

Lack of 26 kDa globulin accompanies increased free amino acid content in rice (*Oryza sativa* L.) grains

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Abstract

Seed nitrogen content, protein composition and free amino acid content were determined in rice mutant lines with altered storage protein composition and their parental cultivars. The *Lgc1* gene, which causes low glutelin content and high content of 13 kDa prolamin and 26 kDa globulin, did not affect grain weight, nitrogen content, or free amino acid content. The *glb1* gene, which lacks the 26 kDa globulin, did not affect grain weight or nitrogen content, but in mutant lines with *glb1* gene the content of the major free amino acids was significantly (1.3–1.5 times) higher than those of their parental cultivars. These results suggest that absence of 26 kDa globulin is accompanied by an accumulation of a high level of free amino acids in rice grains.

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Keywords: Seed storage protein; Rice mutant; Free amino acid; 26 kDa globulin

1. Introduction

Rice (*Oryza sativa* L.) is an important source of nutrients and energy for almost 50% of the world's population. The protein content of rice is about 7% (w/w), which is relatively low compared with other cereals (Shih, 2004).

Most seed-stored proteins are accumulated in special vacuoles called protein bodies. Two kinds of protein bodies exist in rice: protein body-I (PB-I) and protein body-II (PB-II). PB-I contains prolamins and constitutes approximately 20% of milled rice protein, whereas PB-II contains glutelins and a 26 kDa globulin that together constitute approximately 60% of milled rice protein (Ogawa et al., 1987; Yamagata et al., 1982). The morphometric features of intact PB-I are similar to the indigestible remains of rice found in faeces of monogastric animals and the two protein particles are considered to be identical (Barber et al., 1998; Ogawa et al., 1987), however, the

results of immunocytochemical analysis make the link between PB-I and faecal protein particles less clear (Barber et al., 1998). In any case, humans are not able to digest rice seed storage proteins completely (Tanaka et al., 1975).

Altering the compositions of rice storage proteins by inducing mutations may modify their digestibility and nutritional qualities. Rice with poorly digestible protein is expected to be useful for low-protein diets of patients with chronic renal failure whose protein intake should be restricted, and so decreased protein digestibility is one target for rice improvement programs (Iida et al., 1993). Kumamaru et al. (1988) screened seeds of numerous rice mutant lines by comparing profiles obtained using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and found both decreases and increases in polypeptides. Also Iida et al. (1993) produced a mutant line, LGC1, with a low content of glutelin and high contents of 13 kDa prolamin and 26 kDa globulin. The mechanism for lowering the glutelin content in LGC1 has been described: in the dominant mutation at the *Lgc1* locus there is a 3.5 kb deletion between two highly similar glutelin genes that forms a tail-to-tail inverted repeat. This repeat produces a double-stranded RNA molecule that induces RNA silencing (Kusaba et al., 2003). A second mutant line, 88KG30-913, which lacks a glutelin a-2 subunit, was found to be controlled by a single recessive gene, *glu2*, on chromosome 10 (Iida et al., 1997). A third mutant line that lacks a glutelin a-3 subunit, 90WPKE0.1-561, is controlled by a single recessive gene on chromosome 1, *glu3* (Iida et al.,

Abbreviations γ -ABA, γ -amino-*n*-butyric acid; CBB, Coomassie Brilliant Blue; PB-I, protein body-I; PB-II, protein body-II; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

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The integration of mutations involving the major seed storage proteins of soybean, glycinin and α -conglycinin, produced a mutant line, QF2, which accumulated high levels of free amino acids (Takahashi et al., 2003). The free amino acid contents were about three to eight times higher than those of wild-type varieties; Arg was especially enriched in the seeds of the mutant line. The accumulation of free amino acids and other proteins in the seeds of the integrated mutant line was suggested to be a compensation for the decreased glycinin and α -conglycinin contents. In maize, *opaque-2* mutation improved the lysine content and appears to be related to the decreased synthesis of zein storage proteins, which contain no lysine, and the increased synthesis of lysine-containing, non-zein proteins in the mutant endosperm (Moro et al., 1996). The *Opaque-2* gene encodes a leucine-zipper transcriptional activator bound to promoter element(s) of zein storage protein(s) (Schmidt et al., 1990). The *opaque-2* mutation is also associated with an increased level of free amino acids (Mirsa et al., 1975). Quantitative trait loci mapping (Wang and Larkins, 2001) identified loci influencing pools of free amino acids in *opaque-2* mutants, and concluded that alteration of amino acid and

Although the mechanism of mutation that induces the decrease in some polypeptide(s) remains unclear, their decrease in rice, soybean, and maize is sometimes accompanied by an increase of other polypeptide(s) and this has been described by [Kusaba et al. \(2003\)](#) as a compensatory phenomenon. In addition to the increases in some seed protein(s), several soybean and maize mutants had increased contents of free amino acids. Thus it was of interest to enquire whether an increase in free amino acids also accompanied alteration of storage protein composition in grains of rice mutant lines.

2.1. Materials

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Six rice cultivars/lines were used in this study: Nihonmasari and Koshihikari, the parental *japonica* cultivars; LGC1, a low glutelin content mutant line produced by backcrossing of Nihonmasari with a mutant NM67 that shows low glutelin content (Iida et al., 1993); K $\times$ 433, a mutant line lacking 26 kDa globulin derived from backcrossing Koshihikari with the 89WPKG30-433 mutant, which lacks 26 kDa globulin (Iida et al., 1998); LGC-Jun, a crossbred double mutant line that shows low glutelin content and lack of 26 kDa globulin (Nishimura et al., 2005); QA28, a crossbred quadruple mutant line, which shows low glutelin content, and lacks the glutelin a-2 subunit, glutelin a-3 subunit and 26 kDa globulin. The pedigrees of the lines used are shown in Fig. 1. The cultivars/

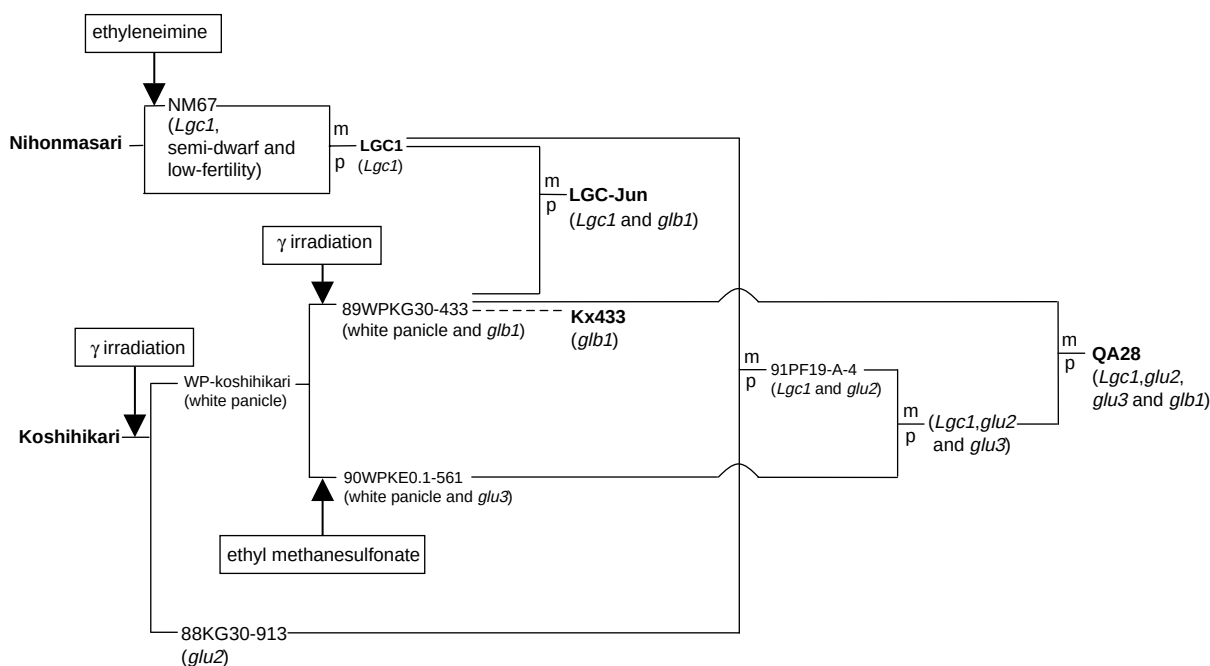


Fig. 1. Pedigree of rice mutant lines that lack storage proteins. Mutagen treatments are indicated by squares. Broken line represents backcross. Backcrossing of the mutant 89WPKG30-433 to cultivar Koshihikari produced K×433. Cultivars and lines used in this study are indicated in bold typeface. *Lgl1*, mutant allele for low glutelin content; *glb1*, mutant allele for lack of 26 kDa globulin; *glu2*, allele for lack of glutelin a-2 subunit; *glu3*, allele for lack of glutelin a-3 subunit. m, maternal line; p, paternal line.

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