

Establishment of the *Fraxinus mandshurica* gene speedy editing technology system by using the *Agrobacterium*-mediated transformation method and identification of the drought resistance function of the *FmbHLH1* gene

Li lei Yang Fan Li weiqi Wang qiang Xu xuefei Ji xiaoyu*

(Shenyang Agricultural University, Shenyang 110000)

Abstract: The lack of an effective genetic transformation system for *Fraxinus mandshurica* restricts the study of its gene functions, and improving its traits through genetic engineering methods is challenging. We here employed an *Agrobacterium*-mediated efficient transient transformation system, with the growing points of *F. mandshurica* as explants. By using a combination of different *Agrobacterium* concentrations and infection times, we established an efficient genetic transformation system for the growing points. Furthermore, a tissue culture system was developed for clustered buds by adding different hormones with varying concentrations. Accordingly, *FmbHLH1* gene-edited chimeric plants were generated through the CRISPR target cutting efficiency assay by using the developed genetic transformation system for growing points. Homozygote plants were screened using the developed clustered bud system. Among 100 randomly transformed growing points, 18 chimeras were obtained, which further yielded 12 homozygotes, indicating an editing efficiency of 18%, which confirmed that the fabricated gene editing technology system was efficient. On analyzing the phenotypes and drought resistance physiological indicators of *FmbHLH1* knockout and wild-type lines, we inferred that *FmbHLH1* is positively correlated with peroxidase and superoxide dismutase activities. In addition, we found that the gene positively regulates the drought resistance of *F. mandshurica* by adjusting its ability to scavenge reactive oxygen species and osmotic potential. This study developed an efficient *Agrobacterium*-mediated gene editing technology system for *F. mandshurica*, using which the drought resistance gene *FmbHLH1* was identified. This study offers an effective method and candidate resistance genes for the molecular breeding of *F. mandshurica*.