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Brief Communication

Fast track to obtain heritable transgenic sweet potato inspired by its evolutionary history as a naturally transgenic plant

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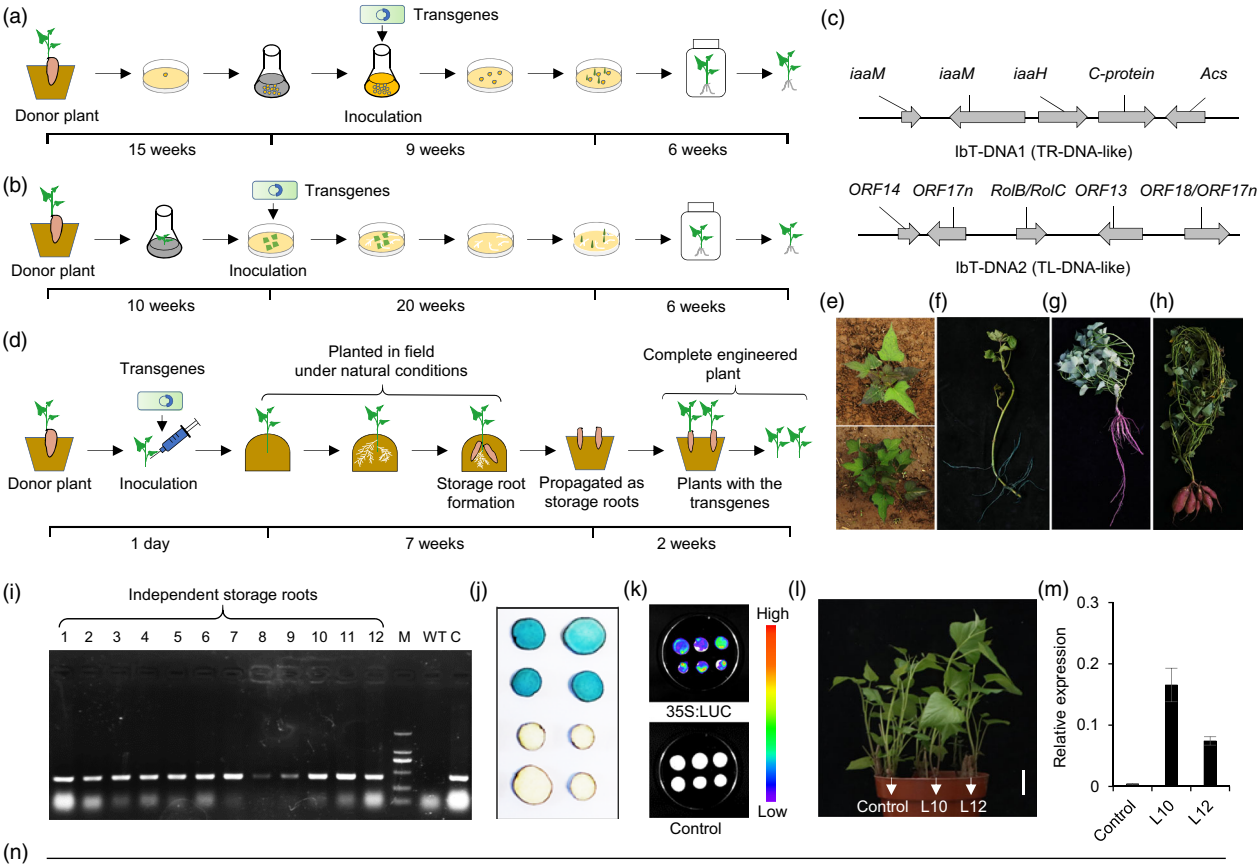
Sweet potato (*Ipomoea batatas* [L.] Lam.) is one of the most important crops in the world. Conventional genetic engineering requires delivering genetic changes to plant cells usually using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* (Otani *et al*., 1993; Yu *et al*., 2007). Both systems through tissue culture are time-consuming, require highly trained individuals and often cause unintended changes to the genome, strongly hampering its research using genetic approaches (Figure 1a,b).

Previous studies from our laboratory and collaborators showed that sweet potato is a naturally transgenic plant that contains two *Agrobacterium* transfer DNAs (T-DNAs) called IbT-DNA1 and IbT-DNA2 (Kyndt *et al*., 2015). *Agrobacterium rhizogenes* contains two transferable T-DNA regions: TR-DNA corresponds to IbT-DNA1 (harbouring the auxin biosynthesis genes *iaaH* and *iaaM*) and TL-DNA (harbouring the various *Rol* genes) to IbT-DNA2 (Figure 1c). At least four of the IbT-DNA1 genes and two of the IbT-DNA2 genes are expressed in every tissue type tested and IbT-DNAs appear to have a role in the evolution, suggesting that this bacterium-plant relationship should not be regarded as a merely parasitic interaction, but rather as a symbiosis (Kyndt *et al*., 2015; Quispe-Huamanquispe *et al*., 2017).

Agrobacterium rhizogenes is a relative of *Agrobacterium tumefaciens* and can be used to induce adventitious roots named 'hairy roots' upon wounding and infection of plant leaves or stems. Once the root-inducing T-DNA is inserted into the host genomic DNA, the new hormonal balance regulates the infected cells inducing the formation of proliferating roots, called hairy roots, emerging at the points of infection. When *Agrobacterium rhizogenes* harbours a binary vector, the hair roots may contain the T-DNA of the binary vector if it is cotransferred (Phelep *et al*., 1991). Nevertheless, hairy root cells are not expected to transfer T-DNA to subsequent generations of plants in the natural condition. Notably, the storage roots of sweet potato result from secondary thickening of the adventitious roots and have functions in both carbohydrate storage and vegetative propagation. Together, this phenomenon is suggestive of the hairy roots as a kind of adventitious root with the potential to develop into storage roots to achieve heritable genetic modifications.

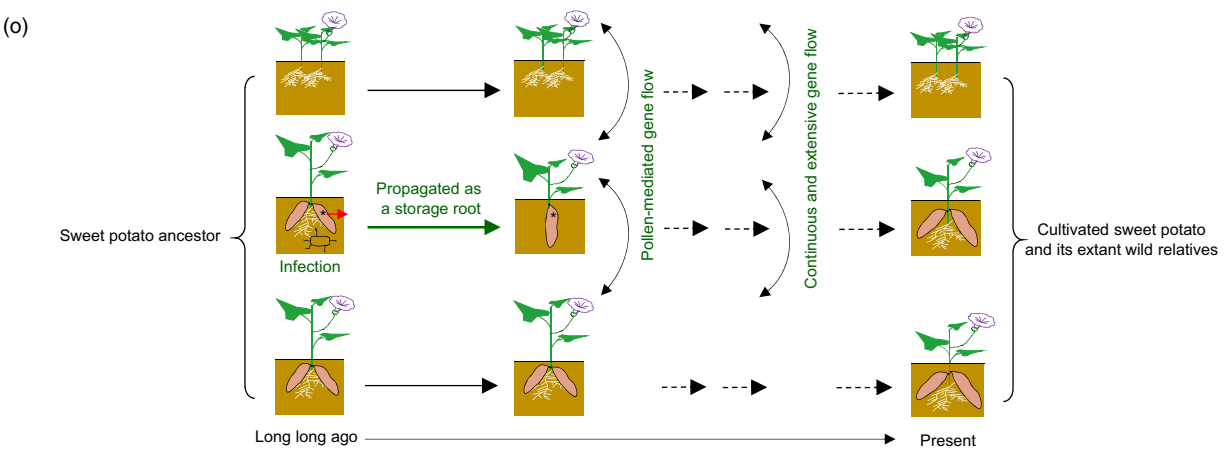
To test this hypothesis, we first determined whether one-step *Agrobacterium rhizogenes*-mediated transformation could apply to obtain heritable transgenic sweet potato plants via overexpression of β-glucuronidase (*GUS*) gene. The recombinant *IbRPS5a:GUS* plasmid was introduced into *Agrobacterium rhizogenes* strain K599 by freeze-thawing and used for plant transformation. Shangshu 19, a widely cultivated sweet potato cultivar in China, was employed in this study (Figure S1). Briefly, sweet potato vine cuttings were infected with *Agrobacterium rhizogenes* K599-IbRPS5a:GUS by wounding the nodes with a syringe. Subsequently, the inoculated vine cuttings were then directly planted in the field under natural conditions (Figure 1d, Appendix S1). The induction of hairy roots and the growth of plant were finished in the field (Figure 1e–h). PCR detection of the hygromycin phosphotransferase gene demonstrated that nearly 100% of the infected vine cuttings could produce transgenic positive storage roots (Figure 1i, Figure S2). As shown in Figure 1j, transgenic plants exhibited a high level of GUS activity, indicating the stable integration of binary vector T-DNA in the genome of storage roots. Other transgenes are conducted in the same way (Figure 1k,l). Whole plants were regenerated from storage roots within 2 weeks. The overexpression of transgene in the seedlings grown from storage roots was also assessed by quantitative reverse transcription-PCR (qRT-PCR) analysis (Figure 1m). Using this method, 90%–100% of the infected plants form positive storage roots within 2 months from the start of the experiments (Figure 1n). Consequently, the *Agrobacterium rhizogenes*-mediated method is faster, simple and more efficient than the conventional transformation methods. Furthermore, because every root meristem derives from a single cell, hairy root lines established from single root meristems are cellular clones (Costantino *et al*., 1984). Consequently, every transgenic storage root represents an independent transformation event.



(n)

Transgene vectors	No. of inculated plants	Total storage roots per plants	Positive storage roots per plants	Positive storage roots per plants	Transformation efficiency
IbRPS5a:GUS	15	3.20 ± 1.08	2.53 ± 1.25	75.3%	93%
35S:LUC	18	3.11 ± 1. 02	2.33 ± 0.97	76.8%	94%
35S:IbLOG2	12	2.75 ± 1.05	2.42 ± 1.08	84.7%	92%
35S:IbFBox	9	2.70 ± 0. 95	1.81 ± 0.92	67.5%	90%
35S:IbNramp4	10	3.30 ± 1. 06	2.80 ± 1.13	84.2%	100%

Average values±SD are shown.



Recently, using *Agrobacterium* T-DNA-encoded proteins as queries against sequenced plant genomes and transcriptomes shows naturally transgenic plant species occur on an unexpectedly large scale (Matveeva and Otten, 2019). Interestingly, the vast majority of these horizontal gene transfer events were mediated by *Agrobacterium rhizogenes* (Matveeva, 2021). Our results support the hypothesis that an *Agrobacterium rhizogenes* (or an ancestral related species) infection at some point in the past resulted in a clone (storage root) that possessed an interesting trait, perhaps a compact plant phenotype or/and

Figure 1 Fast track to obtain completely genetically engineered sweet potato. (a) Conventional *Agrobacterium tumefaciens*-mediated sweet potato transformation using embryogenic suspension cultures. (b) Traditional transformation of sweet potato plants by *Agrobacterium rhizogenes*. (c) Two T-DNAs found in the cultivated sweet potato genome. (d) Overview of one-step generation of heritable genetically modified sweet potato with *Agrobacterium rhizogenes*. (e) Inoculated explant (upper) and wild-type control (bottom) were planted in the field under natural conditions. (f) GUS staining of the roots of K599-IbRPS5a:GUS strain infected plant after 14 days post-inoculation. (g) Representative phenotype of K599-IbRPS5a:GUS strain infected plant during storage root expansion period. (h) Representative K599-IbRPS5a:GUS strain infected plant at harvest. (i) Identification of hygromycin phosphotransferase gene in the storage roots via PCR; C, empty pMDC162 plasmid as a control; M, DL2000 DNA marker; WT, wild type. (j) GUS staining of storage root slices of *IbRPS5a:GUS* transgenic lines (upper) and negative control (bottom). (k) The luminescence images of storage root slices of *35S:LUC* transgenic lines (upper) and negative control (bottom) were captured using a CCD imaging system. (l) Morphological phenotypes of control (empty vector) and the representative *35S:IbNramp4* plants. Bar = 5 cm. (m) Relative mRNA levels of *IbNramp4* were quantified by qRT-PCR in 2-week-old seedlings shown in 'l'. Error bars indicate SD (n = 3). (n) Transformed efficiency of one-step *Agrobacterium rhizogenes*-mediated systems. (o) A proposed model of how *Agrobacterium rhizogenes* (or an ancestral related species) transfers T-DNAs into the cultivated sweet potatoes.

better adaptability, which was selected by humans, and vegetatively propagated. During the long process of cultivation, domestication, dispersal and diversification, many wild relatives might be admixed with sweet potato through pollen-mediated gene flow (Figure 1o). This might explain why genome-integrated IbT-DNAs are not restricted to the cultivated sweet potato but are also present in the related species.

Together, we develop an efficient *Agrobacterium rhizogenes*-mediated method sidestepping the need for tissue culture can be used to quickly obtain genetically modified sweet potato plants for biotechnology and research uses, such as functional characterization of genes involved in nutrient uptake and hormone transport, a means to improve production of phytochemicals and recombinant proteins. We envisage that this one-step *Agrobacterium rhizogenes*-mediated transformation might apply to other root propagating or these naturally transgenic plants. However, this strategy may still have limitations, especially when studying tightly controlled developmental processes that may be deregulated by the Ri T-DNA. In these situations, the disarmed variants of *Agrobacterium tumefaciens*-mediated transformation systems are still preferred.

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Conflict of interest

The authors have not declared a conflict of interest.

Authors' contributions

S.G., H.Zhai and R.X. conceived the idea and designed the experiments. S.G., W.Z., Z.Z, Y.Z., H.Zhao, Y.F. and Y.W. performed experiments. S.G. R.X. and H.Zhai analysed and interpreted data. H.Zhai, H.Zhang, N.Z., S.H. and Q.Liu contributed to funding acquisition and supervised the project. S.G. drafted the manuscript. All authors discussed the results and contributed to the final article.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 IbT-DNA1 and IbT-DNA2 in the genome of Shangshu 19 confirmed via PCR.
Figure S2 Validation of TL-DNA and TR-DNA of root-inducing plasmid in transgenic storage roots.
Table S1 A list of primers used in this study.
Appendix S1 Materials and methods.

