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REGULAR ARTICLE

CHANGES OF PROTEIN PROFILES IN AMARANTH MUTANT LINES

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ABSTRACT

The avalaibility of simple and efficient techniques for inducing genetic variation, such as use of radiation for inducing of mutation and selection for desired traits is an essential component of any plant breeding programme. The goal of the mutation breeding in selected *Amaranthus* spp. was to enhance quality and quantity of amaranth grain.

We have found some promising mutant lines with high coefficient of nutritional quality. Considering overall nutrition values, the lines C15/3, C27/5 and C82/1 are most promising genotypes, which could be possibly used in a future breeding programme.

Keywords: amarath, mutagenesis, protein fraction, nutritional quality

INTRODUCTION

Current interest in amaranth resides in the fact, that they have high nutritional value due to a higher amount of protein with balanced essential amino acid contents **(Gamel et al., 2004; Mnkeni et al., 2007)**, high photosynthetic efficiency, low input requirements,

high yield potential for grain, vegetable and fodder production, and relatively high tolerance to drought, disease and pests **(Lee et al., 2008).**

The objective of our study was to compare the amaranth mutant seeds with untreated control samples on the base of nutrition quality.

MATERIAL AND METHODS

Plant material

The seeds of two genotypes of grain amaranth *Amaranthus cruentus* genotype „Ficha" and hybrid K-433 (*A. hypochondriacus x A. hybridus*) were irradiated by γ radiation dose 175 Gy and positive selection was performed during 8 mutant generations (1998-2008) **(Gajdošová et al., 2008).** In this work we analysed 9 amaranth mutant lines and 2 untreated control samples from the year 2007.

Biochemical characterization

The total nitrogen content was analysed according to Kjeldahl **(Michalík et al., 2006)** and fractional composition of protein was done using the Golenkov method **(Michalík, 2002)**. Percentage of crude protein was calculated from total nitrogen content, which was multiplied by the conversion rate (% N x 5,7). On the base of representation of the protein fraction was calculated coefficient of nutritional quality.

Protein separation by SDS-PAGE

Storage proteins were extracted and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to ISTA methods **(Wrigley, 1992).** Profiles from individual lines were analyzed using 1D GelWorks system for Windows.

Statistical analysis

Statistical evaluation of results was carried out using 5.0 Statgraphic using basic statistical methods (minimum, maximum, standard deviation and coefficient of variation).

RESULTS AND DISCUSSION

The percentage of protein and fractional protein composition

Crude protein content from pale-seeded grain types is substantially higher than most cereal grains and has been reported to range from 12,5 to 22,5 % on a dry matter basis, with an average of about 15 % (**Mallory et al., 2008**). The crude protein content of amaranth mutant lines (Table 1) was in average 13,58 %, which was 10,60 % less than non-mutated control samples. The highest content of crude protein was detected in lines C 15/3 and C 82/1 (14,39 %), on the other hand the lowest content was detected lines C 236/1 and D 54/1 (12,80 %).

Seed proteins are generally classified into four types based on solubility: albumin, globulin, prolamin, and glutelin. **Cai et al. (2004)** reported that the major fraction of amaranth proteins was albumin (48,9 - 56,0 %), followed by glutelin (22,4 – 42,3 %), globulin (13,7 – 18,1 %), and prolamin (1,0 – 3,2 %).

The nutritional quality of seeds is mainly influenced by fractional representation of the protein, while protoplasmic proteins are characterized by highest content of essential amino acids **(Gálová et al., 2008)**. The content of cytoplasmic proteins in amaranth mutant lines ranged from 45,34 % to 62,49 %, in average 51,10 %, which was 3,37 % less than in non-mutated control samples (Table 1). In mutant line D 54/1 was detected a higher value compared to the control sample. On the other hand, the content of storage proteins (prolamin and glutelin) ranged from 25,27 % to 30,79 %, which was in average only about 0,18 % less than in control samples.

Table 1 The percentage of protein and fractional composition of proteins in seeds

	N_T, %	CP, %	alb+glo, %	pro, %	glu, %	rest	CNQ, %
A	2,72	15,51	54,13	3,60	26,28	15,47	1933,33
B	2,61	14,87	51,63	1,61	24,19	21,50	4542,24
x ± σ	2,67±0,08	15,19±0,45	52,88±1,77	2,61±1,41	25,24±1,48	18,49±4,26	3237,79±1844,77
ν (%)	2,97	2,97	3,34	54,02	5,86	23,07	56,98
C 26/2	2,30	13,12	48,76	4,87	24,38	21,95	1451,95
C 15/3	2,53	14,39	49,47	2,77	22,77	23,88	2648,01
C 27/5	2,27	12,96	53,06	2,46	24,68	19,75	2959,76
C 82/1	2,53	14,39	52,79	2,77	23,88	20,00	2627,80
C 236/1	2,25	12,80	46,86	5,61	24,99	21,87	1225,13
C 26/3	2,47	14,07	52,29	2,84	23,29	21,02	2581,34
D 54/1	2,25	12,80	62,49	1,87	26,86	8,11	3775,40
D 279/1	2,44	13,91	48,83	2,29	22,98	25,28	3236,25
D 282/1	2,41	13,75	45,34	5,80	24,99	22,67	1172,59
x ± σ	2,38±0,12	13,58±0,67	51,10±5,03	3,48±1,51	24,31±1,27	20,50±4,97	2408,69±924,37
ν (%)	4,90	4,90	9,84	43,54	5,23	24,24	38,38

Legend: A, B – control samples, N_T- total nitrogen content, CP – crude proteins, alb – albumins, glo – globulins, pro – prolamins, glu – glutelins, CNQ– coefficient of nutritional quality, x – average, σ – standard deviation, υ – coefficient of variation.

Coefficient of nutritional quality of mutant lines was in average about 25,61 % lower than controls, but four lines (C15/3, C26/3, C27/5, C82/1) had highest coefficient compared to control samples.

Electophoretic separation of storage proteins by SDS-PAGE

Electrophoretic patterns of the different protein fractions in amaranth seeds were obtained under denaturing conditions. With help of SDS-PAGE method were identificated high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), from which can be able to predict the technological quality of amaranth. The content of HMW-GS in irradiated samples was in average 0,82 %, which was in contrast with control samples. The highest content of HMW-GS was detected in sample C 27/5 (2,34 %). **Palenčárová et al. (2010)** reported, that content of HMW-GS in analysed genotypes of amaranth ranged from 0,37 % to 4,4 %. Irradiated samples had in average higher content of LMW-GS than non-mutated samples. Results are shown in the table 2.

Table 2 Storage proteins analysis by SDS-PAGE

	HMW-GS	LMW-GS	rest. alb+glo
A	0,32	36,64	63,04
B	0,12	34,47	65,41
x ± σ	0,22±0,14	35,56±1,53	64,23±1,67
ν (%)	64,40	4,31	2,61
C 26/2	0,94	39,42	59,64
C 15/3	0,00	45,49	54,51
C 27/5	2,34	40,89	56,77
C 82/1	1,09	42,17	56,74
C 236/1	0,77	32,61	66,62
C 26/3	0,32	36,02	63,65
D 54/1	0,00	44,55	55,45
D 279/1	0,15	37,89	61,97
D 282/1	1,81	24,02	74,17
x ± σ	0,82±0,82	38,12±6,66	61,06±6,36
ν (%)	100,11	17,48	10,42

Legend: A, B – control samples, rest. alb + glo – residual albumins, globulins, HMW-GS - high-molecular-weight glutenin subunits, LMW-GS - low-molecular-weight glutenin subunits

CONCLUSION

The goal of presented work has been to evaluate the influence of induced mutagenesis on the base of protein content, fractions and electrophoretic profile in comparison amaranth mutants seeds with untreated control samples. The protein composition of amaranth seeds can be changed and their nutrition values significantly influenced through radiation mutagenesis.

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