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LETTER

From elite germplasm to transformation platform: Breaking recalcitrance in Tartary buckwheat

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Highlights

- An elite germplasm, G253, with superior morphogenic callus induction and proliferation capacity was identified.
- A stable *Agrobacterium*-mediated transformation platform enabling transgenic plant generation was established.
- An efficient protoplast transient transformation system was developed from the morphogenic callus.

Buckwheat is an important functional food resource due to its various bioactive compounds (Zhang *et al.* 2021; He *et al.* 2023). Tartary buckwheat is primarily produced in China, where it thrives in the northern arid regions and high-altitude areas of southern China and plays a crucial role in the agricultural economy in mountainous regions like the Himalayas (He *et al.* 2024). Molecular breeding in buckwheat faces several challenges, including an underdeveloped regeneration system and low genetic transformation efficiency (Tomasiak *et al.* 2022). For example, Dong *et al.* (2019) successfully obtained transgenic callus from hypocotyl explants but could not regenerate full plants. Recently, Pinski and Betekhtin (2023) established a stable transformation system using morphogenic callus (MC) from immature zygotic embryos of Tartary buckwheat accession K17, and they achieved around 20% transformation efficiency and successfully created albino *Ftpds* knockout mutants *via* CRISPR-Cas9. However, the poor ecological adaptability and limited fertility of the K17 accession hinder its use in breeding programs.

China is the center of buckwheat germplasm diversity and the origin of cultivated buckwheat, so it boasts abundant buckwheat resources (Zhang *et al.* 2021). However, a systematic analysis of MC induction and transformation potential among accessions is lacking, and no superior recipient lines have been identified. To address this limitation, our team conducted a phylogenetic analysis of 100 Tartary buckwheat accessions from wild and cultivated germplasm in northern and southern China and the Himalayan region (He *et al.* 2024). We selected 20 core accessions representing distinct phylogenetic clades and evaluated their capacity for regeneration through the induction rate of immature zygotic embryo-derived callus, induction rate of proembryogenic cell complexes (PECCs) (Rumyantseva *et al.* 2003; Betekhtin *et al.* 2017), and PECC proliferation capacity (Fig. 1-A; Appendix A). Immature zygotic embryos at 10–20 days post-anthesis, measuring 0.5–1.5 mm, were selected as explants for MC induction (Fig. 1-B). After surface sterilization and removal of the seed coats and endosperm, zygotic embryos were cultured on RX medium (Appendix B) in the dark at room temperature for callus induction. Within 10 days, significant swelling and irregular surface protrusions emerged, leading to initial callus formation. By day 20, the embryos were covered by “soft” callus, and small white PECCs appeared. Continuous culture for 30 days resulted in the multiplication of PECCs. Mature PECCs undergo cyclical disintegration, producing “soft” callus and new PECCs (Rumyantseva *et al.* 2003; Betekhtin *et al.* 2017) (Fig. 1-B). The zygotic embryo-derived initial callus induction rates were generally high among the accessions (80–100%), but significant variation was found in the induction frequency and proliferative capacity of PECCs (Fig. 1-B; Appendix A). Accessions such as G253, G262, H399, and Y482 from the southwest, along with S136, H58, and H389 from the north, produced

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well-formed and highly proliferative PECCs that retained embryogenic competence during prolonged subculture on high-auxin medium, indicating strong regeneration potential. In contrast, accessions S208 and S337 exhibited low induction rates and poor proliferation, quickly senescing and converting to brownish "soft" callus, while losing morphogenic capacity (Fig. 1-B; Appendix A). Notably, G253 showed the strongest capacity for MC induction and proliferation among the 20 tested Tartary buckwheat accessions (Fig. 1-B; Appendix A). Consistent with our comparative analysis of 20 Tartary buckwheat accessions, the genotype remains a major constraint in genetic transformation for many crops, with significant variations in regeneration capacity observed among genotypes (Chen *et al.* 2022). Histological studies

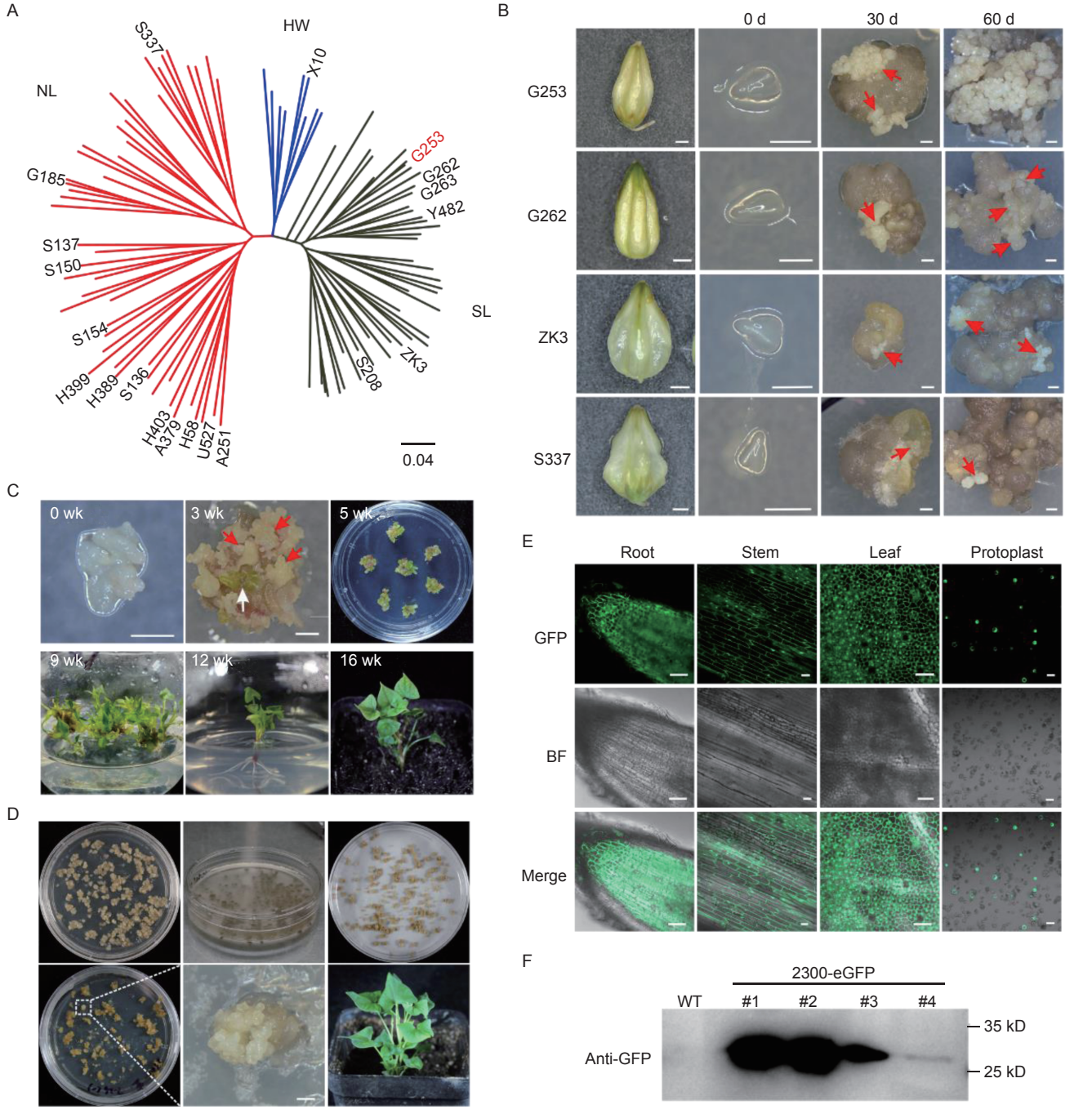


Fig. 1 Transient and stable genetic transformation of Tartary buckwheat morphogenic callus. A, phylogenetic tree of buckwheat germplasm resources from Southwest landrace (SL), Northern Landrace (NL), and Himalayan wild accessions (HW), with the varieties labelled. Scale bar, 0.04 substitutions per 100 bp. B, developmental stages of immature embryos from four varieties on RX medium. proembryogenic cell complexes (PECCs) are marked with red arrows. Scale bar, 1 mm. C, schematic of the regeneration process from MC with time points of the PECCs. Somatic embryos are marked with red arrows, and the bud is marked with a white arrow. Scale bar, 1 mm. D, schematic of *Agrobacterium*-mediated transformation using MC as the recipient. Scale bar, 1 mm. E, confocal microscopy images of the roots, stems, and leaves of positive transgenic buckwheat lines and transformed protoplasts. GFP, green fluorescent protein; BF, bright field. Scale bar, 50 μm. F, Western blot of positive transgenic buckwheat plants using the Rabbit anti GFP-Tag mAb (ABclonal-AE078, China). WT, wild type.

have confirmed that PECCs originate from less-differentiated cells within the embryonic cotyledon — specifically, the spongy mesophyll and procambium cells beneath the adaxial epidermis (Kostyukova *et al.* 2018). The exceptional PECC induction and proliferation capabilities of G253 in this study may be attributed to its genetic background, potentially involving heightened meristematic cell maintenance, enhanced auxin signaling efficiency, superior epigenetic stability, and increased stress tolerance, so using G253 as a model warrants further investigation.

Given the strong proliferative capacity of the G253 MC, we evaluated its capability for complete plant regeneration based on the morphogenetic system defined by Pinski and Betekhtin (2023). PECCs that had been subcultured for 2 wk were inoculated onto a regeneration medium (RM, Appendix B) and cultured at 24°C with a 16 h light (60 μmol m^{-2} s^{-1})/8 h dark photoperiod. After 1 wk, the PECCs expanded and gradually turned partially green; and after 2 wk, somatic embryos and shoot primordia formed on the callus surface (Fig. 1-C). The callus was then transferred to fresh RM medium for further culture until somatic embryos or shoots developed. Mature somatic embryos and young shoots were transferred to Murashige and Skoog (MS) medium without growth regulators to promote shoot and root development. After 6–7 wk, healthy seedlings were obtained and acclimatized for transplantation into nutrient-rich soil, resulting in completely regenerated plants (Fig. 1-C).

Using this regeneration system, G253 MC was also employed for *Agrobacterium*-mediated genetic transformation. We determined the optimal kanamycin resistance concentration for screening as 50 mg L^{-1} based on sensitivity tests (Appendix C). Two weeks after subculturing, MCs were immersed in an *Agrobacterium* strain GV3101 suspension containing the pCAMBIA2300-eGFP plasmid and co-cultivated for 72 h. Following two rounds of selection on RX selection medium (RXS, Appendix B), new PECCs emerged and only the new PECCs were carefully selected (Fig. 1-D). Given the single-cell origin of PECCs (Rumyantseva *et al.* 2003), callus lines emerging from the same position were treated as a single cell line. Molecular analysis of 13 callus lines isolated from a single transformation experiment revealed successful integration of the T-DNA fragment in 12 of the lines, indicating a positive rate exceeding 90% (Appendix D). Persistent eGFP fluorescence was observed in the subcultured progeny of positive callus (Appendix D), further demonstrating stable integration of the transgene. The positive PECCs were subsequently induced to undergo morphogenesis, resulting in transgenic buckwheat plants, with further verification of target gene integration and expression by fluorescence analysis and Western blotting (Fig. 1-D–F).

Using MC as a recipient for transient transformation offers significant advantages due to its rapid preparation. We obtained protoplasts with up to 95% viability from 2-wk cultured MC *via* sucrose density gradient centrifugation based on the method by Zaranek *et al.* (2023) (Appendix B). PEG-mediated transient transformation yielded an efficiency rate of nearly 20% (Fig. 1-E; Appendices B and E). These results demonstrate that G253 MC has excellent regenerative capacity and is suitable for an efficient protoplast transformation system.

In conclusion, using morphogenic callus for genetic transformation represents a significant advancement in overcoming the challenges with buckwheat species. This strategy offers a uniform, actively dividing cell population with high regenerative capacity, which improves the efficiency of gene infection, integration, and regeneration. By screening the superior Tartary buckwheat variety G253 and establishing an efficient morphogenic callus induction system, we provide critical germplasm and technological support for establishing robust genetic transformation platforms and accelerating molecular breeding progress in Tartary buckwheat.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used DeepSeek to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of this article.

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