

Review

Genetic diversity in *Aloe* and its breeding strategies

GALAGUKANNAN and S GANESH

Faculty of Agriculture and Animal Husbandry
Gandhigram Rural Institute (Deemed University)
Dindigul 624302 Tamil Nadu, India
Email for correspondence: gakannan@rediffmail.com

ABSTRACT

Aloe vera gained its commercial importance in medical and cosmetics industry recently. Currently it is being collected from the wild sources posing a serious problem of depletion of its resources in its natural stands. Therefore it is important to bring *A vera* into the mainstream of cultivation by developing improved varieties and its agronomic practices as there is no improved or notified varieties across the country for its commercial cultivation. As genetic divergence is the pre-requisite for any crop improvement programme, an attempt has been made to review the genetic diversity reported by various workers to arrive at an assessment of genetic diversity available in *Aloe* both at inter- and intra-specific levels. The review revealed that there is a quantifiable huge genetic diversity existing in *Aloe* for its growth, yield and quality parameters. The practical utility of using molecular marker analysis like RAPD, AFLP and ISSR to estimate the genetic diversity at molecular level has also been reviewed. The primers having high polymorphism information content value for diversity studies and primer with the highest discrimination index used for identification of different accessions were also suggested. The intra- and inter-cluster distances worked out by D^2 statistics and cluster analysis also revealed the existence of substantial diversity among the accessions and hence there is a wide scope to initiate necessary programmes to evolve high yielding varieties and/or hybrids adopting methods such as clonal selection, hybridization, mutation breeding etc.

Keywords: *Aloe vera*; diversity; crop improvement; cultivation; wild

INTRODUCTION

World Health Organization (WHO) has estimated that about 80 per cent of the world's population relies on plant based traditional systems of medicine for its primary health needs. There are around 20000 species of plants which have been documented worldwide for their medicinal value of which approximately 5000 species

are phytochemically studied. There is a growing demand for herbal products as phytochemicals, nutraceuticals, food supplements and cosmetics in both domestic and international markets (Tewari 2005). Considering the growing demand for raw materials of medicinal plants by the pharmaceutical companies and their depleting resource base due to unscientific gathering from the wild, it is of utmost

necessity to take up ex situ cultivation and conservation of these medicinal plant species and *Aloe vera* is one among them (Hussain and Hore 2007).

Aloe vera Linn (2n= 14) is a succulent belonging to the family Liliaceae. It grows naturally in Africa, America, Europe and Asia. It has fibrous, spreading and shallow root system. Its bright green gelatinous delicate leaves contain a very small quantity of viscous yellow fluid known as *Aloe* juice or *Aloe* latex which is embedded in the pericyclic cells of the vascular bundles of *A vera* leaves. The main constituents of the latex are anthraquinones including the hydroxyanthracene derivatives, aloins A and B, barbaloin, isobarbaloin and emodin (Bradley 1992). The dried latex otherwise known as aloin has been primarily used as a laxative or cathartic agent in traditional medicines for over two centuries and products containing aloes/aloin have been in market in major western countries (Joshi 1998). It is an official drug in the US pharmacopeia (Grindlay and Reynolds 1986). *A vera* gel is the colourless mucilaginous substance obtained from the paranchymatous cells of fresh leaves. The constituents of gel include mono and polysaccharides, vitamins, minerals, amino acids and enzymes (Bruneton 1995). The leaf gel has been known to possess important biological properties such as anti-inflammatory, antibacterial, anti-tumour, anti-allergic, infected wound healing by immuno enhancement and general tonic. Based on

these biological properties of aloe gel and pharmacological properties of aloin a number of formulations are widely available in the market especially in USA for the treatment of skin disorders, wound healing, inflammation-based disorders and as general skin care, cosmetics and health food (Joshi 1998).

The current global turnover of raw *Aloe* leaves amounts up to US \$70-80 million which is expected to grow at a rate of 35 per cent in the next five years. The current global trade is estimated at around US \$1 billion for processed derivatives and 25 billion for value added products of *A vera*. USA supplies the major bulk of *Aloe* in world market having a share of 60-65 per cent whereas Latin American countries share is 20-25 per cent and Australia, China and India cumulatively have a market share of only 10 per cent (Das and Chattopadhyay 2004). The area under *A vera* cultivation worldwide is 23589 ha of which American countries possess 19189 ha, Australia 4170 ha, Africa 300 ha and India possess only negligible area. India is among the few countries endowed with the unique geographical and climatological features essential for the cultivation of *A vera*. Because of its huge demand and vast utility, now it is widely collected indiscriminately from the wild source and thus this species is becoming commercially threatened due to over and destructive harvesting from natural stands (Ghate et al 2002). So far its commercial cultivation is not popular

among the farming community and the reason is simple: lack of improved varieties or technical know-how on local strains or land races and its package of practices including processing. This is the need of the day to bring the plant into mainstream of cultivation by developing and releasing improved varieties suitable for both gel and latex yield and quality besides standardizing agronomic practices in order to meet its ever growing demand and to prevent the pressure on wild population. Hence an understanding of the extent of existing genetic diversity is imperative for germplasm collection and to take up crop improvement programmes.

Nomenclature, species diversity and its geographical origin and distribution

Several scientists considered *A vera* as a species of the family Liliaceae (Wang and Strong 1993, Femenia et al 2003, Saha et al 2005). Liliaceae is the large and heterogeneous family comprising over 250 genera with 3700 species. Most of the species classified under this family are perennial herbs with rhizomes and bulbs. Hence the family has recently been reclassified and divided into smaller and more homogenous families namely the Asparagaceae, Alliaceae, Asphodelaceae, Dracanaeaceae, Eriospemiaceae and Hyacinthaceae. Further the Asphodelaceae family was divided into two sub-families viz Asphodeloideae and Aloeideae. The genera *Aloe* has been placed in the sub-family Aloeideae. On the other hand some workers consider the above two sub-families

(Asphodeloideae and Aloeideae) as distinct families viz Asphodelaceae and Aloaceae (Yenesew et al 1994). Dahlgren et al (1989) stated that the order Asparagales houses the family Asphodelaceae which is subdivided into two sub-families the Asphodeloideae and the Aloeideae. The Aloeideae consists of six genera of which the *Aloe* is the largest. According to Smith and Van Wyk (1998) both the sub-families should be included in a single family the Asphodelaceae.

Until late sixties, 314 species of aloes have been described in classic Monographs of Reynolds (1966). However Harding (1979) identified more than 360 species. Some of them are tree like with their leaves at ground level. They occur over most of Africa, southern Arabia and Madagascar. A few species have been cultivated in and around the Mediterranean region and from there reached in Japan in Far East and Latin America in west thus the plant was introduced throughout the world (Reynolds 1985a). Since it is widely distributed starting from Japan in east to Latin America in west its origin appears to be speculative. It has been introduced and naturalized throughout the tropics and warmer regions of the world including West Indies and Bahamas, Southern USA, Mexico, Central America, Arabia, India and other parts of Asia.

The nomenclature of *A vera* has been very confused and the plant has also been known as *A barbadensis* Miller and

A vulgaris Lamerck (Reynolds 1966). Until recently the official name of *A barbadensis* Miller was popularly referred to *A vera* but as per the WHO monograph (1999) *A vera* (L) Burm f is accepted as the legitimate name for this species. But United States Department of Agriculture (USDA) has preferred the scientific name *A vera* (L). Singh et al (1995) reported that the genus *Aloe* is indigenous to African continent and Mediterranean countries such as Greece and southern Italy. It grows wild on the islands of Cyprus, Malta, Sicily, the Canary Cape and Cape Verde and has naturalized over arid tracts all over India. Of the 250 species, 42 belong to Madagascar region, 12 to 15 to Arabian Peninsula and rest are distributed over tropical South Africa. In India only 4 species are reported to occur and of these *A barbadensis* (syn: *A vera*) is the most widely naturalized species. This taxa comprises several botanical varieties viz *officinalis*, *chinensis* and *litoralis*. Anon (1962) reported that due to the natural crossing between these botanical varieties a large number of intermediary forms have been found to occur in semi-wild state.

Interspecific diversity in *Aloe*

United States Department of Agriculture (USDA) gave the description of eight species of *Aloe* placed in Aloaceae family viz *A arborescens* (Candelabra aloe), *Aloe ferox* (cape aloe), *A graminicola*, *A perryi* (Perry's aloe), *A schoenlandi*, *A secundiflora*, *A socotrina* (Fynbos aloe) and *A vera* (Barbados aloe).

Considerable diversity was recorded among the different species of *Aloe* for its growth characters and aloin content. Nodushan et al (2004) observed the differences in the number of chiasmata and meiotic behaviour attributed to genomic differentiation between populations of *A litoralis* and *A vera* collected from southern part of Iran and studied for its chromosome behaviour during meiotic cell division.

Groom and Reynolds (1987) examined 68 *Aloe* species for barbaloin levels in their leaf exudates. They found the variability in barbaloin content between 10 to 20 per cent in exudates of different *Aloe* species. *A ferox* recorded a relatively low barbaloin content of 9.7 per cent. Among the 68 species compared, *A perryi* recorded the highest barbaloin content of 23.5 per cent. Van Wyk et al (1995) stated that the content of aloin, aloeresin A and aloesin of leaf exudates varied with the geographical variation. Reynolds (1985b) screened 240 species of *Aloe* for its barbaloin content and found that only 85 species contained barbaloin ranging from 10 to 20 per cent. Though there is a variation in barbaloin content ranging from 10 to 30 per cent, a desirable species should yield around 20 per cent of it.

Considerable variation in terms of different components of gel was also reported from the different species of *Aloe*. Thus polysaccharides containing

galacturonic acid (Ovodova et al 1975) or mannose (Yagi et al 1977) have been described in the gel from *A arborescens*. *A plicatalis* was found to contain a single type of unbranched acetylated glucomannan (Paulsen et al 1978) and a similar substance with a glucose/mannose ratio of 1:3 was described from *A vohambe* (Radjabi et al 1983). According to Yagi et al (1984) *A saponaria* gel had two polysaccharides, one an acetylated linear mannan and the other being acetylated branched glucomannan with a glucose and mannose ratio of 1:19.

Rebecca et al (2003) studied the phytochemical profile and identified the main phenolic compounds from the leaf exudates of *A secundiflora* by HPLC mass spectroscopy. Their study revealed that the exudates of *A secundiflora* comprised mixture of phenolic compounds mainly anthrones like aloenin, aloenin B, isobarbaloin, barbaloin and other aloin derivatives, chromones and phenyl pyrones with a low content of polysaccharides and aliphatic compounds.

Intraspecific diversity in *A vera* for growth characters

Grindlay and Reynolds (1986) described the anatomical and morphological features of *A vera* as a perennial succulent with fleshy leaves arising in a rosette from a short stem. In young plants the leaves appear at ground level but the stem can grow up to 25 cm long in older plants.

Number of leaves may vary from 15 to 30 per plant with more or less erect top young leaves to spreading older lower ones. The length of leaves measures up to 50 cm with a base width of 8 to 10 cm and tapers at the end with saw like serrations along the margins. In transverse section, the leaves are slightly concave on their abaxial surface. In young plants as well as in suckers which arise from the plant base the leaves are bright green in colour with irregular whitish spots on both sides. Fully mature leaves appear grey-green in colour without spots. The inflorescence is a dense raceme borne on a peduncle measuring to a height of 30 to 50 cm arising from the centre of the leaf rosette. The flowers are pendent measuring to a length of 2 cm with a tubular yellow perianth.

A vera represents typical liliaceous flowers with trimerous pentacyclic plan. Flowers in compact raceme appear on 60-90 cm long, slender, simple or 1-2 branched peduncle. Wide range of flower colour from yellow, pale orange to salmon red has been reported by number of earlier workers (Lakshmisarasimhan and Sharma 1991, Sharma et al 1996). Still others considered plants with orange coloured flowers as distinct variety *A vera* var *chinensis* (Bailey 1939, Graf 1981) and its common occurrence has been recorded in the west coast regions of India (Trivedi 1963). However *A vera* and *A vera* var *chinensis* are considered as morphotypes and as such have been merged in a single

species ie *A barbadensis* Mill. The study conducted by Alagukannan (2011) with twenty one accessions of *A vera* collected from different parts of South India also confirms the existence of variation among the accessions in terms of phenotypic characters viz plant stature, leaf orientation, leaf colour, blotches on young leaves, spine colour, branching pattern of flower stalk, flower colour and latex colour. This could be due to the evolution of intermediary types by the natural crossing of three botanical varieties of *A vera* viz *officinalis*, *chinensis* and *litoralis*. The distinguishing features of these botanical varieties are described by Yogeewaran et al (2005).

Aloe vera var *chinensis* Baker is common all over the Deccan in which leaves have a distinct purple colour towards the base and the spines are not sharp.

A vera var *litoralis* koening ex Baker is found on the beach shingles in Madras right up to Rameswaram has smaller leaves with a dentate margin.

Aloe vera var *officinalis* Baker is found in the parts of Maharastra. It has large fleshy green leaves with sharp spines and white specks at the base of the leaves.

Though considerable variation exists among the plants described as *A vera* some of them follow the description given by Grindlay and Reynolds (1986) while the

others have dark green leaves arranged in a fan shape than in a rosette. Ghate et al (2002) studied the flowering and fruiting behaviour of large number of *A vera* both wild and cultivated in two subsequent years from different regions of Pune district of India. They found the wide variation in flower colour and other quantitative floral and fruiting traits. The traits expressed high variations included number of flowering axis, length of flower axis, length of raceme, number of flowers per axis, number of fruit bearing axis, fruit length, fruit diameter, number of seeds per fruit, seed length and seed diameter.

Cytological studies in *A barbadensis* conducted by Abraham and Prasad (1979) showed that the chromosome complement is bimodal and is based on $2n=14$. A triploid ($2n=21$) has also been reported from monazite region and Kanyakumari (Cape Comorin). Ayyangar and Sampathkumar (1976) carried out karyological studies on three varieties of *Aloe* found in India and came to believe that the diminution in chromatin content in the species was possibly the chief method in the evolution of these distinctive cytotypes in nature. A preliminary study conducted by Wang et al (2001) on creation of variation by polyploidy induction using colchicine also revealed that the leaves of polyploidy plants were thicker, larger, darker in colour and with larger stomata and less in number than the normal diploid plants.

Cytological studies showed that the chromosome number of the variant is 28 while in the normal diploid plants the number is 14. Mandal and Das (1980) reported a quite different composition of *A. barbadensis* plants collected from West Bengal, India. The important component of the *A. vera* gel was a pectic substance containing mainly galacturonic acid and was accompanied by lesser amounts of galactan, an arabinon and a non-acetylated glucomannan. They suggested that the apparent chemical differences were due to the existence of different plant types within the species.

Intraspecific diversity in *A. vera* for yield parameters

A study conducted by Srivastava et al (1990) at National Bureau of Plant Genetic Resources (NBPGR) research farm, Issapur, India with the 44 genetic stocks collected from Rajasthan and elsewhere in India revealed considerable variation in terms of agro-botanical (leaf length, leaf breadth, leaf thickness, leaf weight etc) and chemical characters (average volume of latex per leaf and aloin content). Correlation between the agro-botanical and chemical characters was also established. A strong positive correlation amongst the growth character of the foliage and yield of its exudates was also observed. The study has shown that the small size plant with broad and thick leaves was ideal type for domestication as it produced higher yield of aloin. They also suggested some of the

promising lines/accessions for high aloin (IC 111271, IC 111280, IC 111269, IC 111273, IC 111279 and IC 111267) and high gel contents (IC 111267, IC 111266, IC 111280, IC 111272 and IC 111277).

Alagukannan (2011) with his study using twenty one accessions of *A. vera* noticed the considerable variation among the accessions for yield and quality parameters. The study suggested the desirable accessions for gel yield (AV_{16}) and latex yield (AV_{17}). Pareek et al (1999) reported two strains of the Indian *Aloe*. The vegetable type had lower bitter content and the leaves were sweet and were used to prepare vegetable and pickle and also salad. The other having bitter leaves was used in medicines.

Apart from the growth, yield and quality parameters considerable variation was also observed among the twenty one accessions evaluated by Alagukannan (2011) in terms of parameters related to biotic (disease) and abiotic stresses like water logging and drought. Leaf spot disease caused by *Alternaria alternata* is the common disease of *A. vera* under damp weather. Among the accessions evaluated accession named AV_8 was highly susceptible and AV_2 was susceptible while the other nineteen accessions were tolerant. Alagukannan and Ganesh (2013) reported that the genetic variability existed among the accessions of *A. vera* towards water logging for more than 96 hours. The study revealed

that 20 accessions showed tolerance to water logging and only one accession expressed its susceptibility to water logging with the ability to revive. Likewise the variation in terms of drought tolerance related parameters like chlorophyll stability index, stomatal frequency, relative water content, water saturation deficit and proline content of leaf tissues were also reported. Among the accessions studied the accessions AV₁₆ and AV₁₇ surpassed all other accessions in terms of drought tolerance related parameters.

Estimation of genetic diversity by molecular marker analysis

Conventional taxonomic approaches for diversity analysis and phylogenetic relationship mainly rely upon the ability to differentiate morphological characters at macroscopic and microscopic levels. Molecular analysis using molecular markers on the other hand can be used not only to reveal the differences at the genetic and functional level but also to precisely quantify the same. Besides morphochemical characters are dependent on age and environment and therefore it is essential to characterize the *A vera* plants at the genetic level. Shasany et al (2007) systematically characterized the *Aloe* species at the molecular level in relation to morphological variation for estimation of genotypic diversity in the germplasm collected from different geographical regions of India using random amplified polymorphic DNA (RAPD) as well as amplified fragment

length polymorphism (AFLP) techniques. The pattern of phylogeny was visibly parallel in AFLP analysis compared with RAPD pattern. The profiles in relation to growth and morphological traits would be useful in future genetic improvement and authentication of species and genotypes of *A vera*.

Eleven *Aloe* germplasm accessions namely *A vera*, *A perryi*, *A lotus*, *A zeylanicum* and seven strains available at the Defence Agricultural Research Laboratory (DARL), Pithoragarh and Medicinal and Aromatic Plant Research Development Centre (MRDC), Pantnagar, Uttarakhand, India were subjected to RAPD analysis by Nayanakantha et al (2010) for estimating the extent of diversity within and between species. Morphological evaluation of the 11 accessions for selected characters showed qualitative variation among the accessions studied. The RAPD analysis revealed comparable inter- and intra-species variation. A total of 192 bands were amplified with 7 primers. Out of 192 bands amplified 89 per cent were polymorphic and 10.9 per cent were unique to a particular accession which made them distinct from all other accessions. Maximum similarity of 61 per cent was observed between DARL 1 and DARL 3 (*A vera*) and minimum similarity of 6.8 per cent was observed between *A lotus* and *A perryi*. Thus *Aloe* accessions maintained at DARL showed high genetic diversity. They concluded that RAPD profiles would be useful in genetic

improvement and authentication of species and genotype of *Aloe*.

Tripathi et al (2011) employed the AFLP to assess the diversity in 12 elite accessions of *A vera* collected from different locations of Madhya Pradesh, India. Among the twelve genotypes the AFLP primer combinations generated a total of 410 fragments with an average of 51.2 fragments per primer combination. In order to assess the discriminatory power of eight primer combinations used a variety of marker attributes like unique alleles, per cent polymorphism and polymorphism information content (PIC) values were calculated by them. Genotypic data obtained for all polymorphic fragments were used to group the accessions and the cluster analysis showed a considerable level of variability among the collected genotypes of *A vera*.

The study conducted by Nejatizadeh-Barandozi et al (2012) revealed that genetic diversity of 10 accessions of *A vera* collected from different parts of Iran showed significant differences for all horticultural characteristics among the accessions suggesting that selection for relevant characteristics could be possible. Results showed that both environmental and genetic factors were effective in observing variations. The investigation also indicated that the RAPD approach along with horticultural analysis seemed to be best-suited for assessing the genetic relationships

among distinct *A vera* accessions with high accuracy.

Nejatizadeh-Barandozi (2013) evaluated phenotypic and molecular diversity among ten Iranian *Aloe* accessions. In this study a combined analysis of variance was used to evaluate the accessions over the period of two years. Traits investigated included leaf weight, root weight and aloin content. RAPD markers were also used to evaluate molecular diversity. Cluster analysis based on agromorphological/phytochemical traits or RAPD markers did not reveal a clear relationship between diversity pattern and geographical origin. The author concluded that genetic diversity of Iranian accessions of *Aloe* should play a critical role in future selection and breeding of *Aloe*.

Panwar et al (2013) evaluated forty six accessions of *Aloe* collected from different parts of India and maintained at Issapur farm of National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The accessions were studied for molecular diversity using RAPD markers. Ten RAPD primers amplified total 56 bands across 46 accessions out of which 54 (96.4%) were polymorphic. The number of bands generated by RAPD primers across 46 accessions varied from 2 (OPL-03) to 9 (OPM-11 and OPM-14) bands with an average of 5.6 bands per primer. Polymorphic information content (PIC) was calculated for all 10 primers and PIC values

varied from 0.13 to 0.44. Based on PIC value maximum accessions were separated by primer OPC-17 (PIC-0.44). Pair-wise genetic similarities among the accessions were determined using Jaccard's similarity coefficient (0.31 to 1.0) and cluster analysis was performed by employing unweighted pair group method with arithmetic mean (UPGMA) method for all 56 RAPD bands generated. All the 46 accessions were grouped into five separate clusters except IC527342, IC524197, IC436191, IC112521 and IC520361 which were ungrouped. The grouping of samples did not show any geographical isolation except in cluster 5 where out of five accessions from Delhi four were grouping together. The authors concluded that the accessions studied had good genetic diversity and could be used in future breeding programmes.

An investigation with 12 elite accessions of *A vera* collected from different geographical regions of India were evaluated by Bhaludra et al (2014) using RAPD and inter-simple sequence repeat (ISSR) markers. Molecular polymorphism was 71.8 per cent with 64 RAPD primers and 80.9 per cent with 25 ISSR primers indicating high level of genetic variation among the accessions and the mantel test revealed positive correlation between the two marker systems. The accession IC111279 and IC111272 appeared to be more divergent with 14.3 per cent similarity while high similarity of 84.7 per cent was recorded between IC111280 and

IC111279. The study indicated the genetic divergence existing in the accessions evaluated and RAPD and ISSR marker profiles were best suited for assessing genetic relationships among *A vera* accessions.

Chandra and Choudhary (2014) collected 10 accessions of *A vera* from Rajasthan and were characterized through RAPD. RAPD revealed 32.08 per cent polymorphic bands detecting 11.9 per cent average diversity among the accessions studied. The diversity ranged from 4.3 to 20.4 per cent. The primers like osteoprotegerin (OPG)-15 having high PIC value (0.346) were considered important for diversity studies whereas OPG-14 had highest discrimination index (0.911) and might be used for identification of different accessions. The clustering analysis resulted in the formation of one group only consisting of eight accessions while Nagour collections remained out of cluster. The diversity pattern did not show any correlation with the site of collection indicating that original introduction consisted of small sample size and its spread was random.

Pushpa and Samantaray (2015) investigated genetic diversity in 38 elite accessions of *A vera* collected from different geographical regions of India using RAPD and inter-simple sequence repeat (ISSR) markers. Molecular polymorphism was 85.39 per cent with 14 RAPD primers and 72.5 per cent with 06 ISSR primers

indicating high level of genetic variation among the accessions and the mantel test revealed positive correlation between the two marker systems. Dendrogram was constructed based on pair-wise genetic similarities and three-dimensional principal coordinate analysis using data from RAPD and ISSR marker systems showed similar clustering pattern and separated accessions into two major groups. They suggested that both the markers techniques (RAPD and ISSR) had been shown to be useful in detecting small genetic variations within and among *A vera* populations.

Estimation of genetic divergence using D² analysis

The study of genetic variability in the available germplasm is a prerequisite for any crop improvement. The genetic diversity of selected plants is not always based on factors such as geographical diversity. Hence characterization of genetic divergence for selection of suitable and diverse genotypes should be based on sound statistical procedures such as D² statistics and cluster analysis. These procedures characterize genetic divergence using the criteria of similarity or dissimilarity based on the aggregate effect of a number of economically important characters. In view of this twenty one *A vera* accessions were evaluated by Alagukannan (2011) to determine the magnitude of variability in the population for yield and yield contributes and to identify genotypically diverse and economically desirable genotypes for exploitation in a breeding programme aimed

at improving leaf yield in *A vera*. The twenty one accessions could be grouped into five clusters. The first cluster consisted of 15 accessions, second and third clusters consisted of two accessions each and fourth and fifth clusters consisted of one genotype each. All the six accessions collected from Indian Institute of Horticultural Research, Bangalore fit into the same cluster. Most of the accessions collected from different districts of Tamil Nadu also fit into the same cluster ie in cluster I. They were found to be very close in genetic configuration based on the characters studied. Genotypes from different states grouped in the same cluster revealed that germplasm distribution did not necessarily determine genetic divergence (Wahab and Gopalakrishnan 1993). The possible reason for grouping of accessions of different states in one cluster could be the free exchange of germplasm among the breeders of different regions or unidirectional selection practiced by breeders in tailoring the promising lines from different regions (Varalakshmi et al 1994). The accession AV₁₆ collected from Tiruvannamalai district of Tamil Nadu and AV₁₇ collected from Trichy district of Tamil Nadu were found to form a separate cluster for each of them and were most divergent. In this investigation the inter-cluster distance was higher than that of intra-cluster which indicated substantial diversity among the accessions evaluated.

The relative contribution of individual characters towards the expression of genetic diversity estimated over

character-wise D^2 values revealed that the characters like leaf yield per plant and leaf weight were the major forces of discrimination among the accessions tested as they contributed 32.37 per cent and 16.31 per cent towards genetic diversity respectively (Alagukannan 2011). It was also suggested to exploit the accessions AV₁₆ for gel yield and AV₁₇ for latex yield and barbaloin content. These two accessions could very well be employed in hybridization programmes and also to create further variability by mutation breeding or polyploidy breeding to select and commercialize the best progenies after conducting the multi-location trials (MLTs).

Possibilities of fruit setting and embryo rescue in *A vera*

Ghate et al (2002) clearly brought out the floral polymorphism, fruiting and germination behaviour of *A vera* seeds. Chandrasekaran and Parthasarathy (1975) reported that *A vera* exhibited heteromorphy and in heteromorphic flowers cross pollination and fertilization leading the fruit setting was possible only between two compatible flowers. Propagation through seeds also has been reported with the comment “It is not true to the name and does not ensure the progeny” (Bailey 1939, Anon 1985). In contrary to that during the later period with the advent of technological developments Ghate et al (2002) succeeded in germination studies of *A vera* true seeds with the use different growth media such as wet blotting paper (80%

germination), MS media (60% germination) and red soil and rice husk (50% germination). From this it could be concluded that *A vera* can also be propagated through seed. Therefore hybridization between the compatible parents from diverse cluster or clusters showed that maximum inter-cluster distance may also be possible and the resultant embryo can be rescued through biotechnological tools. The breeding strategies employed for sexually propagated crop plants can also be applied in *A vera* crop improvement for developing the best varieties through sexual propagation and to fix them as clones, its perpetuation especially through asexual means.

From the preceding discussion on assessment of genetic diversity of *Aloe* at inter- and intra-specific levels it could be concluded that there is considerable variation existing among the different species of *Aloe* and different accessions of *A vera* for growth, yield and quality parameters. The presence of genetic diversity in *Aloe* was well documented by conventional taxonomic approaches as well as modern techniques like use of molecular markers analysis such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeat (ISSR). Among them the molecular techniques viz RAPD and ISSR have been shown to be useful in detecting even small genetic variation within and among the *A vera*

populations. To conclude there is an ample scope to evolve high yielding varieties and/or hybrids with the available genetic diversity employing the crop breeding methods like clonal selection, mutation breeding, hybridization etc to meet the ever growing demand of *A vera* for its commercial value.

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