Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Efficient Agrobacterium tumefaciens-mediated genetic transformation of Aloe vera

Alka Jangra
Guru Jambheshwar University of Science & Technology
Siddhant Chaturvedi
NABI: National Agri-Food Biotechnology Institute
Garima Sharma
NABI: National Agri-Food Biotechnology Institute
Sonia Sihag
Guru Jambheshwar University of Science & Technology
Siddharth Tiwari
NABI: National Agri-Food Biotechnology Institute
Vinod Chhokar (vinodchhokar@gmail.com)
Guru Jambheshwar University of Science & Technology https://orcid.org/0000-0003-1638-7767

Research Article

Keywords: Aloe vera, Agrobacterium, GUS, transformation, secondary metabolites

Posted Date: September 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1968269/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Genetic transformation of plants has emerged as a core research tool for functional characterization of genes and cultivar improvement in the field of plant biology. Effectually high transformation efficiency is a prerequisite for accomplishing the stated targets. *Aloe vera*, a tropical cactus plant in distinction to the family Liliaceae, has seized a long history of secular acceptance as a therapeutic agent and is reasonably the most popular herbal remedy these days. However, the genetic transformation of *Aloe vera* is relatively low and seldom reported formerly. This study aims to optimize *Agrobacterium*-mediated transformation in *Aloe vera* by refining several parameters like explant selection, the extent of explant injury during infection, *Agrobacterium* concentration, co-cultivation pH, and duration and desiccation of plant tissue to improve the infection efficiency. The results showed that an infection efficiency of about 92% was attained by suspending the *Agrobacterium* cells at a concentration of OD_{600}: 0.4 in co-cultivation media at pH-5.6 to infect the shoot base of aloe by desiccation followed by 3 days of co-cultivation. Desiccation during infection had proved to enhance T-DNA delivery, whereas a higher extent of explant injury was found to curtail the infection efficiency. Thereupon, GUS Histochemical assay, PCR analysis, and Southern blotting were used to substantiate the authentication of positive transgenic plants and analyze the copy number of the *nptII* gene in the *Aloe vera* genome. Transformation efficiency of over 7% was obtained, which is higher than the previous reports. This study bestows an improved *Agrobacterium*-mediated transformation protocol based on desiccation in *Aloe vera,* which might help in facilitating various gene expression studies and regulation in the aloe plant, eventually allowing the modification of aloe species in an effective medicinal manner.

Key Message

The present study deliberates various factors affecting the *Agrobacterium*-mediated transformation of *Aloe vera* and introduces an efficient method to facilitate the bioengineering of aloe species in an effective salutary way.

1. Introduction

The primeval written affirmation for the utilization of plants in medication can be found on Sumerian clay slabs from Nagpur, India estimated to be 5000 years old. Some other written shreds of evidence were also reported in the Greek, Egypt, and Mesopotamia civilizations (Süntar 2020). One such medicinally important plant used since the former age is *Aloe vera* (*Aloe barbadensis* Miller), a key candidate for therapeutic research studies in the new century. The expansion in the world of aloe emerged during the 90s owning to its increased global demand by consumers for a healthy lifestyle The pertinence of *Aloe vera* is not limited to remedial purposes only but spread over cosmetic, food, and pharmaceutical industries, generating the world landscape of the million-dollar market around its products, which was around $1.60 billion in 2018 (Martinez-Burgos et al. 2022). Presently, various industrial attempts are bustling to explore the immense biological potential of this miracle plant. Until now, the literature reports various biochemical, transcriptomics, and metabolomics analysis of the aloe plant and its application in

curing various health disorders such as asthma, diabetes, skin problem, microbial infection, etc. (Choudhri et al. 2018; Jangra et al. 2022). These medicinal properties of the plant are attributed to the active secondary metabolites it retains; *Aloe vera* possesses more than 75 active compounds including various efficacious secondary metabolites like polysaccharides, anthraquinones, alkylbenzene, dehydroabietic acid derivatives, lectin, lignin, saponins, etc. (Sharon and Suvarna 2017). Some of the metabolites, particularly contributing to its prime medicinal properties, are found in relatively minor concentrations as low as < 1% dry weight (Bechtold and Bouchez 1995). At the same time, a few of them have been reported to be responsible for various adverse conditions (Grimminger and Witthohn 1993; Brusick and Mengs 1997; Jangra et al. 2022).

Metabolic engineering comes in a role where the concentration of some medicinally important metabolites needs to be enhanced, or the proportion of some toxic metabolites needs to be controlled. To accomplish this objective of metabolic engineering, there is a need to bring about some modifications at the molecular level in the genome, which necessitate some standard genetic transformation techniques. Until now only a few reports are there carrying the objective of genetic transformation in *Aloe vera* that too with very low transformation rate creating a bottleneck in the genetic modification of this plant. *Agrobacterium tumefaciens*-mediated transformation has proven to be a very efficient technique with various monocot plant species, ranging from cereal crops to ornamental plants (Cheng et al. 2004). In this transformation protocol, *Agrobacterium tumefaciens* is transformed with the desired genetic construct (T-DNA). This customized *Agrobacterium* strain is then used to infect the plant and transfer the T-DNA into the plant. One of the significant advantages of using *Agrobacterium* for transformation is that even a small number of copies (one or two) of relatively large T-DNA segments (> 10kb) with definite ends can be integrated into the plant genome with minor rearrangement, producing high-quality transgenic plants (Ishida et al. 2007).

The study focuses on optimizing different parameters and conditions of *Agrobacterium*-mediated transformation in *Aloe vera* to obtain higher transformation efficiency. The highlighted parameters of the study can be started with the selection of explants; the stem of the plant was found to be the most suitable portion of explants as it possesses higher regeneration potential than other plant parts. The second factor for consideration was the extent of injury caused to the plant. Physical wounding of the explant is usually rendered to intensify the transformation efficiency of the plant, where the wound site serves as an entrance for the bacterium. Furthermore, wounding of the explant also results in the discharge of some phenolic substances necessary to activate the *vir* gene (Karami et al. 2009). In the present study, it was observed that more the injury caused to the leaf resulted in the release of more aloe gel, which inhibited the growth of the *Agrobacterium* strain. The literature also found that in an antimicrobial assay, the aloe extract resulted in an 18mm zone of inhibition against *Agrobacterium tumefaciens* (Raineri et al. 1990). Other factors studied in the trials were the pH range of co-cultivation media and the duration for which the plant and *Agrobacterium* strain were kept in the co-cultivation media. The results observed with different combinations of parameters are discussed in the paper further, suggesting a protocol with higher transformation efficiency in *Aloe vera*. The study was also validated with the help of techniques like PCR and southern blotting.

2. Materials And Methods

2.1 Experimental design

In this study, various significant factors affecting the *Agrobacterium*-mediated transformation efficiency, including explant selection and regeneration potential (shoot multiplication), the extent of injury in explant, and antimicrobial activity of aloe gel towards *Agrobacterium tumefaciens*, the concentration of *Agrobacterium* culture for infection, and co-cultivation pH and duration were investigated. These parameters were varied and set to optimize the best possible conditions and obtain souped-up transformation efficiency.

2.2 Vector and *Agrobacterium* strain

Plant transformation binary vector pCAMBIA 1301 containing hygromycin B resistance gene for plant selection and the *gusA* reporter gene (with an intron) was used for plant transformation with AGL1 *Agrobacterium* strain having RecA background and rifampicin resistance for selection.

2.3 Plant material and explant preparation

Aloe vera was used as a transient receptor in the present study for practicing *Agrobacterium*-mediated transformation. Aloe plants were raised in the nursery of Guru Jambheshwar University of Science and Technology, Hisar (India). Young and healthy plants comprising prominent stems were harvested and surface sterilized. The stem portion of the plants was sectioned separately into 1 cm × 1 cm pieces and sterilized in the following sequence: Tween20 for 20 min, 0.1% bavistin for 20 min, 0.1% mercuric chloride for 5 min, 70% ethanol for 30 sec, followed by 2–3 times thorough wash with sterilized distilled water after every treatment. Sterilized explants were cultured on MS basal medium for 4–5 days at 26 ^{0}C under a 16/8 h photoperiod and white fluorescent tube light with an intensity of 200 µmol m^{-2} s^{-1}fitted on culture racks.

2.4 Media composition and *Agrobacterium* culture preparation

Luria broth supplemented with 50 mg/L kanamycin and 50 mg/L rifampicin was used to culture a single colony of the *Agrobacterium* (AGL1/pCAMBIA 1301) until the OD_{600}: 0.6–0.8. The culture was centrifuged, and the cells were suspended in liquid co-cultivation media (CCM) at different OD_{600}viz 0.4, 0.6, and 0.8 and incubated in the shaker incubator for about 30–40 min at 28^{0}C and 150 rpm, to activate the *vir* genes. Liquid CCM comprises 1/10 MS supplemented with 200 µmol/L acetosyringone, 1g/L PVP, and 1% glucose. The use of PVP (polyvinylpyrrolidone) in media decreases the phenolic secretion from the explant. MS media augmented with 4.0 mg/L BAP, 1.0 mg/L IAA, 1g/L PVP, 250 µmol/L cefotaxime, 250 µmol/L augmentin and 25.0 mg/L hygromycin were used to select for the transformants. Rooting was induced on ½ MS containing 1.5 mg/L NAA and 1g/L PVP. All the tissue culture media (except liquid CCM and washing media) were solidified with 0.8% agar and autoclaved after a prior adjustment of pH.

2.5 *Agrobacterium* infection and regeneration of transgenic plants

Pre-cultured explants of *Aloe vera* were sectioned with a sterile scalpel under sterile conditions. Different aloe explants viz. leaf, stem, and root were collected and injured at the stem base at various extents with the help of a sterile needle and immersed in the liquid CCM containing *Agrobacterium* cell suspension. The explants were processed in two batches; Set I and Set II where Set I was subjected to desiccation for 20 mins under sterile conditions and set II was directly transferred to the shaking incubator. The *Agrobacterium* concoction in both sets was shaken for 1 hour at 28^0C and 70 rpm to allow the plants to concede adeptly with the *Agrobacterium* cells. Explants were blot dried, transferred to solid CCM plates on sterile filter paper (5–7 explants per plate), and co-cultured in the dark at 28^0C for 3–5 d.

The explants were co-cultured at different pH values viz. 5.0, 5.4, 5.6, and 5.8 in CCM. After the co-cultivation, the explants were washed with washing media (liquid MS media augmented with 500 µmol/L cefotaxime and 250 µmol/L augmentin) and incubated on MS media fortified with 4.0 mg/L BAP, 1.0 mg/L IAA, 250 µmol/L cefotaxime and 250 µmol/L augmentin (Pre-selective media). The explants were acclimatized under these conditions for 10–15 d or until they start responding. After the instigation of regeneration, explants were transferred onto the selection media (SM-I). A control set of explants (non-transformed) was also cultured along with the experimental sets to optimize the selective concentration of hygromycin sufficient enough to select for the transformants. After 21–30 days of culturing of SM-I, regenerated plants with new vegetative leaves were selected and incubated on SM-II for another 21–30 days. Explants pullulated with fresh shoots were tabbed and excised into individual shoot segments. These newly grown shoots were sub-cultured on SM-III for 21–30 days. The independent shoots that regenerated on SM-III and attained good health and height were transferred to the rooting medium (RM) for 20–25 days. The rooted plantlets were transferred to a sterile soil conditioning mixture (soilrite mix) in plastic containers. The containers were covered with perforated cling film and incubated in the plant growth chamber for 10 days to acclimatize the plantlets in soilrite. The plantlets were irrigated with Hoagland solution. The acclimatized plantlets were then transplanted into vermiculite in plastic pots and transferred to the greenhouse for normalized vegetative growth and multiplication.

2.6 Identification of positive transformants

The following three methods were used to confirm the positive aloe transformants.

2.6.1 GUS Histochemical assay

β-Glucuronidase Reporter Gene Staining Kit from Sigma-Aldrich was used to analyze β-glucuronidase (*E.coli* GUS gene) expression in transformed plants. Infected explants were collected after co-cultivation, washed with sterile water, and submerged in X-GlcA solution. The explants were desiccated for 2 min in a vacuum desiccator to facilitate the uptake of staining solution by the plant tissue and incubated in the dark at 37^0C for 24 hours, followed by destaining in 70% ethanol 2–3 times to remove the chlorophyll

content. Positive transformants were analyzed for the presence of GUS stain (blue color) and viewed under the microscope.

2.6.2 PCR assay

Total genomic DNA was isolated from the aloe transformants using the CTAB method with some modifications. Promega ready to use PCR master mix containing reaction buffer, $MgCl_2$, dNTPs, and Taq DNA polymerase at optimum concentration was used to amplify the DNA at standard conditions. PCR amplification was carried out using *nptII* gene-specific primers in a thermocycler with the following program: one cycle at 94^0C for 5 min (initial denaturation), 35 cycles at 94^0C for 30 s (denaturation), 55^0C for 30 s (annealing), 72^0C for 45 s (extension), 72^0C for 5 min (final extension). The sequence of forward and reverse primers used for PCR amplification was 5'CAGAAGAACTCGTCAAGAAGGCGATAGA3'and5'ATGGGGATTGAACAAGATGGATTGC3' respectively. The amplified PCR was visualized using agarose gel electrophoresis in 1% (w/v) agarose gel containing ethidium bromide and viewed with a UV gel documentation system.

2.6.3 Southern blotting

Two representatives out of the seven plants, which were accorded positive with GUS assay and PCR amplification were analyzed by Southern hybridization. Genomic DNA (10 µg) of these putative positive plants was digested overnight (14–16 h) with *HindIII* (Cat# R0104L, New England Biolabs, USA) at 37°C and separated by electrophoresis in 0.8% agarose gel. The digested DNA was transferred to the Bio Bond-plus nylon membrane (Sigma, USA). After pretreatment, the membrane was hybridized with biotin labelled *npt* probe for 24 h, and the hybridized membrane was conjugated with biotin-linked primary antibody. The probe was prepared by amplifying a part of *nptII* with PCR. Southern blotting analysis was performed using the ECL labeling system.

3. Results

3.1 Effect of explant selection and regeneration potential on transformation efficiency in *Aloe vera*

To optimize the maximum transformation efficiency, different explants from the aloe plant (leaf, stem, and root) were cultured in the shooting media supplemented with a range of auxins and cytokinins. This study found that the aloe leaf and root showed minimal regeneration capability (1.33% and 4.6%, respectively), whereas the stem possessed maximum regeneration potential (91.33%) in MS media augmented with 4.0 mg/L BAP, 1.0 mg/L IAA, and 1g/L PVP. After four weeks of explant (stem) inoculation, numerous shoots were observed at 26 ^{0}C under a 16/8 h photoperiod of white fluorescent light. These results suggested that aloe stem acted as an adequate explant choice for genetic transformation. It withholds wondrous regeneration capability at its base and can beget many new shoots into existence.

3.2 Optimization of physical parameters during infection and co-culturing

An efficient method of *Agrobacterium*-mediated genetic transformation of *Aloe vera* was optimized by varying different physical parameters during the infection and co-cultivation period. Counting the most effective concentration of *Agrobacterium* for infection, the primary culture of *Agrobacterium* was collected at an OD_{600}-0.8, centrifuged, and the cells were suspended in liquid CCM at three different OD_{600} (0.4, 0.6, and 0.8) for the infection of aloe explants. It was found that OD_{600}-0.4 resulted in the maximum infection efficiency compared to OD_{600}-0.4 and 0.8. In our initial small-scale experiments, some other parameters, such as the pH of CCM, extent of tissue injury during infection, co-culture time between 3 and 5 d, and effect of desiccation, were also evaluated. The results showed that co-cultivation of aloe explants with *Agrobacterium* in CCM (in broth and plates) at pH-5.6 aided in maximum infection efficiency (73.33%) compared to pH-5.0 (10.66%) and 5.8 (47.0%).

In the case of tissue injury, the minimal injury was found enough to elevate the infection efficiency as compared to the uninjured, medium, and highly injured explants during infection. An infection efficiency of about 79.66% infection efficiency was obtained with the explants subjected to minimal injury, whereas 21.34% and 38.01% efficiency was attained in case of high and moderated injury levels, respectively. With respect to desiccation, it was found to increase the efficiency of T-DNA delivery in Set I (91.33%), showing *GUS* gene expression in a higher number of explants whereas Set II experienced only 28.6% infection efficiency. The survival rate of transformants after co-cultivation was also enhanced by 15% in desiccation-assisted transformation. Succeeding the *Agrobacterium* infection, the explants were co-cultured on CCM plates on sterile Whatman filter paper for a period of 3–5 d in the dark at 28^0C. A competent transformation score was observed after 3d of co-culturing with 61.35% efficiency. In contrast, 4d and 5d co-culturing resulted in overgrowth of *Agrobacterium* and tissue necrosis in aloe explants with 48.34% and 32.32% efficiency, respectively. Figure 4 represents the outline of the optimized protocol for the *Agrobacterium*-mediated genetic transformation in *Aloe vera.*

3.3 Gus Histochemical assay

Agrobacterium strain AgII containing pCAMBIA 1301 vector was used to infect fresh aloe explants to validate the transient expression of the *GUS* gene. Several distinct blue spots were observed on the explants subjected to GUS histochemical staining of explants after co-cultivation with *Agrobacterium* (Fig. 5). This vector possesses an intron in the *GUS* gene which ensure its active expression in transformed tissue only, eliminating the chance of false positive. Different aloe explants were assessed on the behalf of their regeneration potential and transformation efficiency under various physical parameters through GUS histochemical staining. A transient expression rate of 91.3% and 28.6% was found in Set I and Set II, respectively. Aloe stem (shoot base) exhibits maximum regeneration potential as well as GUS expression. Hence, the detailed experiment was executed by taking aloe stem as an explant for optimizing various conditions and discerning an efficient genetic transformation method for *Aloe vera.*

3.4 Rooting of shoots and hardening of plantlets

The independent shoots regenerated after the selection process were transferred onto the rooting media supplemented with ½ MS, 1.5 mg/L NAA, 1g/L PVP, 250 µmol/L cefotaxime, 250 µmol/L augmentin, and 25.0 mg/L hygromycin. A uniform network or roots was observed after 15–20 days of incubation on rooting media. The rooted shoots were transferred to potted soil conditioning mixture for 10 days and then into vermiculite and incubated in the greenhouse for growth and multiplication (Fig. 6).

3.5 Molecular analysis of transgenic plants

A total of 100 explants were subjected to *Agrobacterium*-mediated transformation, out of which 7 explants were found to exhibit the resistance and shoot multiplication after a 3-month selection procedure comprising 4–5 rounds of selection in selection media with increasing concentration of hygromycin. These hygromycin-resistant plants were confirmed to be putative transgenic by PCR analysis for the *nptII* gene. Results showed the amplification of 800bp fragments from the positive plant DNA (Fig. 7). In contrast, no amplification was observed in control plant DNA with the concerned primer set at the same condition, proposing a successful *Agrobacterium*-mediated transformation in *Aloe vera.* Further, southern hybridization was used to check the copy number of transgene in the transgenic line. Two out of seven putative positive plants were processed for southern blot analysis which showed a single band speculating the integration of a single copy of transgene (*nptII* gene) in the selected transgenic plant genome. Moreover, no hybridization was observed in the control (non-transgenic) plant (Fig. 8).

3.6 Evaluation of transformation efficiency

In the present study, we tried to improvise the transformation efficiency in *Aloe vera* by improving the regeneration and infection efficiency. From 100 explants taken initially, 92 explants showed transient expression of the *GUS* gene in the GUS histochemical assay, out of which 10 plants survived after the hygromycin ($25mg/l^{-1}$) selection process. Among them, 7 plants were found positive by PCR assay with gene-specific primers. The present study accorded 92% infection efficiency and 7% transformation efficiency. The transformants were phenotypically identical to the non-transformed plants.

4. Discussion

Plants have served as an imperative source of medication for many years, and almost 80% of the world's community still gamble on the natural means of remedies for their healthcare. *Aloe vera* enjoys a tremendous global demand and acceptance due to its pervasive medicinal, nutraceutical, and other uses (Rajeswari et al. 2012). Several studies evincing the potential beneficial effect of aloe plants are sufficient enough to warrant the need for the research ensuring the development of improved traits of plants. The genetic transformation of plants is a vital tool for elucidating the gene function and improvising the existing traits via genome manipulation. Genetic transformation via *Agrobacterium* has proved to be a dominant technology in producing genetically modified plants (Tzfira and Citovsky 2006). This study

investigated different parts of aloe plants, viz. stem, leaf, and root, for their regeneration potential where aloe leaf and root exhibited minimal regeneration (1.33% and 4.6%, respectively). Aloe stem showed maximum regeneration of 91.33%. Any conflict in the current findings with the previous studies could be a consequence of variation in experimental conditions and genotype used for the experiment.

Another factor considered in this study was the extent of explant injury during infection. In the current study, it was observed that more wounding reduces the transformation efficiency, which might be attributed to the release of more antibacterial compounds from the injured explant, eventually tampering with the agro infection. It is widely accepted that appropriate wound response in plants leads to the generation of transformation competent cells adjacent to the wound (Potrykus 1991). Leaf and root extract of *Aloe vera* showed potent antibacterial activity against *Agrobacterium tumefaciens* and exhibited an inhibition zone of 18mm and 17.5mm, respectively (Danish et al. 2020). The concentration of *Agrobacterium* cells used for infection was also found to play an essential role in the accomplishment of boon transformation efficiency. *Agrobacterium* culture grown up to an OD_{600}:0.8 and resuspended in liquid CCM at OD_{600}:0.4 followed by 3d co-cultivation in the dark aided in the fruitfulness of the experiment and abetted in avoiding *Agrobacterium* contamination during the selection of transformants. In contrast, a higher concentration of *Agrobacterium* during infection (OD_{600}:0.6 and 0.8) and a long duration of co-cultivation resulted in a decreased survival rate of explants and invasive agro growth during the selection procedure.

Agrobacterium tumefaciens necessitates the activation of *vir* genes in Ti-plasmid (tumor-inducing) under acidic conditions (pH-5.5) to transfer the T-DNA into the plant. The pH of the CCM was varied with a range of pH (5.0, 5.6, and 5.8) during the co-cultivation of explants with *Agrobacterium*, and maximum transformants were obtained at pH 5.6, which is quite near to the favorable condition of *Agrobacterium*-plant interaction. Previous studies revealed that acidic conditions elicit responses in two classes, conserved and general adaptive response and meticulous signaling response associated with plant-agro interaction (Yuan et al. 2008) where conserved response regulates the induction of genes involved in the uptake system, cellular repair, and cell envelope, and the repression of genes associated with the cell metabolism, chemotaxis, and electron transfer. Thus, intending to conceive an *Agrobacterium*-plant interaction, there is a need to mimic the acidic environment of the rhizosphere as the *vir* region is triggered only under acidic conditions (Yuan et al. 2007); acidic conditions can only induce the expression of various virulence factors.

Desiccation of explants during *Agrobacterium*-mediated transformation was also reported to increase stable transformation efficiency and enhance T-DNA delivery in wheat. Desiccation of explants under appropriate conditions could greatly adorn plant cell recovery post-transformation. In the present study, the explants were processed in the two sets before co-cultivation, one with desiccation and the other without desiccation. Desiccation-assisted transformation accorded 92% infection efficiency with a 15% increment in the survival rate of transformants compared to the control where the infection efficiency is 60%, followed by a low survival rate. Multiple stable transformed plants were obtained from desiccation-assisted transformation; however, one plant was obtained from the control set of plants. A survival rate of

100% after co-cultivation in desiccation-assisted transformation compared to the control was reported previously, where the survival rate is 60–80%. In addition, minor browning was observed in desiccated tissue than in the non-desiccated (Cheng et al. 2003).

Aloe barbadensis Miller is considered to be medicinally most potent and therefore is most popular. Thus far, a few reports are concerned about genetic transformation, particularly *Agrobacterium*-mediated transformation in *Aloe vera*. To the best of our knowledge, this is the first most efficient *Agrobacterium*-mediated genetic transformation in the miracle plant *Aloe vera*. The previous report stated high transient expression of the GUS gene but very low transformation efficiency (0.9%) (He et al. 2007). In contrast, the present study proclaims an adequate rate of GUS transient expression showing 92% infection efficiency with an increased rate of transformation efficiency (7%). This study brings forth an ungraded protocol for *Agrobacterium*-mediated genetic transformation in *Aloe vera* that could be utilized for improving the nutritional and therapeutic aura of the plant for human welfare.

Declarations

Acknowledgment

The authors would like to thank Guru Jambheshwar University of Science and Technology, Hisar, Haryana, for providing the necessary facilities and a suitable environment for executing the present study. AJ would like to thank all the co-authors for their assistance throughout all aspects of this study. AJ, SC, GS, and SS acknowledge the Council of Scientific and Industrial Research (CSIR), Department of Biotechnology (DBT), Department of Science and Technology (DST), and University Grant Commission (UGC) for the fellowship.

Author Contributions

Alka Jangra conceptualized the article and designed the experiments. Alka Jangra and Siddhant Chaturvedi performed the experiments and prepared the original draft. Garima Sharma and Sonia Sihag contributed to writing, reviewing, and editing the manuscript. Siddharth Tiwari contributed to data interpretation and curation. Vinod Chhokar contributed to the proofreading and design of the work. All authors have read and approved the final manuscript.

Data availability

All data generated during this study are included in this article.

Statements & Declaration

Funding

Financial support for this work was provided by the Council of Scientific and Industrial Research (CSIR), New Delhi, India, in terms of fellowship.

Competing Interest

The authors have no financial and non-financial conflict of interest to disclose.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

Not applicable.

References

1. Bechtold N, Bouchez D (1995) In planta Agrobacterium-mediated transformation of adult Arabidopsis thaliana plants by vacuum infiltration. Gene transfer to plants. Springer, pp 19–23
2. Brusick D, Mengs U (1997) Assessment of the genotoxic risk from laxative senna products. Environ Mol Mutagen 29:1–9
3. Cheng M, Hu T, Layton J et al (2003) Desiccation of plant tissues post-Agrobacterium infection enhances T-DNA delivery and increases stable transformation efficiency in wheat. Vitr Cell Dev Biol 39:595–604
4. Cheng M, Lowe BA, Spencer TM et al (2004) Factors influencing Agrobacterium-mediated transformation of monocotyledonous species. Vitr Cell Dev Biol 40:31–45
5. Choudhri P, Rani M, Sangwan RS et al (2018) De novo sequencing, assembly and characterisation of Aloe vera transcriptome and analysis of expression profiles of genes related to saponin and anthraquinone metabolism. BMC Genomics 19:1–21. https://doi.org/10.1186/s12864-018-4819-2
6. Danish P, Ali Q, Hafeez MM, Malik A (2020) Antifungal and antibacterial activity of aloe vera plant extract. Biol Clin Sci Res J 2020
7. Grimminger W, Witthohn K (1993) Analytics of Senna Drugs with Regard to the Toxicologicai Discussion of Anthranoids. Pharmacology 47:98–109
8. He C, Zhang J, Chen J et al (2007) Genetic transformation of Aloe barbadensis Miller by Agrobacterium tumefaciens. J Genet Genomics 34:1053–1060
9. Ishida Y, Hiei Y, Komari T (2007) Agrobacterium-mediated transformation of maize. Nat Protoc 2:1614–1621
10. Jangra A, Sharma G, Sihag S, Chhokar V (2022) The dark side of miracle plant-Aloe vera: a review.Mol Biol Rep1–12
11. Karami O, Esna-Ashari M, Kurdistani GK, Aghavaisi B (2009) Agrobacterium-mediated genetic transformation of plants: the role of host. Biol Plant 53:201–212
12. Martinez-Burgos WJ, Serra JL, MarsigliaF RM et al (2022) Aloe vera: From ancient knowledge to the patent and innovation landscape-A review.South African J Bot

13. Potrykus I (1991) Gene transfer to plants: assessment of published approaches and results. Annu Rev Plant Biol 42:205–225
14. Raineri DM, Bottino P, Gordon MP, Nester EW (1990) Agrobacterium–mediated transformation of rice (Oryza sativa L.). Bio/Technology 8:33–38
15. Rajeswari R, Umadevi M, Rahale CS et al (2012) Aloe vera: the miracle plant its medicinal and traditional uses in India. J Pharmacogn Phytochem 1:118–124
16. Sharon K, Suvarna S (2017) Cloning of HVA22 homolog from Aloe vera and preliminary study of transgenic plant development. Int J Pure App Biosci 5:1113–1121
17. Süntar I (2020) Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem Rev 19:1199–1209
18. Tzfira T, Citovsky V (2006) Agrobacterium-mediated genetic transformation of plants: biology and biotechnology. Curr Opin Biotechnol 17:147–154
19. Yuan Z-C, Edlind MP, Liu P et al (2007) The plant signal salicylic acid shuts down expression of the vir regulon and activates quormone-quenching genes in Agrobacterium. Proc Natl Acad Sci 104:11790–11795
20. Yuan Z-C, Liu P, Saenkham P et al (2008) Transcriptome profiling and functional analysis of Agrobacterium tumefaciens reveals a general conserved response to acidic conditions (pH 5.5) and a complex acid-mediated signaling involved in Agrobacterium-plant interactions. J Bacteriol 190:494–507

Tables

Table 1: Media composition

S.No.	Media name	Composition
1.	Shooting media	MS + 4.0 mg/L BAP+1 mg/L IAA+ 1g/L PVP
2.	Rooting media	½ MS + 1.5 mg/L NAA+1g/L PVP
3.	CCM (liquid)	1/10 MS + 200 µmol/L acetosyringone + 1% glucose+1g/L PVP
4.	CCM (plates)	1/10 MS+ 200 µmol/L acetosyringone + 1% glucose+ 4.0 mg/L BAP+1 mg/L IAA + 1g/L PVP
5.	Washing media/ Pre-selective media	MS+500 µmol/L cefotaxime +250 µmol/L augmentin+1g/L PVP
5	SM I	MS+4.0 mg/L BAP+1 mg/L IAA+250 µmol/L cefotaxime+250 µmol/L augmentin+10.0 mg/L hygromycin+ 1g/L PVP
6.	SM II	MS+4.0 mg/L BAP+1 mg/L IAA+250 µmol/L cefotaxime+250 µmol/L augmentin+20.0 mg/L hygromycin+ 1g/L PVP
7.	SM III	MS+4.0 mg/L BAP+1 mg/L IAA+250 µmol/L cefotaxime+250 µmol/L augmentin+25.0 mg/L hygromycin+ 1g/L PVP

Figures

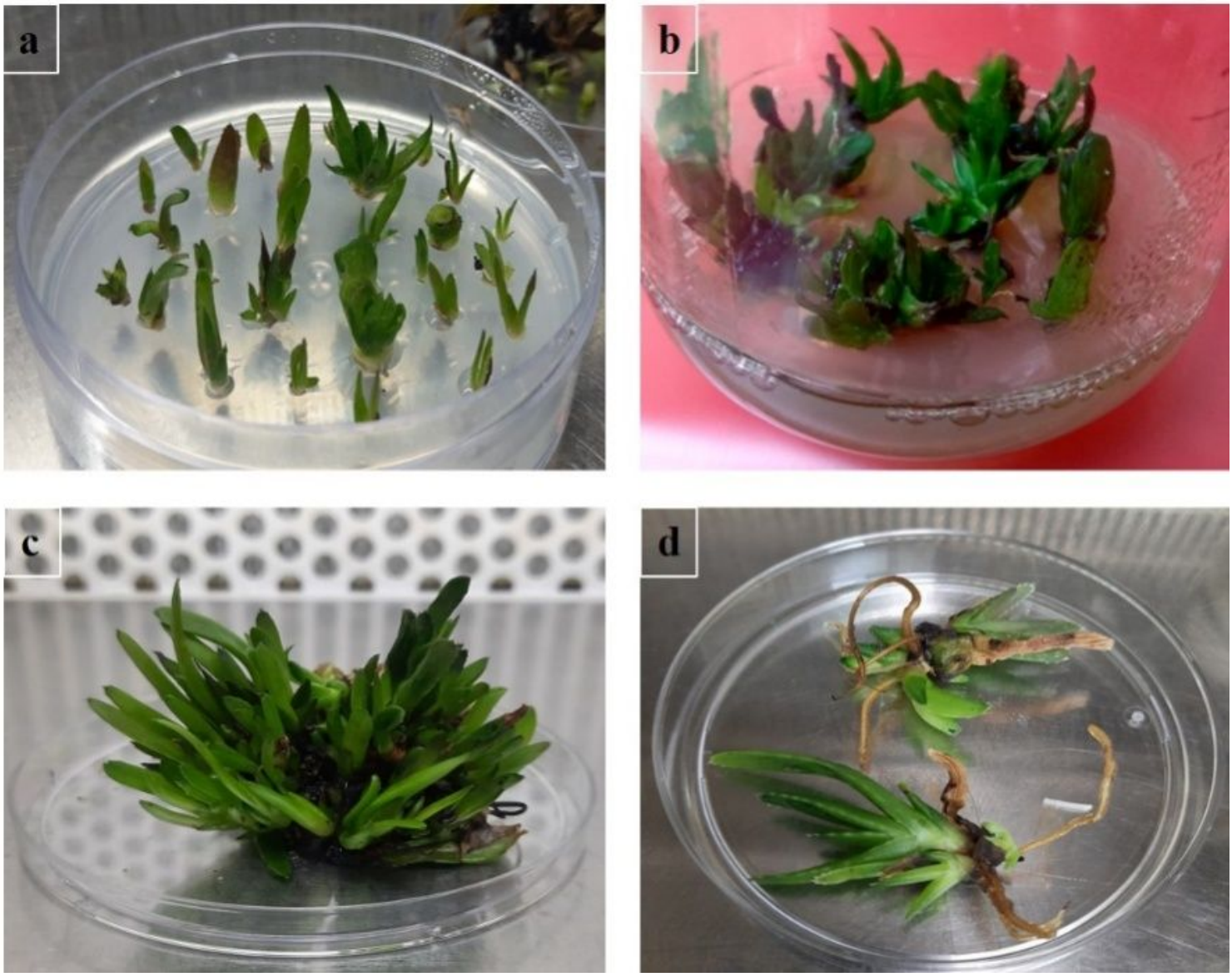


Figure 1

Regeneration potential of aloe stem, (a) Fresh shoot explants on shooting media, (b) initiation of multiplication in shoot segments, (c) shoot multiplication at a later stage, and (d) plantlets with prominent roots

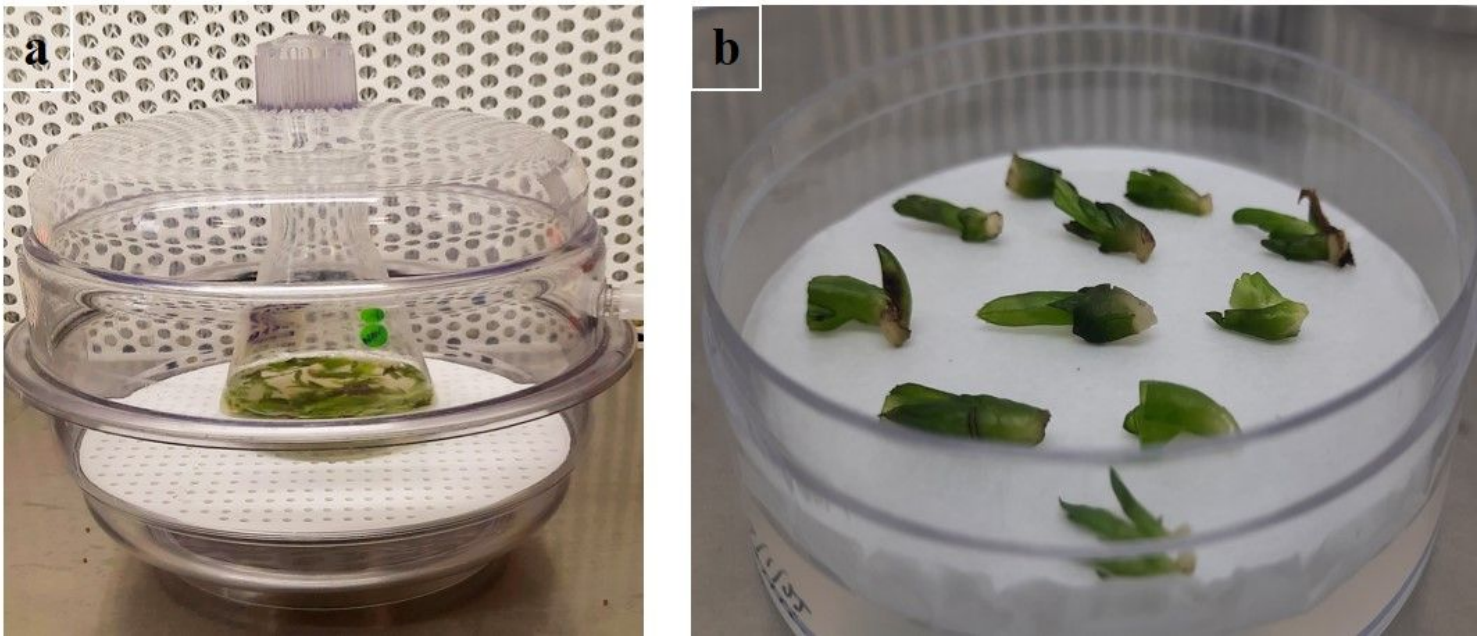


Figure 2

(a) Desiccation of explants during transformation, and (b) explants during co-cultivation on co-cultivation media

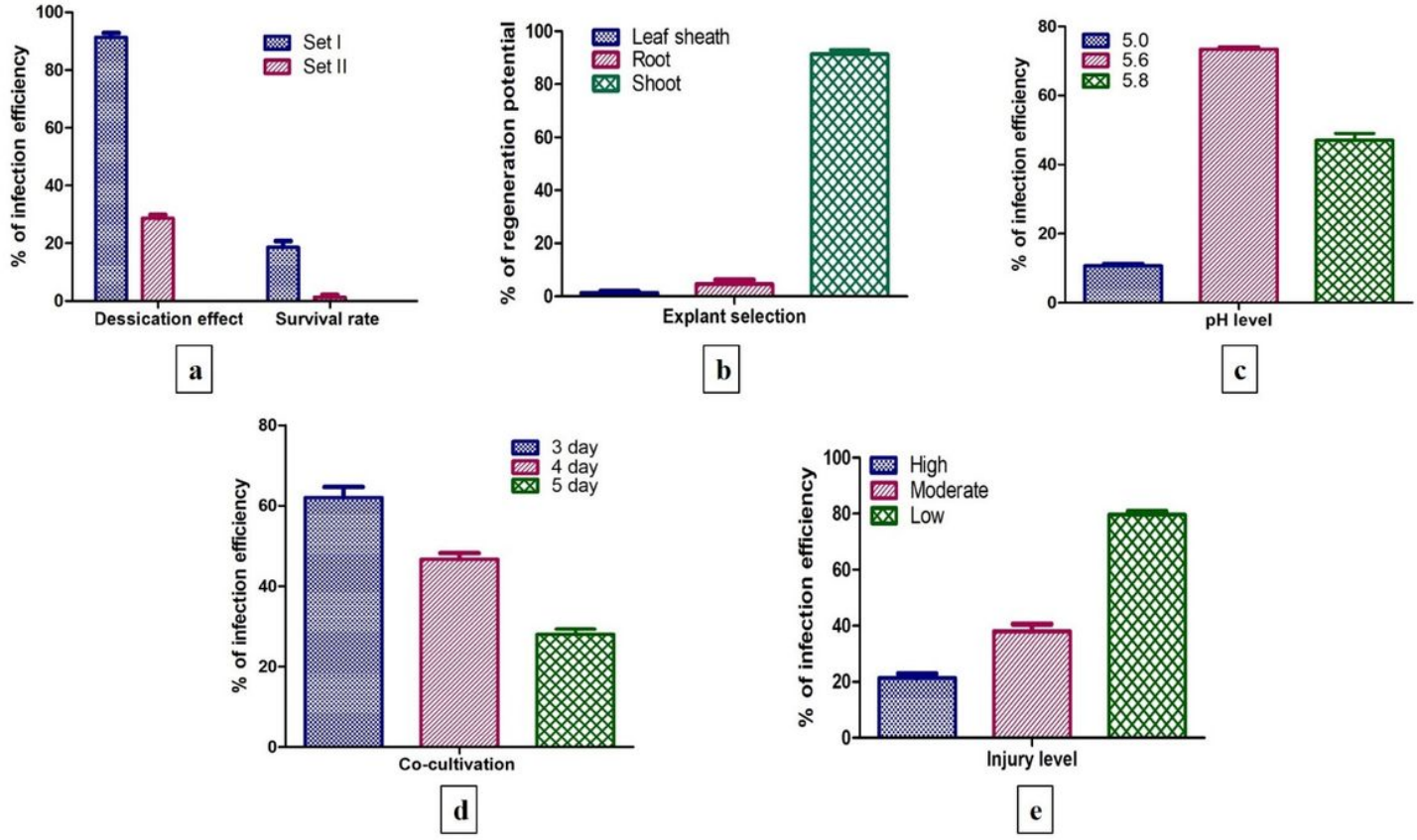


Figure 3

Optimization of different parameters of *Agrobacterium*-mediated transformation of *Aloe vera*, (a) Effect of desiccation on infection efficiency, (b) regeneration potential of different aloe explants, (c) effect of pH during co-cultivation on infection efficiency, (d) effect of co-cultivation duration on infection efficiency, and (e) effect of injury level during transformation on infection efficiency

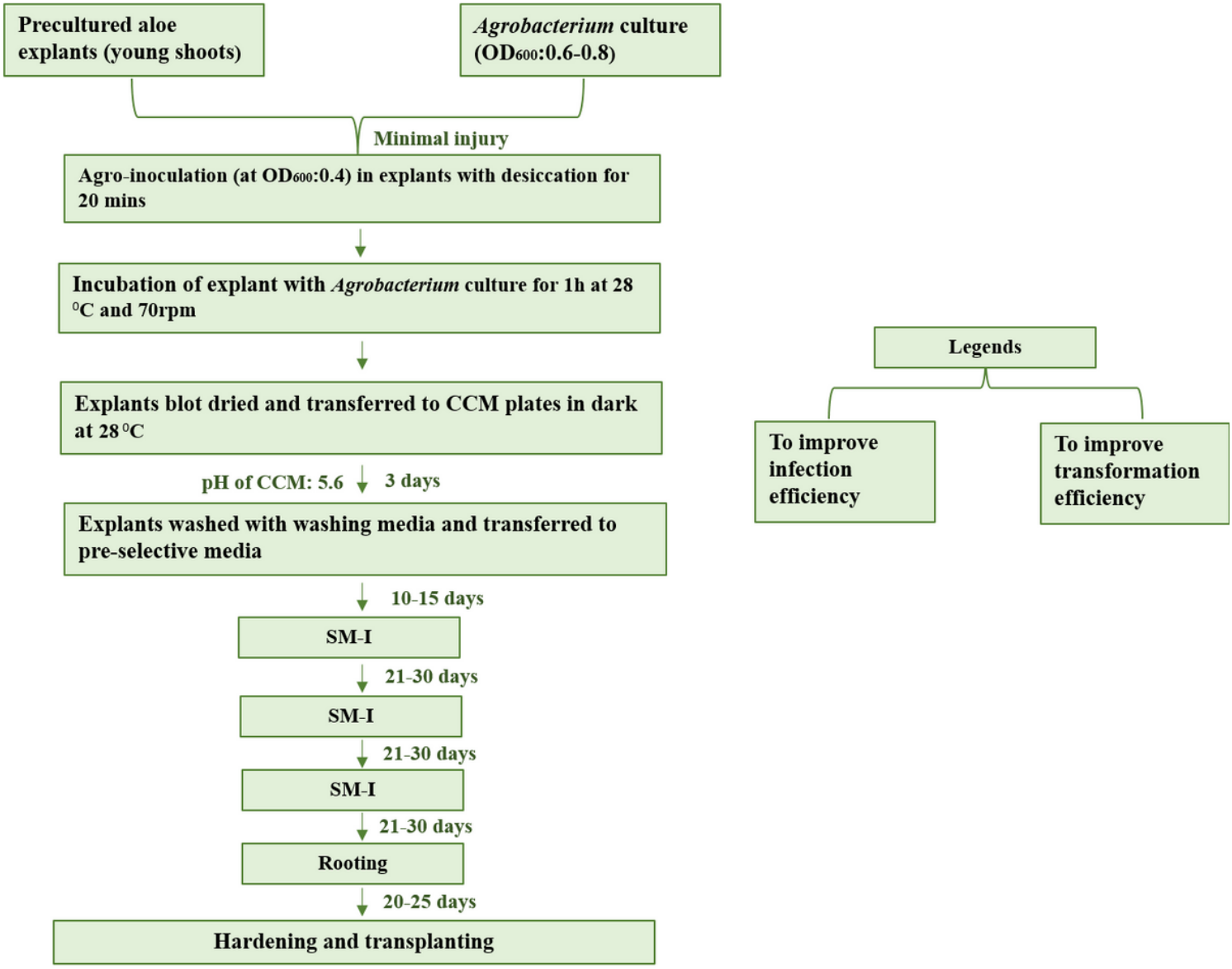


Figure 4

Outline of optimized protocol for the *Agrobacterium*-mediated genetic transformation in *Aloe vera.*

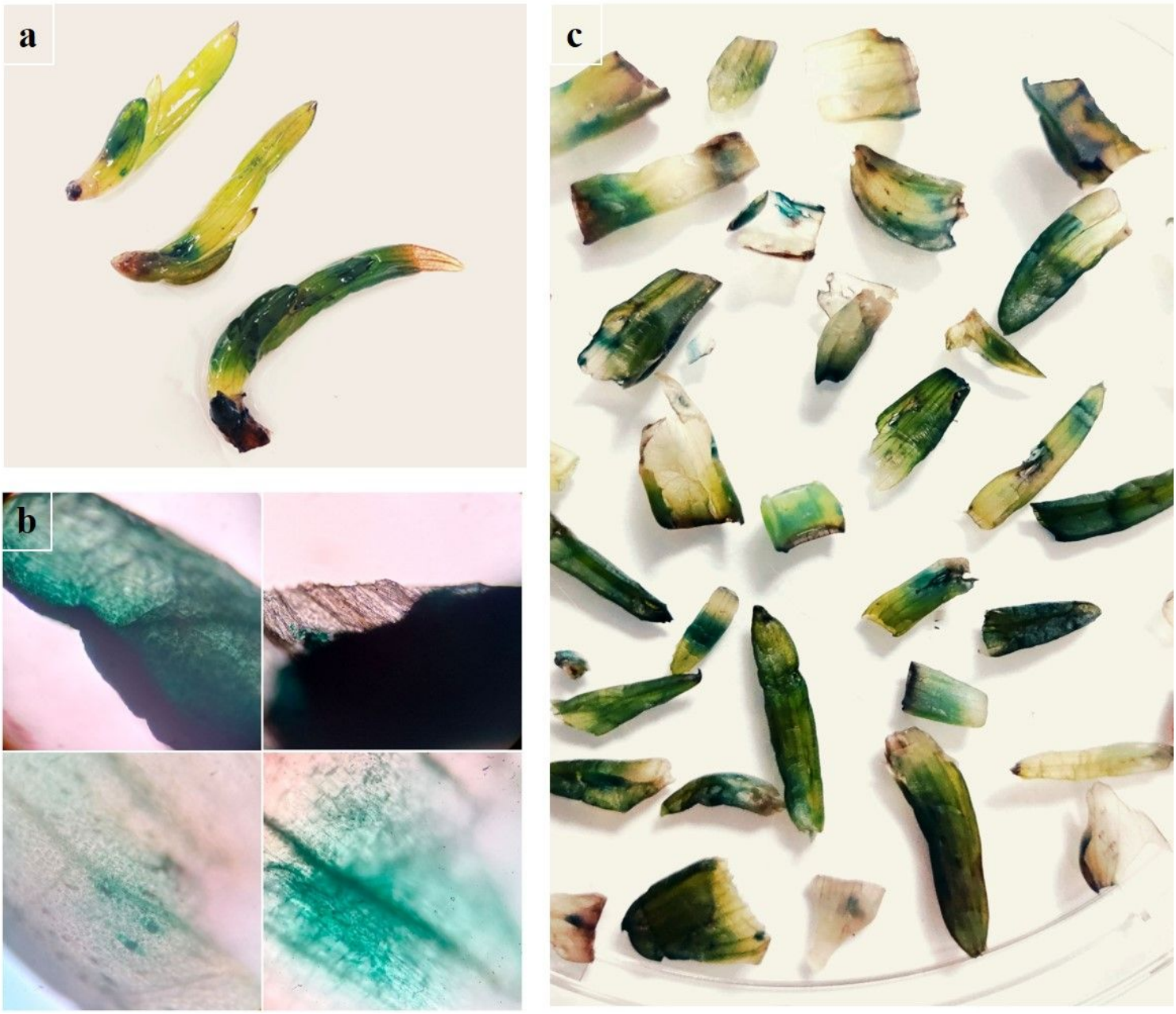


Figure 5

Transient expression of *GUS* gene in aloe explants, (a) intact aloe shoots showing *GUS* gene expression, (b) leaf section showing *GUS* gene expression in cells under the microscope, and (c) different sections of shoots showing expression of *GUS* gene

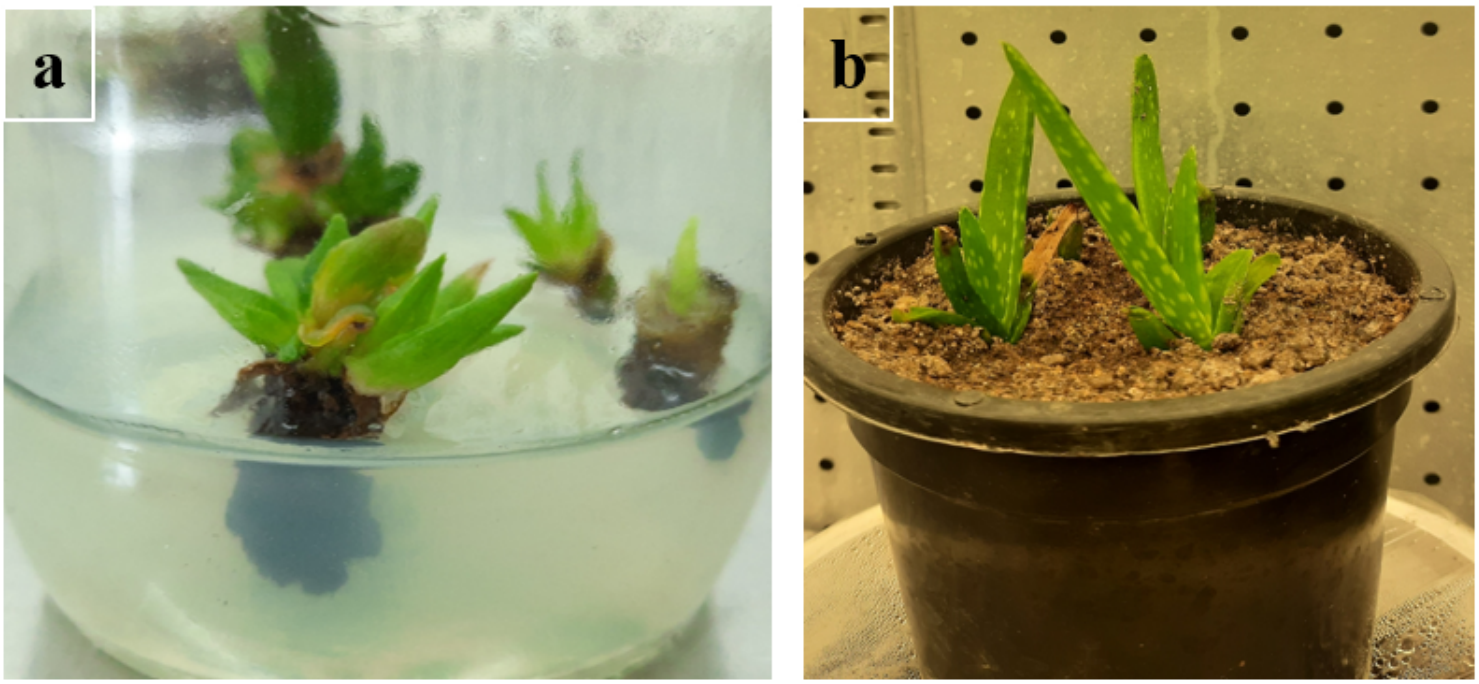


Figure 6

(a) Explant regeneration during the selection procedure, and (b) transgenic aloe plans after hardening and acclimatization in greenhouse

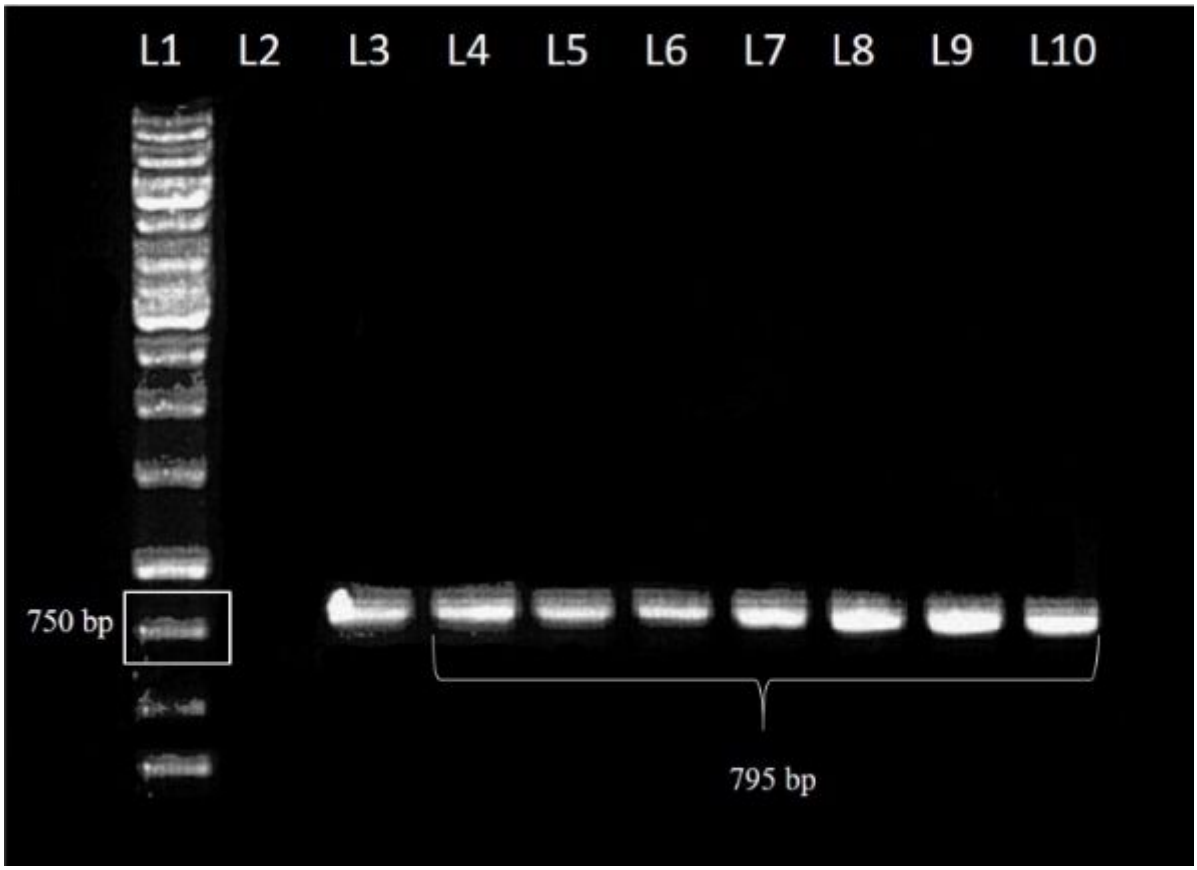


Figure 7

PCR analysis of transformants by *nptII* gene-specific primers (800bp); L1: 1kb gene ruler, L2: Non-template control, L3: positive control, and L4-L10: PCR amplification from putative transformants (800 bp)

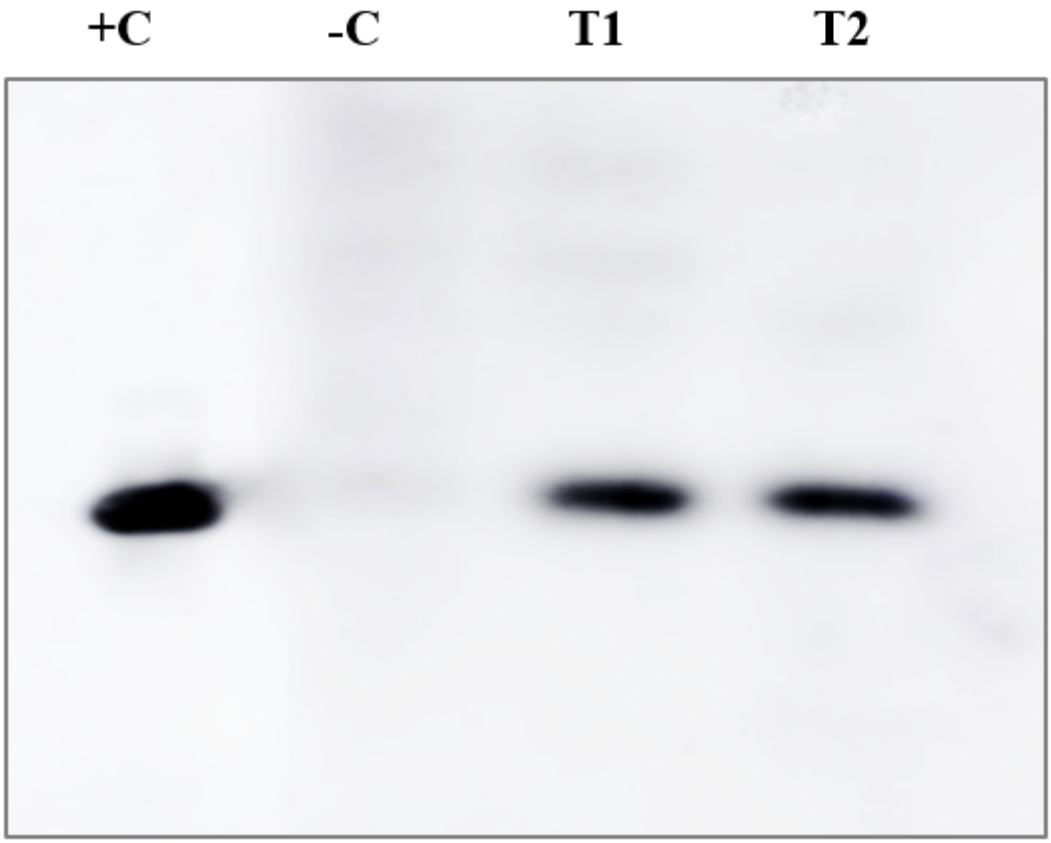


Figure 8

Southern blot analysis of two T_1 aloe transformants probed with *nptII* gene. +C: *nptII* gene (positive control); –C: wild type (negative control); T1 and T2 represents single insertion event in two transgenic lines respectively