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# Limitations, Progress and Prospects of Application of Biotechnological Approaches in Improvement of *Prosopis*: An Important Genus of the Arid Regions

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**Abstract:** Introduction and selection of fast-growing exotic tree and shrub species into the arid regions of Indian subcontinent is imperative to ensure a sustained livelihood under harsh agro-climatic conditions in the region. Large scale mortality of perennials, mechanized agriculture, industrialization and population explosion, increased demand of fuel and fodder, and cutting of trees without adequate reforestation has also fueled the concern for saving such fragile ecosystems from degradation. *Prosopis,* a multipurpose, leguminous, nitrogen fixing genus encompassing approximately 45 species of trees and shrubs remains one of the most widely accepted genera in the arid regions as many of the species of the genus are highly adapted for survival in arid, semi-arid and usar lands. However, there is an urgent need to modify and improve the undesirable features like thorniness, bushy architecture, slow growth rate and high alkaloid content in leaves of important *Prosopis* spp. by developing tailored genotypes suitable for plantation and rapid growth in these dry regions. Limited progress has been made for improvement of the genus through conventional breeding programs due to lack of identified superior parental lines, lack of knowledge about extent of hybridization possible between species, genetic linkage maps and QTL linkage maps. Conventional propagation methods i.e., through seeds and rooting of cuttings are in place, but have limitations for development of genetically uniform planting material on a larger scale. Therefore, application of modern tools of biotechnology need to be standardized for harnessing maximum benefits from this plant. Improvement of this genus through genetic transformation requires an efficient regeneration system, which is yet to be perfected for large scale production of planting material. Genetic diversity studies, taxonomic delineation of species and subspecies and also the breeding programs can be more robustly addressed using molecular markers. An attempt has been made to review the need and the scope of genetic improvement of this important genus using modern tools of biotechnology.

**Key words:** Fabaceae, *Prosopis*, micropropagation, molecular markers, tree improvement.

Arid regions are usually characterized by limited and highly variable annual precipitation, extreme variations in temperature (daily and annual) and high potential evapo-transpiration in addition to poor soil structure. Frequent occurrence of droughts, another characteristic feature of the region, has an adverse effect on agricultural productivity. To evade the adverse effects of these frequent droughts, the native people have developed production systems having both annual and perennial components. This dual component cropping system ensures sustenance of human and livestock requirements even in adverse climatic conditions. Therefore, trees are much more intricately associated with the life of human beings in the arid regions compared to other regions. In addition to providing food, feed, fodder, fuel wood and other products, trees are used as wind breaks, for improving the soil microclimate for growth of under storey crops, to enrich the soil during fallow period and to prevent soil erosion. However, the harsh agro-climatic conditions of the arid regions restrict the number of tree species suitable for growth and are also responsible for their slow growing habit. Therefore, only a limited number of sparsely distributed, slow growing tree species are usually found in the arid regions. Concern for saving fragile ecosystems from degradation has been fueled by large scale mortality of perennials, mechanized agriculture, industrialization and population explosion, increased demand of fuel and fodder, and cutting of trees without adequate reforestation. The dependence of arid populations on agri-

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silvi-pastoral/silvi-pastoral/agri-silvicultural systems for livelihood, especially in drought periods when woody components are the only support, necessitates the strengthening of the available woody germplasm diversity stock. The trees in these arid regions must be drought tolerant, fast growing and multipurpose so that maximum benefits may be harnessed with minimum inputs. Afforestation with these species will also give an added advantage of soil amelioration and sand dune stabilization. Hence, a number of exotic tree species like *Eucalyptus cameldulensis*, *E. melanophloia*, *Acacia tortilis*, *A. cilliata*, *A. raddiana*, *A. senegal*, *A. sieberiana*, *A. aneura*, *A. salicina*, *Colophospermum mopane*, *Dichrostachys glomerata*, *Brasiletta millis*, *Schinus molis* and *Prosopis juliflora* have been introduced into arid regions over the past few decades from other parts of the world, and few introductions have proved very promising for the Indian desert. CAZRI has paid considerable attention to the introduction and selection of fast-growing exotic tree and shrub species from various iso-climatic regions of the world. Among them *Prosopis* and *Acacias* are the most widely accepted genera in the arid regions. An attempt has been made here to analyze the need and scope of improvement of *Prosopis* in arid regions.

*Prosopis* (family Fabaceae, subfamily Mimosoideae) is a multipurpose, leguminous, nitrogen fixing genus encompassing approximately 45 species of trees and shrubs distributed in the drier, warmer areas of America, Africa and Asia. Use of *Prosopis* for afforestation on wastelands, erosion control, soil amelioration and fuelwood production is well documented. The most widely cultivated species of *Prosopis* in the world are *P. juliflora* (Sw.) DC, *P. alba* Griseb., *P. chilensis* (Molina) Stuntz, *P. cineraria* (L) Druce, *P. pallida* (Humb. & Bonpl. ex Willd.) Kunth and *P. tamarugo* Phil. Many of the species of the genus *Prosopis* are highly adapted for survival in arid, semi-arid and usar lands. *Prosopis* pods make excellent food for livestock and wild animals, and the flowers are a source of large quantities of nectar for bees. The wood is strong and durable, is excellent for house construction, and has a high calorific value hence is an excellent fuel wood. Some species are capable of growing rapidly, even on barren and degraded lands, while others have slower growth rates. Most *Prosopis* species have thorns, a bushy architecture and slow growth rates. These characteristics hamper their widespread cultivation. *P. juliflora* is a fast growing, hardy, drought resistant, but frost tender species, having the ability to thrive in hostile climatic and edaphic conditions. It grows in all type of soils, from sandy to saline-alkaline soils. It has proved to be the most versatile species for afforestation on shifting sand dunes, coastal dunes, river beds, saline-alkaline lands, eroded hill slopes, mine-spoiled areas and other wastelands (Muthana and Arora, 1983). About 84% of the 45 species of *Prosopis* described by Allen and Allen (1981) nodulate and fix atmospheric nitrogen, 12 showed rhizobial nodules. However, the efficiency of nodulation varies among the species. Subba Rao *et al.* (1982) classified the *Rhizobium* nodulating *P. juliflora* as belonging to the cowpea group. Plant root exudates and activity of rhizospheric microbes associated with the plant roots play important role in establishment and growth of healthy plants in hostile environment. Nitrogen fixing bacteria associated with tree legumes are important for reclamation of degraded arid lands.

## Introduction of Exotic Species of *Prosopis*

Only one species, *P. cineraria* is endemic to the Indian sub-continent while all others have been introduced. *P. juliflora* which now occurs throughout the arid and semi-arid tropics of the Indian sub-continent was first introduced in 1857 from Latin America. The first systematic plantations were carried out in 1876 in the Kaddapa area of Andhra Pradesh and then it was introduced into parts of Gujarat in 1882 and into Sindh (Pakistan) in 1877. The ruler of the princely state of Marwar introduced *P. juliflora* into Rajasthan in 1913 and declared it a "Royal Plant" in 1940. Since then, the species was gradually introduced in many parts of the country including Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, Maharasthra and Tamil Nadu. Due to its wide ecological amplitude and rapid colonizing ability, the species has spread rapidly over a large part of the arid and semi-arid tracts, with the exception of frost prone areas (Harsh and Tewari, 1993). It is also known as the "miracle tree" for dry zones. At present, *P. juliflora* is thought to inhabit more than 52.64 Mha area, although plant densities vary with different areas and habitats. Introduction of other species of *Prosopis* began only after 1960. In 1962, *P. chilensis* and *P. tamarugo* were introduced into Rajasthan and *P. alba* and *P. pubescens* were introduced at CAZRI, Jodhpur, in 1966. These were simple introductory trials to assess the adaptability of these species to the arid environmental conditions of India. A further trial involving six other exotic provenances of *P. juliflora* was established in 1973, and in 1985, under a FAO/IBPGR programme, *P. pallida* was also introduced at CAZRI, Jodhpur. *P. alba*, *P. chilensis*, *P. glandulosa*, *P. hassleri*, *P. nigra*, and *P. pallida* were introduced into the saline-sodic soils of Uttar Pradesh in 1980 and into the saline-sodic soils at Karnal, Haryana, in 1985. In 1991, under a joint Indo-US research project (PL-480), 232 accessions of several *Prosopis* species (*P. alba*, *P. chilensis*, *P. flexuosa*, *P. nigra*, a *Prosopis* hybrid and Peruvian *Prosopis* sp.) were introduced at CAZRI, Jodhpur (Harsh and Tewari, 1993). Table 1 details the exotic *Prosopis* species evaluated at CAZRI, Jodhpur.

## Improvement of *Prosopis* spp. using Biotechnological Approaches

*P. cineraria* (L) Druce, having a deep root system, a monolayered canopy and the ability to fix atmospheric nitrogen, is compatible with agri-horticultural crops boosting the growth and productivity of companion plants. Trees are also being planted for sand dune stabilization, reclamation and rehabilitation, but its slow growing nature hampers the pace of the reforestation programs. On the other hand, plantations of *P. juliflora* were initially established mainly for the purpose of conservation, but it has now become the main source of fuel in rural and urban areas, fulfilling more than 70% of the firewood requirements in the tropical arid and semi-arid regions of the country. The seed pod is used as a livestock feed, and the wood is also used in charcoal making and occasionally as a timber. Also, this plant retains a lush green color during summer when all other desert trees show signs of drought stress. Despite all these advantages, rural people are against the plantation of this species because of its stiff spines. It grows ever-closer to the edge of rural roads, restricting the path for animal driven carts creating congestion on the main roads and leading to frequent accidents. The stiff thorns often get in the soft foot pads of camels resulting in wounds and subsequently getting septic or even the death of the animal.

With the growing trend towards plantation forestry and genetic improvement of economically important forest trees, it has become necessary to develop improved planting material. Despite their local use, *Prosopis* trees have not received the same

*Table 1. Exotic Prosopis species evaluated at CAZRI, Jodhpur*

| Species | Common name | Native to | Introduced from | Traits of interest |
|---|---|---|---|---|
| *P. alba* Griseb | Algarrobo Blanco | South America | Chile, Israel | Fast growing, thornless, sweet poded |
| *P. chilensis* (Molina) Stuntz (syn *P. siliquastrum* DC) | Chilean mesquite, Algarrobo Chileno | Central America | Chile, Israel | Fodder tree, sugar rich pods |
| *P. flexuosa* DC | Carob tree, Algarrobo Negro | South America, Argentina | Chile | Tolerant to drought, cold and salt |
| *P. glandulosa* Torr. | Honey Mesquite | South western America, Mexico | Mexico | Agroforestry tree |
| *P. juliflora* (Sw.) DC | Bayahonda Blanca, Vilayati babul | Peru | Mexico, USA | Fast growing, protein rich pods |
| *P. nigra* (Griseb.) Hieron (syn *P. algarrobilla*) | Algarrobo Negro, Algarrobo Amarillo, Algarrobo Dulce, Algarrobo Morado | South America | Nigeria | Water logging resistant, sweet and starchy mesocarp |
| *P. pallida* (Humb. & Bonpl. ex Willd.) Kunth | American Carob, Kiawe | Central America, Peru and Ecuador | Latin America | Thornless, sweet pods, salinity tolerant |
| *P. tamarugo* Phil. | Tamarugo | Chile | Chile | Drought and salt tolerant, palatable leaf fodder |

selection and improvement as a major industrial forestry species should receive. Keeping in view the ecological and economic importance of *Prosopis* species in the livelihood security of arid rural people and the need to conserve the arid ecosystems there is an urgent need to modify and improve the undesirable features of *Prosopis* spp. to develop tailored genotypes suitable for plantation and rapid growth in these dry regions. Species can be selected based on the desired end-products/use.

Tree growth is influenced by a number of factors including genotype, agro-climatic conditions and cultural practices. Adequate supply of nutrients is of utmost importance and depends on soil conditions. Availability of most nutrients is higher in low pH rather than high pH soils, and nutrient availability is therefore a limiting factor at sites having alkaline soil. High soil salinity and alkalinity have degraded about 8.5 Mha of once productive land in India. The severity of the problem is increasing, and several million hectare of the canal irrigated area in the arid and semi-arid regions of the country run the risk of being degraded (Singh, 1993). Behl and Goel (1993) studied the potential of six indigenous and exotic *Prosopis* species for short rotation fuelwood forestry on degraded lands and reported that *P. alba* is a promising species for short rotation fuelwood plantations on alkaline soil sites, followed by *P. juliflora* and *P. velutina*. *Prosopis* is moderately tolerant to alkali conditions, although growth may be restricted at very high pH (pH >10.0). In such situations special site preparation techniques and amendments (soil applications) may be needed for raising plantations. Many species of *Prosopis* can tolerate 6,000 mg $L^{-1}$ salinity with no reduction in growth while *P. velutina* can tolerate 12,000 mg $L^{-1}$ salinity, and *P. articlulata*, *P. pallida* and *P. tamarugo* can grow successfully in salinity levels of 18,000-36,000 mg $L^{-1}$ (Singh, 1993). Identification of genes conferring salt tolerance to these species can lead to development of salt tolerant varieties in other crop species also. MSSRF foundation, Chennai, has identified and isolated three metallothiones from *P. juliflora*, which have been successfully transferred to *Nicotiana tabaccum* for heavy metal tolerance (http://www.niam.res.in/pdfs/MSSRF%20-%20 Baramati.pdf). Such transformed genotypes may be very useful in reclamation of degraded lands rich in heavy metals. The use of inter and intra-species grafting can also be used to achieve rapid reforestation of saline/alkaline soils. For example in India, superior plantations could be raised by grafting fast growing, erect and thornless *P. alba* scions onto salt/alkaline tolerant *P. juliflora* rootstocks (Singh, 1993).

The leaves of *P. juliflora* are not suitable livestock feed due to the presence of high levels of alkaloids. There is a need to understand the alkaloid biosynthetic pathway in detail so that transgenics with reduced levels of alkaloids can be created. Development of genotypes having low alkaloid content in leaves and sweet pods using biotechnological interventions, including RNAi or antisense technology, can improve the social acceptance of this species. Similarly, introgression of thornlessness and sweet pod characters into fast growing *P. juliflora* can enhance its social acceptability. Presence of thornless character in *P. pubescens, P. alba* and *P. pallida* and sweet sugary pods/mesocarp in *P. alba, P. chilensis, P. nigra* and *P. pallida* affords the possibility of inculcating thornlessness and sweet pod characters into *P. juliflor*a following conventional or marker based breeding. Similarly, *P. cineraria* is an ideal agroforestry tree, however, its slow growth behavior needs to be modified using fast growth feature available in *P. juliflora* and *P. alba*.

*Prosopis* species is self incompatible, and cross pollination results in a great deal of variation among genotypes with respect to their growth, thorniness and other morphological traits. However, selection of single stemmed, thornless trees followed by their propagation would be desirable for agroforestry purposes (for growth of under storey plants) as well as timber requirements. Clonal planting material is of considerable importance in forestry because it offers the advantage of uniformity of growth and development by eliminating genetic differences between trees and by making immediately available superior individuals for seed orchards and plantations. Multiplication and establishment of cuttings remained an appropriate method to raise homogeneous plantation stands till recently. Felker and Clark (1981) obtained over 70% rooting with stem cuttings of *P. alba*, *P. chilensis*, *P. glandulosa*, *P. pallida* and *P. velutina*. However, only 6 to 40% success was achieved in vegetative propagation of introduced *Prosopis* species using stem

*Table 2. Diversity studies in Prosopis spp.*

| Marker used | Achievement | Reference |
|---|---|---|
| Isozymes | Used isoenzyme electrophoresis to analyze the variability and phenetic relationships among seven American species of *Prosopis* belonging to three different sections: *P. argentina* (Monilicarpa), *P. glandulosa, P. velutina, P. flexuosa, P. ruscifolia, P. kuntzei* (Algarobia), and *P. reptans* (Strombocarpa). The genetic variability in *P. argentina, P. reptans* and *P. kuntzei* was significantly lower than in the rest of the species analyzed. The results suggest that the two North American species (*P. velutina* and *P. glandulosa*) could have originated in different founder events. | Bessega *et al*., 2005 |
| Isozymes, RAPD | Compared allozyme and RAPD techniques for their usefulness for genetic and taxonomic studies in *P. glandulosa* and *P. velutina* populations. Isozymes and RAPDs yielded similarly high estimates of genetic variability. Two isoenzyme systems (GOT and PRX) distinguished between *P. glandulosa* and *P. velutina*, but no diagnostic band for recognition of populations or species studied were detected by RAPD. However, RAPD markers showed higher values for genetic differentiation among conspecific populations of *P. glandulosa* and a lower coefficient of variation than those obtained from isozymes. | Bessega *et al*., 2000 |
| RAPD | Analyzed the taxonomy of 15 *Prosopis* species from Sénégal, India and Argentina. Molecular markers were identified which were present in all species analyzed, only in American species, specific to the sections Strombocarpa and Algorobia, except *P. kuntzei*. RAPD markers clearly distinguished the five sections of the genus. The results suggest that regrouping of species within the section Algarobia may be required. | Ramírez *et al*., 1999 |
| RAPD | Analyzed *P. alba, P. ruscifolia, P. nigra, P. flexuosa* and *P. vinalillo* for obtaining markers for identification of species and hybrids. Five bands provided a tool for their identification, with the exception of *P. vinalillo*. | Ferreyra *et al*., 2004 |
| RAPD | Used RAPD markers to distinguish between *P. juliflora* and *P. pallida*. The genetic similarity observed between *P. juliflora* and *P. pallida* was similar to the value in sympatric *Prosopis* species in North America, Species-specific markers confirmed that material in Burkina Faso was *P. juliflora*, but suggested that material collected in Brazil, Cape Verde and Senegal was *P. pallida*, whereas this was previously identified as *P. juliflora*. | Landeras *et al*., 2006 |
| RAPD | Studied eight exotic species of *Prosopis* (*P. pallida, P. articulata, P. glandulosa, P. chilensis, P. nigra, P. flexuosa, P. alba* and *P. juliflora*) belonging to section Algarobia. Seven RAPD markers could delineate the eight species into two clusters and three species as out groups. | Singh *et al*., 2011b |
| RAPD | Compared six populations of *Prosopis* in Netherlands to determine whether established stands consist of a single or mixture of species. RAPD markers clustered the populations from Marigat, Bura and Isiolo with *P. juliflora*, Bamburi with *P. pallida* and Taveta with *P. chilensis* while the population from Turkwel was a putative hybrid between *P. chilensis* and *P. juliflora*. | Muturi *et al*., 2012 |
| RAPD, ISSR | Studied diversity within and among the populations of *P. cineraria* and *P. juliflora* collected from different locations of Qatar. 21 ISSR bands and only 3 RAPD bands were able to distinguish the two species. | Elmeer and Almalki, 2011 |
| RAPD, SSR | Assessed 12 *Prosopis* species and a putative hybrid *P. pallida* x *P. juliflora* for genetic relationships and concluded that *P. pallida* and *P. juliflora* are not closely related despite some morphological similarities. | Sherry *et al*., 2011 |
| ISSR, DAMD | Determined diversity within and among the populations of *Prosopis cineraria* (L.) accessions collected from different districts of Rajasthan (India). Study concluded that *P. cineraria* is accompanied by high genetic diversity within the population and elevated gene flow showing indications of adaptation to callous and fragile dry conditions of arid environment. | Sharma *et al*., 2011 |

cuttings at CAZRI, Jodhpur, under open nursery conditions (Harsh and Tewari, 1993). Application of improved biotechnological methods like micropropagation can ensure mass multiplication of selected superior genotypes in shorter periods for plantation purposes. Micropropagation under controlled aseptic conditions can enhance the rate of multiplication manifold and will also overcome the limitation of availability of propagules for vegetative propagation. Further, an efficient regeneration system needs to be in place for improvement of this genus through genetic transformation. Two distinct patterns of *in vitro* differentiation, i.e., organogenesis and somatic embryogenesis have been used for micropropagation of *Prosopis*. Organogenesis has been induced through axillary shoot production using explants from seedlings (Batchelor *et al*., 1989; Nandwani and Ramawat, 1992; Rubluo *et al*., 2002) as well as from mature plants (Shekhawat *et al*., 1993; Caro *et al.,* 2002; Kumar and Singh, 2009), while somatic embryogenesis was achieved using immature zygotic embryos as explants (Bundia-Gonzalez *et al*., 2011). Genetic transformation studies have not yet been initiated in *Prosopis* probably due to lack of standardized micropropagation protocols and field trials. The feasibility of micropropagation technology has been demonstrated in *P. glandulosa* var. *torreyana* (Rubluo *et al*., 2002), *P. cineraria* (Goyal and Arya, 1984; Batchelor *et al*., 1989; Nandwani and Ramawat, 1993; Kumar and Singh, 2009), *P. laevigata* (Bundia-Gonzalez *et al*., 2011), *P. juliflora* (Batchelor *et al*., 1989; Nandwani and Ramawat, 1991), *P. alba* (Tabone *et al*., 1986), *P. chilensis* (Batchelor *et al*., 1989; Caro *et al.,* 2002) and *P. tamarugo* (Nandwani and Ramawat, 1992) however; more efforts are required to develop protocols for mass multiplication so that plants may be produced for large scale plantations on a commercial scale. Hardening and acclimatization of tissue culture raised plants remains the major bottle neck in commercialization of this technology, however, use of associated nodulating *Rhizobia* during the procedure can improve the survival percentage as well as health of the tissue culture raised plants. Singh *et al*. (2011a) isolated and identified the root nodulating rhizobia in *P. cineraria* as *Sinorhizobium kostiense* and *S. saheli* using molecular marker techniques. The partial sequences of 16s rDNA were submitted in public database and given accession numbers.

Various fungal pathogens attack *Prosopis* at nursery (Root rot-*Fusarium* sp.; Collar rot-*Macrophomina phaseolina* (Tassi) Goid) and plantation (Stem canker-*Botryodiplodia theobromae;* Leaf blight-*Septoria prosopides;* Twig blight and dieback-*Diplodia prosopides* Stev. & Peir.) stages (Srivastava and Mishra, 1993). Similarly, *Prosopis* is susceptible to many insect pests including *Oxyrachis tarandus* Fabricius, *Aleyrodids* spp., *Taragama siva* Lefevre, *Poekilocerus pictus* Fabricius, *Halys dentatus* Fabricius (Cimex), *Microtermes mycophagus* Desneux, *Eurybrachys tomentosa* fabr. *Homoeocerus signatus* Walker, *Drosicha* spp. (Yousuf and Gaur, 1993). However, no registered pesticides or fungicides are available for the species. The damages caused by the insects and pests can be controlled to some extent by physical, chemical and biological methods, however, development of genetically engineered plants capable to counter such biotic stresses is imperative.

Characterization of germplasm is essential for identifying individual genotype as well as the extent of variability existing among the accessions. Germplasm characterization and evaluation is the first step in a breeding program. The comprehensive information obtained from such an exercise would help breeders, geneticists and conservationists to effectively utilize the valuable genetic resources. Diversity studies have been initiated in localized pockets in *Prosopis* growing regions of the world using isozyme (Bessega *et al*., 2005), Randomly amplified polymorphic DNA (RAPD), directly amplified minisatellite DNA (DAMD), inter-simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers (Ferreyra *et al*., 2004; Elmeer and Almalki, 2011; Sharma *et al*., 2011; Sherry *et al*., 2011; Singh *et al.,* 2011b) (Table 2), which need to be further strengthened.

Identification of superior paternal parents is a prerequisite for achieving the breeding goals. The conventional breeding programs for improving quantitative traits require large inputs of labor, land, and financial resources. Therefore, plant breeders are motivated to identify promising lines as early as possible in the selection process. Marker assisted selection (MAS), which uses DNA markers to select optimal parental genotypes, is an excellent tool for selecting beneficial genetic traits in crops and forest trees at an early stage as well as for assessing the genetic potential of specific genotypes prior to phenotypic evaluation. Molecular markers linked with QTL/major genes for traits of interest must be developed. However, non-availability of mapping populations and substantial time needed to develop such populations may prove to be a major limitation in the identification of molecular markers for specific traits. Additional limitations to this technology include the lack of knowledge about extent of hybridization possible between species and non-availability of linkage maps. Availability of a broad genetic base is must for initiating breeding programs in any given crop. The available genetic base can be broadened using modern tools of biotechnology including *in vitro* selection of somaclonal variations, mutagenesis and transgenics.

Taxonomic delineation of species and subspecies and also the breeding programs can be more robustly addressed using molecular markers. Knowledge of genetic relationships in parental varieties could improve the effectiveness of breeding programs. Breeding programs have not yet been initiated rigorously in this important species. Catalano *et al.* (2008) studied diversification and evolution in 30 *Prosopis* species, representing all sections and series and the complete geographical range of the genus, using an approach combining molecular phylogeny, molecular dating, and character optimization analysis. Phylogenetic relationships were inferred from five different molecular markers (Mat K-trn K, trn L-trn F, trn S-psb C, G3pdh, NIA). They suggested that *Prosopis* is not a natural group. Molecular dating analysis indicated that the divergence between Section Strombocarpa and Section Algarobia plus Section Monilicarpa occurred in the Oligocene, contrasting with a much recent diversification (Late Miocene) within each of these groups. The diversification of the group formed by species of Series Chilenses, Pallidae, and Ruscifoliae was inferred to have started in the Pliocene, showing a high diversification rate. The moment of diversification within the major lineages of American species of *Prosopis* was coincident with the spreading of arid areas in the Americas, suggesting a climatic control for diversification of the group.

*Prosopis* grows under very harsh agro-climatic conditions in the arid regions therefore, must be harbouring many important genes conferring abiotic stress tolerance to the species. Therefore, the germplasm must be conserved and analyzed properly so that these abiotic stress tolerance genes may be identified and harvested for use in other economically important crops and trees in the future. George *et al.* (2007) constructed and analyzed the cDNA library of *P. juliflora* for characterization of genes that contribute to combating abiotic stress. Random expressed sequence tag (EST) sequencing of 1750 clones produced 1467 high-quality reads which were classified into functional categories, and BLAST comparisons revealed that 114 clones were homologous to genes implicated in stress response(s) and included heat shock proteins, metallothioneins, lipid transfer proteins, and late embryogenesis abundant proteins. Of the ESTs analyzed, 26% showed homology to previously uncharacterized genes in the databases. Two of the abundant genes coding for a non-specific lipid transfer protein and late embryogenesis abundant protein were sequenced completely.

Conservation of agronomically important cultivars through *in-vitro* methods and cryopreservation. It will be the most promising answer to biological and climatic hazards which may threaten the germplasm maintained *in situ* in field genebanks and germplasm gardens. Cryopreserved material (stored as seeds, ovules, embryos, callus, etc.) can be used successfully for breeding in future.

## Conclusions and Future Prospects

*Prosopis* is an ecologically important genus introduced in the arid and semi-arid regions of India. Limited progress has been made in improving the taxon through conventional breeding programs, but some traits which need further improvement include yield (pod size and quality), harvesting ease (thornlessness, seed extraction from pods), palatability, local climatic adaptation, and disease and pest resistance. Vulnerability of *Prosopis* spp. to an array of biotic and abiotic stresses, need for elite planting material for commercial plantations, limitations of conventional breeding and propagation methods, lack of proper estimation of available diversity, and conservation strategies necessitates the application of plant biotechniques encompassing plant tissue culture and genetic engineering, and application of molecular markers for its improvement. An outline of tentative biotechnological interventions required in the genus *Prosopis* is summarized in Figure 1. Development of biotic and abiotic stress tolerant improved fast growing, thornless and sweet poded genotypes of *Prosopis* can lead to rapid reforestation of arid degraded

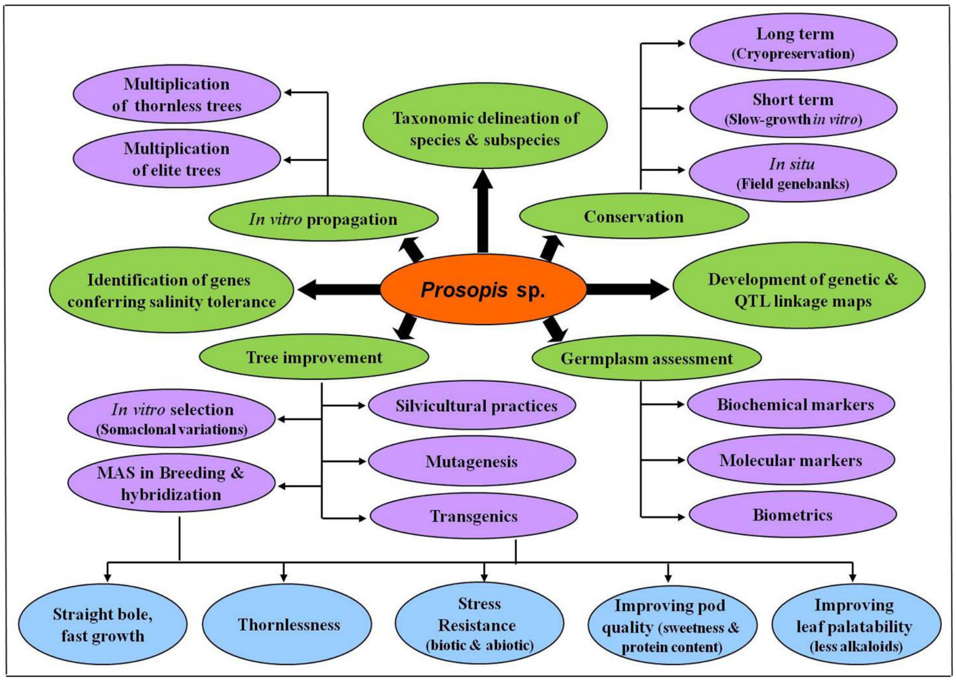


*Fig. 1. Strategies for improvement of Prosopis using conventional and biotechnological tools.*

lands restoring their productivity. *Prosopis* has the capacity to improve the environment and economic development of the regional population if systematic reforestation programs with improved genotypes are undertaken in the region.

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