

## Article

# JrGA20ox1-transformed rootstocks deliver drought response signals to wild-type scions in grafted alfalfa

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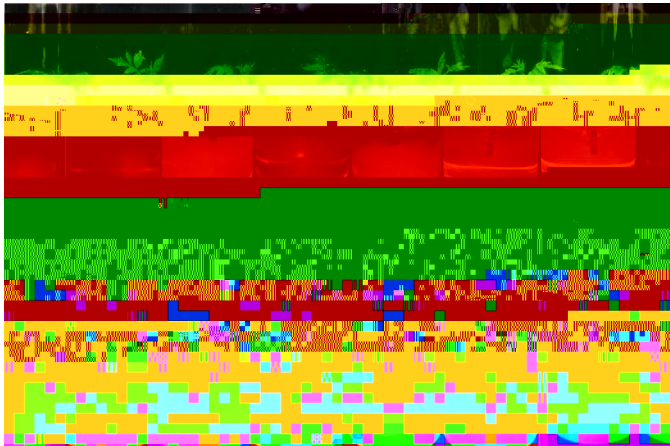
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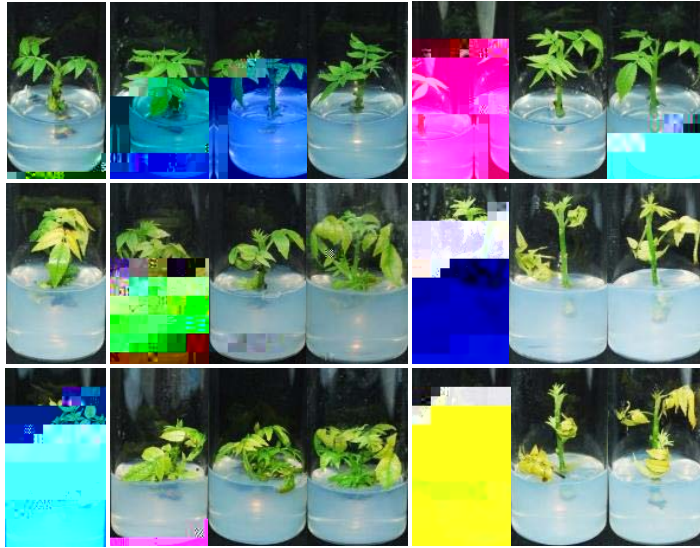
1–6].

The phenotypes of grafted scions vary depending on the rootstock partners [5]. Increasing evidence suggests that various con-





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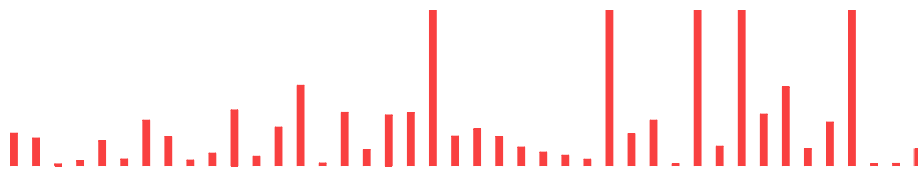






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downregulated in the xjg-treated scions of WT/RNAi (G4) (







respond to GA treatment. These results indicate that drought resistance of the scion is regulated not only by GA signal, but also by other signals, which may be moved from the rootstock. Strikingly, there are two GA-upregulated genes, *S40-3* and *Exo70B1*, and one GA non-responsive gene, *LOL1*, that may partly contribute to the drought resistance of the scion. This is supported by the following reported clues. First, the *AtS40-3* gene showed a much higher expression level in senescent leaves compared with non-senescent ones in *Arabidopsis* WT plants, and delayed senescence was observed in the *AtS40-3a* mutant compared with the WT [35]. Second, it was found that *Exo70B1* negatively regulates stomatal opening in *Arabidopsis* [36]. Third, *LOL1* has been identified as a positive regulator of programmed cell death in *Arabidopsis* through the regulation of ROS homeostasis and Cu/Zn SOD activity [37].

Based on this work, and the work of others, we propose the following model for *JrGA20ox1*-transformed rootstock regulation of drought stress in WT scions (Fig. 7). When WT/OE plants suffer drought stress, the synthesis of active GAs increases in the rootstock. The subsequent  $GA_4$  and unknown signals move from the rootstock to the WT scion, and they can induce the expression of a series of genes, including *S40-3*, *Exo70B1*, and *LOL1*. Then, ROS in leaves are excessively accumulated, leaf stomata open wider, and more water is lost in the scion of WT/OE than in WT/WT plants; thereby, WT/OE is more drought-sensitive than WT/WT (Fig. 7A). When WT/RNAi plants suffer drought stress, the synthesis of active GAs decreases in the rootstock. The sRNAs produced by the RNAi vector expressing short hpRNAs of genomic *JrGA20ox1* move from *JrGA20ox1*-RNAi transformed rootstocks to WT scions, which further leads to a successive decline in *JrGA20ox1* expression and active GA content. Meanwhile, unknown signals move from the rootstock to the WT scion. The decrease in active GAs and unknown signals further lead to the lower expression of a series of genes, including *S40-3*, *Exo70B1*, and *LOL1*. Then, ROS are less accumulated in leaves and massively induced in guard cells, leaf stomata are more closed, and less water is lost in the scion of WT/RNAi than in WT/WT plants; thereby, WT/RNAi is more drought-tolerant than WT/WT (Fig. 7B).

## Materials and methods

### Vector construction

To construct the *JrGA20ox1*-GFP plasmid, the coding sequence of *JrGA20ox1* without stop codon was connected to the pCAMBIA1300-GFP vector. The *JrGA20ox1*-RNAi plasmid was created by inserting a 200-bp cDNA fragment of *JrGA20ox1* into the







