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Protein fractions and their amino acid composition of pseudocereals related to nitrogen supply

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The aim of this study was to investigate the effect of nitrogen (N) supply on seed protein fractions and their amino acid composition in amaranth (*Amaranthus* spp.) and quinoa (*Chenopodium quinoa* Willd). The experiment was carried out at the Institute for Agricultural Chemistry, Georg-August University Göttingen in 2001 and 2002. Two varieties of amaranth and quinoa were treated with three N levels (0, 80 and 120 kg N ha^{-1}). Protein was extracted and separated into five fractions as follows: albumin-1 (ALBU-1), albumin-2 (ALBU-2), globulin (GLOB), glutelin (GLUT) and the insoluble fraction (prolamin and non-protein N compounds). At the higher rates of N supply, lower concentrations of ALBU-1 fractions were found (20.56% and 30.84%, respectively). In ALBU-1 fractions, increased fertilizer rate reduced the histidine (His) content about 16.9% to 18.3% and caused decreased essential amino acid (EAA) content (about 2.9% for each treatment). The N supply did not affect the amino acid composition of the ALBU-2 fractions. In GLOB fractions, the content of methionine (Met) was decreased about 28.1% and 30.9% while phenylalanine (Phe) was increased about 11.6% and 18.4%. In the GLUT fractions, N treatment increased glutamic acid (Glu) and arginine (Arg) contents and decreased threonine (Thr), alanine (Ala), tyrosine (Tyr), valine (Val), lysine (Lys) and leucine (Leu) contents in the seeds. However, the sum of all EAA contents in the seed proteins was not changed. It was concluded that N rates affected the protein fractions and their amino acid composition in the investigated pseudocereals. Increased rates of N decreased Lys and increased Arg in the seeds, whereas the protein and EAA contents in ALBU-1 fractions, which belong to the non-storage proteins, were reduced.

Introduction

Amaranth (*Amaranthus* spp.) and quinoa (*Chenopodium quinoa* Willd) belong to the group of pseudocereals that are dicotyledons. Because of their chemical composition, they are attractive crops for food and pharmaceutical production. They are protein-rich and their amino acid composition is nutritionally good in relation to the recommended dietary amino acid levels reported by FAO/WHO (Ahamed *et al.* 1998). The application of nitrogen fertilizer (N) has been reported to influence seed proteins (Bressani *et al.* 1987). However, research on protein and amino acid composition in relation to nitrogen supply is limited. The aim of this study was to determine the changes in the protein fractions and their amino acid composition of amaranth and quinoa in relation to nitrogen supply.

Materials and methods

The experiment was conducted in 2001 and 2002 at the Institute for Agricultural Chemistry, Georg-August University of Goettingen. The experiment was designed as a split-plot in RCB with three replications. Main plots were cultivars and sub plots were the nitrogen levels.

Two varieties of amaranth [Bärnkraft (A. *cruentus*) and K432 (A. *hypochondriacus* x A. *hybridus*)] and quinoa (Faro and Tango) were cultivated in pots. Each of the pots contained 5 kg of dried sandy loam soil with a nitrogen mineral content in the soil of 0.21 g pot^{-1}. Two plants were grown in each pot and treated with three rates of NH_4NO_3 fertilizer (0, 80 and 120 kg N ha^{-1}). Pre-planting fertilizer was used at the rates of 0, 80 and 80 kg N ha^{-1}, respectively. At flowering growth stage, 40 kg N ha^{-1} top dressing nitrogen fertilizer was added to the treatment applied with 120 kg N ha^{-1}. Plots were harvested after the majority of plants were senescent. All mature seeds of amaranth and quinoa were ground and the meal was defatted with n-hexane for 8 h and stored at 4℃. Protein fractions [albumin-1 (ALBU-1), albumin-2 (ALBU-2), globulin (GLOB) and glutelin (GLUT)] were extracted stepwise according to Delgado *et al.* (1999). All fractions were freeze-dried. Total protein and the residue obtained from the extraction (N x 5.85) were determined by the Dumas combustion method (Sweeney and Rexroad, 1987). Protein contents in each fraction were determined by Bradford method (Bradford, 1976). GLOB content was calculated as the difference between total protein, ALBU-1, ALBU-2, GLUT and residue. Amino acid compositions were analyzed according to Gorinstein *et al.* (2002). All data presented in this study are calculated as percentage difference to the 0 kg N ha^{-1} treatment.

Results

Protein fractions

Effects of N supply on the content of the extracted protein fractions and their amino acid composition were determined (Fig. 1 and 2). The ALBU-1 content significantly decreased when the rate of N supply

increased (19.0 and 28.4%, respectively). Decreasing of ALBU-1 fraction was correlated with increases in the GLOB fraction. The GLOB fraction, as part of the storage proteins, accumulated mainly during the grain filling period while the ALBU-1 fractions (as functional protein) were synthesized only in early stage of seed development (Triboï *et al.* 2003). Therefore, increasing rates of N supply affected the increase of protein contents in GLOB fractions.

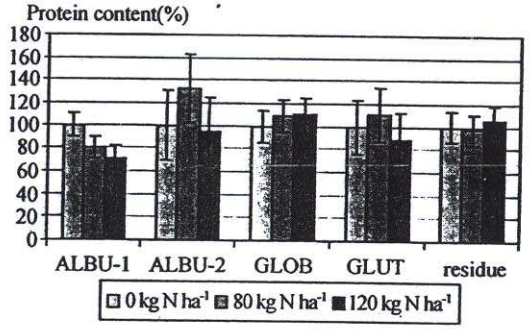

Figure 1. Protein content in each fractions affected by N supply (expressed as relative protein percentage; at 0 kg^{-1} ha = 100%). Bars indicate ± *LSD*, $P \leq 0.05$.

Amino acid composition

The amino acid composition of amaranth and quinoa seed was also affected by N supply. Although Lys was significantly decreased (7.4 and 7.9%, respectively) and the Arg was increased (8.0 and 9.4%) (Fig. 2a), the EAA content was not changed.

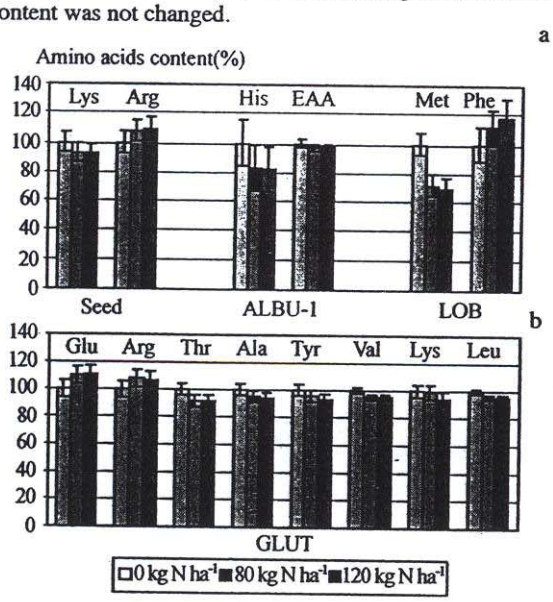

Figure 2. Amino acid content of total seed protein and the protein fractions, ALBU-1, GLOB (a) and GLUT (b) fractions as affected by nitrogen supply (expressed as relative increase/decrease percentage; at 0 kg^{-1} ha = 100%). Bars indicate ± *LSD*, $P \leq 0.05$.

For the amino acid composition, changes in ALBU-1, GLOB, and GLUT fractions were detected (Fig. 2a and b). In the ALBU-1 fractions, the EAA contents decreased about 2.9%. His was affected by N application and decreased about 16.9% and 18.3%, respectively. In the GLOB fractions, the content of Met decreased 28.1% and 30.9% while Phe increased about 11.6% and 18.4% after N application. In the GLUT fractions, the content of several amino acids was changed. Glu and Arg increased with N supply. However, the EAA content of this fraction was not affected.

Conclusion

The N application influenced both concentration and amino acid composition mainly of the water-soluble proteins. Both fractions changed differently in response to increasing supply of N. With raising rates of N, the EAA content in the ALBU-1 decreased, but the N supply did not affected the EAA contents of the other factions.

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