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PhD thesis

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In vitro and *in vivo* functional studies of wheat storage proteins in rice model system

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INTRODUCTION

A unique property of wheat flour is its ability to form dough when it is mixed with water. Dough exhibits viscoelastic and cohesive properties, essential to produce different end products such as bread.

Most of the knowledge about the functional properties of gluten proteins is derived from indirect correlative studies, or from direct reconstitutive experiments. In the first case, populations of samples with different chemical compositions are characterized and then compared using statistical methods. In case of dough reconstitution studies the chemical composition of a base flour is altered systematically by adding/incorporating different compounds such as individual gluten proteins to study their effects on functional properties. One of the limitations of the 'base flour' method is that the supplemented constituents obviously interact with the components of the flour, so depending on the choice of base flour used, certain amount of 'noise' is superposed on the measurements. An ideal solution to avoid this problem would be to use a base flour - such as rice flour - not containing wheat flour components at all.

Rice is one of the most important crops all over the world. In countries where rice production is more suitable than wheat because of the climatic conditions, it could be an interesting approach to partially substitute rice flour for wheat flour in bread and bakery products. However, dough-making quality of rice storage proteins is very poor compared to wheat: rice endosperm lacks the proteins responsible for this trait.

Structure and properties of the storage proteins of wheat and rice are significantly different. While the rice proteins are mostly globulins, the major seed storage proteins in wheat are prolamins. Unlike other cereals which accumulate prolamins as their primary nitrogen reserve, the major storage proteins in rice are the glutelins, which are homologous at the primary sequence level to the 11S globulin proteins. These differences provide the molecular background of the observed physical/functional variations between wheat and rice flour and their utilization in different products.

Supplementing rice flour components with wheat storage proteins – either by *in vitro* methods or by *in vivo* transformation – could improve our basic understanding about the possibilities of improving/altering the functional properties of rice/wheat flour. An interesting aspect of this possible quality improvement is the fact people allergic to wheat globulins and therefore being on strict wheat-free diet could enjoy baking product made from rice flour. (Wheat allergy has to be differentiated from a more serious wheat related health issue: celiac disease,

what is a toxic disorder caused by the prolamin proteins. Introduction of wheat gluten proteins into rice therefore unfortunately does not solve the problem of celiac patients.)

In this study, wheat gluten or its components, such as gliadin and glutenin rich fractions, have been obtained using both *in vivo* and *in vitro* studies. To study the effect of expressed, incorporated wheat proteins for the rice dough it was necessary to develop analytical and functional methods to monitor chemical and functional alterations of the dough system.

THE AIM OF THIS STUDY

1. To study the protein composition and functional properties of rice dough.
2. To get better understanding about the relationships between protein composition of rice flour and its properties to form dough.
3. To develop a reduction/oxidation procedure to incorporate partially purified wheat fractions into the rice dough.
4. To study the effect of added or incorporated glutenin subunits on functional properties of rice dough.
5. To express wheat HMW-GS proteins in transgenic rice plants and to study whether transgenically expressed HMW glutenin protein could be exploited to develop rice with novel dough characteristics
6. To better understand the relationship between the altered composition of rice flour and its properties to form dough.

MATEIALS AND METHODS

-Protein composition of flours from six Australian and five Hungarian rice cultivars were included and analyzed by SDS-PAGE and SE-HPLC in this study..
-A strong Hungarian wheat variety with good breadmaking properties, Mv-Suba, and a set double mutants of Galahad wheat cultivar, containing single Bx6, 7, 8 HMW-GS subunits were selected to isolate gluten using adapted method from wheat protein research..
-Micro scale mixing tests were carried out on a prototype micro z-arm mixer (METEFEM Ltd, Hungary).
-A reduction/reoxidation procedure (incorporation) was adopted for rice dough based on a method originally developed for wheat flour dough.

-The effects of addition and incorporation of wheat storage proteins on the functional properties of rice dough was investigated by micro z-arm mixer.
-Genetic transformation of rice using biolistic methods (GENEBOOSTER, Gödölllő, Hungary)
-Transgenic rice lines were identified by using PCR and RT-PCR. Alcohol soluble proteins, extracted from the endosperm of three independent lines were separated on SDS-PAGE, followed by Western blotting.

RESULTS

1. Protein distribution and functional properties of 11 different rice flours were investigated in this study, introducing several new parameters for the characterisation of rice protein and functional properties of rice dough. Unextractable polymeric protein (UPP%) values, a widely used parameter for characterizing the size distribution of polymerized proteins in wheat flour, were also determined for each rice flour and dough samples. Solubility % as an additional parameter was introduced to characterise the overall extractability of different rice protein classes.

2. While the effect of protein content and composition on mixing properties of wheat flour is well studied, there is no well established published method, so far to characterise the functional properties of dough made of rice flour. Therefore methods had to be developed for these characterizations applying/adopting methodology routinely used for wheat dough studies One of the most significant differences between the mixing properties of wheat and rice flour is the longer time needed for the hydration of rice components in the beginning of the mixing process. To characterise this rice-specific property, a new parameter, called ‘hydration time (Hyd) was introduced to monitor the time required for the hydration of components in the samples before forming the dough. Similarly to the well established relationships at wheat flour doughs, it seems that UPP% can be related to mixing properties: samples with larger amount of unextractable polymeric protein showed larger mixing requirement.

3. A reduction/oxidation procedure was developed to incorporate glutenin subunit type proteins into the polymeric structure of rice dough protein. Because of the slower hydration process in the beginning of the rice mixing action, the application of the reduction/oxidation

procedure to the rice dough preparations produced for z-arm mixer measurement required different conditions compared to those of applied previously for wheat flour. Under the optimized reduction/oxidation conditions, no significant difference could be detected in the size distribution of the proteins from the untreated reduced/reoxidised doughs. So, this procedure was suitable to incorporate partially purified wheat fractions into the rice dough and monitor their effects on the mixing properties of the rice dough.

4. Rice flour, due to the absence of wheat type storage proteins, could provide some advantages over wheat flour to use as base flour studying the characteristics of wheat prolamins. It has been proved that wheat prolamin addition and incorporation has significant effect on rice dough in small scale experiments. After the incorporation treatment using 15 mg glutenin rich fraction in 4g of rice flour, dough samples containing HMW and LMW-GS fraction of cv. Mv-Suba or HMW Bx6, 7 and 8 glutenin subunits of cv. Galahad, showed an increased amount of polymeric proteins and greater mixing requirements.

5. Gene encoding 1Dx5 HMW-GS was introduced into rice by biolistic gene transfer to evaluate functional properties and agronomic performance of such transgenic rice lines. We developed several independent PCR positive transgenic rice lines. It was also confirmed that wheat endosperm specific promoter drives protein expression only in the endosperm of transgenic rice. Stabile expression of wheat 1Dx5 HMW-GS gene in transgenic rice was confirmed in next generations of plants.

Our results are the first published example of significantly changing storage protein composition of rice applying the method of gene technology.

FUNCTIONAL APPLICATIONS

1. This work describes the use of a carefully controlled reduction/oxidation procedure on rice flour-wheat protein systems. Our results demonstrate the potential of using rice flour as a model system in wheat storage proteins structure function studies, determining the contribution of certain wheat proteins to quality attributes.

2. Since rice is one of the most suitable cereal to prepare food for patients suffering allergy reaction (gluten intolerance), this approach could lead to an alternative solution for those who cannot consume wheat products but would like to enjoy bread, pasta or biscuit.

PUBLICATIONS

Oszvald M, Tömösközi S, Tamás L, Larroque O, Kereszténvi E and Békés F. Characterization of Rice Proteins by Size Exclusion High Performance Liquid Chromatography and micro z-arm mixer. *Journal of Cereal Science* 2007. doi:10.1016/j.jcs.2007.08.002.

Oszvald M, Tömösközi S, Jenes B, Békés F and Tamás L. Expression of the Dx5 High Molecular Weight glutenin subunit protein in transgenic rice. *Cereal Research Communication.* 2007. doi: 10.1556/CRC.35.2007.4.1.

Oszvald M, Tömösközi S, Tamás L, Békés F. Preliminary study to investigate the role of rice and added wheat protein in the mixing properties of different rice flours. *Acta Alimentaria.* 2007. *(accepted)*

Oszvald M, Tömösközi S, Tamás L, Békés F. Functional studies of wheat storage proteins in model system, 5th European Young Cereal Scientist and Technologists Workshop, Turkey. 2006.06.04-07.

Oszvald M, Tömösközi S, Tamás L, Békés F. Functional studies of wheat storage proteins in model system. In: Lookhart, G.L., Ng, P.K.W. (Eds.), Gluten Proteins 2006. AACC International, Inc. St. Paul, pp.48-53.

Oszvald M, Tömösközi S, Tamás L, Békés F. Búza tartalékfehérjék vizsgálata modell rendszerben. In: XII. Növénynemesítési Tudományos Napok, Bp. (Ed: Kertész Z.) Összefoglalók pp 58. 2006.

Oszvald M, Jenes B, Tömösközi S, Békés F, Tamás L. 1Dx5 gén expessziója transzgénikus rizsben. VII. Magyar Genetikai Kongresszus. Balatonfüred, 2007. ápr. 15-17.

Oszvald M, Jenes B, Tömösközi S, Tamás L. A fehérje összetétel megváltoztatása gabonafélék szemtermésében géntechnológiai módszerekkel. Mezőgazdasági Biotechnológia Kutatóközpont Tudományos Napok, Gödöllő, 2003.

Jenes B., Pónyi, T., Szilvási A., Oszvald M., Oreifig AS., Bedő Z., Tamás L. Gabonafélék minőségjavítása biotechnológiai módszerrel, különös tekintettel a rizsliszt minőségére. Mezőgazdasági Biotechnológiai Kutatóközpont, Tudományos Napok, Gödöllő, 2001. nov.

Oszvald M, Tömösközi S, Tamás L, Békés F. Gabonafehérjék összetételének in vitro módosítása és hatásai a funkcionális tulajdonságokra. XIII. Növénynemesítési Tudományos Napok, Bp. (Ed: Kiss J, Heszki L) Összefoglalók pp. 117. 2007.

OTHER PUBLICATIONS

Oszvald M, Kang TJ, Tömösközi S, Tamás C, Tamás L, Kim TG, Yang MS. Expression of a synthetic neutralizing epitope of porcine epidemic diarrhea virus fused with synthetic B subunit of Escherichia coli heat labile enterotoxin in rice endosperm *Molecular Biotechnology.* 2007. 35 (3): 215-223.

Oszvald M, Gárdonyi M, Tamás C, Tömösközi S, Jenes B, Tamás L. Characterisation of a wheat endosperm specific HMW glutenin promoter in an intron containing transformation cassette. *In vitro cellural and develp. (accepted).*

Oszvald M, Szilvási A, Jenes B, Tamás L. Effects of new endosperm specific promoters in cereals. 4th International Conference of PhD Studies (Eds: Laszlo Lehoczky and Laszlo Kalmar). University of Miskoc, Innovation and Technology Transfer Center, Hungary, Miskolc. pp 105-108. 2003.

Oszvald M**,** Gárdonyi M, Jenes B, Tömösközi S, Juhász A, Tamás L: Development and improvement of endosperm specific promoters for foreign protein expression in cereal seed. Proceeding offed 10th International Wheat Genetic Symposium. Editors: N.E. Pogma, M. Romano, E.A. Pogna, G. Galterio. 2 volume: 899-901 .2003.

Oszvald M, Kang TJ, Kim TG, Tömösközi S, Jenes B, Tamás L, Yang, MS. Expression of *Escherichia coli* heat-labile enterotoxin in rice endosperm to produce edible vaccine. In: Proc. 2nd Conference on Bioreactor Technology (Eds. Sorvar S. and Toldi O.) FinMed, Finland, Saarisalke, pp 86-94. 2006.

Tamás L, Oszvald M, Tamás C, Tömösközi S, Kang ZJ, Yang MS, Jenes B. Az egyszikű növények alternatív célú alkalmazása-ehető vakcina előállítása rizsben géntechnológiai módszerekkel. XIII. Növénynemesítési Tudományos Napok, Bp. (Ed: Kiss J, Heszki L) Összefoglalók pp.23. 2007.

Oszvald M, Kang TJ, Jenes B, Kim TG, Tamás L, Yang MS. Vakcinák előállítása rizsben. Mezőgazdasági Biotechnológiai Kutatóközpont, Tudományos Napok, Gödöllő, 2006. nov.

Tamás L, Gárdonyi M, Oszvald M, Tamás C, Jenes B. Búza endospermium specifikus kiméra promóterek tanulmányozása tranziens rendszerben. In: XII. Növénynemesítési Tudományos Napok, Bp. (Ed: Kertész Z.) Összefoglalók pp169. 2006.

Oszvald M, Szilvási A, Jenes B, Tömösközi S, Bedő Z, Tamás L. Új típusú endospermium specifikus promóterek létrehozása, hatásának tanulmányozása gabonanövényekben V. Magyar Genetikai Kongresszus, 2003.