J Biochem Tech (2010) 2(5):S83-S84
ISSN: 0974-2328

Expressing of bacterial dihydrodipicolinate synthase in transgenic barley

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Received: 25 October 2010 / Received in revised form: 13 August 2011, Accepted: 25 August 2011, Published: 25 October 2011

Abstract

Nutritional quality of human and animal foodstuffs is determined by the content of essential amino acids. Barley is the fourth most important cereal of the world and the second most important cereal grown in the Czech Republic. Cereal grains such as barley contain insufficient levels of some essential amino acid, especially lysine. The ionex chromatography is very selective and convenient for aminoacids determination such as lysine. In connection with post column derivatization the limit of detection of lysine determination was below 1µM and linearity of calibration curve was R^2=0.995. Thus we were able to determine the rate of expression for transgenic Barley.

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Keywords: Lysine, Barley, DHPS, gene expression

Introduction

Provision of sufficiently quality food and fodders is still present appeal. World food crisis brings to border of famine millions of people. Food supplementation for biologically important compounds, such as vitamins, amino-acids and essential fatty acids may contribute for solving of this problem. Modern methods of molecular biology enable introducing of new properties into plants. Lysine is biologically very important amino-acid, which is essential for human. Adult needs about 1-1.5 g of lysine per day, children about 44 mg of lysine per day. Generally, L-lysine is essential structural amino-acid, which is crucial for all proteins. Lysine plays important role in calcium uptake absorption, participates in biosynthesis of hormones, enzymes and antibodies and its importance is also in metabolism of muscular tissue.

Positive effect of lysine is demonstrated in processes of healing of wounds after surgical interventions or injuries. Due to these properties, supplementation of fodders for livestock by lysine is very important factor for improving of daily increase in weight (Krishnakumar et al. 2010).

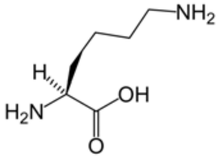

Figure 1: Structure of lysine.

Materials and methods

Transgenic barley plants of the T_0 generation were evaluated by PCR, Real-Time PCR and Western blot. Amino acids content was analyzed by Aminoacid Aminoanalyzer AAA400 with post column derivatization by ninhydrin. Leave samples were prepared by HCl hydrolysis: 0.1g of sample with 2 ml 6M HCl was mineralized for 24 thermoboxes in 85 °C. Glass column with inner diameter 3.7 mm and 350 mm length was filed manually with strong catex ionex in natrium cycle LG ANB with approximately 12 µm particles and 8% porosity. Column was tempered in range 35 - 95°C. Double channel VIS detector with inner cell volume 5 µl worked statically under two wavelengths 440 and 570nm. Solution of ninhydrin (Ingos, Czech Republic) was prepared by dilution in 75 % v/v metylcelosolve (Ingos, Czech Republic) and in 25 % v/v 4M acetic buffer (pH5.5). For reduction $SnCl_2$ (Lachema, Czech Republic) was used. Prepared solution of ninhydrin was stored under inert atmosphere (N_2) in dark and cooled. Elution of Lysine was done by buffer contains natrium citrate 19.6g, NaCl 52.6g and Boric acid 2.05g x $1L^{-1}$ and pH was adjusted to 9.7 by NaOH 0.2M. Flow rate was 0.3 ml/min. Reactor temperature was 120°C.

Results and discussion

Two constructs pBract214::sTPdapA and pBract214::mdapA containing the dapA gene from *Escherichia coli* coding bacterial DHPS were used for transformation of barley. The vector pBract214::sTPdapA in addition includes the transit peptide Rubisco *Hordeum vulgare* ribulose-1,5-bisphosphate carboxylase small subunit, Genbank U43493. An Agrobacterium-mediated technique was used for transformation of immature embryos of barley cv. Golden Promise. Plants were analysed with respect to expression of cloned genes by qRT-PCR technique. On basis of this analysis, four groups of plants were established (low, medium, high and unequal gene expression).

Due to this fact, we decided to carry out analysis of amino-acid lysine as product of gene transcription. Ionex chromatography was used for analysis. Signal of lysine was well-separated with limits of detection about 500 nM and quantification about 5 µM with error determination about 5%. Real samples in 0.025g weight were hydrolyzed by 0.5ml HCl (6M) in microwave under 80W in time 160minutes. Obtained extracts were subsequently applied into chromatographic system in triplicate (R.S.D. was 6.5%). Concentration of lysine in control group of plants was about 4.5% of total content of all amino-acids. From obtained results follows very good positive correlation between rate of expression of cloned gene and lysine concentration (R2 =0.99). We observed that content of lysine was the highest by RT-High expression in compare with control which has the lowest content of Lysine. RT-different expression has similar content of lysine to RT-middle expression and RT-low expression was almost in the middle about content of lysine between Control and RT-midle expression (Fig. 2).

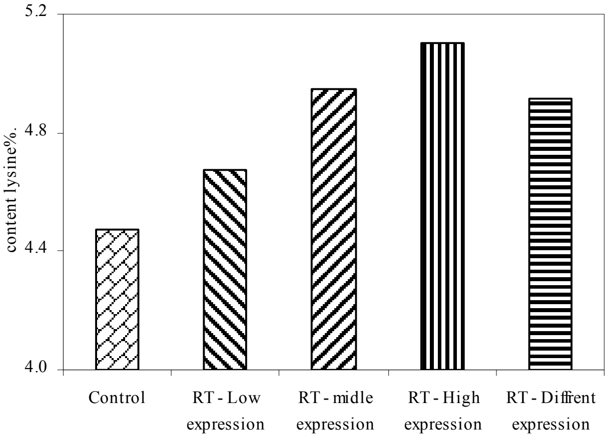

Figure 2: The volume-based lysine (%) on the expression of various genetically modified plants to lysine

Conclusion

Amino acids content was analyzed by Aminoacid Aminoanalyzer AAA400 with post column derivatization by ninhydrin. 30 samples were measured with the gene expression of lysine. The result was converted to % of total lysine. Lysine content had no effect on other amino-acids.

Acknowledgements

This work was supported by project 1M06030 of the Ministry of Education, Youth and Sports of the Czech Republic and REMEDTECH GA ČR 522/07/0692.

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