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This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1633396> since 2017-09-15T10:04:00Z

Published version:

DOI:10.1111/nph.14539

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(Article begins on next page)

This is the author's final version of the contribution published as:

Gennaro Carotenuto ; Mireille Chabaud ; Kana Miyata ; Martina Capozzi ; Naoya Takeda ; Hanae Kaku ; Naoto Shibuya ; Tomomi Nakagawa ; David G. Barker ; Andrea Genre. The rice LysM receptor-like kinase OsCERK1 is required for the perception of short-chain chitin oligomers in arbuscular mycorrhizal signaling. *NEW PHYTOLOGIST*. 214 (4) pp: 1440-1446.
DOI: 10.1111/nph.14539

The publisher's version is available at:

<http://onlinelibrary.wiley.com/doi/10.1111/nph.14539/fullpdf>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/>

1 **The rice LysM receptor-like kinase OsCERK1 is required for the perception of short-**
2 **chain chitin oligomers in arbuscular mycorrhizal signaling**

3

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25 **Total word count: 3107** (Introduction, 792; Materials and methods, 746; Results, 777; Discussion,
26 738; Acknowledgements, 54)

27 **Number of figures: 3**

28 **Supporting information: 3**

29

30 **Heading for social media:** Rice chitin receptor OsCerk1 has a role in the perception of symbiotic
31 fungal signals

32

33 **Summary**

- 34 • The rice LysM receptor-like kinase *OsCERK1* is now known to have a dual role in both
35 pathogenic and symbiotic interactions. Following the recent discovery that the *Oscerk1* mutant
36 is unable to host arbuscular mycorrhizal (AM) fungi, we have examined whether *OsCERK1* is
37 directly involved in the perception of the short chain chitin oligomers (Myc-COs) identified in
38 AM fungal exudates and shown to activate nuclear Ca^{2+} spiking in the rice root epidermis.
- 39 • An *Oscerk1* knock-out mutant expressing the cameleon NLS-YC2.60 was used to monitor
40 nuclear Ca^{2+} signaling following root treatment with either crude fungal exudates or purified
41 Myc-COs.
- 42 • Compared to wild type rice, Ca^{2+} spiking responses to AM fungal elicitation were absent in root
43 atrichoblasts of the *Oscerk1* mutant. In contrast, rice lines mutated in *OsCEBiP*, encoding the
44 LysM receptor-like protein which associates with *OsCERK1* to perceive chitin elicitors of the
45 host immune defense pathway, responded positively to Myc-COs.
- 46 • These findings provide direct evidence that the bi-functional *OsCERK1* plays a central role in
47 perceiving short chain Myc-CO signals and activating the downstream conserved symbiotic
48 signal transduction pathway.

49

50 **Keywords:** Arbuscular mycorrhiza, *Oryza sativa*, Chitin oligomer signaling, LysM RLK receptors,
51 Nuclear calcium spiking Plant-microbe interactions, Root symbiosis

52

53 Introduction

54 Arbuscular mycorrhizal (AM) symbioses with soil borne glomeromycetes are believed to have
55 developed over 400 My ago when the first plant ancestors moved from aquatic to terrestrial
56 environments and are present today in the majority of land plants, including most crops (Fitter et
57 al., 2011; Berruti et al., 2016). This success derives from the ability of obligate mutualistic AM fungi
58 to provide their host plants with privileged access to soil nutrients and water, in return for an
59 ecological niche and host photosynthates (Willis et al., 2013). Metabolite exchange occurs within
60 the root inner cortex, where highly branched hyphal structures known as arbuscules develop
61 within living plant cells (Harrison, 2012). Strong evidence indicates that the establishment of this
62 endosymbiosis requires reciprocal chemical signaling prior to fungal root entry, with plant-exuded
63 strigolactones triggering fungal differentiation and in return fungal signal molecules activating a
64 specific signaling pathway (Delaux et al., 2015) in host epidermal cells (Schmitz and Harrison,
65 2014).

66 The activation of a conserved core module of this signaling pathway is required not only during
67 AM, but also during the establishment of symbiotic nitrogen fixation between rhizobia and
68 legumes as well as between *Frankia* and actinorhizal hosts (Barker et al., 2016). For this reason,
69 the core module is known as the common symbiotic signaling pathway, or CSSP, and mutations in
70 key CSSP components display an early block in either fungal or bacterial penetration of the root
71 epidermis (Kistner et al., 2005). Finally, a characteristic feature of the CSSP is the generation of
72 repetitive nuclear-associated Ca^{2+} oscillations known as Ca^{2+} spiking (Oldroyd and Downie, 2006),
73 which means that the activation of the CSSP can be conveniently monitored in outer root tissues
74 using *in vivo* calcium reporters such as cameleons (Miwa et al., 2006).

75 Studies in legumes have led to the identification of decorated lipo-chitooligosaccharidic (LCO) Nod
76 factors as specific rhizobial signaling molecules recognized by the appropriate host plant. Nod
77 factors are perceived *via* lysin-motif receptor-like kinases (LysM RLKs; Antolín-Llovera et al. 2012),
78 and mutations in these LysM RLKs are defective in nodulation. More recently, chitin-based
79 molecules have also been identified as putative fungal signals perceived by legume host plants
80 during pre-infection stages of the AM association. These include both Nod factor-like Myc-LCOs
81 (Maillet et al., 2011) as well as simpler short-chain chito-oligosaccharides referred to as Myc-COs
82 (Genre et al., 2013). Although both types of molecule are able to trigger CSSP-dependent Ca^{2+}
83 spiking, their respective biological roles still remain to be established. Furthermore, since knock-
84 out mutations in individual legume LysM RLK genes have not yet yielded AM phenotypes with an

85 efficient block in fungal entry, it is currently difficult to evaluate the relationship between Myc-
86 LCO/CO perception and the establishment of the AM association in these species. Part of the
87 reason for this may be due to functional redundancy between members of the very large family of
88 LysM RLKs present in legume genomes (e.g. Arrighi et al., 2006).

89 In contrast, promising advances in this direction have recently come from studies on the monocot
90 rice, where it has been shown that either knock-out (Miyata et al., 2014) or silencing (Zhang et al.,
91 2015) of the *OsCERK1* gene results in a clear defect in AM fungal penetration of the root
92 epidermis. *OsCERK1* was originally identified as a LysM RLK that associates with *OsCEBiP*, a LysM
93 RLP (receptor-like protein) lacking a kinase domain to perceive long-chain chitin oligomers (such
94 as chito-octaose, CO8) as part of a host immune defense signaling pathway responding to fungal
95 pathogen elicitors (Shimizu et al., 2010). Thus, mutations in either *OsCERK1* or *OsCEBiP* fail to
96 activate the chitin-triggered immune defense response (Kouzai et al., 2014; Miyata et al., 2014). In
97 contrast to *Oscerk1*, *Oscebip* mutants establish AM symbiosis normally, suggesting that this
98 particular LysM RLP is probably not essential for the perception of chitin-based AM symbiotic
99 signals (Miyata et al., 2014). In addition to these findings, a recent study has shown that the short-
100 chain Myc-CO chito-tetraose (CO4) is an active elicitor of nuclear Ca^{2+} spiking in rice atrichoblasts
101 (Sun et al., 2015), the non-root hair epidermal cells that are targeted for infection by AM hyphae.
102 In contrast, the same study showed that even high concentrations of Myc-LCOs were unable to
103 trigger Ca^{2+} spiking in rice atrichoblasts.

104 In this paper we have made use of the *Oscerk1* knock-out mutant to directly investigate the role of
105 this rice LysM RLK in perceiving symbiotic AM signals. Experiments using transgenic rice lines
106 expressing a nuclear Ca^{2+} -sensing cameleon probe have revealed that, by comparison with wild
107 type (WT) plants, the *Oscerk1* mutant is unable to respond to crude germinated AM fungal spore
108 exudates. Furthermore, the fact that purified CO4 also fails to trigger Ca^{2+} spiking in the *Oscerk1*
109 background provides additional evidence that Myc-COs present in the fungal exudate are
110 important signals during the initial stages of fungal/host communication.

111

112 **Materials and Methods**

113 **Plant material and cameleon constructs**

114 The nuclear-localized yellow cameleon NLS-YC2.60 (Nagai et al 2004; Suzaki et al 2013) was
115 introduced into the pUB-GW-Hyg vector (Maekawa et al 2008) and used to transform wild type
116 rice (*Oryza sativa* L. *japonica* cv. Nipponbare BL no. 2) using *Agrobacterium*-mediated

117 transformation (Ozawa and Takaiwa, 2010). The expression of NLS-YC2.60 was confirmed in rice
118 seedling primary roots by fluorescence microscopy. NLS-YC2.60 was also introduced into *Oscerk1*,
119 *Osnfr5* and *Oscebip* mutants by crossing with the WT/NLS-YC2.60 line. Genotyping of the F2
120 progenies from these crosses were performed and mutant lines expressing NLS-YC2.60
121 fluorescence were selected.

122 Rice seeds were surface-sterilized as described in Campos-Soriano et al. (2011) and placed on
123 water-agar (0.8% Plant Agar, Duchefa) in 12 cm-square Petri dishes. Dishes were kept in the dark
124 for 3 days to induce germination, and then exposed to a light period of 16h at a constant
125 temperature of 23°C with an aluminium foil wrap to limit light illumination of the root system.
126 Since theameleon fluorescence appeared to be strongly reduced in older roots, 2 cm-long apical
127 segments of primary roots from 7-10 day old plantlets were used for the various treatments and
128 subsequent FRET-based imagery.

129

130 **Fungal signals and root treatments**

131 The AM fungus used in this study was *Gigaspora margarita* isolate BEG 34 (International Bank for
132 Glomeromycota, University of Kent, UK). Germinated spore exudates (GSE) were produced as
133 described in Chabaud et al. (2011). Briefly, batches of 100 surface-sterilized *G. margarita* spores
134 were germinated in 1 ml of sterile distilled water over a 7-day period. The resulting fungal
135 germination medium was then concentrated 10-fold using a Lio5P lyophilizer (Cinquepascal, Milan,
136 Italy) and stored at -20°C. All experiments were performed using the 10-fold concentrated *G.*
137 *margarita* spore exudate.

138 Purified CO₄ was purchased from Megazyme (Libios, France). A CO₄ stock solution was prepared
139 in sterile distilled water at 10⁻³ M and stored at -20°C. Preliminary tests performed on the rice
140 seedling primary roots expressing adequate levels ofameleon fluorescence revealed that a
141 concentration of 10⁻⁵ M CO₄ was required for the efficient induction of Ca²⁺ spiking in root
142 atrichoblasts. (Fig. S1).

143 The protocol for root treatment was modified from that described in Genre et al. (2013). Root
144 segments were placed in a 2 mm-thick microchamber containing sterile distilled water on a
145 microscope slide. The water in the microchamber was rapidly (< 30 s) substituted by 100µl of
146 either GSE, 10⁻⁵ M CO₄ or sterile distilled water (as control) before initiating confocal image
147 acquisition. To prevent a cold-shock response, all solutions were warmed to 25°C before use.

148 Imaging was carried out on atrichoblast-rich areas of the root epidermis located on the part of the
149 root in contact with the agar medium and between 1-2 cm from the root tip.

150

151

152 **Confocal microscopy and data analysis**

153 FRET-based imaging for detecting and plotting relative changes in nuclear Ca^{2+} concentrations
154 corresponding to changes in the ratio of yellow fluorescent protein (YFP) to cyan fluorescent
155 protein (CFP) emission intensity over time was performed using a slightly modified version of the
156 protocol described by Genre et al. (2013). A Leica TCS SP2 AOBS confocal laser-scanning microscope
157 was equipped with a long-distance HCX Apo L NA 40X 0.80 water-immersion objective or a HCX PL
158 APO 40X 0.85 dry objective (Leica Microsystems GmbH, Wetzlar, Germany). Fluorescence
159 intensities corresponding to both the CFP and YFP moieties of the NLS-YC2.60 nuclear cameleon
160 were measured after exciting the probe at 458 nm (80% Ar laser) and recording the emitted
161 fluorescence at 470–500 and 530–570 nm respectively. In order to optimize fluorescence
162 excitation and acquisition, the beam expander was set at 1 and the pinhole diameter at 4-6 Airy
163 units. Transmitted light images were acquired simultaneously to confirm cell identity. Images were
164 scanned at a resolution of 512 x 512 pixels and collected every 5 s over a period of 30 min, starting
165 20 min after the treatment. The reason for this is that, as originally observed by Sun et al. (2015),
166 spiking in rice atrichoblasts generally initiates only after a delay of 20 to 30 min following root
167 treatment.

168 Previous studies using the legume *Medicago truncatula* had shown highly variable Ca^{2+} spiking
169 profiles in root atrichoblasts in response to both AM fungal GSEs and short-chain COs such as CO4
170 (Genre et al., 2013; Russo et al., 2013). Since this also appears to be the case in rice (Sun et al.,
171 2015; this manuscript) comparisons between spiking responses to different AM elicitors and in
172 different host mutant backgrounds have been performed on the basis of the percentage of
173 responding atrichoblasts. As previously (Russo et al., 2013), we have considered two Ca^{2+} peaks
174 within a 30 min period as the minimum threshold for defining a positive spiking response, and for
175 each elicitor condition we present representative profiles for two cells. The total numbers of cells
176 and independent roots analyzed for each experimental condition are presented in Table S1 and
177 statistical tests were carried out using non-parametric analysis of variance (Kruskal-Wallis) with a
178 probability level of $p < 0.05$.

179

180

181 **Results**182 **AM fungal exudates fail to trigger symbiotic Ca²⁺ spiking in the *Oscerk1* mutant background**

183 When concentrated germinated spore exudate (GSE) prepared from the AM fungus *Gigaspora*
184 *margarita* was applied to the roots of the WT rice transgenic line expressing the nuclear-localized
185 NLS-YC2.60 cameleon, nuclear Ca²⁺ spiking was observed in approximately one third of root
186 atrichoblasts (Fig. 1 and 2). These spiking responses are reminiscent of those previously observed
187 on roots of both *Medicago* and *Daucus* (Genre et al. 2013) and the two representative profiles
188 shown in Fig. 1 underline the considerable irregularity and variability of the spiking responses. In
189 contrast, spiking was not observed in control experiments when the GSE was replaced by distilled
190 water (Fig. 1).

191 The NLS-YC2.60 cameleon was then introduced into the *Oscerk1* background, and roots of the
192 transgenic mutant line were treated with the crude fungal exudate. In contrast with WT,
193 atrichoblasts of the *Oscerk1* mutant failed to respond to the *G. margarita* GSE (Fig. 1). This was
194 confirmed statistically by quantifying the percentage of responding atrichoblasts based on the
195 combined results from a total of over 100 atrichoblasts and up to ten independent roots for each
196 line (Fig. 2; Table S1). In conclusion, in the absence of functional OsCERK1, rice roots are no longer
197 able to perceive the fungal signal molecules present in the AM GSE that normally trigger Ca²⁺
198 spiking in WT atrichoblasts.

199

200 **The *Oscerk1* mutant is also defective in responding to Myc-COs**

201 As stated earlier, short-chain chitin oligomers such as chito-tetraose (CO4) are biologically active
202 components of the AM GSE, capable of triggering epidermal Ca²⁺ spiking responses in both
203 *Medicago* and *Daucus* (Genre et al., 2013) as well as in rice (Sun et al., 2015). Furthermore, the
204 fact that strigolactones can boost Myc-CO levels in AM GSEs provides direct evidence for a pre-
205 infection molecular dialogue between symbiotic partners (Genre et al. 2013). CO4 activity was
206 therefore evaluated for both the WT and *Oscerk1* transgenic rice lines. Figure 1 shows that, as
207 expected, 10⁻⁵ M CO4 is able to initiate calcium spiking in root atrichoblasts of WT rice. However,
208 in line with the GSE treatment, *Oscerk1* mutants did not display the sustained Ca²⁺ spiking
209 response observed in the WT line (Fig. 1), and this was again confirmed by quantitative analysis
210 (Fig. 3). These results are therefore consistent with an essential role for OsCERK1 in a receptor

211 complex capable of activating the rice CSSP following the perception of Myc-COs as fungal
212 symbiotic factors.

213

214 **Both GSE and CO4 can elicit Ca^{2+} spiking in *Oscebip* and *Osnfr5* mutants**

215 *OsCERK1* is known to form a receptor complex with the LysM RLP *OsCEBiP* during defense-related
216 perception of long-chain chitin oligomers such as CO8 (Shimizu et al., 2010) and that a knock-out
217 mutation of the *OsCEBiP* gene is only defective in the host immunity response (Miyata et al.,
218 2014). For this reason it was important to examine whether the *Oscebip* mutant is still able to
219 respond to AM fungal signals. Results presented in Fig. 2 and Fig. S2 show that, in contrast to
220 *Oscerk1*, exogenous GSE treatment can trigger Ca^{2+} spiking responses in *Oscebip* root atrichoblasts.
221 Equally, the application of purified 10^{-5} M CO4 to roots of the *Oscebip* mutant elicited Ca^{2+} spiking
222 responses which could not be distinguished quantitatively from those observed with the wild type
223 line (Fig. 3; Fig. S2). These findings are therefore coherent with the lack of an AM phenotype for
224 *Oscebip*, and provide additional evidence that, in rice, distinct receptor complexes are required for
225 perceiving the appropriate chitin oligomers which activate either symbiotic or defense-related
226 downstream signaling pathways (Miyata et al. 2014; Shinya et al., 2015).

227 In the search for a potential LysM RLK/RLP partner for *OsCERK1* in perceiving AM fungal signals,
228 Miyata et al (2016) identified *OsNFR5* (previously known as *OsRLK2*) as the closest rice ortholog of
229 the Nod factor receptor component NFR5 from *Lotus japonicus*. The reasoning behind this was
230 based on the fact that NFR5 associates with a second LysM RLK, NFR1, for which *OsCERK1* is a
231 close ortholog. However, despite the induction of *OsNFR5* expression in rice roots following AM
232 fungal inoculation, knock-out mutants of this gene were colonized normally by AM fungi (Miyata
233 et al., 2016). To examine this further, we also evaluated the capacity of the *Osnfr5* mutant to
234 respond to the exogenous application of GSE/CO4 by introducing the nuclear Ca^{2+} ameleon
235 construct into the mutant background (Methods). The Ca^{2+} spiking data presented in Fig. 2, Fig. 3
236 and Fig. S2 show that, as for *Oscebip*, the percentage of *Osnfr5* root atrichoblasts capable of
237 responding positively to the application of either GSE or 10^{-5} M CO4 is statistically
238 indistinguishable from the WT line. In conclusion, the capacities of the three rice LysM RLK/RLP
239 mutants to respond to GSE/CO4 are fully in line with their corresponding AM phenotypes, thus
240 further underlining the pertinence of the Ca^{2+} spiking assay as a reliable indicator of host
241 perception of the symbiotic fungal signals required for initial root colonization.

242

243 **Discussion**

244 Since the breakthrough discovery of Nod factor LCOs as the key rhizobial signal molecules involved
245 in the initial molecular dialogue leading to successful legume nodulation, it has become a priority
246 to identify the equivalent “Myc factors” produced by endosymbiotic glomeromycota AM fungi.
247 Although several chitin-based molecules (Myc-COs and Myc-LCOs) secreted by AM fungi have
248 emerged as potential Myc factors from research in legumes (Maillet et al. 2011; Genre et al. 2013),
249 the evaluation of their biological significance as signaling molecules has been compromised both
250 by the absence of AM fungal genetic approaches as well as difficulties in identifying legume LysM
251 RLK/RLP receptors essential for initial fungal entry. To circumvent this, attention has turned to the
252 monocot rice, where recent findings have unexpectedly revealed that the *Oscerk1* mutant is not
253 only defective in immune defense responses, but also refractory to AM fungal colonization (Miyata
254 et al. 2014; Zhang et al. 2015). By introducing aameleon Ca^{2+} reporter into the AM-defective
255 *Oscerk1* background, we demonstrate here that this mutant is no longer able to perceive the
256 symbiotic signal molecules present in AM fungal exudates, as revealed by the failure to initiate
257 Ca^{2+} spiking in root atrichoblasts (Figs. 1&2). The triggering of these characteristic nuclear-
258 associated Ca^{2+} oscillations is considered a hallmark for the activation of the conserved CSSP
259 endosymbiotic signaling pathway (Oldroyd and Downie, 2006). Furthermore, these experiments
260 have also revealed that short chain Myc-COs such as CO4, whose concentrations are preferentially
261 enhanced in AM fungal exudates in the presence of host strigolactones (Genre et al. 2013), are no
262 longer able to elicit Ca^{2+} spiking when applied to roots of the *Oscerk1* mutant (Figs. 1&3).
263 Together, these findings argue firstly that the OsCERK1 LysM RLK is necessary for the successful
264 perception/transduction of AM fungal signals in rice, and secondly provide direct evidence for the
265 role of short-chain Myc-COs during this critical stage of host-fungal communication. In the light of
266 these results it will now be important to confirm the significance of Myc-COs during initial AM
267 fungal-host signaling in other plants, including both legumes and dicot non-legumes such as
268 tomato (Buendia et al., 2015) and *Parasponia andersonii* (Op den Camp et al., 2011) for which
269 LysM RLK RNAi knock-down experiments have revealed defective AM phenotypes.

270 What can we infer about the likely role of OsCERK1 in perceiving Myc-COs based on studies of the
271 immune defense receptor complex? Firstly, affinity labeling experiments have shown that OsCEBiP
272 is the major receptor for long-chain chitin oligomers such as CO8 in rice, whereas OsCERK1 does
273 not appear to directly bind chitin oligosaccharides (Kaku et al., 2006; Shinya et al., 2012; Kouzai et
274 al., 2014). Secondly, structural studies have revealed that long-chain chitin oligomers bind to two

275 molecules of *OsCEBiP* *via* the central LysM domains, and that this leads to receptor dimerization
276 (Hayafune et al., 2014; Liu et al., 2016), a requisite for the activation of defense signaling. The
277 current model therefore proposes that *OsCEBiP* homo-dimerization recruits *OsCERK1* to form the
278 active receptor complex (Shinya et al., 2015). This model also explains why CO4/CO5 are unable to
279 trigger defense signaling, since these short-chain oligosaccharides cannot simultaneously bind to
280 two LysM domains. However, they can act as antagonists to CO8-induced receptor dimerization
281 (Liu et al., 2012).

282 Since the *Oscebip* mutant is defective for chitin-triggered immunity, but unaffected for either AM
283 fungal colonization (Miyata et al., 2014) or the capacity to respond to both exogenous AM fungal
284 GSE and 10^{-5} M CO4 (Figs. 2&3), it appears unlikely that *OsCEBiP* has a role in
285 perceiving/transducing the symbiotic AM fungal signals. Bearing in mind that there is currently no
286 evidence for direct binding of the *OsCERK1* co-receptor to chitin oligomers and that all known
287 plant LysM RLP-mediated receptors comprise at least two partners, the most likely scenario at this
288 stage is that *OsCERK1* associates with a second LysM-containing membrane protein. Following this
289 reasoning, the role of one possible LysM RLK partner, *OsNFR5*, the rice ortholog of *LjNFR5/MtNFP*,
290 was examined by creating an *Osnfr5* knock-out mutant. However, this mutant can be successfully
291 colonized by AM fungi (Miyata et al., 2016), and also responds with nuclear Ca^{2+} spiking to both
292 exogenous AM GSE and CO4 (Figs. 2&3). Furthermore, no heterodimerization could be observed
293 between *OsCERK1* and *OsNFR5* in BiFC assays (Miyata et al. 2016). Future research will now need
294 to focus on additional candidate rice LysM-based receptors by evaluating both the AM phenotypes
295 and the GSE/CO4-responsiveness of the respective mutant lines.

296

297 **Acknowledgements**

298 We are grateful to Mara Novero for statistical analyses and assistance in GSE preparation,
299 Valentina Fiorilli for optimizing rice seed sterilization. Financial support was provided to AG by
300 UNITO grant Ricerca Locale 2015 and to MC and DGB by the French National Research Agency
301 grant ANR-12-BSV7-0007-02 and the 'TULIP' Laboratory of Excellence grant ANR-10-LABX-41.

302

303 **Author Contribution**

304 G.C., M.Ch. and M.C. performed experiments and data analysis. N.T., H.K. and K.M. developed the
305 transgenic plant lines. A.G., D.B., M.Ch. and T.N. wrote the manuscript. A.G., D.B., M.Ch., N.S. and
306 T.N. designed the experiments.

307

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445

446 **Figure legends**

447 **Figure 1. Nuclear Ca²⁺ spiking in response to AM fungal signals in both wild type and *Oscerk1***
448 **mutant lines.** The figure shows representative profiles from independent atrichoblasts of wild
449 type and *Oscerk1* mutant roots treated with sterile water (control), 10-fold concentrated *G.*
450 *margarita* germinated spore exudate (GSE) or 10⁻⁵ M chito-tetraose (CO4). Both GSE and CO4
451 trigger a sustained response in the wild type, but not in *Oscerk1* plants, indicating a role for
452 OsCERK1 in the perception of AM fungal signals in *Oryza sativa*. Values on the Y axis represent the
453 ratio between YFP and CFP fluorescence.

454

455 **Figure 2. Percentage of responding atrichoblasts in wild type, *Oscerk1*, *Oscebip* and *Osnfr5***
456 **mutant roots of rice in response to *G. margarita* GSE.** GSE (10-fold concentrated) treatment
457 (grey) elicited a spiking response in 33% of wild type, 40% of *Osnfr5* and 59% of *Oscebip*
458 atrichoblasts, but failed to trigger spiking in the *Oscerk1* background (asterisk indicates a
459 statistically significant difference). Bars on each histogram indicate standard deviations.
460 Differences between water controls (white) were not statistically significant.

461

462 **Figure 3. Percentage of responding atrichoblasts in wild type, *Oscerk1*, *Oscebip* and *Osnfr5***
463 **mutant rice roots in response to 10⁻⁵ M CO4.** Nuclear Ca²⁺ spiking was elicited in a significant
464 proportion of root atrichoblasts from wild type (51%), *Oscebip* (31%) and *Osnfr5* (31%) lines
465 treated with 10⁻⁵ M CO4 (grey), but not in the *Oscerk1* mutant (asterisk indicates a statistically
466 significant difference). Bars on each histogram indicate standard deviation. Differences between
467 water controls (white) were not statistically significant.

468

469 **Supporting Information**

470

471 **Figure S1. Representative Ca^{2+} spiking profiles and percentage of responding atrichoblasts in**
472 **wild type rice roots treated with increasing concentrations of CO_4 .** Based on these results, the
473 concentration of 10^{-5}M CO_4 was chosen as the most appropriate for the study of nuclear Ca^{2+}
474 spiking responses in rice plantlet primary root (see Materials and Methods). Bars represent
475 standard deviation; asterisks indicate statistically significant differences compared to the water
476 control. A minimum of 15 atrichoblasts from two independent roots was used for each condition.

477

478 **Figure S2. Representative Ca^{2+} spiking profiles observed for the *Oscebip* and *Osnfr5* mutants**
479 **treated with either *G. margarita* GSE or 10^{-5} M CO_4 .** As compared to *Oscerk1* (Fig. 1) spiking
480 responses were detected for both rice mutants treated with either fungal elicitor.

481

482 **Table S1. Number of atrichoblasts per experimental condition used for the various statistical**
483 **analyses.** Numbers in brackets indicate the number of independent roots used for each condition.

484

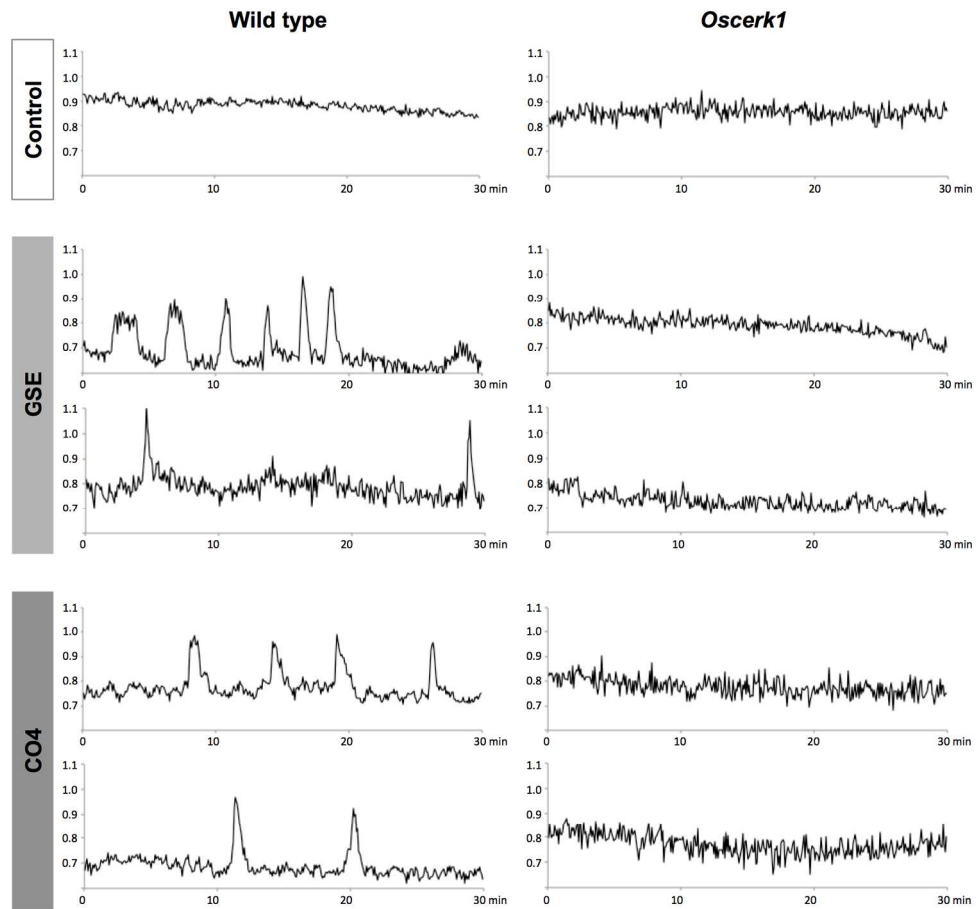


Figure 1. Nuclear Ca^{2+} spiking in response to AM fungal signals in both wild type and *Osker1* mutant lines. The figure shows representative profiles from independent atrichoblasts of wild type and *Osker1* mutant roots treated with sterile water (control), 10-fold concentrated *G. margarita* germinated spore exudate (GSE) or 10^{-5} M chito-tetraose (CO4). Both GSE and CO4 trigger a sustained response in the wild type, but not in *Osker1* plants, indicating a role for *OscERK1* in the perception of AM fungal signals. Values on the Y axis represent the ratio between YFP and CFP fluorescence.

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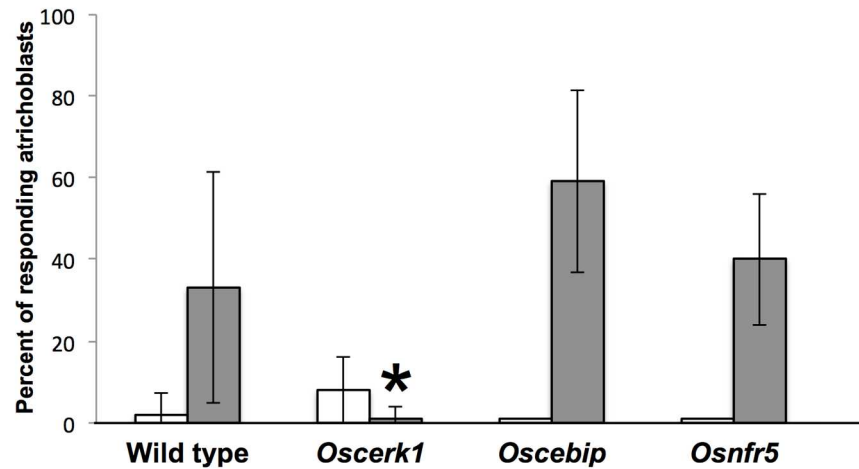


Figure 2. Percentage of responding atrichoblasts in wild type, *Osker1*, *Oscebip* and *Osnfr5* mutant roots in response to *G. margarita* GSE. GSE (10-fold concentrated) treatment (grey) elicited a spiking response in 33% of wild type, 40% of *Osnfr5* and 59% of *Oscebip* atrichoblasts, but failed to trigger spiking in the *Osker1* background (asterisk indicates a statistically significant difference). Bars on each histogram indicate standard deviations. Differences between water controls (white) were not statistically significant.

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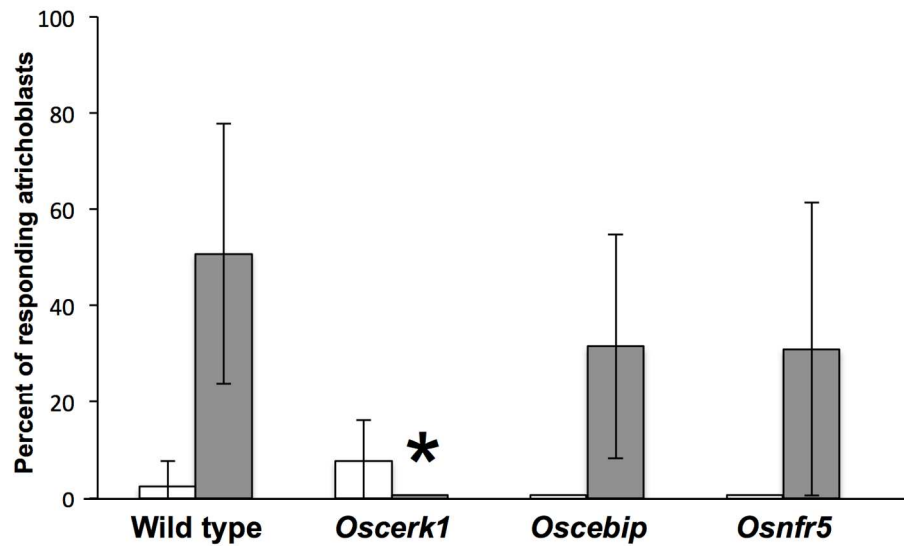


Figure 3. Percentage of responding atrichoblasts in wild type, Oserk1, Oscebp and Osnfr5 mutant roots in response to 10⁻⁵ M CO₄. Nuclear Ca²⁺ spiking was elicited in a significant proportion of root atrichoblasts from wild type (51%), Oscebp (31%) and Osnfr5 (31%) lines treated with 10⁻⁵ M CO₄ (grey), but not in the Oserk1 mutant (asterisk indicates a statistically significant difference). Bars on each histogram indicate standard deviation. Differences between water controls (white) were not statistically significant.

142x90mm (300 x 300 DPI)