Molecular cloning and characterization of five genes from embryogenic callus in *Miscanthus lutarioriparius*

Lingling Zhao¹ · Xiaofei Zeng¹ · Xiaohu Hu¹ · Jiajing Sheng¹ · Fenglin Zhu¹ · Lin Zhong¹ · Fasong Zhou¹ · Surong Jin² · Zhongli Hu¹ · Ying Diao¹

Received: 12 December 2018 / Revised: 21 April 2020 / Accepted: 24 April 2020 / Published online: 7 May 2020 © The Author(s) 2020

Abstract

The regeneration from embryogenic callus of higher plants in tissue culture is regulated by explants types and developmental stage and also regulated by some genes. In *Miscanthus lutarioriparius*, five candidate genes were selected to decide the differential expression between embryogenic and non-embryogenic calli, including *MlARF-GEP* (guanine nucleotide-exchange protein of ADP ribosylation factor), *MlKHCP* (kinesin heavy chain like protein), *MlSERK1* (somatic embryogenesis receptor-like kinases 1), *MlSERK2* (somatic embryogenesis reporter-like kinases 2), and *MlTypA* (tyrosine phosphorylation protein A) with Genbank accession numbers KU640196–KU640200. Multiple sequence alignment analysis showed that five genes were highly conserved among members of their gene families respectively. Phylogenetic relationship analysis showed that five genes were closest with homologous genes of *Zea mays* and *Sorghum*. The qRT-PCR results showed significant differences of five genes expression pattern between two different callus types, the relative expression in embryogenic callus was detected to exceed in non-embryogenic callus. Furthermore, simple sequence repeats (SSR) marker statistics results via Chi-square showed a significant correlation between *MlSERK1* genotype and induction of embryogenic callus in *M. lutarioriparius*. This study may lay the foundation of the molecular mechanism on the embryogenic callus induction of *M. lutarioriparius* and perhaps provide some gist for further study on genetic manipulation.

Keywords *Miscanthus lutarioriparius* · Embryogenic callus · cDNA · Simple sequence repeat · Relative expression

Introduction

The establishment of the in vitro system through embryogenic callus of higher plants is a bottleneck step for production and genetic breeding. The solution of the embryogenic callus induction depends on the cell totipotent, and some of the plant cells may become totipotent when they treated with proper conditions (Fehér 2019). With the deepening research, early in twentieth century somatic embryogenesis was achieved in *Arabidopsis thaliana* (Mordhorst et al. 1998), *Oryza sativa* L. (Karthikeyan et al. 2009), *Zea mays* L. (Armstrong and Green 1985; Vasil and Vasil 1986), *Hordeum vulgare* L. (Pasternak et al. 1999), *Gossypium hirsutum* (Zheng et al. 2014), as well as in two *Miscanthus* species including *Miscanthus sinensis* and *Miscanthus × giganteus* (Gawel et al. 1990; Holme and Petersen 1996). There are probably several reasons affect the *Miscanthus × giganteus* regeneration including explant types, developmental stages, and culture media (Holme and Petersen 1996). From previous study, somatic embryogenesis had been accomplished in a number of plants with different hormone concentrations or culture conditions; however, the molecular mechanism has not been deciphered clearly. To elucidate the molecular mechanism, the additional research of somatic embryogenesis should be carried out.
It was reported that some genes and transcriptional factors played key roles in somatic embryogenesis formation (Méndez-Hernández et al. 2019; Namasivayam 2007). Among those genes, the somatic embryogenesis receptor kinase (SERK) genes must be mentioned first, which was recognized to be the most important one, involving in a series of developmental processes such as induction and differentiation of callus and cell totipotency in plant (Méndez-Hernández et al. 2019; Pilarska et al. 2016). SERK had been first considered to be an important marker to identify embryogenic cells in carrot (Schmidt et al. 1997). In Arabidopsis thaliana, the loss of SERK was demonstrated to result in abnormal embryo development (Li et al. 2019). The homolog genes of SERK, associated with somatic embryogenesis, have also been detected in some other species, such as in rice (Singla et al. 2009), maize (Baudino et al. 2001), wheat (Singh and Khurana 2012), Medicago truncatula (Nolan et al. 2009), and Citrus unshiu Marc. (Shimada et al. 2005). Three critical cDNA fragments including ZmARF, ZmKHCP, and ZmTypA in the embryogenic callus induction were isolated by cDNA amplified fragment length polymorphism analysis in maize (Sun et al. 2012). All of the three genes showed a higher expression pattern in embryogenic callus than in non-embryogenic callus. In addition, the Arabidopsis leafy cotyledon (LEC) genes, AtLEC1 and AtLEC2, promoted the somatic embryogenesis when they transferred into tobacco (Guo et al. 2013) and also considered to be a key regulators of somatic embryogenesis in cassava (Brand et al. 2019). Previous study also found that carrot homolog of Arabidopsis LEC1 only expressed in somatic embryos (Yamamoto et al. 2005).

Miscanthus lutarioriparius, originated in China and a genus of C4 plant, has great potential as a biomass energy crop (Zhao et al. 2013). High efficiency regeneration from embryogenic callus system is a key bottleneck step for its transgenic engineering. Because of the wild distribution of M. lutarioriparius, it is hard for finding the suitable samples for tissue culture. Molecular marker-assistant breeding has focused on the use of random markers for quantitative trait locus (QTL) analysis of traits, such as single-nucleotide polymorphism (SNP), simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP) markers (Amini et al. 2016; Feng et al. 2016; Lu et al. 2015).

In this study, a total of 37 M. lutarioriparius samples were used for induction of callus. To investigate the mechanism of somatic embryogenic callus formation in M. lutarioriparius, five candidate genes were used to assess the differential expression between embryogenic and non-embryogenic calli which were differentiated by the morphology of the paraffin section with optical microscope. Three full-length candidate genes and two partial sequence genes were cloned based on the maize and sorghum genome sequence database. In addition, one of the genes showed a significant correlation between genotype and induction of embryogenic callus in M. lutarioriparius. This study may lay the foundation of the molecular mechanism on the embryogenic callus induction of M. lutarioriparius and, perhaps, provide some gist for further study on genetic manipulation.

**Materials and methods**

**Preparation of biological materials**

Thirty seven samples of M. lutarioriparius were grown in Ezhou, Hubei Province of China, and used for this study. The information of samples is accessioned in Table S1 and the distribution is shown in Fig S1. Their immature inflorescence tissues were harvested in the middle of September and were then surface-sterilized by washing with 75% ethyl alcohol for 30 s, 0.1% (w/v) mercuric chloride (HgCl2) for 1.5 min, and rinsed thoroughly (at least three times) with sterile distilled water. Immature inflorescence tissues were placed onto the induction medium (4 mg/L 2, 4-D) according to the methods used by Zhao et al. (2013). The induced calli were transferred to the same fresh medium after 21 days and subcultured every 3 weeks. The characteristic of the calli induced from 37 samples were recorded after two rounds of subculturing. The induction frequencies were calculated by SPSS (Statistical Program for Social Sciences) 14.0. The samples with higher frequencies of embryogenic callus induction, i.e., I065 and I093, were used for RNA extraction, gene cloning, and expression study.

**RNA extraction and cDNA synthesis**

Plant materials were stored at −80 °C and total RNA was extracted from embryogenic calli using RNAprep pure Plant Kit (TIANGEN BIOTECH., LTD, Beijing, China). DNase treatment was conducted prior to column purification of RNA according to the manufacturer’s instructions (TIANGEN BIOTECH). cDNAs were synthesized using M-MLV Reverse Transcriptase (Promega Corporation, USA). PCR was carried out with KOD-Plus-Neo DNA Polymerase (TOYOBO CO., LTD, Osaka, Japan) with cDNAs as templates. PCR primers were designed from sequences’ data in the NCBI of Zeamays and Sorghum, which are shown in Table 1. The reaction mixture of cDNA synthesis contained 5 μl of 2 mM dNTPs, 5 μl of 10× PCR Buffer for KOD Plus-Neo (TOYOBO, Osaka, Japan), 3 μl of 25 mM MgSO4, 15 pmol of a primer pair, 2 μl of cDNAs template, 2 μl of dimethylsulphoxide (DMSO), and 1 μl of KOD-Plus-Neo and the last supplementary 29 μl of ddH2O with a total volume of 50 μl. PCR amplifications were set to 36 cycles of amplification: pre denaturation at 94 °C for 2 min, and then denaturation at 98 °C with 10 s, annealing for 2–5 min
at 68 °C (the criterion: 1000 bp about 1 min) and extension at 16 °C for 1 min as the last step. T4 ligase was used for PCR products cloning in pGEM-T vectors (Promega), following transformation to E. coli DH5α competent cells (TransGen, Beijing, China). Ten positive clones of E. coli in each species were selected to sequence. The plasmids of each gene were isolated using a plasmid extract kit (Tiangen) and stored at − 20 °C for standby application in other experiments.

**Sequence alignment and phylogenetic analysis**

We used online BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to detect the identity of the candidate genes. ORF Finder (https://www.ncbi.nlm.nih.gov/projects/gorf/) was selected for open-reading frames (ORFs) prediction. We performed sequence alignments including DNA and protein by ClustalX 2.1 Program (Larkin et al. 2007). The phylogenetic analysis of candidate genes conducted by MEGA 5.1 (Tamura et al. 2011). To identify the conserved domains and functional sites, InterPro (https://www.ebi.ac.uk/interpro/) was selected as the tool. ARF-GEPs, KHCPs, and TypAs from other species, including both monocots and dicots (Table S2, S3, and S5), were chosen for phylogenetic analyses. SERKs from other species, including monocots, dicots, mosses, and ferns, were chosen for phylogenetic analyses (Table S4). A basic neighbor-joining tree was built by MEGA 5.1.

**Expression profiles of five genes at different developmental stages on two types of callus**

The embryogenic callus of accession I093 and non-embryogenic callus of A061 were used for analysis of expression level after 3 weeks of callus induction. The relative expression pattern files of MIARF-GEP, MIKHCP, MISERK1, MISERK2, and MITypA in M. lutarioriparius were calculated by qRT-PCR using the comparative C_T (2−ΔΔC_T) method (Schmittgen 2008). The total RNA isolation was obtained from two callus types at different developmental stages (the 1st, 2nd, 3rd, and 4th weeks) with their morphology observed under optical microscope (Fig. 1). The qRT-PCR primers for amplification of five genes (MIARF-GEP forward: 5’- AGCAGATATCATGCTGTTACCTTT TA-3’, reverse: 5’- CCTGTCTGATCTTGCACACTAA -3’; MIKHCP forward: 5’- GGAAGCAGAAGCAGATAG CAT -3’, reverse: 5’- GCAGCCTTCACCTCAAGTTCC -3’; MISERK1 forward: 5’- TGCCGGCCGTAGGTCAGG -3’, reverse: 5’- CTCATTTGGCCGTCCCT -3’; MISERK2 forward: 5’- GCAGTCTGTGGGTATCTCC -3’, reverse: 5’- AGGACTTGGCCAGTTGGACTAC -3’; MITypA forward: 5’- CATACCAAGCCAGCGATGCG -3’, reverse: 5’- GTC ACCAGACTAACAGATCT -3’) were designed from a conservative sites. 18SrRNA1 was chosen as a reference gene according to Huang et al. (Huang et al. 2014). The primers are: forward, 5’- CTACCACATCAGAGAAG-3’; reverse, 5’- CAAATTACCAGACACTAG -3’. The expected size of amplified fragments of MIARF-GEP, MIKHCP, MISERK1, MISERK2, MITypA, and 18SrRNA1 are 109, 154, 106, 121, 238, and 105 bp, respectively. The specificity of the primers and reference primers were confirmed by qRT-PCR amplification efficiency, using geometric dilution method (100, 10−1, 10−2, 10−3, and 10−4) of embryogenic callus as template. C_T value of different concentration cDNA was used for standard curve. The amplification efficiency is (1−k) √(10 − 1) x 100%

**Table 1** Names and sequences of primers used for PCR amplification of five genes

| Gene name | Primer sequence (5’-3’) |
|-----------|------------------------|
| MIARF-GEP | F-GCCCGCCAGATCCAGATCCACC R-TCAGCATCAGCAGAGATGGACATC |
| MIKHCP | F-GTGAGTAGGATCTGAGTCTGT R-TCAGCAGGCACTATTTGGCCCT |
| MISERK1 | F-GTCGGCGGATGCGCGCTGCTG R-CTACCTTCGGCCGGAMGTTGGAC |
| MISERK2 | F-GGCCCGTTTGCCGTAGTGGGA R-TCTTTCTATGAATGGTGAAGG |
| MITypA | F-CCCTCYACCWCCGGCCACCATGCA R-GGTTCTTGACATCGGATGCTT |

R: A, G; M: A, C; W: A, T; Y: C, T

Fig. 1 Two types of callus and their paraffin section, optical microscope (10×100). a Embryogenic callus, b embryogenic callus paraffin section, c non-embryogenic callus, and d non-embryogenic callus paraffin section

| R: A, G; M: A, C; W: A, T; Y: C, T |
(k was the slope of standard curve). qRT–PCR was carried out in a total volume of 20 μl, and the reaction mixture contained 10 μl of TUNDERBIRD SYBR qPCR Mix (TOYO-OBO), 6 pmol of primer pair, and 2 μl of cDNA and 6.8 μl of ddH₂O according to the manufacturer’s recommendation. ABI StepOneTM Real-Time PCR System (Applied Biosystems, Foster City, California, USA) was used for qRT-PCR amplification, setting with 95 °C for 1 min, 95 °C for 15 s with 40 cycles, and 60 °C for 30 s. Three biological repeats along with three technical repeats were used in this study. StepOne™ Software v2.3 (Applied Biosystems) used to analyze the experimental data.

**PCR amplification and polymorphism analysis of SSR markers**

The DNeasy Plant Mini Kit (TIANGEN BIOTECH., LTD, Beijing, China) was used to total genomic DNA extraction from dried leaf tissue in *M. lutarioriparius*. According to the genome information of *Sorghum* in the NCBI, DNA sequences of five genes were amplified. The SSR loci of five genes were detected using SSR Hunter 1.3. PCR amplification-specificity of the designed SSR primers (Table 2) and the presence of a polymorphism were tested on 37 individuals from *M. lutarioriparius*. PCR amplifications were performed in a total volume of 10 μl, containing 0.2 μl Taq polymerase (0.2 U/ml), 1 μl genomic DNA (50 ng/ml), 1.25 μl 10×PCR buffer, 1 μl MgCl₂ (20 mol m⁻³), 0.4 μl dNTP (10 mol m⁻³), and 0.2 μl (25 mol m⁻³) of each reverse primer and supplementary with 5.75 μl ddH₂O. The PCR reaction was set to 95 °C for 5 min, 95 °C for 30 s with 35 cycles, 58 °C for 45 s, and 72 °C for 30 s, following a final elongation step for 10 min at 72 °C. Then, we performed the PCR products for electrophoresis on 6% denaturing polyacrylamide sequencing gels. DNA stripes were silver stained and recorded.

Data analysis of the SSR marker was performed using SPSS 14.0. The number of alleles per locus and observed results of induction characterization were estimated via Chi-square test.

**Results**

**Cloning of MIARF-GEp, MIHKCP, MISERK1, MISERK2, and MITypA**

By degenerate primerr amplification designed on sequences of *Sorghum* and maize, we cloned the full-length ORF of cDNA sequences of three genes and named them *MIARF-GEp*, *MISERK1*, and *MISERK2* (GenBank accession numbers KU640196, KU640198, and KU640199) based on the obtained cDNA. However, the other two genes were only got the partial ORF of cDNA sequences and named *MIHKCP* and *MITypA* (GenBank accession numbers KU640197 and KU640200) based on the obtained cDNA. All the genes of the PCR amplification results are shown in Fig. 2. The complete ORF sequence of *MIARF-GEp* is 5385 bp long.

| Primer name | Primer sequence 5′-3′ | Repeat motif |
|-------------|-----------------------|--------------|
| MIARF-GEp   |                       |              |
| MIKHCP-1    | F1: ATGCTATCTGCTGCTGCTT | (TC)₅       |
|             | R1: GCTTGTGTGTGTGCTGTT |              |
| MIKHCP-2    | F2: GAACCTTCAATGGAGCAGCCA | (AC)₆     |
|             | R2: CGAAGGGTGTTGTTGATCA |              |
| MIKHCP-3    | F3: GAATGTGGAAACAGCAACTGGA | (AG)₉ |
|             | R3: TCTACTAAATCCTGCTGTCAG |              |
| MIKHCP-4    | F4: CCAAGATGACACAGCAAGG | (TCA)₅     |
|             | R4: TTTGAGCCAGGGAGACCAA |              |
| MIKHCP-5    | F5: ACTTGTATTTAATGCAACCT | (AT)₅      |
|             | R5: AGCAGATCTTAGTCTTTCGC |              |
| MISERK1-1   | F1: CGCTGATGATGATCAAGCC | (TA)₄      |
|             | R1: ACCCTTCTGTGGCATAAGG |              |
| MISERK1-2   | F2: CAATGGTAGAATTTGAGCGC | (TA)₄      |
|             | R2: TTGGGAACGTGAGGAAAGA |              |
| MISERK1-3   | F3: TCCGCTTGGACTACTCAT | (ACC)₄     |
|             | R3: GGACCTCGTCTACAGTCAGC |              |
| MISERK1-4   | F4: CGCGCTTCTCGGGAGCCCA | (GA)₄      |
|             | R4: AAACCTCCTCGTCTTCGG |              |
| MISERK2     | F1: ATTTGAAATTTTCAAAACAGGTAAGG | (GA)₁₂ |
|             | R1: GTTCTCCATAATTAGGAGTTCACA |              |
| MITypA      |                       |              |
and encoding a protein of 1794 amino acids. The cDNA sequence of MISERK1 is 1956 bp long and contains 1872 bp ORF encoding a protein of 624 amino acids. The partial 5′-UTR and 3′-UTR are 9 and 75 bp long, respectively. The cDNA sequence of MISERK2 is 2119 bp long and contains 1878 bp ORF encoding a protein of 626 amino acids. The partial 5′-UTR and 3′-UTR are 130 and 111 bp long, respectively. The partial ORF sequence of MIKHCP is 1172 bp long and encoding a protein of 387 amino acids. The partial ORF sequence of MITypA is 1962 bp long and encoding a protein of 654 amino acids.

**Sequence analysis of MIARF-GEp, MIKHCP, MISERK1, MISERK2, and MITypA**

The protein sequence and length of the MIARF-GEp was quite conserved, compared to the other monocots (Brachypodium distachyon, Oryza brachyantha, Oryza sativa Japonica Group, Oryza sativa, Setaria italic, Sorghum bicolor, and Zea mays) and dicots (Arabidopsis thaliana, Brassica napus, Malus domestica, and Ricinus communis) by multiple alignment analysis (Fig S2). The highest identity was 99% (SbARF-GEp) as compared to MIARF-GEp. The neighbor-joining tree built by MEGA 5.1 showed that MIARF-GEp was separated into monocots and dicots groups, respectively (Fig 3a). It means that MIARF-GEp evolved independently after differentiation of monocot and dicot plants. In addition, MIARF-GEp exhibited a closest genetic relationship with SbARF-GEp (Fig 3a). Multiple alignment analysis of MIKHCP was conserved with both in the monocots and dicots (Fig S3), and the neighbor-joining tree showed a similar characteristic with MIARF-GEp, MITypA exhibited a closest genetic relationship with SbTypA (Fig 3d).

**Expression patterns of five genes in two types of embryogenic calli and non-embryogenic calli**

The primers amplification ability of MIARF-GEp, MIKHCP, MISERK1, MISERK2, MITypA, and 18S rRNA1 was examined specifically (Fig. S6). The six primer amplification efficiencies were 107.18%, 92.64%, 94.84%, 98.03%, 100.33, and 106.00%, and their standard curves are shown in Fig. S6. The R^2 values of the six standard curves were all between 0.99 and 1.0 (Fig. S7). Five genes were expressed in all developmental stage in two types of calli. As we expected, the similarities of the five genes expression pattern were obtained in four callus developmental stages. The highest expression levels of MIARF-GEp, MIKHCP, MISERK1, MISERK2, and MITypA were observed in the third week after induction in embryogenic callus (Fig. 4). Furthermore, the lowest expression levels were found in the fourth week. In addition, the relative expression levels in embryogenic calli were detected to exceed than in non-embryogenic calli among four developmental stages, the expression of MIARF-GEp, MIKHCP, MISERK1, and MISERK2, and MITypA in embryogenic calli exhibited approximately several hundred folds higher than in non-embryogenic calli. Interestingly, the expression of five genes plummeted in the fourth week.

**Polymorphism analysis of SSR markers in five genes**

The induction frequencies and descriptive of calli from 37 individuals of M. lutarioriparius are shown in Table S1. The embryogenic calli were induced in only nine individuals. According to the five genes in Sorghum genome database, there were no SSR loci detected by SSR Hunter 1.3 analysis in MIARF-GEp and MITypA genes. Although five SSR loci in MIKHCP and one SSR loci in MISERK2 were detected, no polymorphism was observed. Interestingly, two SSR loci, MISERK1-3 (Fig S8) and MISERK1-4, amplified three alleles in 37 M. lutarioriparius individuals. The variation from MISERK1-3 showed significance with the induction characteristic, but there is no significance from MISERK1-4 (Table 3). The individuals which could be
induced embryogenic calli were all observed in part of the heterozygotes and all homozygotes were induced non-embryogenic calli by the MlSERK1-3 amplification.

Discussion

Immature inflorescence tissues are frequently used as to the explants in *Miscanthus* species tissue culture (Holme and Petersen 1996; Zhang et al. 2012); however, the genotype of the materials, the cultural condition of the calli, and the concentration of the plant growth regulator in the medium were simultaneously affected the growth and development of the embryogenic calli (Glowacka et al. 2010). The same results were also exhibited in rice (Niroula et al. 2005) and turfgrass (Salehi and Khosh-Khui 2005), and inflorescence length was one of the reasons of embryogenic calli induction in orchard grass (Çeliktas et al. 2014). In this study, a total of 37 individuals of *M. lutarioriparius* were used for callus induction; we examined very different induction frequencies (Table S1). The induction results were affected by the different genotypes. Some genes, especially *SERKs*, maybe play a very important role in embryogenesis. Our study evaluated two *SERK* genes, *MlSERK1* and *MlSERK2* ORF and other *SERKs* form dicots and monocots. *MlTypA* ORF and other *TypA* form dicots and monocots. The different color represents the clustering in a branch.
above results, MlSERK1 showed significance between genotype and callus characteristic. The genotypes which could be induced embryogenic calli were all heterozygotes of MlSERK1, this result maybe implies heterozygotes of MlSERK1 which were more easily used for induction as compared to homozygotes. Our study provided a recommendation that the MlSERK1 protein may play an important role in the formation process of embryogenic calli and following studies should be carried to identify the accurate roles which it makes. The relative expression had an extreme elevation in the third week of MlSERK1 and MlSERK2, it may be caused by the hormone accumulation in medium. The relative expression plummeted in the fourth week maybe because of weakening of the hormone. Many reports suggested that the Miscanthus species subcultured in tissue culture every 3 weeks (Holme and Petersen 1996; Zhao et al. 2013), and thus, the expression pattern of MlSERK1 and 2 demonstrated the above method from molecular level.

ZmARF-GEF is reported to belong to the ADP ribosylation factor (ARF) family; it plays function relays on the alternation between GDP and GTP (Morinaga et al. 1996). In maize, ZmARF-GEF was deduced to associate with the auxin flowing and polarization, which further influenced the formation of embryogenic calli (Sun et al. 2012). MlARF-GEF was closely related to ZmARF-GEF. In addition, according to the expression pattern in two types of callus of MlARF-GEF, it implied that MlARF-GEF in M. lutarioriparius and ZmARF-GEF in Zea mays played a similar role.

KHCP may be a kinesin heavy chain like protein, that can move along cytoskeletal fibers by hydrolyzing ATP to generate energy (Vallee and Shpetner 1990), and they play a significant role in chromosome segregation, assisting the spindle to play function during mitosis and meiosis (Sawin...
and Endow 1993). The MIKHCP homolog protein ZmKHCP was demonstrated to account for hydrolyzing ATP to generate force to separate the chromosome during cell division in embryogenic calli (Sun et al. 2012).

Homologous sequence alignment analysis showed that the MtTypA was highly similar with TypA (Tyrosine phosphorylation protein A) proteins from the other species. Phylogenetic analysis showed that MtTypA is closed to SbTypA and ZmTypA. TypA is a new protein of the ribosome-binding GTPase superfamily, which involved in various regulatory pathways (Margus et al. 2007). AtTypA genes are involved in pollen tube growth in Arabidopsis (Lalanne et al. 2004). A TypA cDNA fragment is isolated from embryogenic calli in maize; they suggest that ZmTypA may perform and regulate similar roles with other species (Sun et al. 2012).

Our study suggested that MiARF-GEP, MIKHCP, MISERK1, MISERK2, and MtTypA genes participated in the process of embryogenic calli formation. In conclusion, the qRT-PCR technique provided valuable information on distinguishing of the gene expression levels between embryogenic calli and non-embryogenic calli. Five candidate genes involved in the formation of embryogenic calli were cloned. The identification of these candidate genes helped to understand the underlying mechanisms related to somatic embryogenesis. However, the clear functions of these candidate genes remain unclear. An effective plant regeneration system has been established, the transformation system is developing in our lab. Further work, the exact role of the five genes should be explored by RNA interference or overexpression technology in M. latuarioparvus.

Acknowledgements This study was financially supported by the National Natural Science Foundation of China (31371691) and the National High-Tech R&D Program (2012AA101801).

Author contributions LZ performed the experiments, analyzed the results, and wrote the manuscript. XH, JS, FZ, and LZ collected experiments materials and provided assistance to the experiments. XZ prepared Fig. 1. FZ, ZH, SJ, and YD offered scientific advice, guided the experiments, and revised the manuscript.

Compliance with ethical standards Conflict of interest We declare that we have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Amini F, Mirlohi AF, Majidi MM (2016) The possibility of use of AFLP molecular markers and phenotypic traits to increase forage yield in tall fescue (Festuca arundinacea Schreb.) breeding. J Agric Sci Technol 18:1419–1429

Armstrong CL, Green CE (1985) Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline. Planta 164:207–214. https://doi.org/10.1007/BF00396083

Baudino S et al (2001) Molecular characterisation of two novel maize LRR receptor-like kinases, which belong to the SERK gene family. Planta 213:1–10. https://doi.org/10.1007/s004250000471

Brand A, Quimbaya M, Tohme J, Chavarriaga-Aguirre P (2019) Arabidopsis LEC1 and LEC2 orthologous genes are key regulators of somatic embryogenesis in Cassava. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00673

Çeliktaş N, Can E, Hatipoglu R (2014) Effect of inflorescence length on the callus induction and plant regeneration in orchardgrass (Dactylis Glomerata L.). Biotechnol Biotech Equip 17:29–32. https://doi.org/10.1080/131102818.2003.10819191

Fehr A (2019) Callus, de-differentiation, totipotency, somatic embryogenesis: what these terms mean in the era of molecular plant biology? Front Plant Sci. https://doi.org/10.3389/fpls.2019.00536

Feng Y et al (2016) Genome wide association mapping for grain shape traits in indica rice. Planta 244:819–830. https://doi.org/10.1007/s00425-016-2548-9

Gawel NJ, Robacker CD, Corley WL (1990) vitro propagation of Miscanthus sinensis. HortScience 25:1291–1293

Glownacka K, Jezowski S, Kaczmarek Z (2010) The effects of genotype, inflorescence developmental stage and induction medium on callus induction and plant regeneration in two Miscanthus species. Plant Cell Tissue Organ Cult 102:79–86. https://doi.org/10.1007/s11240-010-9708-6

Guo F et al (2013) Induced expression of AtLEC1 and AtLEC2 differentially promotes somatic embryogenesis in transgenic tobacco plants. PLoS ONE 8:e71714–e71714. https://doi.org/10.1371/journal.pone.0071714

Holme IB, Petersen KK (1996) Callus induction and plant regeneration from different explant types of Miscanthus x giganteus Honda ‘Giganteus’ Plant Cell. Tissue Organ Cult 45:43–52. https://doi.org/10.1007/bf00043427

Huang L et al (2014) Evaluation of candidate reference genes for normalization of quantitative r-t pc-r in switchgrass under various abiotic stress conditions. BioEnergy Res 7:1201–1211. https://doi.org/10.1007/s12155-014-9457-1

Karthikeyan A, Pandian STK, Ramesh M (2009) High frequency plant regeneration from embryogenic callus of a popular indica rice (Oryza sativa L.). Phys Mol Biol Plants 15:371–375. https://doi.org/10.1007/s12298-009-0042-6

Lalanne E et al (2004) Analysis of transposon insertion mutants highlights the diversity of mechanisms underlying male progamic development in Arabidopsis. Genetics 167:1975. https://doi.org/10.1534/genetics.104.030270

Larkin MA et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Li H et al (2019) SERK receptor-like kinases control division patterns of vascular precursors and ground tissue stem cells during embry development in Arabidopsis. Mol Plant 12:984–1002. https://doi.org/10.1016/j.molp.2019.04.011

Lu X et al (2015) Segregation analysis of microsatellite (SSR) markers in sugarcane polyploids. Genet Mol Res 14:18384–18395. https://doi.org/10.4238/2015.December.23.26
Margus T, Remm M, Tenson T (2007) Phylogenetic distribution of translational GTPases in bacteria. BMC Genomics 8:15. https://doi.org/10.1186/1471-2164-8-15
Méndez-Hernández HA et al (2019) Signaling overview of plant somatic embryogenesis. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00077
Mordhorst AP, Voerman KJ, Hartog MV, Meijer EA, van Wient J, Koornneef M, de Vries SC (1998) Somatic embryogenesis in Arabidopsis thaliana is facilitated by mutations in genes repressing meristematic cell divisions. Genetics 149:549–563
Morinaga N, Tsai SC, Moss J, Vaughan M (1996) Isolation of a bre-feldin A-inhibited guanine nucleotide-exchange protein for ADP ribosylation factor (ARF) 1 and ARF3 that contains a Sec7-like domain. Proc Natl Acad Sci USA 93:12856–12860. https://doi.org/10.1073/pnas.93.23.12856
Namasiyayam P (2007) Acquisition of embryogenic competence during somatic embryogenesis. Plant Cell Tissue Organ Cult 90:1–8. https://doi.org/10.1007/s11240-007-9249-9
Niroula RK, Sah BP, Bimb HP, Nayak S (2005) Effect of genotype and culture media on callus induction and plant regeneration from mature rice grain culture. J Inst Agric Anim Sci 26:21–26. https://doi.org/10.3126/jias.v26i0.607
Nolan KE, Kurdyukov S, Rose RJ (2009) Expression of the SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 (SERK1) gene is associated with developmental change in the life cycle of the model legume Medicago truncatula. J Exp Bot 60:1759–1771. https://doi.org/10.1093/jxb/erp046
Pasternak TP, Rudas VA, Lörz H, Kumlehn J (1999) Embryogenic callus formation and plant regeneration from leaf base segments of barley (Hordeum vulgare L.). J Plant Physiol 155:371–375. https://doi.org/10.1016/S0176-1617(99)0119-9
Pilar ska M, Malec P, Salaj J, Bartnicki F, Konieczny R (2016) High expression of SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE coincides with initiation of various developmental pathways in vitro culture of Trifolium nigrescens. Protoplasma 253:345–355. https://doi.org/10.1007/s00709-015-0814-5
Salehi H, Khosh-Khui M (2005) Effects of genotype and plant growth regulator on callus induction and plant regeneration in four important turfgrass genera: A comparative study. Vitro Cell Dev Biology Plant 41:157–161. https://doi.org/10.1079/IVP2004614
Sawin KE, Endow SA (1993) Meiosis, mitosis and microtubule motors. BioEssays 15:399–407
Schmidt ED, Guzzo F, Toomen MA, de Vries SC (1997) A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. Development 124:2049
Schmittgen TD (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3(6):1101
Shimada T, Hirabayashi T, Endo T, Fujii H, Kita M, Omura M (2005) Isolation and characterization of the somatic embryogenesis receptor-like kinase gene homologue (CitSERK1) from Citrus unshiu Marc. Sci Hortic 103:233–238. https://doi.org/10.1016/j.scienta.2004.07.005
Singh A, Khurana P (2012) Genome wide functional characterization of erk genes in wheat, Triticum aestivum. In: International conference on plant biotechnology for food security: new frontiers (ICPBFS). National Agricultural Science Centre Pusa, New Delhi, India
Singla B, Khurana JP, Khurana P (2009) Structural characterization and expression analysis of the SERK/SELR gene family in rice (Oryza sativa). Int J Plant Genomics. https://doi.org/10.1155/2009/539402
Sun L et al (2012) Differential gene expression during somatic embryogenesis in the maize (Zea mays L.) inbred line H99. Plant Cell Tissue Organ Cult (PCTOC) 109:271–286. https://doi.org/10.1007/s11240-011-0093-6
Tamara K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. https://doi.org/10.1093/molbev/msr121
Vallee RB, Shpetner HS (1990) Motor proteins of cytoplasmic microtubules. Annu Rev Biochem 59:909–932. https://doi.org/10.1146/annurev.bi.59.070190.004401
Vasil V, Vasil IK (1986) Plant regeneration from friable embryogenic callus and cell suspension cultures of Zea mays L. J Plant Physiol 124:399–408. https://doi.org/10.1016/S0176-1617(86)80196-1
Yamamoto N, Kobayashi H, Togashi T, Mori Y, Kikuchi K, Kuriyama K, Tokui Y (2005) Formation of embryogenic cell clumps from carrot epidermal cells is suppressed by 5-azacytidine, a DNA methylation inhibitor. J Plant Physiol 162:47–54. https://doi.org/10.1016/j.jplph.2004.05.013
Zhang QX et al (2012) Micropropagation and plant regeneration from embryogenic callus of Medicago sinensis. Vitro Cell Dev Biol Plant 48:50–57. https://doi.org/10.1007/s11627-011-9387-y
Zhao L, Hu H, Zhan H, Diao Y, Jin S, Zhou F, Hu Z (2013) Plant regeneration from the embryogenic calli of five major Miscanthus species, the non-food biomass crops. Vitro Cell Dev Biol Plant 49:383–387. https://doi.org/10.1007/s11627-013-9503-2
Zheng W et al (2014) AtWuschel promotes formation of the embryogenic callus in Gossypium hirsutum. PLoS ONE 9:e87502–e87502. https://doi.org/10.1371/journal.pone.0087502

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.