Bamboo, the “Green Gold” of the 21st century is commonly known as “Poor man’s timber”. It has been playing a significant role in human society since time immemorial. Presently, it is contributing towards the subsistence needs of over a billion people worldwide. Bamboo has the potential to solve many of India’s developmental problems. It is an ideal environmental as well as economic investment which can be utilized in a variety of ways. Bamboo is already generating energy in some places and acting as a wood substitute in paper and other allied industries. In recognition of its enormous worth, there has been an overdrive in R&D activities worldwide to find new applications for the plant. Since improvement of bamboos through conventional breeding is not possible, genetic transformation can play an important role.

In this regard, the present study concentrated on developing an efficient and reproducible system of genetic transformation of a new plant like bamboo either through Agrobacterium or biolistic mediated approaches. It was recognised that *D. hamiltonii* showed recalcitrance both towards direct and indirect genetic transformation methods. Hence, the study focussed on (i) identifying the major blocks that prevent *Agrobacterium* infection of *D. hamiltonii* somatic embryos and then (ii) devising methods for successful plant-*Agrobacterium* interaction and hence, transformation.

For this, a highly synchronous regeneration system was the primary pre-requisite for optimization of genetic transformation system. Therefore, the existing regeneration system was further modified as per the requirements of genetic transformation. In this regard, the use of 1 mg/l BA and 0.5 mg/l NAA improved the germination percentage of somatic embryos of *D. hamiltonii* to 85%. Somatic embryos were preferable targets for genetic transformation because of their high reproducibility, germination and development of complete plants within a short period. Moreover, a large number of explants could be targeted at one go. Modified ½ strength B5 medium supplemented with IBA and NAA allowed faster multiplication of transgenic and control shoots followed by 100% rooting and rhizome formation.

*D. hamiltonii* was found to possess the typical characteristics that generally make many monocots un-amenable to *Agrobacterium* infection. These were found to be: (i) waxy surface of the somatic embryos that prevented *Agrobacterium* attachment, (ii) cell wall thickening at wound sites which cordoned off the transformed sectors and
(iii) necrosis due to tissue polyphenols and PPO activity resulting in the loss of probable transformants. Measurement of polyphenol contents and PPO activity revealed significantly higher polyphenol oxidation when treatments that induced higher necrosis were used, and this increased by about 1.5 folds when the treatments were combined with *Agrobacterium* infection. The presence of *Agrobacterium* was so stressful that the somatic embryos were finally killed on increasing the duration of co-cultivation beyond 2 days.

Necrosis of plant tissues due to hypersensitive reaction and oxidative burst generally require detoxification with anti-oxidants and adsorbents that scavenge and quench reactive oxygen species. Thus, treating the somatic embryos with PVP (500 mg/l) for 15 min during infection was extremely useful in overcoming the necrosis completely.

Specific wound response(s) is a major factor responsible for the resistance of monocots to *Agrobacterium* mediated genetic transformation. Thus, epidermal cells of treated (pricked or immersed) *D. hamiltonii* somatic embryos were observed to be elongated and closely packed at the sites of injury. Wound sites in the treated somatic embryos were sealed within 24 h making the accumulated polyphenols within them un-available for PPO activity. Therefore, necrosis due to polyphenol oxidation was also low/negligible. Since each of the three infection methods (i.e., immersion, vacuum infiltration and pricking) exhibited some kind of negative influence on the somatic embryos, the least damaging and ‘*Agrobacterium* infection supporting’ method was identified. Vacuum infiltration was the most injurious followed by immersion and pricking therefore, making it totally unsuitable. Although necrosis was lowest, a 50 µm layer of closely packed elongated cells developed at the epidermal regions of pricked somatic embryos and this prevented further cell division or differentiation. As a result, successful transformation was hampered. In contrast, necrosis in immersed somatic embryos was easily overcome with time and the single 25 µm layer of elongated epidermal cells did not hinder cell division and further differentiation. Therefore, immersion for 15 min followed by co-cultivation for 2 days was selected as the most suitable method for genetic transformation of *D. hamiltonii*.

In order to overcome the inhibitory effect of somatic embryo surface-waxes in *Agrobacterium* attachment, a surfactant i.e., 0.01% Tween-20 was used during infection. The inhibition was overcome because it served as a wetting agent and facilitated attachment of a large number of agrobacteria to the somatic embryo surface.
Once the transformation blocks were overcome, parameters varying from vector/strain combinations, *Agrobacterium* densities, presence of acetosyringone, co-cultivation medium, temperature, pH and light conditions were further optimized. Use of optimized parameters improved the transient transformation efficiency (TTE) of *D. hamiltonii* somatic embryos to a maximum of 74.67%.

Among the studied binary vectors, pBI121 responded best for *D. hamiltonii* genetic transformation as confirmed from the high level of transient GUS expression. In contrast, only small blue spots were obtained with p35SGUSINT. The *A. tumefaciens* strain GV3101 harbouring pBI121 was reported to be the best strain/vector combination for the transformation studies in *D. hamiltonii*. The transformed somatic embryos showed positive transient as well as stable GUS expression. The transgene integration in the transformed somatic embryos was further confirmed by PCR, slot blot and Southern hybridization analysis. Though the transformants germinated on PNB medium supplemented with selection antibiotic, their germination efficiency was reduced to 15% and of the total shoots obtained, 95% were complete albinos. The plants showed strong GUS expression as well as positive signals in PCR. Albino shoots multiplied and rooted on BIN medium but failed to survive under polyhouse conditions.

Besides the *Agrobacterium* mediated approach, *D. hamiltonii* somatic embryos were also successfully transformed by biolistic mediated transformation. As reported in *Agrobacterium* mediated transformation, the vector pBI121 also attributed optimum response during biolistic method and resulted in high levels of transient as well as stable transformation efficiencies. Therefore, the binary vector pBI121 harbouring *gus* and *nptII* genes was used for optimization of parameters for biolistic mediated transformation of *D. hamiltonii*. Thereafter, a construct was prepared by replacing *gus* with *tlp* gene and used for developing transgenic plants for abiotic stress tolerance.

Different parameters were thus, extensively optimized. In this regard, concentration of gold particles played an important role during optimization. Use of lower concentrations resulted in sparse coverage over the target tissue while higher concentrations resulted in agglutination of the particles thereby, resulting in greater injury to explants. RDP was another important factor, wherein, an increase in TTE to 46.67% was recorded when the DNA-coated gold particles were propelled at 1100 psi and each Petri-plate was bombarded twice. While 900 psi RDP was unable to facilitate appropriate gold particles penetration into the target tissue; greater tissue injury due to
increased penetration force of 1350 psi RDP was probably responsible for lower TTE. Number of bombardments also affected the transformation efficiency significantly. However, a significant decline in TTE was observed when the explants were bombarded either once or thrice, irrespective of the RDPs tested. Together with the above parameters, variations in GD and TD resulted in significant variations in TTE. Maximum expression was obtained when *D. hamiltonii* somatic embryos were bombarded at ¼ inches of GD and 6 cm TD. Gradual decline in TTE was recorded whenever TD of 9 and 12 cm were used. The TTE of *D. hamiltonii* somatic embryos was also significantly influenced by the concentration of plasmid DNA. While lower concentrations (0.3 µg/bombardment) of plasmid DNA resulted in a TTE of 74%, it increased to 80.67% when gold particles were coated with 2 µg plasmid DNA/bombardment using the above optimized parameters.

*D. hamiltonii* somatic embryos bombarded as per the above optimized parameters, showed TTE of 80.67%. When the somatic embryos were bombarded twice with 4 µg plasmid DNA (2 µg/bombardment x 2) coated onto 1.5 mg gold particles (0.75 mg/bombardment x 2) using (PC2) combination of parameters i.e. 1100 psi RDP, ¼ inches GD, 6 cm TD, 16 mm MFD, the expression reduced to 40.67% after 30 days of bombardment.

The optimized parameters were further used for transferring *tlp* gene into the somatic embryos of *D. hamiltonii* by biolistic method. Both the *gus* as well as *tlp* transformed somatic embryos germinated on kanamycin supplemented PNB medium and yielded transgenic plants. All the *tlp*-shoots were healthy, green whereas, 90% of the *gus*-shoots obtained were complete albinos. Transgenic lines obtained were confirmed by PCR, slot blot and Southern hybridization analysis. Although southern hybridization analysis of *tlp*-transgenic lines revealed insertion of multiple copies of transgene, these did not interfere with the expression of *tlp* protein in the transformed shoots as confirmed by RT-PCR. The cDNAs from all the four *tlp*-transgenic lines gave specific amplification of 449 bp with specific internal primers. The plants not only showed better growth under *in vitro* conditions but also grew well under contained conditions. However, flow cytometric analysis of the *tlp*-transgenic and the CNB lines did not show any detectable differences in the DNA 2C-value. The CNB control and the *tlp*-transgenic lines were further evaluated for resistance against cold stress (4 and 10°C for 48 h). Both the RWC and chlorophyll content of CNB and *tlp*-transgenics was observed to decrease.
with the increase in the duration of cold stress. However, the decrease in the tlp-transgenics was significantly lower than CNB, irrespective of the duration and temperature treatment. Faster recovery of the tlp-transgenics (cold stressed for 48 h) was also observed. This was indicated by a comparatively higher increase in the chlorophyll content and RWC after transfer to ambient temperature of 25°C. This was further confirmed by lower electrolyte leakage and MDA content in the cold stressed tlp-transgenics as compared to CNB. Even upon transfer to ambient temperature (25°C), the tlp-transgenics exhibited lower contents of these parameters. In fact, the proline content in tlp-transgenics was always lower than CNB during stress (4 and 10°C) as well as after transfer to ambient temperature conditions.

ROS scavenging enzyme assays of CNB and tlp-transgenics showed increased SOD activity up to 24 h but declined thereafter. On the other hand, the APX activity continued to increase with the duration of stress period (4 and 10°C). The SOD and APX activity in both the CNB and tlp-transgenics also declined upon transfer to ambient temperature conditions of 25°C. Irrespective of the duration or temperature treatment, SOD and APX activities of tlp-transgenics were invariably higher than CNB.

Studies were also undertaken to understand the difference between the gus transformed albino and the green shoots. The chlorophyll contents in the green shoots i.e. CNB as well as transformed ones were 2.054-2.068 mg/g Fw while that of albino shoots ranged between 0.243-0.260 mg/g Fw. This indicated that the albino plants were not completely devoid of chlorophyll. Rather, they had lower content of chlorophyll as compared to normal green-gus and CNB plants. Further, the protein profile of the albino plant in SDS PAGE and two-dimensional electrophoresis revealed that the proteins from both albino as well as green plants were significantly different. The protein profiles of gus transformed normal green and albino plants were compared. Among the total protein spots resolved on the gel, the content of 40 proteins of gus transformed green plant was compared with that of albino plant. Up- or down-regulation of 22 proteins were observed as compared to 18 proteins in the gus-albino plant transformed by the biolistic mediated method. These 18 proteins either appeared or disappeared in the gus-albino plant. Of the 22 proteins, 13 were up-regulated, whereas, 9 were down-regulated in the gus-albino plant as compared to gus-green plant.

In conclusion, the study reports the first successful complete genetic transformation protocol by biolistic as well as Agrobacterium tumefaciens mediated
approaches for *D. hamiltonii*. Transgenic bamboo expressing *tlp* has the potential for wide commercial applications. It also paves the way for breaking the hitherto experienced blocks in transgenic bamboo production. The study shows the leads obtained can be further used for genetic transformation of other industrially important bamboos as well.