

α -Amylase Expression under Anoxia in Rice Seedlings: An Update*

E. Loreti*, A. Alpi**, and P. Perata***

*Institute of Biology and Agricultural Biotechnology, CNR, Pisa, Italy

**Department of Crop Plant Biology, University of Pisa, Via Mariscoglio 34, Pisa, Italy

***Department of Agricultural Sciences, University of Modena and Reggio Emilia,

Via Kennedy 17, 42100 Reggio Emilia, Italy;

fax: +39-050-221-6541; e-mail: perata.pierdomenico@unimo.it

Received January 15, 2003

Abstract—Rice grains can germinate under anoxia, while most other cereals fail to behave similarly. The ability of rice grains to degrade starch, thanks to the successful production of α -amylase even in the absence of oxygen is likely one of the factors allowing rice to germinate anaerobically. In this paper we review the most recent results concerning the physiology and molecular biology related to the expression of α -amylase genes under anoxia. The current view is that expression of sugar-starvation induces isoforms of α -amylase playing a major role during germination under anoxia, while gibberellin induces α -amylase predominating under aerobic conditions.

Key words: *Oryza sativum* - α -amylase - anoxia - rice - starch degradation

THE ROLE OF α -AMYLASES DURING RICE GRAIN GERMINATION

Starch represents the main reserve compound in cereal grains. This polysaccharide is stored in the endosperm where it is hydrolyzed during germination to provide soluble sugars to the germinating seedling. Starch degradation is a complex biochemical process, which is modulated by both hormonal and metabolic regulation ([1] and references therein). A set of enzymes are needed to carry on starch breakdown: α -amylase, β -amylase, debranching enzyme, and α -glucosidase [2, 3]. Both α -glucosidase and α -amylase are able to degrade native starch granules, but the latter enzyme plays a major role in this process, and it is therefore the key enzyme for starch degradation [2, 3]. Furthermore, Akazawa *et al.* [4] demonstrated that starch phosphorylases play a minor role in starch degradation in germinating rice seedlings while α -amylase predominates during this physiological process.

Rice α -amylases are encoded by at least ten genes, located on five different chromosomes [5]. The α -amylase genes have been classified into three subfamilies: *Amy1* (A-B-C), *Amy2* (A), and *Amy3* (A-B-C-D-E-F). *Amy1A* induction by gibberellins (GA) and repression by abscisic acid are well documented under aerobic conditions [6, 7], but information about the mechanisms regulating other α -amylase genes in rice is scant.

Sugar modulation of α -amylase genes have been described [8, 9]. Indeed, *Amy3D* and *Amy3E* are sugar-repressed in rice embryos [8], and sugar repression of *Amy3D* transcription in anoxic rice aleurones has also been observed [10].

α -Amylase genes are expressed in two different grain tissues, namely the scutellar epithelium and the aleurone layer [11, 12]. The expression pattern of α -amylase genes in these two tissues have been described for *Amy1A* and *Amy3D*, showing that both genes are initially expressed in the epithelium and, at a latter stage, in the aleurone [11]. The expression of the GA-induced *Amy1A* gene largely predominates over that of the *Amy3D* gene, and overall expression of *Amy1A* in the aleurone is likely responsible for producing the majority of total α -amylase mRNA [11, 13].

The GA-deficient mutant (*Tan-ginbozu*), in which one of the steps in the GA-biosynthetic pathway is genetically blocked, allows the evaluation of the importance of GA-induced α -amylases on rice grain germination. The level of the major GA produced during rice grain germination (GA_1) is strongly reduced in the shoots of 10-day-old mutant seedlings [14]. Although no data is available about the GA content in *Tan-ginbozu* grains during the first days of germination, Mitsu-naga and Yamaguchi [15] have shown that α -amylase production is drastically reduced in *Tan-ginbozu* grains germinating in the absence of exogenous GA, while wild-type rice grains do not require exogenous GA for α -amylase production [16], thus indirectly indicating the absence of enough GA to trigger α -amylase induction in this mutant. The *Tan-ginbozu* and other GA-

*This article was submitted by the authors in English.

Abbreviations: GA—gibberellins; GA_3 —gibberellic acid.

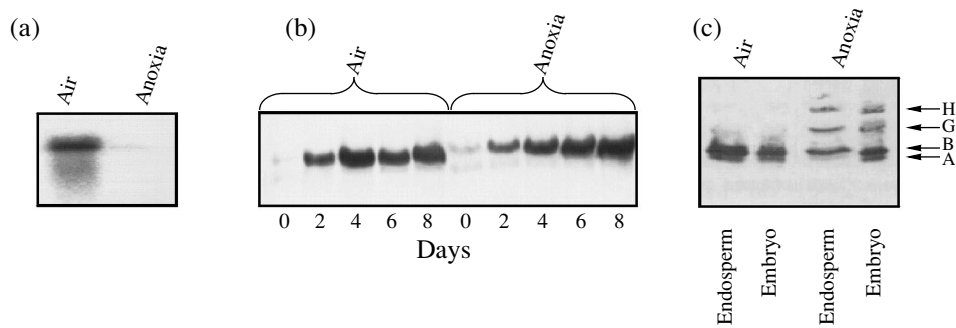


Fig. 1. Effects of anoxia on α -amylase mRNA induction by GA_3 , α -amylase protein accumulation, and isoforms pattern.

(a) Effects anoxia on the induction of α -amylase mRNA in embryoless half-grains. Half-grains were incubated in the presence of gibberellic acid for three (air) and six (anoxia) days, followed by Northern blot analysis with a rice α -amylase probe. See [1] for the details. (b) Effects of anoxia on α -amylase protein accumulation. Rice grains germinating under aerobic or anaerobic conditions were extracted for proteins, subjected to SDS-PAGE and immunoblot, probed with an antibody against α -amylase. See [19] for the details. (c) Pattern of aerobic and anaerobic α -amylase isoforms. α -Amylase isoforms are indicated as A, B, G, and H. Isoforms A and B are encoded by the rice *Amy1A* gene while isoforms H and G are encoded by *Amy3D*. Rice grains germinating under aerobic or anaerobic conditions for 8 days were extracted for proteins, subjected to IEF and immunoblot then probed with an antibody against α -amylase. See [1] for the details.

deficient rice mutants germinate promptly (our unpublished observation), suggesting that other α -amylase genes not GA-modulated are sufficient for triggering the production of enough α -amylase to efficiently degrade enough starch to allow germination.

THE ROLE OF α -AMYLASE IN RICE TOLERANCE TO ANOXIA

Rice is among the few plant species that can germinate under anoxia [1, 17]. The production of α -amylase in rice is likely playing a crucial role for the successful degradation of starch in the anoxic rice endosperm since other starch degrading enzymes are unlikely to be able to initiate the process of starch degradation. Induction of α -amylase takes place even under anoxia in rice grains, while wheat, barley, and oat (anoxia-intolerant cereals) fail to produce this enzyme [18, 19]. Furthermore, other enzymes needed for starch degradation are present in the anoxic rice grain, while they are either absent or inactive in the other cereals [19]. Efficient starch degradation is thus observed in rice under anoxia, while the anoxia-intolerant cereals fail to degrade starch, and therefore cannot utilize their starchy reserves. As a consequence, anoxia-intolerant cereals, such as wheat and barley, suffer from sugar starvation, and eventually die [20]. Exogenous addition of glucose to anoxic wheat grains strongly enhances tolerance to anoxia, as demonstrated by the rapid recovery of glucose-treated anoxic grains when transferred to aerobic conditions [18].

The mechanisms underlying the successful production of α -amylase under anoxia in rice seeds is largely unknown, but the ability of rice grains to respond to GA even under anoxia have been documented [21]. Indeed, anoxic rice embryoless half-grains respond to exogenous gibberellic acid (GA_3), while anoxia-intolerant

cereals fail to produce α -amylase even if exogenously GA-treated. Response to GA in rice under anoxia is however extremely slow when compared to the rapid induction of α -amylase mRNA accumulation triggered by GA under aerobic conditions (Fig. 1a). Intriguingly, the appearance of the α -amylase protein appears to be unaffected by anoxia. The immunoblot analysis shows that α -amylase accumulates in aerobic and anoxic grains without significant differences [19] (Fig. 1b). The discrepancy between the timing of appearance of GA-induced α -amylase mRNA and α -amylase protein accumulation is possibly explained on the basis of the evidence of the production of α -amylase isoforms not observed in aerobic rice seedlings [1] (Fig. 1c). Comparison of the isoelectric point of these anaerobically-induced α -amylase isoforms with that of known rice α -amylase isoforms [22, 23] indicates that isoforms such as isoforms G and H are observed only under anoxia, while isoforms A and B are observed in extracts from both aerobic and anaerobic seedlings. Isoforms A and B are encoded by the *Amy1A* gene, while isoforms G and H are encoded by the *Amy3D* gene, thus suggesting that differences in α -amylase gene expression may occur in aerobic and anaerobic rice seedlings. Indeed, a recent report by Hwang *et al.* [13] demonstrated that while *Amy1A* appears to be repressed by anoxia, *Amy3D* is anoxia-induced. *Amy1A* and *Amy3D* differ in their respective mechanisms of regulation, the former being hormonally modulated and the latter sugar modulated, suggesting that the pattern of expression of amylases should be addressed using gene-specific probes to differentiate between the role of each single α -amylase gene under either aerobic or anaerobic conditions.

RESPONSE TO GIBBERELLINS IN CEREAL GRAINS UNDER ANOXIA

Evidence has been published showing that barley grains kept under anaerobic conditions do not produce α -amylase, as a consequence of the inability of barley aleurone to respond to GA. On the contrary, rice half-grains treated with gibberellic acid produce α -amylase [21]. In barley and other anoxia-intolerant cereal grains no α -amylase genes are transcribed and translated into protein under anaerobic conditions, as demonstrated by the absence of α -amylase activity or α -amylase immunoreactive proteins in extracts from grains of anoxia-intolerant cereals [21]. Among the hypotheses proposed to explain the different response of rice when compared to barley in terms of ability to respond to GAs, we can recall the following [7]:

(1) the lack of oxygen in barley may negatively affect energy-dependent processes, of which α -amylase production depends on;

(2) the fermentative metabolism could lead to the production of an ethanol amount repressing the ability to produce α -amylase in barley but not in rice;

(3) anoxia may affect GA perception in barley but not in rice;

(4) anoxia treatment may result in an increased ABA content in barley but not in rice.

A lack of energy in barley, when compared to rice under anoxia, is not unlikely some days after imbibition, when rice grains show α -amylase activity and degrade starch, thus producing enough glucose to fuel the fermentative metabolism, while barley would suffer from sugar starvation. However, feeding barley grains with exogenous sugars does not result in α -amylase induction and ethanol production in barley half-grains indicates that ATP production through fermentation proceeds at a higher rate in barley than in rice [7], thus suggesting that the ATP level in barley is compatible with α -amylase production.

ATP production through fermentation results in the production of ethanol [24] and its presence negatively affects the induction of α -amylase by GA in barley aleurones when used at concentrations higher than 37 mM [20, 25]. Indeed, barley half-grains produce ten times more ethanol than rice half-grains, but treatment of barley and rice half-grains with ethanol (up to 20 mM, a concentration higher than that found in the incubation media of anaerobic barley) does not repress the induction of α -amylase, indicating that ethanol does not play a role in anoxia repression of α -amylase [7].

We have demonstrated that barley half-grains from a constitutive GA-response mutant (*slender*) do not produce α -amylase under anaerobic conditions, suggesting that repression occurs downstream of GA perception [7]. In the *slender* barley, α -amylase production is independent of GA₃ presence, but normally repressed by ABA [26]. A possible role of differential ABA accumulation in barley and rice under anoxia

could be proposed, but we have shown that no significant differences can be observed in the ABA content when comparing barley and rice under either aerobic or anaerobic conditions [7]. It is therefore at present unknown why barley fails to produce α -amylase under anoxia. Recent experimental evidences suggest that an explanation may come from the study of the different genes encoding α -amylase in cereals [13].

 α -AMYLASE GENES EXPRESSION UNDER AEROBIC AND ANOXIC CONDITIONS

Hwang *et al.* [13] have recently published an interesting description of the pattern of expression of several α -amylase genes in rice grains under either aerobic or anaerobic conditions. In the aleurone isolated from germinating grains kept under aerobic conditions, the highest expression level was observed when the RNA blots are probed with an *Amy1A* gene-specific probe, and to a much lower extent, with *Amy3B/C* and *Amy3E* probes. Under anoxia this pattern changed, with a drop in the expression of *Amy1A* to equal the expression of *Amy3E* (whose expression is unaffected by oxygen availability) and a minor expression of *Amy3B/C* and *Amy3D*. The expression pattern of α -amylase genes in the scutellar epithelium under aerobic conditions mirrored that of the aleurone: *Amy1A* showed the highest expression level, while *Amy3B/C*, *Amy3D*, and *Amy3E* were expressed, but at a low level. Anoxia changed the expression pattern by lowering the mRNA level of *Amy1A* and increasing that of the *Amy3* subfamily [13]. Overall, the effects of anoxia on the expression pattern of the α -amylase gene family can be summarized as follows:

(1) anoxia reduces the expression of *Amy1A*, which is the gene predominantly expressed under aerobic conditions;

(2) the expression of the *Amy3* subfamily is enhanced by anoxia.

As underlined by Hwang *et al.* [13], starch breakdown catalyzed by the isoforms encoded by the *Amy3* α -amylases may be one of the factors allowing rice grain germination and survival under anoxia. This conclusion challenges the importance of GA-induction of α -amylase under anoxia, since only the *Amy1A* gene is known to be modulated by this hormone.

GIBBERELLINS ARE NOT REQUIRED FOR RICE GERMINATION UNDER ANOXIC CONDITIONS

We proposed that GA can induce α -amylase in rice, while anoxia-intolerant cereals fail to produce α -amylase because they are unable to respond to this hormone [24]. Experimental evidences (see [24]) have been presented supporting this model, but additional considerations are needed. GA cannot be synthesized *de novo* in the absence of oxygen, since membrane-bound monooxygenases that convert ent-kaurene to GA₁₂ require NADPH and oxygen [27]. The hypothesis of

the possible existence of stored GA (or precursors) in the dry grain of rice is reasonable, since some GA₁ have been detected in dry rice grains [28]. However, this proposal would imply that release from the stored pool is anoxia-dependent, since aerobic GA-dependent α -amylase gene expression requires exogenous GA₃ when GA biosynthesis was blocked using inhibitors [15].

The GA-dependent *Amy1A* gene is expressed under anoxia [13], thus further confirming the ability of rice to respond to GAs under anoxia. However, anoxia exerts a negative effect on the expression of the *Amy1A* gene and suggests that response to GA in the aleurone could not represent a key factor for α -amylase production. Results obtained by Hwang *et al.* [13] indicate that expression of the *Amy1A* gene in the aleurone likely plays a major role in α -amylase production under aerobic conditions, while expression of the *Amy3* subfamily in the embryo is predominant under anoxia. Expression of the *Amy3* genes in the embryos takes place as early as 12 h after imbibition and peaks 2 days later, well before the induction of *Amy1A* in the aleurone, reaching a comparable level of expression only 4–6 days after imbibition [13].

The germination of rice grain under anoxia could be a GA-independent process, and this is supported by the following evidences [29]:

- (1) the GA-deficient rice mutant (*Tan-ginbozu*) germinates successfully under anoxia;
- (2) exogenous GA does not affect anaerobic germination of *Tan-ginbozu* grains;
- (3) the *Tan-ginbozu* rice mutant shows an extremely low level of *Amy1A* transcript;
- (4) expression of *Amy3D*, whose expression does not require GA, in the *Tan-ginbozu* rice mutant predominates over that of *Amy3B/C* (whose expression appears to require GA [4]) under anoxia.

The overall conclusion is that GA are not required for rice germination under anoxia. This conclusion does not challenge the role of α -amylases under anoxia because the vigorous expression of *Amy3D* (and to a minor extent *Amy3B/C*) compensates for the lower expression of the GA-modulated *Amy1A*-encoded enzyme [29].

SUGAR SENSING UNDER ANOXIA AND α -AMYLASE GENE REGULATION

Assuming that starch degradation is of importance for rice survival under anoxia, and that α -amylase is a prerequisite for this process to take place, it is likely that production of this starch-degrading enzyme during the first days after imbibition under anoxia is crucial for survival. Strong expression of *Amy3D* under anoxia is intriguing, also as far as the mechanisms of regulation of this gene are concerned. The *Amy3D* gene is not GA-induced, but is instead sugar-modulated [8]. This evi-

dence is of importance, since some genes modulated by anoxia are also sugar modulated [30].

We recently demonstrated that sugar repression of *Amy3D* under anoxia occurs at the level of transcription, as demonstrated previously for aerobic rice embryos [10, 31]. Transient expression experiments performed using a *Amy3D*-GUS constructs demonstrated that 100 mM glucose was able to repress 70% transcription of *Amy3D* [10], a repression only slightly less effective than under aerobic conditions (see [31]). This evidence demonstrates that sugars are able to elicit gene regulation also under anoxia.

The *Amy3D*-encoded isoenzyme is likely responsible for starch degradation in anaerobic rice embryos during the first 2–3 days of germination [10, 13]. We indeed detected α -amylase isoforms under anoxia, which are absent from the samples of aerobic rice seedlings (Fig. 1; [9]). As recently discussed [29], α -amylase have distinct functional roles in starch breakdown during grain germination [32]. The *Amy1A* enzyme has a much higher activity to starch granules, while *Amy3D* degrades preferentially oligosaccharides [32]. The relatively low expression of *Amy1A* in anaerobic rice grains may therefore lead to a slow rate of starch degradation, thus not allowing glucose to accumulate to levels repressing *Amy3D* expression. Expression of *Amy3D* under anoxia results in the production of an isoform that catalyzes relatively slower starch degradation, thus not allowing glucose to accumulate at levels able to repress expression of *Amy3D* itself. The rise of *Amy3D* expression in *Tan-ginbozu* [29] is however not observed with wild type M202 grains, since some starch degradation by the *Amy1A* enzyme can be expected in this cultivar, even under anoxia ([13]; our unpublished observation). Overall, the evidences obtained using the *Tan-ginbozu* mutant evidenciate that expression of GA-dependent amylase genes is not necessary for anoxic starch degradation. It should be, however, underlined that wild-type rice varieties take advantage of the expression of the *Amy1A* gene, thus suggesting that *Amy3* genes and *Amy1A* cooperate in the process of anoxic starch degradation, with *Amy3* genes allowing the grains to degrade starch during the initial stages of germination when induction of *Amy1A* is slowed down by GA-unavailability and/or insensitivity.

CONCLUDING REMARKS

Rice grains possess a complex multigene family encoding α -amylases. At least four out of ten α -amylase genes are expressed during rice grains germination, namely *Amy1A*, *Amy3B/C*, *Amy3D*, and *Amy3E*. Recent experiments have demonstrated that a GA-deficient mutant can germinate under anoxia, and that feeding exogenous GA does not enhance anaerobic germination [29]. In this rice mutant kept under anoxia *Amy1A* and *Amy3E* are not expressed during the first days of anoxic germination, while vigorous expression of *Amy3D* is observed [29]. *Amy3D* may therefore be

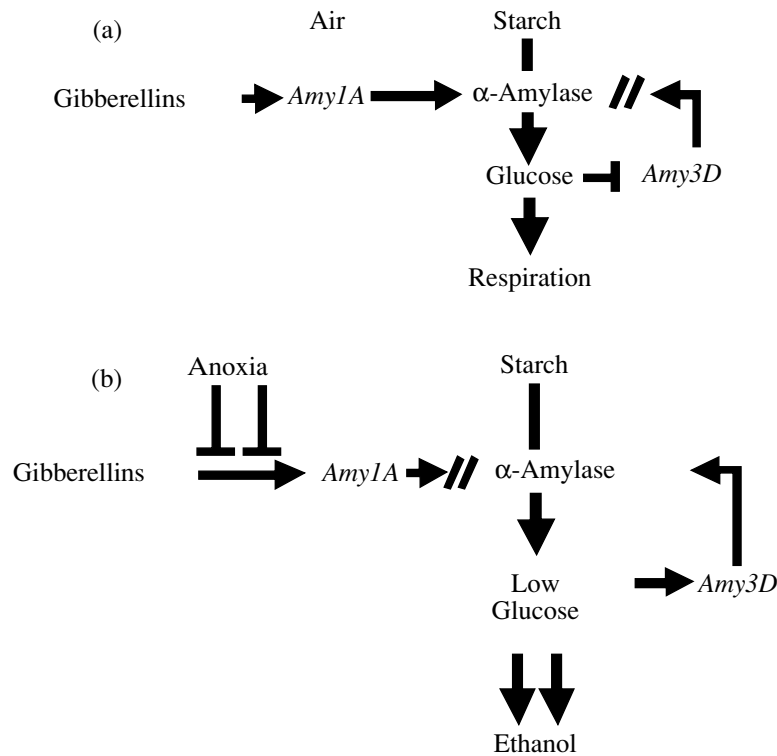


Fig. 2. Proposed mechanism of regulation of *Amy1A* and *Amy3D* genes under either aerobic or anaerobic condition in rice grains. (a) Under aerobic conditions gibberellins can be produced and elicit expression of the *Amy1A* gene. This results in rapid and efficient starch degradation through the action of the *Amy1A* isoform. The elevated level of glucose present in the germinating grain represses *Amy3D*. (b) Under anoxia, oxygen unavailability does not allow GAs to be produced and/or reduce their ability to induce *Amy1A*. This rapidly results in sugar starvation, since starch degradation cannot take place. The low glucose content induces the expression of the *Amy3D* gene, which allows starch degradation to take place, at a rate near to that of glucose utilization through fermentation.

sufficient to allow α-amylase to be produced in rice grains kept under anoxia. The recent evidences of the ability of rice embryos to sense sugars also under anoxia is intriguing, and suggests that sugar modulation of genes could be of importance for survival under anoxia (see Fig. 2). The lack of oxygen results in the switch from mitochondrial respiration to fermentative metabolism. An increased glycolytic flux is needed to allow the production of enough ATP to keep rice cells alive. Strong sugar utilization through fermentation may easily lead to sugar starvation of the cells, but induction of a starch-mobilizing enzyme, such as α-amylase, can avoid a decline in cell metabolism due to sugar shortage. *Amy3D* is repressed by sugars and induced by starvation, and it is very likely that induction of *Amy3D* by anoxia is mediated by the sensing of a low carbohydrate concentration in the anoxic cells. Expression of *Amy1A* under aerobic conditions may instead prevent *Amy3D* expression. Under anoxia, response to GA, which are likely present in minute amounts, is delayed, resulting in negative effects on the expression of *Amy1A*. Vigorous sugar utilization through fermentation, together with the absence of *Amy1A* leads to sugar starvation, which activates *Amy3D* expression during the earlier stages of germina-

tion. Continuous fuelling of glucose units coming from starch thanks to the expression of *Amy3D* may, therefore, allow ATP production through the fermentative metabolism.

REFERENCES

1. Perata, P., Guglielminetti, L., and Alpi, A., Mobilization of Endosperm Reserves in Cereal Seeds under Anoxia, *Ann. Bot.*, 1997, vol. 79A, pp. 49–56.
2. Dunn, G., A Model for Starch Breakdown in Higher Plants, *Phytochemistry*, 1974, vol. 13, pp. 1341–1346.
3. Sun, Z. and Henson, C.A., A Quantitative Assessment of the Importance of Barley Seed α-Amylase, Debranching Enzyme, and α-Glucosidase in Starch Degradation, *Arch. Biochem. Biophys.*, 1991, vol. 284, pp. 298–305.
4. Murata, T., Akazawa, T., and Fukuchi, S., Enzymic Mechanism of Starch Breakdown in Germinating Rice Seeds: 1. An Analytical Study, *Plant Physiol.*, 1968, vol. 43, pp. 1899–1905.
5. Rodriguez, R.L., Huang, N., Sutliff, T.D., Ranjhan, S., Karrer, E., and Litts, J., Organization, Structure and Expression of the Rice α-Amylase Multigene Family. In *Rice genetics II: Proceedings of the Second International Rice Genetics Symposium*, Los Banos (Philippines): Int. Rice Res. Inst., 1992, pp. 417–429.

6. Itoh, K., Yamaguchi, J., Huang, N., Rodriguez, R.L., Akazawa, T., and Shimamoto, K., Developmental and Hormonal Regulation of Rice α -Amylase (*Ramy1A*)-*gusA* Fusion Genes in Transgenic Rice Seeds, *Plant Physiol.*, 1995, vol. 107, pp. 25–31.
7. Loreti, E., Vernieri, P., Alpi, A., and Perata, P., Repression of α -Amylase Activity by Anoxia in Grains of Barley Is Independent of Ethanol Toxicity or Action of Abscisic Acid, *Plant Biol.*, 2002, vol. 4, pp. 266–272.
8. Yu, S.M., Lee, Y.C., Fang, S.C., Hwa, S.F., and Liu, L.F., Sugars Act as Signal Molecules and Osmotica to Regulate the Expression of α -Amylase Genes and Metabolic Activities in Germinating Cereal Grains, *Plant Mol. Biol.*, 1996, vol. 30, pp. 1277–1289.
9. Perata, P., Matsukura, C., Vernieri, P., and Yamaguchi, J., Sugar Repression of a Gibberellin-Dependent Signaling Pathway in Barley Embryos, *Plant Cell*, 1997, vol. 9, pp. 2197–2208.
10. Loreti, E., Yamaguchi, J., Alpi, A., and Perata, P., Sugar Modulation of α -Amylase Genes under Anoxia, *Ann. Bot.*, 2003, vol. 91, pp. 143–148.
11. Ranjan, S., Karrer E.E., and Rodriguez, R.L., Localizing α -Amylase Gene Expression in Germinated Rice Grains, *Plant Cell Physiol.*, 1992, vol. 33, pp. 73–79.
12. Sugimoto, N., Takeda, G., Nagato, Y., and Yamaguchi, J., Temporal and Spatial Expression of the α -Amylase Gene during Seed Germination in Rice and Barley, *Plant Cell Physiol.*, 1998, vol. 39, pp. 323–333.
13. Hwang, Y.S., Thomas, B.R., and Rodriguez, R.L., Differential Expression of Rice α -Amylase Genes during Seedling Development under Anoxia, *Plant Mol. Biol.*, 1999, vol. 40, pp. 911–920.
14. Kobayashi, M., Sakurai, A., Saka, H., and Takahashi, N., Quantitative Analysis of Endogenous Gibberellins in Normal and Dwarf Cultivars of Rice, *Plant Cell Physiol.*, 1989, vol. 30, pp. 963–969.
15. Mitsunaga, S. and Yamaguchi, J., Induction of α -Amylase Is Repressed by Uniconazole, an Inhibitor of the Biosynthesis of Gibberellin, in a Dwarf Mutant of Rice, *Wai-to-C*, *Plant Cell Physiol.*, 1993, vol. 34, pp. 243–249.
16. Mitsunaga, S., Tashiro, T., and Yamaguchi, J., Identification and Characterization of Gibberellin-Insensitive Mutants Selected from among Dwarf Mutants of Rice, *Theor. Appl. Genet.*, 1994, vol. 87, pp. 705–712.
17. Vartapetian, B.B. and Jackson, M.B., Plant Adaptation to Anaerobic Stress, *Ann. Bot.*, 1997, vol. 79A, pp. 3–20.
18. Perata, P., Pozueta-Romero, J., Akazawa, T., and Yamaguchi, J., Effect of Anoxia on Starch Breakdown in Rice and Wheat Seeds, *Planta*, 1992, vol. 188, pp. 611–618.
19. Guglielminetti, L., Yamaguchi, J., Perata, P., and Alpi, A., Amyolytic Activities in Cereal Seeds under Aerobic and Anaerobic Conditions, *Plant Physiol.*, 1995, vol. 109, pp. 1069–1076.
20. Perata, P., Guglielminetti, L., and Alpi, A., Anaerobic Carbohydrate Metabolism in Wheat and Barley, Two Anoxia-Intolerant Cereal Seeds, *J. Exp. Bot.*, 1996, vol. 47, pp. 999–1006.
21. Perata, P., Geshi, N., Yamaguchi, J., and Akazawa, T., Effect of Anoxia on the Induction of α -Amylase in Cereal Seeds, *Planta*, 1993, vol. 191, pp. 402–408.
22. Yamaguchi, J., Geshi, N., Mitsunaga, S., Itoh, S., Umemura, T., Masui, H., and Mitsui, T., Expression of RAmy3D-Protein (Isoform H) in Rice Seedlings, *Seventh International Symposium on Preharvest Sprouting in Cereals*, Noda, K. and Mares, D.J., Eds., Osaka: Center for Academic Societies Japan, 1995, pp. 405–410.
23. Mitsui, T. and Itoh, K., The α -Amylase Multigene Family, *Trends Plant Sci.*, 1997, vol. 2, pp. 255–261.
24. Perata, P., Loreti, E., Guglielminetti, L., and Alpi, A., Carbohydrate Metabolism and Anoxia Tolerance in Cereal Grains, *Acta Bot. Neerl.*, 1998, vol. 47, pp. 269–283.
25. Jones, R.L. and Varner, J.E., The Bioassay of Gibberellins, *Planta*, 1967, vol. 72, pp. 53–59.
26. Chandler, P.M., Hormonal Regulation of Gene Expression in the “Slender” Mutant of Barley, *Planta*, 1988, vol. 174, pp. 115–120.
27. Hedden, P. and Kamiya, Y., Gibberellin Biosynthesis: Enzymes, Genes and Their Regulation, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1997, vol. 48, pp. 431–460.
28. Choi, Y.H., Kobayashi, M., Fujioka, S., Matsuno, T., Hirose, T., and Sakurai, A., Fluctuation of Endogenous Gibberellin Levels in the Early Development of Rice, *Biochem. Biotechnol.*, 1995, vol. 59, pp. 285–288.
29. Loreti, E., Yamaguchi, J., Alpi, A., and Perata, P., Gibberellins Are Not Required for Rice Germination under Anoxia, *Plant Soil*, 2003 (in press).
30. Koch, K.E., Ying, Z., Wu, Y., and Avigne, W.T., Multiple Paths of Sugar-Sensing and a Sugar/Oxygen Overlap for Genes of Sucrose and Ethanol Metabolism, *J. Exp. Bot.*, 2000, vol. 51, pp. 417–427.
31. Morita, A., Umemura, T., Kuroyanagi, M., Futsuhara, Y., Perata, P., and Yamaguchi, J., Functional Dissection of a Sugar-Repressed α -Amylase Gene (*Ramy1A*) Promoter in Rice Embryos, *FEBS Lett.*, 1998, vol. 423, pp. 81–85.
32. Terashima, M., Katoh, S., Thomas, B.R., and Rodriguez, R.L., Characterization of Rice α -Amylase Isozymes Expressed by *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.*, 1995, vol. 43, pp. 1050–1055.