Brief Communication

An in vivo GA- and ABA-responsive dual-luciferase reporter system for simultaneous detection of GA and ABA responses, hormone crosstalk and heat stress response in rice

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Gibberellic acid (GA) and abscisic acid (ABA) are the primary phytohormones that play antagonistic roles in the control of the transition from embryogenesis to germination in seeds (Finch-Savage and Leubner-Metzger, 2006). Given the low concentrations of GA and ABA in seeds and plants, biosynthesis inhibitors (Toh et al., 2008) or deficient mutants (Debeaujon and Koornneef, 2000) have been used to investigate the relative importance of their physiological functions. However, biosynthetic inhibitors may have pleiotropic effects in plants, and the use of GA- or ABA-deficient mutants does not permit simultaneous monitoring of dynamic changes in GA and ABA during seed development or germination (Dejonghe et al., 2018). Analytical determination provides accurate quantification of GA and ABA contents, but the extraction procedure is time-consuming, and the antagonism and dynamic changes between GA and ABA may not be detected.

Reporter genes directed by an ABA- or a GA-responsive promoter have been used to study the concentration or signalling of GA or ABA. The ABA-responsive complex (ABRC) has been manipulated in the control of several stress-tolerance genes expressed in transgenic plants (Chen et al., 2015; Garg et al., 2002; Shen and Ho, 1995). In addition, the α-amylase32b promoter has been known to be highly induced by GA during seed germination in barley (Gomez-Cadenas et al., 2001; Lanahan et al., 1992). An individual ABRC or Amy32b promoter has been used to direct the expression of reporter genes. However, a simple and versatile system for rapid and simultaneous determination of antagonistic and dynamic changes in GA and ABA remains elusive. In this study, an in vivo GA/ABA dual-luciferase reporter system was established (Figure 1a).

Transgenic rice harbouring a firefly luciferase (firefly LUC) directed by two copies of the ABRC together with a Renilla luciferase (Renilla LUC) directed by the Amy32b promoter was generated. In developing seeds, firefly LUC activity was notably enhanced 323-fold at 28 days after flowering (DAF), which indicates that the ABA response was strongly and positively correlated with seed development. In contrast, Renilla LUC activity showed 30%–40% reduction at 7–21 DAF and increased slightly at 28 DAF (Figure 1b). During seed germination, a 30%–40% decrease in firefly LUC activity was observed at 12–48 hours after imbibition (HAI). In contrast, Renilla LUC activity significantly increased at 12–48 HAI (Figure 1c). In the embryo, firefly LUC activity declined significantly at 24 and 48 HAI. However, the decline in firefly LUC activity at 24 and 48 HAI was less pronounced than that in the embryo (Figure 1d). In contrast, Renilla LUC activity increased greatly in the embryo and endosperm at 12-48 HAI (Figure 1e). These data indicate that the activation of the GA response preceded that of the ABA response after imbibition. Therefore, the dual-luciferase reporter system faithfully reflects the effects of ABA and GA in transgenic rice during seed germination.

To assess the sensitivity of the ABA-responsive promoter to ABA, luciferase activities were measured in roots of rice seedlings due to high ABA concentrations in mature seeds. Measurement of firefly LUC activity revealed that 0.5, 1 and 5 μM ABA significantly increased promoter activity with treatment for 12–24 h. However, ABA treatment for 48-h attenuated firefly LUC activity (Figure 1f). The sensitivity of the Amy32b promoter in transgenic rice to GA was determined using embryoless half-seeds. Measurement of Renilla LUC activity revealed that GA concentrations higher than 10−3 μM significantly activated Amy32b promoter activity with treatment for 12 h. The GA response was increased strongly by GA treatment for 48 h (Figure 1g).

The effects of methyl jasmonate (MJ), kinetin (KT) and salicylic acid (SA) on LUC activity in the roots were measured to examine the potential crosstalk of phytohormones in rice seedlings. After testing a range of phytohormone concentrations, rice seedlings were treated with or without 100 μM MJ, 100 μM KT or 1000 μM SA for 24 and 48 h. The firefly LUC activity was increased significantly by MJ at 24 h, but was strongly suppressed by MJ at 48 h. In contrast, Renilla LUC activity was increased by MJ at 24 and 48 h. Kinetin treatment markedly reduced firefly LUC activity at 24 and 48 h. However, Renilla LUC activity was notably enhanced at 24 and 48 h. The activation of Renilla LUC activity by
Kinetin is possibly caused by two putative cytokinin responsive elements in the Amy32b promoter. Treatment with SA significantly increased firefly LUC activity at 24 h but suppressed activity at 48 h. Renilla LUC activity was increased by SA at 24 h (Figure 1h and i). These results provide evidence for crosstalk between exogenous and endogenous phytohormones. Nevertheless, a significant difference in luminescent signal was observed within the control group without exogenous hormone at the different time points. This indicates that this system is quantitative but time-dependent in root tissues.

Few studies have focused on the correlation between ABA and heat stress. Incubation of rice seeds at 27–37 °C results in 90%–97% germination. However, above this temperature range the germination percentage declines markedly (Fujino et al., 2004). The dual-luciferase reporter system was applied to investigate the changes in GA and ABA responses during seed germination under suboptimal temperature (27 °C), supraoptimal temperature (37 °C) and heat stress (40 °C). The expression of OsRab16A and OsAmy7, which are known to be highly induced by ABA and GA, respectively, was determined by qRT-PCR to compare with luciferase signal. After imbibition for 24 h, seed germination was observed only at 37 °C. At 36 h, 98%, 64% and 6% seed germination was observed at 37 °C, 27 °C and 40 °C, respectively (Figure 1j). Simultaneous detection of the changes in GA/ABA-responsive complex from barley HVA22 (GenBank accession: X05166); LUC: Luciferas; Amy32b: barley Amy32b (GenBank accession: AAA16094); HPT: hygromycin phosphotransferase. Firefly and Renilla LUC activity during seed development (b) and seed germination (c). Firefly (d) and Renilla (e) LUC activity in the embryo and endosperm during seed germination. (f) Response of firefly LUC activity to exogenous ABA in roots. (g) Response of Renilla LUC activity to exogenous GA in embryoless half-seeds. (h) Response of firefly and (i) Renilla LUC activity to exogenous phytohormones in roots. Data are mean ± SD (n = 9). (j) Germination percentage of rice seeds incubated under 27 °C, 37 °C and 40 °C. Data are mean ± SD (n = 150). Firefly (k) and Renilla (l) LUC activity during seed germination. Data in (b) to (g), (k) are mean ± SD (n = 6). Values with the same letter are not significantly different at $P < 0.05$. Significance analysis is performed by Duncan’s multiple range tests.
ABA responses revealed that firefly LUC activity notably declined with imbibition for 8 and 2 h at 37 °C and 40 °C, respectively, compared with that observed at 27 °C. Almost no firefly LUC activity was detectable at 8 and 48 h of imbibition at 37 °C and 40 °C, respectively (Figure 1k). In contrast, firefly LUC activity declined slowly after seed germination at 27 °C (Figure 1l). These data indicate that the sensitivity of these plants to ABA is hampered or the degradation of ABA was strongly correlated with the increasing temperature during seed germination in rice. Renilla LUC activity increased significantly during the first 4 h of heat treatment (37°C and 40°C) compared with that observed at 27°C. With imbibition for 12–48 h, Renilla LUC activity gradually increased at 27°C and markedly increased at 37°C, whereas the GA response was suppressed under heat stress (40°C) (Figure 1l). Therefore, the GA response gradually increased followed by gradual degradation of ABA under suboptimal and supraoptimal temperatures. In contrast, both the ABA and GA responses were suppressed under heat stress. The GA/ABA dual-luciferase reporter system provides a simple, sensitive and reliable method to elucidate the role of genes crucial for signalling and antagonism of GA and/or ABA by stable transformation or transient expression in protoplasts. This system is also useful for studying the effect of exogenous environmental factors and endogenous factors on the dynamic changes in GA and ABA responses simultaneously. Our system measures transcriptional responses to GA and ABA, in contrast to the FRET reporters which directly measure GA and ABA concentration changes. These tools are complementary to address different biological questions.

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**Conflict of interest**

The authors declare no competing financial interests.

**Author contributions**

CYW and CYH designed the experiments and wrote the manuscript. CYW performed the experiments and analysed the data. Both authors participated in data interpretation.

**References**

Chen, Y.S., Lo, S.F., Sun, P.K., Lu, C.A., Ho, T.H. and Yu, S.M. (2015) A late embryogenesis abundant protein HVA1 regulated by an inducible promoter enhances root growth and abiotic stress tolerance in rice without yield penalty. *Plant Biotechnol. J.*, 13, 105–116.

Debeaujon, I. and Koornneef, M. (2000) Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiol.* 122, 415–424.

Dejonghe, W., Okamoto, M. and Cutler, S.R. (2018) Small molecule probes of ABA biosynthesis and signaling. *Plant Cell Physiol.* 59, 415–424.

Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytol.* 171, 501–523.

Fujino, K., Sekiguchi, H., Sato, T., Kiuchi, H., Nonoue, Y., Takeuchi, Y., Ando, T. et al. (2004) Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 108, 794–799.

Garg, A.K., Kim, J.K., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V. and Wu, R.J. (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci. USA*, 99, 15898–15903.

Gomez-Cadenas, A., Zentella, R., Sutliff, T.D. and Ho, T.H. (2001) Involvement of multiple cis-elements in the regulation of GA responsive promoters: Definition of a new cis-element in the Amy32b gene promoter of barley (*Hordeum vulgare*). *Physiol Plant.* 112, 211–216.

Lanahan, M.B., Ho, T.H., Rogers, S.W. and Rogers, J.C. (1992) A gibberellin response complex in cereal alpha-amylase gene promoters. *Plant Cell*, 4, 203–211.

Shen, Q. and Ho, T.H. (1995) Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel cis-acting element. *Plant Cell*, 7, 295–307.

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A. et al. (2008) High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. *Plant Physiol.* 146, 1368–1385.