A mutant gibberellin-synthesis gene in rice

New insight into the rice variant that helped to avert famine over thirty years ago.

The chronic food shortage that was feared after the rapid expansion of the world population in the 1960s was averted largely by the development of a high-yielding semi-dwarf variety of rice known as IR8, the so-called rice 'green revolution'[1–3]. The short stature of IR8 is due to a mutation in the plant's sd1 gene, and here we identify this gene as encoding an oxidase enzyme involved in the biosynthesis of gibberellin, a plant growth hormone. Gibberellin is also implicated in green-revolution varieties of wheat, but the reduced height of those crops is conferred by defects in the hormone's signalling pathway[4].

There are various reasons for the dwarf phenotype in plants, but gibberellin (GA) is one of the most important determinants of plant height[5–7]. To investigate whether the sd1 gene in semi-dwarf rice (Fig. 1a) could be associated with malfunction of gibberellin, we tested the response of this mutant to the hormone. We found that sd1 seedlings are able to respond to exogenous gibberellin, which increases their height to that of wild-type plants (results not shown).

Gibberellin is synthesized from geranylgeranyl diphosphate in higher plants, with an aldehyde intermediate being converted by a sequence of oxidase-catalysed reactions to a series of gibberellin precursors (designated here by GA subscripts). We found that the GA_{20} intermediate was depleted in the sd1 mutant relative to the wild type, but that GA_{53} (produced earlier in the pathway) was accumulating (results not shown). These results indicate that the activity of GA_{20} oxidase (GA20ox), a key enzyme in the biosynthesis of gibberellin that catalyses the three steps GA_{53}• GA_{44}• GA_{19}• GA_{20}, is not functioning effectively in the mutant.

A gene encoding a GA20ox isoenzyme (GA20ox-1) has been isolated from rice[8], but this does not correspond to the sd1 locus (results not shown). However, we isolated a new GA20ox gene (GA20ox-2) by using degenerate primers based on the conserved domain of the GA20ox genes in rice (GA20ox-1)[8] and Arabidopsis (GA5)[9], and found that GA20ox-2 was located on the long arm of chromosome 1, tightly linked to the sd1 locus[10] (Fig. 1b).

The deduced amino-acid sequence of GA20ox-2 showed 47.8% identity to GA20ox-1 and 49.5% identity to Arabidopsis GA5 (results not shown). When we compared the GA20ox-2 gene sequences from four sd1 mutants to that in the wild type, we found that one sd1 allele contains a 383-base-pair deletion (the semi-dwarf rice strain 'dee-geo-woo-gen' and IR8 both carry the same sd1 allele), which induces a frameshift that creates a stop codon, and that the other three sd1 alleles encode proteins with amino-acid substitutions (Jikkoku, Calrose 76 and Reimei strains; Fig. 1c). Introducing the GA20ox-2 gene from the wild-type plant rescued the semi-dwarf phenotype of sd1; furthermore, a recombinant GA20ox-2 protein catalysed the conversion of GA_{53} to GA_{20} (results not shown). We conclude that the wild-type SD1 gene encodes the biosynthesis enzyme GA20ox.

The rice genome carries at least two GA20ox genes (GA20ox-1 and GA20ox-2). SD1 corresponds to GA20ox-2, which is strongly expressed in the leaf blade, stem and unopened flower, whereas GA20ox-1 is predominantly expressed in the unopened flower (Fig. 1d). The increased expression of GA20ox-2 in the leaf blade and stem of the wild type would be expected to result in a semi-dwarf phenotype in the enzyme-defective sd1 mutants, which indeed have shorter leaves and stems. Surprisingly, however, flower formation and fertilization are normal in the mutants, although active gibberellins are important for these events. It is likely that the other GA20ox, which is encoded by GA20ox-1 and is preferentially expressed in the reproductive organs, enables the flowers in sd1 plants to develop and be fertilized normally, explaining why plant height is reduced without seed yield being affected.

The wheat green-revolution gene Rht (for 'reduced height')[4] is a gain-of-function allele caused by a mutation in a transcription factor that is associated with the gibberellin signalling pathway. As wheat has a hexaploid genome, it does not contain recessive alleles such as sd1 in rice that might otherwise be used to produce a semi-dwarf strain of wheat. Although the genetic and biochemical functions of the rice SD1 and wheat RHT proteins are completely different (that is, recessive versus dominant, loss-of-function versus gain-of-function events, enzyme versus transcription factor, respectively), the products of both genes are linked with gibberellin malfunction. Consequently, manipulation of the biosynthesis or signalling pathways of this growth hormone may offer a means of regulating the height of other important crop plants.

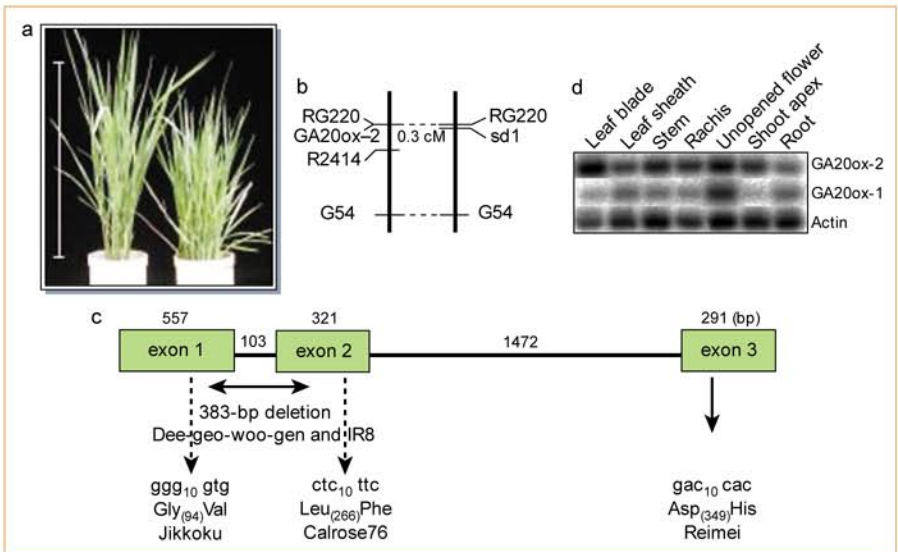

Figure 1 Effect of a mutant gibberellin-biosynthesis gene in rice. a, Morphology of *sd1*-mutant rice plants: left, taichung 65 (wild type); right, IR8 (*sd1*). Scale bar, 60 cm. b, Linkage between *GA20ox-2* and *sd1* in the rice genome. Left, chromosomal location of *GA20ox-2*; *GA20ox-2* co-segregates with *RG220* on chromosome 1. Right, map position of *sd1*; *sd1* is tightly linked to *RG220* on chromosome 1 (ref. 10). cM, centimorgans. c, Mutation sites of the four *sd1* alleles. The *GA20ox-2* gene consists of three exons and two introns. The mutation in each allele is indicated by either an arrow (single-nucleotide substitution) or a line (internal deletion). d, Expression of *GA20ox-2* in different organs. Amplification was by polymerase chain reaction with reverse transcription using first-strand complementary DNA derived from different organs. Products were detected by Southern blot DNA analysis; actin DNA was used as a loading control[11]. The nucleotide sequence of *GA20ox-2* has been deposited in GenBank under accession number AB077025.

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