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| Corresponding Author | Family Name | **Chen** |
| | Particle | |
| | Given Name | **Kegui** |
| | Suffix | |
| | Division | Department of Agronomy |
| | Organization | University of Wisconsin |
| | Address | 53706, Madison, WI, USA |
| | Email | kchen@desu.edu |
| Author | Family Name | **Tian** |
| | Particle | |
| | Given Name | **Shulan** |
| | Suffix | |
| | Division | Department of Plant Pathology |
| | Organization | University of Wisconsin |
| | Address | 53706, Madison, WI, USA |
| | Email | |
| Author | Family Name | **Yandell** |
| | Particle | |
| | Given Name | **Brian S.** |
| | Suffix | |
| | Division | Department of Horticulture and Statistics |
| | Organization | University of Wisconsin |
| | Address | 53706, Madison, WI, USA |
| | Email | |
| Author | Family Name | **Kaeppler** |
| | Particle | |
| | Given Name | **Shawn M.** |
| | Suffix | |
| | Division | Department of Agronomy |
| | Organization | University of Wisconsin |
| | Address | 53706, Madison, WI, USA |
| | Email | |
| Author | Family Name | **An** |
| | Particle | |
| | Given Name | **Yong-Qiang Charles** |
| | Suffix | |

| | | |
|---|---|---|
| | Division | Department of Agriculture, Agricultural Research Service |
| | Organization | Cereal Crops Research |
| | Address | 53726, Madison, WI, USA |
| | Email | |

| | | |
|---|---|---|
| Schedule | Received | 4 October 2009 |
| | Revised | 28 December 2009 |
| | Accepted | 31 December 2009 |

| | |
|---|---|
| Abstract | Gibberellic acid (GA) is an important signaling molecule that participates in many aspects of plant growth and development. While the importance of this hormone is clear, the transcriptional regulatory networks involved are still being characterized. The cereal aleurone, particularly the barley aleurone, has been used as a classic model to study GA and GA signaling for many years, and these studies have significantly contributed to our understanding of GA in plant biology. The objective of this study was to characterize the transcripts regulated through the DELLA protein SLN1, a negative regulator of the GA signaling pathway. To detect the transcripts, Affymetrix Barley 1 GeneChips were hybridized with RNA extracted from barley aleurone treated with GA or aleurone of the DELLA mutant *sln1c* without GA treatment. The transcripts detected, in term of both expressed genes and their function, were highly similar between the GA-treatment and the s*ln1c* mutant. These results from a genome-wide transcript analysis provide evidence that SLN1 in the GA signal transduction pathway controls almost all GA-induced genes in the barley aleurone. |

| | |
|---|---|
| Keywords (separated by '-') | Aleurone - Gibberellic acid - DELLA - SLN1 - *Hordeum vulgare* - Transcripts |
| Footnote Information | Communicated by S. Abe.<br>**Electronic supplementary material** The online version of this article (doi:10.1007/s11738-009-0458-1) contains supplementary material, which is available to authorized users. |

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Acta Physiol Plant
DOI 10.1007/s11738-009-0458-1

ORIGINAL PAPER

# Loss-of-function of DELLA protein SLN1 activates GA signaling in barley aleurone

**Kegui Chen · Shulan Tian · Brian S. Yandell · Shawn M. Kaeppler · Yong-Qiang Charles An**



Received: 4 October 2009 / Revised: 28 December 2009 / Accepted: 31 December 2009


**Abstract** Gibberellic acid (GA) is an important signaling molecule that participates in many aspects of plant growth and development. While the importance of this hormone is clear, the transcriptional regulatory networks involved are still being characterized. The cereal aleurone, particularly the barley aleurone, has been used as a classic model to study GA and GA signaling for many years, and these studies have significantly contributed to our understanding of GA in plant biology. The objective of this study was to characterize the transcripts regulated through the DELLA protein SLN1, a negative regulator of the GA signaling pathway. To detect the transcripts, Affymetrix Barley 1 GeneChips were hybridized with RNA extracted from barley aleurone treated with GA or aleurone of the DELLA mutant *sln1c* without GA treatment. The transcripts detected, in term of both expressed genes and their function, were highly similar between the GA-treatment and the s*ln1c* mutant. These results from a genome-wide transcript analysis provide evidence that SLN1 in the GA signal transduction pathway controls almost all GA-induced genes in the barley aleurone.

**Keywords** Aleurone · Gibberellic acid · DELLA · SLN1 · *Hordeum vulgare* · Transcripts

**Abbreviations**

| | |
|---|---|
| ABA | Abscisic acid |
| GA | Gibberellic acid |
| MAP | Mitogen-activated protein |
| SAM | Significance analysis of microarray |

## Introduction

The phytohormone gibberellic acid (GA) is well known to promote seed germination in plants. One of its functions is to stimulate the production of hydrolytic enzymes in the aleurone and their secretion to the adjacent endosperm. The storage in the endosperm is thus degraded by these hydrolases into small molecules, which are utilized as nutrients for embryo growth to establish the young seedling (Fincher 1989). In cereal, GA is usually synthesized de novo in the embryo when the seed is placed in favorable conditions with water, oxygen, temperature and light (Kaneko et al. 2002, 2003; Radley 1967). GA-deficient mutants of *Arabidopsis* and tomato (*Solanum lycopersicum*) cannot initiate the process of seed germination even though the embryos of some plants can start germination

Communicated by S. Abe.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11738-009-0458-1) contains supplementary material, which is available to authorized users.

K. Chen (✉) · S. M. Kaeppler
Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA
e-mail: kchen@desu.edu

S. Tian
Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, USA

B. S. Yandell
Department of Horticulture and Statistics, University of Wisconsin, Madison, WI 53706, USA

Y.-Q. C. An
Department of Agriculture, Agricultural Research Service, Cereal Crops Research, Madison, WI 53726, USA





Journal : Large 11738 | Dispatch : 5-1-2010 | Pages : 12
Article No. : 458 | ☐ LE | ☐ TYPESET
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when providing nutrients for their growth (Koornneef and Veen 1980; Liu et al. 1994).

In barley, a mutant with tall and slim phenotype, known as *sln1*, was identified in genetic research many years ago (Chandler 1988; Foster 1977). The mutant actually has a mutation in a gene encoding a protein in the GA response pathway (Chandler et al. 2002; Chandler and Robertson 1999). Such a mutant was also isolated in rice (*Oryza sativa*) and named *slr1* (Ikeda et al. 2001). In the aleurone tissues of these slender mutants, hydrolytic enzymes such as α-amylase are produced and secreted without GA, in contrast with wild type. The protein is characterized by a DELLA domain in its N-terminal region and conserved in plants as a negative regulator of GA signaling (Dill et al. 2001; Peng 1997; Peng et al. 1999; Silverstone et al. 1998). In response to GA treatment, the DELLA protein disappears rapidly, further supporting the notion that it is a negative regulator of GA signal transduction (Fu et al. 2002; Gubler et al. 2002; Itoh et al. 2002; Silverstone et al. 2001). In most recent reports, the DELLA protein has proven to be a conserved repressor of GA signaling that acts immediately downstream of the GA receptor to modulate all aspects of GA-induced growth and development in plants (Griffiths et al. 2006; Nakajima et al. 2006; Ueguchi-Tanaka et al. 2005).

In *Arabidopsis* five DELLA proteins, GAI, RGA, RGL1, RGL2 and RGL3, have been identified with overlapping but distinct functions in the GA signaling pathway. GA-induced vegetative growth and floral initiation are repressed by RGA and GAI (Dill et al. 2001; King et al. 2001). RGL2 is the main regulator of seed germination, while RGA, GAI, RGL1 and RGL2 only play minor roles in this process (Cao et al. 2005; Lee et al. 2002; Tyler et al. 2004; Wen and Chang 2002). RGA, RGL1 and RGL2 redundantly function in flower and fruit development (Cheng et al. 2004; Tyler et al. 2004; Yu et al. 2004). Recently, 14 early GA-responsive genes were identified as early DELLA-responsive genes, and eight of them could be putative DELLA target genes (Zentella et al. 2007).

The discovery of the GA receptor, first reported in rice and subsequently confirmed in *Arabidopsis*, represents a significant advance in our understanding of the role of GA in plant growth and development (Griffiths et al. 2006; Iuchi et al. 2007; Nakajima et al. 2006; Willige et al. 2007). The receptor, GID1 in rice, interacts directly with SLR1 through the DELLA domain in a GA-dependent manner, which triggers the association of the activated SLR1 with the F-box protein GID2 of an SCF ubiquitin ligase complex, leading to destruction of the SLR1 protein (Itoh et al. 2005; Sasaki et al. 2003). In *Arabidopsis*, three orthologs of rice GID1 (GID1a, GID1b and GID1c) have the capacity to interact with the F-box protein SLY1, subsequently resulting in the degradation of DELLA proteins via the ubiquitin–proteasome pathway (Fu et al. 2004; Griffiths et al. 2006; McGinnis et al. 2003). Thus, the GA receptor, DELLA proteins and F-box protein function together at the start of GA signaling to detect and transfer the GA signal, and as a consequence, to relieve the DELLA-dependent repression and allow for GA-dependent growth and development in plants.

GAMYB is a transcription factor involved in GA signaling identified first in the barley aleurone. The expression of *GAMYB* is induced by GA, and as a consequence, the translated GAMYB protein then directly binds to the promoters of many hydrolase genes, such as α-amylase, inducing hydrolase gene expressions in the aleurone (Gubler et al. 1995, 1999; Huttly and Phillips 1995). Loss-of-function mutations of *GAMYB* impair alpha-amylase expression in the aleurone and flower development, suggesting that GAMYB is a critical downstream transcription factor in the GA signaling pathway (Kaneko et al. 2004). In *sln1* or *slr1* mutants, *GAMYB* is also highly expressed in the aleurone and floral organs, such as the anther (Aya et al. 2009; Gubler et al. 2002), indicating that the DELLA proteins repress *GAMYB* expression in the GA signaling pathway (Murray et al. 2003). However, *GAMYB* is unlikely to be a direct target of the DELLA proteins because of a 1-h lag time between GA-dependent DELLA protein degradation and *GAMYB* mRNA induction (Gubler et al. 2002).

In rice, the DELLA protein SLR1 was reported to control all GA response genes in the aleurone (Tsuji et al. 2006). In *Arabidopsis*, about one-half GA-regulated genes are apparently regulated in a DELLA-dependent fashion (Cao et al. 2006). So far, several direct target genes of DELLA proteins have also been reported (Hou et al. 2008; Zentella et al. 2007). As DELLA proteins play a central role in modulating GA responses in plants, we performed this study to elucidate the transcriptome regulated by the DELLA protein SLN1 in barley aleurone.

## Results and discussion

### The transcript profiles induced by GA and of the *sln1c* mutant are highly similar

The *sln1c* mutation is a loss-of-function allele due to a G–A nucleotide substitution, which truncates the protein at amino acid 602 in barley (Chandler et al. 2002). The mutant typically grows faster than wild type, developing the slender phenotype. α-amylase production by the mutant half-grain without the embryo can be induced without GA supplementation (Chandler et al. 2002). In de-embryonic *sln1c* aleurone tissues, α-amylase activities were detected at a level equivalent to the level in the GA-treated wild







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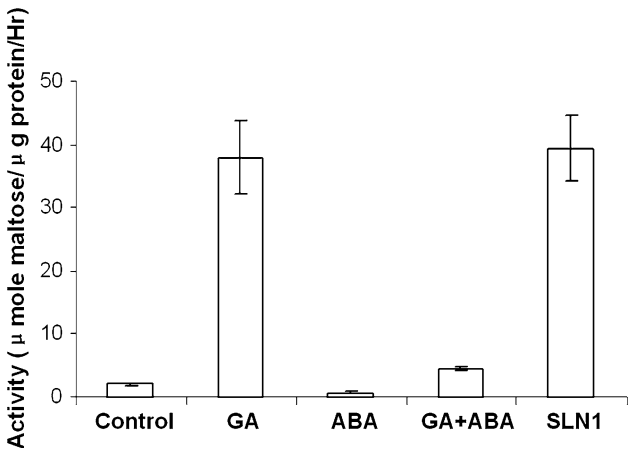


**Fig. 1** α-amylase activities in the treated aleurone tissues used for microarray experiments. The aleurone tissues from de-embryonic half-grains of barley cv. Himalaya and *sln1c* mutant were incubated at 25°C for 15 h without any hormone (Control), with 1 μM $GA_3$ (GA), with ABA 50 μM (ABA), with 1 μM $GA_3$ and ABA 50 μM (AG), and *sln1c* without any hormone (SLN1)

type (Fig. 1). Thus, the experimental system was well established for further analysis and comparison of gene expression between GA treatment and *sln1c* mutant. The genome-wide transcripts were then quantified by using 22K Barley1 GeneChip (Close et al. 2004), which was developed by Affymetrix based on 350,000 high-quality ESTs from 84 cDNA libraries, in addition to 1,145 barley (*H. vulgare*) gene sequences from the National Center for Biotechnology Information.

In our microarray experiments, three independent biological replicates were conducted. Statistic analysis of slope and $R^2$ as goodness-of-fit across three replicates (Schmid et al. 2005) showed high levels of reproducibility and reliability for all of the treatments (Fig. 2). The expression of α-amylase genes identified in this experiment was further confirmed by northern blotting (Chen and An 2006).

By a SAM statistic calculation (Tusher et al. 2001) with the threshold of a threefold change, 1,328 genes (GA-regulated genes) were significantly regulated by GA (Chen and An 2006), and 1,448 genes (SLN1-dependent genes; Supplemental Table 1) were significantly changed in the *sln1c* mutant without GA supplementation. While 683 genes were up-regulated and 645 genes were down-regulated among the GA-regulated genes, 906 and 542 genes were identified as up- and down-regulated in the SLN1-dependent genes, respectively.

Interestingly, the fold changes of the up-regulated genes (Table 1) were larger than those of the down-regulated genes in both the GA treatment and in the *sln1c* mutant, suggesting that both GA and the loss of function of SLN1 highly induce overall gene expression in the barley aleurone. Moreover, genes in the *sln1c* mutant experiments also displayed larger fold changes in either up- or down-regulated genes. Thus, the SLN1 mutation was more efficient than the GA treatment in term of both the number and magnitude of genes that were induced or repressed.

Of the significantly regulated genes, 704 genes were shared by both treatments, 624 genes were only in the GA-regulated genes and 744 genes only in the SLN1-dependent genes. However, a further analysis of all of the 2,072 (704 + 624 + 744) genes together revealed that transcript levels in the *sln1c* mutant without GA treatment actually were very similar with those in the wild type with GA treatment. The Pearson Correlation Coefficient of the gene expression levels was 0.89 between the GA treatment and the *sln1c*, much higher than the correlation of the same genes between the GA treatment and the control (0.43) and between the GA treatment and the abscisic acid (ABA) treatment (0.35). This observation suggests that the GA-induced genes and SLN1-dependent genes have highly similar expression profiles even though only some of them are shared in the lists of significantly regulated gene by the highly stringent statistical threshold used in our analysis.

## The GA-regulated genes of hydrolytic enzymes are SLN1-dependent

In this study, α-amylase was used as markers as it had been well established in GA and GA signaling research in cereal

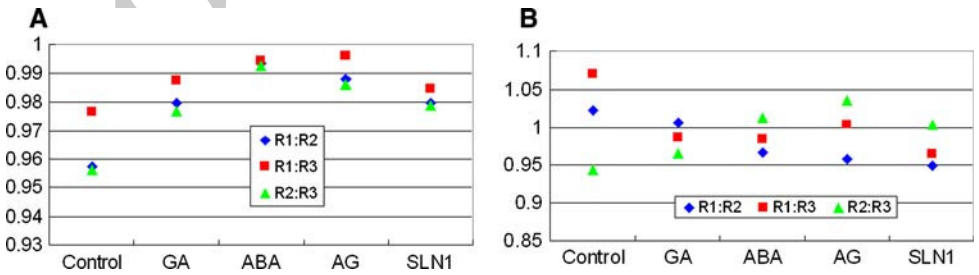


**Fig. 2** "Goodness-of-fit" statistics of microarray data. Normalized intensity is used to calculate $R^2$ (**a**) and slope (**b**) of three replicates in the treatment. *Control* Himalaya aleurone without any hormone, *GA* 1 μM/L $GA_3$, *ABA* 50 μM ABA, *AG* 1 μM $GA_3$ plus 50 μM ABA, *SLN1* *sln1c* aleurone without any hormone, *R1* Replicate 1, *R2* Replicate 2, *R3* Replicate 3





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**Table 1** Statistics of gene expression in the GA treatment and in the *sln1c* mutant

| | Gene number (%) | | AVG of fold change | | STDEV of fold change | |
|---|---|---|---|---|---|---|
| | Up | Down | Up | Down | Up | Down |
| Significant in both GA and *sln1c* mutant | | | | | | |
| GA | 432 (61.5) | 270 (38.4) | 20.8 | −9.8 | 43.8 | 14.9 |
| SLN1 | | | 42.5 | −28.8 | 96.4 | 50.9 |
| Detected in Barley 1 GeneChip | | | | | | |
| GA | 5,761 (51.3) | 5,464 (48.7) | 3.3 | −2.0 | 13.4 | 3.5 |
| SLN1 | 6,675 (55.1) | 5,432 (44.9) | 5.7 | −3.2 | 28.8 | 13.9 |

There is one dehydrin gene (DHN7), Contig1709_at, is up-regulated by GA, but down-regulated in *sln1c*

*Up* up-regulated genes, *Down* down-regulated genes, *AVG* average, *STDEV* standard deviation



aleurone (Gubler et al. 1995; Zentella et al. 2002). In the GA treatment, 83 hydrolase genes were identified as GA-regulated (Supplemental Table 2), while 80 hydrolase genes were SLN1-dependent (Supplemental Table 3). A total of 48 genes were shared among the two (Table 2), in which 22, 18, 6 and 2 genes were predicted, respectively, to function in the degradation of polysaccharides, proteins, nucleic acids and lipids. Among these, 44 genes, including six α-amylase genes, were up-regulated in both the GA treatment and the *sln1c* mutant. Only four genes that were down-regulated by GA were also down-regulated in *sln1c*, suggesting that they may be suppressed by GA through SLN1 degradation. Interestingly, genes that showed significant regulation, either up or down, in either the GA treatment or *sln1c* mutant, were consistently up-regulated or down-regulated in both treatments, even though some were GA-regulated only or some were SLN1-dependent only. These results indicate that the signal transduction pathway of GA-induced hydrolases is SLN1-dependent.

## The transcription factor genes regulated by GA are also dependent on SLN1

Transcriptional regulation is a major aspect in the regulation of gene expression in the GA signaling pathway. The activation of *GAMYB* or *GAMYB*-like genes has been well documented in the GA-induced α-amylase pathway in the aleurone tissue (Gubler et al. 1995), and floral initiation and development (Aya et al. 2009; Gocal et al. 2001; Millar and Gubler 2005; Tsuji et al. 2006). *WRKY* (Zhang et al. 2004; Zou et al. 2008), *Dorf* (Mena et al. 2002; Washio 2003) and *GMPOZ* (Woodger et al. 2004) were also found in GA signaling pathway. Among the significantly regulated genes in this study, 70 (Supplemental Table 4) and 90 genes (supplemental Table 5) were, respectively, identified as GA-regulated and SLN1-dependent transcription factor genes. Among them, 39 genes (Table 3) appear in both lists. Several genes in the MYB family, including *HvGAMYB* (X87690_s_at and HS18K19u_s_at), were significantly induced in the GA treatment and/or in the *sln1c* mutant. X87690_s_at was up-regulated more than fourfold in both the GA treatment and *sln1c* mutant, even though it was not in the SLN1-dependent list. Furthermore, all of the genes up- and down-regulated by GA were also consistently up- and down-regulated in the *sln1c* mutant, and vice versa. These results support that GA regulates transcription factor gene expression through SLN1 in the barley aleurone, which further suggests a fundamental role of SLN1 in GA-regulated gene expression.

## The genes for phosphorylation and dephosphorylation regulated by GA are consistent with those in the *sln1c* mutant

In eukaryotes, protein phosphorylation and dephosphorylation is one of the most important post-translational regulatory events by which the activities of proteins are switched on or off. DELLA protein is phosphorylated, though the role of the phosphorylation has not yet been determined (Itoh et al. 2005; Ueguchi-Tanaka et al. 2007). In addition, the phosphorylation of sugars is often the first stage of their catabolism. In this study, 43 and 39 kinase genes were regulated by GA (Supplemental Table 6) and in the *sln1c* mutant (Supplemental Table 7), respectively. These include various protein kinases, such as MAP kinases, receptor kinases and sugar kinases. Sixteen genes were regulated by GA, and also in the *sln1c* mutant (Table 4), and these genes could be involved in signal transduction or sugar metabolism. Two diacylglycerol kinases (Contig5427_at and Contig5428_s_at) were up-regulated, supporting their role in the phosphorylation of lipids (Wattenberg et al. 2006), which is recognized to be a major mode in the production of second messengers in GA signal transduction. Additionally, all of the identified genes, either up- or down-regulated in either treatment, were consistently up- or down-regulated in both, suggesting that GA-regulated kinase gene expression is SLN1 dependent.





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**Table 2** Hydrolase genes regulated by both GA and SLN1

| Probe Set ID | Intensity | | | Fold change | | Putative annotation |
|---|---|---|---|---|---|---|
| | Control | GA | SLN1 | GA | SLN1 | |
| Contig14542_at | 38 | 135 | 413 | 3.6 | 10.9 | Alpha-amylase |
| Contig22899_at | 1,098 | 31,572 | 60,788 | 28.8 | 55.4 | Alpha-amylase |
| Contig3952_at | 420 | 27,776 | 31,333 | 66.1 | 74.5 | Alpha-amylase |
| Contig3953_s_at | 1,329 | 29,726 | 29,100 | 22.4 | 21.9 | Alpha-amylase |
| Contig7087_at | 698 | 19,341 | 24,059 | 27.7 | 34.5 | Alpha-amylase |
| Contig7088_at | 1,988 | 43,686 | 49,969 | 22.0 | 25.1 | Alpha-amylase |
| Contig11648_at | 329 | 9,924 | 10,552 | 30.1 | 32.1 | Pullulanase, starch debranching enzyme |
| Contig7937_s_at | 3,240 | 53,918 | 74,827 | 16.6 | 23.1 | Alpha-glucosidase 1 (AGLU1) |
| Contig7938_at | 1,101 | 9,103 | 10,199 | 8.3 | 9.3 | Alpha-glucosidase 1 (AGLU1) |
| Contig11243_at | 11 | 131 | 108 | 12.4 | 10.2 | Glycoside hydrolase family 28 protein |
| Contig2736_s_at | 2,294 | 655 | 310 | −3.5 | −7.4 | Glycosyl hydrolase family 1 protein |
| Contig16010_at | 249 | 14,419 | 17,691 | 57.9 | 71.1 | Glycosyl hydrolase family 10 protein |
| Contig13792_s_at | 44 | 5,021 | 6,858 | 115.4 | 157.6 | Glycosyl hydrolase family 10 protein |
| Contig2834_at | 1,854 | 22,016 | 24,019 | 11.9 | 13.0 | Glycosyl hydrolase family 17 protein |
| HU14A02u_at | 90 | 503 | 462 | 5.6 | 5.2 | Glycosyl hydrolase family 17 protein |
| Contig13674_at | 9 | 1,960 | 1,862 | 227.3 | 216.0 | Glycosyl hydrolase family 3 protein |
| Contig5703_at | 787 | 10,745 | 13,404 | 13.7 | 17.0 | Glycosyl hydrolase family 3 protein |
| Contig5995_at | 547 | 100 | 64 | −5.5 | −8.6 | Acidic endochitinase (CHIB1) |
| Contig7811_s_at | 497 | 2,920 | 5,054 | 5.9 | 10.2 | Cell wall invertase |
| Contig11583_at | 24 | 2,922 | 6,724 | 120.0 | 276.1 | Beta-galactosidase, lactase |
| Contig13013_at | 42 | 462 | 963 | 11.1 | 23.2 | Polygalacturonase, pectinase |
| Contig2672_at | 92 | 781 | 611 | 8.5 | 6.6 | Xyloglucan endotransglycosylase |
| Contig2555_at | 129 | 695 | 2,219 | 5.4 | 17.2 | Cysteine proteinase |
| Contig2556_s_at | 2,857 | 14,750 | 25,180 | 5.2 | 8.8 | Cysteine proteinase |
| Contig17638_at | 4,047 | 33,565 | 44,538 | 8.3 | 11.0 | Cysteine proteinase |
| Contig2403_at | 543 | 3,846 | 5,093 | 7.1 | 9.4 | Cysteine proteinase |
| Contig5278_at | 77 | 13,792 | 15,591 | 178.2 | 201.4 | Cysteine proteinase |
| Contig5281_at | 5,113 | 47,626 | 40,417 | 9.3 | 7.9 | Cysteine proteinase |
| U19359_s_at | 718 | 29,788 | 25,044 | 41.5 | 34.9 | Cysteine proteinase |
| Contig86_at | 1,814 | 9,171 | 13,734 | 5.1 | 7.6 | Cysteine proteinase |
| Contig3900_at | 234 | 2,327 | 3,696 | 10.0 | 15.8 | Cysteine proteinase |
| Contig600_at | 9,211 | 45,940 | 33,198 | 5.0 | 3.6 | Serine carboxypeptidase III, putative |
| Contig6685_at | 4,483 | 24,635 | 24,971 | 5.5 | 5.6 | Serine carboxypeptidase S10 family protein |
| Contig6686_s_at | 4550 | 28,554 | 30,740 | 6.3 | 6.8 | Serine carboxypeptidase S10 family protein |
| Contig9219_at | 446 | 1,380 | 2,616 | 3.1 | 5.9 | Serine carboxypeptidase |
| Contig2681_at | 25 | 198 | 189 | 7.8 | 7.5 | Cathepsin B-like cysteine protease |
| Contig2683_s_at | 1,599 | 13,002 | 15,104 | 8.1 | 9.4 | Cathepsin B-like cysteine protease |
| Contig11268_at | 464 | 1,726 | 1,770 | 3.7 | 3.8 | OTU-like cysteine protease |
| Contig9418_at | 814 | 238 | 104 | −3.4 | −7.8 | Aspartyl protease family protein |
| Contig20999_at | 214 | 48 | 34 | −4.5 | −6.3 | Acyl-peptide hydrolase |
| Contig4111_at | 792 | 7,795 | 13,338 | 9.8 | 16.8 | Bifunctional nuclease, putative |
| Contig4112_at | 80 | 542 | 1,385 | 6.8 | 17.3 | Bifunctional nuclease, putative |
| Contig4113_at | 340 | 14,377 | 21,671 | 42.2 | 63.7 | Bifunctional nuclease, putative |
| Contig3691_at | 54 | 9,819 | 18,754 | 182.0 | 347.6 | Ribonuclease 1 (RNS1) |
| Contig7478_at | 218 | 1,113 | 1,890 | 5.1 | 8.7 | Ribonuclease 2 (RNS2) |
| Contig14247_at | 147 | 58 | 45 | −2.5 | −3.2 | Exodeoxyribonuclease |
| Contig19422_at | 8 | 180 | 522 | 21.5 | 62.6 | Lipase class 3 family protein |
| Contig8049_at | 250 | 1,896 | 3,185 | 7.6 | 12.7 | Glycerophosphoryl diester phosphodiesterase |







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**Table 3** Transcription factor genes regulated by both GA and SLN1

| Probe Set ID | Intensity | | | Fold change | | Putative annotation |
|---|---|---|---|---|---|---|
| | Control | GA | SLN1 | GA | SLN1 | |
| Contig20506_at | 5 | 71 | 162 | 14.3 | 32.8 | bHLH family protein |
| Contig15975_at | 28 | 706 | 1,777 | 25.6 | 64.5 | bHLH protein |
| Contig8163_at | 4,092 | 979 | 991 | −4.2 | −4.1 | bZIP transcription factor |
| Contig14342_at | 726 | 17,533 | 23,393 | 24.1 | 32.2 | Chloroplast DNA−binding protein |
| Contig8986_at | 185 | 22 | 20 | −8.6 | −9.2 | DNA-binding family protein |
| Contig20055_at | 296 | 85 | 76 | −3.5 | −3.9 | DNA-binding protein |
| Contig15377_at | 273 | 2,688 | 2,440 | 9.8 | 8.9 | Dof-type zinc finger protein |
| Contig9071_at | 12 | 426 | 726 | 36.0 | 61.3 | Dof-type zinc finger protein |
| Contig4395_at | 657 | 176 | 193 | −3.7 | −3.4 | Ethylene-insensitive3-like1 (EIL1) |
| HVSMEa0017I09r2_s_at | 2,714 | 625 | 598 | −4.3 | −4.5 | Ethylene-insensitive3-like1 (EIL1) |
| Contig15595_at | 79 | 5 | 4 | −14.9 | −20.1 | Heat shock transcription factor |
| Contig10555_at | 28 | 112 | 545 | 4.0 | 19.4 | Myb family transcription factor |
| Contig14220_at | 19 | 336 | 76 | 17.5 | 3.9 | myb family transcription factor |
| Contig15670_at | 39 | 181 | 199 | 4.7 | 5.1 | myb family transcription factor |
| HS18K19u_s_at | 1,071 | 4,386 | 5,123 | 4.1 | 4.8 | GAMYB |
| X70876_at | 26 | 154 | 403 | 6.0 | 15.7 | myb family transcription factor |
| Contig13658_at | 111 | 1,356 | 1,482 | 12.2 | 13.3 | No apical meristem family protein |
| Contig6233_s_at | 3,295 | 1,171 | 726 | −2.8 | −4.5 | No apical meristem family protein |
| Contig6235_s_at | 1,543 | 481 | 305 | −3.2 | −5.1 | No apical meristem family protein |
| Contig9031_at | 128 | 1,058 | 1,173 | 8.3 | 9.2 | No apical meristem family protein |
| Contig9418_at | 814 | 238 | 104 | −3.4 | −7.8 | DNA-binding protein |
| Contig15230_at | 97 | 660 | 1,062 | 6.8 | 10.9 | Telomere-binding protein |
| Contig6484_at | 122 | 1,586 | 2,444 | 13.0 | 20.0 | NAC transcription activator |
| Contig8519_at | 28 | 582 | 961 | 21.1 | 34.9 | Trihelix DNA-binding protein |
| Contig8572_s_at | 74 | 1,137 | 1,484 | 15.4 | 20.1 | Two-component regulator |
| Contig3395_at | 198 | 936 | 1,400 | 4.7 | 7.1 | WD-40 repeat family protein |
| Contig4386_at | 146 | 18 | 10 | −8.2 | −14.9 | WRKY transcription factor |
| Contig23823_at | 15 | 697 | 2,666 | 46.9 | 179.6 | Zinc finger family protein |
| Contig11443_at | 310 | 4,237 | 4,240 | 13.7 | 13.7 | Zinc finger family protein |
| Contig14351_at | 150 | 24 | 32 | −6.4 | −4.6 | Zinc finger family protein |
| Contig24933_at | 54 | 1,675 | 5,537 | 31.0 | 102.4 | Zinc finger family protein |
| Contig2830_at | 95 | 329 | 654 | 3.5 | 6.9 | Zinc finger family protein |
| Contig8204_at | 249 | 1,041 | 2,055 | 4.2 | 8.3 | Zinc finger family protein |
| HVSMEg0010A16r2_s_at | 359 | 4,061 | 6,035 | 11.3 | 16.8 | Zinc finger family protein |
| Contig20287_at | 1,143 | 11 | 10 | −105.8 | −117.3 | Zinc finger family protein |
| Contig5214_at | 425 | 2,529 | 5,737 | 5.9 | 13.5 | Zinc finger family protein |
| Contig7881_at | 140 | 689 | 937 | 4.9 | 6.7 | Zinc finger family protein |
| Contig17684_at | 283 | 1,021 | 1,130 | 3.6 | 4.0 | Zinc finger family protein |
| Contig12869_at | 306 | 96 | 40 | −3.2 | −7.6 | Zinc finger homeobox |

On the other hand, 18 phosphatase genes were significantly regulated by GA and/or in the *sln1c* mutant (Table 5). Some of these were protein phosphatase genes and the others are sugar phosphatase genes. All of the genes up- (5 genes) or down-regulated (13 genes) in the GA treatment showed up- or down-regulation in the *sln1c* mutant, suggesting that the phosphatase genes were consistently expressed in both the GA-treatment and the *sln1c* mutant. Therefore, there was no large difference in the transcript profiles of kinase and phosphatase genes between the GA treatment and the *sln1c* mutant, and phosphorylation and dephosphorylation are active parts of the GA response in the barley aleurone.





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**Table 4** Kinase genes regulated by both GA and SLN1

| Probe set ID | Intensity | | | Fold change | | Putative annotation |
|---|---|---|---|---|---|---|
| | Control | GA | SLN1 | GA | SLN1 | |
| Contig17642_at | 294 | 2421 | 2638 | 8.2 | 9.0 | Adenylylsulfate kinase |
| Contig8678_s_at | 159 | 14 | 12 | −11.1 | −13.6 | Bifunctional aspartate kinase |
| Contig15997_at | 60 | 1,533 | 1,752 | 25.5 | 29.1 | Calcium-dependent protein kinase |
| Contig15820_at | 54 | 1,891 | 5,905 | 34.7 | 108.4 | CBL-interacting protein kinase |
| Contig5427_at | 317 | 2,612 | 4,413 | 8.2 | 13.9 | Diacylglycerol kinase |
| Contig5428_s_at | 202 | 1,879 | 4,113 | 9.3 | 20.4 | Diacylglycerol kinase |
| Contig8087_at | 2287 | 15,527 | 30,154 | 6.8 | 13.2 | Galactokinase |
| Contig12296_at | 954 | 112 | 93 | −8.5 | −10.3 | Hexokinase |
| Contig19027_at | 17 | 163 | 270 | 9.6 | 16.0 | Leucine-rich repeat protein kinase |
| Contig9077_at | 218 | 1,212 | 2,629 | 5.6 | 12.1 | Leucine-rich repeat protein kinase |
| Contig4711_s_at | 113 | 508 | 1,332 | 4.5 | 11.8 | Mitogen-activated protein kinase |
| Contig14879_at | 516 | 49 | 30 | −10.4 | −17.3 | Protein kinase |
| Contig16082_at | 28 | 125 | 261 | 4.5 | 9.4 | Protein kinase |
| Contig7326_at | 54 | 264 | 507 | 4.9 | 9.4 | Protein kinase |
| Contig16137_at | 775 | 130 | 35 | −6.0 | −21.9 | Pyruvate kinase |
| Contig8995_at | 184 | 1,144 | 2,147 | 6.2 | 11.7 | Serine/threonine protein kinase |

**Table 5** Phosphatase genes regulated by GA and/or SLN1

| Probe set ID | Intensity | | | Fold change | | Putative description |
|---|---|---|---|---|---|---|
| | Control | GA | SLN1 | GA | SLN1 | |
| HS01M21w_s_at | 668 | 2,044 | 1,745 | 3.1 | 2.6 | Protein phosphatase 2C |
| Contig10323_at | 891 | 95 | 39 | −9.4 | −22.8 | Protein phosphatase 2C |
| Contig11720_at | 45 | 209 | 140 | 4.7 | 3.1 | Protein phosphatase 2C |
| Contig20457_at | 126 | 417 | 1,252 | 3.3 | 9.9 | Inositol monophosphatase |
| Contig7453_at | 646 | 175 | 251 | −3.7 | −2.6 | Inositol monophosphatase |
| Contig7382_at | 91 | 328 | 265 | 3.6 | 2.9 | Fructose-1, 6-bisphosphatase |
| Contig7382_s_at | 324 | 1,217 | 921 | 3.8 | 2.8 | Fructose-1, 6-bisphosphatase |
| **Contig2964_at** | **177** | **2,750** | **4,538** | **15.5** | **25.6** | **Fructose-1, 6-bisphosphatase** |
| **Contig7617_at** | **58** | **1,166** | **2,500** | **20.2** | **43.4** | **Tyrosine specific protein phosphatase** |
| **Contig7672_at** | **240** | **26** | **9** | **−9.4** | **−27.6** | **Protein phosphatase 2C** |
| **HA11O05u_at** | **30** | **204** | **393** | **6.7** | **12.9** | **Inositol monophosphatase** |
| **HA11O05u_s_at** | **67** | **283** | **629** | **4.2** | **9.4** | **Inositol monophosphatase** |
| Contig7098_at | 1,016 | 60 | 33 | −17.1 | −30.8 | Purple acid phosphatase |
| Contig14920_at | 38 | 95 | 700 | 2.5 | 18.3 | Purple acid phosphatase |
| Contig12732_at | 94 | 139 | 283 | 1.5 | 3.0 | Protein tyrosine phosphatase |
| Contig18582_at | 303 | 128 | 39 | −2.4 | −7.8 | Protein phosphatase 2C |
| Contig4453_at | 102 | 14,708 | 30,639 | 144.8 | 301.7 | Acid phosphatase type 5 |
| Contig2434_at | 44 | 59 | 400 | 1.3 | 9.1 | Acid phosphatase |

Font in bold indicates the genes regulated by both GA and SLN1

### F-box protein genes up-regulated by GA are also up-regulated in the sln1c mutant

Regulated proteolysis plays an essential role in the development of all organisms. One of the most widely studied, and arguably the most important, proteolysis system in plants is the ubiquitin/26S proteasome system. In *Arabidopsis,* an estimated 694 SCF F-box proteins are involved in these pathways (Vierstra 2003). An F-box protein, GID2 in rice and SLY1 in *Arabidopsis,* is involved in the GA





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**Table 6** F-box protein genes regulated by GA and/or by SLN

| Probe set ID | Intensity | | | Fold change | | Putative annotation |
|---|---|---|---|---|---|---|
| | Control | GA | SLN1 | GA | SLN1 | |
| Contig12152_at | 786 | 2,176 | 2,343 | 2.8 | 3.0 | F-box family protein (FBX3) |
| Contig18568_at | 39 | 185 | 62 | 4.7 | 1.6 | F-box family protein (FBL3) |
| Contig20398_at | 36 | 146 | 128 | 4.1 | 3.6 | F-box family protein (ORE9) |
| **Contig10649_at** | **90** | **763** | **1,643** | **8.5** | **18.3** | **Kelch F-box family protein** |
| **Contig12407_at** | **345** | **5,146** | **2,520** | **14.9** | **7.3** | **Kelch F-box family protein** |
| **Contig13530_at** | **24** | **665** | **2,635** | **27.6** | **109.4** | **F-box family protein** |
| **Contig21207_at** | **6** | **94** | **154** | **15.8** | **25.8** | **Kelch F-box family protein** |
| **Contig2179_at** | **40** | **320** | **1,174** | **8.0** | **29.3** | **F-box family protein** |
| **Contig6385_at** | **999** | **3,724** | **4,769** | **3.7** | **4.8** | **Kelch F-box family protein** |
| Contig10992_at | 240 | 469 | 973 | 2.0 | 4.1 | Kelch F-box family protein |
| Contig11386_s_at | 334 | 548 | 1,591 | 1.6 | 4.8 | F-box family protein |
| Contig16042_at | 76 | 150 | 461 | 2.0 | 6.1 | F-box family protein |
| Contig19651_at | 147 | 299 | 772 | 2.0 | 5.3 | F-box family protein |
| Contig6301_at | 128 | 264 | 717 | 2.1 | 5.6 | F-box family protein |
| Contig6534_at | 1,287 | 3,506 | 7,592 | 2.7 | 5.9 | Kelch F-box family protein |
| Contig6590_at | 501 | 959 | 2,348 | 1.9 | 4.7 | F-box family protein |
| HV_CEb0009I14r2_s_at | 493 | 1,661 | 2,271 | 3.4 | 4.6 | Kelch F-box family protein |

Font in bold indicates the genes regulated by both GA and SLN1

signaling pathway and directly interacts with DELLA proteins (McGinnis et al. 2003; Sasaki et al. 2003). In this study, nine F-box genes were up-regulated by GA while the *sln1* mutant de-repressed the expression of 14 F-box genes (Table 6). Among them, six F-box genes were regulated by GA and in the *sln1c* mutant, as well. Consistent with the above observation of a substantial overlap between the effects of GA and the *sln1c* mutation, all of the genes showed a slight up-regulation in both the GA treatment and the *sln1c* mutant, even though some were missed in GA-regulated list or in SLN-dependent list. The GID2 ortholog from barley was not present on the gene chip so that its expression could not be evaluated. However, a total of 17 F-box protein genes showed a consistent up-regulation in both the GA treatment and the *sln1c* mutant, suggesting that the expression of these F-box genes were also SLN1-dependent and ubiquitin-dependent protein degradation plays important roles in GA signaling and the GA response in the barley aleurone.

## Concluding remark

There is no doubt that the DELLA proteins are repressors in the GA signaling pathway, as evidenced by its direct interaction with GA receptor, and plants require GA to overcome the effects of these proteins on plant growth and development. The evidence described here from barley and other from rice (Tsuji et al. 2006) demonstrate that the DELLA proteins controls almost all the GA-induced genes in the aleurone tissues. However, in *Arabidopsis*, only about half of the GA-regulated genes are apparently regulated in a DELLA-dependent fashion (Cao et al. 2006). This complexity from the *Arabidopsis* research may result from the more complicated tissues or organs used in the study. Of course, the five DELLA proteins in *Arabidopsis* may also reflect that complexity of GA signaling, as only one DELLA protein is present in cereal crops, such as rice and barley. In *Arabidopsis*, the loss-of-function mutant of the F-box protein SLY1 has a 100% seed germination rate and the DELLA protein RGL2 accumulates in large amount, and thus GA signaling may function in a proteolysis-independent manner (Ariizumi et al. 2008; Ariizumi and Steber 2007). In rice, de-repression of the SLR1 repressive activity can be accomplished by GA and GID1 alone, and does not require the function of the F-Box protein GID2 (Ueguchi-Tanaka et al. 2008). Most recently, a study revealed that cytosolic SPY and GA regulate cytokinin responses via a DELLA-independent pathway(s) (Maymon et al. 2009). On the other hand, DELLA proteins also can be regulated via routes that do not directly involve GA (Achard et al. 2007; Fukao and Bailey-Serres 2008; Oh et al. 2007). The greater induction of gene expression in the SLN1 mutant than in the GA treatment revealed here might imply that, in the barley aleurone, the DELLA protein could be regulated by factors other than GA.





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## Methods

### Plant material and treatment

Barley seeds (*Hordeum vulgare* L. cv Himalaya), harvested in 1998 (Department of Agronomy, Washington State University, Pullman, WA, USA), were used for the GA and ABA treatments. The mutant *sln1c* in the Himalaya background was kindly provided by Dr. Peter M. Chandler, CIRSO (Canberra, Australia), and the homozygous grains harvested in a greenhouse here were used. The seeds were cut in half by excision above the embryo perpendicular to the length of the kernel. The half-seeds without embryos were surface-sterilized and then imbibed in 10 mmol/L $CaCl_2$-saturated paper tissues for 3 days in darkness at 25°C. The aleurones from the half-seeds were isolated by gently removing the starchy endosperm and seed coat (Chrispeels and Varner 1967), and then incubated in 10 mmol/L $CaCl_2$ (control), or in the 10 mmol/L $CaCl_2$ solution containing 1 μmol/L $GA_3$ (GA treatment) or 50 μmol/L ABA (ABA treatment). To select *sln1c* homozygotes, the half-seeds with embryos were germinated and transferred to soil to identify the slender phenotype. The selected homozygous *sln1c* half-seeds without the embryo were imbibed in the same conditions as the wild type but with 5 μmol/L ABA. After imbibition for 3 days, the aleurones were isolated (Chrispeels and Varner 1967) and washed 3–4 times with 10 mmol/L $CaCl_2$. The isolated aleurones were treated in Petri dishes with continuously shaking (60 rpm) in darkness at 25°C, and harvested in 15 h. Three replicates for each treatment were conducted in parallel. The harvested aleurones were frozen immediately in liquid nitrogen and stored at −80°C for the α-amylase activity assay and RNA isolation.

### α-amylase assay

The α-amylase activity was conducted as described before (Skadsen 1993). Briefly, the aleurones were ground in liquid nitrogen. The extracts were incubated at 69°C for 15 min, and 10 μL of the supernatant was transferred to 490 μL of phosphate buffer (20 mmol/L $Na_2{\cdot}HPO_4$, 10 mmol/L NaCl, pH 6.9) with 0.5% starch (Sigma, St. Louis, MO, USA) and incubated at 30°C for 30 min. Then, 500 μL of reaction regent [1% (W/V) 3, 5-dinitrosalicylic acid, 30% (W/V) NaK tartrate and 1.6% (W/V) NaOH] was added and incubated for 15 min at 100°C. Maltose (Sigma) was used as a standard to calculate the enzyme activity. The amount of maltose in the reaction was measured at 547 nm. The total soluble proteins in the extraction were determined using a Protein Assay Kit (Bio-Rad Laboratories Inc., Hercules, CA, USA).

### RNA extraction

The aleurones were ground in liquid nitrogen, and extracted with a mixture of equal amounts of extraction buffer [4% (W/V) p-aminosalicylic disodium, 1% (W/V) 1, 5-naphthalenedisulfonic acid] and phenol. After mixing well, chloroform was added in the same volume as phenol. The supernatant separated by centrifugation was precipitated with ethanol. The pellet was dissolved in water and the RNA was separated from the solution using a LiCl precipitation method. Total RNA was further purified using RNeasy kits (Qiagen GmbH, Germany). The RNA quality and quantity in the samples were measured using a NanoDrop (Agilent Technologies, Palo Alto, CA, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies).

### Probe labeling and hybridization to Barley 1 GeneChip

The Affymetrix (Santa Clara, CA, USA) 22K Barley1 GeneChip (Close et al. 2004) was used. The probe labeling and hybridization were conducted as described in the Affymetrix manual. Total RNA (10 μg) was used for the cDNA synthesis. Purified double-stranded cDNA (5 μL) was used to generate the biotinylated cRNA target. The labeled cRNA was purified, and 20 μg of the cRNA at a final concentration 0.5 μg/μL was fragmented. The fragmented cRNA (15 μg per hybridization) was used to make up the hybridization cocktail and 10 μg equivalents were hybridized to each GeneChip. The hybridization was performed in an Affymetrix hybridization oven model 640. The chips were washed and stained with streptavidin–phycoerythrin in the Affymetrix GeneChip fluidics station model 400. The stained chips were immediately scanned with an Agilent 2500A GeneArray scanner.

### Data acquisition and analysis

The original spot intensities from the microarray chip were normalized using GeneChip RMA (Wu and Irizarry 2004) in GeneSpring (Agilent Technologies) and Microsoft Excel (www.microsoft.com) was used to calculate the slope and $R^2$ of replicates for the "goodness-of-fit" (Schmid et al. 2005) and the Pearson Correlation Coefficient of gene expression. To remove the genes with unreliable signal, the Microarray Suite 5.0 in GCOS (Affymetrix, Inc.) was used to assign present calls ($P \leq 0.065$, detected) or absent calls ($P > 0.065$, undetected) for genes detected in the gene chip. A gene with more than two present (or absent) calls among the three replicates was finally defined as detected (or undetected) in the treatment. The genes expressed at undetectable levels in both treatments were removed and the remaining genes were used for a further significance analysis of microarray (SAM) analysis (Tusher et al. 2001)





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to identify the genes significantly regulated in the *sln1c* mutant with the threshold of a threefold change.

The microarray design and experimental data are available in the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/projects/geo/index.cgi) under series GSE18758.

**Acknowledgments** The authors thank Stacey Madson, and Sandra BonDurant for their technical help, Dr. Lishuang Shen (Virtual Reality Applications Center, Iowa State University, Ames, IA, USA) for searching BarleyBase, Dr. Peter M. Chandler (Commonwealth Scientific and Industrial Research Organization Plant Industry, Canberra, Australia) for kindly providing *sln1* mutants and treatment method, and Dr. Ron Skadsen and Dr Li Lin for their critical reading and thoughtful comments in the manuscript. This research was partially funded by USDA ARS, Cereal Crops Research Unit CRIS funds.



## References

Achard P, Baghour M, Chapple A, Hedden P, Van Der Straeten D, Genschik P, Moritz T, Harberd NP (2007) The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. Proc Natl Acad Sci USA 104:6484–6489. doi:10.1073/pnas.0610717104

Ariizumi T, Steber CM (2007) Seed germination of GA-insensitive *sleepy1* mutants does not require RGL2 protein disappearance in *Arabidopsis*. Plant Cell 19:791–804. doi:10.1105/tpc.106.048009

Ariizumi T, Murase K, Sun T-P, Steber CM (2008) Proteolysis-independent downregulation of DELLA repression in *Arabidopsis* by the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1. Plant Cell 20:2447–2459. doi:10.1105/tpc.108.058487

Aya K, Ueguchi-Tanaka M, Kondo M, Hamada K, Yano K, Nishimura M, Matsuoka M (2009) Gibberellin modulates anther development in rice via the transcriptional regulation of GAMYB. Plant Cell 21:1453–1472. doi:10.1105/tpc.108.062935

Cao D, Hussain A, Cheng H, Peng J (2005) Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in *Arabidopsis*. Planta 223:105–113. doi:10.1007/s00425-005-0057-3

Cao D, Cheng H, Wu W, Soo HM, Peng J (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in *Arabidopsis*. Plant Physiol 142:509–525. doi:10.1104/pp.106.082289

Chandler PM (1988) Hormonal regulation of gene expression in the "slender" mutant of barley (*Hordeum vulgare* L.). Planta 175:115–120. doi:10.1007/BF00402888

Chandler PM, Robertson M (1999) Gibberellin dose-response curves and the characterization of dwarf mutants of barley. Plant Physiol 120:623–632. doi:10.1104/pp.120.2.623

Chandler PM, Marion-Poll A, Ellis M, Gubler F (2002) Mutants at the *slender1* locus of barley cv Himalaya. Molecular and physiological characterization. Plant Physiol 129:181–190. doi:10.1104/pp.010917

Chen K, An Y-QC (2006) Transcriptional responses to gibberellin and abscisic acid in barley aleurone. J Integr Plant Biol 48:591–612. doi:10.1111/j.1744-7909.2006.00270.x

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. Development 131:1055–1064. doi:10.1242/dev.00992

Chrispeels MJ, Varner JE (1967) Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley and aleurone layers. Plant Physiol 42:398–406. doi:10.1104/pp.42.3.398

Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, Wing RA, Muehlbauer GJ, Kleinhofs A, Wise RP (2004) A new resource for cereal genomics: 22 k barley GeneChip comes of age. Plant Physiol 134:960–968. doi:10.1104/pp.103.034462

Dill A, Jung H-S, Sun T-P (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc Natl Acad Sci USA 98:14162–14167. doi:10.1073/pnas.251534098

Fincher GB (1989) Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. Annu Rev Plant Physiol Plant Molec Biol 40:305–346. doi:10.1146/annurev.pp.40.060189.001513

Foster CA (1977) Slender: an accelerated extension growth mutant of barley. Barley Genet Newslett 7:24–27

Fu X, Richards DE, Ait-ali T, Hynes LW, Ougham H, Peng J, Harberd NP (2002) Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLN1 repressor. Plant Cell 14:3191–3200. doi:10.1105/tpc.006197

Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP (2004) The Arabidopsis mutant sleepy1gar2–1 protein promotes plant growth by increasing the affinity of the SCFsly1 E3 ubiquitin ligase for DELLA protein substrates. Plant Cell 16:1406–1418. doi:10.1105/tpc.021386

Fukao T, Bailey-Serres J (2008) Submergence tolerance conferred by SUB1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci USA 105:16814–16819. doi:10.1073/pnas.0807821105

Gocal GFW, Sheldon CC, Gubler F, Moritz T, Bagnall DJ, MacMillan CP, Li SF, Parish RW, Dennis ES, Weigel D, King RW (2001) *GAMYB*-like genes, flowering, and gibberellin signaling in *Arabidopsis*. Plant Physiol 127:1682–1693. doi:10.1104/pp.010442

Griffiths J, Murase K, Rieu I, Zentella R, Zhang Z-L, Powers SJ, Gong F, Phillips AL, Hedden P, Sun T-P, Thomas SG (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. Plant Cell 18:3399–3414. doi:10.1105/tpc.106.047415

Gubler F, Kalla R, Roberts JK, Jacobsen JV (1995) Gibberellin-regulated expression of a MYB gene in barley aleurone cells: evidence for MYB transactivation of a high-pl α-amylase gene promoter. Plant Cell 7:1879–1891. doi:10.1105/tpc.7.11.1879

Gubler F, Raventos D, Keys M, Watts R, Mundy J, Jacobsen VJ (1999) Target genes and regulatory domains of the GAMYB transcriptional activator in cereal aleurone. Plant J 17:1–9. doi:10.1046/j.1365-313X.1999.00346.x

Gubler F, Chandler PM, White RG, Llewellyn DJ, Jacobsen JV (2002) Gibberellin signaling in barley aleurone cells. Control of SLN1 and GAMYB expression. Plant Physiol 129:191–200. doi:10.1104/pp.010918

Hou X, Hu W-W, Shen L, Lee LYC, Tao Z, Han J-H, Yu H (2008) Global identification of DELLA target genes during *Arabidopsis* flower development. Plant Physiol 147:1126–1142. doi:10.1104/pp.108.121301

Huttly AK, Phillips AL (1995) Gibberellin-regulated plant genes. Physiol Plant 95:310–317. doi:10.1111/j.1399-3054.1995.tb00843.x

Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J (2001) Slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating





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gene *GAI/RGA/RHT/D8*. Plant Cell 13:999–1010. doi:10.1105/tpc.13.5.999

Itoh H, Ueguchi-Tanaka M, Sato Y, Ashikari M, Matsuoka M (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. Plant Cell 14:57–70. doi:10.1105/tpc.010319

Itoh H, Sasaki A, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Hasegawa Y, Minami E, Ashikari M, Matsuoka M (2005) Dissection of the phosphorylation of rice DELLA protein, SLENDER RICE1. Plant Cell Physiol 46:1392–1399. doi:10.1093/pcp/pci152

Iuchi S, Suzuki H, Kim Y-C, Iuchi A, Kuromori T, Ueguchi-Tanaka M, Asami T, Yamaguchi I, Matsuoka M, Kobayashi M, Nakajima M (2007) Multiple loss-of-function of arabidopsis gibberellin receptor atgid1s completely shuts down a gibberellin signal. Plant J 50:958–966. doi:10.1111/j.1365-313X.2007.03098.x

Kaneko M, Itoh H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M (2002) The alpha-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. Plant Physiol 128:1264–1270. doi:10.1104/pp.010785

Kaneko M, Itoh H, Inukai Y, Sakamoto T, Ueguchi-Tanaka M, Ashikari M, Matsuoka M (2003) Where do gibberellin biosynthesis and gibberellin signaling occur in rice plants? Plant J 35:104–115. doi:10.1046/j.1365-313X.2003.01780.x

Kaneko M, Inukai Y, Ueguchi-Tanaka M, Itoh H, Izawa T, Kobayashi Y, Hattori T, Miyao A, Hirochika H, Ashikari M, Matsuoka M (2004) Loss-of-function mutations of the rice *GAMYB* gene impair α-amylase expression in aleurone and flower development. Plant Cell 16:33–44. doi:10.1105/tpc.017327

King KE, Moritz T, Harberd NP (2001) Gibberellins are not required for normal stem growth in *Arabidopsis* thaliana in the absence of GAI and RGA. Genetics 159:767–776

Koornneef M, Veen JH (1980) Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) heynh. Theor Appl Genet 58:257–263. doi:10.1007/BF00265176

Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng J (2002) Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a *GAI/RGA*-like gene whose expression is up-regulated following imbibition. Genes Dev 16:646–658. doi:10.1101/gad.969002

Liu Y, Bergervoet JHW, Vos CHR, Hilhorst HWM, Kraak HL, Karssen CM, Bino RJ (1994) Nuclear replication activities during imbibition of abscisic acid- and gibberellin-deficient tomato (*Lycopersicon esculentum* Mill.) seeds. Planta 194:368–373. doi:10.1007/BF00197537

Maymon I, Greenboim-Wainberg Y, Sagiv S, Kieber JJ, Moshelion M, Olszewski N, Weiss D (2009) Cytosolic activity of spindly implies the existence of a della-independent gibberellin-response pathway. Plant J 58:979–988. doi:10.1111/j.1365-313X.2009.03840.x

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun T-P, Steber CM (2003) The *Arabidopsis SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell 15:1120–1130. doi:10.1105/tpc.010827

Mena M, Cejudo FJ, Isabel-Lamoneda I, Carbonero P (2002) A role for the DOF transcription factor BPBF in the regulation of gibberellin-responsive genes in barley aleurone. Plant Physiol 130:111–119. doi:10.1104/pp.005561

Millar AA, Gubler F (2005) The *Arabidopsis GAMYB*-like genes, *MYB33* and *MYB65*, are microRNA-regulated genes that redundantly facilitate anther development. Plant Cell 17:705–721. doi:10.1105/tpc.104.027920

Murray F, Kalla R, Jacobsen J, Gubler F (2003) A role for HvGAMYB in anther development. Plant J 33:481–491. doi:10.1046/j.1365-313X.2003.01641.x

Nakajima M, Shimada A, Takashi Y, Kim Y-C, Park S-H, Ueguchi-Tanaka M, Suzuki H, Katoh E, Iuchi S, Kobayashi M, Maeda T, Matsuoka M, Yamaguchi I (2006) Identification and characterization of arabidopsis gibberellin receptors. Plant J 46:880–889. doi:10.1111/j.1365-313X.2006.02748.x

Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, Lee H-S, Sun T-P, Kamiya Y, Choi G (2007) PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the *GAI* and *RGA* promoters in *Arabidopsis* seeds. Plant Cell 19:1192–1208. doi:10.1105/tpc.107.050153

Peng J (1997) The *Arabidopsis GAI* gene defines a signalling pathway that negatively regulates gibberellin responses. Genes Dev 11:3194–3205. doi:10.1101/gad.11.23.3194

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261. doi:10.1038/22307

Radley M (1967) Site of production of gibberellin-like substances in germinating barley embryos. Planta 75:164–171. doi:10.1007/BF00387132

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong D-H, An G, Kitano H, Ashikari M, Matsuoka M (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299:1896–1898. doi:10.1126/science.1081077

Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Scholkopf B, Weigel D, Lohmann JU (2005) A gene expression map of *Arabidopsis thaliana* development. Nat Genet 37:501–506. doi:10.1038/ng1543

Silverstone AL, Ciampaglio CN, Sun TP (1998) The *Arabidopsis RGA* gene encodes a transcriptional regulator repressing the gibberellin signal-transduction pathway. Plant Cell 10:155–169. doi:10.1105/tpc.10.2.155

Silverstone AL, Jung H-S, Dill A, Kawaide H, Kamiya Y, Sun T-P (2001) Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in *Arabidopsis*. Plant Cell 13:1555–1566. doi:10.1105/tpc.13.7.1555

Skadsen RW (1993) Aleurones from a barley with low α-amylase activity become highly responsive to gibberellin when detached from the starchy endosperm. Plant Physiol 102:195–203. doi:10.1104/pp.102.1.195

Tsuji H, Aya K, Ueguchi-Tanaka M, Shimada Y, Nakazono M, Watanabe R, Nishizawa NK, Gomi K, Shimada A, Kitano H, Ashikari M, Matsuoka M (2006) GAMYB controls different sets of genes and is differentially regulated by microRNA in aleurone cells and anthers. Plant J 47:427–444. doi:10.1111/j.1365-313X.2006.02795.x

Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA 98:5116–5121. doi:10.1073/pnas.091062498

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun T-P (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. Plant Physiol 135:1008–1019. doi:10.1104/pp.104.039578

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow T-Y, Hsing Y-Ic, Kitano H, Yamaguchi I, Matsuoka M (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. Nature 437:693–698. doi:10.1038/nature04028

Ueguchi-Tanaka M, Nakajima M, Katoh E, Ohmiya H, Asano K, Saji S, Hongyu X, Ashikari M, Kitano H, Yamaguchi I, Matsuoka M (2007) Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. Plant Cell 19:2140–2155. doi:10.1105/tpc.106.043729





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MS Code : ACPP-D-09-00479 | ☑ CP | ☑ DISK

Ueguchi-Tanaka M, Hirano K, Hasegawa Y, Kitano H, Matsuoka M (2008) Release of the repressive activity of rice DELLA protein SLR1 by gibberellin does not require SLR1 degradation in the GID2 mutant. Plant Cell 20:2437–2446. doi:10.1105/tpc.108.061648

Vierstra RD (2003) The ubiquitin/26s proteasome pathway, the complex last chapter in the life of many plant proteins. Trends Plant Sci 8:135–142. doi:10.1016/S1360-1385(03)00014-1

Washio K (2003) Functional dissections between GAMYB and Dof transcription factors suggest a role for protein-protein associations in the gibberellin-mediated expression of the *RAmy1A* gene in the rice aleurone. Plant Physiol 133:850–863. doi:10.1104/pp.103.027334

Wattenberg BW, Pitson SM, Raben DM (2006) The sphingosine and diacylglycerol kinase superfamily of signaling kinases: localization as a key to signaling function. J Lipid Res 47:1128–1139. doi:10.1194/jlr.R600003-JLR200

Wen C-K, Chang C (2002) *Arabidopsis RGL1* encodes a negative regulator of gibberellin responses. Plant Cell 14:87–100. doi:10.1105/tpc.010325

Willige BC, Ghosh S, Nill C, Zourelidou M, Dohmann EMN, Maier A, Schwechheimer C (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of *Arabidopsis*. Plant Cell 19:1209–1220. doi:10.1105/tpc.107.051441

Woodger FJ, Jacobsen JV, Gubler F (2004) GMPOZ, a BTB/POZ domain nuclear protein, is a regulator of hormone responsive gene expression in barley aleurone. Plant Cell Physiol 45:945–950. doi:10.1093/pcp/pch100

Wu Z, Irizarry RA (2004) Preprocessing of oligonucleotide array data. Nat Biotech 22:656–658. doi:10.1038/nbt0604-656b

Yu H, Ito T, Zhao Y, Peng J, Kumar P, Meyerowitz EM (2004) Floral homeotic genes are targets of gibberellin signaling in flower development. Proc Natl Acad Sci USA 101:7827–7832. doi:10.1073/pnas.0402377101

Zentella R, Yamauchi D, Ho T-hD (2002) Molecular dissection of the gibberellin/abscisic acid signaling pathways by transiently expressed RNA interference in barley aleurone cells. Plant Cell 14:2289–2301. doi:10.1105/tpc.003376

Zentella R, Zhang Z-L, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E, Kamiya Y, Sun T-P (2007) Global analysis of DELLA direct targets in early gibberellin signaling in *Arabidopsis*. Plant Cell 19:3037–3057. doi:10.1105/tpc.107.054999

Zhang Z-L, Xie Z, Zou X, Casaretto J, Ho T-hD, Shen QJ (2004) A rice *WRKY* gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells. Plant Physiol 134:1500–1513. doi:10.1104/pp.103.034967

Zou X, Neuman D, Shen QJ (2008) Interactions of two transcriptional repressors and two transcriptional activators in modulating gibberellin signaling in aleurone cells. Plant Physiol 148:176–186. doi:10.1104/pp.108.123653





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