SHORT COMMUNICATION

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Tomato strigolactones
A more detailed look

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Strigolactones are plant signaling molecules that induce germination of parasitic plant seeds, initiate host plant - arbuscular mycorrhizal fungus symbiosis and act as plant hormones controlling shoot branching and root architecture. To date four unique strigolactones (e.g., orobanchol, didehydroorobanchol isomers 1 and 2 and the aromatic strigolactone solanacol) have been reported in the root exudates and extracts of tomato (Solanum lycopersicum). Here we report on the presence of several additional strigolactones in tomato root exudates and extracts, orobanchyl acetate, two 7-hydroxyorobanchol isomers, 7-oxoorobanchol and two additional didehydroorobanchol isomers and discuss their possible biological relevance.

Strigolactones are plant hormones involved in the regulation of above and below ground plant architecture[1-3] and rhizosphere signaling[4,5] and have been identified in many plant species.[6-10] Strigolactones are derived from carotenoids[11] and therefore belong to the chemical class of the apocarotenoids. The strigolactone chemical structure consists of four rings (ABCD), with a tricyclic lactone (ABC part) and butenolide group (D-ring) connected by a characteristic enol ether bridge (**Fig. 1A**). It has been reported that this enol ether bridge is not only essential for parasitic seed germination,[12,13] but is also required for the induction of AM branching[14] and their hormonal activity in planta.[15] Although the main structure of strigolactones is rather similar, their A- and B-ring decoration and stereochemistry can vary substantially (**Fig. 1B**).[5,16] It is clear that different functional groups and stereochemistry lead to different biological specificity in strigolactones.[5,14,16,17] We recently reported on the role of orobanchol, solanacol, and two putative didehydroorobanchol isomers in tomato.[18] In this addendum we report the presence of several additional strigolactones in tomato and discuss their possible biological relevance.

Tomato plants were grown under controlled conditions, their exudates collected, purified and analyzed as previously described.[9,18,19] All strigolactones previously reported in tomato[7,20] were present in the samples analyzed (**Fig. 2A**). In addition, orobanchyl acetate was also detected in tomato root exudates and its identity confirmed by comparison with an authentic standard (**Figs. 2B and 3A**). Orobanchyl acetate was recently detected in the xylem sap of tomato,[18] but its presence in root exudates has not been reported before. In addition, 7-oxoorobanchol and two 7-hydroxyorobanchol isomers were detected in purified tomato exudates (**Fig. 2A and C**). The relatively low levels (based on MS signal intensities) of these strigolactones is likely the reason why they have not been detected before in crude tomato root exudates.

The compound eluting at RT 3.75 min was identified as 7-oxoorobanchol, based on comparison of its RT and MS/MS fragmentation spectra with that of an authentic 7-oxoorobanchol standard[21] (**Figs. 2C and 3B**). The RT of 2.57 min (**Fig. 2C**) of one of the putative 7-hydroxyorobanchol isomers, as well as its MS/MS fragmentation spectrum obtained at collision energies of[5,10,15,20] and 25 eV were identical to those of an authentic 7α-hydroxyorobanchol standard (kindly provided by K. Yoneyama) (**Fig. 3C**, data not shown) and similar to an authentic 7α-hydroxyorobanchyl acetate standard (**Fig. 3C**), identifying this compound as 7α-hydroxyorobanchol. The MS/MS fragmentation spectrum of the less polar 7-hydroxyorobanchol isomer (RT 3.09 min, **Fig. 2C**) was very similar to that of the authentic 7α-hydroxyorobanchol (**Fig. 3C**) and 7β-hydroxyorobanchyl acetates standards 16 (data not shown). Therefore, this compound is tentatively identified as 7β-hydroxyorobanchol. Co-injection of authentic 7α-hydroxyorobanchol and 7-oxoorobanchol standards further confirmed their presence in the tomato root exudates (data not shown).

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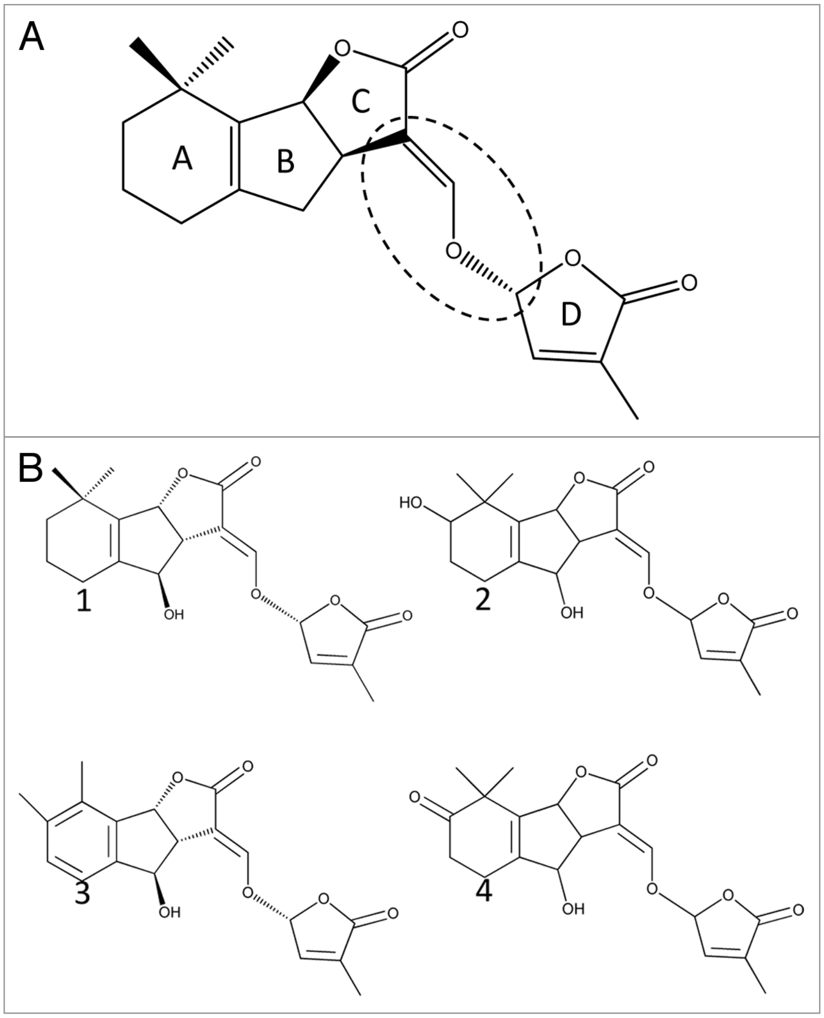

Figure 1. Structures of strigolactones. (**A**) Structure of 5-deoxystrigol highlighting the core strigolactone structure (ABCD indicate the four ring stucture, dashed line indicates the enol ether bridge) (**B**) Structure of some naturally occurring strigolactones (**1**, orobanchol; **2**, 7-hydroxyorobanchol; **3**, solanacol; **4**, 7-oxoorobanchol).

Four putative didehydroorobanchol isomers with m/z 345—eluting at RTs 6.45, 6.85, 7.01 and 7.15 min—were detected (**Fig. 2A**). The accurate mass for their protonated molecular ions [M + H]+ was m/z 345.1333 ± 0.0005 (determined using LC-LTQ/Orbitrap-MS). This is in accordance with the theoretically calculated mass for $C_{19}H_{21}O_6$, m/z 345.1338. The collision induced fragmentation spectra of the protonated molecular ions of all four isomers were obtained with triple quad MS (**Fig. 3D**). Upon fragmentation the [M + H]+ ion is converted to an ion with m/z 327 [M + H - H_2O]+ through a loss of water. A further loss of the D-ring gives the ion at m/z 231 [M + H - H_2O - D-ring]+. Several consecutive losses of water, CO and/or acetylene lead to ions at m/z 203, 187, 175 and 161 (**Fig. 3D, 1–4**). The abundance of most of these fragments is rather similar for all isomers. The main difference between the isomers is the fragment with m/z 187, which is relatively low in MS/MS fragmentation spectra of two of the isomers (RTs 6.45 and 7.01 min). Based on these observations it seems likely that all four compounds are isomers. Unfortunately, the isolation and purification of these isomeric compounds was not possible due to partially overlapping RTs.

To test the biological activity of the tomato strigolactones in *Phelipanche ramosa* seed germination, tomato root exudates were fractioned by HPLC as previously described 9 and the fractions in which the strigolactones eluted determined by MRM-LC-MS/MS (**Fig. 4**). *P. ramosa* germination corresponded, to some extent, to the elution of known tomato strigolactones. It is plausible that the germination inducing activity detected in the earlier eluting fractions (Fraction 9–14), can be attributed to the presence of the newly identified 7-hydroxy- and 7-oxoorobanchol isomers (**Fig. 4**). However, the germination inducing activity of several other fractions cannot be directly explained. It is possible that in some of these, minor concentrations of several strigolactones co-elute and that their accumulated activity leads to reasonably high seed germination while remaining below the detection level of MRM-LC-MS/MS. But we can also not exclude that even more unknown strigolactones (or germination stimulants of other chemical classes) are secreted by tomato roots. Fraction 22 is of interest as it was also detected in the root exudate of Arabidopsis.[9]

The identification of orobanchyl acetate, 7-oxoorobanchol, two 7-hydroxyorobanchol isomers and two additional didehydroorobanchol isomers in tomato root exudate expands the number of tomato strigolactones to ten. The aromatic strigolactone solanacol has been postulated to be derived from orobanchol through a series of enzymatic hydroxylation/dehydroxylation reactions with migration of a methyl group and double bonds.[5,16] Several of the tomato strigolactones identified here were postulated to be intermediates in this conversion[16] and their identification in tomato seems to support this hypothesis. In addition, the stereochemistry of the revised solanacol structure[22,23] matches the stereochemistry of orobanchol which was recently unambiguously determined.[24] The configuration of these two compounds does, however, not match the proposed stereochemistry of 7-oxoorobanchol[21] and 7-hydroxyorobanchol.[13,16] However, in these reports the stereochemistry of the latter compound was not unambiguously determined. Technical advancements, in combination with an increasing interest in the stereochemistry of strigolactones have resulted in debate on the structural reliability of authentic standards (personal communication Prof. Dr. Binne Zwanenburg) and led to several revisions to proposed structures of naturally occurring strigolactones already[23,24] and additional revisions in the near future are likely. Further research and structure identification will be needed to prove this and confirm the postulated pathway. A better understanding of this will in the near future

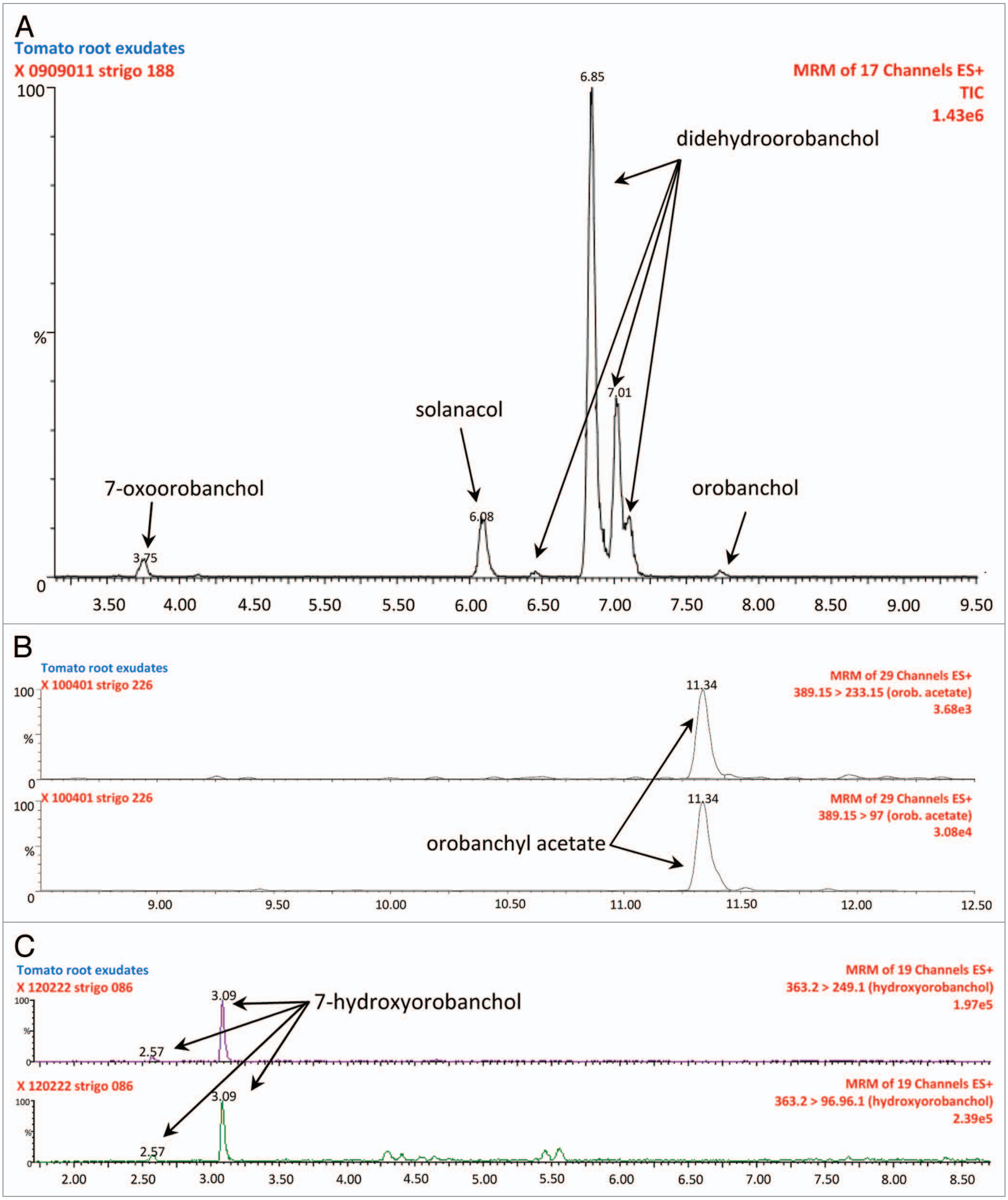

Figure 2. MRM-LC-MS/MS analysis of tomato (cv Moneymaker) root exudates. (**A**) Total ion current (TIC) chromatogram of tomato root exudates. (**B**) Transitions 389.15 > 233.15 and 389.15 > 97 for orobanchyl acetate. (**C**) Transitions 363.2 > 249.1 and 363.2 > 96.96 for 7-hydroxyorobanchol isomers.

prove instrumental for designing strategies to fine-tune the strigolactone composition in a plant. This would enable plant breeders to optimize strigolactones for biological activity and to select varieties that, for example, do produce root exudates that

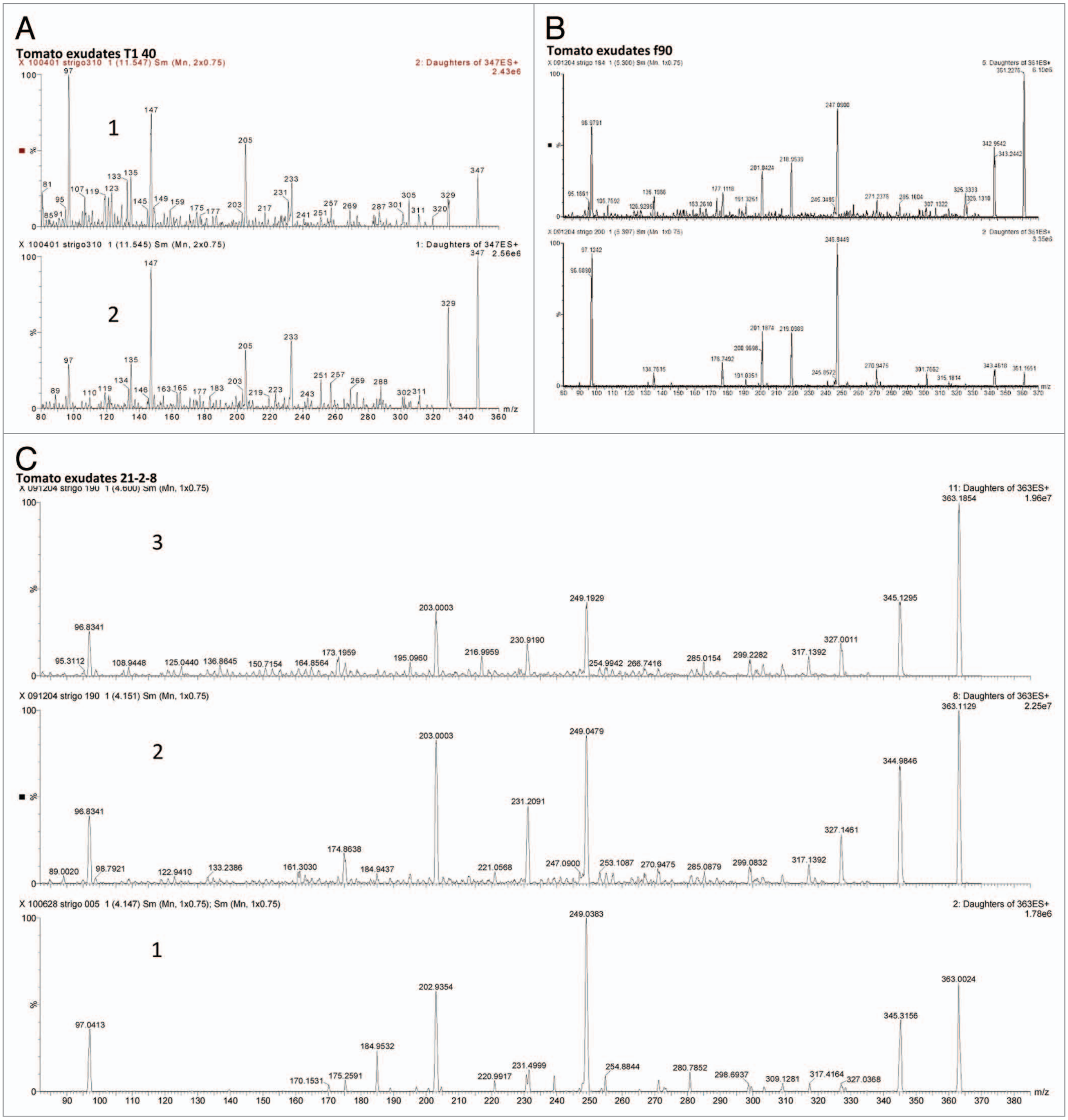

Figure 3. MS/MS fragmentation spectra of the newly identified tomato strigolactones recorded during online separation of tomato root exudates. (**A**) MS/MS fragmentation spectra of orobanchyl acetate in tomato root exudates (**1**) and authentic orobanchyl acetate (**2**) at a collision energy of 18 eV. (**B**) MS/MS fragmentation spectrum of 7-oxoorobanchol in tomato root exudates (**1**) and authentic 7-oxoorobanchol (**2**) at a collision energy of 15 eV. (**C**) MS/MS fragmentation spectra of an authentic 7α-hydroxyorobanchol (**1**), the putative 7α-hydroxyoorbanchol isomer ([M+H]+, m/z 363) in tomato root exudates (**2**), the tentative 7β-hydroxyoorbanchol isomer ([M+H]+, m/z 363) in tomato root exudates (**3**), at a collision energy of 15 eV.

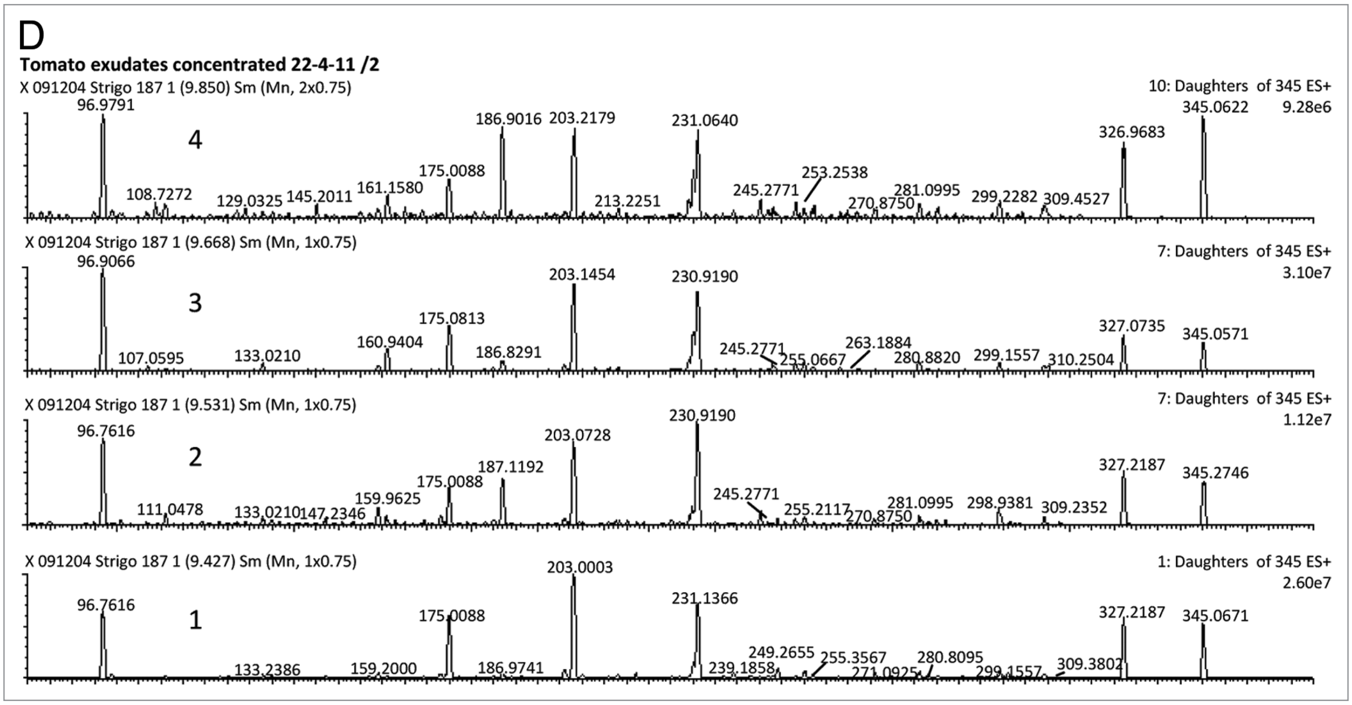

Figure 3. MS/MS fragmentation spectra of the newly identified tomato strigolactones recorded during online separation of tomato root exudates. (**D**) MS/MS fragmentation spectra of the four putative didehydroorobanchol in tomato root exudates (**1–4**) at a collision energy of 15 eV.

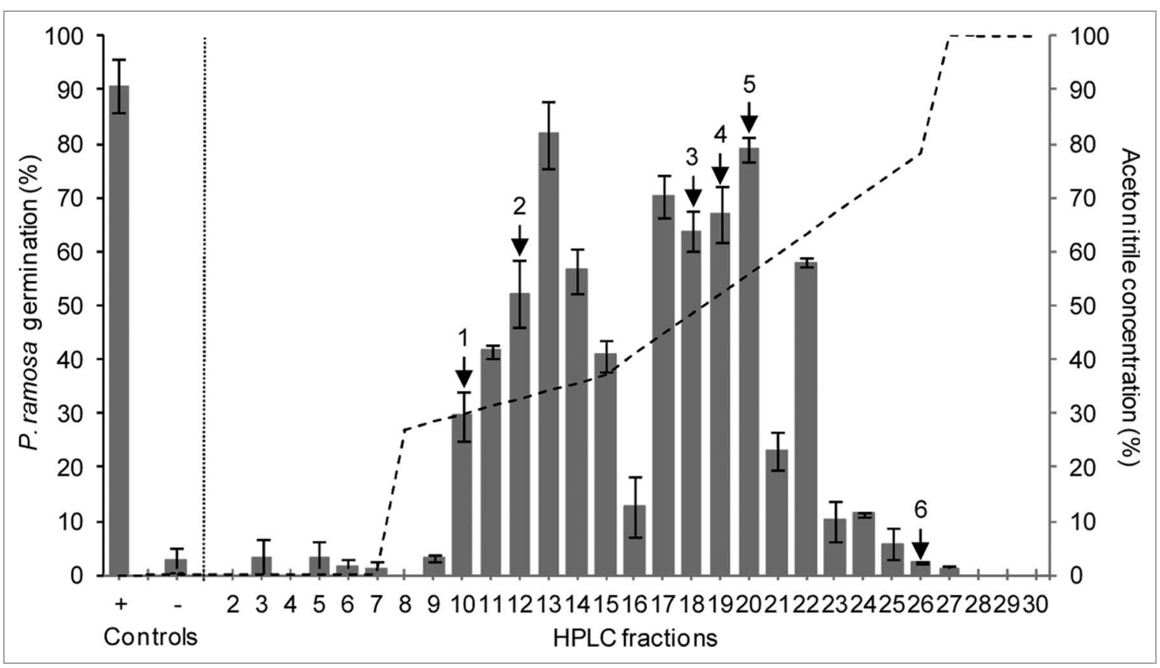

Figure 4. Germination of Phelipanche ramosa seeds induced by HPLC fractions of tomato (cv Moneymaker) root exudate. Bars represent the average of three independent biological replicates ± SE. Dashed line indicates HPLC gradient (acetonitrile concentration), arrows point to main fractions in which strigolactone standards elute: 7-hydroxyorobanchol (**1**), 7-oxoorobanchol (**2**), solanacol (**3**), didehydroorobanchol isomers (**4**), orobanchol (**5**) and orobanchyl acetate (**6**).

facilitate AM symbiosis and control shoot branching, but do not induce parasitic plant seed germination.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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