

IN SILICO ANALYSIS OF GIBBERELLIN-20 OXIDASE-2

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Gibberellins (GAs) constitute a large group of natural tetracyclic diterpenoids. Active Gibberellins function as hormones in plants, controlling many aspects of development, including stem extension, fruit set, and seed germination. Gibberellin-20 oxidase-2 enzyme catalyzes the sequential oxidation of GA53 to GA20. The semidwarfing gene in rice (*sd-1*) is one of the most important genes deployed in modern rice breeding. Its recessive character results in a shortened culm with improved lodging resistance and a greater harvest index. In an attempt to identify the homologs of GA20ox2 in other crop species sequence database scanning was performed. Several homologues were obtained with varied degree of identity between them. The deduced amino acid sequences were aligned and phylogenetic trees were constructed to determine the consensus pattern of sequences and for establishing the relationship among the GA20ox2 of rice and other crops. On the basis of alignment, proteins were designated to their respective groups. *In silico* analysis has identified several homologues of GA20ox2 in various species and provided insight into their evolutionary relationships.

Keywords: GA20ox2, Gibberellic Acids, GA, phylogenetic analysis, *sd-1*, semidwarf-1.

Gibberellins (GAs) constitute a large group of natural tetracyclic diterpenoids. Biologically active GAs have a profound effect on plant growth and development (Reid and Howell 1995). Numerous studies with GA-deficient mutants have shown that GAs are involved in processes such as stem elongation, leaf expansion, photoperiodic induction of flowering, flower development, seed development, and germination (Crozier 1983, Swain *et al.* 1997).

GA 20-oxidase (GA 20-ox) is a multifunctional enzyme that catalyzes the sequential oxidation of GA53 to GA20 (Fig. 1). The product of the reaction GA20 catalyzed by GA20-ox, is then hydroxylated by GA 3b-hydroxylase (GA 3b-hy) to produce the active GA, GA1. GA 20-ox is encoded by a small multigene family whose members are differentially regulated (Phillips *et al.* 1995, Garcı́a-Martı́nez *et al.* 1997, Rebers *et al.*

1999). The semidwarfing gene in rice (*sd-1*) is one of the most important genes deployed in modern rice breeding. Its recessive character results in a shortened culm with improved lodging resistance and a greater harvest index, allowing for the increased use of nitrogen fertilizers. The *sd-1* gene was first identified in the Chinese variety Dee-geo-woo-gen (DGWG), and was crossed in the early 1960s with Peta (tall) to develop the semidwarf cultivar IR8, which produced record yields throughout Asia and formed the basis for the development of new high-yielding, semidwarf plant types (IRRI 1966). In rice plant architecture is mainly affected by plant height, tillering and panicle morphology (Wang *et al.* 2005). In rice, short stature plants were developed by altering the plant architecture. Short stature in rice was developed by a recessive semidwarf gene *sd-1*, responsible for High Yielding Varieties (Nagano *et al.* 2005).

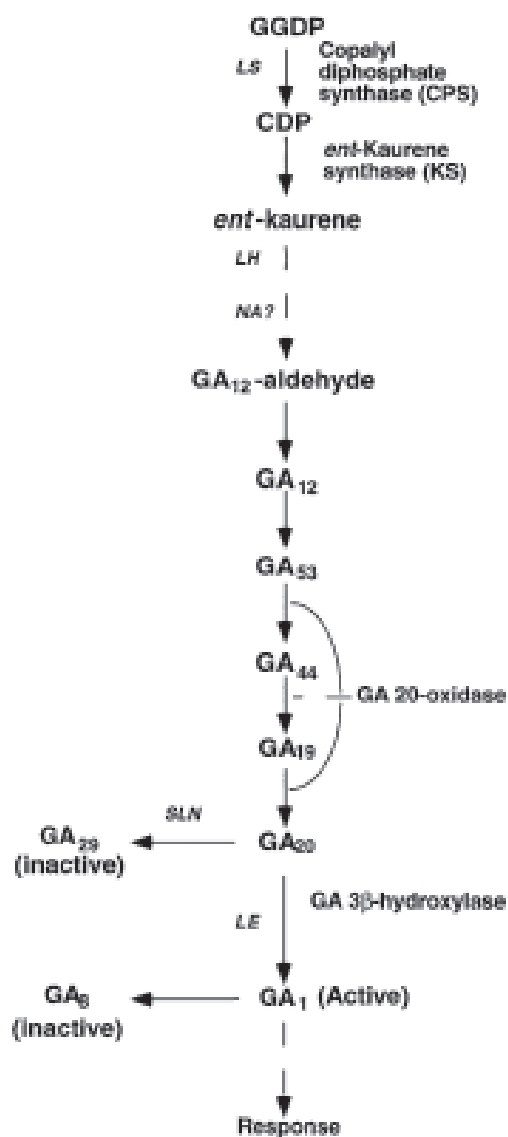


Figure 1: The sequential oxidation of GA_{53} to GA_{20} catalysed by GA20ox-2

GA20ox2 is tightly linked to the sd-1 locus of rice chromosome 1. Semidwarfing gene inhibits the elongation of lower internodes which makes rice resistant to lodging (Ogi *et al.* 1993). The short stature of IR8 is due to a mutation in the plant's sd1 gene, encoding an oxidase enzyme involved in the biosynthesis of gibberellin, a plant growth hormone (Sasaki *et al.* 2002). Ga20ox2 catalyses the conversion of GA_{53} to GA_{20} . DGWG sd-1 mutants have 383 bp deletion in genomic sequence which encode

a non-functional protein in an *indica* semidwarf IR8 (Monna *et al.* 2002). However, a substitution of Leu-266 that is highly conserved residue resulted in the loss of function in *japonica* semidwarf. By making the use of sequence similarity/ homology genetic relationships among cereal crops can be established (Ishii *et al.* 1996).

Comparative sequence analysis revealed that the sdw1/denso gene of barley is located in the syntenic region of the rice semidwarf gene *sd1* on chromosome 1 (Jia *et al.*, 2009). In this paper, using the sequences of homologues of *sd1*, we have performed *in silico* analysis for identifying the evolutionary relationships and conserved sequence patterns among the GA20ox2 from crop species. We also report the phylogenetic relationship among different homologues of *sd-1*.

METHODS

Protein sequence database was surveyed for the known sequences of Gibberellin-20 oxidase-2 from various species available at ncbi. The sd-1 protein P0C5H5.1, a Gibberellin-20 oxidase-2 sequence from *Oryza sativa indica* obtained from [http:// www. ncbi.](http://www.ncbi.)

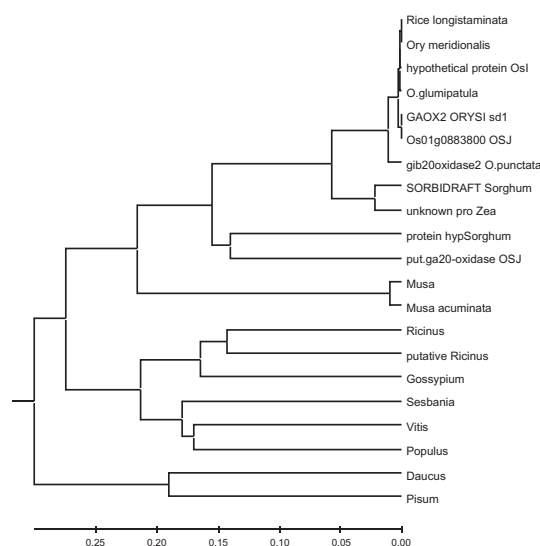


Figure 2 : Phylogenetic tree to show the evolutionary relationship of GA-20 oxidase enzyme between various crops. The tree was generated through MEGA4 program using 500 bootstrap replications.

All amino acid sequences of proteins were aligned and phylogenetic tree was constructed to determine the relationship

amongst the proteins. The alignment of all 21 proteins was done through ClustalX2 program. The alignment obtained is shown in the Fig.2. A '*' represents aligned identical residues, ':' represents conserved substitutions residues and '.' represents semi-conserved substitutions. The leucine residue originally present at 266 position in semidwarf variety of rice is highly conserved in all the proteins (position-319 in the multiple sequence alignment) which implements that it may be critical for the active protein. Several other residues were also conserved which may be the part of the conserved domain of the protein.

The phylogeny was traced through MEGA4 program using UPGMA method. Most of the proteins formed clusters according to their taxonomic classification. All cultivars of rice clustered together indicating the close relationship in their GA-20 oxidase enzyme. An unknown protein from *Zea mays* clustered with the gibberellin-20 oxidase protein of *Sorghum* indicating that this hypothetical protein shares close relationship to sorghum protein. The closer relationships between a hypothetical protein from *Sorghum* and putative gibberellins-20 oxidase protein of *Oryza sativa japonica* suggest that these members share not a too distant common ancestor. All clusters obtained in the phylogeny for proteins have been shown in Fig.2.

CONCLUSION

Phylogenetic comparisons of gene and protein sequences between related species are often used to identify evolutionary conserved elements that are important for protein expression and regulation. The semidwarf protein of rice was taken as the query sequence and the similar proteins from crop species with 55% or above identity were taken for the analysis. All sequences were deduced in fasta format and subjected to multiple sequence

alignment and the phylogenetic UPGMA tree was constructed. A phylogenetic comparison between the sequences from different species provided an insight to their genetic and evolutionary relationship. A much less variation has occurred in different rice cultivars than the other crops. Several homologues for Ga20ox2 have been identified from various crop species which after characterization could help in crop improvement.

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