

Establishment of in vitro shoot tips regeneration system of foxtail millet and obtainment of transgenic plants of *SiSERK1*

Wu Guofan (✉ wugf1971@163.com)

Northwest Normal University <https://orcid.org/0000-0002-2844-0820>

Tian Nongfu

Northwest Normal University

Li Wenbo

Northwest Normal University

Wu Wangze

Northwest Normal University

Zheng Sheng

Northwest Normal University

Yang Ning

Northwest Normal University

Research Article

Keywords: foxtail millet, genetic transformation, explant, shoot tip, SiSERK1

Posted Date: September 19th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1795578/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Foxtail millet (*Setaria italica*) would be suitable as a model plant of C4 plants given its small genome (about 470 MB) and diploid self-pollination. However, the study of foxtail millet faces the problem of low efficiency of explant regeneration and genetic transformation. In this study, a new genetic transformation system of Yugu1 foxtail millet is established with *in vitro* shoot tips as the explant, and, the concentrations of 6-BA and kanamycin are optimized. It is found 0.5 mg L⁻¹ 6-BA and 25 mg L⁻¹ kanamycin are the most suitable in terms of the differentiation rate of shoot tips and survival rate of differentiated seedlings. In addition, 12 transgenic foxtail millets of *SiSERK1* are identified by resistance screening and PCR. The insertion site of one line of the transgenic plants chosen at random is further identified. The results of qRT-PCR show that the expression of *SiSERK1* gene in transgenic plants is significantly higher than that in wild-type plants. A new method of generation of material for further study of *SiSERKs* is provided for foxtail millet genetics and breeding.

Introduction

Plant tissue culture and genetic transformation technology have been applied to many crops, and each crop has its unique regeneration and transformation materials. The optimal explants for regeneration and genetic transformation of rice [1], wheat [2] and potato [3] are seed, mature embryo and the internodal, respectively. Foxtail millet is one of the oldest crops in the world with a wide planting range [4-7]. The tissue culture of foxtail millet first began in the 1970s. The anthers of foxtail millet were cultured by Y. Ban in Blaydes medium containing 2,4-D and KT and the regenerated plants were successfully obtained [8]. Subsequently, when scientists studied the viability of downy mildew mycelium of foxtail millet seeds, it was found that the callus induced by foxtail millet seeds could also be induced to differentiate into a mature plant [9]. Since then, the tissue culture system of foxtail millet had been established. With the development of tissue culture, the research on genetic modified plant (GMP) of foxtail millet has also begun. In 2007, the callus induced by young panicle of foxtail millet was used as the receptor material for GMP research for the first time [10], and the factors affecting the transformation efficiency were analyzed. Successively, the induced by inflorescence [11], mature embryo [12-15], seeds [16] and young embryo [10] were used for genetic transformation research. A kind of mini millet was studied as the C4 model plant and it only took 4-5 months to get from callus to mature seed [17]. So far, the progress has been made in the research on the tissue culture and genetic transformation of foxtail millet. However, compared with rice (*Oryza sativa*) [18], wheat (*Triticum aestivum*) [19, 20] and other crops [21], the genetic transformation efficiency of foxtail millet is still very low. In this study, aiming at improving the regeneration efficiency, the shoot tips of foxtail millet are taken as the explant for genetic transformation materials.

Somatic embryogenesis receptor-like kinase (SERK) genes widely exist in the plant kingdom. It is a family of structurally conserved genes with functions such as plant embryonic development, male development, disease defense and signal transduction [22, 23]. They have been verified from *Arabidopsis thaliana* [24], rice [25], wheat [26] and other plants [27, 28]. In our early study, *Setaria italica* *Somatic embryogenesis receptor-like kinase 1 (SiSERK1)* engineering strain has been constructed and overexpressed in a brassinosteroid (BR) receptor mutant of *Arabidopsis thaliana* WS2 (*bri1-5*) [29]. It was found that *SiSERK1* was involved in BR signaling pathway and plant immune regulation. Therefore, it is speculated that *SERK* genes may have their own unique function in each crop, and its functions in foxtail millet need to be further studied. Therefore, in order to improve the genetic transformation efficiency of foxtail millet, the *in vitro* shoot tips of foxtail millet were used as explants for tissue culture to establish a new *in vitro* regeneration system of foxtail millet in this study, and *SiSERK1* gene is transferred into foxtail millet.

Materials And Methods

2.1 Plant material and tissue culture

In 2011, the transgenic plant of *Pennisetum glaucum* (L.) R. Br was obtained using shoot apices as explant source by P. Jha [30]. In this study, the *in vitro* regeneration system of foxtail millet is established according to P. Jha and regenerated plants are obtained. The mature seeds of foxtail millet are sterilized in 0.1% mercuric chloride for 20 min. And then rinsed with 80% alcohol for 2 min and with sterile distilled water five times, each time for 2 min. After that, the surface sterilized seeds are blot dried using sterile filter paper on a plastic petri dish. Then 16 seeds are inoculated on MS medium (4.33 g·L⁻¹ MS + 30 g·L⁻¹ sucrose + 10 g·L⁻¹ agar) in a culture dish and cultured in the dark for 4 days at 25 °C. The shoot tips, about 5 mm of the top of seedlings, are cut and placed on MS medium to induce differentiation. After continuous culturing for 2 weeks, the differentiated seedlings are transferred into a culture bottle containing 1/2 MS medium for rooting. The seedlings growing up to 3 cm are moved into nutrient soil after one day of acclimatization.

2.2 GUS histochemical assay and expression analysis

The shoot tips of foxtail millet cultured for 4 days are infected by the *Agrobacterium tumefaciens* LBA4404 harboring pCAMBIA-1305.1. Then GUS staining is performed after co-cultivating for 3 days according to the prescribed method [31]. The shoot tips are made a small incision in the middle with a scalpel, and then they are put into a 1.5 mL EP tube. GUS staining solution (0.5 M Na₂EDTA (pH 8.0), 50 mM K₄Fe (CN)₆·3H₂O, 50 mM K₃Fe (CN)₆, 0.1 g·mol⁻¹ X-Gluc, 10% Triton X-100, 0.05 M PBS (pH 7.0)) is added for dying at 37 °C for 16 h. The gradient decolorization is conducted by using ethanol until the material has no obvious green, then photos are taken by a microscope.

2.3 The concentration screening of 6-BA and kanamycin

6-BA, an important plant hormone, is used to promote cell differentiation. Therefore, The shoot tips of seedlings cultured on MS medium for 4 days in the dark are taken out and put on the induced differentiation medium containing 6-BA concentration gradient (0, 0.25, 0.5, 0.75, 1.0 mg·L⁻¹). The differentiation rates of shoot tips are counted after 2 weeks of culturing.

Because the pBIB vector transformed contains kanamycin resistance gene, the resistant seedlings are screened by kanamycin. Kanamycin concentration gradient (0, 10, 15, 20, 25, 30, 35 and 40 mg·L⁻¹) is set to find the suitable resistance concentration.

2.4 Establishment of genetic transformation system

In our early work, the CDS region of *SiSERK1* gene has been constructed into the plant overexpression vector pBIB by Gateway technology and transferred into *Agrobacterium tumefaciens* LBA4404 [29]. Therefore, only the strain needs to be activated and identified in this experiment.

The system is established according to P. Jha [30]. The shoot tips cultured for 4 days in the dark are cut and put into the prepared *Agrobacterium* immersion solution (4.33 g·L⁻¹ MS + 30 g·L⁻¹ sucrose + 100 μM·L⁻¹ AS + 0.5 mg·L⁻¹ 6-BA + OD₆₀₀ = 1.0 bacterial solution) for 20 min. After that, the shoot tips are placed on the filter paper for 5 min to dry the surface water. Then the shoot tips are put into the co-culture medium (4.33 g·L⁻¹ MS + 30 g·L⁻¹ sucrose + 100 μM·L⁻¹ AS + 0.5 mg·L⁻¹ 6-BA + 10 g·L⁻¹ agar) covered with a layer of filter paper and cultured at 25 °C in the dark. After 3 days, they are transferred to the screening differentiation medium (4.33 g·L⁻¹ MS + 30 g·L⁻¹ sucrose + 250 mg·L⁻¹ cephalosporium + 0.5 mg·L⁻¹ 6-BA + 10 g·L⁻¹ agar + 25 mg·L⁻¹ kanamycin) for two consecutive screening for 2 weeks each time. After screening, the differentiated seedlings are transferred to 1/2 MS medium for rooting. The seedlings growing to 3 cm are transferred to the soil for culturing after one day of acclimatization.

2.5 Identification of transgenic plants

The DNAs are extracted from transgenic plants and wild-type foxtail millet growing in soil for 4 weeks by plant genomic DNA extraction kit (TIANGEN BIOTECH (BEIJING) CO, China). The PCR reaction to identify transgenic plants is performed according to the procedure of template 2 μL, ddH₂O 9.9 μL, 10 × Taq buffer 1.5 μL, dNTPs 0.3 μL, the upstream and downstream primers 0.6 μL, enzyme 0.1 μL respectively, taking the extracted DNA as the template, *SiSERK1*-F and *SiSERK1*-R as upstream and downstream primers. The PCR program is as follows: 94 °C for 5 min; 36 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 3 min; and 72 °C for 5 min. The primer sequences are as table 1.

2.6 Expression analysis of *SiSERK1* gene

Total RNA is extracted using RNA simple total RNA kit (TIANGEN BIOTECH (BEIJING) CO, China). Complementary DNA (cDNA) is synthesised using HiScript® II 1st Strand cDNA Synthesis Kit (+Gdna wiper) (Nanjing Vazyme Biotech Co, China). The qRT-PCR is performed in a 96-well plate using a Bio-Rad iQ5 (Bio-Rad, USA). The PCR primers are designed by Primer Premier 5.0 software and the sequences are listed in table 1. The qRT-PCR reaction procedure is performed using 2 × SYBR Premix ExTaq Tm II (Takara, Dalian, China). The 20 μL reaction system contains 10 μL 2 × SYBR, 1 μL each of forward and reverse primers, 2 μL cDNA template and 6 μL ddH₂O. Reactions are carried out as the following cycling program: 95 °C for 5 min, 40 cycles of 95 °C for 15 s and 60 °C for 30 s, 1 cycle of 95 °C for 15 s, 60 °C for 1 min and 95 °C for 1 s. Actin is taken as an internal control. The 2^{-ΔΔCt} method is used to calculate relative changes in gene expression.

Table 1 Primer sequences

Primer	Sequence (5'-3')	Usage
<i>SiSERK1</i> -F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCAATATCTGGAACCTTACAGTA	Gene identification
<i>SiSERK1</i> -R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCCTCGGGCCGGACAGCTC	Gene identification
<i>SiSERK1</i> -qRT-F	TTCAATCCCCAACACCAAC	qRT-PCR
<i>SiSERK1</i> -qRT-R	ATGCTCTTCAGGCTTACGCC	qRT-PCR
Actin-F	TGCTCAGTGGAGGCTCAACA	qRT-PCR
Actin-R	CCAGACACTGTACTTGCGCTC	qRT-PCR
YFP-F	ATGGTGAGCAAGGGCGAG	Site detection
YFP-R	TCACTTGACAGCTCGTCCATG	Site detection
Detection primer-R	GCCAGGCTGCACGAATC	Site detection

2.7 Identification of insertional site of *SiSERK1* in transgenic plant

The insertion site of *SiSERK1* gene in transgenic millet is determined by YFP-F and YFP-R. The PCR reaction is performed DNA 2 μL, ddH₂O 9.9 μL, 10 × Taq buffer 1.5 μL, dNTPs 0.3 μL, YFP-F 0.3 μL and YFP-R 0.006 μL, enzyme 0.1 μL respectively, taking the extracted DNA as the template. The PCR program is as follows: 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min; and 72 °C for 5 min. The primer sequences are as table 1.

The PCR product (about 1000 bp) is sequenced with YFP-F. The YFP is about 700 bp and the other 300 bp is compared with the millet genome sequence to determine its insertion site. The downstream primers of single strand complementary strand are designed according to the sequencing results, and the transgenic millet DNA is identified by YFP-F and detection primer-R to ensure the correct location.

Results

3.1 Establishment of regeneration system

The sterilized Yugu 1 seeds are seeded in MS medium with 16 seeds in each petri dish (Fig. 1a) and cultured in the dark at 25 °C for 4 days (Fig. 1b). The shoot tips are taken and put into MS medium for differentiation (Fig. 1c). After two weeks of continuous culturing (Fig. 1d), the differentiated seedlings are moved to the culture tank containing 1/2 MS medium to induce rooting. After a week (Fig. 1e), the differentiated seedlings begin to take root. Two weeks later of culturing, the differentiated seedlings grow well and the taproots become longer (Fig. 1f) and then they are moved into nutrient soil for culturing.

3.2 GUS assay for co-cultivated shoot tips

To ensure that *in vitro* shoot tips of foxtail millet can be used as the direct receptor material for genetic transformation, in this study, the shoot tips explant co-cultured for 3 days after immersion of *Agrobacterium tumefaciens* containing pCambia-1305.1 (Fig. S1) are put into a 1.5 mL centrifuge tube and subjected to GUS staining assay. The results are schematically shown in Fig. 2, and the GUS activity can be observed obviously (Fig. 2S1 and 2S2). The results indicate that *in vitro* shoot tips are capable be used as explants for genetic transformation of foxtail millet.

CK: GUS staining results of shoot tips explants not infected by *Agrobacterium tumefaciens*, CK1: one of CK. S1 and S2: GUS staining results of shoot tips explants impregnated with *Agrobacterium tumefaciens*, S1-1 and S2-2: one of S1 and S2.

3.3 Determination of hormone concentration for optimal differentiation

3.3.1 Determination of 6-BA concentration

The *in vitro* shoot tips are inoculated on the induced differentiation medium added 6-BA concentration gradient to induce differentiation. The differentiation rate of shoot tips is counted and analyzed after 2 weeks of culturing (Fig. 3A). When the 6-BA concentration is 0.5 mg·L⁻¹, the differentiation rate of shoot tips is the highest. Therefore, 6-BA concentration of 0.5 mg·L⁻¹ is selected for transformation.

3.3.2 Determination of kanamycin concentration

Kanamycin can interfere with the synthesis of chloroplast and mitochondrial proteins in plant cells [32, 33] and cause yellowing and whitening of plant green organs. In the early stage of this study, the *Agrobacterium tumefaciens* containing pBIB-BASTA-35S-*SiSERK1*-GFP is constructed for foxtail millet genetic transformation. Because the pBIB vector transferred contains kanamycin resistance gene and kanamycin could damage the seedlings in the screening medium. In this study, the appropriate concentration of kanamycin is screened by setting the kanamycin concentration gradient (Fig. 3B). A suitable kanamycin concentration of 25 mg·L⁻¹ is selected for screening transgenic seedlings. The phenotype of wild-type plants treated with kanamycin is shown in Fig. 3C.

3.4 The screening of transgenic seedlings

Shoot tips that has been co-cultured for 3 days are placed into screening differentiation medium for induction differentiation. One week later, most of the differentiated shoot tips survive (Fig. 4a). After the first subculturing, some differentiated seedlings have albino and die (Fig. 4b), and after the second subculturing, most differentiated seedlings die (Fig. 4c). The surviving seedlings are transferred to the rooting medium for culturing. However, there are two phenotypes of surviving seedlings (Fig. 4e). One is that all differentiated shoot tips in the early stage of screening are albino and died, and new shoot tips are redifferentiated in the later stage of screening. The other phenotype is that differentiated shoot tips are always green throughout the screening process. Subsequently, the screened positive seedlings are transferred to 1/2 MS medium for cultivation for 14 days (Fig. 4d and 4h), followed by moved into nutrient soil for culturing after one day for acclimatization (Fig. 4f and 4g).

3.5 Identification of transgenic plants and Expression analysis of *SiSERK1* gene

The DNA of the positive screening seedlings grown for 4 weeks is extracted and identified by PCR (Fig. 5B). The results obtained are similar to the identified results of *SiSERK1* engineering bacteria (Fig. 5A) (1654 bp). Hence, it is considered that *SiSERK1* gene is successfully transferred into foxtail millet.

The qRT-PCR is used to detect and evaluate the expression of *SiSERK1* gene in wild-type plants and transgenic plants (Fig. 5C). The results indicate that the expression of *SiSERK1* gene in four transgenic materials is remarkably higher than that in wild-type plants. Consequently, it is determined that *SiSERK1* gene has been transferred to foxtail millet and 12 *SiSERK1* transgenic plants are successfully obtained. The total transformation rate reached 4.5%.

3.6 Identification of *SiSERK1* insertion sites

The single strand is amplified by asymmetric PCR (Fig. 6A), and its size is about 1000 bp. The sequencing result is compared in the millet database on NCBI. It is found that the sequence is aligned to 37529335-37529661 bp on chromosome 9 of millet. Single stranded sequences and transgenic plants are identified using YFP-F and detection primer-R (Fig. 5b). The size is about 1000 bp, which is consistent with the target size. Therefore, we can determine that *SiSERK1* has been inserted in front of chromosome 37529335 bp (Fig. 6C).

Discussion

According to the improved experimental methods of P. JHA [30], the *in vitro* shoot tips regeneration system of foxtail millet has been established, and *SiSERK1* transgenic foxtail millet is identified by resistance screening and molecular detection. Eventually, 12 transgenic foxtail millets of *SiSERK1* have been successfully obtained. It provides a theoretical guidance for the future study of foxtail millet genetic transformation system and the function of *SiSERK1* gene.

This study proves that the *in vitro* shoot tips of foxtail millet can be induced to differentiate into a new mature plant. Whereafter, the *in vitro* shoot tips are transferred into GUS reporter gene [34-36]. GUS staining is performed after 3 days of co-culturing, and the results show that the *in vitro* shoot tips of foxtail millet can be directly used as the receptor for genetic transformation. Then, the gradient screening is carried out on the concentration of 6-BA and kanamycin. It is determined that the concentration of 6-BA with the highest differentiation rate of shoot tips is $0.5 \text{ mg}\cdot\text{L}^{-1}$, which is consistent with the results of Cesar [37], and the kanamycin concentration suitable for screening transgenic seedlings is $25 \text{ mg}\cdot\text{L}^{-1}$. After transferring *SiSERK1* gene, the positive transgenic seedlings are preliminarily obtained by kanamycin screening and PCR identification. The qRT-PCR is used to detect the expression of *SiSERK1* gene in the materials transformed with *SiSERK1* gene. The results show that *SiSERK1* gene is successfully transferred into foxtail millet. *In vitro* genetic transformation of millet stem tips, three-day millet stem tips are directly transfected with *Agrobacterium tumefaciens*, and the transformation rates are 2.5% and 1.5% [38]. Our method has higher transformation about 4.5% and shorter screening cycle when using the isolated stem tip of millet. Compared with the previous research about genetic transformation of foxtail millet, this experiment is obviously innovative. Firstly, the receptor materials used are very different, the main receptor materials of foxtail millet genetic transformation in the previous stage were the callus induced by inflorescences [11], seeds [16] and mature embryos [12-15]. In this study, the *in vitro* shoot tips of foxtail millet cultured in the dark for 4 days are mainly used, and the differentiation and rooting need to be induced in the culture medium. Secondly, the presented phenotypic of differentiated seedlings during screening and differentiation are analyzed. It is preliminarily speculated that the first phenotype is due to the fact that the kanamycin resistance gene of transformed plants is not expressed immediately at the initial stage of screening. The second phenotype is due to the high efficiency of shoot tips transformation, and the transgenic plant show resistance to kanamycin soon after transformation. Finally, the transformed plants are detected by molecular detection to ensure the successful transformation of transgenic materials.

According to the existing reports, *SERK* genes have many functions, mainly involved in plant immune response and regulating plant growth and development [19]. In this study, the transgenic foxtail millet of *SiSERK1* is obtained. It provides materials for further studying the function of *SiSERK1* gene, especially in improving the disease resistance.

In further study, we will continue to improve the *in vitro* shoot tips regeneration system of foxtail millet and optimize the genetic transformation efficiency. In addition, some questions about whether transgenic foxtail millet of *SiSERK1* has disease resistance, whether there are significant differences between *SiSERK1* gene and *AtSERK1* gene, and whether they are involved in a new signaling pathway will be studied, as well as whether the same gene has similar functions in C4 plant and C3 plant [39].

Declarations

Acknowledgements

We would like to thank the reviewers, editors for their comments and suggestions.

Funding

This research is funded by National Natural Science Foundation of China (No. 31860113), (No.31960061).

Author information

Guofan Wu

Laboratory of the research for molecular mechanism and functional genes of plant stress adaptation, College of life sciences, Northwest Normal University, Lanzhou 730070, China. ORCID: 0000-0002-2844-0820.

Nongfu Tian, Wenbo Li, Wangze Wu, Sheng Zheng, Ning Yang

Laboratory of the research for molecular mechanism and functional genes of plant stress adaptation, College of life sciences, Northwest Normal University, Lanzhou 730070, China

Contributions

Author contributions: writing and data analysis, Nongfu Tian and Wenbo Li; review and editing, Guofan Wu; funding acquisition, Wangze Wu (No. 31860113) and Ning Yang (No.31960061); paper inspection, Sheng Zheng. All authors read and agree to publish version of the manuscript.

Declarations

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Lee H-J, Abdula SE, Jee M-G, et al (2011) High-efficiency and Rapid *Agrobacterium*-mediated genetic transformation method using germinating rice seeds. *J Plant Biotechnol* 38:251–257. <https://doi.org/10.5010/jpb.2011.38.4.251>
2. Kim D, Hager M (2020) Optimize Highly Efficient Genetic Transformation Method of Spring Wheat (*Triticum aestivum* L.) Cultivars Using Mature Embryo. *BIO-PROTOCOL* 10:e3816. <https://doi.org/10.21769/BioProtoc.3816>
3. A. Bakhsh, Development of Efficient, Reproducible and Stable *Agrobacterium*-Mediated Genetic Transformation of Five Potato Cultivars, *Food Technology and Biotechnology* 58 (2020) 57-63. <https://doi.org/10.17113/ftb.58.01.20.6187>.
4. Peng R, Zhang B (2021) Foxtail Millet: A New Model for C4 Plants. *Trends Plant Sci* 26:199–201. <https://doi.org/10.1016/j.tplants.2020.12.003>
5. Ding ZS, Huang SH, Zhou BY, et al (2013) Over-expression of phosphoenolpyruvate carboxylase cDNA from C4 millet (*Setaria italica*) increase rice photosynthesis and yield under upland condition but not in wetland fields. *Plant Biotechnol Rep* 7:155–163. <https://doi.org/10.1007/s11816-012-0244-1>
6. Swain A, Behera D, Karmakar S, et al (2021) Morphophysiological alterations in transgenic rice lines expressing PPK and ME genes from the C4 model *Setaria italica*. *J Plant Physiol* 264:153482. <https://doi.org/10.1016/j.jplph.2021.153482>
7. Lata C, Gupta S, Prasad M (2013) Foxtail millet: A model crop for genetic and genomic studies in bioenergy grasses. *Crit Rev Biotechnol* 33:328–343. <https://doi.org/10.3109/07388551.2012.716809>
8. Ban, Y., T. Kokubu, Y. Miyaji, (1971) Production of haploid plant by anther-culture of *Setaria italica*. *Kagoshima Univ.Fac.Agr.Bull.* 21: 77–81. <https://agris.fao.org/agris-search/search.do?recordID=US201302281330>.
9. Prabhu, M, Safeeulla, K. M, Shetty, H. S. (1983). Tissue culture technique to demonstrate the viability of downy mildew mycelium in pearl millet seeds. *Current Science*, 52(21), 1027-1029. [https://doi.org/10.1016/0361-3658\(83\)90004-8](https://doi.org/10.1016/0361-3658(83)90004-8)
10. Liu YH, Yu JJ, Ao GM, Zhao Q (2007) Factors influencing *Agrobacterium*-mediated transformation of foxtail millet (*Setaria italica*). *Chin J Biochem Mol Biol*: 531-536. <https://doi.org/10.3969/j.issn.1007-7626.2007.07.004>
11. Wang MZ, Pan YL, Li C, et al (2011) Culturing of immature inflorescences and *Agrobacterium*-mediated transformation of foxtail millet (*Setaria italica*). *African J Biotechnol* 10:16466–16479. <https://doi.org/10.5897/AJB10.2330>
12. Nguyen DQ, Van Eck J, Eamens AL, Grof CPL (2020) Robust and Reproducible *Agrobacterium*-Mediated Transformation System of the C4 Genetic Model Species *Setaria viridis*. *Front Plant Sci* 11:. <https://doi.org/10.3389/fpls.2020.00281>
13. Finley T, Chappell H, Veena V (2021) *Agrobacterium* -Mediated Transformation of *Setaria viridis* , a Model System for Cereals and Bioenergy Crops. *Curr Protoc* 1:. <https://doi.org/10.1002/cpz1.127>
14. Ramadevi R, Rao K V, Reddy VD (2014) *Agrobacterium tumefaciens*-mediated genetic transformation and production of stable transgenic pearl millet (*Pennisetum glaucum* [L.] R. Br.). *Vitr Cell Dev Biol - Plant* 50:392–400. <https://doi.org/10.1007/s11627-013-9592-y>
15. Ceasar SA, Ignacimuthu S (2011) *Agrobacterium*-mediated transformation of finger millet (*Eleusine coracana* (L.) Gaertn.) using shoot apex explants. *Plant Cell Rep* 30:1759–1770. <https://doi.org/10.1007/s00299-011-1084-0>
16. Sood P, Singh RK, Prasad M (2020) An efficient *Agrobacterium*-mediated genetic transformation method for foxtail millet (*Setaria italica* L.). *Plant Cell Rep* 39:511–525. <https://doi.org/10.1007/s00299-019-02507-w>
17. Yang Z, Zhang H, Li X, et al (2020) A mini foxtail millet with an *Arabidopsis*-like life cycle as a C4 model system. *Nat Plants* 6:1167–1178. <https://doi.org/10.1038/s41477-020-0747-7>
18. Sundararajan S, Sivaraman B, Rajendran V, Ramalingam S (2017) Tissue culture and *Agrobacterium*-mediated genetic transformation studies in four commercially important indica rice cultivars. *J Crop Sci Biotechnol* 20:175–183. <https://doi.org/10.1007/s12892-017-0045-0>
19. Gaponenko AK, Mishutkina Y V., Timoshenko AA, Shulga OA (2018) Genetic Transformation of Wheat: State of the Art. *Russ J Genet* 54:267–283. <https://doi.org/10.1134/S1022795418030043>
20. Wang K, Shi L, Liang X, et al (2022) The gene TaWOX5 overcomes genotype dependency in wheat genetic transformation. *Nat Plants* 8:110–117. <https://doi.org/10.1038/s41477-021-01085-8>
21. Silva MR de, Didoné DA, Ceccon CC, et al (2017) Genetic transformation of the Brazilian BR 451 maize variety by the *Agrobacterium tumefaciens* method. *Ciência Rural* 47:. <https://doi.org/10.1590/0103-8478cr20141247>
22. Brandt B, Hothorn M (2016) SERK co-receptor kinases. *Curr Biol* 26:R225–R226. <https://doi.org/10.1016/j.cub.2015.12.014>
23. Fan M, Wang M, Bai M-Y (2016) Diverse roles of SERK family genes in plant growth, development and defense response. *Sci China Life Sci* 59:889–896. <https://doi.org/10.1007/s11427-016-0048-4>

24. Karlova R, Boeren S, Russinova E, et al (2006) The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 protein complex includes BRASSINOSTEROID-INSENSITIVE1. *Plant Cell* 18:626–638. <https://doi.org/10.1105/tpc.105.039412>
25. Zuo S, Zhou X, Chen M, et al (2014) OsSERK1 regulates rice development but not immunity to *Xanthomonas oryzae* pv. *oryzae* or *Magnaporthe oryzae*. *J Integr Plant Biol* 56:1179–1192. <https://doi.org/10.1111/jipb.12290>
26. Singh A, Khurana P (2017) Ectopic expression of *Triticum aestivum* SERK genes (TaSERKs) control plant growth and development in *Arabidopsis*. *Sci Rep* 7:12368. <https://doi.org/10.1038/s41598-017-10038-1>
27. Jerard, A., Jayasankar, S., Jayanthi, & M., et al. (2014). Molecular characterization of somatic embryogenesis receptor-like kinase (serk) genes from plum (*prunus salicina*) and peach (*prunus persica*). *Indian Journal of Horticulture*, 71(4), 560-563. https://www.researchgate.net/publication/273204836_Molecular_characterization_of_somatic_embryogenesis_receptor-like_kinase_SERK_genes_from_plum_Prunus_salicina_and_peach_Prunus_persica
28. Ma J, He Y, Hu Z, et al (2014) Characterization of the third SERK gene in pineapple (*Ananas comosus*) and analysis of its expression and autophosphorylation activity in vitro. *Genet Mol Biol* 37:530–539. <https://doi.org/10.1590/S1415-47572014000400009>
29. Wu G, Li W, Tian N, et al (2022) Cloning and functional identification of *setaria italica* somatic embryogenesis receptor-like kinase1 gene (SiSERK1). *Gene* 813:146119. <https://doi.org/10.1016/j.gene.2021.146119>
30. Jha P, Shashi, Rustagi A, et al (2011) Efficient *Agrobacterium*-mediated transformation of *Pennisetum glaucum* (L.) R. Br. using shoot apices as explant source. *Plant Cell Tissue Organ Cult* 107:501–512. <https://doi.org/10.1007/s11240-011-0001-0>
31. Yan J, Fang L, Yang L, et al (2020) Abscisic acid positively regulates l-arabinose metabolism to inhibit seed germination through ABCISIC ACID INSENSITIVE4-mediated transcriptional promotions of MUR4 in *Arabidopsis thaliana*. *New Phytol* 225:823–834. <https://doi.org/10.1111/nph.16149>
32. Rashid A (2017) Comparison of a kanamycin versus hygromycin resistance gene in transgenic plant selection of *Arabidopsis thaliana* L. *J Cell Sci Mutat* 01:1–3. <https://doi.org/10.35841/Cell-Science.1000101>
33. Eu YJ, Lee MH, Chang HS, et al (1998) Chlorophyll fluorescence assay for kanamycin resistance screening in transgenic plants. *Plant Cell Rep* 17:189–194. <https://doi.org/10.1007/s002990050376>
34. Lopez, E, Proano, K, Jadan, M, Mihai, R. (2015). Callus tissue induction and analysis of gus reporter gene expression in tomato (*solanum lycopersicum* L.) transformed with *agrobacterium tumefaciens*. *Romanian Biotechnological Letters*, 20(2), 10205-10211. https://www.researchgate.net/publication/282269550_Callus_tissue_induction_and_analysis_of_GUS_reporter_gene_expression_in_tomato_Solanum_lycc
35. M. M. HAKKAK, N. MOSHTAGHI A. BAGHERI, (2020) OPTIMIZED REGENERATION OF *Kalanchoe* (*Kalanchoe blossfeldiana* R.) AND *Agrobacterium tumefaciens*-MEDIATED Gus GENE TRANSFORMATION, *PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY* 1–16. <https://www.ikppress.org/index.php/PCBMB/article/view/5427>.
36. Кириєнко АВ, Кучук МВ, Щербак НЛ, et al (2020) Expression of gus and gfp genes in amphidiploid spelt wheat (*Triticum spelta* L.) after *Agrobacterium*-mediated transformation. *Plant Var Stud Prot* 16:103–113. <https://doi.org/10.21498/2518-1017.16.1.2020.201377>
37. Ceasar SA, Baker A, Ignacimuthu S (2017) Functional characterization of the PHT1 family transporters of foxtail millet with development of a novel *Agrobacterium*-mediated transformation procedure. *Sci Rep* 7:14064. <https://doi.org/10.1038/s41598-017-14447-0>
38. Yang L, Liu Y, Xiang Y, et al (2015) Establishment and Optimization of a Shoot Tip-based Genetic Transformation System for Foxtail Millet. *Chinese Bull Bot* 56:71–79. <https://doi.org/10.11983/CBB20119>
39. Salaj J, von Recklinghausen IR, Hecht V, et al (2008) AtSERK1 expression precedes and coincides with early somatic embryogenesis in *Arabidopsis thaliana*. *Plant Physiol Biochem* 46:709–714. <https://doi.org/10.1016/j.plaphy.2008.04.011>

Figures

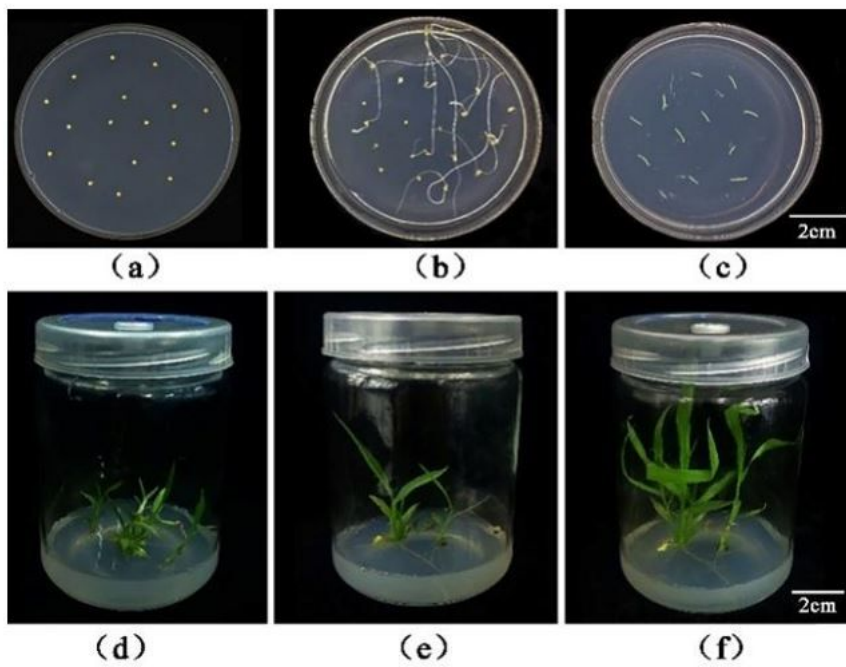


Figure 1
Culture process of tissue culture seedlings

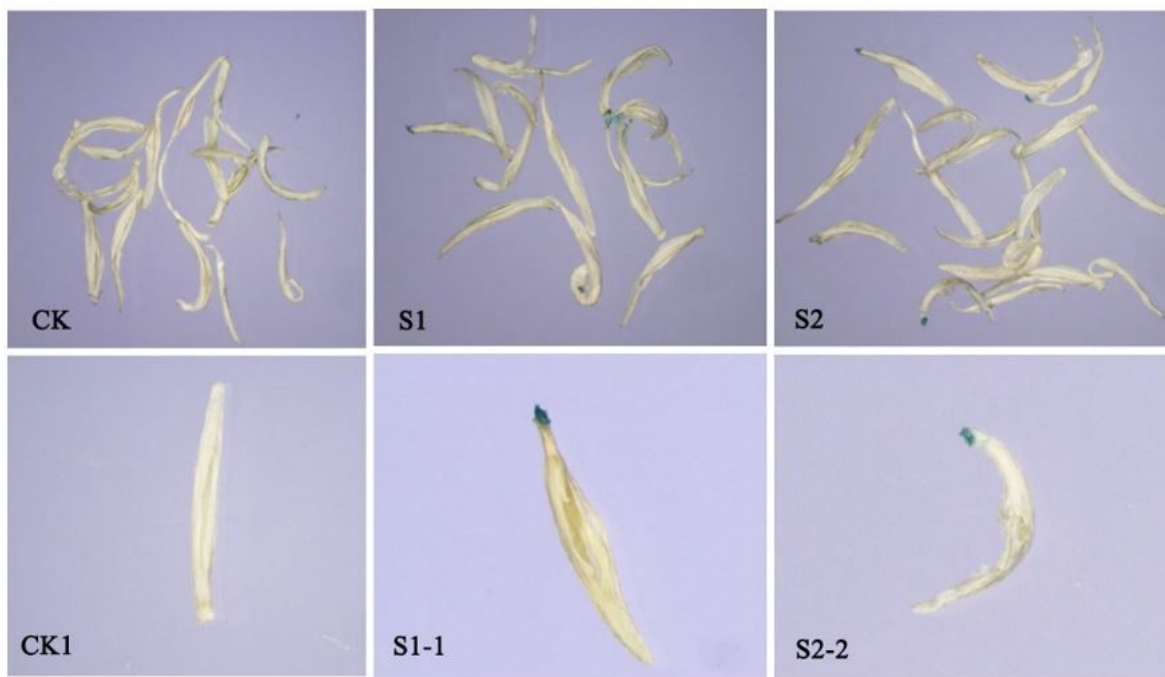


Figure 2
GUS staining results

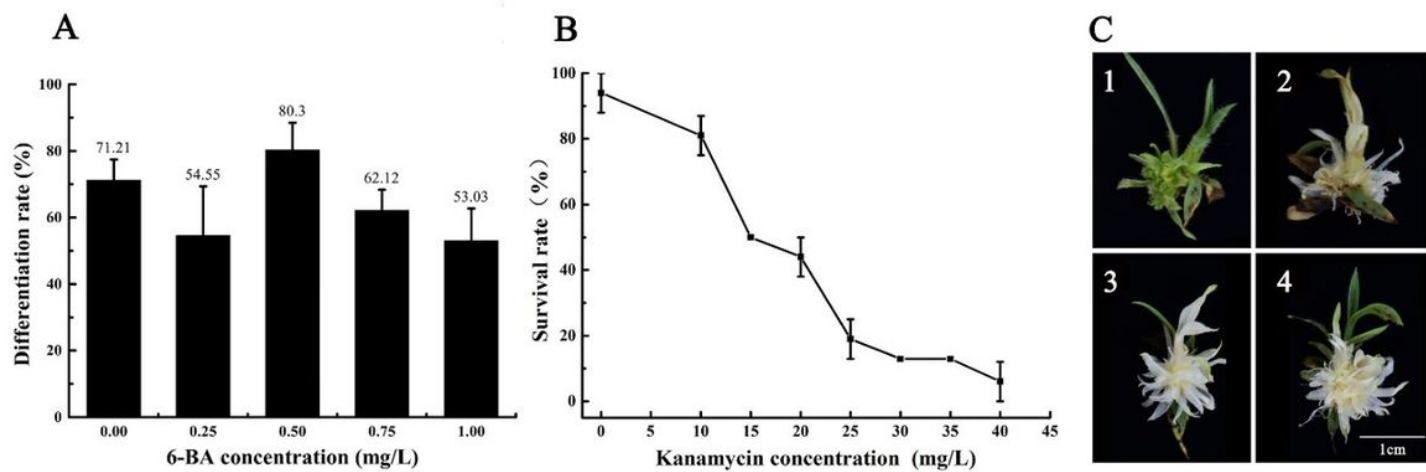


Figure 3

The concentration screening of 6-BA and kanamycin

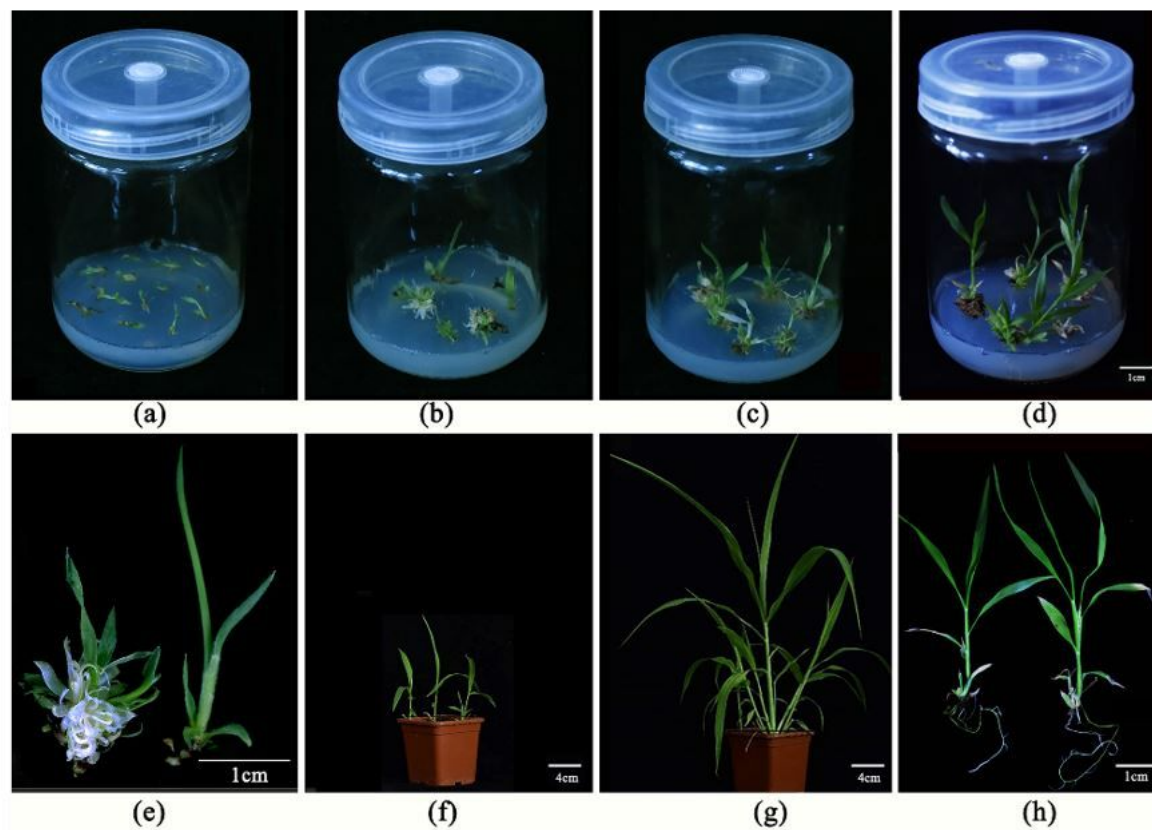


Figure 4

The screening of transgenic seedlings

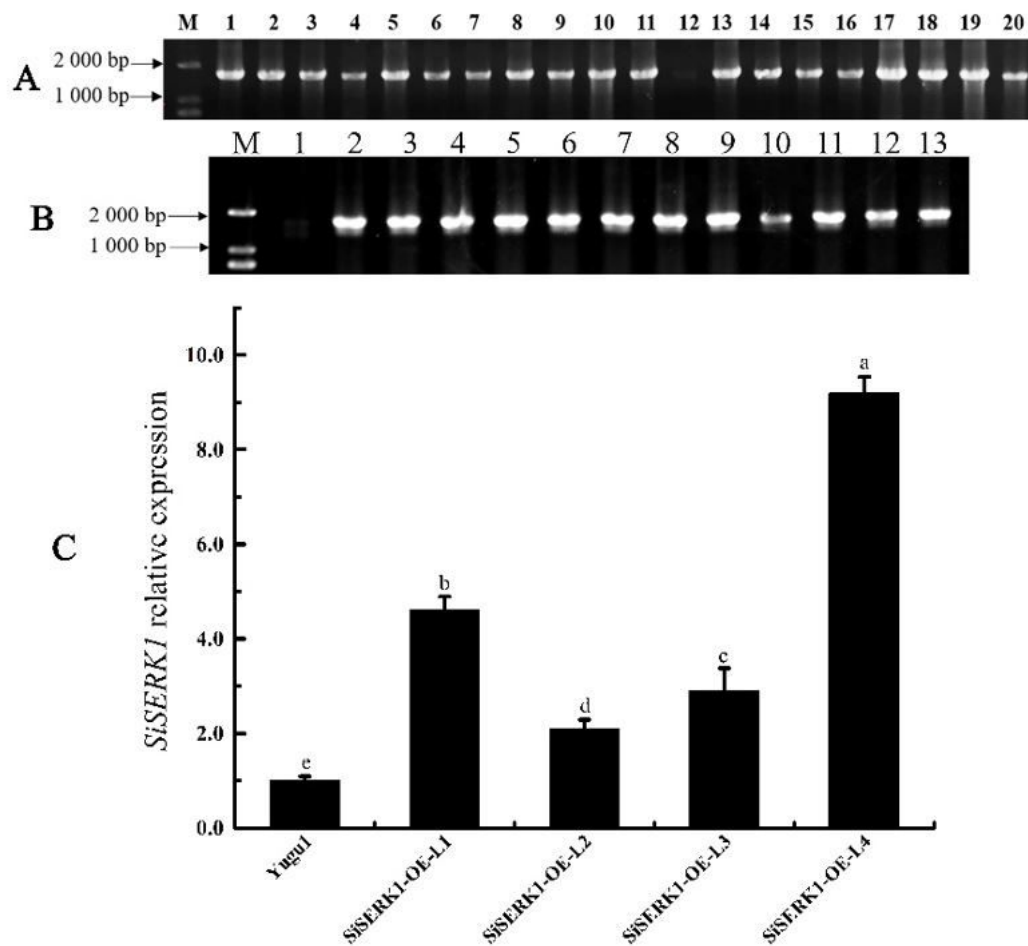


Figure 5

Identification and expression analysis of *SiSERK1* transgenic plants

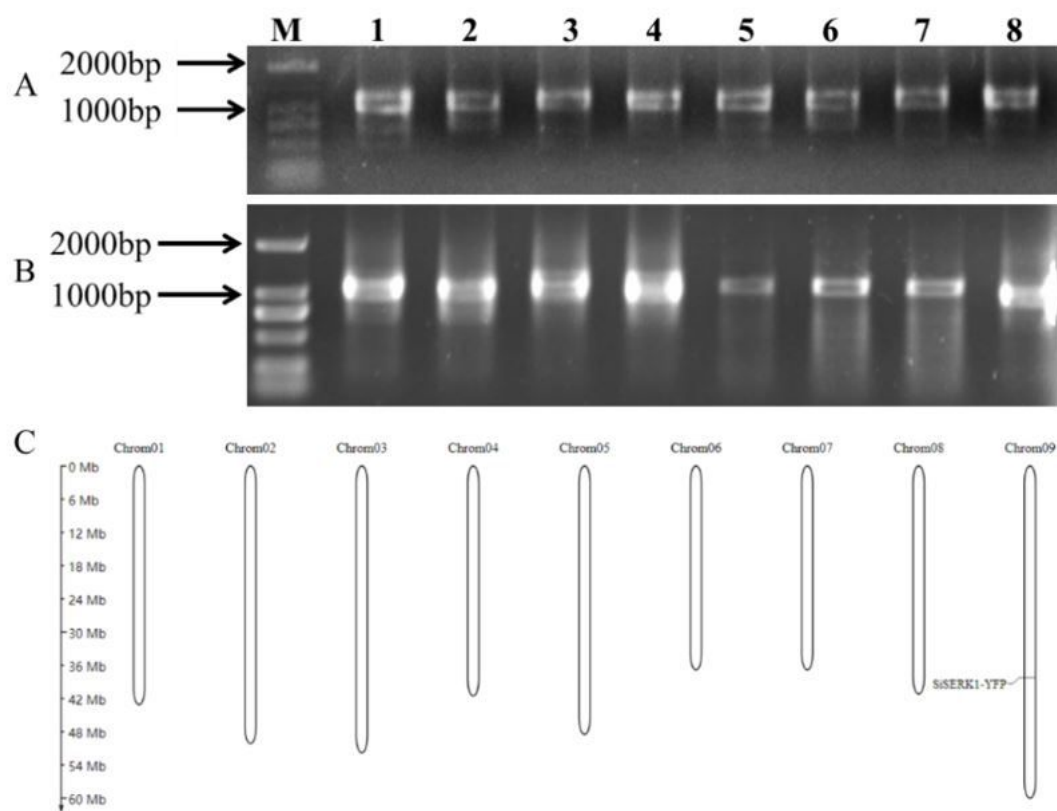


Figure 6

Detection of insertion site of *SiSERK1* in transgenic plant

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AppendixA.docx](#)