

Figure 1. Effects of volatile compounds (VCs) from *Bacillus spp.* on growth and root development in *Arabidopsis*. A, Three-day-old *Arabidopsis* Col-0 seedlings were grown with or without seven selected *Bacillus spp.* strains in bi-compartmented Petri dish for 8 d, avoiding direct contact or solute exchange between the seedlings and the bacteria. Scale bar, 2 cm. B to E, Quantification of primary root (PR) elongation (B), lateral root (LR) number (C), LR density (number of emerged lateral roots cm⁻¹) (D), and total biomass production per *Arabidopsis* seedling (E) after 8d of co-cultivation with the *Bacillus spp.* strains. Error bars indicate \pm SD of the mean, n = 24, One-way ANOVA.

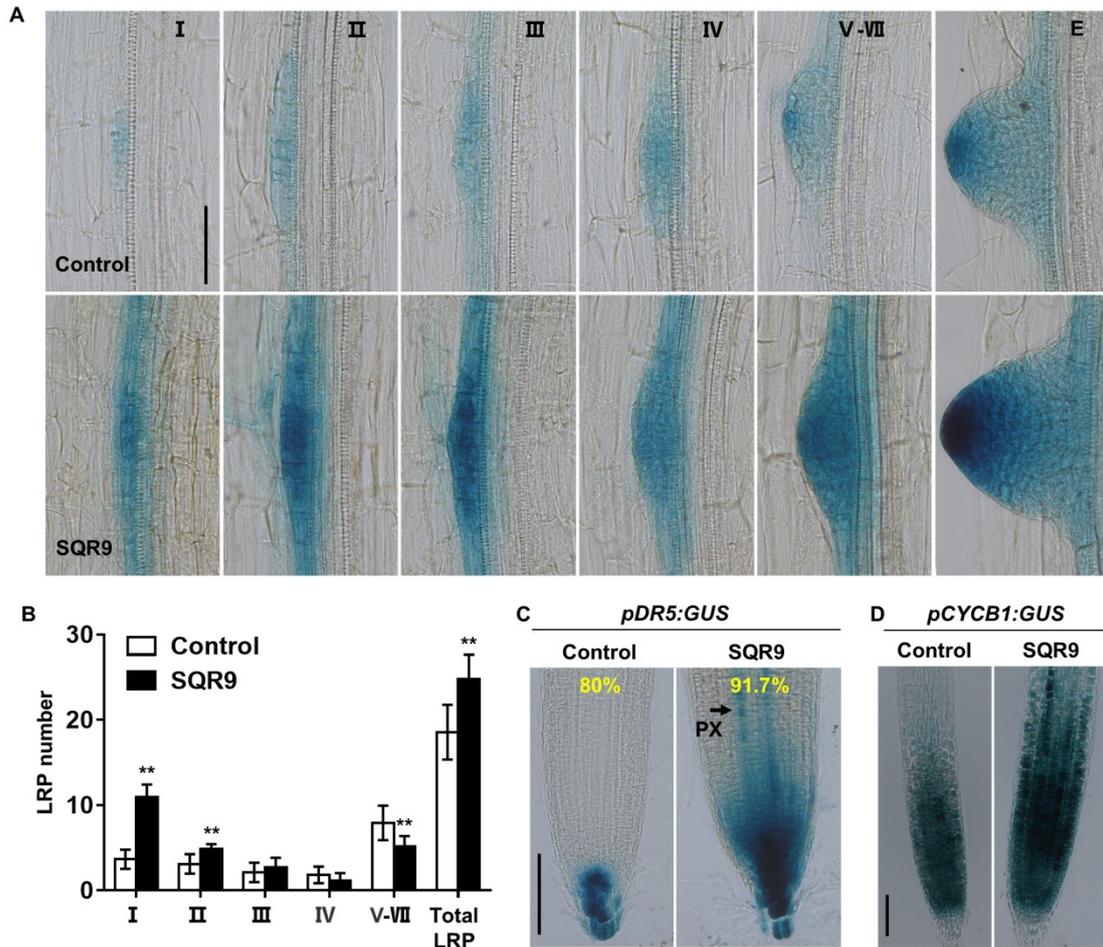


Figure 2. Effects of SQR9 VCs on LR formation, auxin response, and cell division. A, Expression patterns of *pDR5:GUS* during primordium development after 6 d co-cultivation with SQR9. Analogous results were observed in at least eight LRPs at the same stage. E, emergence. Scale bar, 100 μ m. B, Distribution of LRP in seven developmental stages as marked by the *pCYCB1:GUS* activity after 6 d co-cultivation with SQR9. Error bars indicate \pm SD of the mean. $n = 12$. **, $P < 0.01$; *, $P < 0.05$ (Two-way ANOVA). C and D, Expression pattern of *pDR5:GUS* (C) and *pCYCB1:GUS* (D) in the root tips of 3-day-old *Arabidopsis* seedling after 6 d co-cultivation with SQR9. Percentages indicate the proportion of seedlings showing the identical *DR5* expression pattern within a population. PX, protoxylem pole. Scale bar, 100 μ m.

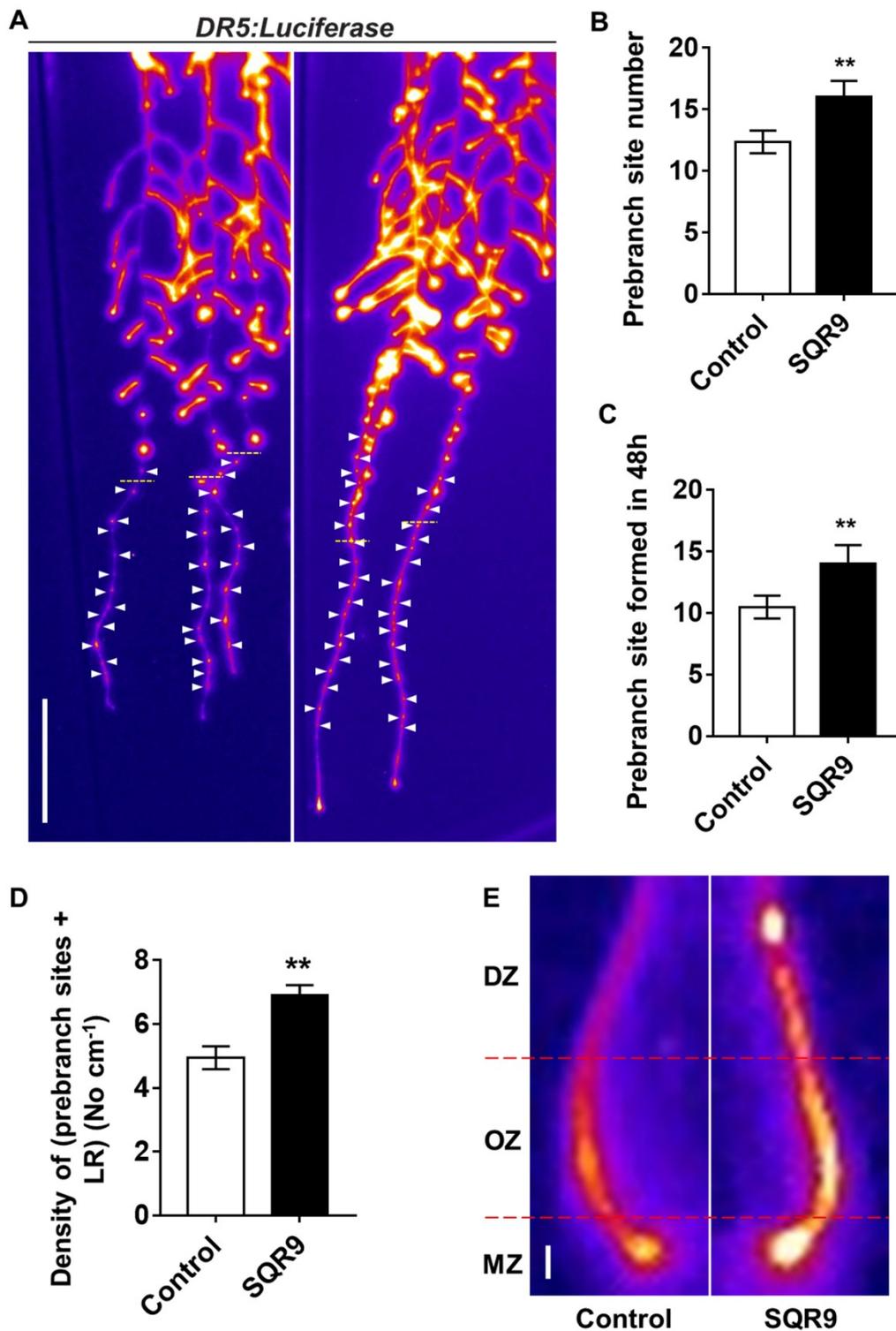


Figure 3. A, Luciferase imaging of *DR5:Luciferase* seedlings after 6 d co-cultivation with SQR9. White arrow indicates prebranch site revealed by statically expressed *DR5:Luciferase* signal. Dotted line indicates the position of root tip after 4 d co-cultivation. Scale bar, 1 cm. B, Quantification of prebranch site number of

DR5:Luciferase seedlings after 6 d co-cultivation with SQR9. $n \geq 10$. **, $P < 0.01$; *, $P < 0.05$ (Student's *t*-test). C, Quantification of prebranch site number of *DR5:Luciferase* seedlings within 48 h under SQR9-treated condition. D, Density of prebranch sites and LR along the PR. $n \geq 10$. **, $P < 0.01$; *, $P < 0.05$ (Student's *t*-test). E, Expression pattern of *DR5:Luciferase* in the OZ after 6 d co-cultivation with SQR9. MZ, meristem zone; OZ, oscillation zone; DZ, differentiation zone. Scale bar, 100 μm . Error bars indicate \pm SD of the mean.

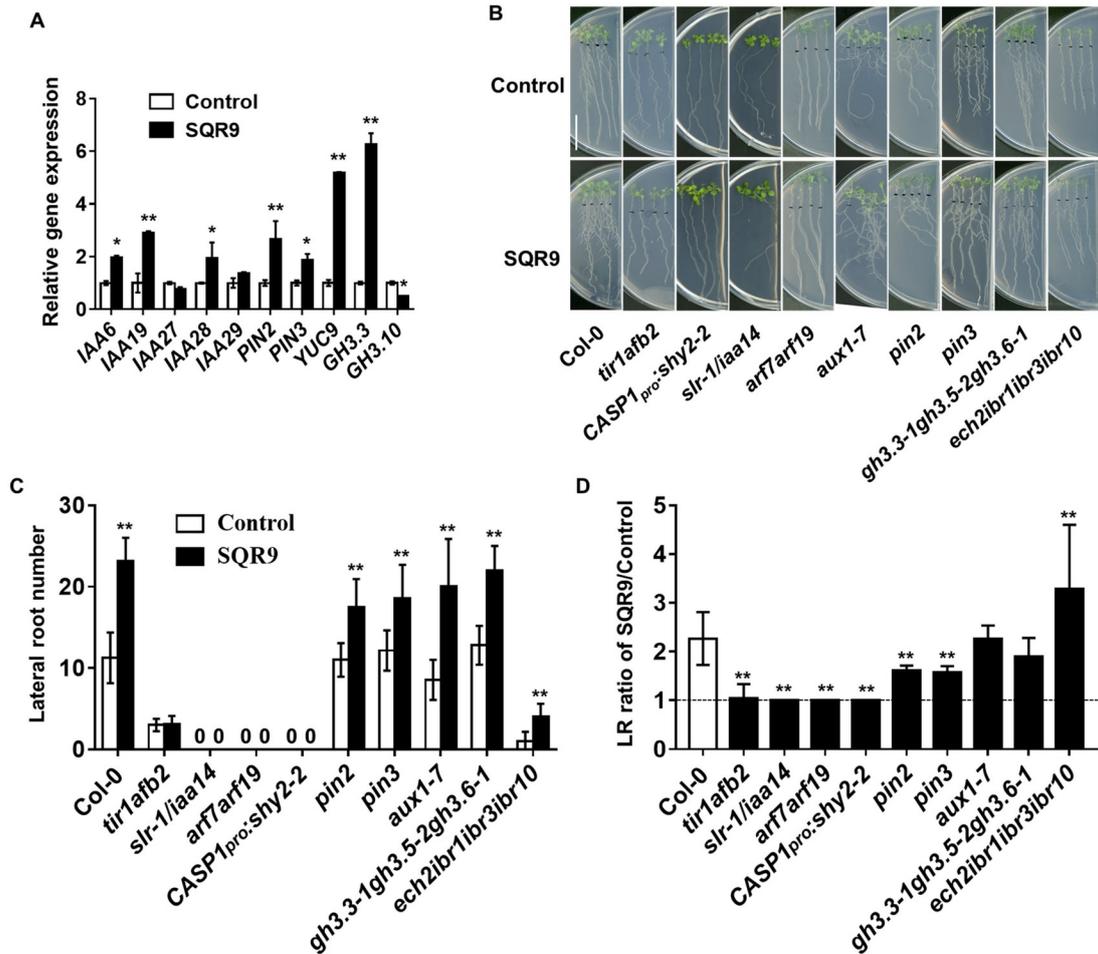


Figure 4. Auxin related genes function in SQR9 VCs-induced LR formation. A, Relative expression level of auxin-related genes selected from RNA-Seq data under control or SQR9 VCs-treated conditions for 4 d. $n = 3$. **, $P < 0.01$; *, $P < 0.05$ (Student's *t*-test). B and C, Representative photographs (B) and quantification of lateral root number (C) of *Arabidopsis* wild-type (Col-0) and auxin response (*tir1afb2*, *CASP_{pro:shy2-2}*, *slr-1/iaa14*, *arf7arf19*), transport (*pin2*, *pin3*, *aux1-7*), homeostasis (*gh3.3-1gh3.5-2gh3.6-1*) and conversion (*ech2ibr1ibr3ibr10*) mutants treated with SQR9 VCs for 8 d. $n = 12$. **, $P < 0.01$; *, $P < 0.05$ (Two-way ANOVA). Scale bar, 2 cm. D, Ratio of LR number of plants showed in B with SQR9 VCs-treated versus control plants. $n = 12$. **, $P < 0.01$; *, $P < 0.05$ (One-way ANOVA). Error bars indicate \pm SD of the mean.

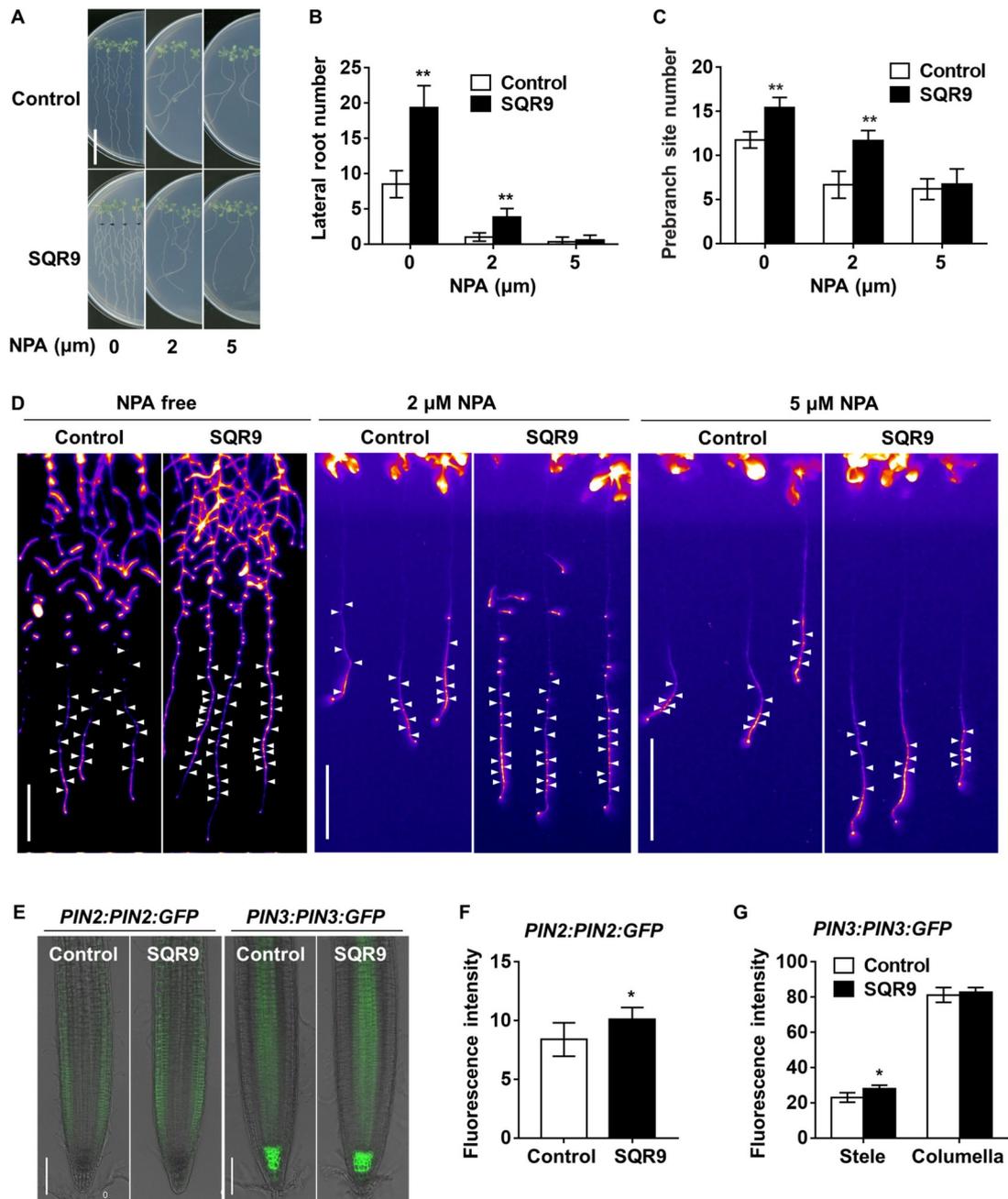


Figure 5. Effects of auxin transport on SQR9 VCs-mediated LR formation in the *Arabidopsis*. A and B, Representative photographs (A) and quantification of LR number (B) after 6 d co-cultivation with SQR9 in presence of varying concentrations of NPA. $n = 20$. **, $P < 0.01$; *, $P < 0.05$ (Two-way ANOVA). Scale bar, 2 cm. C and D, Luciferase imaging (D) and quantification of prebranch site number (C) of *DR5:Luciferase* seedlings after 6 d co-cultivation with SQR9 in presence of varying concentrations of polar auxin transport inhibitor NPA. White arrow indicates

prebranch site revealed by statically expressed *DR5:Luciferase* signal. $n \geq 10$. **, $P < 0.01$; *, $P < 0.05$ (Two-way ANOVA). Scale bar, 1 cm. E to G, Confocal images and quantification of *pPIN2:PIN2:GFP* and *pPIN3:PIN3:GFP* signal in the primary root tips of 3-day-old seedlings grown with or without SQR9 VCs for 6 d. $n \geq 8$. **, $P < 0.01$; *, $P < 0.05$ (F, Student's *t*-test; G, Two-way ANOVA). Scale bar, 100 μm . Error bars indicate \pm SD of the mean.

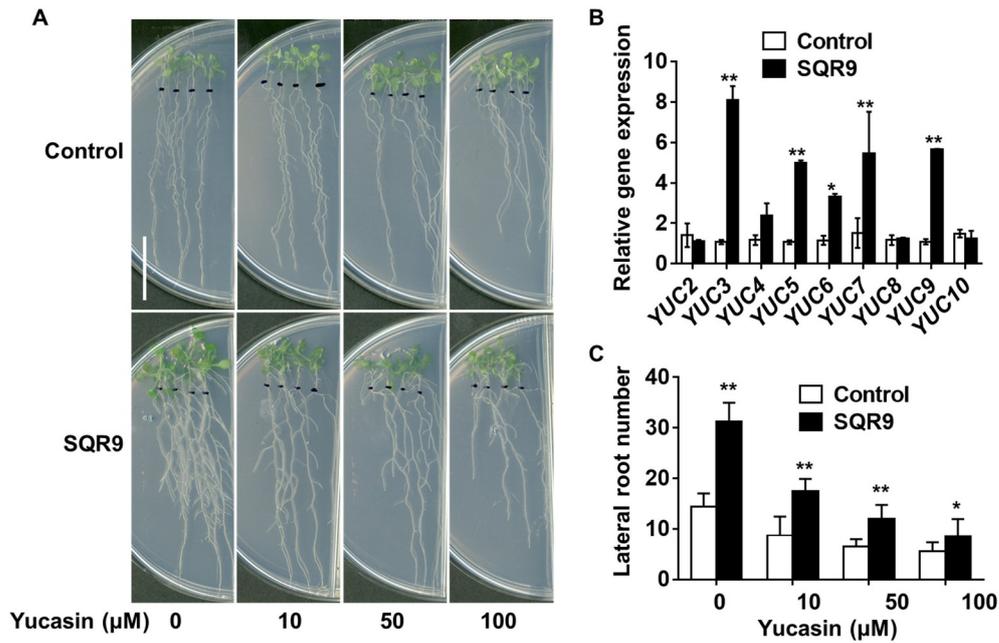


Figure 6. Effects of YUCs-mediated auxin biosynthesis on SQR9 VCs-mediated LR formation in the *Arabidopsis*. A and C, Representative photographs (A) and quantification of LR number (C) of *Arabidopsis* seedlings grown with or without SQR9 VCs in presence of varying concentrations of yucasin. n = 16. **, P < 0.01; *, P < 0.05 (Two-way ANOVA). Scale bar, 2 cm. B, Relative expression level of YUCs gene under control or SQR9 VCs-treated conditions for 4 d. n = 3. **, P < 0.01; *, P < 0.05 (Student's *t*-test). Error bars indicate \pm SD of the mean.

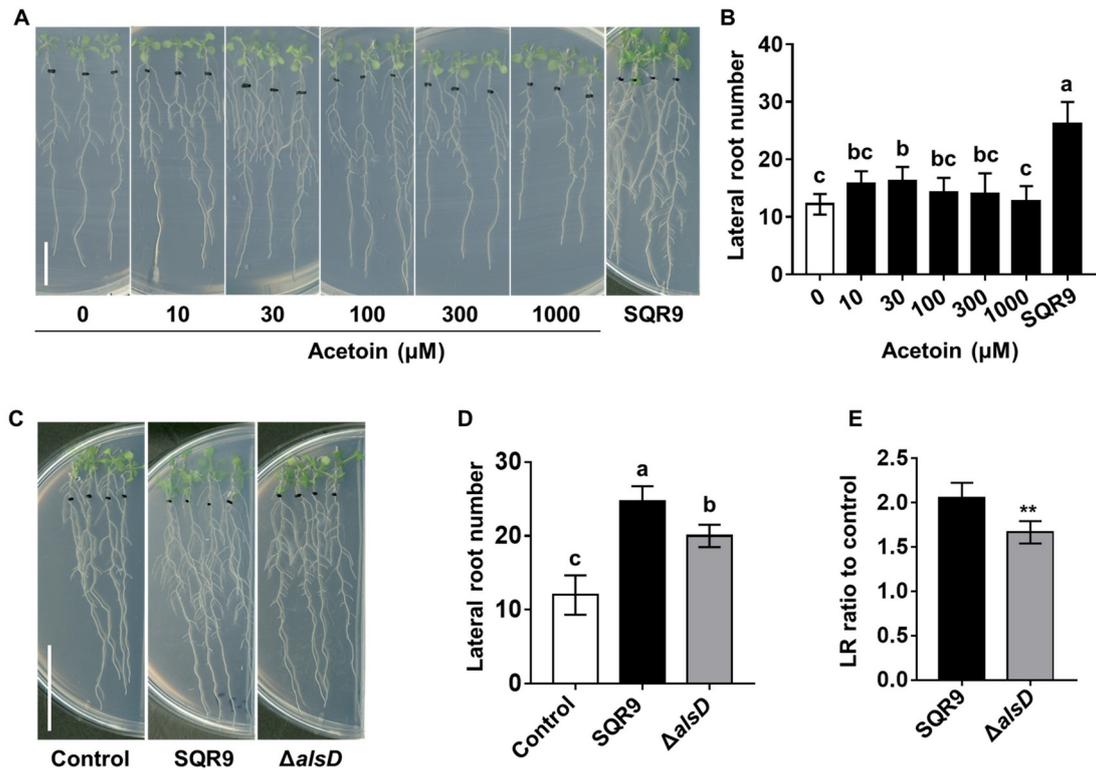


Figure 7. Acetoin slightly induces LR formation, but its effect is not comparable with the pool of SQR9 VCs. A and B, Representative photographs and quantification of LR number in 3-old-day *Arabidopsis* seedlings treated with 0, 10, 30, 100, 300, 1000 μM acetoin and SQR9 VCs for 8 d. Scale bar, 2 cm. n = 18. **, P < 0.01; *, P < 0.05 (One-way ANOVA). C and D, Representative photographs and LR formation of *Arabidopsis* seedlings under control, SQR9 VCs-treated, and $\Delta alsD$ VCs-treated conditions. Scale bar, 2 cm. n = 20. **, P < 0.01; *, P < 0.05 (One-way ANOVA). E, The LR number ratio of plants with SQR9 and $\Delta alsD$ VCs treatment versus control. n = 12. **, P < 0.01; *, P < 0.05 (Student's t-test). Error bars indicate \pm SD of the mean.

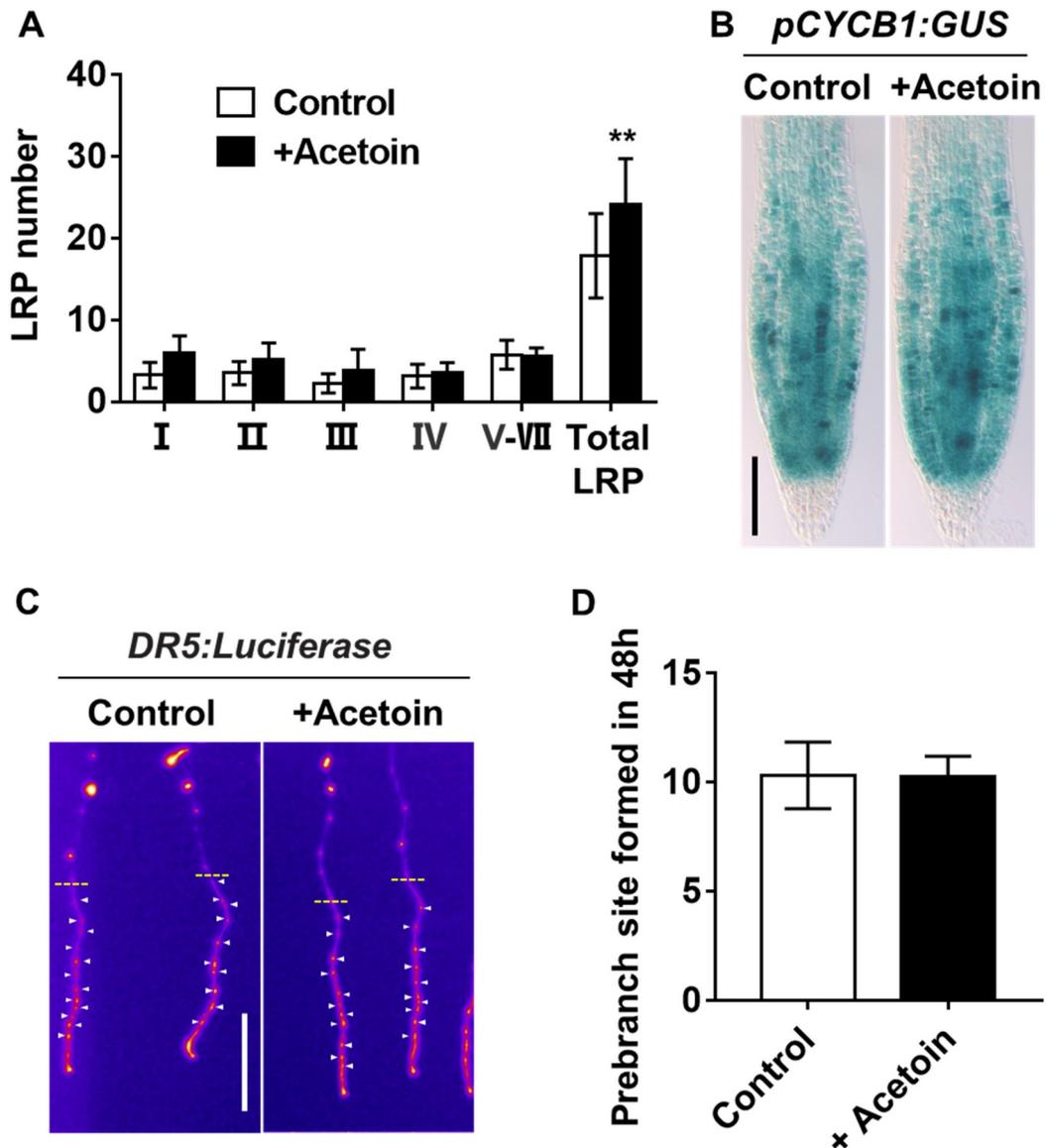


Figure 8. Effects of acetoin on LRP distribution, cell division, and prebranch sites formation in *Arabidopsis*. A, Distribution of LRP in seven developmental stages of the seedlings treated with or without 30 μ M acetoin for 6 d. $n=8$. **, $P < 0.01$; *, $P < 0.05$ (Two-way ANOVA). B, Expression pattern of *pCYCB1:GUS* in the root tips of 3-day-old *Arabidopsis* seedling treated with 30 μ M acetoin for 6 d. Scale bar, 100 μ m. C and D, Luciferase imaging of *DR5:Luciferase* seedlings treated with 30 μ M acetoin for 6 d and quantification of prebranch site number formed in 48 h. Dotted line indicates the position of root tip after 4 d treatment. $n = 18$. **, $P < 0.01$; *, $P < 0.05$ (Student's *t*-test). Scale bar, 1 cm. Error bars indicate \pm SD of the mean.

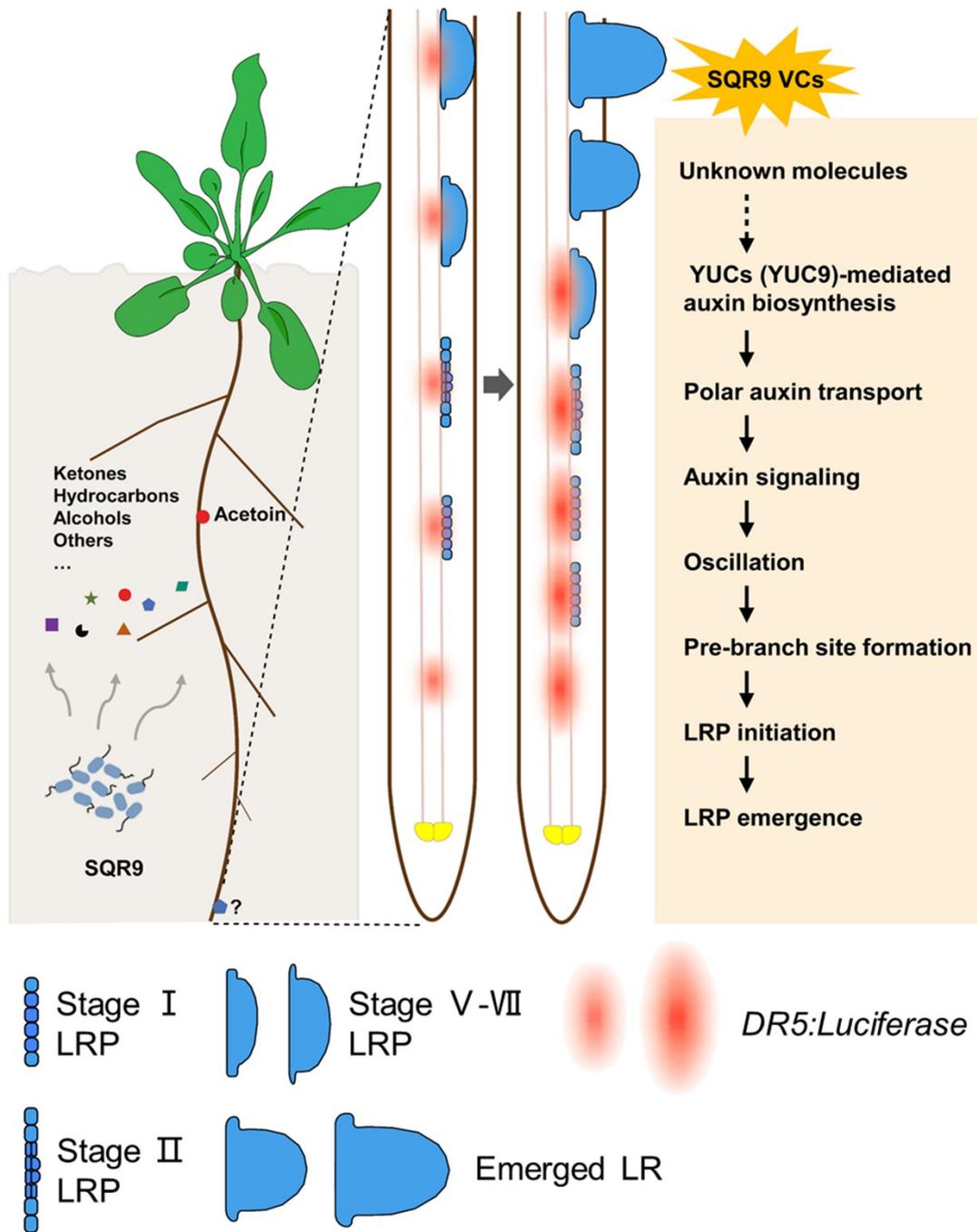


Figure 9. A hypothetical model for SQR9 VCs-induced lateral root LR formation. VCs from *B. amyloliquefaciens* SQR9, containing unknown active ingredients, increases the oscillation periodicity and enhances the oscillation intensity in an auxin-dependent manner, resulting in denser prebranch sites along the primary root (PR). Subsequently, SQR9 VCs induces LR initiation, and stimulates LR emerged in advance to form more LRs.