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Primer Designing and PCR Amplification of Glutelin Complete Promoter Region in Sri Lankan Rice Variety (Japonica Group)

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ABSTRACT

The long-term goal of the project in progress is to develop a metabolically engineered rice variety possessing flour qualities that would be suitable for making dough-in effect, 'wheat-like' rice. The objective of the present study is to amplify the complete promoter region of glutelin of rice. In this study, rice DNA was extracted by using a modified CTAB procedure and designed the new primers for the complete promoter region (glutelin) of rice. An attempt was taken to isolate glutelin complete promoter region of rice with several PCR optimizations.

KEYWORDS: Glutelin complete promoter, Japonica group, *O. sativa.*

INTRODUCTION

Rice (*Oryza sativa L.)* which belongs to family Poaceae, is the single most important crop occupying 34 per cent (0.77/ million ha) of the total cultivated area in Sri Lanka. On average 560,000 ha are cultivated during *maha* and 310,000 ha during *yala* making the average annual extent sown with rice to about 870,000 ha.

Rice provides 45% of the total calorie and 40% of the total protein requirement of an average Sri Lankan. Sri Lanka currently produces 2.7 million tones of raw rice annually and satisfies around 95 percent of the domestic requirement. The per capita consumption of rice fluctuates around 100 kg per year depending on the price of rice, bread and wheat flour (Anon, 2007).

Glutelin is the major seed storage protein of rice and it accounts for about 80% of the total endosperm proteins. It is an oligomeric protein (Quddus and Ma, 2003). A multigene family encodes this protein. About six known genes encoding these proteins are classified into two subfamilies, known as GluA and GluB. Members within each subfamily share more than 80% homology and homology between the two subfamilies share about 65% homology based on their DNA sequences. The subfamily GluA contains *GluA* -1, *GluA* -2 (Gt-1), *GluA* -3 (Gt-3) and *GluA* -4 (Chuan – Yin *et al.*, 1998).

Gluten proteins are the major storage proteins that accumulate in wheat endosperm cells and are important for the unique suitability of wheat flour for bread making. Gluten consists mainly of two types of seed storage proteins, namely, glutenins and gliadins. These glutenins are polymerized through intermolecular disulfide bonds, which are important to the properties of wheat flour dough (Gupta and Sheperd, 1990). The glutenins could be divided into two main groups. These two groups are high molecular weight glutenin (HMW glutenin) and low molecular weight glutenin (LMW glutenin), based on the mobilities in SDS – polyacrylamide gel electrophoresis. The variations on bread – making quality among different varieties are explained by the variation in HMW glutenin composition and the LMW glutenin with a smaller proportion of gliadins. The interactions of these gluten proteins play an important role in the determination of gluten strength and bread making quality.

Wheat flour has its unique property of being able to form dough, a property that is required for making bread and other related food products. Quality of rice proteins is comparatively low since they lack some of the essential amino acids such as lysine. The protein content of rice is also lower than is some cereals such as wheat. In spite of being a major rice consuming country, Sri Lanka has a relatively high wheat consumption of around 900,000 kg /year [Grain report number CE 1001 (2001)]. This is attributed to the convenience in production and versatility of wheat flour based food products. As wheat cannot be grown successfully in Sri Lanka, increasing wheat consumption means increase in foreign exchange expenditure and heavy dependence on imports.

The long-term goal of the project in progress is to develop a metabolically