Proteome Analysis of Rice Root Proteins Regulated by Gibberellin

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To gain an enhanced understanding of the mechanism by which gibberellins (GAs) regulate the growth and development of plants, it is necessary to identify proteins regulated by GA. Proteome analysis techniques have been applied as a direct, effective, and reliable tool in differential protein expressions. In previous studies, sixteen proteins showed differences in accumulation levels as a result of treatment with GA_3, uniconazole, or abscisic acid (ABA), and/or the differences between the GA-deficient semi-dwarf mutant, Tan-ginbozu, and normal cultivars. Among these proteins, aldolase increased in roots treated with GA_3, was present at low levels in Tan-ginbozu roots, and decreased in roots treated with uniconazole or ABA. In a root elongation assay, the growth of aldolase-antisense transgenic rice was half of that of vector control transgenic rice. These results indicate that increases in aldolase activity stimulate the glycolytic pathway and may play an important role in the GA-induced growth of roots. In this review, we discuss the relationship among GA, aldolase, and root growth.

Key words: aldolase, gibberellin, proteome, rice

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world. It is the main staple food of more than half of the world's population. Since rice has a genome that is significantly smaller than those of other cereals, it is an ideal model plant for genetic and molecular studies, particularly among the monocots (*1*). Draft sequences of rice genomes have been reported for subspecies *indica* (*2*) and *japonica* (*3*). Furthermore, the complete map-based genome sequences of chromosomes 1 (*4*) and 4 (*5*) for cultivar Nipponbare have been reported. The challenge ahead for the plant research community is to identify the functions, post-translational modifications, and the regulation of proteins encoded by the plant's genes. Understanding the biological functions of novel genes is a more difficult proposition than merely obtaining the nucleotide or peptide sequences. This is because the existing information on amino acid sequences of known proteins in the database is derived primarily from genetic and biochemical studies, which are by nature focused and labor intensive; the use of a large number of plant species as experimental systems; and the extensive range of unique plant-produced secondary metabolites. Thus, the cumulative knowledge of functions of known and unidentified proteins does not match the wealth of nucleotide sequence information being generated through genome sequencing projects (*6*). The analysis of proteins using high-resolution, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the most direct, first-order approach to define gene functions.

Root architecture in plants is determined by interactions between their intrinsic developmental programs and external biotic and abiotic stimuli (*7*, *8*). Root systems of plants growing in the field are marvelously successful at foraging for nutrients and water in a hostile, competitive environment where supplies of them are very limited, local and variable (*9*). The acquisition of soil resources by plant root systems is a subject of considerable interest in agriculture and ecology, as well as a complex and challenging problem in basic plant biology. Thus, root growth regulation is a critical function of terrestrial plants and is closely associated with the production, transport, and response to plant hormones. To understand the mechanism by which plant hormones regulate the root growth and development of plants, it is necessary to identify and characterize proteins regulated by plant hormones.

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Gibberellins (GAs) are essential endogenous regulators of plant growth and developmental processes (*10*, *11*). Significant progress has been made in understanding the pathways involved in GA biosynthesis and on the mechanisms by which GA levels are regulated in plants (*12*, *13*). The plant response to GA applied to intact root systems appears to be concentration-dependent, and can have rather variable effects. For example, a low concentration of exogenous GA plays a role in normal elongation of roots by maintaining the extensibility of the cell wall in GA-sensitive dwarf pea (*14*). GA is a regulator of cell elongation in roots, and there is an apparent correlation between low levels of GA *in planta* and short roots (*15*). Since root system architecture influences water and nutrient absorption, the GA regulation of root growth is essential for plant survival. Plant cells with defects in vacuole expansion do not expand (*16*), and the uptake of water by expanding vacuoles and the rapid osmoregulation between cytosol and vacuole are regulated by tonoplasts (*17*). Vacuole expansion and cell elongation are dependent on several factors, such as rates of cell component biosynthesis, metabolite concentration, and pH gradient across the tonoplast. GA is likely to be associated with the tonoplast function.

In previous studies, proteome analysis was used to investigate the effects of GA and aldolase increases in roots treated with GA_3 (*18*). Konishi *et al* (*19*) have reported that increases in the expression of aldolase by exogenous GA_3 were reversed in roots treated with uniconazole, an inhibitor of GA biosynthesis, and the abscisic acid (ABA) and aldolase levels were low in the roots of the GA-deficient mutant Tan-ginbozu. The GA deficiency in this mutant is due to the blocking of the metabolic steps from *ent*-kaurene to *ent*-kaurenoic acid, catalyzed by *ent*-kaurene oxidase (*20*). Since the increase of aldolase caused by GA activates the glycolytic pathway, the acceleration of root growth may be a direct result of these actions. Furthermore, antisense-transgenic rice plants were used to clarify the role of aldolase regulated by GA (*21*), and aldolase regulated the V-ATPase-mediated control of cell elongation, which determines root growth (*22*). In this review, the regulation of rice root proteins by GA as revealed by proteome analysis is discussed.

Proteome Analysis of Rice Root by 2D Gel Electrophoresis

Root protein identification using 2D electrophoresis

Proteins were extracted from roots (*23*) and separated by 2D-PAGE (*24*) using IEF and IPG tube gels (*25*) in the first dimension, with IEF in the low pI range (4.0–7.0) and IPG in the high pI range (6.0–10.0). After detection with CBB staining, protein spots were analyzed using Image-Master 2D Elite software. The 2D maps of the low and high pI ranges overlapped at around pI 6.0. A total of 508 proteins from rice roots were detected in the 2D-PAGE patterns. Although clear images were obtained in the range of pI 3.5–6.0 from 2D-PAGE with the IEF tube gel, including ampholytes in the first dimension, it was difficult to effectively isolate the basic proteins. Therefore, electrophoresis using IPG tube gels (pI 6.0–10.0) was carried out for improved resolution of basic proteins. About 90% of the visible proteins detected by 2D-PAGE were present in the pI 4.0–7.5 range. Proteins separated under these conditions were reproducibly observed in their relative 2D map positions. Computer analyses using Image-Master 2D Elite software revealed 508 individual rice root protein spots. After 2D-PAGE, amino acid sequences were determined by mass spectrometry and/or Edman sequencing (*26*).

A total of 38 N-terminal sequences of the 94 root proteins separated by 2D-PAGE were determined by Edman sequencing. A further 35 separated proteins excised from gels were analyzed by mass spectrometry. Using these approaches, at least some sequence information was determined for 73 individual proteins. The N-terminal amino acid sequences of 56 (59.6%) proteins of the total could not be determined by the Edman technique likely due to a blocking group at the N-terminus. This result is consistent with a report in which 134 rice proteins were subjected to sequencing, of which 79 (59%) were found to have blocked N-termini (*27*).

Functional classification of root proteins

Twenty-six percent of the identified proteins were involved in plant defense, indicating that the rice plant produces more disease-resistance and defense-related proteins than any other type. This functional category involves metallothionin, glutathione S-transferases, chitinases, NBS-LRR-type resistant proteins, antifungal protein R, thaumatin-like proteins, superoxide dismutase (Cu-Zn) [(Cu/Zn)SOD], type-1 pathogenesis-related (PR-1) protein, and SalT