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Original Article

Cloning, transformation and expression of cell cycle-associated protein kinase *OsWee1* in indica rice (*Oryza sativa L.*)

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ABSTRACT

The development process of seed in plants is a cycle of cells which occur gradually and regularly. One of the genes involved in controling this stage is the *Wee1* gene. *Wee1* encode protein kinase which plays an important role in phosphorylation, inactivation of cyclin-dependent kinase 1 (CDK1)-cyclin (CYC) and inhibiting cell division at mitotic phase. The Overexpression of *Wee1* leads to delaying entry into mitotic phase, resulting in enlargement of cell size due to suppression of cell division. Accordingly, the cloning and overexpressing of *Wee1* in rice plant is important aim of this research in achieving better quantity and quality of future rice. The main objective of this present study is to cloning and generate transgenic rice plants overexpressing of *Wee1* gene. *Wee1* was isolated from cDNA of indica rice (*Oryza sativa*), called *OsWee1*. The full length of *OsWee1* was 1239 bp in size and successfully inserted into plant expression vector pRI101ON. Seven-day-old rice seedlings were prepared for transformation of *OsWee1* gene using Agrobacterium-mediated transformation method. Four positive transgenic lines were identified through the presence of kanamycin resistance gene (*nptII*) using genomic PCR analysis. Southern blot analysis result provides evidence that four independent rice transformants contained one to three rearranged transgene copies. Further screening in transgenic rice generation is needed in order to obtain stable expression of *OsWee1*.

1. Introduction

One of the parameters to increase the yield of rice is the seed sizes, which regulated by the seed development processes after pollination. The process is a part of cell cycle which occur gradually and regularly during its life cycle. The cycle is divided into four phases, the mitotic (M) phase which includes mitosis and cytokinesis, G1 (first gap) phase, S (synthesis) phase, and G2 (second gap) phase. The cell develops larger in the G1 phase, then in S phase, cell replicates the chromosome inside the cell. Furthermore, the cell will continue its growth in G2 phase, and divided. The daughter cells can repeat the cycle [1]. The cell cycle progression is controlled at distinct checkpoints which major checkpoints are synthesis phase (G1-S checkpoint), mitosis (G2-M checkpoint) and the spindle checkpoint [2].

In eukaryotes, the cell cycle is controlled by family of conserved cyclin-dependent protein kinases (CDKs). The activity of CDK fluctuates regularly during the cell cycle, triggering important processes [3]. Phosphorylation and dephosphorylation of the CDK catalytic subunit, threonine 14 and tyrosine 15, are able to regulate and determine the timing of G2 and mitosis [4]. Previous study reported that phosphorylation of CDKs at tyrosine 15 in *Schizosaccharomyces pombe* is mediated by *Wee1*, which causes a delay in mitosis by phosphorylating the M-phase promoting factor on tyrosine 15 [5].

Wee1 is a gene encoding protein kinase located in the nucleus. The expression of this gene in plants is strongly induced by DNA damage which can be caused by radiation, ionization, chemicals and other stresses [6,7]. When DNA is damaged, ataxia-telangiectasia mutated (ATM) or ATM- and Rad3-related (ATR) kinases will be expressed depending on the genotoxic type of stress. Futhermore, the ATM and ATR signals will phosphorylate and activated Chk1 and further phosphorylates the *Wee1*. Activation of Chk1 caused cell cycle delay in G2-M phase by increasing *Wee1* regulation and decreasing regulation of phosphatase (Cdc25) which

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controls tyrosine-15 phosphorylation inhibitors on cyclin-dependent kinase (Cdc25) resulting in G2 phase arrest [8–10].

In fission yeast (*S. pombe*), loss of Wee1 activity causes unsufficient growth cells early enter to mitosis phase and cytokinesis, therefore it causes cells to produce two abnormally small daughter cells [5,11]. However, increasing expression of *Wee1* causes delayed entry into mitosis and increase in cell size, this indicates that the activity levels of Wee1 plays a role in ensuring the entry time of the mitotic phase and having strong effect on cell size [12]. Sun et al. [13] reported that expression of *ZmWee1* was observed in endosperm tissue at 15 day after pollination, where this gene shows its role in endoreduplication in the endosperm and supposes to be a potential regulator of seed development. The similar result was reported in *Wee1* tomato [14] and *AtWee1* from Arabidopsis [15], that the expression levels of *Wee1* gene was found higher in the generative organs such as seed, fruit and flower compared to that the vegetative organs. In previous study, the expression of rice *OsWee1* was almost found in all the tissues; roots, stem, tiller, flowers, leaves and seeds. The highly expression of rice *OsWee1* was found in 5 day after pollination of the seeds [16]. These results revealed that besides having an important role in seed developments, *Wee1* has also influence in the growth and developments of plants.

Considering the important role of *Wee1* in the development of seed, cloning and transformation of *OsWee1* was conducted in order to have understanding of the superior potential of *OsWee1* overexpressing in rice. In this study, we present results of *OsWee1* cloning and overexpression of this gene in rice.

2. Materials and methods

2.1. Plant materials

The mature seeds of indica rice (cv. Mekongga) were used in this research. Dehulled seeds were sterilized with 70% ethanol for 2 min followed by 5.25% sodium hypochlorite for 10 min and then washing with sterile distilled water for 3–5 times. The sterilized seeds were placed on MS basal salt media (Table 1) pH 5.8, supplemented with 3% (w/v) sucrose, 100 mg/L L-glutamine, 0.25% phytagel, and cultured under continuous light at ±22 °C within a period of 7 days.

2.2. Plasmid construct

Cloning of *OsWee1* consists of 2 steps, first step was cloning *OsWee1* into pGEMT easy vector (Promega), and the second was cloned into plant expression vector pRI101ON vector (TaKaRa) (Fig. 1A). The amplification fragment of *OsWee1* was conducted from DNA recombinant *pGEMT.·.OsWee1* which obtained from previous study [16] and deposited in GeneBank under Accession no. KX758541. PCR analysis were performed using the following a set of primer contain *NdeI* and *BamHI* sites (Table 2) overhang to ensure compatibility with pRI101ON vector. The fragment of *OsWee1* was amplified as follows PCR Core Kit (Roche) manufacture's procedure, initial denaturation at 94 °C for 2 min, each with 25 cycles of denaturation at 94 °C for 15 sec, annealing at 57 °C for 20 sec, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. Fragment obtained was then purified using GeneAll Expin^{tm} Combo GP and quantified using nanodrop (NanoVue Plus spectrophotometer, BioLab). The DNA fragment of *OsWee1* was ligated into pRI101ON and the recombinant *pRI101ON.·.OsWee1* was then transformed into *E. coli XL10 gold* competent cells through heat shock method [15].

Table 1
MS basal salt media content (in mg/L media).

Components	mg/L
NH_4NO_3	1650.0
KNO_3	1900.0
$MgSO_4{\cdot}7H_2O$	370.0
$MnSO_4{\cdot}4H_2O$	22.3
$ZnSO_4$	10.6
$CuSO_4{\cdot}5H_2O$	0.025
$CaCl_2{\cdot}H_2O$	440.0
KI	0.83
$CoCl_2{\cdot}6H_2O$	0.025
KH_2PO_4	170.0
H_3BO_3	6.2
$Na_2MoO_4{\cdot}2H_2O$	0.25
$FeSO_4{\cdot}7H_2O$	27.85
$Na_2EDTA{\cdot}2H_2O$	37.25
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	1.0
Glycine	2.0

2.3. Flanking analysis of OsWee1

A recombinant of *pRI101ON.·.OsWee1* was amplified and confirm the correct size by digestion using *HindIII*, *EcoRI*, *NdeI* and *BamHI* restriction enzymes (NEBr Inc.). The flanking frame of *OsWee1* in pRI101ON was checked and analyzed using Sanger dideoxy sequencing technology (The 1st BASE, Malaysia). The sequence was then analyzed using BLAST (www.ncbi.nlm.nih.gov/blast).

2.4. Transformation into Agrobacterium

DNA recombinant of *pRI101ON.·.OsWee1* was transferred into *Agrobacterium* cells by heat shock method [17]. Aliquot of 100 μL freshly prepared competent cells and 1 μL of DNA recombinant were mixed, keep on ice for 5 min and chilled into liquid nitrogen. Heat shock was immediately conducted by heated at 42 °C in waterbath for 90 sec. Added 1 ml of YEP medium (10 g/L yeast extract, 10 g/L peptone, 5 g/L $NaCl_2$) and gently shaked at 28 °C for 1 h to allow bacteria and harboring DNA replication. Cultured bacteria was then collected by centrifuge at 5000 rpm for 3 min, colected pellet was disolved and spread on YEP agar medium supplement with 50 mg/L kanamycin, 100 mg/L rifampicin and 12.5 mg/L gentamicin. The bacteria cells were incubated at 28 °C for 2 d and the colony grown was identified using PCR with several specific primers (Table 2).

2.5. Agrobacterium-mediated transformation and putative rice transformants selection

The *Agrobacterium* harboring the binary construct *pRI101ON.·.OsWee1* was inoculated in YEP medium supplemented with 100 mg/L rifampicin, 12.5 mg/L gentamicin and 50 mg/L kanamycin, then incubate at 28 °C by gently shake at 110 rpm for 48 h. The growth of *Agrobacterium* was checked for its optical density by spectrophotometer (OD_{600nm} = 0.3). Seven-day-old rice seedlings were soaked in the *Agrobacterium* suspension for 20 min. To reduce the growth of excessive bacteria, the infected seedlings were dried using sterilized filter papers for 5 min. The infected seedlings were grown into co-cultivation medium (MS basal salt (Table 1), 3% sucrose, 100 mg/L acetosyringone, 0.3% phytagel; pH 5.2) and incubated for 2 d in the dark condition. The co-cultivated rice seedlings were thoroughly washed with 500 mg/L cefotaxime followed by sterilized water for three times. Furthermore, the seedlings were cultured on selection medium (MS basal salt, 3% sucrose, 0.25% phytagel, 50 mg/L kanamycin and 250 mg/L cefotaxime; pH 5.8) under a 16/8-h (day/night) light cycle at 22 °C and periodically sub-cultured every 4 weeks into fresh media. The screening

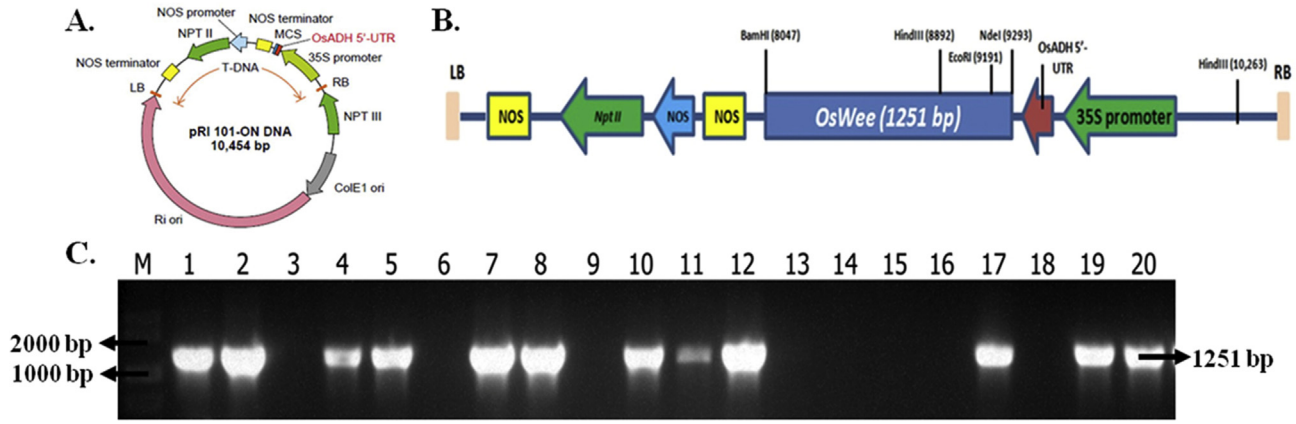

Fig. 1. Cloning *OsWee1* into expression vector pRI101ON. A. A Map of pRI101ON vector; B. Schematic representation of *pRI101ON.:.OsWee1* construct; C. Colony PCR analysis of *OsWee1* amplified using 146-FNdeI and 147-RBamHI primers.

Table 2
List of primers used in this study and corresponding sequences.

Primer name	Sequence (5′–3′)	GC content (%)
OsWee-F	ATGGCACTTGGAATTAGTTGTGGTC	44.0
OsWee-R	TTATCGTGGCAAACCAACTGAGG	47.8
FndeI	GCCATATGGCACTTGGAATTAGTTGT	42.3
RBamHI	GCGGATCCTTATCGTGGCAAACCAA	52.0
CaMV-F	GAAGACGTTCCAACCACG	55.6
RV-F	CAGGAAACAGCTATGACC	50.0
nptII-F	GTCATCTCACCTTGCTCCTGCC	59.1
nptII-R	GTCGCTTGGTCGGTCATTTCG	57.1
OsActin-F	TCCATCTTGGCATCTCTCAG	50.0
OsActin-R	GTACCCGCATCAGGCATC	61.1

putative transformants were conducted by screening of rice explants on the selection media containing 50 mg/L kanamycin.

2.6. Molecular analysis of putative transformed plants by PCR and Southern blotting

Genomic DNA was isolated from 4 g of leaves of *wildtype* and putative transformants rice [18] with minor modification. To confirm the presence of transgene, the putative transformants and non-transformant rice samples were analyzed by PCR analysis using nptII-F and nptII-R primers (Tabel 2). About 50–100 ng of total genomic DNA from independent putative transformant lines and non-transformant were mixed with 50 µl of reaction mix (KAPA Taq Extra HotStart ReadyMix) and subjected for PCR analysis under pre-denaturation condition at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 sec, 58 °C for 30 sec, 72 °C for 1 min and a final extension at 72 °C for 5 min. The results were visualized by loading on 1% (w/v) agarose gel electrophoresis.

Southern blot analysis was performed by method previously described [19]. Twenty µg of the genomic DNA was digested with the restriction enzymes *BamHI* (Promega) at 37 °C overnight. The digested DNA was separated on a 1% agarose gel and shifted to an Amersham Hybond N+ membrane (GE Healthcare, UK) using capillary transfer. The membrane was hybridized with a DIG-labelled DNA probe (Roche, Germany) and incubated overnight at 42 °C with gentle agitation. The DNA probe was prepared by amplification of *pRI101ON.:.OsWee1* by PCR using FNdeI and RBamHI primers and the PCR product was then labelled with DIG. The processes of probe preparation and washing of the membrane to remove the unbound probe were performed according to the manufacturer's instructions (Roche). Hybridization was visualized by exposing the membrane to X-ray (Fuji Film).

2.7. RNA isolation and reverse transcriptase PCR (RT-PCR)

The total RNA was isolated from 100 mg of young leaves of putative transformant lines and non-transformant 30-day-old rice plants using RNAprep pure plant kit (Tiangen, Beijing). The first-strand cDNA was prepared from 1 µg of total RNA using iScript™ cDNA Synthesis Kit (BIO-RAD) according to the manufacturer's instructions. The synthesized cDNA was then used as template to check the expression of *OsWee1* using OsWee-F and OsWee-R primers (Table 2). The RT-PCR conditions were 95 °C for 3 min, followed by 25 cycles at 95 °C for 30 sec, 56 °C for 30 sec, 72 °C for 1 min and a final extension at 72 °C for 5 min. The primer pairs of OsActin (Table 2) was used to amplify the *OsActin* reference gene, at 95 °C for 3 min, followed by 25 cycles at 95 °C for 30 sec, 48 °C for 30 sec, 72 °C for 1 min and a final extension at 72 °C for 5 min. The results were visualized by loading on 1% (w/v) agarose gel electrophoresis.

3. Results and discussion

3.1. Cloning of OsWee1

The cloning of full length *OsWee1* gene (1239 bp) in the expression vector (pRI101-ON) was performed on the *NdeI* and *BamHI* restriction sites. Clone of *OsWee1* that obtained in previous study [14] was amplified using a pair of FNdeI and RBamHI primers (Table 2) in order to add those restriction sites on the open reading frame (ORF) of *OsWee1* before cloning. The construct of *OsWee1* gene in the expression vector was shown in Fig. 1A. The construct of *OsWee1* was then transformed into *E. coli* strain XL10 gold competent cells and selected using antibiotic kanamycin on the growth media. To verify the positive clones, 20 recombinant colonies were randomly selected and prepared for the colony PCR analysis using FNdeI and RBamHI primers (Table 2). As shown in Fig. 1B, the presence of 1251 bp of *OsWee1* gene was found in 12 clones which were confirmed by colony PCR and visualized on agarose gel. For further analysis, clone no. 7 was selected.

In order to evaluate the correct size and flanking of the *OsWee1* clones, PCR analysis, restriction enzymes digestion and sequencing were performed. The PCR reaction was conducted using 4 pairs of primers, CaMV-F/RV-R, FNdeI/RBamHI, nptII-F/nptII-R, CaMV-F/OsWee-R (Table 2). The PCR result showed that expected bands appear at the appropriate sizes, 1868 bp, 1251 bp, 524 bp and 1504 bp, respectively (Fig. 2 A). Digestion was performed by single or combination of *HindIII*, *BamHI* and *EcoRI* restriction enzymes. Open reading frame of *OsWee1* contains *BamHI* and *EcoRI* enzymes,

while *HindIII* was present two sites in the construct. Then, this clone was further conformed by sequencing (*data not shown*). The correct band sizes of *OsWee1* after digestion was shown in Fig. 2B. The nucleotide sequence analysis showed a full length region of *OsWee1* about 1239 bp and a 100% homolog with cDNA of *OsWee1* in GeneBank (KX758541). It suggested that the desired recombinant *pRI101ON.:.OsWee1* was successfully prepared to expressed in *Agrobacterium*.

3.2. Transformation of OsWee1 into Agrobacterium

The recombinant construct of *pRI101ON.:.OsWee1* was transformed into *Agrobacterium* strain GV3101 and selected on YEP solid medium supplemented with 50 mg/L kanamycin, 100 mg/L rifampicin and 12.5 mg/L gentamicin antibiotic (Fig. 3A). Twelve colonies were randomly selected for PCR analysis using FNdeI and RBamHI primers and 4 positive clones harboring *OsWee1* were obtained by PCR (Fig. 3B). This results clearly confirm that recombinant *pRI101ON.:.OsWee1* was transformed into *Agrobacterium,* and it can be used for transformation in rice.

The successful Agrobacterium-mediated transformation in rice has been achieved using various methods. However, in monocot plants remains limited because it is not a natural host for *Agrobacterium* [20]. Many researchers have developed methods for Agrobacterium-mediated transformation in monocotyledons, especially in utilizing of different *Agrobacterium* strains [21,22], piercing method and vacum infiltration [23], choosing of different cultivars [24] and type of explants [25,26]. The basic protocol of Agrobacterium-mediated transformation [27] was develop for induction of callus derived from scutella seeds. However, since most of indica rice genotypes have less regeneration potential [28], we conducted Agrobacterium-mediated transformation in rice using 7-day-old rice sprouts as explants.

A hundred explants were infected by *Agrobacterium* suspension for 20 min and co-cultivation in dark condition for 2 d followed by cefotaxime-antibiotic treatment. Transformation was carried out twice with total 200 explants. However the percentage of rice transformation efficiency is very low (2%) (Table 3). Previous study reported [29] numerous factors that can improve transformation efficiency, was lighting condition, temperature, co-cultivation periods and *Agrobacterium*-density during co-cultivation step. The most critical factor reported was the period of co-cultivation. The successful integration of target gene into the plant genome occurs mainly during co-cultivation. Sahoo and Tuteja [29] found that suitable method for rice transformation was infection for 20 min followed by co-cultivation for 2 day. An extension of co-cultivation period, caused arising of excessive bacterial growth which results in inhibiting explants growth, reduce the number of shoots generation and finally causing death of explant. Rashid *et al.* [30] reported that to anticipate those problems, reducing the density of bacteria into 0.1 – 0.2 OD prevents overgrowth bacteria during co-cultivation.

The co-cultivated explants were transferred in the selection medium, to inhibit the formation of non-transformant explants and eliminate the residual of *Agrobacterium*. The first shoots produced from the explants were subjected to three successive propagation cycles with the same level concentration of antibiotic. This method is applied to select putative transformants and reduce false positive transformants or eliminate the chimeric of transgenic explants [31]. After three sub-cultures, the non-transformant plantlet were turned into white (chlorosis), while the putative transformants were able to survive and grow normally (Fig. 4A). The lacking of *nptII* gene in the non-transgenic plants caused inhibing of chlorophyll development and induce chlorosis at the shoot of rice plantlets. In contrast to the transgenic overexpressing *OsWee1* showed normal green at the shoots. The absence of *nptII* gene will suppress the growth of non-transformant roots caused by unability of root to inactivated the kanamycin in the media (Fig. 4A). Similar results were also obtained in previous study [32] that increased concentration of kanamycin may lead to inhibition of root growth. Kanamycin inhibits the synthesis of protein in plastid and mitochondrial. Kanamycin acts as an inhibitor, active destroy the function of ribosome following by inhibition of translational initiation. Another way, the response was automatically inhibit protein synthesis and effects on reducing the growth and development of plants.

3.3. Expression of OsWee1 in rice

The putative transgenic plants which survived in selection *in vitro* medium were then transferred to soil and placed in the greenhouse under agronomic conditions (Fig. 4B). These plants were then analyzed for their transgenic status by PCR analysis. PCR analysis showed that the *nptII* gene 550 bp in size was found in 4 of putative transgenic lines (Fig. 4C). To determine the expression of *OsWee1* at the RNA levels, we performed a RT-PCR analysis. The quantity of RNA level is a reflection of the level of transcription. As shown in Fig. 5A, the transcript levels of rice overexpress-

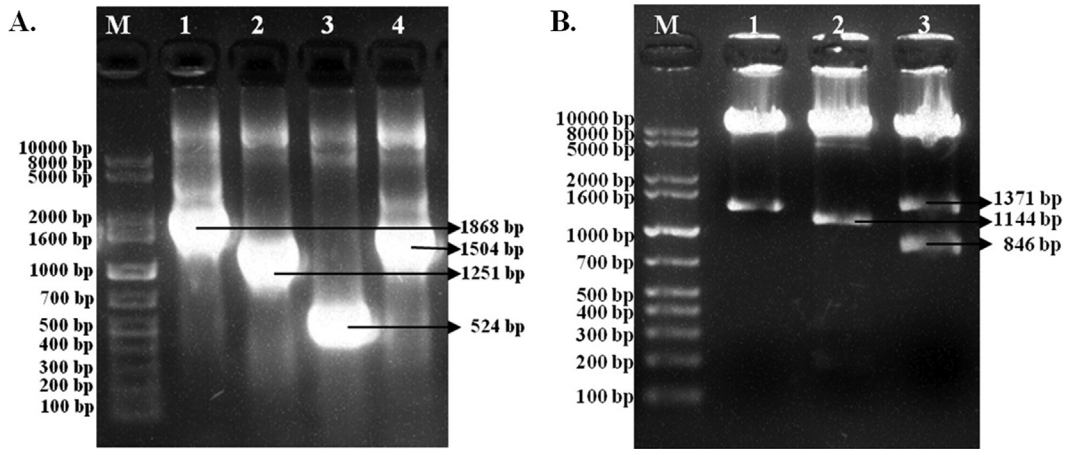

Fig. 2. Analyze of recombinant *pRI101ON.:.OsWee1*. A. PCR analysis of positive clones using several primers (M: 1 kb Tiangen Ladder, line 1–4: CaMV-F/RV-R primer, FndeI/RBamHI primer, nptII primer, and CaMV-F/OsWee-R, respectively); B. Restriction digestion with *HindIII* (line 1), *EcoRI*/*BamHI* (line 2) and *HindIII*/*BamHI* (line 3).

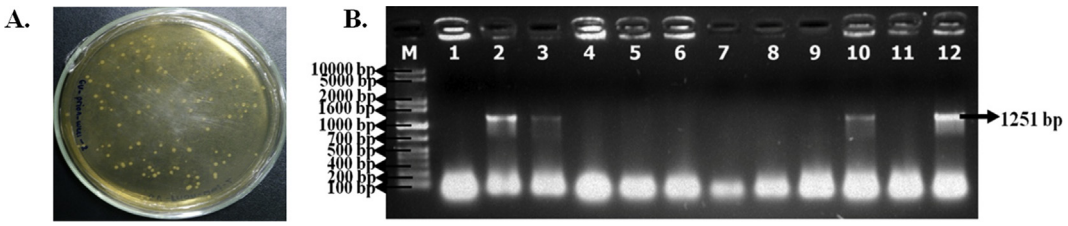

Fig. 3. Transformation *pRI101ON.:.OsWee1* into *Agrobacterium*. A. *Agrobacterium* colonies strain GV 3101 harboring recombinant DNA of *OsWee1*; B. PCR analysis of *OsWee1* using a set of FNdeI and RBamHI primers and the estimation size of *OsWee1* fragment.

Table 3
The percentage of rice transformation efficiency.

Transformation number	Number of infected seedling	Number of selected plant	Number of transformant[a]	Transformation efficiency (%)
1	100	3	3	3%
2	100	2	1	1%
Total	200	5	4	2%

[a] Confirmation by PCR analysis.

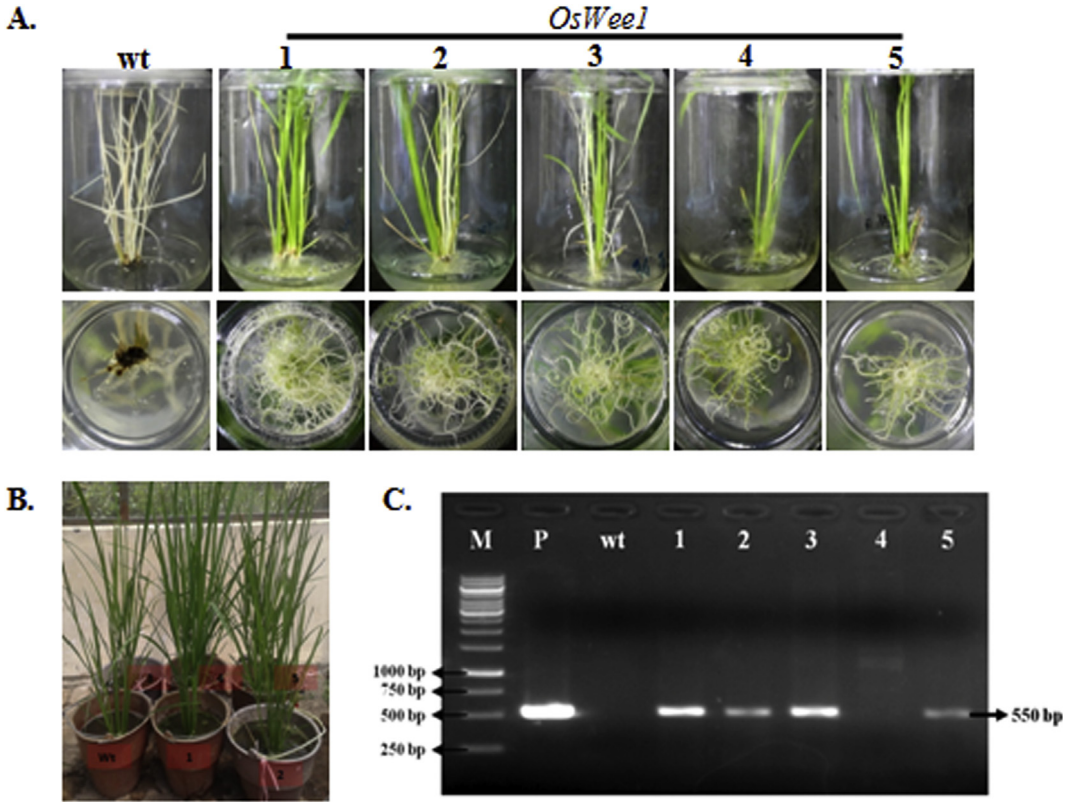

Fig. 4. Transgenic rice overexpressing of *OsWee1* gene. A. Phenotypic selection of transgenic rice overexpressing *OsWee1*, rice planlet (upper) and root (lower) of wildtype (wt) and transgenic (lane 1–5); B. Transgenic plants after acclimatization in green house; C. PCR analysis of the T0 transgenic plants (line 1–5), *wildtype* (WT), pRI101ON.:.*OsWee1* (P) as positive control, and DNA marker (M).

ing *OsWee1* were higher compared to the *wildtype*. The data indicates that *OsWee1* driven by CaMV35S was expressed in rice.

Southern blot analysis of transgenic rice was performed to prove integration of the transgene into the plant genome and to determine copy number of the T-DNA. Southern blot analysis was conducted using a gDNA isolated from leaves of four transgenic rice lines. The Southern blot analysis showed that the transgenic rice displayed one to three hybridized DNA copy with a difference in molecular size suggesting the independent transformation events. The hybridized DNA was not found in the genome of the WT plant (Fig. 5B). These results confirmed that the copy of the *OsWee1* gene was integrated into the genome of the transgenic rice.

The transgenic plants required stability of expression to be used in seed production [33], as well as *OsWee1* overexpression plants. In the present study, four among five independent transformation events have been shown to carry multiple copies of the T-DNA. Multiple copies of the transgene are prone for transgene inactivation, silencing, and likely to cause a high frequency of insertional mutagenesis [34]. To obtained stability of expression, the selection will be carried out through anther culture in the second generation

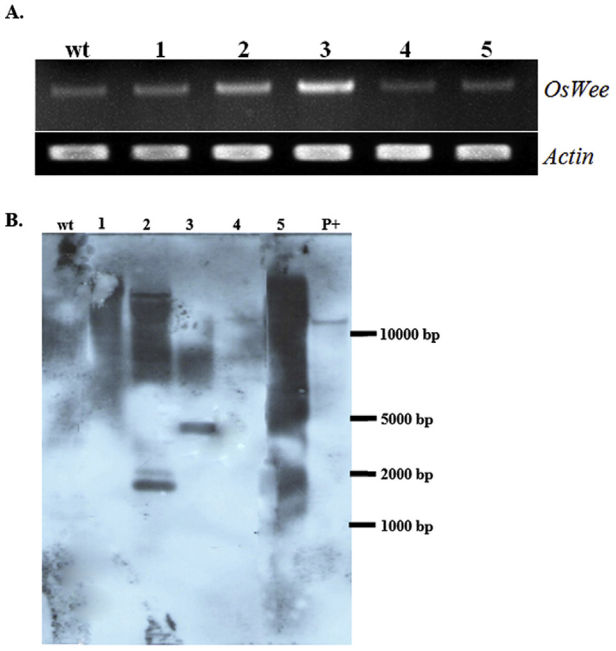

Fig. 5. Analysis of the first generation of transgenic plants (T0). A. RT-PCR analysis of the T0 transgenic plants overexpressing *OsWee1* using full length *OsWee1* primer (upper) and *Actin* gene as a control (lower); B. Southern blot analysis of transgenic plants (line 1–5), *wildtype* (WT), pRI101ON::*OsWee1* (+P), and DNA marker (M).

of transgenic rice. Regeneration of haploid plants from anther culture followed by chromosomal doubling can produce double haploid or pure line of plants. This result will provide an opportunity to accelerate the time for the formation of inbreed line which is normally through several inbreeding cycles [35].

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References

[1] Dewitte W, Murray JAH. The plant cell cycle. Annu. Rev. Plant Biol. 2018;54 (2003):235–64. doi: https://doi.org/10.1146/annurev.arplant.54.031902.134836 [May 29, 2018].
[2] Harashima H, Dissmeyer N, Schnittger A. Cell cycle control across the eukaryotic kingdom. Trends Cell Biol. 2013;23(7):345–56. doi: https://doi.org/10.1016/j.tcb.2013.03.002 [May 29, 2018].
[3] Nurse P, Thuriaux P. Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. Genetics 1980;96(3):627–37 [September 21, 2018] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1214365/.
[4] Shiozaki K, Russell P. Cell-cycle control linked to extracellular environment by MAP kinase pathway in fission yeast. Nature 1995;378(6558):739–43. doi: https://doi.org/10.1038/378739a0 [September 21, 2018].
[5] Nurse P. Genetic control of cell size at cell division in yeast. Nature 2018;256 (1975):547–51. doi: https://doi.org/10.1038/256547a0 [May 29, 2018].
[6] Do K, Doroshow JH, Kummar S. Wee1 kinase as a target for cancer therapy. Cell Cycle 2013;12(19):3159–64. doi: https://doi.org/10.4161/cc.26062 [May 29, 2018].
[7] Kalhorzadeh P, Hu Z, Cools T, Amiard S, Willing E, De Winne N, et al. Arabidopsis thaliana RNase H2 deficiency counteracts the needs for the WEE1 checkpoint kinase but triggers genome instability. Plant Cell 2018;26 (2014):3680–92. doi: https://doi.org/10.1105/tpc.114.128108 [May 29, 2018].
[8] Sørensen CS, Syljua RG. Safeguarding genome integrity: the checkpoint kinases ATR, CHK1 and WEE1 restrain CDK activity during normal DNA replication. Nucleic Acids Res 2012;40(2):477–86. doi: https://doi.org/10.1093/nar/gkr697 [May 29, 2018].
[9] Caparelli ML, O'Connell MJ. Regulatory motifs in Chk1. Cell Cycle 2013;12 (6):916–22. doi: https://doi.org/10.4161/cc.23881 [May 29, 2018].
[10] Su C, Zhao H, Zhao Y, Ji H, Wang Y, Zhi L, et al. RUG3 and ATM synergistically regulate the alternative splicing of mitochondrial nad2 and the DNA damage response in Arabidopsis thaliana. Sci Rep 2018;7(2017):1–14. doi: https://doi.org/10.1038/srep43897 [May 29, 2018].
[11] Yu Z, Zhang M, Wang G, Xu D, Keifenheim D, Franco A, Cansado J, Masuda H, Rhind N, Wang Y, Jin Q. Fission yeast nucleolar protein Dnt1 regulates G2/M transition and cytokinesis by downregulating Wee1 kinase. J Cell Sci 2014;126 (21):4995–5004. doi: https://doi.org/10.1242/jcs.132845 [May 29, 2018].
[12] Russell P, Nurse P. Negative regulation of mitosis by weel*, a gene encoding a protein kinase homolog. Cell 2018;49(1987):559–67. doi: https://doi.org/10.1016/0092-8674(87)90458-2 [May 29, 2018].
[13] Sun Y, Dilkes BP, Zhang C, Dante RA, Carneiro NP, Lowe KS, Jung R, Gordon-Kamm WJ, Larkins B. Characterization of maize (Zea mays L.) Wee1 and its activity in developing endosperm. Proc Natl Acad Sci 1999;96:4180–5. doi: https://doi.org/10.1073/pnas.96.7.4180 [May 29, 2018].
[14] Gonzalez N, Gevaudant F, Hernould M, Chevalier C, Mouras A. The cell cycle-associated protein kinase WEE1 regulates cell size in relation to endoreduplication in developing tomato fruit. Plant J 2007;51(4):642–55. doi: https://doi.org/10.1111/j.1365-313X.2007.03167.x [May 29, 2018].
[15] Sorrell DA, Marchbank A, McMahon K, Dickinson JR, Rogers HJ, Francis D. A WEE1 homologue from Arabidopsis thaliana. Planta 2002;215(3):518–22. doi: https://doi.org/10.1007/s00425-002-0815-4 [May 29, 2018].
[16] Ermawati N, Wibisono Y. Early isolation of cell cycle-associated protein kinase (OsWee) gene in rice (Oryza sativa L.). Pak. J. Biotechnol. 2017;14(1):71–6 [May 29, 2018] http://www.pjbt.org/uploads/PJBT-VOL-14-NO-1-OF-YEAR-2017%20%2811%29.
[17] Sambrook J, Russell. Molecular cloning a laboratory manual. third ed. Cold Spring Harbor Laboratory Press; 2001.
[18] Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: version II. Plant Mol Biol Rep 1983;1:19–21. doi: https://doi.org/10.1007/BF02712670 (September 19, 2018).
[19] Apriasti R, Widyaningrum S, Hidayati WN, Sawitri WD, Darsono N, Hase T, et al. Full sequence of the coat protein gene is required for the induction of pathogen-derived resistance against sugarcane mosaic virus in transgenic sugarcane. Mol Biol Rep 2018;1(2018):1–10. doi: https://doi.org/10.1007/s11033-018-4326-1 [September 19, 2018].
[20] Aldemita RR, Hodges TK. Agrobacterium tumefaciens-mediated transformation of *japonica* and *indica* rice varieties. Planta 2018;199 (1996):612–7. doi: https://doi.org/10.1007/BF00195194 [May 29, 2018].
[21] Balaji V, Rajamuni P, Sridevi G, Veluthambi K. Agrobacterium-mediated Transformation Efficiency in Blackgram and Rice Enhanced by Multiple Copies of pTiBo542 virB and virG. Indian J Biotechnol 2003;2:138–46 [May 29, 2018] http://nopr.niscair.res.in/handle/123456789/11283.
[22] Ratanasut K, Rod-In W, Sajipuli K. In planta agrobacterium-mediated transformation of rice. Rice Sci 2017;24(3):181–6. doi: https://doi.org/10.1016/j.rsci.2016.11.001 [May 29, 2018].
[23] Lin J, Zhou B, Yang Y, Mei J, Zhao X, Guo X, Huang X, Tang D, Liu X. Piercing and vacuum infiltration of the mature embryo: a simplified method for Agrobacterium-mediated transformation of indica rice. Plant Cell Rep 2009;28:1065–74. doi: https://doi.org/10.1007/s00299-009-0706-2 [May 29, 2018].
[24] Saika H, Toki S. Mature seed-derived callus of the model *indica* rice variety Kasalath is highly competent in *Agrobacterium*-mediated transformation. Plant Cell Rep 2010;29(12):1351–64. doi: https://doi.org/10.1007/s00299-010-0921-x [May 29, 2018].
[25] Manimaran P, Kumar GR, Reddy MR, Jain S, Rao TB, Mangrauthia SK, et al. Infection of early and young callus tissues of indica rice BPT 5204 enhances regeneration and transformation efficiency. Rice Sci 2018;20(2013):415–26. doi: https://doi.org/10.1016/S1672-6308 (13)60153-5 [May 29, 2018].
[26] Dey M, Bakshi S, Galiba G, Sahoo L, Panda SK. Development of a genotype independent and transformation amenable regeneration system from shoot apex in rice (*Oryza sativa* spp. *indica*) using TDZ. Biotech 2012;2(3):233–40. doi: https://doi.org/10.1007/s13205-012-0051-y [May 29, 2018].
[27] Hiei Y, Ohta S, Komari T, Kumashiro T. Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J 1994;6(2):271–82. doi: https://doi.org/10.1046/j.1365-313X.1994.6020271.x [May 29, 2018].
[28] Nishimura A, Ashikari M, Lin S, Takashi T, Angeles ER, Yamamoto T, Matsuoka M. Isolation of a rice regeneration quantitative trait loci gene and its application to transformation systems. PNAS 2005;102(33):11940–4. doi: https://doi.org/10.1073/pnas.0504220102 [May 29, 2018].
[29] Sahoo RK, Tuteja N. Development of Agrobacterium-mediated transformation technology for mature seed-derived callus tissues of indica rice cultivar IR64. GM Crops Food: Biotechnol Agric Food Chain 2012;3(2):123–8. doi: https://doi.org/10.4161/gmcr.20032 [May 29, 2018].
[30] Rashid H, Afzal A, Khan MH, Chaudhry Z, Malik SA. Effect of bacterial culture density and acetosyringone concentration on *Agrobacterium* mediated transformation in wheat. Pak J Bot 2010;42(6):4183–9 [May 29, 2018] https://www.researchgate.net/publication/260156533_Effect_of_bacterial_culture_density_and_acetosyringone_concentration_on_Agrobacterium_mediated_transformation_in_wheat.
[31] Nap JP, Bijvoet J, Stiekema WJ. Biosafety of kanamycin-resistant transgenic plants. Transgenic Res 1992;1(6):239–49 [May 29, 2018] https://link.springer.com/article/10.1007/BF02525165.

[32] Bibi N, Fan K, Yuan S, Ni M, Ahmed IM, Malik W, Wang X. An efficient and highly reproducible approach for the selection of upland transgenic cotton produced by pollen tube pathway method. Aust J Crop Sci 2013;7 (11):1714–22 [May 29, 2018] https://search.informit.com.au/documentSummary;dn=644871058289920;res=IELHSS.

[33] Takaiwa F, Wakasa Y, Takagi H, Hiroi T. Rice seed for delivery of vaccines to gut mucosal immune tissues. Plant Biotechnol J 2015;13(8):1041–55. doi: https://doi.org/10.1111/pbi.12423 [May 29, 2018].

[34] Finn TE, Wang L, Smolilo D, Smith NA, White R, Chaudhury A, Dennis ES, Wang M. Transgene expression and transgene-induced silencing in diploid and autotetraploid arabidopsis. Genetic 2011;187(2):409–23. doi: https://doi.org/10.1534/genetics.110.124370 [September 22, 2018].

[35] Silva TD. Indica rice anther culture: can the impasse be surpassed? Plant Cell Tissue Organ Culture 2010;100(1):1–11. doi: https://doi.org/10.1007/s11240-009-9616-9 [May 29, 2018].