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Brief Communication

Synthetic biosensor for mapping dynamic responses and spatio-temporal distribution of jasmonate in rice

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Jasmonate (JA) critically regulates plant development and stress response, but its spatio-temporal distribution at the cellular level remains unclear. A JA biosensor consisting of a JA degron motif Jas9 fused with the fluorescent protein VENUS was developed in *Arabidopsis* (Larrieu *et al*., 2015), but its *35S* promoter has low activity in reproductive tissues and does not express well in monocotyledons, thus limiting its application in crops and reproductive development.

To develop a JA biosensor in rice, we generated a synthetic construct based on Jas9-VENUS (Figure 1a), containing (i) a single optimal maize ubiquitin-1 (Ubi-1) promoter (Cornejo *et al*., 1993), (ii) a nuclear-localized JA sensor module (Jas-VENUS) with an optimized JA-dependent degradation sequence, VENUS, a N7 nuclear localization signal (NLS) (Cutler *et al*., 2000) and a 6x Hemagglutinin (HA) tag, (iii) a nuclear normalization element (H2B-mCherry) containing a fusion of the Histone H2B protein and the red fluorescent protein mCherry (Shaner *et al*., 2004) and (iv) a F2A ribosomal skipping peptide as linker, allowing stoichiometric co-production of Jas-VENUS-HA and H2B-mCherry (Liu *et al*., 2017). JA responses can thus be inferred ratiometrically by comparing fluorescence signals of VENUS and mCherry.

We selected OsJAZ3 and OsJAZ6 as sensor constructs as they were expressed ubiquitously and were JA sensitive (Figure 1b), and they interacted with OsCOI1b in the presence of coronatin (COR, JA analog) (≥0.5 μM; Figure 1c). We used their Jas degron sequences to make J3V-HM and J6V-HM (*Ubi-1:Jas3/6-VENUS-6HA:F2A:H2B-mCherry*) (Figure 1b). Jas motif mutants (mJas), having two amino acids substitutions (RK->AA) that block JA-dependent Jas degradation, were used as controls (Figure 1a) (Cai *et al*., 2014). For each construct, we obtained at least three independent transgenic lines, in which the sensor and the normalization element proteins were properly expressed and translated in tandem (Figure 1d). Robust VENUS fluorescence signals were only observed in the J6V-HM transgenic lines, with line 6 chosen for further analysis.

We next assessed whether the J6V-HM transgenic lines are suitable as JA indicators in rice. We first characterized JA content in the root tip since the fluorescence was clearest in this region. Upon MeJA treatment, VENUS fluorescence was rapidly (20 min) suppressed in J6V-HM seedling roots, but not in mJ6V-HM roots (Figure 1e). Immunoblot analyses confirmed that the decrease in fluorescence correlated with the degradation of the J6V-VENUS protein (Figure 1f). Treatment with MG132, an inhibitor of the 26S proteasome, blocked the fluorescence change in J6V-HM (Figure 1e), demonstrating that the J6V-HM response was due to JA-induced protein degradation through the 26S proteasome. Further analyses revealed that J6V-HM degradation responded to four active jasmonic molecules (Figures 1g,h). Finally, fluorescence quantification showed that the relatively rapid decrease in J6V-HM fluorescence was induced by bioactive JA, and to a lesser extent by GA3 (Figure 1i), confirming cross talks between JA and GA signalling (Hou *et al*., 2010).

We next explored whether J6V-HM can measure cellular JA responses upon environmental challenges. VENUS fluorescence, and thus JA, was significantly reduced in all root cells 8 min after the addition of 200 mM NaCl, which continued until 60 min after treatment (Figure 1j). These results correlated well with JA levels, that is JA and JA-Ile levels (Figure 1k), analysed via high-performance liquid chromatography–tandem mass spectrometry (HPLC-QQQMS), and the expression of the JA-responsive gene *OsOPR7* (Figure 1l). We also tested the efficiency of J6V-HM in response to wounding at the root tip after damaging the roots 1 cm above the root tip with tweezers. Here, the VENUS signal was significantly reduced within 30 min (Figure 1m), and the expression of the wounding marker gene *OsAOS2*, which encodes a JA biosynthetic enzyme, was induced (Figure 1n).

We next assessed JA content in different root tip cells. Since degradation of J6V-HM is proteasome-dependent and OsCOI1-mediated, and that most of the OsJAZ proteins only interact with OsCOI1b (Cai *et al*., 2014), we generated OsCOI1b-GFP plants as control. By comparing the fluorescence of OsCOI1b-GFP and mJ6V-HM (Figures 1o,p), we found higher JA levels in root epidermis, root cap and the quiescent centre, and relatively lower JA levels in stele and cortex cells, especially in exodermis (Figure 1q).

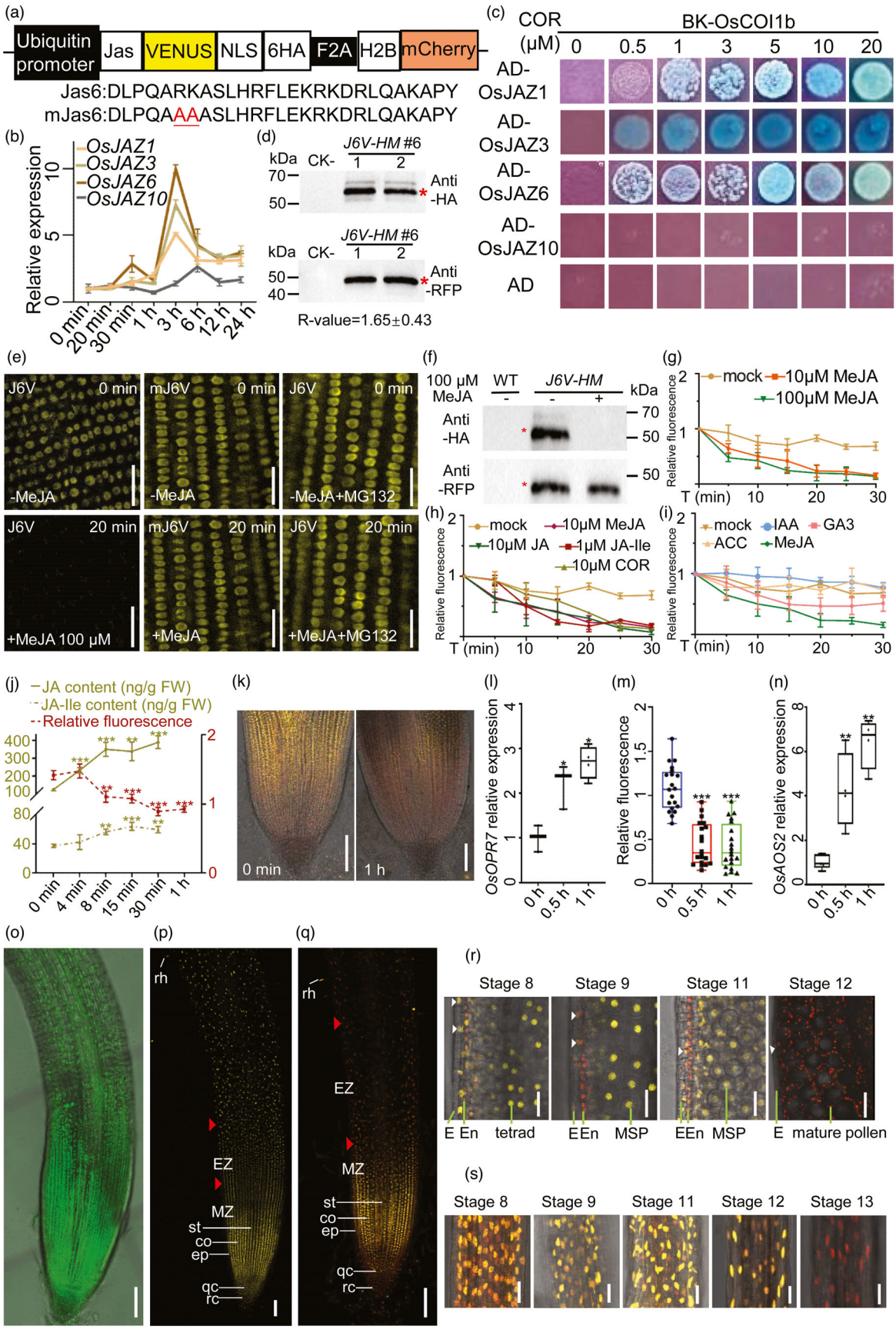

Figure 1 J6V-HM serves as an effective JA biosensor in rice. (a) Schematic representation of the J6V-HM construct. (b) qRT-PCR analysis of *OsJAZ* genes after 50 μM MeJA treatment. (c) Y2H assays to detect COR-dependent OsJAZ-OsCOI1b interactions. (d) Immunoblot analysis of J6V-HM transgenic lines. Asterisk, the target band. *R*-value is the ratio of the expression levels between Jas6-VENUS and H2B-mCherry and presented as mean ± SD ($n > 3$). (e) VENUS fluorescence in the root after MeJA treatments. Scale bars, 25 μm. (f) Immunoblot analysis of wild-type and J6V-HM seedlings treated with 100 μM MeJA for 4 h. (g) Degradation of J6V-HM fluorescence after MeJA treatment ($n > 3$). (h–i) Time-course quantification of VENUS fluorescence normalized to mCherry signals after treatments of various JAs (h) and other plant hormones in 100 μM (i). $n > 3$. (j) Stress response of J6V-HM treated with 200 mM NaCl, red line means quantification of J6V-HM fluorescence by normalization to mCherry signals in root tip, and green lines mean time-course quantification of JA and JA-Ile levels in the root. Data are presented as mean ± SD ($n > 4$). FW, fresh weight. (k) Time-course imaging of J6V-HM fluorescence in rice root tip following treatment with 200 mM NaCl. Scale bars, 100 μm. (l, n) Relative expression of *OsOPR7* and *OsAOS2* after NaCl (l) or wounding (n) treatment. Data represent mean ± SD ($n > 3$). Expression level at 0 h was set as 1. (m) Quantification of J6V-HM fluorescence normalized to mCherry signals in root apices after wounding. (o) Expression of COI1bpro:COI1b-eGFP in root tip. Scale bars, 100 μm. (p–q) mJ6V-HM (p) and J6V-HM (q) fluorescence map in a root tip. Scale bars,100 μm. co, cortex cell; ep, epidermis; EZ, elongation zone; MZ, meristem zone; qc, quiescent centre; rc, root cap; rh, root hair; st, stele. (r–s) Time-course imaging of J6V-HM fluorescence during rice anther (r) and filament (s) development. E, epidermis; En, endothecium; Msp, microspore. Overlays of VENUS and mCherry are presented. Scale bars, 25 μm.

Jasmonate is pivotal for reproduction (Acosta and Przybyl, 2019); however, JA distribution in anthers during reproduction is unclear. We observed J6V-HM fluorescence in the anther at stage 8, with the strongest signals in tetrads. At stage 9, the signals subsided in epidermis (E) and endothecium (En), but remained strong in microspore (MSP). Fluorescence decreased in E, En and MSP at stage 11, and no above-background fluorescence was detected in any anther cells at stage 12 (Figure 1r). The VENUS fluorescence was homogenous in all filament cells, increased continuously from stages 8 to 11 and started to decrease at stage 12 before completely disappearing at stage 13 (Figure 1s). These results demonstrate that JA levels peaked at stage 13 in filaments and at stage 12 in anthers during rice anthesis. It should be noted that some cell and tissue types may be better suited to visualize the JA content via J6V-HM, largely due to protein and expression levels. Nevertheless, J6V-HM is a powerful tool to detect JA levels during reproduction and to monitor dynamic JA responses in rice.

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Conflict of interests

The authors declare no competing interests.

Author contributions

Z.Y. and S.P. designed the project. S.Q.L., L.C.C., X.F.C., Y.L.L., M.J.C. and Z.B.C. conducted the experiments. L.C.C., J.P.H., D.B. Z., S.P. and Z.Y. co-wrote the manuscript.

References

Acosta, I.F. and Przybyl, M. (2019) Jasmonate signaling during *Arabidopsis* stamen maturation. *Plant Cell Physiol.* **60**, 2648–2659.

Cai, Q., Yuan, Z., Chen, M., Yin, C., Luo, Z., Zhao, X., Liang, W. *et al.* (2014) Jasmonic acid regulates spikelet development in rice. *Nat. Commun.* **5**, 3476.

Cornejo, M.-J., Luth, D., Blankenship, K.M., Anderson, O.D. and Blechl, A.E. (1993) Activity of a maize ubiquitin promoter in transgenic rice. *Plant Mol. Biol.* **23**, 567–581.

Cutler, S.R., Ehrhardt, D.W., Griffitts, J.S. and Somerville, C.R. (2000) Random GFP:cDNA fusions enable visualization of subcellular structures in cells of *Arabidopsis* at a high frequency. *Proc. Natl Acad. Sci. USA*, **97**, 3718–3723.

Hou, X., Lee, L.Y., Xia, K., Yan, Y. and Yu, H. (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Dev. Cell*, **19**, 884–894.

Larrieu, A., Champion, A., Legrand, J., Lavenus, J., Mast, D., Brunoud, G., Oh, J. *et al.* (2015) A fluorescent hormone biosensor reveals the dynamics of jasmonate signalling in plants. *Nat. Commun.* **6**, 6043.

Liu, Z., Chen, O., Wall, J.B.J., Zheng, M., Zhou, Y., Wang, L., Ruth Vaseghi, H. *et al.* (2017) Systematic comparison of 2A peptides for cloning multi-genes in a polycistronic vector. *Sci. Rep.* **7**, 2193.

Shaner, N.C., Campbell, R.E., Steinbach, P.A., Giepmans, B.N., Palmer, A.E. and Tsien, R.Y. (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. *Nat. Biotechnol.* **22**, 1567–1572.

