

Biolistic transformation of sugarcane (*Saccharum officinarum* L.) with the *Oryza sativa* L. H⁺-PPase gene for improved salt stress tolerance

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Abstract

Several biotic and abiotic stresses adversely affect sugarcane crop. Amongst the abiotic stresses, salinity causes substantial losses to sugarcane yield. Lack of conducive environment for sugarcane flowering and unstable genome are the main constraints in sugarcane crop improvement via conventional breeding. Therefore, genetic engineering of sugarcane using *Oryza sativa* L. H⁺-PPase gene (OVPI) to confer salt tolerance is the preferred alternative strategy. In the present research, tissue culture and biolistic transformation protocols for sugarcane were optimized. For callus induction, Dichlorophenoxyacetic acid (2,4-D) was most effective. Maximum callus induction (50.22%) was achieved on CM-3 augmented with 2.5 mgL⁻¹ 2,4-D. The induced calli was subjected to biolistic transformation. For biolistic transformation, the gold particles (0.6 and 1.0 μm) and plasmid DNA (100 and 50 ng) were used for 1X and 2X bombardment. Significant differences (P≤0.05) were observed amongst the gold particles and plasmid DNA concentrations. Maximum transformation frequencies were observed when calli were bombarded 2X with 0.6 μm gold particles coated with 100 ng of DNA (23.60%). Selection and regeneration of transgenic calli were performed on MS media augmented with BAP (2.5 mgL⁻¹), NAA (1 mgL⁻¹) hygromycin (25 mgL⁻¹) and 2% coconut water. Regenerated plants after root induction on MS media containing NAA (2 mgL⁻¹) and 2% coconut water were transferred to loam and clay (1:1) and acclimatized to greenhouse condition. The transgenic plants were confirmed through PCR and RT-PCR.

Keywords: biolistic transformation; OVPI; sugarcane; salinity stress; tissue culture

Introduction

Sugarcane is amongst the major cash crops in the world. Sugarcane processing yields many important by products of daily life such as sugar, gur, bioethanol etc (Guerzoni *et al.*, 2014). Crop yield potential is affected up to 70% by abiotic stresses (Agrawal *et al.*, 2006). Amongst abiotic stresses, salinity is the major threat to crop yield. About 22% of the world's agricultural land is saline (FAO, 2004). Like other glycophytes, sugarcane is also suffering severe damages due to high salinity in the soil. Damages due to salt stress in sugarcane can be

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demonstrated by symptoms such as imbalance in nutritional, growth reduction low biomass, and low sprout emergence (Akhtar *et al.*, 2003). Sucrose accumulation in sugarcane is also affected by salinity due to the accumulation of toxic ions in the rhizospheric regions (Lingle and Wiegand, 1997). Salinity also reduces plant's ability to uptake water which negatively impacts plant metabolism. Studies on plants undergoing salt stresses showed significant reduction in shoot numbers (Ahire *et al.*, 2013). High concentrations of Na⁺ and Cl⁻ in chloroplasts also inhibit photosynthesis.

Certain defence strategies such as sequestration of ions and the accumulation of compatible solutes help the plant to overcome salinity stresses. However, excessive accumulation of toxic ions may cause severe damages to cell membrane. Tonoplast of plant cells has two different pump systems i.e., V-ATPase and V-PPase which are believed to enhance vacuolar sequestration of Na⁺ through electrogenic proton pump (Sakakibara *et al.*, 1996). These pumps establish the electrochemical gradient which internally helps in the translocation Na⁺ across the membrane. V-PPases have been extensively characterized in different plants for their role in Na⁺ sequestration in different plants (Zhang *et al.*, 2010). Transgenic sugarcane harboring *Arabidopsis thaliana* vacuolar pyrophosphatase 1 gene (AVP1) has shown improved tolerance to salinity stress (Kumar *et al.*, 2014). *Oryza sativa* genes codes for V-PPase proteins which confer tolerance to high level of salinity and water deficit conditions. Over expression of *Oryza sativa* vacuolar pyrophosphatase 1 (OVP1) gene in rice has shown improved vacuolar sequestration of Na⁺.

The development of efficient methods for transformation has enabled the transformation of sugarcane with the beneficial agronomic characteristics. Though the genetic transformation of sugarcane via agrobacterium is possible due to the discovery of acetosyringone, however; the efficiency of transformation is low. In contrast, biolistic transformation has high transformation efficiencies. Most of the published research on sugarcane transformation is based on biolistic transformation of sugarcane (Jackson *et al.*, 2013; Matroodi *et al.*, 2013). Altpeter and Oraby (2010) preferred biolistic technique because of its applicability to a large number of species. Low DNA quantity for biolistic technique can lead to the simpler integration pattern in sugarcane (Kim *et al.*, 2011).

Since biofuel impacts the world's economy enormously, there is a dire need of an intense research to develop new sugarcane varieties with improved characteristics against salinity stress. To neutralize the harmful effects of salinity sugarcane must have improved salt stress tolerance strategies. Conventional strategies to improve sugarcane might not be effective due to several factors such as no flowering nature of sugarcane, high level of heterozygosity and perennial nature etc. Therefore, alternative strategies such as genetic transformation of sugarcane must be focused for the speedy varietal improvement of sugarcane. In the present studies callus induction and biolistic mode of genetic transformation for sugarcane has been optimized for the development of large number of transgenic sugarcane lines harboring salt tolerant OVP1 gene.

Materials and Methods

Plant material

Sugarcane variety 'CP 77-400' was kindly provided by Sugar Crop Research Institute (SCRI), Mardan. The setts were cultivated in the fields at IBGE, The University of Agriculture, Peshawar. Sugarcane plants of about 6 months old were used for explant collection for tissue culture.

MS media and explant preparation

Callusing media (CM) was prepared in dH₂O using MS basal salt medium (Murashige and Skoog, 1962) supplemented with different concentrations of 2,4-D (Table 1). Adjustment of the pH was done using 0.1 N NaOH or HCl and kept at 5.7 ± 0.1. After pH adjustment, 0.8% agar was added to the media and was autoclaved at 121 °C for 15-20 min and 15 PSI pressure. The media was then poured into Petri plates. The

immature leaf whorls were used as explants and were collected from the 6-month-old field grown sugarcane plants and were sterilized according to the procedure reported (Ullah *et al.*, 2022). Thin slices of the leaf whorls were placed on callus induction medium and were incubated under 2000 lux light at 28 ± 0.2 °C. The explant inoculated plates were exposed to 16 hrs photoperiod and observed on daily basis for callus induction. The calli induction was calculated by the following formula;

$$\text{Frequency of callus induction} = \frac{\text{Number of explants inducing calli}}{\text{Total explants cultured}} \times 100$$

Table 1. MS Media supplemented with different concentration of 2,4-D for callus induction in sugarcane

S. No.	Callus Media (CM)	Composition
1	Control	MS + 0.0 mg/L 2,4-D
2	CM-1	MS + 1.0 mg/L 2,4-D
3	CM-2	MS + 2.0 mg/L 2,4-D
4	CM-3	MS + 3.0 mg/L 2,4-D
5	CM-4	MS + 4.0 mg/L 2,4-D

Construct preparation

The cDNA clones of OVP1 were searched in KOME data base and ordered from NIAS, Japan. The OVP1 cDNA (3.2 Kb) was cloned in pGreenII0129 expression vector through sticky end cloning using restriction enzymes i.e., HindIII and BamHI. The T-DNA construct is shown in Figure 1.



Figure 1. T-DNA region of pGreenII0129 vector harboring OVP1 under the control of Ubiquitin promoter and Hygromycin selectable marker

Biolistic transformation

Preparation of gold particle

The gold particles of different sizes (0.6 and 1.0 μm) were used in the experiment. The gold particles (30 mg) were taken in an Eppendorf tube followed by the addition of 1 ml 70% ethanol. The tubes were vortexed for 15 min and the gold particles were incubated at room temperature for 5 min. The supernatant was discarded after centrifugation at 10,000 rpm for 5 sec. Then 1 ml ddH₂O was added to the pellet and were vortexed for 1 min. After centrifugation at 10,000 rpm for 2 min, pellet was taken and the supernatant was discarded. The above steps were repeated 3 times followed by the dissolution of pellet in 1 ml 50% glycerol.

Coating gold particles with DNA

The gold particles were mixed by vortexing and 40 μl was transferred to a new 1.5 ml Eppendorf tube and different concentrations of plasmid DNA (pGreenII0129) were transferred to the gold particles. After vortexing, 40 μl of CaCl₂ was added to the gold particles followed by the addition of 16 μl spermidine and vortex thoroughly for 5 min. The supernatant was discarded after centrifugation at 10,000 rpm for 2 min and the pellet was washed twice with 100% ethanol and finally dissolved in 40 μl of 100% ethanol followed by vortexing for 1 min.

Bombardment of calli

The calli were bombarded with the DNA coated gold particles. Prior to bombardment, the osmoticum medium was prepared using CM-3 having 0.2 M sorbitol and Mannitol respectively. Before bombardment, the calli were placed on the osmoticum medium for 3 hrs. The calli were then subjected to bombardment with gold

particles coated with the desired construct (pGreenII0129), at 1100 psi and vacuum pressure 25 Hg using BIO-RAD model PDS-1000/He biolistic particle delivery system. The bombarded calli were incubated for 24 hrs at 28 °C.

Selection and regeneration of putative transgene calli

Following bombardment, the putative transgenic calli were placed on CM-3 augmented with 2% coconut water for recovery for 1 week. After 1 week of recovery, the calli were transferred to selection media (CM-3 + 250 mgL⁻¹ cefatoxime + 25 mgL⁻¹ hygromycin + 2% coconut water) for two sub culture (each of 10 days). After selection, the calli were transferred to regeneration media (2.5 mgL⁻¹ BAP + 1 mgL⁻¹ NAA + 25 mgL⁻¹ hygromycin + 2% coconut water) for plantlets regeneration.

Molecular characterization of the transgenic plants

The transgenic sugarcane plants were confirmed through specific PCR primers. Expression analysis was performed using reverse transcriptase (RT-PCR).

PCR confirmation of the transgenic sugarcane plants

Genomic DNA was extracted from both the non-transgenic and transgenic sugarcane lines via CTAB DNA extraction method as previously reported by (Rogers and Bendich, 1988). The transgenic sugarcane plants were confirmed using ubi F1 (ACTGAATACAAGTATGTTGGACTAT-3) and OVP1 R228 (5'-ATCCTCTTCAAGTGGTTTTAG-3') primers.

Expression analysis of the transgenic sugarcane

After successful establishment of the transgenic lines under *in vitro* conditions, the plants were acclimatized to the soil under growth room conditions. For RNA isolation, the plants were exposed to 75 mM NaCl stress to trigger the OVP1 gene expression. TRizole method was used for RNA extraction. The isolated RNA was subjected to cDNA synthesis. A total of 5.0 ng RNA was used for cDNA synthesis. OVP1 Gene specific primers (5'-CATGAAGAGTGTTGGCAGCG-3'); 5'-ACCAGAAACGAGAGCACCAG-3') were used for the amplification specific regions from the cDNA. *Glyceraldehyde-3-phosphate dehydrogenase (GADPH)* gene used as an internal control was amplified from the cDNA using specific primers (F: 5'-TTGGTTTCCACTGACTTCGTTR-3'; R: 5'-CTGTAGCCCCACTCGTTGT-3').

Statistical analysis

The data (n=3) was analyzed using statistical package Statistix 8.1. The ANOVA was constructed for factorial experiments. Status of the main effects and interactions were noted. To compare all pairwise interactions, the Least Significant Test (LSD) was performed at probability value of 0.05 (P≤0.05).

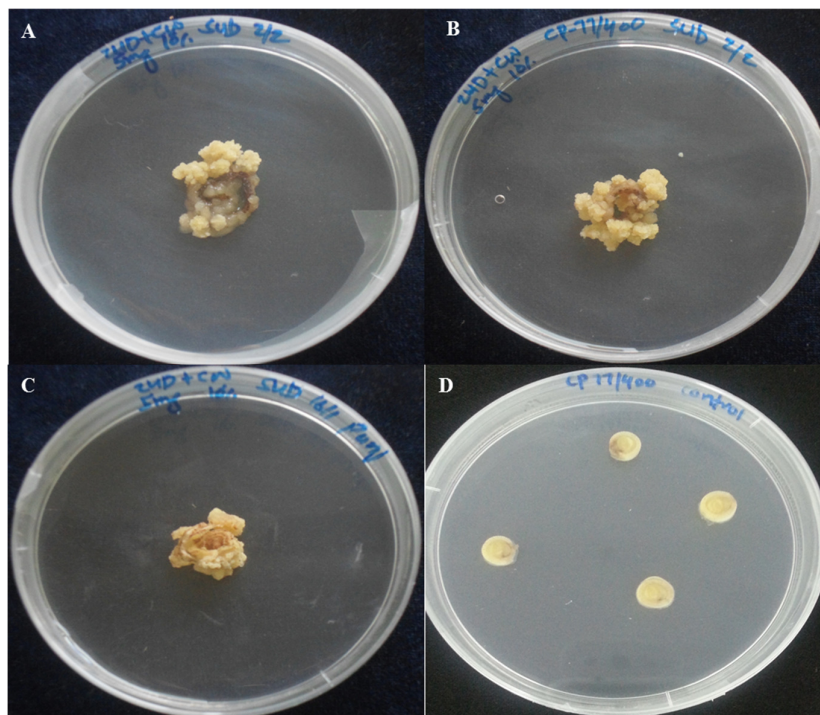
Results

Callus induction

The data on callus induction is given (Table 2). Callus induction increased with increase in 2,4-D concentration. Maximum callus induction i.e 85% was observed when MS media was observed on CM-3 followed by callus induction capabilities of 65.0% on CM-4. While minimum callus induction of 48 % was observed on CM-1. On control media no callus induction was observed (Figure 2).

Table 2. Callus induction on different callusing media (CM) in CP 77-400

S. No.	Callus Media (CM)	Callus Induction Frequency (%)
1	Control	0.0000 ± 0.00
2	CM1	48.440 ± 2.81
3	CM2	51.773 ± 2.32
4	CM3	85.553 ± 1.99
5	CM4	65.550 ± 2.02

**Figure 2.** Callus induction in CP 77-400 on CM-4. A-C= 28 days old callus and D= Control*Biolistic transformation*

Efficiency of biolistic transformation by subjecting induced calli to 1X bombardment using different sizes of gold particles and plasmid DNA concentration is shown (Figure 3). Significant differences ($P \leq 0.05$) were observed amongst the different sizes of gold particles. The gold particles of 0.6 μm caused minimum damages to the induced calli during bombardment and resulted in maximum transformation frequencies of 16.445% as compared to 1.0 μm size gold particles i.e 12.566%. The ANOVA suggested significant differences ($P \leq 0.05$) amongst the different plasmid DNA concentration used. Plasmid DNA concentration of 100 ng was proved to be optimum for the maximum transgenic plant generation of 15.977% as compared to 150 ng (13.034%) of plasmid DNA concentration. The data on interactions was also statistically significant ($P \leq 0.05$). Maximum transformation efficiencies of 19.076% were observed when gold particles of 0.6 μm were coated with 100 ng of plasmid DNA. However, when gold particle sizes were increased to 1.0 μm and coated with 150 ng of plasmid DNA, the transformation efficiencies were decreased to 12.254%.

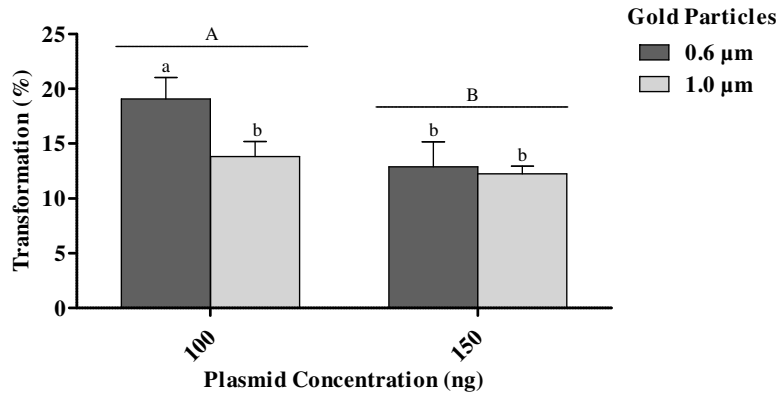


Figure 3. Frequency of 1X biolistic transformation of sugarcane calli with 0.6 and 1.0 μm of gold particles coated with 100 and 150 ng of plasmid DNA respectively. Values are means $\text{SD} \pm$ ($n=3$).

Similar to 1X bombardment, gold particles of 0.6 μm and DNA concentration of 100 ng were also productive for 2X bombardment (Figure 4). The ANOVA showed significant differences ($P \leq 0.05$) amongst the different sizes of gold particles and plasmid DNA concentrations used ($P \leq 0.05$). Gold particles of 0.6 μm sizes showed higher transformation frequencies of 17.654% as compared to 1.0 μm gold particles with 9.579%. The efficiency of low plasmid DNA concentration used was high i.e., 17.479% as compared to high plasmid DNA concentration (9.754%). In case of interactions, significant differences were observed ($P \leq 0.05$). The gold particles of 0.6 μm coated with 100 ng of plasmid DNA showed higher transformation efficiencies (23.601%). However, the transformation efficiencies were lower i.e., 7.802% when the huge particles of size 1.0 μm were coated with higher plasmid DNA concentrations.

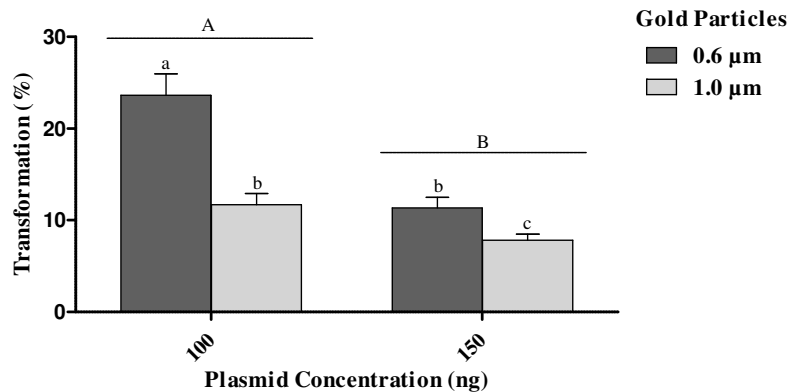


Figure 4. Frequency of 2X biolistic transformation of sugarcane calli with 0.6 and 1.0 μm of gold particles coated with 100 and 150 ng of plasmid DNA respectively. Values are means $\text{SD} \pm$ ($n=3$).

Selection and regeneration of the transgenic calli

Following an overnight incubation on the osmoticum media, the bombarded calli were transferred to CM-3 media augmented with 2% coconut water without selection pressure. Successful selection of the putative transgenic calli was performed on CM-3 augmented with 25 mgL^{-1} hygromycin and 2% coconut water (Figure 5C). The viable portions of the calli were carefully trimmed off and were continuously transferred to fresh media to ensure the supply of nutrients to the growing cells. After two subculture (each of 10 days), only the viable calli cells were taken, while the dead calli were completely trimmed off. Following selection, the putative transgenic calli were placed on MS media supplemented with BAP (2.5 mgL^{-1}) and NAA (1 mgL^{-1}) 25 mgL^{-1}

hygromycin and 2% coconut water to promote organogenesis (Figure 5D). After two subcultures (each of 10 days) on regeneration media, the development of leaf primordia was observed (Figure 5E). At the end of the fourth subculture, plantlet regeneration was observed on BAP (2.5 mgL⁻¹) and NAA (1 mgL⁻¹) supplemented MS media (Figure 5F and G). The presence of any chimeric escapes and false positive plants were successfully avoided by subjecting the putative transgenic plants to high concentration of hygromycin (35 mgL⁻¹). After fourth subculture the fully grown plantlets were observed.

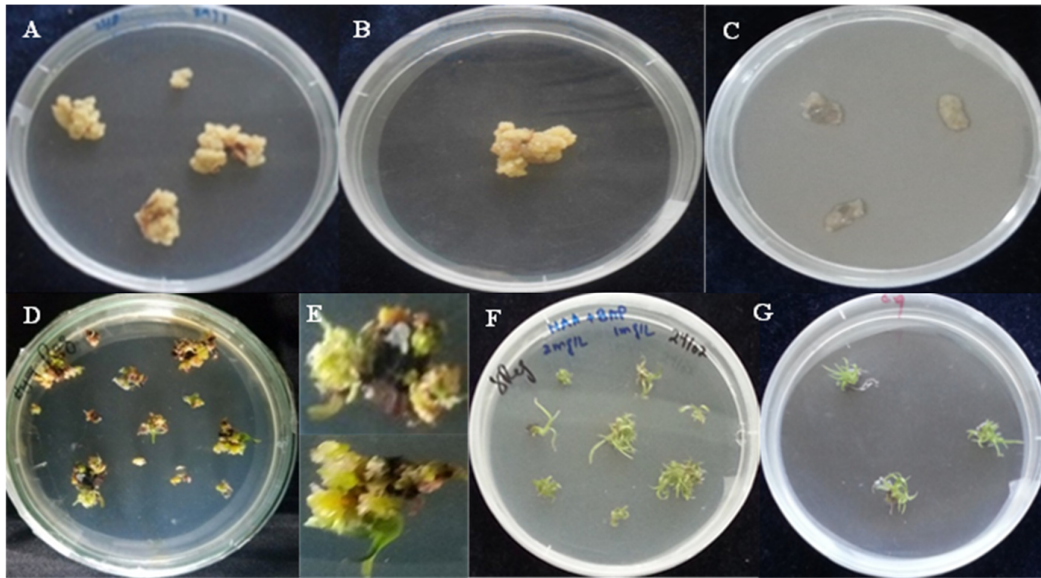


Figure 5. Biolistic transformation of sugarcane calli with recombinant *pGreenII0129* plasmid harboring *OVPI* gene. A= Calli on CM-2, B= Calli on osmoticum media, C= Calli undergoing selection, D and E= development of leaf primordia on regeneration media and F and G= Plantlet regeneration.

Acclimatization of putative transgenic plantlets

Root induction in the putative transgenic plantlets was successfully achieved on ½ MS media augmented with 2 mgL⁻¹ NAA and 2% coconut water (Figure 6A and B). The putative transgenic plants were acclimatized from *in vitro* to growth room in polythene bags and finally to greenhouse conditions in pots (Figure 6C and D).

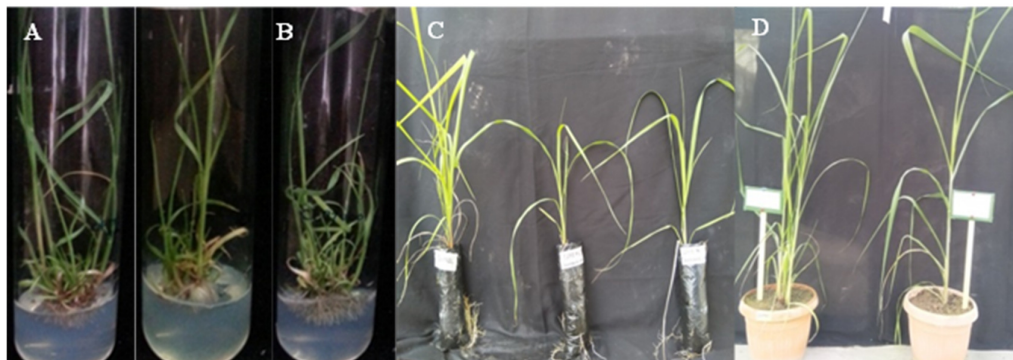


Figure 6. Stepwise acclimatization of the putative transgenic sugarcane plantlets from *in vitro* to greenhouse conditions
 A= Plantlets undergoing selection process, B= Root induction in putative transgenic plantlets, C= Acclimatization of the transgenic plants in polythene bags and D= Transgenic sugarcane plants in greenhouse.

Molecular confirmation of the transgenic sugarcane plants

PCR confirmation

After the successful establishment, the transgenic sugarcane lines were confirmed with PCR which showed the successful integration of the T-DNA insert into the plant genome. The amplified PCR product was approximately 400 bp (Figure 7).

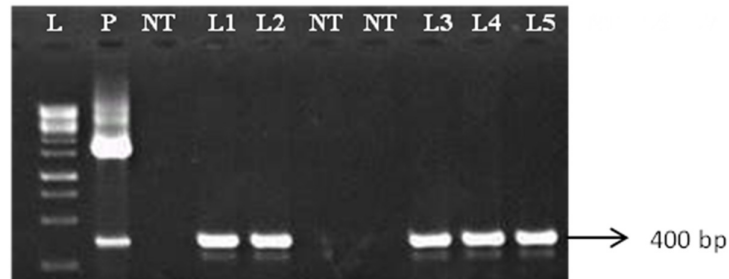


Figure 7. PCR confirmations of the transgenic sugarcane lines
L= 1Kb+ ladder, NT= Non transgenic lines, L1-L7= Transgenic lines, P= Positive control.

RT-PCR of transgenic sugarcane lines

Expression analysis of the transgenic sugarcane lines was performed through reverse transcriptase PCR which indicates the successful integration and transcription of *OVP1* gene in the transgenic line. *Glyceraldehyde-3-phosphate dehydrogenase (GADPH)* was selected as reference gene under salinity stress according to the previous recommendations in literature. The amplified fragment of *OVP1* and *Glyceraldehyde-3-phosphate dehydrogenase (GADPH)* reference gene from the cDNA of transgenic sugarcane line is shown (Figure 8). The RT-PCR result confirms the expression of *OVP1* gene. The independent transgenic lines were named as L1, L2, L3, L4, L5.

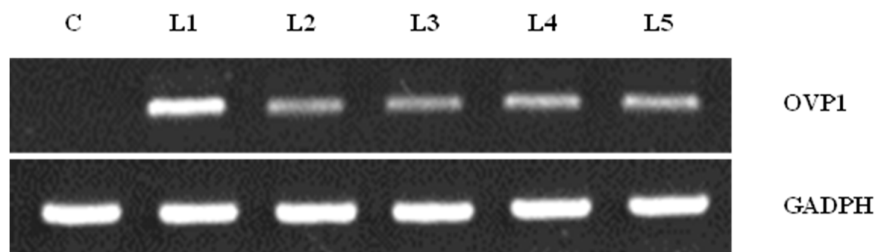


Figure 8. Expression analysis of the putative transgenic plants harboring *OVP1* gene against salinity stress
C= Control, L1-L6 = Transgenic lines and *GADPH* = Sugarcane reference gene.

Discussion

Due to high ploidy level of sugarcane, conventional methodologies for sugarcane crop improvement may not be affective. There for alternative approaches such as biolistic transformation of sugarcane must be focused for future crop improvement of sugarcane. Various studies on the biolistic transformation of sugarcane have been reported previously (Basnayake *et al.*, 2011; Khan *et al.*, 2021). Sugarcane is assumed to lack cellular mechanisms essential for transformation (Potrykus, 1991). Though, reports on successful genetic manipulations are available; however, the success rate is low (Jackson *et al.*, 2013). A number of biological and physiological parameter affects the biolistic transformation process (Matroodi *et al.*, 2013). As seed production is not possible in most of the region due to the lack of suitable; therefore, the induction of embryogenic calli

for transformation is demanded. In the present study the induced embryogenic calli cells induced on 4 mg/L 2,4-D of sugarcane had competency for transgene integration and subsequent regeneration. Previously 2,4-D has been extensively used for callus induction in sugarcane (Ullah *et al.*, 2022). The competency of embryogenic calli for DNA uptake was previously reported (Bower and Birch, 1992). In the present study, factors such as particles size, number of bombardments and plasmid concentration were optimized for the improved efficiency of biolistic transformation. Other studies also declared these factors as indispensable for the biolistic transformation of sugarcane (Taparia *et al.*, 2012).

In the present research, the gold particles were used as microcarriers for their biologically inert behavior, uniformity in shape, size and non-toxic nature. Efficiency of gold particles in comparison to tungsten particles as microcarriers has also been reported (Matroodi *et al.*, 2013). Gold particles of 0.6 μm size resulted in maximum transformation efficiency in contrast to 1 μm particle size. Large size particles may be lethal and can cause severe damages to the already exposed callus cells as compare to seeds and other explant used. Ramasamy *et al.* (2011) suggested small size gold particles as favorable microcarriers for biolistic transformation. Randolph-Anderson *et al.* (1995) correlated large size particles to cellular damage which can adversely affects the transgenic events. The present results are in complete alignment with the previously reported results of Sanford *et al.* (1993) by declaring tungsten particles as involved in cell acidification and breakage of DNA bonds which can be lethal for cellular survival. An efficient osmotica with non-metabolization ability is recommended to prevent cytoplasmic leakage from the target cell. The present study suggests the combination of sorbitol and mannitol for effective for transgene development. The present results on the use of osmoticum in biolistic transformation have been extensively reported (Matroodi *et al.*, 2013; Khan *et al.*, 2021). Spermidine, a polyamine known to have a role in plant growth regulation and stress tolerance was used for overcoming the cellular damage during bombardment and incubation on osmoticum media. Spermidine has been extensively in biolistic transformation (Khan *et al.*, 2021). Spermidine is important for binding DNA to a gold particle. In the present study, a combination of spermidine and CaCl_2 was found to be vital for binding plasmid DNA to gold particles. These results are in complete alignment with the results of Zuraid *et al.* (2010). The Ca^+ enhances the binding of plasmid DNA to the gold particles. It also helps in the aggregation of particles. Perl *et al.* (1992) reported a decrease in transient gene expression upon exclusion of spermidine which confirms the findings of the present research. The fidelity of spermidine as a precipitating agent has also been reported by Xiong *et al.* (2013). However, other studies suggested protamine as efficient for prolonged plasmid DNA protection against DNases (Sivamani *et al.*, 2009). Multiple bombardments were assumed to have positive impact on the transformation frequency. Multiple bombardment increased callus bombardment area which leads to the increased number of transgenic events in the present research. Our results confirm the findings of Matroodi *et al.* (2013). Sreramanan *et al.* (2005) also achieved multiple transgenic events via two bombardments which confirm the present results. However, it is worth mentioning that an increased bombardment numbers may cause injuries to the cells which can affect the stability of transformation. Lonsdale *et al.* (1990) also reported potential damages to cell during multiple bombardments. Kartha *et al.* (1989) and Reggiardio *et al.* (1991) contradict the findings of the present results suggesting multiple bombardments as a strategy to increase transformation efficiency. Increasing DNA concentration from 100 to 150 ng showed non-significant effects on transformation frequencies. This effect was surprising as an increased plasmid concentration was assumed to have positive impact on the transformation frequencies. Most of the studies revealed 90 ng as a routine plasmid concentration used for biolistic transformation (Jackson *et al.*, 2013). Matroodi *et al.* (2013) observed adverse effect of increased DNA concentration which strongly correlates with the findings of the present study. This effect is may be due to the increased aggregation of particles which in turn may reduce cellular penetration. Furthermore, it is also assumed that an increased plasmid concentration may cause complexities in the transgene integration patterns. Complexities in DNA integration patterns due to the use of high DNA concentration has also been reported (Lowe *et al.*, 2009) which is in complete

agreement with the present research. Non-significant effect of an increased DNA concentration on transformation frequencies has also been reported (Musavi *et al.*, 2009). However, Khan *et al.* (2021) observed increased transformation frequencies by increasing plasmid concentration which contradicts the findings of the present study. The use of complete plasmid may result in the integration of prokaryotic sequences which may not be desirable. Therefore, the use of minimal cassettes may result in integration of desired transgene free of prokaryotic sequences termed as free insertion technology (Fu *et al.*, 2000). The use of full plasmid in the present research had probably affected the transformation frequency and the stability of the transgene expression. The efficiency of minimal cassettes in biolistic transformation has also been reported (Taparia *et al.*, 2012). The present study agrees in the use of minimal cassettes in biolistic transformation is also suggested previously (Taparia *et al.*, 2012; Wu *et al.*, 2015).

Molecular confirmation of the transgene is an important step prior to physiological and biochemical analysis of the transgenic plants. The transgenic plants were analyzed both through PCR which confirmed the successful integration of the transgene. Previously Damayanti *et al.* (2017) and Saravanan *et al.* (2018) successfully confirmed transgenic sugarcane with PCR. Though PCR is an effective tool to confirm transgene; however, it cannot give an idea about expression of the gene. Therefore, RT-PCR was performed for expression analysis of the transgenic sugarcane plants. The OVP1 gene was successfully expressing in the transgenic sugarcane lines. Numerous studies have been reported on the fidelity of RT-PCR for the transgenic plant confirmation (Kumar *et al.*, 2013). Other studies suggest southern blot as an effective molecular tool for transgenic plant confirmation.

Conclusions

For improved production of sugarcane, development of varieties with improved salinity tolerance is prerequisite. Reproducible callus induction protocol requires optimum level of 2,4-D. Biolistic transformation technique can be a suitable approach for the production of large number of transgenic lines in a polyploid crop like sugarcane. Biolistic transformation efficiency is affected by the plasmid concentration and number of bombardments. However, plasmid concentration and number of bombardments beyond certain level may adversely affect the transformation efficiency. OVP1 gene can be a suitable candidate gene against salinity stress in sugarcane.

Authors' Contributions

MU conducted the research, data analysis and manuscript preparation, MSK and AJ designed the experiment and supervised the research; NI, SUD and SUAS assisted in experimental work; IM critically checked the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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