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A mini review on morphological, biochemical and molecular characterization of *Aloe vera* L.

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Abstract
Aloe vera is a widely known medicinal plant prominent in pharmaceutical as well as corporate sector. Geographical conditions influence the genetic diversity that eventually alters the phytoconstituents and medicinal properties of it. Traditionally, diversity is estimated by measuring variation in phenotypic or qualitative traits. There is a reduction in the ability of these morphological markers for the estimation of genetic diversity in plants, of which the main factor is its high dependence on the environment for expression, yet they can provide a base for genetic variation. Biochemical and molecular markers avoid many of the environmental affects acting on characters by directly observing variation controlled by gene or the genetic material. Molecular techniques have been proven useful in the investigation of the origin and domestication of species and information on evolutionary relationships. This review is mainly focused on utilization of these markers in *Aloe vera*.

Keywords: *Aloe vera*, medicinal plant, genetic diversity, markers, phytochemical constituents

Introduction
The aloe species has been studied, evaluated and utilized for a period of time for its several properties namely Antiulcer activity, Antidiabetic, Antihypercholestermic, Antioxidative Effect, Antibacterial activity, Antiviral activity, Antifungal activity, Antiacne, Cardiac stimulant, Nutraceutical, Moisturizer, Immunomodulator, Protection of skin from UV-A & UV-B rays and Wound healing property. Aloe vera could be used in various conditions like Mild to moderate burns, Erythema, Genital herpes, Seborrheic dermatitis, Psoriasis vulgaris, Skin moisturizer, Type 2 diabetes, Oral lichen planus infections, Angina pectoris, Ulcerative colitis, UV-induced erythema, Kidney stones and Alveolar osteitis (Bhuvana *et al*. 2014) [1] showing vast benefits in health, beauty, medicinal and skin care industries. Aloe vera is a shrubby or arborescent, perennial, xerophytic, succulent, pea- green color plant. The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds (Tyler, 1993) [2]. The Aloe vera plant belongs to Asphodelaceae (Liliaceae) family and has over 300 species which are native in the dry regions of Africa, Asia, Europe and America. Its botanical name is *Aloe barbadensis* Miller (Klein and Penneys, 1988) [3]. The gel or mucilage obtained from the flesh of the leaf contains quite different compounds from the bitter latex extracted from the leaf lining such as polysaccharides, glucomannan, anthraquinone glycosides, salicylic acid (Sharrif Moghaddasi and Verma, 2011) [4].

- Polymorphism in biology and zoology is the existence of two or more clearly divergent morphs or forms, also cited as alternative phenotypes, in the population of a species. To be classified as such, morphs must lived in the same habitat at the same time and belong to a panmictic population (one with random mating) (Ford, 1965) [5]. Three mechanisms may cause polymorphism (Leimar, 2005) [6]:
- Genetic polymorphism-where the phenotype of each individual is genetically determined
- A conditional development strategy, where the phenotype of each individual is set by environmental cues.
- A mixed development strategy, where the phenotype is randomly assigned during development.

Genetic polymorphism in medicinal plants has been widely studied, which helps in distinguishing plants at inter and/or intraspecific level.
The diversity is estimated conventionally by measuring variation in phenotypic or qualitative traits such as growth habit, color of flower etc.

Because of several defects there is a reduction in the ability of these morphological markers for the estimation of genetic diversity in plants, of which the main factor is its high dependence on the environment for expression, yet they can provide a base for genetic variation. On the other hand both biochemical and molecular markers avoid many of the environmental affects acting on characters by directly observing variation controlled by gene or by observing the genetic material.

Modern advances in molecular biology have supervened upon traditional ones in providing with robust techniques which can be used for the screening, characterization and evaluation of genetic diversity, primarily the development of the polymerase chain reaction (PCR) for amplifying DNA, identification, phylogenetic analysis, population studies, DNA sequencing and genetic linkage mapping (Willium *et al*. 1990) [7]. The extensive number of research articles currently appearing in the literature, describing the use of these techniques in a wide range of plant species and diversity problems, is testimony to their increasing impact in this field. Nevertheless, there are still many problems to be addressed before universal strategies for their wide-spread use can be recommended. Comparative studies in which different approaches have been contrasted in specific germplasms, some of which are discussed in this article, provide extremely valuable insights into the relative strengths and weaknesses of the different technologies.

This article briefly reviews and is a venture to consolidate and cite information on the morphological, biochemical and molecular characterization of the *Aloe vera* L. and highlight the need for research.

Morphological Studies

Morphology deals with the study of forms and features of different plant organs. Different plant characters like plant height and girth, leaf shape and size, fruit size, shape and color, seed size, pigmentation and other visually identifiable characters are known as morphological markers. As there exist immense number of morphological diversities, which can be successfully and traditionally used in species characterization. Hence morphological characterization is a conventional technique used for evaluating the plant diversity as a tool for breeding and improvement.

Morphological studies in *Aloe vera* L.

Ganesh and Alagukannan (2006) [8] studied morphology of twenty one *Aloe vera* ecotypes collected from the wild state and from research institutions across south India. Considerable variation in terms of growth, yield and quality parameters was noted from the 21 ecotypes tested. An ecotype TCY showed maximum values for plant height (79.31 cm), plant spread in North-South direction (85.38 cm), growth rate in terms of height gain month^{-1} (8.64 cm), growth rate in terms of number of leaves produced month^{-1} (3.94), number of leaves plant^{-1} (29.50), leaf length (63.10 cm), leaf width (10.92 cm), leaf weight (434.30 g) and aloin yield leaf^{-1} (0.32 g). Ecotype named TVM recorded the maximum values for the traits such as leaf thickness (2.74 cm), leaf volume (429.27 cm^3), leaf yield plant^{-1} (1597.37 g), gel yield leaf^{-1} (282.57 g), total solids (0.81%) and soluble solids in gel (0.71%). Though the ecotype TCY recorded the maximum values for growth characters, ecotype TVM showed its superiority in yield and quality parameters.

Kaur and Saggoo (2010) [9] screened 60 North Indian accessions of *Aloe vera* on the basis of morphogical, cytological, biochemical and molecular variability. Morphological studies divided 60 accessions into 3 groups on the basis of height (*viz.* short, medium and long) and color (*viz.* dark green, green and light green) and orientation (*viz*. spiral, parallel and nearly parallel) and into 2 groups on the basis of color of spine (*viz.* green and red).

Nayanakantha *et al.* (2010) [10] estimated morphometric parameters of eleven *Aloe* germplasm accessions for the extent of diversity within and between species. Characters such as plant height, leaf length, leaf width and leaf thickness were recorded in all *Aloe* accessions for comparative studies. Leaf colour was recorded with the help of New Ornamentals Society (NOS) colour chart. Morphological evaluation of the 11 accessions for selected characters showed qualitative variation among the accessions studied. *Aloe zeylanicum* was found to be the tallest (53 cm) as it possesses a distinct stem (caulescent) with long internodes. The leaf shape was linear in this species compared to that of linear-lanceolate shaped leaves in others except *A. lotus*. Minimum leaf thickness (0.4 cm) and wideness (1.0 cm) were recorded in this species among all *Aloe* accessions and therefore, it contained a lesser amount of gel. *Aloe lotus* was the shortest plant type with a height of 17 cm. It more or less resembles the common cactus plant in appearance than other *Aloe* accessions in possessing the ovate-lanceolate shaped and broadest (4.5 cm) leaves. Among all *Aloe* accessions, *A. perryi* was considered as a blue green variety because of its blue-green appearance in leaf. It is also a caulescent type plant but the internodal length was low compared to that in *A. zeylanicum*. Leaf width and size were slightly deviated from other *A. vera* strains. Among *A. vera* strains, conspicuous variations were observed in plant height, leaf size, thickness and width while very little variations were observed in leaf color. In terms of leaf thickness, DARL-3 showed the highest thickness (1.1 cm) than other accessions. These accessions were further subjected to RAPD analysis for the assessment of genetic variation at molecular level.

The investigation was carried out to study the genetic variability, h^2, genetic advance for 8 traits in *Aloe vera* by Kiran and Tirkey (2018) [11]. The experimental materials comprised of 13 genotypes of *Aloe vera (Aloe barbadensis)*, were evaluated to study the PCV, GCV, GA% and diversity pattern. Wider variability as observed for plant height (cm), leaf length (cm), number of spines per leaf, number of leaves per plant, number of suckers, gel content per leaf(g). The PCV value was slightly higher than the GCV showed the slight influence of environment in the expression of the Accession. High h^2 coupled with high genetic advance was recorded in plant height (cm), leaf length (cm), number of spines per leaf, number of suckers and gel content per leaf (g). Hence selection will be effective for these traits. The genotype was studies for the diversity and D^2 statistic was done and the Accessions were grouped into four cluster. The distributing pattern indicates that the maximum numbers of genotypes (5) were into cluster IV Followed by cluster II, cluster I and cluster III. The maximum inter cluster distance was observed in between I and IV (5.350) followed by cluster IV and III (3.569) these suggest that wider diversity among the group and hybridization programme involved parents from these cluster is expected to give higher frequency of better segregator, therefore Accession 1, 2, 3, 12, 13 can be used as potential donors for hybridization programme to develop variety with higher yield.

Biochemical Studies

Aloe vera is a distinctive plant which constitute many chemical compounds and plays an important role in the international market. Chemistry of the plant revealed the presence of more than 200 different biologically active substances including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Chauhan *et al*. 2007) [12]. Rowe and Parks (1941) [13] were probably first to take vital steps in the chemical analysis of the plant. Reynolds and Dweck (1999) [14] reported 16 different polysaccharides extracted from *Aloe vera* leaf gel. Anthraquinones are the phenolic compounds which are found in the sap were first to identify the principal active substance of the plant and Smith and Smith (1851) [15] named it, Aloin.

Aloe vera leaf has been alienated in two major parts namely the external green rind including the vascular bundles and the internal colorless parenchyma containing the aloe gel. Nearly 98.5% of water content is present in the raw pulp of *Aloe vera*, while 99.5 percentile was shown in the mucilage or gel. It has the pH of 4.5. The residual solid material approximately 0.5 to 1% consists of a range of compounds which include vitamins which are water-soluble and fat soluble, minerals, enzymes, polysaccharides, phenolic compounds and organic acids as reported by Boudreau and Beland (2006) [16] and Hamman (2008) [17].

Waller *et al*. (1978) [18] determined free amino acids, free monosaccharides and total saccharides, sterols and triterpenoides released upon hydrolysis of the leaves of *Aloe barbadensis* Miller. Some seventeen amino acids, D-glucose and D-mannose were present in the water soluble fraction. Cholesterol, campesterol, β-sitosterol and lupeol were found in substantial amounts in the lipid fraction.

Phenolic anthraquinones were separated and characterized using TLC, HPLC and column chromatography by Rajendran *et al*. (2007) [19]. Thirteen phenolic components namely, aloesin, 8-C-glucosyl-7-Omethyl-(S)-aloesol, neoaloesin A, 8-O-methyl-7- hydroxyaloin A and B, 10- hydroxyaloin A, isoaloeresin D, aloin A and B, aloeresin E, *Aloe*-emodin, aloenin and aloenin B in *A. barbadensis* and *A. arborescens* were separated and quantified by the HPLC method.

Kaur and Saggoo (2010) [9] subjected different accessions of North Indian germplasm to biochemical analysis to evaluate the quality of *Aloe*, making quantitative estimation of total carbohydrates, chlorophyll, proteins, phenols, aloin, gel and stabilized gel. To know the impact of maturity on the quantity of these constituents, these estimations have been made twice from each accession representing different level of maturity and season i.e. pre-flowering phase (October - November) and post-flowering phase (March-April). The range of carbohydrate content in the leaves of various accessions of *Aloe* germplasm under study was 0.96±0.0031 mg/g to 3.30±0.0018 mg/g during pre-flowering phase and 1.20±0.004 mg/g to 3.33±0.002 mg/g during post-flowering phase. The range of protein content in the leaves of various accessions of *Aloe* was 0.229±0.001 mg/g to 1.49±0.0017 mg/g during vegetative phase and 0.462±0.0097 mg/g to 1.58± 0.0022 mg/g during post-flowering phase. Phenol content in the leaves of all the collected accessions was estimated seasonally. The range of total phenol content during pre-flowering phase (October to November) was 87.2±5.40 mg/100 gm to 149.0±2.07 mg/100 gm and it ranged between 98.2±2.80 mg/100 gm to 152.3±1.07 mg/100 gm in post-flowering phase.

Kohli *et al*. (2011) [20] investigated phytochemical constituents of *Aloe barbadensis* whole leaf using aqueous, ether and solvent extracts. The extracts were tested for the presence of carbohydrates, saponins, flavonoides, tannins, alkaloids, anthraquinons and resins. The gel part of the Aloe leaf obtained after peeling of outer rind was used for anti - inflammatory activity and crude sap was used to evaluate the antimicrobial activity. The results indicated that the gel of *Aloe* leaf possessed significant anti-inflammatory activity while crude sap possessed antimicrobial activity.

Ravi *et al*. (2011) [21] identified and characterized the phenolic anthroquinones (Aloin- A and B) from *Aloe vera* samples. Among the different samples forms of *Aloe vera* the *Aloe vera* sap contain more aloin of 4 hydroxy aloin. *Aloe vera* leaf, gel, root commercial gel and commercial soap samples were characterized by FT-IR and UV Spectroscopy techniques.

Ahmed and Hussain (2013) [22] conducted a study to determine chemical composition and biochemical activity of *A. vera* leaves. Proximate composition (moisture, ash, crude protein, crude lipid and crude fibre), ascorbic acid, superoxide dismutase, catalase, peroxidase, amylase, reducing sugars and total soluble sugars were determined. Moisture content of 97.42 ± 0.13% was observed, while average percent ash, fiber, protein and fat contents were 16.88±0.04%, 73.35 ± 0.30%, 6.86 ± 0.06% and 2.91 ± 0.09% respectively along with traces of ascorbic acid (0.004 ± 0.05%). Variable levels (IU/mg) of superoxide dismutase (802.14 ± 55.6-2830.19 ± 37.09), peroxidase (1.46 ± 0.06-3.72 ± 0.19), catalase (1.56 ± 0.14-2.8 ± 0.19) and amylase (0.97 ± 0.82-24.02 ± 1.5) were observed in the extracts. Total soluble and reducing sugars accounted for 120.68 ± 7.24-363.03 ± 9.25 mg/mL and 97.23 ± 0.05-123.33 ± 0.74 mg/mL.

The study by Gangwar *et al.* (2017) [23] determined biochemical composition and enzymatic activity of *Aloe vera* leaves. Five genotypes of *Aloe vera* leaves were analysed biochemically and enzymatically. Germplasm IC-112517 showed maximum gel percentage (97.00%), maximum moisture percentage (97.03) and IC-112527 contain maximum per cent of carbohydrate (64.20%), total mineral (11.10%) and total sugar content (30.07%) and total chlorophyll content (0.20 mg/gm). The germplasm IC-285626 showed maximum per cent of crude fibre content (11.43%). Germplasm IC- 112527 showed maximum catalase enzyme activity (1.92unit/ml) and peroxidase enzyme activity (1.67unit/ml). The investigation provided a succinct resume of information regarding the biochemical composition and enzymatic activity of *Aloe vera* leaves.

DNA isolation

Dellaporta *et al*. (1983) [24] first reported a rapid method for large scale isolation of plant genomic DNA. The DNA being of moderately high molecular weight and satisfactory for most restriction endonucleases was found to be suitable for blot analysis and for PCR based DNA polymorphisms.

A rapid method for DNA isolation in small amount was developed by Edwards *et al*. (1991) [25]. This method was applicable to a variety of plant species and had the advantage of not requiring phenol or chloroform extraction.

Khanuja *et al*. (1999) [26] observed that the presence of certain metabolites interfere with DNA isolation procedures and downstream reactions such as DNA restriction, amplification and cloning. The chemotypic heterogeneity among species may not permit optimal DNA yields with a single protocol, and thus, even closely related species may require different isolation protocols. They described the essential steps of a

rapid DNA isolation protocol that can be used for diverse medicinal and aromatic plants, which produce essential oils and secondary metabolites such as alkaloids, flavanoids, phenols, gummy polysaccharides, terpenes and quinones. The procedure is applicable to dry as well as fresh plant tissues. This protocol permitted isolation of DNA from tissues of diverse plant species and produced fairly good yields. The isolated DNA proved amenable to PCR amplification and restriction digestion.

Khan *et al.* (2007) [27] performed isolation of genomic DNA from fresh and dry roots of medicinal plants. It involved a modified CTAB procedure using 3% CTAB, 4% b-mercaptoethanol, 2 M NaCl and 5% PVP. The extraction was carried out at 70 °C. A slight increase in the concentrations of these chemical components and temperature helped in the removal of secondary metabolites and polysaccharides from the DNA preparation. The quantity and purity of isolated DNA was higher when compared with DNA extracted by the methods of CTAB by Doyle and Doyle (1987). The DNA yield ranged from 33 to 68 µg per g of root samples and it was 1.47 times greater in dried than fresh samples. The DNA samples were found suitable for analysis with restriction enzyme digestion and Random Amplification of Polymorphic DNA (RAPD). The total duration for DNA extraction from roots of medicinal plants using this protocol was 135 min as compared to 225 min with existing protocol.

Vural and Eri (2009) [28] had done the optimization of DNA isolation protocols and PCR conditions for RAPD analysis of selected medicinal plants of conservation concern from Turkey, containing high levels of polysaccharides, polyphenols and secondary metabolites. These methods involved a modified CTAB extraction employing polyvinyl pyrrolidone while grinding, successive long-term Chloroform-lsoamylalcohol extractions. The yield of DNA ranged from 1-2 g of the leaf tissue and the purity (ratio) was between 1.7 - 1.8 indicating minimal levels of contaminating metabolites. The DNA isolated was used for Randomly Amplified Polymorphic DNA (RAPD) analysis. RAPD protocol was optimized based on the use of 50 ng of template DNA and annealing temperature of 37 °C, resulted optimal amplification. Thus, the results indicated that the optimized protocol for DNA isolation and PCR was amenable to plant species belonging to different genera which is suitable for further work on diversity analysis.

The article by Singh *et al.* (2010) [29] deals with optimization of DNA isolation and PCR conditions for RAPD analysis of medicinal plants, *Asparagus* and *Aloe vera*. The method involves a modified CTAB extraction including addition of PVP, 0.3M NaCl along with CTAB. The DNA isolated was used for randomly amplified polymorphic DNA (RAPD) analysis. RAPD protocol was optimized using different concentrations of $MgCl_2$, T*aq* polymerase, genomic DNA, primer annealing temperature. Reproducible amplifiable products were observed in PCR reactions.

Molecular Marker Studies

DNA based molecular marker techniques have become powerful and accurate tools for assessment of genetic diversity and genotype identification (Reddy *et al.* 2002) [30]. To this day, only few studies on genetic diversity of *Aloe* species were reported in literature.

Molecular markers in *Aloe sp*

He-sheng *et al.* (2001) [31] investigated the phylogenetic relationship of nine Aloes, including *Aloe vera var. chinese, Aloe arborescens var. natalensis bgr., Aloe barbadensis* L., *Aloe arborescens, Aloe saponaria, Aloe vera hybrid 1,Aloe vera hybrid 2, Aloe aculeata* and *Aloe juvenna* by RAPD analysis. 18 single primers were selected out of 40 random primers. The amplified fragments of 18 primers were analyzed by the Phylip software packet, and the phylogenetic trees were constructed by UPGMA method and N-J method respectively. The results indicated that: *Aloe vera var. chinese, Aloe vera hybrid 1* and *Aloe aculeata* had close phylogenetic relationship. While *Aloe arborescens var. natalensis bgr. Aloe barbadensis* L. and *Aloe arborescens* had close phylogenetic relationship. The phylogenetic relationship between *Aloe arborescen var. natalensis bgr.* And *Aloe arborescens* was much closer. *Aloe saponaria* and *Aloe vera* hybrid 2 have close phylogenetic relationship too. The phylogenetic relationship of *Aloe juvenna* was far away from the others.

Darokar *et al.* (2003) [32] carried out genetic analysis using RAPD and AFLP profiling of *Aloe* germplasm (*Aloe vera, A. perryii, A. arborescens* and *A. saponaria*) available at consideration, they further analyzed these accessions at molecular level using RAPD and AFLP profiling for genetic diversity. The RAPD analysis revealed comparable inter-and intra-specific variations with AFLP results. The pattern of phylogeny was visibly parallel in AFLP analysis as compared to RAPD pattern. But the degree of diversity revealed by AFLP showed zooming effect over RAPD analysis.

Twenty seven North Indian accessions *Aloe* germplasm which were morphologically and biochemically different were analyzed by Kaur and Saggoo (2010) [9] and an attempt has been made to find out the diversity at molecular level and to identify the putative duplicate accessions using two primers. Two primers used for amplification were 5.8S19R (5'TCTTCATCGACGCGAGAGC3') and 18S20F (5'AAGTCGTAACAAGGTTTCCG3') which were designed based on sequences alignment of the ITS region of *Aloe* 18SrDNA and 5.8SrDNA in several *Aloe* species. The amplicons produced by these ITS markers were analyzed for analysis of genetic diversity among the accessions. The dendrogram prepared on the base of similarity coefficient. Among the investigated accessions of *Aloe vera* germplasm the analysis revealed that there are two distinct main groups showing only 0.46% similarity. The investigated accessions PBM2 and PBJ1, RJH1 and HPU2, PBMO2 and PBFZ1, PBK1 and UKD1 though collected from different places were apparently genetically duplicates of each other. The closely placed accessions probably belonged to same ancestral clones. The distribution of these clones under human preference has also been revealed. This limited study has clearly indicated the presence of genetic variability among different clones being maintained separately by different individuals in their kitchen gardens, pots or plantations.

Nayanakantha *et al.* (2010) [10] subjected eleven *Aloe* germplasm accessions; *A. vera*, *A. perryi*, *A. lotus*, *A. zeylanicum* and seven strains of *A. vera* available at the Defense Agricultural Research Laboratory (DARL), Pithoragarh, and Medicinal and Aromatic Plant Research Development Centre (MRDC), Pantnagar, Uttaranchal, India to Random Amplified Polymorphic DNA (RAPD) analysis in relation to morphometric parameters for estimating the extent of diversity within and between species. 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% was polymorphic and 10.9% was unique to a particular accession which made it distinct from all other

accessions. Maximum similarity of 61% was observed between DARL 1 and DARL 3 (*A. vera*) and minimum similarity of 6.8% was observed between *A. lotus* and *A. perryi*. *Aloe* accessions maintained at DARL showed high genetic diversity.

Sanmukhiya *et al.* (2010) [33] compared the phytochemical, antimicrobial and DNA profiles of *Aloe* endemic to Mauritius and Reunion with the profiles of *A. vera*. In *g*enetic diversity studies using RAPD markers showed that Mascarene *Aloe* species were very different from *A. vera*. This study was the first report highlighting the differences between *Aloe* sp. from Mascarene and *Aloe vera* at the metabolic and genomic level.

Tripathi *et al.* (2011) [34] applied AFLP to assess the diversity in 12 elite accessions of *Aloe vera* collected from different locations of Madhya Pradesh, India. Among the 12 genotypes the AFLP primer combinations generated a total of 400 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, percent polymorphism and Polymorphism Information Content (PIC) values were calculated. The PIC genotypic data obtained for all polymorphic fragments were used to group the accessions analyzed using UPGMA-phenogram and Principle Component Analysis (PCA). The PIC value of the primer combination was ranged from 0.246 to 0.458 with an average of 0.364+0.0257. The neighbor joining tree based on all AFLP fragments grouped 12 Aloe germplasm accessions into two major clusters. The cluster analysis showed a considerable level of variability among the collected genotypes of the *Aloe vera*.

Nejatzadeh-Barandozi *et al.* (2012) [35] studied genetic diversity of ten accessions of *Aloe vera*, collected from different parts of Iran, using horticultural and Random Amplified Polymorphic DNA (RAPD) data. Statistical analysis showed significant differences for all horticultural characteristics among the accessions, suggesting that selection for relevant characteristics could be possible. For the analysis of molecular diversity ten random primers of 10-mer oligonucleotides were used. Out of the ten primers, five were polymorphic, producing 269 DNA bands, 189 of which were polymorphic among accessions. A dendrogram was prepared on the basis of a similarity matrix using the Unweighted Pair Group Method Arithmetic Mean (UPGMA) algorithm, separating the 10 accessions into two groups. Results showed that both environmental and genetic factors are effective in observing variations. The results also indicated that the RAPD approach, along with horticultural analysis, seemed to be best-suited for assessing with high accuracy the genetic relationships among distinct *A. vera* accessions.

Panwar *et al.* (2013) [36] studied 46 accessions of Aloe and maintained at Issapur farm of NBPGR using RAPD markers. Out of 32 primers only 10 showed amplification. From a total of 56 generated bands, 54 were polymorphic i.e. 96.4% polymorphism was given by 10 primers. Polymorphic Information Content (PIC) for all the 10 primers was also calculated, values of which varied from 0.13 to 0.44. Pairwise genetic similarity among accession using Jaccard's similarity coefficient was determined 0.31 to 1. On the basis of UPGMA these 46 accessions were grouped into 5 separate clusters.

In the investigation by Bhaludra *et al.* (2014) [37], genetic diversity in 12 elite accessions of *Aloe vera*, collected from different geographical regions of India, were evaluated using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. Molecular polymorphism was 71.8% with 64 RAPD primers and 80.9% 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. Dendrogram was constructed based on pair wise genetic similarities and two-dimensional principal coordinate analysis using data from RAPD and ISSR marker systems showed similar clustering pattern and separated accessions into two major groups. The accession IC111279 and IC111272 appeared to be more divergent with 14.3% similarity, while high similarity of 84.7% was recorded between IC111280 and IC111279. The study indicated that RAPD and ISSR marker profiles were best-suitable for assessing genetic relationships among *Aloe vera* accessions.

Aloe vera (L.) Burm. f. collected from 12 states covering all the different agro-climatic zones of India were investigated for its genetic diversity analysis by using SSR marker assay by Yadav *et al*. (2015) [38]. Total genomic DNA was isolated from young leaf samples using CTAB method. Twenty primers were selected which were used for *Asparagus officinalis* L. a related species of *A. vera* and others were developed from available *Aloe vera* plant sequences with the help of primer 3 software. Similarity matrices and dendrogram were constructed by using NTSys software to show a phenetic representation of the genetic relationship. Polymorphic Information Content (PIC), the effective multiplex ratio (EMR) and Marker Index (MI) were calculated for the assessment of genetic diversity. The neighbor-joining tree based on all SSR fragments of twelve *Aloe vera* germplasm accessions grouped into three major clusters. The similarity value ranged from 46% to 100%. The highest 100% similarity was noted between Haryana and Uttar Pradesh accessions followed by 93% similarity between Haryana and Punjab accessions with Rajasthan. Minimum similarity was noted between Gujarat and Kerala accessions. This study revealed the rich genetic diversity among *Aloe vera* accessions from different agro-climatic zones of India. It is also concluded that SSR marker analysis can be a useful tool for the assessment of genetic diversity of the medicinal plants.

In order to facilitate reasoned scientific decisions on *Aloe vera* L. conservation and for selective breeding programme, aloin content and genetic diversity analysis of 55 genotypes were performed by Kumar *et al.* (2016) [39]. Aloin content in the leaves of 55 genotypes varied from 3.29 to 276.76 mg/g of dry wt. Twenty six RAPD and fourteen ISSR primers amplified a total of 236 and 111 scorable bands, of which 86.44 and 72.07% were polymorphic, respectively. Analysis of molecular variance (AMOVA) indicated high genetic variation among genotypes. Genetic variation among genotypes grouped into low, intermediate and high aloin content was negligible, 5.4% (RAPD) and 4.08% (ISSR). The dendrogram obtained from Neighbor-joining and STRUCTURE analysis revealed splitting of genotypes into four clusters with no clear distinction between low, intermediate and high aloin content genotypes. Results showed that genetic variability using RAPD and ISSR, was not associated with aloin content. However, both the markers revealed high genetic variation among genotypes, which is important in the conservation and exploitation of A. vera genetic resources.

The study by Rana and Kanwar (2017) [40] was undertaken for the assessment of genetic diversity in *Aloe vera* L. genotypes from different provinces of Himachal Pradesh using morphological, biochemical, Random Amplified Polymorphic

DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers. On the basis of morphological and biochemical characters very low variation was found among selected genotypes. A total of 30 RAPD and 6 ISSR primers were screened and only 24 RAPD and all the 6 ISSR primers gave amplification. The similarity coefficient value ranged from 0.62 to 0.91 in RAPD. C1 genotype showed its distinct nature at 0.62 similarity index value leaving behind 65-91% genetic similarity among the remaining genotypes. Cluster analysis, using UPGMA, SAHN clustering (NTSYS-pc ver. 2.0) grouped the genotypes into 4 and 2 main clusters in RAPD and ISSR markers respectively. In ISSR markers 57-100% of genetic similarity was obtained, depicting 80% similarity in minor and 65% similarity in major cluster.

Conclusion and future prospective

Medicinal plants are the source of a large number of essential drugs of herbal medicine, which is not only the primary source of health care for most of the world's population living in developing countries but also enjoys growing popularity in developed countries. They also play a vital role to preserve our health. *Aloe vera* L. is one of such medicinal plant which is in enormous demand in the medicinal and cosmetic industries. Authentic identification of taxa is necessary for breeders to ensure protection of intellectual property right and also for propagators and consumers. This review divulged the importance of various markers which are sufficiently informative and powerful tool to access genetic variability of natural populations of *Aloe vera*. The results of investigations on genetic diversity provide estimates on level of genetic variation among diverse materials that can be used in assessing the purity and stability of genotypes entering into a breeding or multiplication programs. This indicates the power of molecular marker system, presumably covering the entire genome. Thus, marker analysis can be useful for designing collection strategies and germplasm conservation of medicinal plants. In future, the extent and distribution of variation in the species gene pool of this plant species need to be studied in controlled manner which will prove the effectiveness of *Aloe vera* under various conditions.

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