

Genetic Diversity: A Tool for Assessing Potential of Genepool

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Abstract

Diversity is the essence of biological world. Any couple of living organism; even maternal twins are not exactly similar to each other at genetic level. The importance of plant genetic diversity (PGD) is now being recognized as a specific area since exploding population with urbanization and decreasing cultivable lands are the critical factors contributing to food insecurity in developing world. A gene pool refer to the set of *genes* within an interbreeding population, *featured by* increased biological *fitness* through its ability to survive throughout pressures caused by rapid changes in respect of range of biotic and abiotic factors. The variation within the population at genetic level may be characterized by allele differences with regard to the level of frequency and the relative frequency of each phenotype expressed in population highlighting the fact that the phenotype of an individual is expression of its own genotype. Population having narrow genetic base containing low diversity and subsequently prone to extinction due to reduced fitness and adversely affected by stresses which may be the cause of a low availability of alleles that are necessary for an organism to survive under certain conditions, or may be ill consequences of *inbreeding*.

Genetic diversity is the prime factor for many important phenomena of crop genetics viz., heritability, heterosis and transgressive segregation. In Breeding the need of diverse lines always thrust area for defect correction at genetic level of commercial varieties and development of novel cultivars to fulfil the specific objectives of crops. Therefore, the identification of diverse and potent lines or creation of diversity and its subsequent utilization in improvement programs are the prime targets of crop breeder community. In this context, the knowledge regarding the genetic diversity viz., factors for genetic diversity, techniques of diversity analysis, their estimation and the software for carrying statistical analysis becomes imperative in order to efficient utilization in field of plant breeding.

Marker technology based on morphological, cytological, biochemical or molecular markers are applied to assess the degree of diversity, whereas the statistical techniques viz., metroglyph, D^2 analysis, cluster analysis, PCA, PCoA, Canonical analysis etc., used to convert the diversity into numerical, which are handled by the appropriate software viz., SAS, SPAR, PAST, NTSYSp, GenAlEx, POPGENE, POWERMAKER etc., for their proper presentation and interpretation. The diversity estimates obtained by application of different analysis techniques related to statistical tools and computer softwares can further be utilized in formulation experiments of heterosis breeding, transgressive breeding and intergression of alien genes for specific traits and much more in respect of utilization of genepool.

Keywords: Genetic Diversity, Genepool, Morphological Markers, Biochemical Markers, Molecular Markers, Statistical tools/software.

Introduction

Globally, India is known as the second most populated nation, broadly categorized into three geological regions viz., the Himalayas and eastern Hills, the Indo-Gangetic plains and the peninsular shield. The Indian subcontinent is very rich in biological diversity, harboring around 49,000 species of plants, including about 17,500 species of higher plants (Anonymous, 2007).

India accounts a total geographical area of about 329 million hectares including a coastline of over 7500 km. In India, the diversity at

level of ecosystem is enormous, ranging from sea level to the highest mountainous ranges in the world; hot and arid conditions in the northwest to cold arid conditions in the trans-Himalayan region; tropical wet evergreen forests in Northeast India and the Western Ghats; mangroves of Sundarbans and fresh water aquatic to marine ecosystems (Sharma & Singh, 2000). India has twelve bio-geographical provinces, five biomes and three bioregion domains (Cox & Moore, 1993). India highlights the diverse array of habitats or ecosystems such as forests, grasslands, wetlands, coastal, marine and desert with its richness and unique floristic diversity features, which enhanced by the geographic location of the country at the confluence of three major global bio-geographic realms, viz. Indo-malesian, Eurasian and Afro-tropical, thus allowing the intermingling of floristic elements

from these regions as well and making it one of the seventeen mega-diversity countries in the world, recognised by the World Conservation Monitoring Centre in 2000 (Anonymous, 2014). The floral diversity in India is majorly concentrated in the four biodiversity hotspots, namely Eastern Himalayas, Western Ghats (and Sri Lanka), Northeast India and Andaman Islands (Indo-Burma) and Nicobar Island (Sundaland), out of thirty-four biodiversity hotspots recognised in the world (Anonymous, 2014).

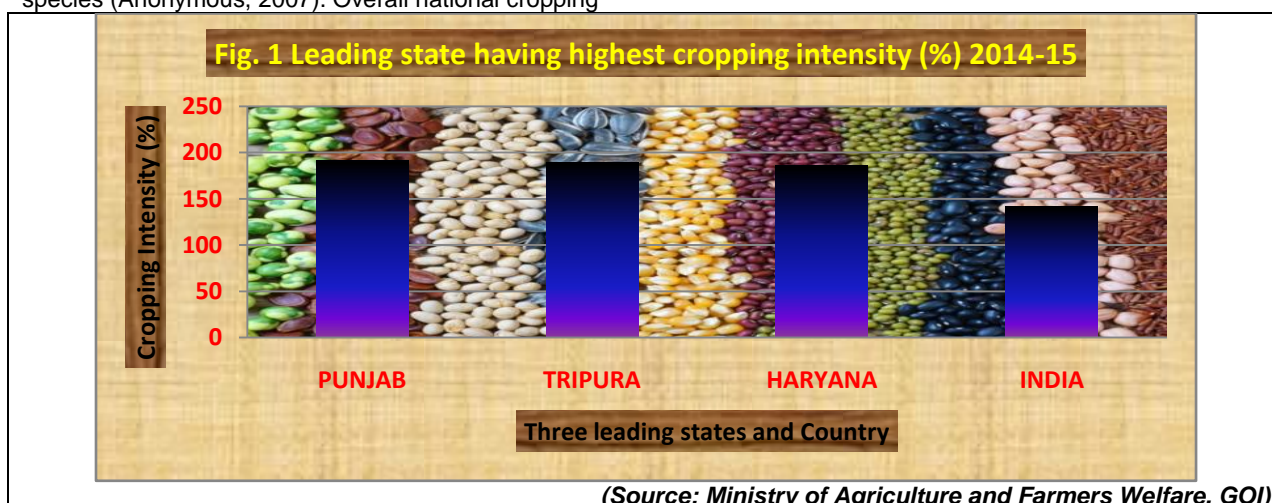
These floristically significant areas exhibit exceptional concentration of endemic species and also experiencing loss of habitat with higher occurrence of threatened plant species. The comparative status of biodiversity of India and world is given in Table 1.

Table 1: Comparative biodiversity status of India with World (Anonymous, 2014)

No.	Type	Number of known Species		Percentage of Occurrence in India	Number of Endemic Species	Number of Threatened Species
		World	India			
I	Flowering Plants					
1.	Gymnosperms	1021	74	7.35%	8	7
2.	Angiosperms	268600	18043	6.72%	ca. 4036	1700
III	Non-flowering Plants					
1.	Bryophytes	16236	2523	15.54%	629	ca. 80
2.	Pteridophytes	12000	1267	10.57%	47	414
III	Others					
1.	Virus and Bacteria	11813	986	8.77%	Not Known	Not known
	Algae					
2.	Fungi	40000	7284	18.21%	1924	Not known
3.	Lichens	98998	14883	15.09%	ca. 4100	ca. 580
4.		17000	2401	14.12%	ca. 520	Not known
Total		465668	47513	-	11273	2781

Source: Chapman (2009) and Singh & Dash (2014). intensity of India recorded of 141.6 per cent. (Anonymous, 2015)

Rich crop diversity is available in India in terms of both numbers of species and within the species (Anonymous, 2007). Overall national cropping



(Source: Ministry of Agriculture and Farmers Welfare, GOI)

In India, the agriculture is the largest private enterprise which is the lifeline of Indian economy. It contributes nearly 22% of the national GDP and sustains livelihood of about two-thirds of its population (Anonymous, 2007). The Indian floral diversity is

composed of rich genetic wealth comprises of native as well as introduced flora types. In respect of crop diversity, it is well represented as developed cultivars (hybrids, straight varieties, composites, segregating

lines etc.), landraces or as folk varieties in different phyto-geographical regions of the country.

The Indian gene centre holds a prominent position among the twelve mega-gene centres of the world and identified as a one of the Vavilovian centers of origin and diversity of crop plants. Two out of the twenty-five global hotspots of biodiversity, namely the Indo-Burma and Western Ghats / Sri Lanka, are located here (Myers et al., 2000). India possesses about twelve per cent of world flora with 5,725 endemic species of higher plants belonging to about one hundred forty one endemic genera and over forty seven families. About 166 species of crops including twenty-five major and minor crops have originated and/or developed diversity in this part of the world. Moreover, the 320 species of wild relatives of crop plants are also known to occur here (Arora and Nayar, 1984 and Arora, 1991).

Presently, the Indian diversity is composed of rich genetic wealth of native as well as introduced types, that are India is a primary as well as a secondary centre of diversity for several crops, and has also rich regional diversity for several South/Southeast Asian crops as described below (Salgotra and Gupta, 2015):

Primary centre of diversity for crops

Rice, black gram, moth bean, pigeonpea, cucurbits (like smooth gourd, ridged gourd and

pointed gourd), tree cotton, capsularis jute, jackfruit, banana, mango, *Syzygium cumini* (Jamun), large cardamom, black pepper and several minor millets and medicinal plants like *Rauvolfia serpentina* and *Saussurea costus*.

Secondary centre of diversity for African crops

Finger millet, pearl millet, sorghum, cowpea, cluster bean (transdomesticated), okra, sesame, niger and safflower; tropical American types such as maize, tomato, muskmelon/Cucumis species, pumpkin/Cucurbita species, chayote/ chou-chou, chillies and Amaranthus; and

Regional (Asiatic) diversity for crops

Maize, barley, amaranth, buckwheat, proso millet, foxtail millet, mungbean/green gram, chickpea, cucumber, bitter melon, bottle gourd, snake gourd and some members of Tribe Brassicaceae.

Whereas, the crops of Indian origin are listed in Table 2. Other important crops grown in India include onion, groundnut, rapeseed mustard, soybean, tea, coffee, sunflower and among horticultural crops-banana, citrus, grapes, cashew and vegetables of European origin. The indigenous plant wealth has been supplemented by introduction of species and forms that have greatly enriched the local flora. These introduced species also diversified in India due to isolation over time and space, and diversity in climate and human intervention.

Table 2: Major crop species of Indian Origin (Anonymous, 2007)

Crop Group	Crops (Botanical names)
Cereals and millets	Rice (<i>Oryza sativa</i>), little millet (<i>Panicum sumatrense</i>), kodo millet (<i>Paspalum scrobiculatum</i>)
Grain legumes	Black gram (<i>Vigna mungo</i>), moth bean (<i>V. aconitifolia</i>), pigeonpea (<i>Cajanus cajan</i>), horse gram/kulthi (<i>Macrotyloma uniflorum</i>), velvet bean (<i>Mucuna utilis</i>)
Fruits	Mango (<i>Mangifera indica</i>), banana (<i>Musa spp.</i>) jamun (<i>Syzygium cumini</i>), jackfruit (<i>Artocarpus heterophyllus</i>), Citrus group, lime and others, karonda (<i>Carissa congesta</i>), khirni (<i>Manilkara hexandra</i>), phalsa (<i>Grewia asiatica</i>), bael (<i>Aegle marmelos</i>), wood apple (<i>Feronia limonia</i>), kokam (<i>Garcinia indica</i>)
Vegetables	Eggplant (<i>Solanum melongena</i>), ridged gourd and smooth gourd (<i>Luffa spp.</i>) round gourd/tinda (<i>Praecitrullus fistulosus</i>), pointed gourd/parval (<i>Trichosanthes dioica</i>), taro/arbi (<i>Colocasia esculenta</i>), yam (<i>Dioscorea spp.</i>), jimikand (<i>Amorphophallus campanulatus</i>), kundri (<i>Coccinia indica</i>), cucumber (<i>Cucumis sativus</i>), rat tailed radish/mungra (<i>Raphanus caudatus</i>)
Oilseeds	Rai, sarson and toria types (<i>Brassica spp.</i>)
Fibres	Jute (<i>Corchorus capsularis</i>), cotton (<i>Gossypium arboreum</i>), sunnhemp (<i>Crotalaria juncea</i>)
Medicinal and aromatic	<i>Rauvolfia serpentina</i> , <i>Saussurea lappa</i> , Indian belladonna (<i>Atropa acuminata</i>), Indian barberry (<i>Berberis aristata</i>), <i>Commiphora wightii</i>
Spices and condiments	Turmeric (<i>Curcuma domestica</i>), ginger (<i>Zingiber officinale</i>), cardamom (<i>Elettaria cardamomum</i>), Bengal/large cardamom (<i>Amomum aromaticum</i>), long pepper (<i>Piper longum</i>), black pepper (<i>Piper nigrum</i>), betle leaf (<i>Piper betel</i>), cinnamon (<i>Cinnamomum spp.</i>)
Other crops	Sugarcane (<i>Saccharum officinarum</i>), bamboos (<i>Bambusa arundinacea</i> , <i>Dendrocalamus hamiltoni</i> , <i>Sinocalamus giganteus</i>), <i>Sesbania sesban</i> , tea (<i>Camellia sinensis</i>)

The geographical proximity with the Indo-Chinese-Indonesian, the Chinese-Japanese, the Central and West Asian centres of diversity has helped in considerably augmenting our crop plants resources. The influx of genetic material from the Mediterranean, African centre, the European and

American regions in the past has also resulted in accumulation and diversification of enormous genetic variability. The ancient travelers, traders and religious missionaries contributed significantly towards enriching the agro-biodiversity in the Indian gene centre. Presently, India has more than 480

species of agricultural crops as native and introduced species (Roshini Nayar et al, 2003)

In respect of Genetic diversity of crop plants, it may be either natural or human-directed, is primarily based on existing genetic diversity in the population. Genetic diversity may be elaborated as the degree of differentiation between or within species. The objective of any improvement programe target towards the identification of diverse lines (if available), creation of diversity (if not available or limited) and its subsequent utilization. Hence, the knowledge on all aspects of genetic diversity viz., factors affecting genetic diversity, different methods of diversity analysis, their determination in respect of magnitude along with the softwares for carrying statistical analysis becomes imperative for its efficient utilization.

In common parlance, genetic variability and genetic diversity are considered synonym to each other which is erroneous. Genetic variability is the variation in alleles of genes or variation in DNA/RNA sequences in the gene pool of a species or population (Bhandari et al., 2017). This expresses itself in terms of alternate forms in phenotype. Genetic diversity, on the other hand, is a broad term encompassing all the variability occurring among

different genotypes with respect to total genetic make-up of genotypes related to single species or between species. Genetic diversity can be measured by counting the number of different genes in a gene pool, but genetic variation can only be expected to occur and cannot be measured. Genetic variability thus, can be considered as the building blocks of genetic diversity

Basis of Diversity Analysis

Genetic diversity analysis can be estimated based on morphological, cytological, biochemical and molecular characterization. Since the evolution of breeding techniques, morphological markers were used for diversity analysis and are still in use effectively with an aspect that phenotype or morphology is the representation of genes carried by an organism. There are naturally occurring variants of a particular plant species. Later on the discoveries of chromosomes as a physical carrier of genes, cytological and biochemical differences occurring in the genotypes of a species started to be used in genetic diversity assessment. Moreover, the advance of genomic tools, molecular markers became the method of choice for genetic diversity assessment. Different markers are employed for analysis of diversity are reviewed in Table 3

Table. 3: Various techniques for diversity analysis

Marker	Description
Morphological markers	These analyses are carried out by raising germplasm lines, purelines, improved varieties etc. in a particular experimental design. This involves morphological characterization of different entries grown in the field as the morphological characteristics are the strongest determinants of the agronomic value and taxonomic classification of plants
Cytological markers	Study of cytological features like chromosome size, secondary constriction in chromosomes, position of centromere, arm ratio, constitutive heterochromatic patterns, banding characteristics (G, Q, R and N banding), DNA content, total genomic chromosome length, chromosome volume etc
Biochemical markers	Study involves separation of proteins or their variants (isozymes) into specific banding patterns. The isozymes reflect products of different alleles and not the genes. These isozymes can be mapped onto chromosomes and can be used genetic markers for mapping other genes
Molecular markers	These include the characterization/documentation of variation among genotypes at nucleic acid level ie. DNA/RNA. Range of molecular markers has different characteristics making them suitable for different purposes, which are primarily classified as hybridization-based and PCR-based. Recently, a new generation of markers based on sequence or array-platforms have been developed. They can also be classified as neutral markers, genes markers and functional markers based on their activity and expression. Further, these markers may be based on variation in genomic DNA/ RNA, ribosomal RNA or organelle genome sequences.

Whereas, the many studies conducted by the plant scientists on genetic diversity have been reported to use both morphological- and molecular- markers simultaneously.

Measures of Genetic Diversity

Beside above discussed marker techniques of diversity assessment has to accompany with the techniques to measure the diversity in terms of figures. The diversity can be measured at two levels viz., genetic and allelic (Table 4)

Table. 4: Measures of Diversity

Measures of Diversity	Description
Gene Level	Genetic base of any crop expressed in terms Coefficient of Parentage (COP) or Coefficient of Correlation'. These indicate how frequently a line appears in the commercial varieties of a particular crop and is revealed by pedigree records of varieties released. COP is defined as the probability that alleles of two individuals are identical by descent. The segregating generations resulting from a cross between individuals with high COP will exhibit less variability and <i>vice versa</i> .

Genetic Distance	Genetic distance was first defined by (Nei, 1973) as the difference between two entities that can be described by allelic variation. In other words "extent of gene differences among populations that are measured using numerical values"
Allelic Diversity	Allels may be defined as an alternative form of a gene. The allelic diversity may be used in case where, the genetic marker data or molecular marker data can be interpreted by locus/allele model. In such cases, data is used to generate binary matrix for further analysis

Estimation of Genetic Diversity

Multivariate statistics are used to assess the magnitude of genetic diversity among different strains/varieties/entries or members of gene pool of plant species. These statistical techniques have a very strong theoretical basis to provide most reliable information regarding the real genetic distances between and among genotypes. Therefore these techniques may be used for assessment of genetic diversity (Singh and Pawar, 2005). These above discussed laboratory techniques may be deployed for the assessment of genetic diversity, classification or grouping of germplasm into different groups based on diversity level and their further utilization in selection of diverse parents with an ultimate objective to develop the transgressive segregants. Some of the multivariate techniques being used are discussed below in Table 5.

Table 5: Statistical Tools for assessment of Genetic Diversity

Statistical Tool	Description
Metroglyph analysis	Anderson (1957) developed a semi-graphical approach for displaying genetic diversity among a number of lines referred to as 'Metroglyph analysis'. This method represents each genotype by a circle of fixed radius (called glyph) with rays emanating from its periphery. Each variable is assigned a position on the glyph. The length of the ray represents index score of the variate. This method uses a range of variations arising from trait such that extent of trait variation is determined by the length of rays on the glyph. The performance of a genotype is adjudged by the value of the index score of that genotype. The score value determines the length of ray which may be small, medium or long.
D ² Statistics	This technique also called Mahalanobis generalized distance was developed by Mahalanobis (1936). This technique reduces the number of comparisons among genotypes by classifying them into different clusters. D ² values are estimated by transforming correlated variables into uncorrelated variables using pivotal condensation method. In general, the Mahalanobis distance is a measure of distance between two points in the space defined by two or more correlated variables
Cluster analysis	Cluster analysis assumes discontinuities within the data. It depicts the pattern of relatedness between genotypes based on evolutionary relationships or phenotypic performance. It is used to group similar lines/germplasm in one group and differentiate other groups. It is based on methods namely (i) Unweighted paired group method using arithmetic mean (UPGMA), (ii) Unweighted paired group method using centroid (UPGMC), (iii) Weighted paired group method using arithmetic mean (WPGMA), (iv) single linkages (SLCA), (v) complete linkage (CLCA) and (vi) Median linkage (MLCA). UPGMA and UPGMC provide more accurate grouping information on breeding materials used in accordance with pedigrees and calculated results found most consistent with known heterotic groups than the other clusters (Aremu et al., 2007)
Principal component analysis (PCA)	Principal components analysis (PCA) can be defined as a data reduction technique applicable to quantitative type of data. PCA transforms multi-correlated variables into another set of uncorrelated variables for further study. These new set of variables are linear combinations of original variables. It is based on the development of eigen-values and mutually independent eigen-vectors (principal components) ranked in descending order of variance size. Such components give scatter plots of observations with optimal properties to study the underlying variability and correlation.
Principal coordinate analysis (PCoA)	It is another ordination method, somewhat similar to PCA, was developed by Schoenberg (1935). The PCoA routinely finds the eigen- values and eigen-vectors of a matrix containing the distances between all data points, measured with the Gower distance or the Euclidean distance. It produces a 2 or 3 dimensional scatter plot of the samples such that the distances among the samples in the plot reflect the genetic distances among them with a minimum of distortion
Canonical analysis	The concept of canonical analysis is given by Bartlett (1938 and 1947) It assumes additively in all characters and improves prediction by eliminating linear correlations between characters. Hotelling (1935 and 1936)proposed the technique to describe the dependencies between two sets of variants. Seal (1964) defined it as 'a procedure of discriminating as clearly as possible between two or more multivariate normal universes with the same variance-covariance matrix'
Factor analysis	Technique of Factor analysis reduces data into smaller meaningful groups based on

	their inter-correlations or shared variance. It is based on the assumption that correlated variables measure a similar factor or trait. It is used to describe the covariance relationships among many variables in terms of few underlying random quantities called factors
Correspondence Analysis (CA)	Correspondence Analysis or CA may be termed as an ordination method, somewhat similar to PCA, but for counted or discrete data. It uses Chi-square distance between the objects/components under investigation. Correspondence analysis can compare associations containing counts of taxa or counted taxa across associations

Different methods of genetic diversity analysis have been found to give similar results and hence can be used interchangeably and applied by plant scientist, specially the plant breeders to group the material based on diversity estimates to reap the potential in form of hybrids and transgressive segregants in almost all crop plants.

Software for Genetic Diversity Analysis

Many types of software have been developed for analyzing genetic diversity. Most of these software(s) are based on multivariate statistics. Most of the software(s) are freely available on internet and suitable for PCs (Tanavar et al., 2014). The different statistical programs/software are available (Table 6)

Table. 6: Software(s) available for assessment of Genetic Diversity

Software	Details
SAS	SAS offers the package for different multivariate techniques. It involves canonical correlation, correspondence analysis, cluster analysis, factor analysis, principal component analysis etc. Principal component analysis can be performed using PROC PRINCOMP or PROC PRINQUAL. PROC CORRESP, PROC CANCORR and PROC FACTOR can be used for performing correspondence analysis, canonical correlation analysis and factorial analysis, respectively. (Bhandari et al., 2017)
SPAR 3.0	IASRI, New Delhi have designed Statistical Package for Agricultural Research (SPAR). Apart from other modules, it is also capable of carrying out multivariate statistics (Bhandari et al., 2017)
PAST	Paleontological Statistics software was developed by Hammer et al. (2001). It is a free, user friendly and comprehensive package. Functions found in PAST include parsimony analysis with cladogram plotting, detrended correspondence analysis, principal component analysis, principal coordinates analysis, time-series analysis, geometrical analysis etc.
NTSYSpc	NTSYSpc (Numerical Taxonomy System for personal computer) It is a popular program used to analyze genetic diversity from molecular marker data and has been used in different areas of science. It is based on similarity indices and works on 0, 1 matrix of genotypic data. It is used for several applications namely cluster analysis, principal component analysis, principal coordinate analysis, etc., (Rohlf, 1998).
GenALEx: (Genetic Analysis in Excel)	Excel-based and user-friendly program. It was designed for the use of SSR, SNP, AFLP, allozyme, multi locus markers and sequencing DNA data in diversity genetics analyses. It accepts three types data viz., co-dominant data, dominant, and geographic data. GenALEx analysis include frequency by Locus, observed and expected heterozygosity, marker index, fixation index, Allelic Patterns, Allele list, Private alleles list, Haploid diversity by Population, Haploid diversity by Locus, Haploid disequilibrium and Pairwise Fst), Nei's Genetic Distance, Principal component analysis, Shannon index etc. (Bhandari et al., 2017)
Popgene	The package for the analysis of genetic diversity among and within natural populations. It enables to perform complex analysis and produce scientifically sound statistics and analyze population genetic structure using the target markers/traits. It accepts three types of data viz., codominant data, dominant and quantitative traits. The analysis include gene frequency, allele number, effective allele number, polymorphic loci, gene diversity, Shannon index, homozygosity test, F-statstics, gene flow, genetic distance (based on Nei cofficent) and dendrogram (based on UPGMA and neighbor-joining method) and neutrality. (Bhandari et al., 2017)
Power marker	Power Marker is a newly designed program, specifically for the application for analysis of SSR/ SNP data in population genetics experiments. Data can be imported from Excel or other formats, making data set-up very easy. Available options include summary statistics (allele number, gene diversity, inbreeding coefficient; estimation of allelic, genotypic and haplotypic frequency; Hardy-Weinberg disequilibrium and linkage disequilibrium), population structure, phylogenetic analysis, association analysis and tools (Utility tools such as SNP simulation and identification, Mantel test and exact p-values for contingency tables). (Bhandari et al., 2017)

Gene banks across the world maintain a large number of germplasm (about 6 million) of important crop plants (Hammer et al., 2003). Of them, less than 1% has been utilized by breeders (Upadyaya, 2006). This is because of lopsided approach of plant breeding aiming at only few important traits contributing towards yield at the cost of other traits. Many other germplasm accessions possessing diverse traits remain unutilized. This leads to narrow genetic base of crop varieties leading to genetic vulnerability which may be devastating in context of changing climatic conditions

Aim of Study

To discuss the various available statistical tools/techniques and software of diversity analysis in life science

Conclusion

Presence of genetic variability in crops is essential for its further improvement by providing options for the breeders to develop new varieties and hybrids. Morphological data in conjunction with molecular data are used for precise characterization of germplasm resources. With the advent of high throughput molecular marker technologies it is possible to characterize larger number of germplasm with limited time and resources. The analysis is based on statistical tools for better interpretation. The most used statistical tools for morphological data are D^2 statistics and PCA because of their easy interpretation. PCoA is very much in use for molecular diversity analysis. POWERMARKER and GenAIEX are mostly used software because of their high informativeness. Whereas, the morphological diversity estimates along with advanced laboratory techniques of molecular markers can speed up the evaluation by skipping field evaluation.

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