EjBZR1 represses fruit enlargement by binding to the *EjCYP90* promoter in loquat

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Abstract: Loquat (*Eriobotrya japonica*) is a subtropical tree that bears fruit that ripens during late spring. Fruit size is one of the dominant factors inhibiting the large-scale production of this fruit crop. To date, little is known about fruit size regulation. In this study, we first discovered that cell size is more important to fruit size than cell number in loquat and that the expression of the *EjBZR1* gene is negatively correlated with cell and fruit size. Virus-induced gene silencing (VIGS) of *EjBZR1* led to larger cells and fruits in loquat, while its overexpression reduced cell and plant size in *Arabidopsis*. Moreover, both the suppression and overexpression of *EjBZR1* inhibited the expression of brassinosteroid (BR) biosynthesis genes, especially that of *EjCYP90A*. Further experiments indicated that *EjCYP90A*, a cytochrome P450 gene, is a fruit growth activator, while EjBZR1 binds to the BRRE (CGTGTG) motif of the *EjCYP90A* promoter to repress its expression and fruit cell enlargement. Overall, our results demonstrate a possible pathway by which EjBZR1 directly targets *EjCYP90A* and thereby affects BR biosynthesis, which influences cell expansion and, consequently, fruit size. These findings help to elucidate the molecular functions of BZR1 in fruit growth and thus highlight a useful genetic improvement that can lead to increased crop yields by repressing gene expression.

Key words: Fruit size, Brassinosteroid, BZR1, Cell expansion, Loquat, Transcription factor, Plant size.

Figure. 1

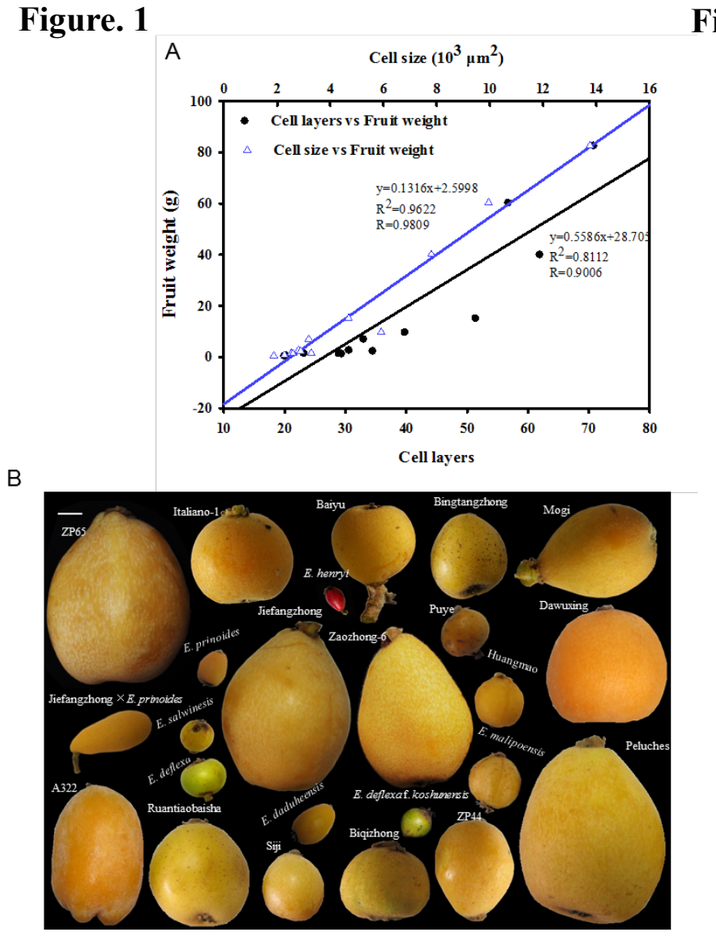

Figure. 2

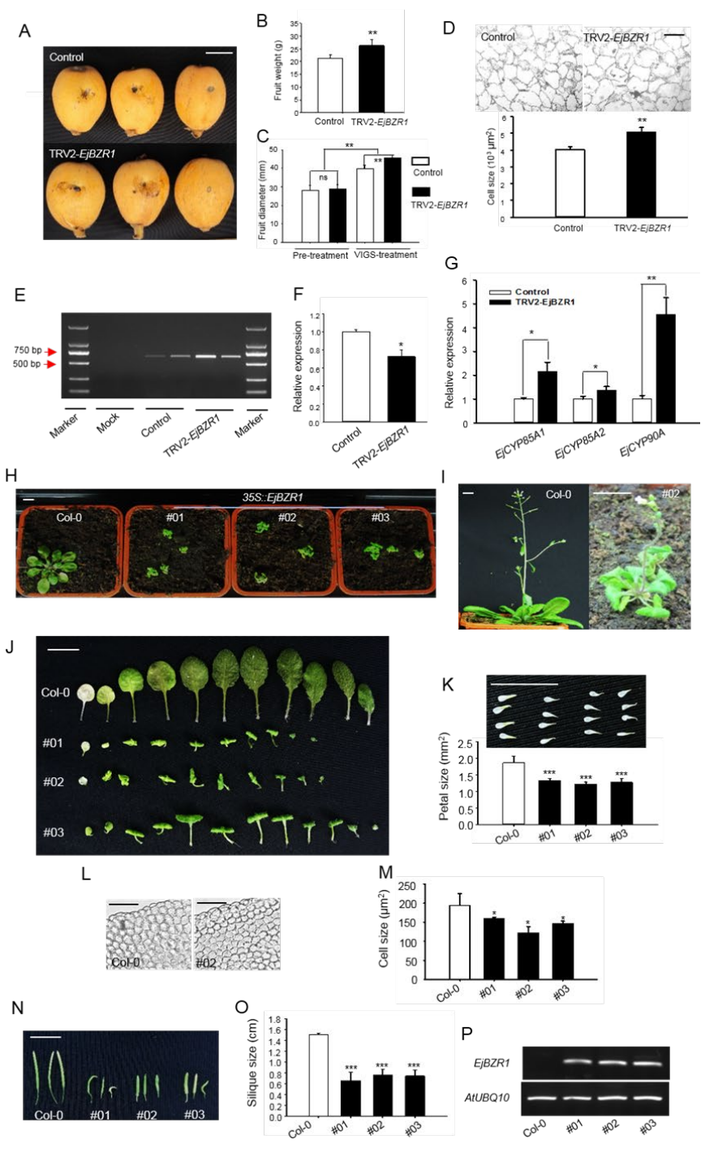

Figure. 3

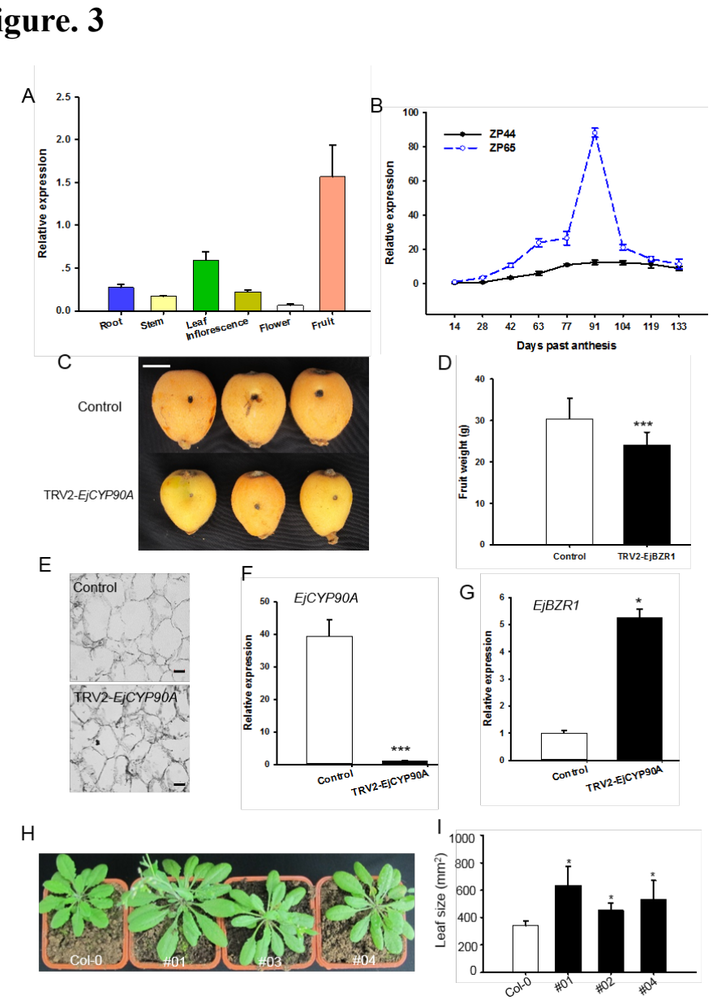

Figure. 4

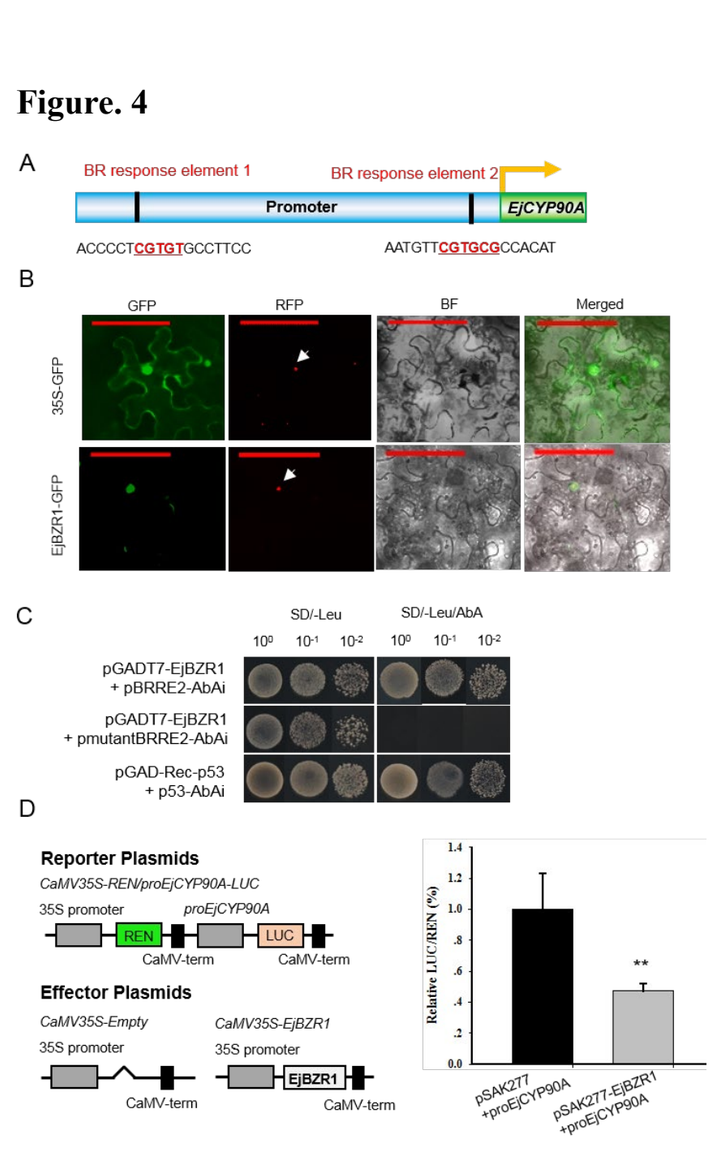

Figure. 1 Cell size contributes greater to fruit size than cell number. (A) Cell size is more strongly positively correlated than cell number with fruit weight among 13 loquat accessions. (B) Mature fruits of various sizes, shapes and colorations across the *Eriobotrya* genus.

Figure. 2 *EjBZR1* functions as a repressor of fruit and organ size development. (A) VIGS of *EjBZR1* promoted fruit growth. VIGS treatment increased fruit weight (B) as well as fruit diameter (C) and cell size (D). (E) Amplification of the 615 bp-length coat protein cDNA confirmed a working VIGS system in the treated fruits. (F) VIGS reduced *EjBZR1* expression in loquat fruit. (G) Reducing the *EjBZR1* expression level promoted BR biosynthesis gene expression in VIGS-treated fruits. Ectopic expression of *EjBZR1* decreased plant size in Arabidopsis (H-I). (J) Extremely small and curly rosette leaves in the transgenic lines. (K) Smaller petals in the transgenic lines. (L) Epithelial petal cells of Col-0 and line 2. (M) Petal cell size decreased in all overexpression lines. (N-O) Smaller and abnormal siliques in the OE lines. (P) Semiquantitative RT-PCR of *EjBZR1* in overexpression lines.

Figure. 3 *EjCYP90A* promotes fruit and organt growth. (A) Specific expression pattern of *EjCYP90A*. (B) Comparisons of *EjCYP90A* expression patterns in ZP44 and ZP65 fruits. (C) Fruits under VIGS treatment for EjCYP90A. (D) Reduction *in EjCYP90A* expression decreased fruit weight. (E) Flesh sections of TRV2-EjCYP90A. (F) VIGS treatment significantly reduced *EjCYP90A* transcript abundance. (G) The repression of *EjCYP90A* transcript abundance induced higher EjBZR1 expression levels. (H) Overexpression of *EjCYP90A* promoted Arabidopsis plant growth. (I) Larger leaf size in EjCYP90A overexpression lines.

Figure. 4 Direct targeting of *EjCYP90A* by EjBZR1. (A) EjCYP90A promoter. (B) Subcellular localization of EjBZR1. (C) Yeast one-hybrid assays of EjBZR1 binding to the EjCYP90A promoter. (D) EjBZR1 represses EjCYP90A expression in N. benthamiana leaves through the dual-luciferase (LUC) system.