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Research Article

# Introduction of the *OsGERLP* Gene into Potato cv. IPB CP3 to Develop Aluminum Stress-Tolerant Potato Lines

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## ABSTRACT

The *OsGERLP* gene is an aluminum (Al) stress tolerance gene. Potato cv. IPB CP3 is a horticultural crop that has not been proven to be tolerant to Al; therefore, enhancing its tolerance through genetic transformation is necessary. This research aims to obtain transgenic potatoes cv. IPB CP3 contains the *OsGERLP* gene and is tolerant to Al stress. Experimental methods include transforming potatoes with the *OsGERLP* gene via *Agrobacterium tumefaciens*, transgene integration testing, *in vitro* assays of transgenic plants under low pH and Al stress, and analysis of transgene expression. The results showed that the transformation efficiency achieved was relatively high at 47.03%, with a regeneration efficiency of 42.19%. The transgenic clones had longer roots and more roots than the non-transgenic ones under aluminum stress. The transgenic clones GERLP2, GERLP3, and GERLP4 exhibited the greatest root growth enhancement under stress conditions and the highest *OsGERLP* gene expression levels. These clones have the potential to be developed into Al-tolerant potato varieties. Future research is required to evaluate aluminum stress tolerance, tuber yield performance, and transgene stability across the three clones under greenhouse and field conditions of the three clones.

## 1. Introduction

Marginal land is sub-optimum land for agricultural production because it has low-fertility soil quality (Shortall 2013). One of the marginal lands that can be used to support food crop production is acid soil. The main factor that inhibits plant production in acid soil is abiotic stress in the form of aluminum (Al) toxicity. In acidic soils with a pH value <5, Al can be toxic in a form that can leach into the soil, thereby inhibiting root growth and function (Samac and Tesfaye 2003). Nutrients such as phosphorus (P) will be chelated by Al so that they cannot be absorbed by the roots, causing plants to lack these nutrients, and the growth will be stunted (Delhaize and Ryan 1995).

Plants have evolved tolerance mechanisms to survive under stressful conditions. Several local rice cultivars in Indonesia that are tolerant to Al stress, such as Grogol, Krowal, and Hawara Bunar, exhibit strong Al tolerance by maintaining root growth under high Al concentrations (Miftahudin *et al.* 2007, 2008). In rice, Al tolerance has been extensively associated with genes encoding membrane transporters, such as ALMT (Al-activated malate transporter) and MATE (multidrug and toxic compound extrusion), which facilitate the exudation of organic acids to chelate and detoxify $Al^{3+}$ ions in the rhizosphere (Delhaize *et al.* 2004; Yokosho *et al.* 2011). Other regulatory genes, including *ART1, STAR1,* and *STAR2,* are known to coordinate transcriptional and cell wall modification responses that enhance Al detoxification (Huang *et al.* 2009; Yamaji *et al.* 2009).

Recently, *OsGERLP* was isolated and characterized from the Al-tolerant rice cultivar Hawara Bunar, and

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its expression was found to be induced by Al exposure (Roslim 2011). Unlike structural or transporter genes such as *ALMT* and *MATE*, *OsGERLP* appears to act as an upstream regulatory factor whose expression correlates with *ART1, STAR1, STAR2*, and *OsFRDL4* (Miftahudin *et al.* 2021). This suggests that *OsGERLP* may function as a signaling or transcriptional regulator that coordinates multiple Al-tolerance pathways rather than directly mediating ion transport. Despite this potential, the function of *OsGERLP* has not yet been validated in non-rice species. Therefore, introducing *OsGERLP* into potato (*Solanum tuberosum* L.) provides an opportunity to evaluate whether its regulatory role can enhance Al tolerance in eudicot crops, thereby addressing a key gap in understanding cross-species functionality of Al-tolerance genes.

Potato (*Solanum tuberosum* L.) is a major horticultural crop with significant economic importance and a popular dietary carbohydrate source. Despite increasing domestic demand, Indonesia's potato production remains relatively low, at approximately 1.27 million tons in 2024 (BPS 2025). Production efforts are constrained by limited highland cultivation areas, which compete with other vegetable crops. Therefore, expanding potato cultivation to sub-optimal lands, such as acidic former tea plantations with high Al solubility, represents a promising strategy to increase production.

Genetic transformation has been widely used to improve potato traits, including quality enhancement (e.g., suppression of vacuolar invertase to reduce cold-induced sweetening) (Bhaskar *et al.* 2010) and to optimize transformation methods across cultivars (Chakravarty and Wang-Pruski 2010). Several studies have demonstrated successful *Agrobacterium*-mediated transformation in various potato genotypes, confirming the reliability of this method for introducing stress-responsive genes (Bakhsh 2020). Previous studies have reported the successful transformation of the Indonesian potato cv. IPB CP3 was developed by the Biotechnology Center, IPB, which introduced *LYZ-C* using *Agrobacterium tumefaciens* strain LBA4404 (Pasmawati *et al.* 2021), *FPBase/CIRan1* using strain EHA105 (Wijayanti 2022), and *MmCu/ZnSOD* using strain LBA4404 (Puteri 2022). However, the IPB CP3 cultivar has not been evaluated for Al tolerance, and no transformation study has introduced an Al stress tolerance gene into IPB CP3. Therefore, this study aims to establish the transgenic potato cv. IPB CP3 containing the *OsGERLP* gene from rice to enhance tolerance to aluminum (Al) stress, expanding the potential of this high-value crop to acid soils.

## 2. Materials and Methods

### 2.1. Preparation of *Agrobacterium tumefaciens* LBA4404 containing pH35YG-*OsGERLP*

The binary vector pH35YG–*OsGERLP* (Figure 1) was initially transformed into *Escherichia coli* TOP10 competent cells using the heat shock method (Sambrook *et al.* 1989) with amounts of 5 µL plasmid DNA. The verified plasmid was subsequently mobilized into *Agrobacterium tumefaciens* strain LBA4404 through triparental mating (TPM) using the helper plasmid pRK2013 in *E. coli* DH1, following Wise *et al.* (2006). To ensure the success of the TPM process, *A. tumefaciens* LBA4404 bacteria carrying pH35YG-*OsGERLP* were verified by colony PCR using the GERLP primer (forward): 5'-CAC CAT GGC GGC GGC GGG TTG TC-3' (reverse): 5'-TTA TGA GCT TGA GTC GCC GGG GTT CCC T-3'). The PCR reaction was performed in a total volume of 25 µL, consisting of 100 ng of template DNA, 0.5 µM each of primer, 12.5 µL of MyTaq™ HS Red Mix (Bioline, UK), and nuclease-free water to make up the final volume. Amplification was performed with an initial denaturation at 95°C for 3 minutes; 35 cycles of 95°C for 15 seconds, 65°C for 15 seconds, and 72°C for 15 seconds; and a final extension at 72°C for 10 minutes.

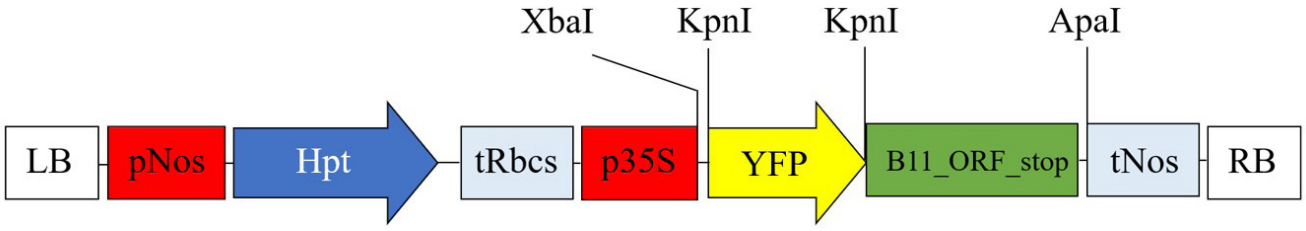


Figure 1. Linear map of the pH35YG plasmid containing the *OsGERLP* gene. LB: left border, pNos: nopaline synthase promoter, Hpt: hygromycin phosphotransferase, tRbcs: ribulose bisphosphate carboxylase small subunit gene terminator, p35S: 35S *CaMV* promoter, YFP: Yellow fluorescent protein, B11_ORF_stop: B11/*OsGERLP* gene, tNos: nopaline synthase terminator, RB: right border (Fendiyanto 2019)

### 2.2. Plant Materials and *Agrobacterium tumefaciens* Propagation

*In vitro* propagation of potato cv. IPB CP3 variety was conducted using single-node stem segments containing one bud, cultured *in vitro* on Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962) with modified hormone concentrations (Appendix). Plantlets were maintained at 20-24°C under a light intensity of 2000-3000 lux with an 18-hour photoperiod for 4 weeks.

The *A. tumefaciens* harboring the pH35YG-*OsGERLP* plasmid was grown in liquid Luria Bertani (LB) medium supplemented with selective antibiotics (Appendix). The bacterial culture was incubated at 28°C with shaking at 150 rpm in the dark for 8-12 hours until the optical density at 600 nm ($OD_{600}$) reached 0.2-0.5. Bacterial cells were harvested by centrifugation at 4,000 rpm for 10 minutes and resuspended in liquid co-cultivation medium as described in the Appendix.

### 2.3. Potato Transformation

The explants used were stem segments (internodes) without buds, measuring 0.5–1.0 cm from four-week-old potato plants. The explants were pre-cultured on modified MS medium (Appendix) for 24 h, then inoculated with *Agrobacterium tumefaciens* LBA4404 ($OD_{600}$ 0.2-0.4) carrying the pH35YG–*OsGERLP* plasmid. Bacterial infection and co-cultivation were conducted following the method of Yokota *et al.* (2013) with minor modifications in hormone concentrations and antibiotic treatments (Appendix). The combination of zeatin, indole-3-acetic acid (IAA), and gibberellic acid ($GA_3$) was used to induce callus formation and shoot regeneration. The concentrations of IAA and $GA_3$ were specifically optimized for the potato cv. IPB CP3 to improve regeneration efficiency under selective culture conditions.

After co-cultivation, explants were washed with sterile distilled water containing cefotaxime to eliminate bacterial contamination and subsequently cultured on callus induction medium (Appendix). Putative transgenic shoots that regenerated on the selection medium were propagated through stem cuttings on MS basal medium supplemented with 10 mg/L hygromycin. Stepwise selection was performed by gradually increasing the hygromycin concentration from 10 to 20 mg/L in each subsequent subculture. The number of developed calli and hygromycin-resistant calli was recorded to determine transformation and regeneration efficiencies, following the method of Sahoo *et al.* (2011).

### 2.4. Molecular Analyses of Transformed Potato Plants

Putative transgenic potato plantlets subcultured on selection medium were analyzed for transgene integration. Total genomic DNA was extracted using the CTAB method (Doyle and Doyle 1987). DNA purity and concentration were measured using a spectrophotometer (MaestroGen, Taiwan), and only samples with an A260/A280 ratio of 1.8–2.0 and a concentration above 50 ng $\mu L^{-1}$ were used for PCR amplification. DNA integrity was further verified by electrophoresis on a 1% agarose gel.

PCR amplification was conducted to verify DNA integrity using actin gene primers, Tact-qF (5'-ACA TCG TCC TTA GTG GTG GA-3') and Tact-qR (5'-GTG GAC AAT GGA AGG ACC AG-3'), producing an expected fragment of approximately 400 bp. PCR amplification was carried out with the following program: pre-denaturation at 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 55°C for 45 seconds, and 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes.

Integration of the *OsGERLP* gene was confirmed using specific primers 35s-F (forward): 5'-AAA CCT CCT CGG ATT CCA TT-3' and primer GERLP_R (reverse): 5'-TTA TGA GCT TGA GTC GCC GGG GTT CCC T-3'), yielding a target amplicon of approximately 1,500 bp. PCR conditions were: pre-denaturation at 95°C for 3 minutes; 35 cycles of 95°C for 15 seconds, 65°C for 15 seconds, and 72°C for 15 seconds; followed by a final extension at 72°C for 10 minutes. The amplification results were analyzed using electrophoresis with a 1% agarose gel in TAE buffer at a voltage of 70 volts for 28 minutes. Plasmid pH35YG–*OsGERLP* served as a positive control, and non-transgenic IPB CP3 genomic DNA as a negative control.

### 2.5. Aluminum Stress Tolerance Assay

The aluminum stress tolerance assay was conducted using a completely randomized design with two treatment factors and three replications. The samples used for this assay were explants derived from putative transgenic plants. The first factor was genotype, consisting of non-transgenic IPB CP3 (CP3NT) and four transgenic clones: CP3GERLP1, CP3GERLP2, CP3GERLP3, and CP3GERLP4. The second factor was the culture medium, comprising (1) pH 4 + 2 mM Al, (2) pH 4 without Al, and (3) pH 5.8 without Al (control). The Al concentration and pH levels were determined based on prior optimization tests using several Al concentrations

(0–3 mM), where 2 mM at pH 4 induced consistent stress responses without fully inhibiting growth. These conditions also reflect the Al solubility typical of acid soils. Each replicate consisted of one culture bottle containing three uniformly sized plantlets, treated as a single experimental unit to minimize environmental variation. After four weeks of cultivation, total root length, root number, and shoot length were measured using ImageJ software.

### 2.6. Gene Expression Analysis

Gene expression analysis consists of three steps: total RNA isolation, cDNA synthesis, and Quantitative Real-Time PCR (qPCR). The first stage is the isolation of total RNA from transgenic potato plants. RNA was isolated using the GENEzol reagent (Geneaid, Taiwan), and the quality of total RNA was evaluated by agarose gel electrophoresis. Residual genomic DNA was eliminated using the gDNA removal step included in the cDNA synthesis kit. First-strand cDNA was synthesized using 1 µg of total RNA with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Quantitative real-time PCR (qPCR) was performed using SensiFAST SYBR Lo-ROX Kit (Meridian Bioscience, USA) on a QuantStudio 5 instrument (Applied Biosystems, USA). The *OsGERLP*-specific primers were GERLP (forward: 5'-CAC CAT GGC GGC GGC GGG TTG TC-3'), reverse: 5'-TTA TGA GCT TGA GTC GCC GGG GTT CCC T-3'), and specificity was verified by melt curve analysis. Actin was used as the internal reference gene for normalization, as it showed stable expression across treatments in preliminary tests. The qPCR program was initiated at 95°C for 30 seconds, followed by 50 cycles of denaturation at 95°C for 10 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. Each qPCR reaction was performed in duplicate (two technical replicates) using cDNA obtained from three independent biological replicates per genotype. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

## 3. Results

### 3.1. Introduction of pH35YG-*OsGERLP* Plasmid into *A. tumefaciens*

The pH35YG–*OsGERLP* plasmid was successfully transformed into *Escherichia coli* TOP10 and verified by colony PCR, producing the expected 339 bp fragment of the *OsGERLP* insert (Figure 2). The confirmed construct was subsequently introduced into *A. tumefaciens* strain LBA4404 via triparental mating. Transformant colonies grew on a selective medium containing marker antibiotics, and the presence of the *OsGERLP* gene was further confirmed by colony PCR (Figures 3 and 4).

### 3.2. Genetic Transformation of Potato cv. IPB CP3

Transformation of 367 potato cv. IPB CP3 explants using *A. tumefaciens* harboring pH35YG–*OsGERLP* yielded 162 hygromycin-resistant calli and 71 regenerated shoots (Figure 5, Table 1). The average transformation and regeneration efficiencies were 47.03% and 42.19%, respectively. Six vigorous hygromycin-resistant shoots (CP3GERLP1–6) were selected for molecular confirmation.

### 3.3. Molecular Analysis of Transgenic Potato cv. IPB CP3

PCR analysis confirmed the integration of the *OsGERLP* gene into all six transgenic clones (CP3GERLP1–6). Amplification of the actin gene verified the quality of genomic DNA, while specific

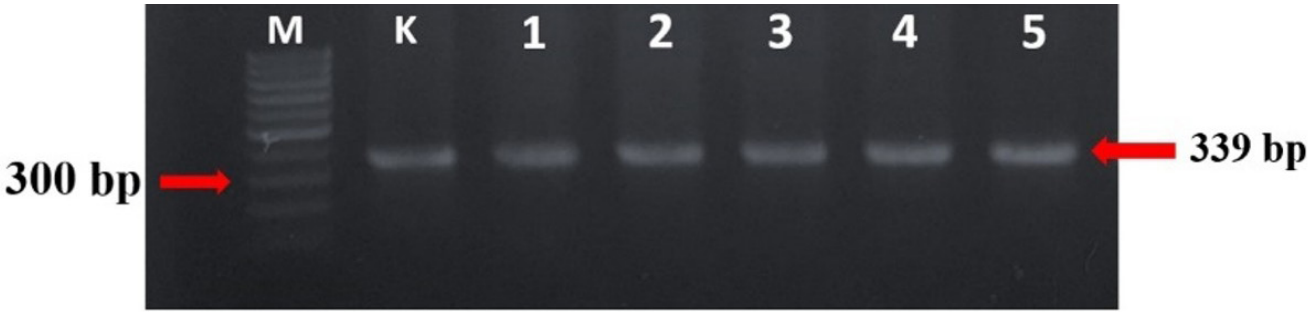


Figure 2. Electropherograms of colony PCR product by using *E. coli* TOP10 with pH35YG-*OsGERLP* plasmid. M = 100 bp marker, K = plasmid (positive control), 1-5 = colony 1-5

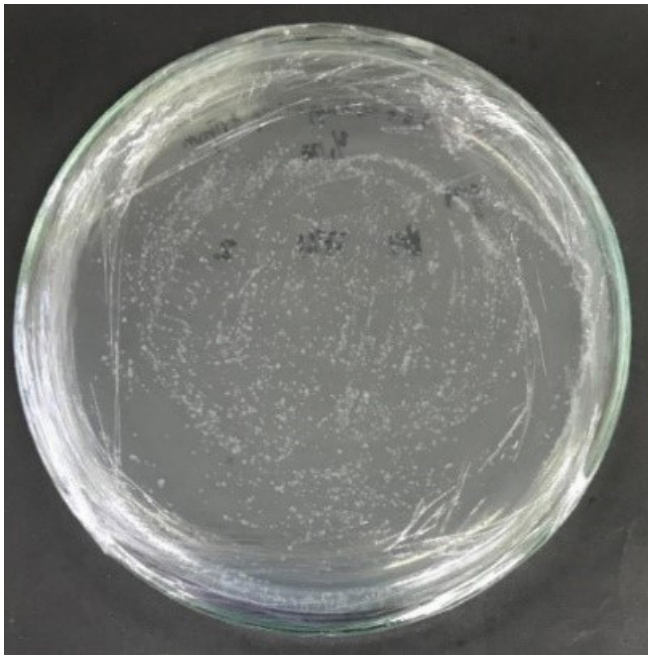

Figure 3. Bacterial colonies from TPM results were cultured on a selective medium

amplification of an approximately 1,500 bp fragment confirmed the presence of the *OsGERLP* transgene in each clone. No amplification was detected in the non-transgenic control (CP3NT) (Figures 6 and 7).

### 3.4. *In Vitro* Evaluation of Aluminum Stress Tolerance in Transgenic Potato Plants

Under aluminum stress (2 mM Al, pH 4), both transgenic and non-transgenic plants showed reduced growth compared to controls (Figure 8). However, transgenic clones, particularly CP3GERLP2, CP3GERLP3, and CP3GERLP4, showed significantly greater total root length than the non-transgenic control (Table 2). Differences in root number and shoot length were not statistically significant. These results indicate that *OsGERLP* expression primarily enhances root tolerance to Al toxicity, with limited effects on aboveground growth.

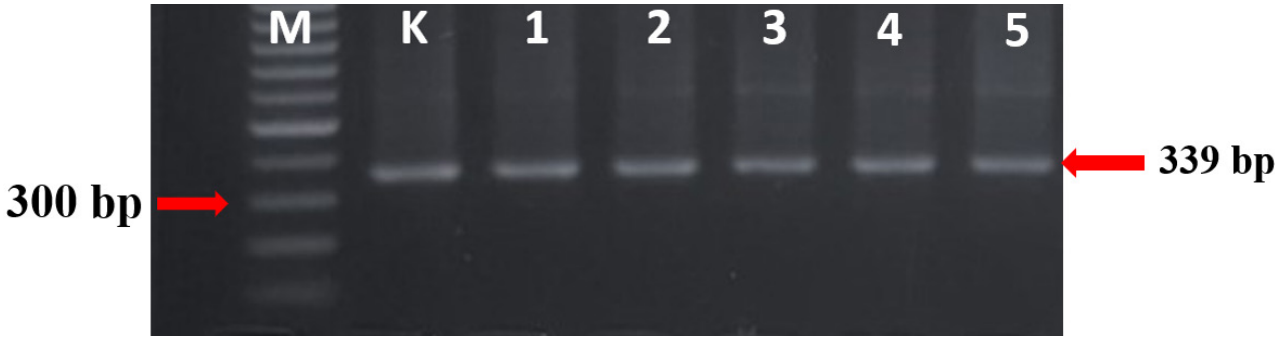


Figure 4. Electropherograms of the colony PCR product were done using A. tumefaciens LBA4404, which was transformed with pH35YG-*OSGERLP* plasmid. M = 100 bp marker, K = plasmid (positive control), 1-5 = colony 1-5

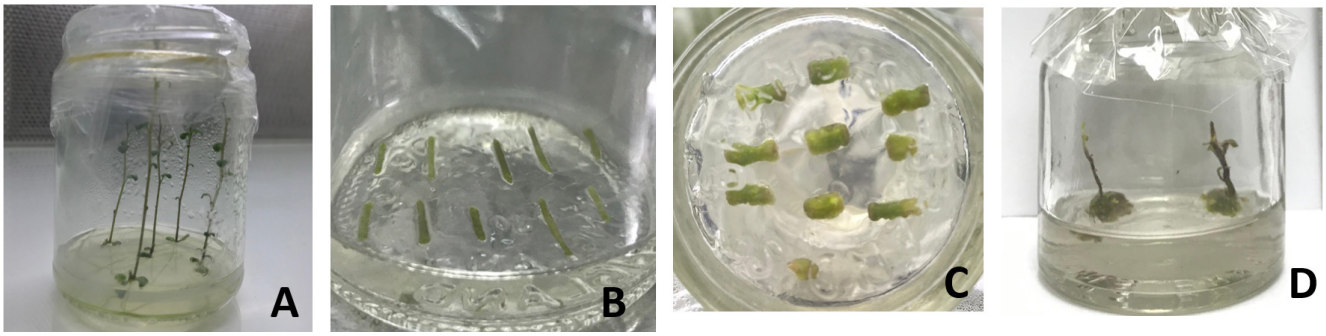


Figure 5. (A) Potato cv. IPB CP3 plants four weeks after planting, (B) explant of internode in the pre-culture medium, (C) callus formation 14 days after planting, (D) shoot regeneration on the selection medium

Table 1. The transformation and regeneration efficiency of the potato transgenic putative

| Experiment | Total explant | Hygromycin resistant | | Transformation efficiency (%) | Regeneration efficiency (%) |
|---|---|---|---|---|---|
| | | Total resistant callus | Total regenerated callus | | |
| 1 | 100 | 46 | 21 | 46.00 | 45.65 |
| 2 | 160 | 43 | 13 | 26.88 | 30.23 |
| 3 | 107 | 73 | 37 | 68.22 | 50.68 |
| Averages | 122 | 54 | 24 | 47.03 | 42.19 |
| St. Dev | - | - | - | 16.89 | 8.70 |

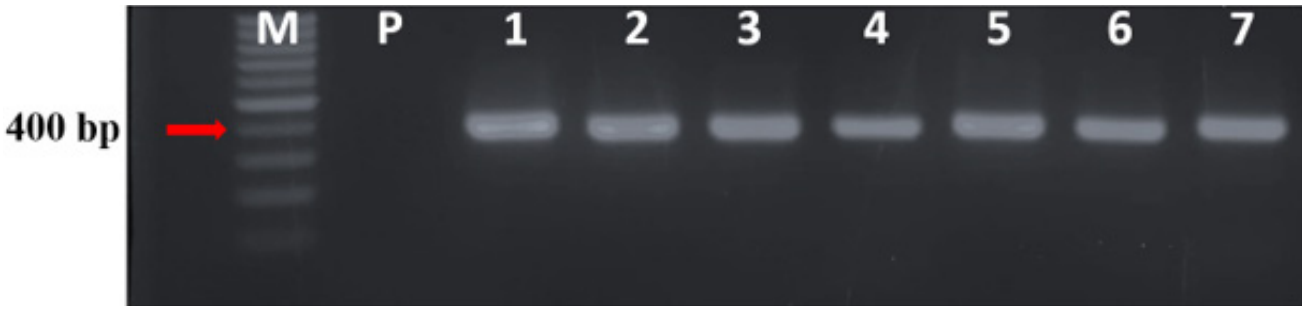


Figure 6. Electropherograms of the PCR actin gene product from the DNA of the potato cv. IPB CP3. M = 100 bp marker, P = Plasmid pH35YG-*OsGERLP*, 1 = CP3NT, 2-7 = CP3GERLP1-6

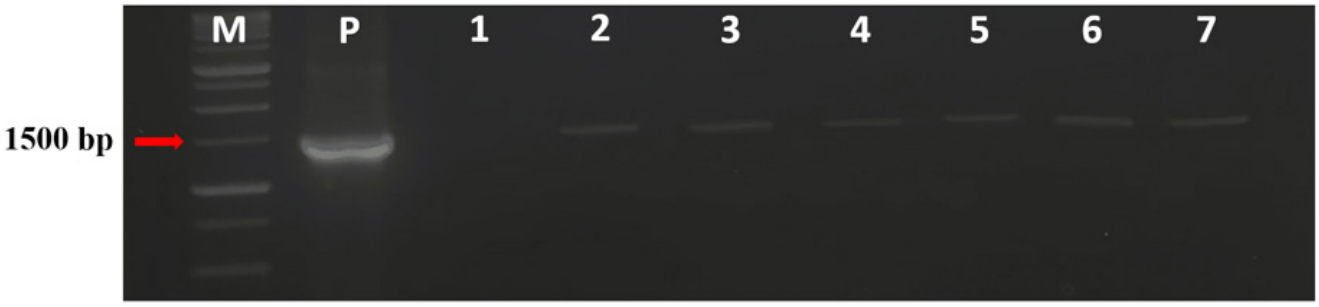


Figure 7. Electropherograms of the PCR *OsGERLP* gene product in the DNA of the potato cv. IPB CP3. M = 1 kb marker, P = Plasmid pH35YG-*OsGERLP*, 1 = CP3NT, 2-7 = CP3GERLP1-6

### 3.5. Gene Expression Analysis of *OsGERLP* in Transgenic Potato Plants

RNA integrity was evaluated prior to cDNA synthesis to ensure the reliability of downstream gene expression analysis. Agarose gel electrophoresis showed clear and distinct 18S and 5.8S rRNA bands, confirming the high quality of total RNA isolated from both transgenic and non-transgenic potato plants (Figure 9A). Total cDNA was then successfully synthesized from purified RNA. Amplification of the actin gene confirmed the integrity of the cDNA samples (Figure 9A). The *OsGERLP* gene was successfully detected in transgenic plants but not in CP3NT (Figure 9B). Quantitative RT-PCR showed that *OsGERLP* expression was upregulated in all transgenic lines under Al stress, with clones CP3GERLP2 and CP3GERLP3 exhibiting approximately twofold higher expression than CP3NT (Figure 10).

## 4. Discussion

The successful introduction of the *OsGERLP* gene into potato cv. IPB CP3, through *Agrobacterium*-mediated transformation, demonstrates the feasibility of extending aluminum (Al) tolerance mechanisms characterized in rice to other crop species. The introduction of the *OsGERLP* gene into the potato plant genome was carried out using four-week-old stem explants, mediated by *A. tumefaciens* carrying the pH35YG-*OsGERLP* plasmid (Figure 4A). Stem segment explants are widely used in potato plant transformation because they have better shoot regeneration effectiveness than leaves and petioles (Kamrani *et al.* 2015). The explants were grown in pre-culture media for 1 day, and slight swelling was observed (Figure 4B). Callus formation occurred 14

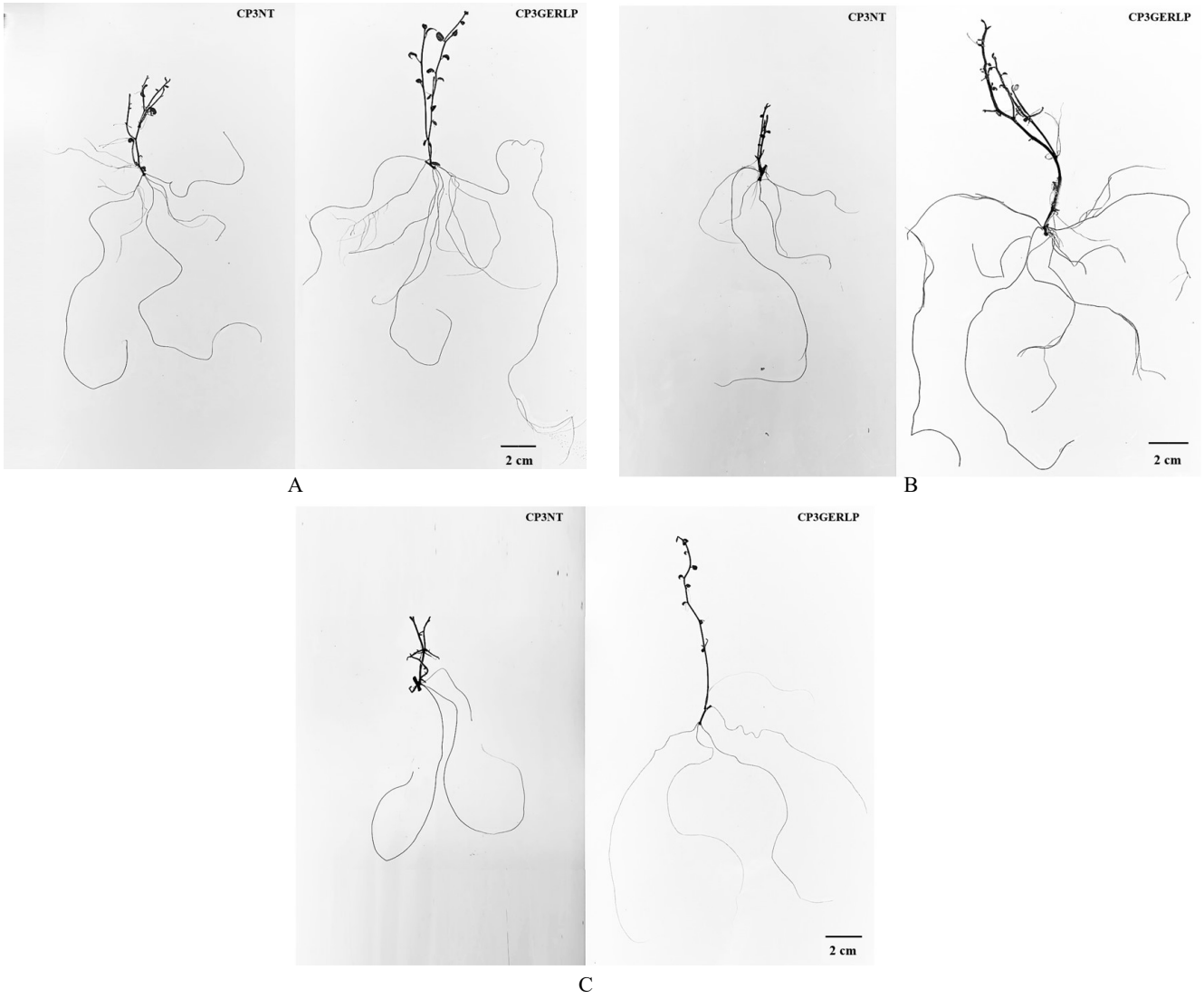


Figure 8. Aluminum stress tolerance assay of non-transgenic and transgenic potato cv. IPB CP3. (A) pH 5.8 medium, (B) pH 4 medium, (C) pH 4 + Al medium. CP3NT = non-transgenic potato cv. IPB CP3, CP3GERLP = transgenic potato cv. IPB CP3. Scale bar = 2 cm

Table 2. Comparison of root length, root number, and shoot length of non-transgenic and transgenic potato cv. IPB CP3 in different media

| Clones | Total root length (cm) | | | Root number | | | Shoot length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH 5.8 | pH 4 | pH 4 +Al | pH 5.8 | pH 4 | pH 4 +Al | pH 5.8 | pH 4 | pH 4 +Al |
| CP3NT | 57.10$^{ab}$ | 52.60$^{ab}$ | 22.70$^{d}$ | 3.67$^{a}$ | 4.77$^{ab}$ | 3.13$^{b}$ | 8.90$^{abc}$ | 9.20$^{abc}$ | 5.23$^{bc}$ |
| CP3GERLP1 | 54.93$^{ab}$ | 59.00$^{ab}$ | 35.23$^{cd}$ | 3.57$^{a}$ | 4.57$^{ab}$ | 3.23$^{b}$ | 12.13$^{a}$ | 11.13$^{a}$ | 4.67$^{c}$ |
| CP3GERLP2 | 59.13$^{ab}$ | 46.07$^{bc}$ | 51.50$^{ab}$ | 4.53$^{a}$ | 4.20$^{ab}$ | 4.70$^{ab}$ | 10.40$^{a}$ | 11.10$^{a}$ | 9.83$^{ab}$ |
| CP3GERLP3 | 52.93$^{ab}$ | 49.43$^{abc}$ | 57.57$^{ab}$ | 3.87$^{a}$ | 4.13$^{ab}$ | 5.00$^{a}$ | 10.77$^{a}$ | 8.03$^{abc}$ | 7.90$^{abc}$ |
| CP3GERLP4 | 66.50$^{a}$ | 66.40$^{a}$ | 43.80$^{bc}$ | 3.57$^{a}$ | 5.00$^{a}$ | 4.57$^{ab}$ | 10.17$^{a}$ | 12.13$^{a}$ | 7.47$^{abc}$ |

Numbers followed by the same letter indicate no significant difference in DMRT results (α=0.05). CP3NT: non-transgenic plants; CP3GERLP1-4: transgenic plants

days after inoculation. The formed callus has a compact, dark-green structure (Figure 4C). The callus was then grown on a selection medium containing 10 mg/L hygromycin. The resistant callus remains green, while the non-resistant callus turns brown. Callus resistant to hygromycin regenerated to form putative transgenic plantlets 6-10 weeks after planting (Figure 4D). Each of the putative transgenic plantlets, which represents one clone, was propagated for further analysis.

The transformation and regeneration efficiencies obtained in this study (47.03% and 42.19%, respectively) were higher than previously reported for IPB CP3

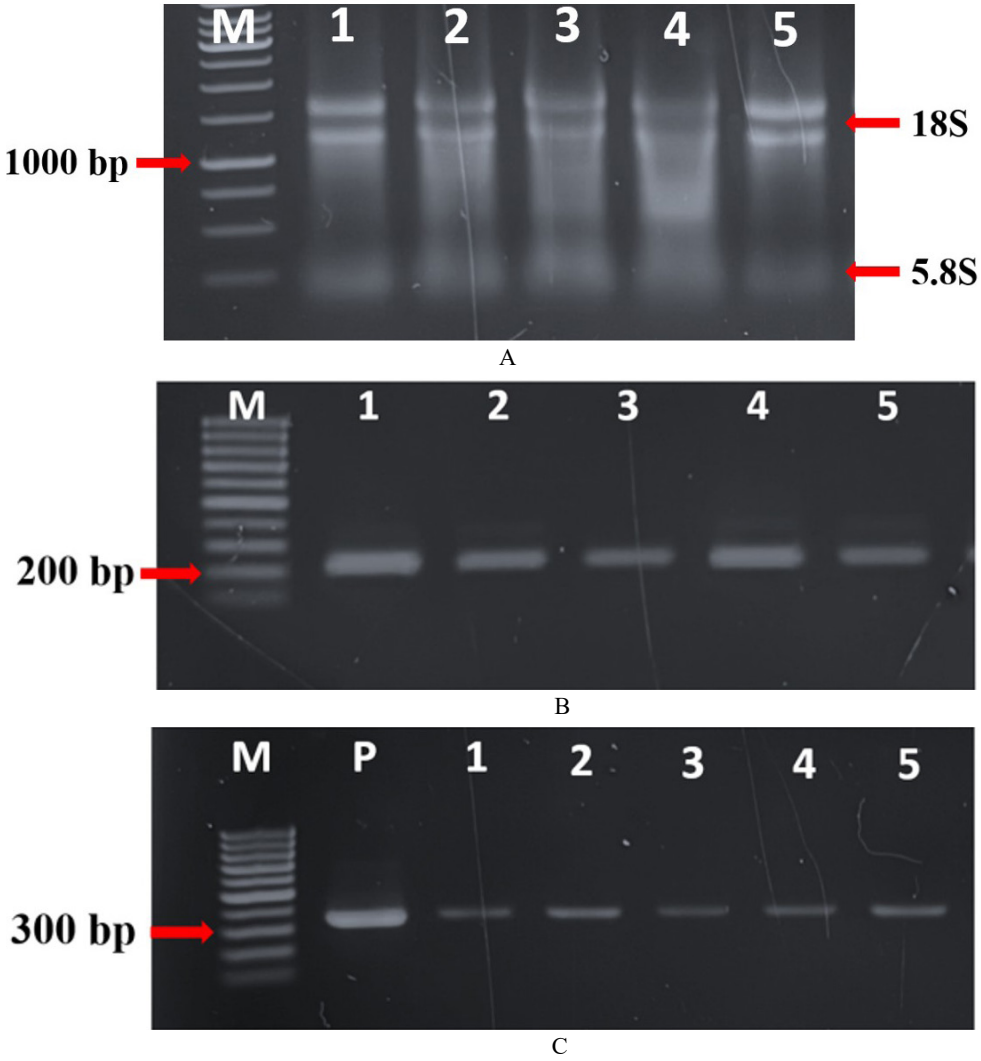


Figure 9. (A) Electropherograms of RNA total extraction, (B) PCR cDNA potato cv. IPB CP3 actin gene product, (C) *OsGERLP* gene. M = 1 kb and 100 bp marker, P = pH35YG-OsGERLP plasmid, 1 = CP3NT, 2-5 = CP3GERLP 1-4

transformations with other genes such as *FBPase/CIRan1* Wijayanti (2022). These differences may reflect optimization of explant type, culture conditions, and hormone concentrations used during transformation, which strongly influence plant regeneration (Wang *et al.* 2020). Different explant types, such as embryo axis, cotyledonary node, and axillary meristems, exhibit varied transformation efficiencies due to differences in their regenerative potential (Sadhu *et al.* 2022).

The success of genomic DNA isolation and the assessment of DNA integrity was evaluated through amplification of the conserved actin gene, commonly used as a reference or housekeeping gene due to its stable expression compared to other reference genes such as β-tubulin, elongation factor, initiating factor, ubiquitin, and glyceraldehyde 3-phosphate dehydrogenase (GADPH) in plant tissues (Nasreena *et al.* 2023). PCR amplification using actin-specific primers produced a 400 bp amplicon in all samples, including the six putative transgenic clones and the non-transgenic clone (CP3NT), confirming the quality of isolated DNA (Figure 5). As expected, the plasmid pH35YG-*OsGERLP*, used as a transformation vector, did not yield a band for the actin gene because it does not carry this endogenous plant gene.

PCR verification confirmed stable integration of *OsGERLP* into the potato genome. Similar molecular confirmation approaches have been applied in other transgenic studies for traits such as Al tolerance and disease resistance (Haverkort *et al.* 2016; Ratnasari *et al.* 2016; Farhanah *et al.* 2017). The confirmation of gene presence alone, however, does not fully ensure

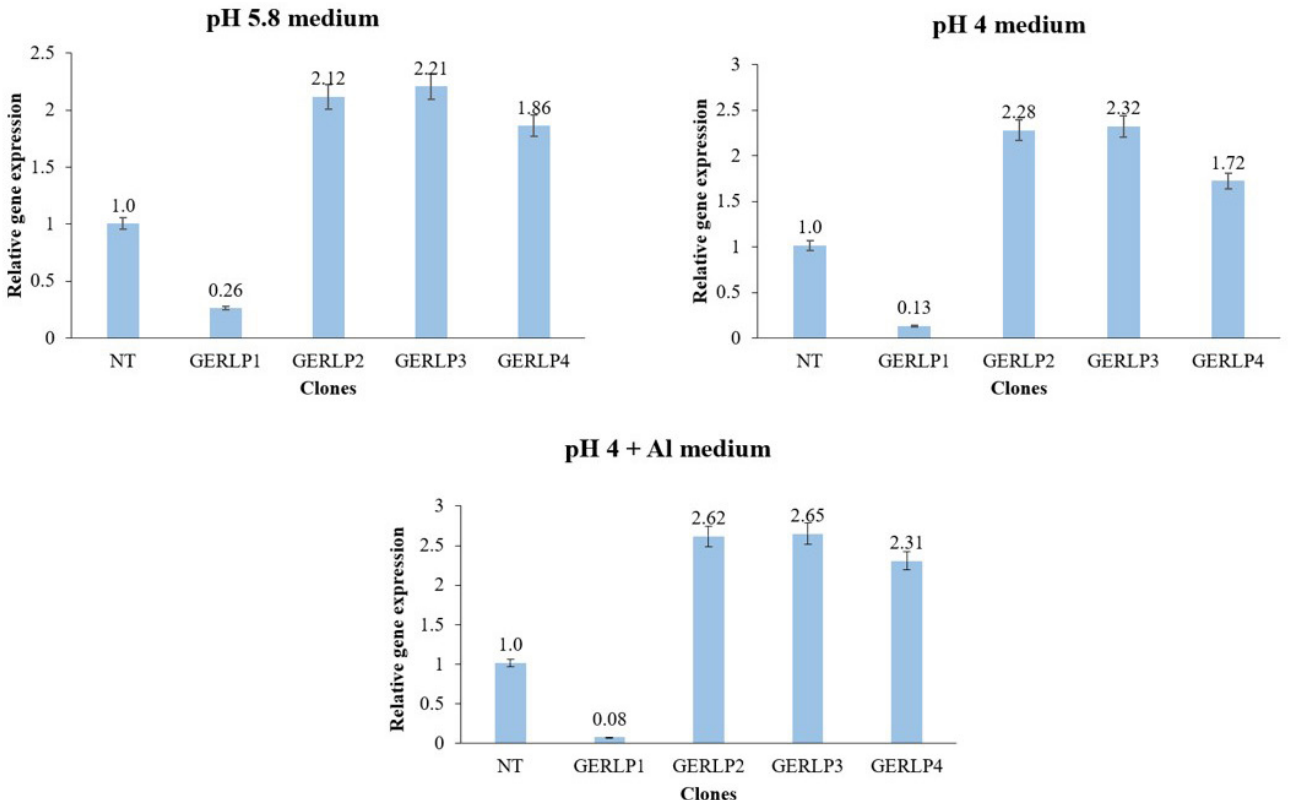


Figure 10. Relative gene expression of the *OsGERLP* in transgenic IPB CP3 potato plants in pH 5.8 media, pH 4 media, and pH 4 + Al media

stable transgene expression, as positional effects, copy number, or silencing phenomena can alter transgene behavior over time (Nagaya *et al.* 2005). Hence, subsequent expression analysis and phenotypic assays are crucial for establishing functional transgene performance.

The *in vitro* evaluation revealed that transgenic potato clones exhibited greater tolerance to Al stress than non-transgenic plants, as indicated by improved root elongation and number under acidic conditions. This finding aligns with previous studies showing that enhanced root growth is a key indicator of Al tolerance (Wei *et al.* 2021). Similarly, Siska *et al.* (2017) reported that transgenic rice displayed increased Al tolerance, as evidenced by root growth parameters, Al accumulation, membrane lipid peroxidation, and root tip cell structure. In this study, root growth responses were more pronounced than shoot responses, suggesting that *OsGERLP* activity may be primarily localized or functionally significant in root tissues. The limited shoot improvement under Al stress indicates that enhanced root tolerance does not necessarily translate into overall plant vigor, reflecting the complexity of Al toxicity effects on whole-plant physiology. These results are consistent with those of Wang *et al.* (2023), who demonstrated that Al accumulates predominantly in root apices, particularly in the transition zone, where it activates specific signaling cascades and transcriptional responses associated with Al tolerance.

Although root performance improved under Al stress, this did not translate into significant differences in shoot growth, suggesting that enhanced root tolerance alone may not fully compensate for the overall physiological impact of Al toxicity. Moreover, since this evaluation was conducted under controlled *in vitro* conditions, the response of transgenic plants may differ under greenhouse or field environments, where interactions with soil, microbes, and fluctuating pH levels are more complex. Variations among transgenic clones may also be influenced by transgene copy number or insertion sites, which could affect *OsGERLP* expression stability and lead to variable tolerance levels. Further evaluation under realistic growth conditions would therefore be necessary to confirm the functional role of *OsGERLP* in conferring Al stress tolerance.

This functional role of *OsGERLP* can be further contextualized by comparing *OsGERLP* with other well-characterized genes associated with aluminum

tolerance. Previous studies have identified MATE (e.g., *OsFRDL4*) and ALMT1 transporters that facilitate organic acid exudation to chelate $Al^{3+}$ ions, thereby reducing toxicity in the rhizosphere (Delhaize *et al.* 2004; Yokosho *et al.* 2011). Other genes, such as *ART1, STAR1*, and *STAR2*, regulate transcriptional and cell-wall modifications that enhance Al detoxification (Huang *et al.* 2009; Yamaji *et al.* 2009). In contrast, *OsGERLP* appears to function as an upstream regulatory component whose expression correlates with *ART1, STAR1, STAR2*, and *OsFRDL4*, suggesting its role in coordinating downstream tolerance pathways rather than directly mediating ion transport (Miftahudin *et al.* 2021). This distinction indicates that *OsGERLP* may act as a signaling or transcriptional regulator that integrates multiple Al-response mechanisms across tissues.

Under Al stress, clones CP3GERLP2, CP3GERLP3, and CP3GERLP4 demonstrated the highest tolerance levels relative to CP3GERLP1 and the non-transgenic control (CP3NT). Quantitative RT-PCR analysis further revealed that these clones exhibited higher *OsGERLP* transcript levels across all tested conditions, Al stress, low pH, and control media, with CP3GERLP2 and CP3GERLP3 expressing approximately twice as much as CP3NT. This positive correlation between *OsGERLP* expression and Al tolerance supports the hypothesis that transgene expression level is a major determinant of stress response efficiency. Expression variability among clones is a common occurrence in plant transformation studies and may result from chromosomal position effects, repeat sequences, or transgene copy number variation (Nagaya *et al.* 2005). Future transformation work could minimize such variability by using single-copy insertion screening, site-specific integration systems (e.g., CRISPR/Cas-mediated targeted insertion), or marker-free vector designs to ensure more uniform transgene expression.

In conclusion, the *OsGERLP* gene was successfully introduced and functionally expressed in potato cv. IPB CP3, conferring enhanced tolerance to Al stress as reflected by improved root traits and elevated transgene expression levels. The transformation and regeneration efficiencies achieved were relatively high (47.03% and 42.19%, respectively), indicating an effective genetic modification system. Further in vivo evaluations under greenhouse and field conditions are required to assess Al tolerance stability, tuber yield, and agronomic performance. Additionally, future studies should address biosafety, regulatory compliance, and public acceptance to ensure that the development of Al-tolerant transgenic potatoes aligns with sustainable and responsible agricultural practices.

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