted in similar values of 164 and 166 g kg^{-1}, respectively. Rising protein content in grain of later sown plants and in those fertilized up to target levels 200 and 260 kg N ha^{-1} was paralleled by an increase in non-essential amino acids in protein. Since nitrogen application up to the N_{min} target value of 140 kg N ha^{-1} (stated at the time of plant sowing) raised protein concentration in the grain, and maintained the content of essential amino acids in protein, thus this target value seems optimal. Considering N_{min} status at the time of amaranth sowing (58 to 147 kg N ha^{-1}), the quantity of nitrogen applied to achieve the recommended target value was 0 to 82 kg N ha^{-1}, and therefore within the range suitable for a single application of mineral nitrogen (80 kg N ha^{-1}). The average of summed EAA evaluated in grain fertilized up to the target value of 140 kg N ha^{-1} was higher (397 g kg^{-1}) than the standard requirement for preschool children (339 g kg^{-1}). LEU was the limiting amino acid in grain protein of *A. cruentus* cv. 'G6'.

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