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Review article

APPLICATION OF BIOTECHNOLOGICAL TOOLS FOR EVALUATION OF GENETIC DIVERSITY, *IN VITRO* PROPAGATION AND GENETIC TRANSFORMATION IN *PARKIA TIMORIANA* (DC.) MERR. : A REVIEW

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ABSTRACT: *Parkia timoriana* (DC.) Merr., commonly known as tree bean, is a multipurpose leguminous tree commonly found throughout the northeastern Indian region. Its pods are consumed as vegetables and fetch high market price during the season. However in recent years the tree is reported to be associated with various pests and infestations accompanied with die-back symptoms leading to mass death in many locations. Thus there is an urgent need to address the issues for the multiplication and conservation of this tree. The rapid multiplication and improvement through conventional means are difficult and limited. The use of biotechnological tools for the evaluation of the genetic status, mass production and genetic improvement provides a viable option. Advances in the studies of genetic diversity, in vitro regeneration and genetic transformation in tree beans are discussed in this paper.
Key words: *Parkia timoriana*, tree bean, pest, production, conservation, biotechnology.

INTRODUCTION

Parkia timoriana (DC) Merr. Commonly known as the tree bean belonging to the family Leguminosae and sub-family Mimosoidae. It is the most widespread species of *Parkia* in the Indo-Pacific region distributed from northeast India to Irian Jaya [1]. It is a highly branched and medium height (10-12 m) multipurpose tree having potential commercial and ecological significance in the region [2]. The pods and the seeds are consumed as vegetable, salad and *chutney* in all its developmental stages or sun-dried for future use during off-season. The tree is well adapted in different agro-climatic regions from the colder hilly regions to the hotter plains. It has high nutritional and medicinal value [3, 4]. Traditional system of classifying the varieties was based on morphological and eating qualities. Trees bearing narrow and uniform pods having light green colour are superior in flavour than the others and accordingly thirteen cultivars were identified [5]. Similarly nine varieties of tree beans were reported from Manipur based on their palatability and other eating qualities [6]. Conventionally the tree is propagated through seeds and vegetative cuttings. However, vegetative cuttings are not feasible in view of low rooting percentage. Propagation using seeds is sometimes associated with with severe fungal and pest infestation during storage. There were many instances of mass-death of tree bean trees have been observed in the northeast Indian region due to the die-back symptoms. This seriously affects the socio-economic situation of the growers. Infestation of *Cadra cautella* in the pods collected from field and storage have been well documented [7]. The problems of die-back symptoms in this tree have also been found to be associated with the infestation of *Anoplophora glabripennis* (Motchulsky) commonly known as Asian longhorned beetle [8]. For a sustainable commercial cultivation of this tree, it would require large amount of superior quality planting materials that may be difficult to obtain by conventional methods of propagation. Further, the genetic improvement of any plant species requires the development of an efficient transformation and regeneration procedure for further transfer of gene of interest. In recent years, the biotechnological interventions for the sustainable production, genetic improvement and conservation of plant species is gaining popularity because of its scientific advancement and reliability. Various biotechnological tools are being used for the studies in tree beans and are highlighted as under.

Evaluation of genetic variations

Knowledge on the level, structure and origin of genetic variation within and between plant populations is a pre-requisite for the effective utilisation and conservation of the species. The factors determining the level and structure of genetic variation within plant species include evolutionary history characteristics, population density, mating system, and mechanisms of gene flow [9].

This intraspecific genetic diversity plays a critical role in the ability of populations to respond to specific adaptations such as resistance to disease or insect, tolerance to certain soil conditions or other attributes that may be of current or future value in forest tree breeding programmes [10]. Thus, the conservation of plant genetic resources involves not only preventing extinction but also ensuring the availability of resources for future use through adaptation to the changing environments [11]. Although morphological characters and agronomic traits have been used traditionally to characterise levels and patterns of diversity, these traits alone represent only a small portion of the plant genome and are also influenced by environmental factors, thereby limiting their utility in describing the potentially complex genetic structures which may exist within and between taxa [12]. To overcome these constraints, various polymerase chain reaction (PCR) based DNA markers such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and inter-simple sequences repeats (ISSRs) have been widely used for the detection of genetic variation in many crops [13]. These 'markers' of genetic variation are independent of environmental factors and more numerous than phenotypic characters, thereby providing a clearer indication of the underlying variation in the genome of an organism [14]. Using randomly amplified polymorphic DNA (RAPD) markers genetic variation of eight genotypes of tree beans grown in Manipur, India was investigated at the DNA level revealing considerable genetic variations among the genotypes collected from the different geographical locations [15]. The genetic variations in three tree bean populations in Manipur was also analyzed using inter-simple sequence repeat (ISSR) markers which showed the variations ranging from 33.33% to 18.92% [16]. The overall genetic differentiation (Gst) among populations was estimated to be 0.29 and the number of gene flow (Nm) was estimated to be 1.23 per generation between populations. Of the total genetic variance, 70.04% were attributed to within-population diversity while 4.72% differences to the among-populations

In vitro regeneration and genetic transformation

For a sustainable commercial cultivation of this tree, it would require large amount of superior quality and genetically improved planting materials that may be difficult to obtain by conventional methods of propagation. Thus, there is an urgent need for biotechnological interventions using tissue culture techniques for mass production of quality planting materials and genetic modification using gene(s) coding for insect resistance or proteinase inhibitors. The genetic improvement of any plant species requires the development of an efficient transformation and regeneration procedure for further transfer of gene of interest.

Micropropagation refers to *in vitro* mass production of plant propagules from any plant part or cell. Such propagules are used to raise whole plants. Through somatic embryogenesis individual cultured cells or small groups of cells undergo development resembling that of the zygotic embryo and the embryoids produced can be used to produce whole plants. Thangjam and Singh [17] reported the potential for *in vitro* rapid regeneration of tree bean, using cotyledon explants cultured on MS and B5 basal media supplemented with various concentrations of 2,4-D, NAA and BAP. Successful callus induction was observed in all the treatments. Maximum percentage of callus induction was obtained in the 2,4-D supplemented basal media. However, the overall response of MS medium was found to be superior to that of B5 medium. Explants cultured on MS medium fortified with combinations of 2,4-D and BAP induced rapidly proliferating calli that turned more friable and nodular. These protuberances eventually developed into somatic embryos when transferred in basal MS suspension medium without growth regulators. *In vitro* regeneration and genetic transformation were also achieved using cotyledonary node explants [18]. The ability to produce multiple shoots has been evaluated using semi-solid Murashige and Skoog (MS) basal medium and Gamborg's B-5 basal medium supplemented with various concentrations of α-naphthalene acetic acid (NAA) and 6-benzylaminopurine (BA) either in single or in combinations. The explants cultured in MS medium supplemented with combinations of 2.7 µM NAA and 11 µM BA showed the maximum frequency of multiple shoots (96.66%) formation and number of shoots per explants (6.60) respectively. For rooting full and half strength MS medium supplemented with various concentrations of indole-3-butyric acid (IBA) and NAA were studied and the highest number of root formation was observed in full strength MS supplemented with 9.8 µM IBA. Using *Agrobacterium tumefaciens* strain EHA105 pCAMBIA2301 various optimum conditions for efficient transformation were determined by recording the percentage of GUS^+ explants. Following the optimized conditions, the co-cultured explants were cultured on semi-solid shoot regeneration medium (SRM) containing MS medium + 2.7 µM NAA + 11 µM BA + 100 mg/l kanamycin + 500 mg/l cefotaxime. After 8 weeks of culture, the regenerated shoots were rooted in rooting medium (RM) containing MS medium + 9.8 µM indole-3-butyric acid (IBA), 3% sucrose, 7.5 mg/l kanamycin and 500 mg/l cefotaxime. Successful transformation was confirmed by histochemical GUS activity of the regenerated shoots, *npt*II gene PCR analyses of the regenerated kanamycin resistant plantlets and Southern analysis of putative transgenic PCR^+ plants.

Thus, the advances made on the application of biotechnological tools can be further applied to identify the superior genotypes, mass propagation and genetic improvement of this multipurpose tree legume.

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