**19. *Glup4* gene encodes small GTPase, Rab5a in rice.**

M. SATOH-CRUZ[1,2)], M. FUKUDA[1)], M. OGAWA[3)], H. SATOH[1)] and T. KUMAMARU[1)]
1) Faculty of Agriculture, Kyushu University, Fukuoka 812-8581 Japan
2) Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340 USA
3) Yamaguchi Prefectural University, Yamaguchi 753-8502 Japan

The rice storage proteins, glutelin and prolamin, are synthesized on the rough endoplasmic reticulum (ER; Muench and Okita, 1997) prior to their packaging into separate protein bodies. Prolamins assemble into intracisternal inclusion granules within the ER lumen, forming spherical protein bodies (prolamin PB or PB-I). Glutelins are initially synthesized as a precursor form on the ER membrane and then accumulated as processed subunits in the protein storage vacuole (PSV). To elucidate the function of genes participating in glutelin biosynthesis, we identified eight mutants generated by N-methyl-N-nitrosourea (MNU) treatment (Kumamaru et al., 1987, Satoh et al., 1994, 1995, 1999 Tian et al., 2001), which accumulated excess amounts of unprocessed glutelin precursor. Genetic and biochemical studies of these mutants suggested that the glutelin precursor accumulating mutations affected events from the translation of glutelin on the ER to the deposition of glutelin within the PSV. Here, we describe the results of genetic linkage analysis of the *glup4* mutants amongst them.

Previously, we reported that the *glup4* gene is located within a 6.5 cM region on chromosome 12 (Sato et al., 2003). In this study, to investigate the key gene of *glup4* mutants, we constructed a more detailed genetic linkage map of the *Glup4* gene on chromosome 12 (Fig. 1). A linkage analysis was conducted using 58 homozygous plants for *glup4* from $F_2$, a cross between EM956 and an indica rice variety, Kasalath. The locus of *glup4* was mapped within a 1.8 cM region, between RFLP markers R1759 (107.4 cM from short arm end) and Y2824R (109.2 cM). The search of the BAC clone database revealed that the region between R1759 and Y2824R corresponded to eight overlapping BAC clones. The *Glup4* gene sequence is contained within these BAC clones. Within the 1.8 cM region, more than 200 genes were annotated. Among them, a sequence was highly homologous with the sequence of small GTPase Rab5a, which is reported to participate in vesicular transport and ER structuring in mammalian cells (Audhya et al., 2007). The sequence was considered as a candidate for the *Glup4* gene.

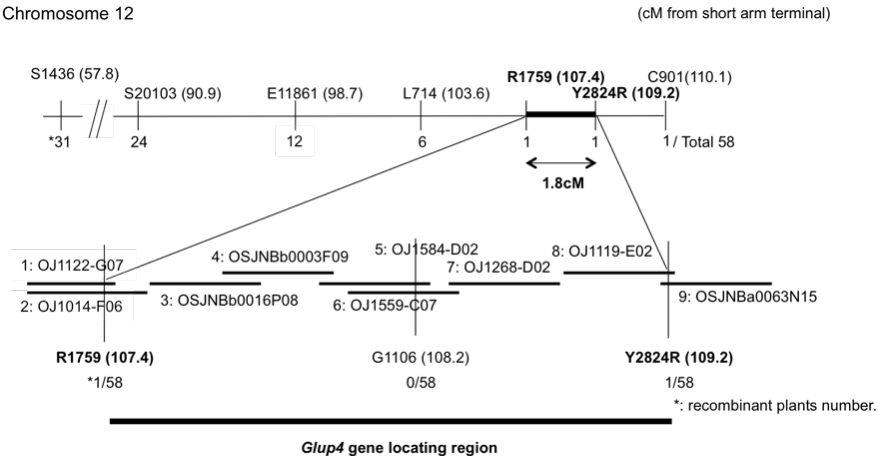


Fig. 1 Linkage map of *Glup4* gene on chromosome 12.
The upper figure is showing gene map of *Glup4* and the lower is a physical map of the BAC clone. Numbers in parenthesis and under the linkage map indicate the genetic distance (cM) from the short arm terminus and the recombinant plant number, respectively. The bold line denotes the *Glup4* candidate gene region.

Genomic DNA sequence analysis of the Rab5a gene in three *glup4* allelic lines showed that Gly-45 and Gln-176 were replaced by Asp and a stop codon in EM960 and EM425, respectively, while EM956 had a G to A substitution at nucleotide positions 43, a conserved splicing site. These results indicate that the *Glup4* gene encodes the structural gene of the small GTPase, Rab5a.

Rab5a may play a role in coordinating the transport and localization of glutelin precursor to the PSV.

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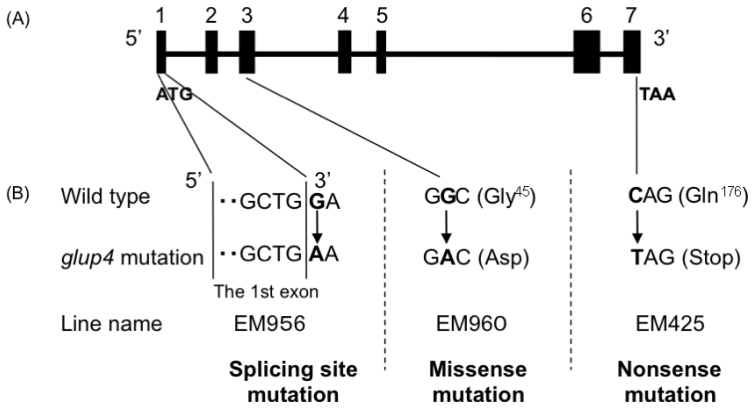


Fig. 2 Mutation sites of *Rab5a* gene in three *glup4* mutants.

(A) Structure of the GTPase Rab5a gene. ATG and TAA indicate the initiation and termination codons. Black boxes indicate the 7 exons. The Rab5a reading frame spans 612 bps and codes for 203 amino acids.

(B) Mutation sites in three *glup4* alleles. Genomic sequence analysis demonstrated that Gly45Asp and Gly176Stop replacements are observed in EM960 and EM425, respectively, while EM956 has a G to A substitution in a conserved splicing site at nucleotide positions 43. These indicate that the *Glup4* gene encodes the structural gene of the small GTPase, Rab5a.

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