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Difference in *Striga*-susceptibility is reflected in strigolactone secretion profile, but not in compatibility and host preference in arbuscular mycorrhizal symbiosis in two maize cultivars

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Summary

• Strigolactones released from plant roots trigger both seed germination of parasitic weeds such as *Striga* spp. and hyphal branching of the symbionts arbuscular mycorrhizal (AM) fungi. Generally, strigolactone composition in exudates is quantitatively and qualitatively different among plants, which may be involved in susceptibility and host specificity in the parasite-plant interactions. We hypothesized that difference in strigolactone composition would have a significant impact on compatibility and host specificity/preference in AM symbiosis.

• Strigolactones in root exudates of *Striga*-susceptible (Pioneer 3253) and -resistant (KST 94) maize (*Zea mays*) cultivars were characterized by LC–MS/MS combined with germination assay using *Striga hermonthica* seeds. Levels of colonization and community compositions of AM fungi in the two cultivars were investigated in field and glasshouse experiments.

• 5-Deoxystrigol was exuded exclusively by the susceptible cultivar, while the resistant cultivar mainly exuded sorgomol. Despite the distinctive difference in strigolactone composition, the levels of AM colonization and the community compositions were not different between the cultivars.

• The present study demonstrated that the difference in strigolactone composition has no appreciable impact on AM symbiosis, at least in the two maize cultivars, and further suggests that the traits involved in *Striga*-resistance are not necessarily accompanied by reduction in compatibility to AM fungi.

Introduction

The root parasitic weeds, witchweeds (*Striga* spp.) occur mainly in sub-Saharan Africa and pose the greatest economic threat to agriculture in the area (Parker, 2012). The seeds of *Striga* spp. germinate only when chemical stimulants from plant roots are perceived, and among known stimulants, strigolactones are one of the strongest; they induce seed germination at a concentration as low as 10^{-12} M (Kim *et al.*, 2010). Since the isolation and structural elucidation of the first strigolactone, strigol, from cotton root exudates (Cook *et al.*, 1966, 1972), more than 20 strigolactones have been characterized across the plant kingdom (Kisugi *et al.*, 2013; Xie *et al.*, 2013). *Striga* spp. widely prevail in nutrient-poor soils, and fertilizer application generally decreases their emergence (Raju *et al.*, 1990; Jain & Foy, 1992; Cechin & Press, 1993; Mumera & Below, 1993; Abu Irmaileh, 1994). It has now been widely accepted that deficiency in mineral nutrients, especially phosphorus (P) and nitrogen (N), promotes strigolactone exudation (Yoneyama *et al.*, 2007a,b, 2012; López-Ráez *et al.*, 2008; Jamil *et al.*, 2012).

Most terrestrial plants associate with arbuscular mycorrhizal (AM) fungi and take up a significant proportion of P via the fungi, particularly under P-deficient conditions (Smith & Read, 2008). Strigolactones stimulate not only the seed germination of the parasites but also hyphal branching of AM fungi (Akiyama *et al.*, 2005), which increases probability of contact between the fungi and the roots. Therefore, plants increase strigolactone exudation under conditions of nutrient deficiency as they need to attract AM fungi.

Quantitative and qualitative differences in strigolactone composition are likely to be critical for triggering interactions with the parasitic/symbiotic organisms (Jamil *et al.*, 2011a,b). In AM associations, strigolactone-deficient pea (Gomez-Roldan *et al.*, 2008) and tomato (Koltai *et al.*, 2010) mutants were less compatible to AM fungi i.e. showed significantly lower levels of colonization than the wild types. Not only quantitative differences but also structural differences in strigolactone critically affect hyphal branching-stimulatory activity; 5-deoxystrigol, a nonhydroxy-strigolactone, was 30-fold more active than sorgomol, a hydroxy-strigolactone, in an AM fungus *Gigaspora margarita* (Akiyama *et al.*, 2010). In the parasite-plant interactions, *Striga*-susceptible sorghum cultivars mainly exuded 5-deoxystrigol (Awad *et al.*, 2006; Yoneyama *et al.*, 2010), whereas sorgomol was the major strigolactone exuded by a *Striga*-resistant cultivar (Yoneyama *et al.*, 2010).

These observations led us to raise an important question; whether susceptibility to the parasitic plants correlates with compatibility to AM fungi. That is, more resistant genotypes that attract *Striga* spp. to lesser extents may attract AM fungi to lesser extents, which would potentially have a negative impact on crop productivity, particularly in nutrient-poor soils.

Although AM fungi show no apparent host specificity/preference in single species-based inoculation experiments (Smith & Read, 2008), different plant species harbor different AM fungal assemblages in the field (Sanders, 2003). On the other hand, it has been well documented that strigolactone composition in root exudates is qualitatively and quantitatively different among plant species/cultivars (Yoneyama *et al.*, 2008, 2011, 2012; Jamil *et al.*, 2011a,b), and in several holoparasitic *Orobanche* spp. that show strict host specificity, seed germination occurs only when the seeds are exposed to the root exudates from their host plants (Fernández-Aparicio *et al.*, 2009). These observations suggest that strigolactones, at least in part, mediate host-parasite specificity (Xie *et al.*, 2010), leading us to hypothesize that strigolactones also act as a selective factor in the process of AM fungal colonization, that is, strigolactones mediate host specificity/preference in the symbiosis. To examine this hypothesis, the same plant species that exude different strigolactones are ideal material.

In the present study, the hypothesis that qualitative and quantitative differences in strigolactone composition are involved in compatibility and host preference in AM symbiosis, particularly under P-deficient conditions in which strigolactone exudation is enhanced, was addressed. To test the hypothesis, strigolactones exuded by two maize cultivars that differ in *Striga*-susceptibility were first characterized qualitatively and quantitatively by means of *S. hermonthica*-germination assay and LC–MS/MS. Secondly, responses of AM fungal community to the two contrasting cultivars were investigated using three natural AM fungal populations originated from arable soils with distinct P fertility.

Materials and Methods

Plant material and chemicals

Pioneer 3253 and KST 94 are *Striga*-susceptible and -resistant cultivars, respectively, of maize (*Zea mays* L.) selected by the International Maize and Wheat Improvement Center

(CIMMYT) in Kenya. The seeds of *Striga hermonthica* (Del.) Benth. were generously supplied by Prof. A.G.T. Babiker (Sudan University of Science and Technology, Sudan). Sorgomol was purified from root exudates of sorghum (Xie *et al.*, 2008). 5-Deoxystrigol was a generous gift from Prof. Kohki Akiyama (Osaka Prefecture University). [3a,4,4,5,5,6'-$^{2}H_6$]-5-Deoxystrigol (Ueno *et al.*, 2010) was a generous gift from Prof. Tadao Asami (The University of Tokyo). The other chemicals of analytical grade and HPLC solvents were obtained from Kanto Chemical Co. Ltd. and Wako Pure Chemical Industries Ltd.

Characterization of strigolactones in root exudate

Seeds of *Striga*-susceptible Pioneer 3253 and -resistant KST 94 maize cultivars were surface-sterilized, germinated in autoclaved sand, grown for 10 d in a glasshouse or a growth chamber, transplanted to 5 l of half-strength Tadano and Tanaka (1/2 TT) medium (Tadano & Tanaka, 1980) without phosphate in a container (28.5 × 23.5 × 11 cm, W × L × H), and grown in the growth chamber with a 14-h photoperiod at 120 µmol photons m^{-2} s^{-1} at 28/25°C day/night temperature (n = 3). The culture media were replaced every other day. After 2 wk of acclimatization, strigolactones exuded in the media were adsorbed separately onto activated charcoal by using a circulation pump for 24 h, extracted, and subjected to LC–MS/MS analysis. Full details of LC–MS/MS analysis are presented in Supporting Information Methods S1. A portion of the extracts was fractionated by reversed-phase (RP)-HPLC under the same conditions as for the LC–MS/MS analysis, and the fractions collected every 30 s were examined for *S. hermonthica* seed germination stimulation (Yoneyama *et al.*, 2007a).

To quantify strigolactones exuded under various nutrient conditions, 10-day-old seedlings grown in autoclaved sand in the growth chamber was transplanted to the 1/2 TT medium (500 ml) and to the medium from which either N or P was withheld in plastic cups (9.5 cm in diameter, 17 cm in height) and grown in the growth chamber for 10 d (n = 3). The growth media were collected at 10, 13, 16, and 19 d after transplanting for 24 h, followed by the addition of 5-deoxystrigol-d_6 (300 pg) as internal standard, and extracted three times with an equal volume of ethyl acetate. The ethyl acetate solutions were combined, washed with 0.2 M K_2HPO_4 (pH 8.3), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*, and known strigolactones in the extracts were quantified as described in Supplementary Information Methods S1.

Responses of arbuscular mycorrhizal fungal community

To examine responses of diverse AM fungal communities, a glasshouse experiment using two arable soils that differ in P fertility was conducted in addition to a field experiment. For the glasshouse experiment, 120 kg of soil was collected from each of the experimental fields in Hokkaido University (H-soil) in Sapporo and Utsunomiya University (U-soil) in Utsunomiya, Japan. The level of available P in the U-soil was an order of magnitude lower than that in the H-soil (Table S1). The H-soil and U-soil were mixed with autoclaved river sand at ratios of 2:3 and 1:1 (v/v), respectively, and used as culture media. Seeds of the susceptible and resistant cultivars were surface-sterilized, sown in the soil-sand mixtures in plastic pots (18 cm in diameter, 15 l in volume), thinned to one plant per pot a week after sowing, and grown for 5 wk in a temperature/humidity/light-controlled glasshouse (26°C, 60% relative humidity, 14-h day length) (n = 4). The roots were harvested from each pot separately and washed with tap water. For the field experiment, seeds of the two cultivars were sown in a plot (1 × 5 m) at a 50 : 30 cm (interrow : intrarow) spacing on 9th June 2011 (four plants per row, ten rows for each cultivar) in the experimental field of Utsunomiya University. The available P level of the field soil was four-fold higher than that of the H-soil (Table S1). After 52 d, a plant was randomly chosen from each of the 10 rows (ten samples). The root system was collected from an area of 30 cm in diameter × 20 cm in depth, and about 30 g of fine roots was collected from each plant separately and washed with tap water.

In both experiments, the root samples were cut into 2–3 cm segments immediately after harvest, randomized in water, collected on a sieve, and divided into two subsamples. One-half of the sample was frozen in liquid nitrogen, freeze-dried for 2 d, and stored at –30°C for DNA extraction, and the other half was stained with trypan blue for the assessment of mycorrhizal colonization by the gridline intersect method (Giovannetti & Mosse, 1980). DNA was extracted from the freeze-dried roots with DNeasy Plant Mini Kit (Qiagen), and a partial sequence of fungal large subunit (LSU) ribosomal RNA gene (rDNA) was amplified with the eukaryote-universal forward and the fungi-specific reverse primers (Table S2) according to Kawahara & Ezawa (2013). In the glasshouse experiment, each PCR product (n = 4) was cloned separately to construct a clone library, and the nucleotide sequences of randomly chosen clones from each library were determined by dideoxy-cycle sequencing method

(Sangar sequencing). In the field experiment (ten samples), two PCR products were combined (n = 5), tagged, and sequenced using the 454 GS-FLX Titanium system (Roche Diagnostics, Tokyo) at Hokkaido System Science Co., Ltd., Sapporo, Japan (454 sequencing). The sequences obtained by Sangar and 454 sequencing were combined, and AM fungal phylotypes were defined based on ≥ 95% sequence similarity with ≥ 97% bootstrap support in phylogenetic analysis. The accession numbers of the representative sequences of each phylotype, including those newly obtained in this study, are listed in Table S3. All statistical analyses were performed with R 2.15.0 (R Development Core Team, 2012). Full details of sequencing, phylotype definition, and statistical analysis are presented in Supporting Information Methods S2.

Results

Characterization of strigolactones in *Striga*-resistant and -susceptible cultivars

The two maize cultivars, *Striga*-susceptible Pioneer 3253 and resistant KST 94, grown hydroponically showed no apparent differences in growth characteristics (data not shown). A four-channel MRM chromatogram and the distribution of germination stimulation activity on *S. hermonthica* after RP-HPLC separation of the root exudates are shown in Fig. 1. Both cultivars exuded SL2 (arrow 1), SL1 (arrow 2), and sorgomol (arrow 3), while 5-deoxystrigol (arrow 4) was found only in the susceptible cultivar Pioneer 3253. Germination stimulation activities on *S. hermonthica* seed in the fractions corresponding to SL1, sorgomol, SL2, and 5-deoxystrigol could be identified in reference to the retention times in the LC–MS/MS analysis. Although there were several unidentified active fractions, the results in the germination assay were generally in agreement with those in the LC–MS/MS analysis; one distinct difference between the two cultivars is that strong activity corresponding to 5-deoxystrigol was observed only in Pioneer 3253.

Profiling of strigolactone exudation with respect to nutrient deficiency

Strigolactone levels in the root exudates were quite low or below the detection limit under the normal nutrient conditions in both cultivars (Fig. 2). Although SL2 exudation was promoted

by N deficiency as well as by P deficiency in both cultivars, the amounts of SL2 exuded in response to the nutrient deficiencies were much greater in the susceptible cultivar Pioneer 3253. SL1 exudation was highly enhanced by P deficiency in Pioneer 3253. P deficiency, but not N deficiency, enhanced exudation of sorgomol, particularly in the resistant cultivar KST 94. 5-Deoxystrigol was exuded only in Pioneer 3235 under the P-deficient conditions. SL2 and SL1 exudations in Pioneer 3235 were generally higher in the early stage (i.e. at 10-d after transplanting) than in the later stage, whereas the exudation of sorgomol in KST 94 and that of 5-deoxystrigol in Pioneer 3235 gradually increased in the later stage.

Responses of arbuscular mycorrhizal fungal communities to *Striga*-susceptible and -resistant cultivars

The levels of mycorrhizal colonization significantly differed among different growth conditions (glasshouse with two soils and field) (F = 4.697, P = 0.019), but not between the cultivars (F = 2.480, P = 0.128), although the levels seemed slightly but consistently higher in the susceptible cultivar Pioneer 3235 than in the resistant cultivar KST 94 (Fig. 3). The interaction between growth condition and cultivar was not significant (F = 0.004, P = 0.996).

Fungal LSU rDNA was successfully amplified from all of the root samples; AM fungal sequences obtained in Pioneer 3235 and KST 94 were 7,229 and 6,727 reads, respectively, in the field (454 sequencing), 178 and 172 clones, respectively, in the glasshouse with the H-soil (Sanger sequencing), and 152 and 158 clones, respectively, in the glasshouse with the U-soil (Sanger sequencing). In total, 109 phylotypes were defined across seven families: 10 phylotypes in Gigasporaceae, 51 phylotypes in Glomeraceae, 14 phylotypes in Paraglomeraceae, 14 phylotypes in Claroideoglomeraceae, 3 phylotypes in Diversisporaceae, 11 phylotypes in Acaulosporaceae, and 4 phylotypes in Archaeosporaceae, in addition to two phylotypes that could not be assigned to any of the known families. Detailed information about the reference sequences and frequencies (clone/read numbers) of the individual phylotypes is presented in Table S3. Rarefaction curves showed signs of leveling off, suggesting that our sampling provides reasonable coverage of AM fungal richness (Fig. S1). Phylotype richness (total number of phylotype) and Shannon diversity index were not significantly different between the two cultivars in all experiments (Table S4). The DCA sample plot indicated that the communities in the field and those of the two soils in the

glasshouse experiments were clearly separated along axis 1 that explained 23.5% of total variance, but separation between Pioneer 3235 and KST 94 was ambiguous in all experiments (Fig. 4). Two-way PERMANOVA confirmed that growth conditions significantly affected the community compositions (Pseudo-F = 64.903, P = 0.001), but cultivars (Pseudo-F = 0.755, P = 0.491) and their interaction (Pseudo-F = 0.405, P = 0.840) did not.

Discussion

The present study for the first time demonstrated that the difference in strigolactone composition has no appreciable impact on compatibility to AM fungi. 5-Deoxystrigol was exuded exclusively in the susceptible maize cultivar that also exuded much larger amounts of SL2 and SL1, whereas the resistant cultivar exuded mainly sorgomol. These results are consistent with the observations in sorghum (Awad *et al.*, 2006; Yoneyama *et al.*, 2010), suggesting that 5-deoxystrigol may play a key role in the parasite-plant interactions at least in some maize and sorghum cultivars. However, no differential responses of AM fungi to the susceptible and resistant cultivars were observed in the present study, even in the highly P-deficient soil (U-soil) in which differences in the composition and amount of strigolactones might be distinct between the cultivars. These observations suggest that AM fungi respond to structurally different strigolactones similarly, inconsistent with the observations that the hyphal branching-stimulatory activity of 5-deoxystrigol was much higher than sorgomol in the *in vitro* assay system (Akiyama *et al.*, 2010). It seems likely that the action of strigolactones in soil, in which the compounds may be degraded much more rapidly than in the *in vitro* system, is more complex than expected, and a technical breakthrough for the assessment of the activity in the soil system is necessary for understanding of the interactions mediated by strigolactones.

One might expect, however, that AM fungi also differentially respond to the two cultivars as *Striga* spp.; the fungi colonized the resistant cultivar to a lesser extent at the initial phase, but after the establishment of association the colonies extended to the same levels as those in the susceptible cultivar. If this is the case, the communities in the resistant cultivar would be less diverse, because the root exudate would activate fewer spores i.e. attract stochastically fewer species. However, given that AM fungal diversity (richness and Shannon index) was not different between the cultivars, this possibility seems unlikely.

The community compositions of AM fungi were not different between the cultivars, even under the highly P-deficient conditions (i.e. in the U-soil), suggesting that the differences in strigolactone composition are not involved in host specificity/preference in AM symbiosis. This observation further implies that responsiveness to structurally diverse strigolactones did not differentiate among AM fungal species, even during the long history of coevolution with land plants (Phipps & Taylor, 1996; Redecker *et al.*, 2000). The absence of the differentiation may have important implications in the evolution of the fungi; the lack of specificity to particular strigolactones has maintained the broad host range in individual species, which in turn reduced a risk for extinction and thus has maintain species diversity in the fungi, rather than increasing specificity to particular strigolactones produced by a narrow range of plant species.

We demonstrated that the *Striga*-resistant maize cultivar KST 94 is a compatible host for AM fungi under the field conditions. In Africa, soil fertility is poor, while fertilizers are more expensive than in developed countries. Therefore, the enhancement of nutrient acquisition via AM associations is essential to improve crop productivity. In this context, the present study has significant implications for agricultural production in Africa; of the traits involved in *Striga*-resistance would not necessarily be accompanied by reduction in compatibility to AM fungi, ensuring that the breeding program is a promising strategy for protection of crops against *Striga* spp. Whereas the involvement of difference in strigolactone composition in susceptibility/resistance to *Striga* spp. has not been fully understood yet and to be elucidated.

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Figures

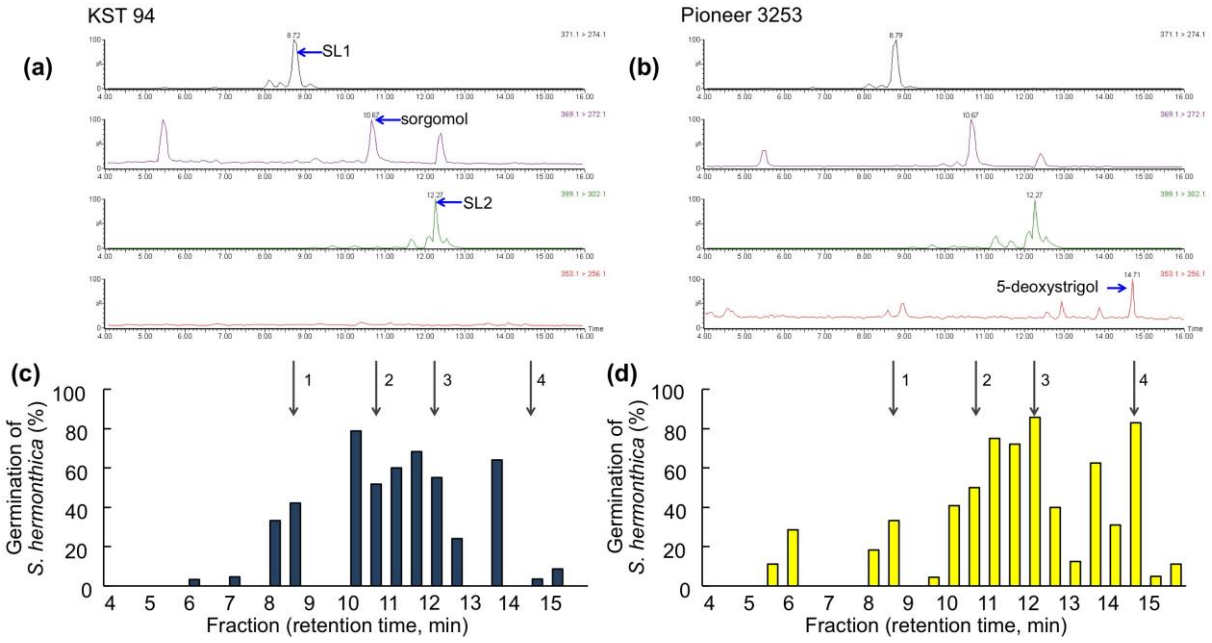

Figure 1. Four-channel multiple reaction monitoring (MRM) chromatograms of the root exudates from *Striga*-resistant maize (*Zea mays*) cultivar KST 94 (a) and -susceptible maize cultivar Pioneer 3253 (b), where the transitions of *m/z* 371→274, 369→272, 399→302, and 353→256 were monitored for SL1 (and isomers, arrow 1), sorgomol (arrow 2), SL2 (and isomers, arrow 3), and 5-deoxystrigol (arrow 4), respectively. Distribution of germination-stimulation activities on *S. hermonthica* seeds in the root exudates of *Striga*-resistant cultivar KST 94 (c) and -susceptible cultivar Pioneer 3253 (d) after separation by reversed-phase high-performance liquid chromatography. The eluate was collected for 30 s into 5-cm Petri dishes lined with a filter paper (e.g., fraction 8.5 contained eluate collected from 8.50 min to 8.99 min). After evaporation of the solvent, three discs carrying conditioned *S. hermonthica* seeds were placed and wetted with 650 µl of sterile Milli-Q water. The seeds were incubated in the dark for 24 h and then germination rates were measured. The percent germination of the seeds treated with sterile Milli-Q water and 1 µM GR24 (synthetic strigolactone) was 0% and 78%, respectively.

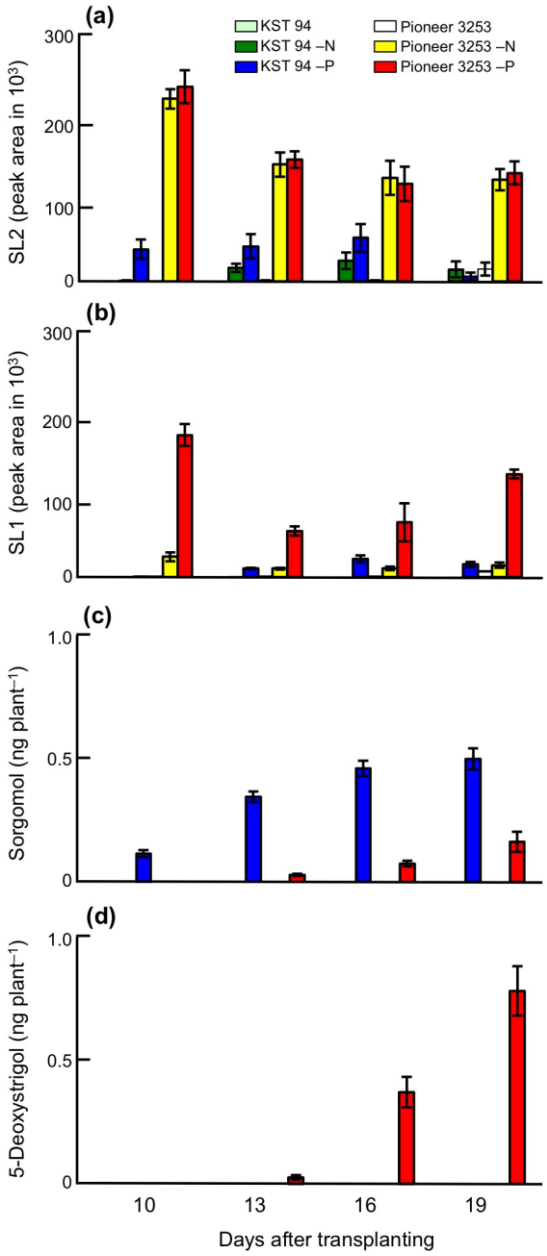

Figure 2. Time-course characteristics of SL2 (a), SL1 (b), sorgomol (c), and 5-deoxystrigol (d) exudation by the roots of *Striga*-resistant maize (*Zea mays*) cultivar KST 94 (pale blue, green, and dark blue columns) and -susceptible maize cultivar Pioneer 3253 (white, orange, and red columns) grown with 1/2 Tadano and Tanaka medium (control, pale blue and white) and in the absence of either nitrogen (–N, green and orange) or phosphorus (–P, dark blue and red) in hydroponic culture (n = 3). The root exudates were collected from the culture media 10, 13, 16 and 19 d after transplanting. nd, not detected. Vertical bars indicate means ± *s.e.*

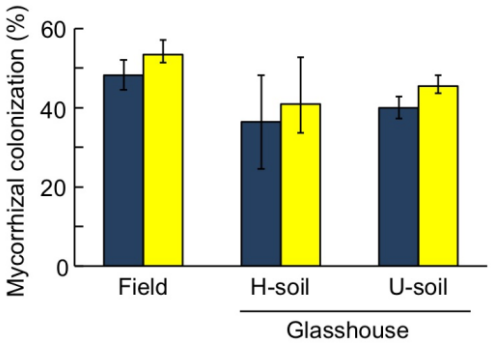

Figure 3. Percentage mycorrhizal colonization in *Striga*-resistant maize (*Zea mays*) cultivar KST 94 (dark blue columns) and -susceptible maize cultivar Pioneer 3253 (yellow columns) grown in the field for 7 wk (n = 10) and in the glasshouse for 5 wk using the soils collected from Hokkaido University (H-soil) and Utsunomiya University (U-soil) (n = 4). Vertical bars indicate means ± *s.e.*

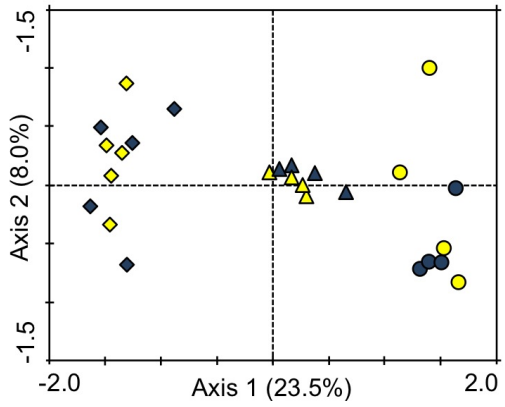

Figure 4. Sample plot of detrended correspondence analysis on the AM fungal communities in the roots of *Striga*-resistant maize (*Zea mays*) cultivar KST 94 (dark blue symbols) and -susceptible maize cultivar Pioneer 3253 (yellow columns) grown in the field (diamonds) and in the glasshouse using the soil collected from Hokkaido University (H-soil, triangles) and Utsunomiya University (U-soil, circles).

436 **Supporting Information**

437

438 **Methods S1** LC-MS/MS analysis

439

440 HPLC separation was conducted with a LaChromUltra UHPLC instrument (Hitachi, Tokyo,
441 Japan) fitted with an ODS (C_{18}) column (LaChromUltra C_{18}, 2 × 50 mm, 2 μm; Hitachi,
442 Tokyo, Japan). The crude extracts dissolved in acetonitrile (50 □l) were filtered through spin
443 columns (Ultra-Free MC, 0.45 μm pore size; Millipore, Tokyo, Japan), and 3 □l of the
444 solutions was injected into the HPLC column connected to a tandem mass spectrometer. The
445 mobile phase was a water–methanol gradient. Separation started at 30% methanol (v/v),
446 followed by a 3-min gradient to 45% methanol, by a 5-min gradient to 50% methanol, by a
447 4-min gradient to 70% methanol, and then by a 3-min gradient to 100% methanol, which was
448 maintained for 5 min, followed by a 1-min gradient back to 30% methanol. The column was
449 equilibrated at this solvent composition for 2 min before the next run. The total run time was
450 23 min. The flow rate was 0.2 ml min^{-1} and the column temperature was set to 40°C.
451 Mass spectrometry was performed with a Quattro LC mass spectrometer (Micromass,
452 Manchester, UK) equipped with an electrospray source as previously described (Yoneyama *et*
453 *al.*, 2011). The transitions of *m/z* 399 to 302, 371 to 274, 369 to 272, 353 to 256, and 359 to
454 262 were monitored for SL2, SL1 (Jamil *et al.*, 2012), sorgomol, 5-deoxystrigol, and
455 5-deoxystrigol-d_6, respectively. Retention times of strigolactones under these analytical
456 conditions were 8.7, 10.6, 12.2, and 14.7 min for SL1, sorgomol, SL2, and 5-deoxystrigol,
457 respectively. Quantification of sorgomol was conducted using natural standard in a manner
458 similar to that described previously (Yoneyama *et al.*, 2007a,b). For SL1 and SL2, both were
459 found to be mixtures of isomers, peak areas were used for comparison of their quantities. Data
460 acquisition and analysis were performed with the MassLynx software (ver. 4.1).

461

462 **Methods S2** Sequencing, phylotype definition, and statistical analysis

463

464 For 454 sequencing, two PCR products were combined and diluted 10 times with deionized
465 water, and the sequence adaptors and a multiplex identifier (MID, 10 bp) tag were appended
466 to both ends by amplification with the LR1 primer linked with the 454 adapter B and the
467 FLR2 primer linked with a MID tag followed by the 454 adapter A under the same PCR

conditions, except for the cycle number (10 cycles in this case). Different MID sequences were allocated to each sample (n = 5) for sorting after sequencing. The MID-tagged amplicons were separated by electrophoresis, purified, and sequenced using the 454 GS-FLX Titanium system (Roche Diagnostics, Tokyo) at Hokkaido System Science Co., Ltd. (Sapporo, Japan) (454 sequencing).

Before sequence analysis, the region between either of the consensus sequences, 5'-GTGAAATTG-3' or 5'-GTGAGATTG-3' located at 320–400 bp upstream of the 3' end, and the 3' end (FLR2 sequence) was truncated in all sequences. The sequences data obtained by Sangar and 454 sequencing were combined and subjected to BLAST searches against the NCBI nucleotide sequence database. In this process, the closest relatives of the sequences were selected at a criterion of ≥ 95% similarities and an E value ≤ $10e^{-100}$. The sequences that did not show ≥ 95% sequence similarities to published sequences were grouped using the CD-HIT program (Li & Godzik, 2006) with a minimum similarity of 95%, and singletons were excluded at this step. Representative sequences were then randomly chosen from the individual 95%-similarity groups created by CD-HIT, and the validity of the sequences (groups), *i.e.*, whether they were chimeric or not, was carefully assessed by comparing published sequences. The sequences of the closest relatives and the representative sequences of each 95%-similarity group were combined and aligned using MAFFT version 6.9 (Katoh *et al*., 2002), and a neighbor-joining tree was constructed using TOPALi v2.5 (Milne *et al*., 2004), in which confidence limits of each branch were assessed by 500 bootstrap replications. AM fungal phylotypes were defined based on ≥ 97% bootstrap support, and those that were unlikely to belong to Glomeromycota were excluded at this step.

All statistical analyses were performed with R 2.15.0 (R Development Core Team, 2012). For ordination analysis, the Vegan package (Oksanen *et al.*, 2011) for R was employed. To standardize the sequenced clone/read numbers among the glasshouse and field experiments, the clone/read numbers of individual phylotypes were divided by the total sequenced clone/read number of the samples for ordination analysis. Detrended correspondence analysis (DCA) (Hill & Gauch, 1980) was first applied, and then the significance of difference between the cultivars was assessed by permutation multivariate analysis of variance (PERMANOVA) function adonis using the Morisita-Horn distance (999 permutations). Rarefaction curves were constructed with Analytic Rarefaction v2.0 (http://www.uga.edu/strata/software/index.html).

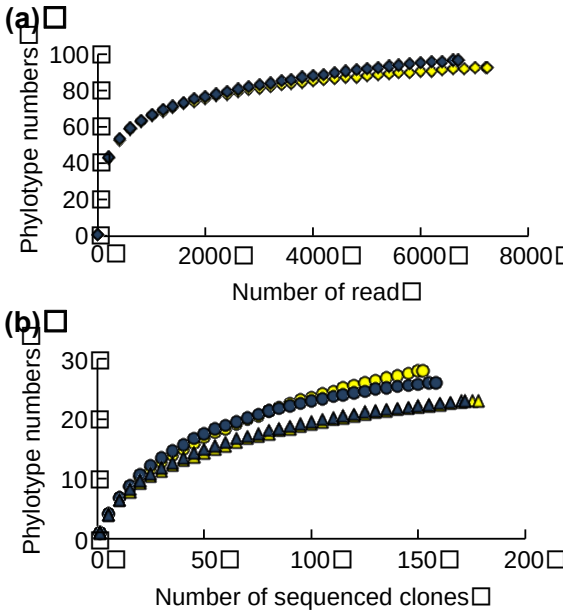

Figure S1. Sampling effort curves of AM fungal phylotype richness in *Striga*-resistant maize (*Zea mays*) cultivar KST 94 (dark blue symbols) and -susceptible maize cultivar Pioneer 3253 (yellow symbols) grown in the field (**a**) and in the greenhouse (**b**) using the soil collected from Hokkaido (triangles) and Utsunomiya Universities (circles). The curves were constructed using Analytic Rarefaction 2.0.

508 **Table S1.** Chemical properties of the soils.

Experiment	pH (H_2O)	Total N (g-N kg^{-1})	Total C (g-C kg^{-1})	Available P (g-P kg^{-1})
Field	5.4	3.8	55.6	99.2
Glasshouse[a]				
H-soil	6.0	0.6	9.8	25.3
U-soil	4.9	1.6	24.8	2.6

509 [a] The soils were collected from the experimental field in Hokkaido University (H-soil) (17 m
510 altitude, 43˚04'N, 141˚20'E) and the university farm in Utsunomiya University (U-soil) (110
511 m altitude, 36˚33'N, 139˚55'E).
512

513 **Table S2.** PCR primers used in this study.

Primer name	Sequence (5' to 3')	Note	Reference
LR1	GCA TAT CAA TAA GCG GAG GA	Eukaryotic LSU rDNA-universal forward primer	van Tuinen *et al.* (1998)
FLR2	GTC GTT TAA AGC CAT TAC GTC	Fungal LSU rDNA-specific reverse primer	Trouvelot *et al*. (1999)
454 adapter A	CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG	Adapter sequence for 454 sequencing	
454 adapter B	CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG	Adapter sequence for 454 sequencing	

514

Table S3. Accession numbers of reference sequences and frequencies (clone/read numbers) of AM fungal phylotypes in individual samples.

			Field experiment										Glasshouse experiment														
													H-soil								U-soil						
			KST 94					Pioneer 3253					KST 94				Pioneer 3253				KST 94				Pioneer 3253				
Accession no.	Family	Phylotype	1	2	3	4	5	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	Total
AF378502	Acaulosporaceae	ACA1	2	3	6	21		7	11	3	3																		56
FJ461804	Acaulosporaceae	ACA2		3	3	3	8	6	6		3	3			1						1								37
JF439093	Acaulosporaceae	ACA3	1	13						1	3	2																	20
AB561121	Acaulosporaceae	ACA4	14	36	31	3	23	11	21	43	23	79																	284
AB369793	Acaulosporaceae	ACA5	4	15	8	3	17	3	13	15	3	28					1												110
AF378437	Acaulosporaceae	ACA6	37	145	32	32	1	42	52	33	22	4																	400
FR750155	Acaulosporaceae	ACA7	4	5	7	2	7	32	11	8	5	7																	88
FR750156	Acaulosporaceae	ACA8	31	95	27	25		39	36	21	11	6																	291
JF717598	Acaulosporaceae	ACA9	5	0	4	2		34	6	8	5	1																	65
FR821675	Acaulosporaceae	ACA10	14	43	13	17		26	17	14	11	1																	156
AB812595	Acaulosporaceae	ACA11	1	1						1																			3
FR750035	Archaeosporaceae	ARC1			2		4					1																	7
AB812596	Archaeosporaceae	ARC2	2	2	2		10	1	3			3																	23
AB812597	Archaeosporaceae	ARC3	1				8	3			1	21																	34
AB812598	Archaeosporaceae	ARC4	1									5																	6
AY541851	Claroideoglomeraceae	CLA1					2					2													1				5
AY639193	Claroideoglomeraceae	CLA2				2					5																		7
AJ271928	Claroideoglomeraceae	CLA3	4	3	29	1	17	5	14	2	17	20																	112
HM485770	Claroideoglomeraceae	CLA4	13	7	51	10	44	20	54	9	49	92														1			350
AB812589	Claroideoglomeraceae	CLA5			1		1																						2
AB812590	Claroideoglomeraceae	CLA6				1				1																			2
AB812591	Claroideoglomeraceae	CLA7	7		1		2	3	3			3																	19
AB812592	Claroideoglomeraceae	CLA8	3		3		3		1	1		1														1			13
AB812593	Claroideoglomeraceae	CLA9			1		10					4																	15
AB812594	Claroideoglomeraceae	CLA10	2		1		2		2		1	2																	10
AB812614	Claroideoglomeraceae	CLA11											1			1	1		1										4
AB812615	Claroideoglomeraceae	CLA12																							1		1		2
AB812616	Claroideoglomeraceae	CLA13																	2		2								4
AB812617	Claroideoglomeraceae	CLA14															1	2						1		1			5
AB640737	Diversisporaceae	DIV1	12	1	3		5				4	7																	32
FR686952	Diversisporaceae	DIV2	5	1	11	1	27	3			5	7																	60
AB812578	Diversisporaceae	DIV3		1			1		1		1														1				5
FN547559	Gigasporaceae	GIG1			9	27	98	12	18	4	15	31	3	3	2	5	4	3	5	5									244
FN547561	Gigasporaceae	GIG2			13	43	89	2	4	10	12	29	3	5	6	5	4	2		2		1		1	3				234
AM040348	Gigasporaceae	GIG3		1	4	9		6	1	4																			25
AF378455	Gigasporaceae	GIG4	5	15	19	75		35	28	9	7																		193
AF378453	Gigasporaceae	GIG5	0	4	4	13		4	1	2	1																		29
FR750177	Gigasporaceae	GIG6		14	9	42		10	12	8																			95
AF378446	Gigasporaceae	GIG7	11	7	10	35		13	3	15	17		1		1				1	1									115
JF816948	Gigasporaceae	GIG8	9	4						3												1		1	1				19
FJ461867	Gigasporaceae	GIG9	2	40	1	44		19	36	21	16																		179
FJ461881	Gigasporaceae	GIG10	9	99	3	103		46	53	40	42												3					1	399
AJ510243	Glomeraceae	GLO1	1	13	15	99	4	39	6	15	6	2															1		201
AY842572	Glomeraceae	GLO2			1							1																	2
FR873160	Glomeraceae	GLO3	3	17	13	4	1	21		22	98																		179
AB665504	Glomeraceae	GLO4		1	1		3	2	2		1	1			2						1		1	1	13			5	34
AM158947	Glomeraceae	GLO5	6	31	16	145	5	77	12	35	22	3																	352
FM865541	Glomeraceae	GLO6	8	51	39	296	17	142	40	82	48	8	1	1	1							4	3				1	1	743
AB640745	Glomeraceae	GLO7					11				8	1					1			2									23
AY541854	Glomeraceae	GLO8				1					1																		2
JF439124	Glomeraceae	GLO9				0	14				16						1												31
AB370889	Glomeraceae	GLO10	10	8	13	3	8	10	14	3	16	4	3		1	2	3		6	3									107
AJ854646	Glomeraceae	GLO11	2	9	10	9	5	14	17	11	9	5	5		6		5	1	5	3		1	1	3	1		2		124
AJ854594	Glomeraceae	GLO12	5	5	19	1	7	10	23	2	10	9			2			1				1	1				2		98
JF439131	Glomeraceae	GLO13	2	1				3		1		15																	22
JF439138	Glomeraceae	GLO14				1		3				8																	12
AY639271	Glomeraceae	GLO15	18	8	42	18	50	17	52	17	34	80																	336
FN547491	Glomeraceae	GLO16	159	82	236	255	223	94	262	146	153	405									2	2	5	2	7	4	1	6	2044
AB561101	Glomeraceae	GLO17	53	31	45	42		77	10	142	24	96	7	21	11	10	12	10	15	15	2	1				1		1	626
JF717616	Glomeraceae	GLO18	3	1	1	3	7		9	1	2	4																	31
AB547176	Glomeraceae	GLO19	6	2	4	3	36	7	28	5	6	14																	111
AB369739	Glomeraceae	GLO20				1			1			1																	3
AY639221	Glomeraceae	GLO21	3	1	8	7	14	10	18	1	5	5	3								3	2	2		1	1		1	85
JF439166	Glomeraceae	GLO22			1				1				1								2	2			1	1			9
AB369767	Glomeraceae	GLO23	20	5	55	7	130	5	8	6	64	30	2	1	3		1				2		6	7	2	1	9	5	369
AB547181	Glomeraceae	GLO24						2	2	2	1																		7
FJ461850	Glomeraceae	GLO25		1		1		7	1	2																			12
JF439144	Glomeraceae	GLO26	6		2			1			2	9																	20
AJ271926	Glomeraceae	GLO27											4	2		1	3	1				3	1	2					17
AB665521	Glomeraceae	GLO28	50	31	71	27	97	55	65	33	102	121		2			1			1									656
JN685290	Glomeraceae	GLO29	3	3	24	3	65	7	6	6	5	6						1	1	1									131
AY541832	Glomeraceae	GLO30	2	14	8	1		4	9	10	7																		55
AY541824	Glomeraceae	GLO31	62	42	45	15	5	61	70	90	41	22		1															454
AB547176	Glomeraceae	GLO32											5	6	4	6	7	3	8	8	1			1		1			50
FM865598	Glomeraceae	GLO33														1	1	1			2	5	2		2	4	3	2	23
FJ461812	Glomeraceae	GLO34														1	1	1											3
JF439201	Glomeraceae	GLO35																			1				1			1	3
FM865601	Glomeraceae	GLO36																			8	15	16	6	7	12	14	5	83
FM865604	Glomeraceae	GLO37																			3	5	5	5	1	5	1	2	27
AB812579	Glomeraceae	GLO38							3		1																		4
AB812580	Glomeraceae	GLO39	7	20	31	8		22	2	56	6	31																	183
AB812581	Glomeraceae	GLO40			3						1																		4
AB812582	Glomeraceae	GLO41				1				1																			2
AB812583	Glomeraceae	GLO42					1			1	1																		3
AB812584	Glomeraceae	GLO43					1	1																					2
AB812585	Glomeraceae	GLO44				1					1																		2
AB812586	Glomeraceae	GLO45				1	1																						2
AB812587	Glomeraceae	GLO46	2	2	7	3		8	2	15	3	5																	47
AB812588	Glomeraceae	GLO47			1	1																							2
AB812610	Glomeraceae	GLO48																			1	2				1		1	5
AB812611	Glomeraceae	GLO49																				1	1						2
AB812612	Glomeraceae	GLO50																			1					3	2		6
AB812613	Glomeraceae	GLO51												2		0	1												3
FR750054	Paraglomeraceae	PAR1	167	151	148	37	193	72	292	143	180	251																	1634
FR750083	Paraglomeraceae	PAR2	12	36	19	1		9	34	13	7		1		1		2									1		2	138
NG027567	Paraglomeraceae	PAR3	190	138	60	98	15	99	169	141	64	55																	1029
AJ271713	Paraglomeraceae	PAR4	7	90	41	24	14	47	64	88	27	18		2	8	5	5	3	3	6		1	1						454
AB812599	Paraglomeraceae	PAR5	12	13	7	8	1	12	14	13	2																		82
AB812600	Paraglomeraceae	PAR6			1			1		2		1																	5
AB812601	Paraglomeraceae	PAR7		9		8		2	3	1																			23
AB812602	Paraglomeraceae	PAR8	5	33	19	10		13	3	17		1																	101
AB812603	Paraglomeraceae	PAR9		1			5				1	6																	13
AB812604	Paraglomeraceae	PAR10				1				1		1																	3
AB812605	Paraglomeraceae	PAR11		1					1	2																			4
AB812606	Paraglomeraceae	PAR12		1		1				1																			3
AB812607	Paraglomeraceae	PAR13		1							1																		2
AB812608	Paraglomeraceae	PAR14				1				1		1																	3
AY639353	Unassigned	UNA1					2															1						1	4
AB812609	Unassigned	UNA2			5					2																			7
Total number of clones/reads			###	###	###	###	###	###	###	###	###	###	40	46	49	37	55	29	47	47	32	48	45	33	43	38	37	34	14616
Total number of phylotypes			54	59	63	62	49	58	58	62	62	57	14	11	14	11	19	12	10	11	15	17	13	12	15	15	11	14	109
Shannon diversity index			2.89	3.22	3.33	3.01	2.92	3.44	3.10	3.22	3.25	2.89	2.48	2.39	2.09	2.23	2.21	2.26	1.88	2.38	2.44	1.84	2.31	2.01	2.58	2.14	1.96	2.03	

516 **Table S4.** Results of Student's *t*-test on total number of phylotypes (richness) and Shannon
517 diversity index of the AM fungal communities in association with the *Striga*-resistant cultivar
518 KST 94 and -susceptible cultivar Pioneer 3253 in the field and glasshouse experiments.

		Glasshouse experiment[a]	
	Field experiment	H-soil	U-soil
Total number of phylotypes			
t-value	5.914	0.248	4.786
P-value	0.500	0.815	0.750
Shannon diversity index			
t-value	0.639	0.645	0.997
P-value	0.559	0.435	0.893

519 [a] The soils were collected from the experimental fields of Hokkaido University (H-soil) and
520 Utsunomiya University (U-soil) and used in the glasshouse experiment.
521

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