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Advances in Breeding Techniques of Bulbous ornamental Crops

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ABSTRACT

Bulbous crops are specialized modified underground stem structures in which food material is stored to overcome the unfavorable environmental conditions. These plants are prized for their magnificent flowers and for being grown for mass effect. Breeding techniques are of two types i.e. conventional (Introduction, Selection, Hybridization, Mutation) and non- conventional (Genetic engineering, Biotechnology). Hybridization is the crossing of two or more plants which are genetically different from each other to produce a new crop. It is used to create genetical variation and to exploit the hybrid vigour. Mutation breeding is the sudden heritable changes occurred in an organism. Mutation may be caused by spontaneous and induced and the result of a change in the gene or chromosome. Anther culture is the process of using anthers to culture haploid plantlets. The technique was discovered in 1964 by Guha and Maheshwari. Embryo culture is to separation or isolation of plant embryo and growing to this in culture medium under controlled condition or lab. Genetic engineering is the artificial transfer of gene(s) from one organism to another to produce novel traits in the recipient living organism. It is of two types Direct or Indirect. Direct gene transfer are Chemical Mediated Gene Transfer, Microinjection, Electroporation, ParticlGun/Particle Bombardment, Liposome Mediated Gene Transfer Or Lipofection and Indirect gene transfer are Agrobacterium tumefacians, Agrobacterium rhizogenes. Molecular markers are classified on the based of basic stragety i.e. PCR and Non PCR based. PCR based are (RAPD, AFLP, SSR, ISSR), Non PCR based (RFLP), Marker assisted selection (MAS) is the selection of plants with desirable traits based on their molecular genotype. It can be used alone or in combination with the classical methods of selection.

Key words: *PCR, Mutation, Bulbous, Microinjection, Electroporation,*

INTRODUCTION

Bulbous flowering plants are prized for their magnificent flowers grown for mass effect and bulb are defined modified underground stem structures in which food material is stored to overcome the unfavorable environmental conditions[1]. Gladioli (9500), Narcissus (7000ha), Lilium (5000ha), Iris (2000ha), Tulips (1600ha) are the top five genera of bulbous plants acerage wise in the world.

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Out of total world floriculture trade (70, 34,922.88MT) bulbous ornamental trade contribute 4.46 % share quantity wise and 5.55 % value wise However, in India total area under bulbous crops are 3500ha and gladiolus contributes maximum area (1200ha) followed by tuberose (800ha). Kalimpong and Sikkim contributing 30-35% of total bulb export. Other areas are Srinagar (J&K) and Katrain, Kullu (HP). Gladiolus, Tulip, Lilium, Dahlia, Alstroemeria and Tuberose are the commercially high valued bulbous crops. These crops get high prices in the market only when they are healthy and appeal to consumers so these objectives can only met by using different breeding techniques.

Breeding techniques are of two types i.e. conventional (Introduction, Selection, Hybridization and Mutation) and non-conventional (Genetic engineering, Biotechnology).

Conventional Techniques:-

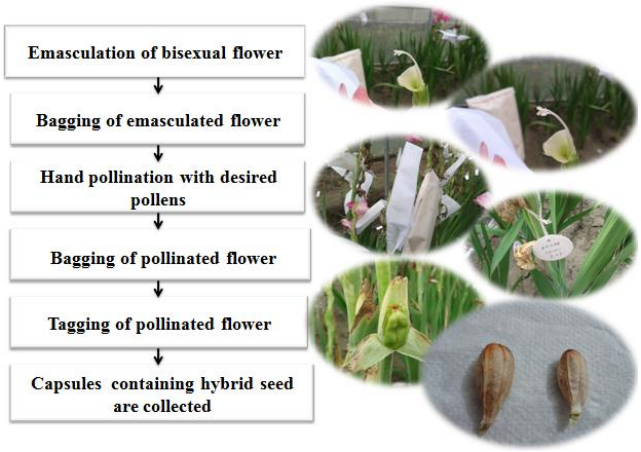
Introduction: is the process of introducing plants from their growing to a new locality. The main objectives of introducing plant material from outside are for use as medicinal or industrial purpose, to study origin and evolution of crop plants and in case of ornamentals to enrich and fulfill the aesthetic values of gardens and for genetical improvement of economic crops through direct release, selection, as breeding materials. Organizations involved in plant introduction are FRI (forest research institute, Dehradun), Botanical Survey of India, NPBGR.

Selection: are the oldest breeding method and the basis of all crop improvements. It is the choosing the best out of one's crop continued over generations for development and retention of already developed varieties.

Hybridization: is the crossing of two or more plants which are genetically different from each other to produce a new genotype. It is effective to combine all the good characters in a single variety to create genetical variation and to exploit the hybrid vigour. There are five methods of hybridization based on relationship between parental plants i.e. intravarietal, intervarietal, interspeific, intergeneric and introgressive hybridization. By using these hybridization techniques different hybrids has been develop.

Role of hybridization in bulbous ornamental crops

1) Gladiolus: - started by William Herbert in England. Colville hybrid was first commercial hybrid developed by James Colville in 1823 in England. Other hybrids are Gandavensis which is cross between *Gladiolus psittacinus x G. cardinalis.* Nanus hybrid cross between *G. cardinalis x G. venustus* and Glandanthera hybrid cross between Filigree x *Acidanthere bicolor* var. *murielae.* The inflorescence of gladiolus is spike and flowers are bisexual, trimerous florets. Protoandry and distyly are occurring. Anthers mature with opening of flowers. Stigma receptive after three days of anthesis. In gladiolus hybridization was done by following ways :-



Hybridization in Gladiolus

Rao and janakiram got fragrant hybrid by crossing between *Gladiolus dalenii x G. callianthus* and also reported that interspecific hybrid with mild fragrance were produced by crossing *Gladiolus grandiflorus* and *G. callianthus*.

2). Lilium:- the inflorescence of lilium is terminal raceme or solitary perianth are 6 and sepals and petals are 3, flowers are erect, spreading, stamens are 6, ovary is superior and stigma three lobed. Batistero (LA), La Reina (LA), Boogie Woogie (OT) are fragrant hybrids. Easter moon (Disease resistance), Pisa (Golden yellow cultivars), Yellito, China (pollen less cultivars). Loffler *et al.*[9], crossed fusarium resistant species *Lilium dauricum* (resistant) and *Lilium longiflorum* var. Gelria (susceptible) got resistant hybrid.

Breeding problems in lilium:

1. **Self incompatibility** (failure of viable pollen to fertilize the flower), a major problem, is of two types:-
   a) Pre fertilization barriers: – when growth of pollen tube inhibited either at base of stigma or in style.
   b) Post fertilization barriers: – fertilization occurs but embryo gets degenerate at later stage of development.
2. **F1 hybrids sterility**
3. **Low multiplication rate**
4. **Long juvenile period**

Techniques for Overcoming Pre-Fertilisation Barriers

- The cut style method: one day after stigmatic receptivity, style of flower is cut 1-2mm above the ovary then pollen applied on cut surface.
- The grafted-style method: Style from pollinated flower after one day attached to the ovary of desired another flower.
- Heat treating of style
- use of plant growth regulators

Techniques for Overcoming Post-Fertilisation Barriers:

- In vitro rescue method
- Embryo culture
- ovary-slice culture
- ovule culture

Integrated Techniques for Overcoming Pre- and Post-fertilisation Barriers

Various combinations of in vitro pollination (cut-style and grafted-style method) and embryo rescue (ovary, ovule and embryo culture, placental pollination).

3) Dahlia :- The inflorescence of dahlia is head or capitulum. Dahlia is a bisexual flower.

Both ray and disc florets of capitates inflorescence open in succession. The opening starts from outer petals. In dahlia ray florets are female flower contain only stigma in the inner base of petals. While disc florets contain both female and male organs which complete flowers.

Hybrids of Dahlia[3]:

- **Swami Vinayananda (Breeder):** Bhikkus Mother, Bhikkus Vivek, Jyotsana Lord Buddha, Prabodh, Sarada Devi, Swami Gauriswarananda, Swamji**.**
- **C. Sen and A.Sen (Breeder):** Basu dev and Jayanti
- **L.N. Singh Thakur (Breeder):** Chitchor
- **R. Mitra (Breeder):** Manjushri
- **S.C. Dey(Breeder):** Disco and Swami vinayananda

4) Tuberose: is an important bulbous ornamental plant of tropical and subtropical areas.

These are commercially cultivated for loose flower and cut flower trade and also for the extraction of its highly valued natural flower oil. Dichogamy and Self-incompatibility are occurring. Perianth are funnel shaped, fragrant, waxy white, 25 mm long. Stamens = six, ovary = 3 locular, ovules numerous, fruits are capsule.

- *Polianthes geminifera x P. bulliana.*
- *Polianthes* x *blissii- Polianthes geminiflora* x *P. tuberosa*.
- *P. bundrantii = P. tuberosa x P. howardii*

5) Tulip: - Flower- Hypogyanous, erect, Perianth (6) segments, stamens (6), ovary superior. Stigma three lobed, Fruit –capsule, Seeds numerous, flat**.** Tuyl and Creij, 2006

reported that by crossing *Tulipa gesneriana* x *Tulipa fosteriana* -Darwin Hybrid (Triploid). *T. gesneriana* and *T. kaufmanniana* - Partial resistance

- **Tulip Breaking Virus resistance:-** Cantata, Princeps
- **New varieties:-** Mughal, Don**,** Prince Victoria and Blenda

6) Iris: -is native of Holland, Spain and England. These are highly suitable for group planting, borders and cut flowers. The bulbs are planted in September – October about 10cm deep. Flowering occurs during February – March. Important varieties are White Excelsior, White Emperor, Sapphire Beauty, and Purple Sensation. Cultivars for year round production areWedgwood, Ideal, Professor, Blue Magic.

Role of Mutation in bulbous ornamental crops[15].

Mutation is a sudden heritable change in a characteristic of an organism. Mutations occur in natural populations at a low rate; these are known as spontaneous mutations. Utilization of induced mutations for crop improvement is known as mutation breeding. Mutation have certain general characteristics are recessive, recurrent, random and deleterious. Flower shape, plant type, early blooming, flower colour, long stem, leaf color, neutrality to photoperiod are the main attributes of the plant which are affected by mutation.

1) Gladiolus :- Four mutants[3]

Cultivar	Mutagen	Parent	Earlier colour	Changed colour
1. Shobha	Gamma rays (10Gy)	Wild Rose	Roseine purple	Shell pink
2. Tambari	Gamma rays	Oscar	Single	Altered flower colour
3. Swarnima	Spontaneous	Dhanvantari	Light yellow	Coppery yellow

Patil[12], studied Influence of gamma radiations from Co-60 was studied in three varieties of gladiolus (*Gladiolus hybrida* L.) namely, American Beauty, Nova Lux and Eurovision irradiated with 1, 2, 3, 4, 5, 6 and 7 kR doses. Percentage of sprouting was affected significantly at1 kR to 4 kR. LD50 was found to be beyond 7 kR dose for both sprouting. Doses of 4 kR and above proved to be detrimental for various vegetative and floral traits. Plants treated with 6 kR and 7 kR did not produced flower spikes in cv. Nova Lux and Eurovision whereas cv. American Beauty had produced few flower spikes. When corms were treated with higher doses, plant height was reduced significantly and leaves become narrow and leathery. Colour variations in florets and whole spike were also increased with increase in dose rate along with increase or decrease in number of floral organs. Radiation treatments at higher doses caused delayed in spike initiation with decrease in number of florets while lower doses responded positively. Three desirable mutants with light colours were isolated from all three varieties in 5 kR whereas one mutant have bifurcated spike at 6 kR from cv. American Beauty.

2) Dahlia:-

Mutation is a natural or artificially induced change of genetic information contained in a cell. It occurs when the order of gene is changed or damaged. This can happen sexually in the ovule through the chance mating of dissimilar chromosomes. However, mutants can be induced by irradiation or chemically interference. Juanita provides a good example of natural mutation. Dohare *et al.*, located a sectorial chimera in White Pearl which has a deep magenta steaked flower and named as Manali. Some Induced mutants in dahlia.

Mutants	Parent
Bichitra	Kenya
Black Beauty	Black Out
Happiness	Croydon Monarch
Jayaprakash	Croydon Apricot
Jubilee	Kenya
Jyoti	Kenya
Netaji	Eagle Stone
Pearl	Eagle Stone

Dwivedi and Banerji, 2008 studied effect of gamma irradiation on dahlia cv " Pinki" in this rooted cuttings of dahlia were irradiated with 0, 250, 500, 1000 and 1500 rads of gamma rays. Reduction in survival percentage, plant height, and flower size was observed after irradiation and with increase in exposure of gamma rays. Morphological abnormalities in leaf increased with increase in exposure to gamma rays. On the basis of his observation they recommend 500 and 1000 rads dose of gamma rays for induction of somatic mutation.

3) Tuberose: - NBRI (Luck now) releases two mutants i.e. **Rajat Rekha (single)-** silvery white streaks along the middle of the blade**.**

Swarna Rekha (double)-golden-yellow streaks are present along the margins of the blade (1 to 5 kr).

4) Tulip[18].

Mutants	Parents	Characters
Faraday (De Mol, 1936)	Fantasy	White, flushed **salmon pink** colour
Estella Rijnveld (1954)	Red Champion	Red flamed, **white flower** colour
Apeldoorn (Mutants)	-	Half of one petal being **yellow**
Bartigon, William Copland, Murillo	-	-

ICAR in 2013 reported that when they treated the varieties of Apeldoorn, Golden Melody and Strong Gold with different doses of 5, 10 and 20 Gy gamma rays and got results are at higher dose large number of flower buds dried before opening, less number of bulbs and bulblets

Non- Conventional Techniques:- are non-traditional method includes biotechnological tools, tissue culture, genetic engineering, molecular markers and takes short time, save labour etc.

- **Anther culture: -** Using anthers to culture haploid plantlets. Discovered by Guha and Maheshwari
- **Embryo rescue:-** is to promote the development of an immature or weak embryo into a viable plant, it produce interspecific and intergeneric hybrids **Northern Beauty, Starbrust Sensation** is developed through embryo rescue in lilium Asano.
- **Genetic engineering: -** artificial transfer of gene(s) from one organism to another to produce novel traits in the recipient living organism.

Methods of gene transfer: there are two methods:-

Direct method: which includes Chemical Mediated Gene Transfer, Microinjection, Electroporation, Particle Gun/Particle, Bombardment Liposome Mediated Gene Transfer Or Lipofection.

Indirect:- *Agrobacterium tumefacians, Agrobacterium rhizogenes*

Direct gene transfer:-

Methods	Description
Chemical mediated gene transfer	Polyethylene glycol (PEG) and Dextran Sulphate induce DNA uptake into plant protoplasts.Calcium phosphate
Microinjection	DNA is directly injected into plant protoplasts or cells (specifically into the nucleus or cytoplasm) fine tipped (0.5 - 1.0 micrometer diameter) glass needle or micropipette.
Electroporation	High voltage electrical pulses to a solution containing a mixture of protoplasts and foreign DNA introduce DNA which enters the cells through reversible pores created in the membrane by the action of short electrical pulses.
Particle gun (microprojectile gun) Biolistics inventor Sanford (1988)	Foreign DNA containing the genes to be transferred is coated onto the surface of minute gold or tungsten particles (1-3 micrometers)
Liposome mediated gene transfer or lipofection	Circular lipid molecules with an aqueous interior that can carry nucleic acids. Encapsulate the DNA fragments and then adher to the cell membranes and fuse with them to transfer DNA fragments. DNA enters the cell and then to the nucleus

In direct gene transfer:-

Agro bacterium-mediated gene transfer : is a soil bacterium helps in transferring a piece of DNA (T-DNA) into the genome of host plants. Genes inserted into the T-DNA region through Ti plasmids are co-transferred and integrated into the host genome.

Ozel and Kamo[11]. studied on *Agrobacterium*-mediated transformation of Easter Lily (*Lilium longiflorum* 'Nellie White'). Plants were grown *In vitro* on Murashige and Skoog's medium they use *A. tumefaciens* strain AGL1 containing pCAMBIA2301 *uidA* gene that codes for β-glucuronidase was cultured with kanamycin. Precultured explant was exposed to Agrobacterium incoluation for 24-25 mins after etoilted for 11 days at 25^0C. extra agrobacterium rinsed from the explant and then cultured on co-cultivation medium containing 500 mg/L cefotaxime. Results are smaller bulb scales from the inner region of the bulb or from a small bulb showed significantly more blue spots than the larger bulb scales. Maximum GUS expression occurred when bulb scales had been obtained from plants that had been grown in the dark for at least 2 months.

Otani *et al.*[10], studied on establishment of *Agrobacterium*-mediated genetic transformation system in Dahlia. They use mass of shoot primordia (MSP) induced on MS medium. The test was Confirmation PCR and Southern blot analyses. *Agrobacterium tumefaciens* strain EHA101 (pIG121-Hm) harboring both β-glucuronidase (GUS) and hygromycin resistant genes.Shoots were successfully regenerated from hormone-free medium without hygromycin and they rooted on hormone-free medium containing hygromycin. Hygromycin-resistant plants thus obtained showed histochemical blue staining for GUS.

Dilip *et al.*[5], studied on Genetic transformation of *Fusarium oxysporum* f sp. *gladioli* with *Agrobacterium.* They use *Agrobacterium tumefaciens* containing using the AGL-1 strain Confirmation test by PCR and Southern blot analyses. Vectors are hygromycin B phosphotransferase (hph) gene and fluorescence reporter genes EGFP (green), EYFP (yellow), ECFP (cyan) Result are

Gladiolus successfully generated several stable transformed lines showing hygromycin B resistance and green, yellow or cyano fluorescence.The transformed Fog lines developed in this study will be useful in molecular and histopathological investigations.

Morphological traits as a target for genetic modification

Crop	Gene	Trait	Reference
Caladium	Maize Lc+C1	Leaf color	Li *et al*, 2005
Lilium	*Rol A,B,C*	*Dwarf*	Mercuri *et al*, 2003
Lilium	Maize Zm401	Pollen-less	Li *et al*, 2005
Lilium	Carotenoids	Flower, leaf color	Azadi *et al*., 2010
Narcissus	Phytoene synthase	Flower color	Lu *et al*., 2007

Virus resistant Genes:-

Type of gene	Resistant Gene	Virus
Coat protein gene	CP-TMV	Tobacco mosaic virus
	CP-AMV&TRV	α –α mosaic virus & Tobacco rattle virus
	CP-CTV	Citrus tristeza virus
	CP-PPV	Plum pox virus
Nucleoplasmid Gene	NC-TSWV	Tomato spotted wilt virus

Azadi *et al*.[2], studied on increased resistance to Cucumber Mosaic Virus (CMV) in *Lilium* transformed with a defective CMV replicase gene**.** Cultivar: Acapulco. They use *Agrobacterium tumefaciens* mediated (CMV2-GDD) gene. They Confirm test by reverse transcription PCR (RT-PCR). Result are increased levels of resistance to CMV was observed in *Lilium*, and CMV-GDD Replicase gene is an effective construct that has protection against CMV in *Lilium*.

Marker is the sign and trait to identify any individual. There are three types of markers[4]. Morphological markers, Protein (Biochemical) markers and DNA (molecular) markers.

NEED OF MOLECULAR TECHNOLOGY

- Save time and other resources: save time compared to phenotypic selection (PS), more popularly known as conventional breeding. In PS, breeders must wait until the plant is fully mature so they can identify desired traits by observing the plant.
- Eliminate unwanted plants
- Expands useful genetic diversity for crop improvement
- Increases favorable gene action
- Independent of environmental factors

1. Morphological markers: Traditionally, diversity within and between populations was determined by assessing differences in morphology. These measures have the advantage of being readily available, do not require sophisticated equipment and are the most direct measure of phenotype, thus they are available for immediate use, an important attribute. However, morphological determinations need to be taken by an expert in the species, they are subject to changes due to environmental factors and may vary at different developmental stages and their number is limited. E.g **Albinism, Plant height, leaf morphology.**

2. Protein (biochemical) markers: To overcome the limitations of morphological traits, other markers have been developed at both the protein level (phenotype) and the DNA level (genotype). Protein markers are

usually named biochemical markers but, more and more; they are mistakenly considered as a common class under the so-called 'molecular markers'.

Protein markers (seed storage proteins and isozymes) are generated through electrophoresis, taking advantage of the migrational properties of proteins and enzymes, and revealed by histochemical stains specific to the enzymes being assayed. Detecting polymorphisms i.e. detectable differences at a given marker occurring among individuals in protein markers is a technique that shares some of the advantages of using morphological ones. However, protein markers are also limited by being influenced by the environment and changes in different developmental stages. Even so, isozymes are a robust complement to the simple morphometric analysis of variation.

3. DNA (molecular) markers

DNA polymorphisms can be detected in nuclear and organelle DNA, which is found in mitochondria and chloroplasts. Molecular markers concern the DNA molecule itself and, as such, are considered to be objective measures of variation. They are not subject to environmental influences; tests can be carried out at any time during plant development; and, best of all, they have the potential of existing in unlimited numbers, covering the entire genome.

Characteristics of good marker:

- ✓ **Polymorphic,** that is, it is variable among individuals. The degree of polymorphism detected depends on the technology used to measure it.
- ✓ **Reproducible** in any laboratory experiment, whether within experimental events in the same laboratory or between different laboratories performing identical experiments.
- ✓ **Co dominant.** Depending on the type of application, the selected technology must be able to detect the marker's different forms, distinguishing between homozygotes and heterozygotes (codominant inheritance). A heterozygous individual shows simultaneously the combined genotype of the two homozygous parents.
- ✓ **Evenly distributed throughout the genome**. The more distributed and dense genome coverage is, the better the assessment of polymorphism.
- ✓ **Discriminating,** that is, able to detect differences between closely related individuals.
- ✓ **Not subject to environmental influences**. The inference of a marker's genotype should be independent of the environment in which the individual lives or its developmental stage.
- ✓ **Neutral.** The allele present at the marker locus is independent of, and has no effect on, the selection pressure exerted on the individual. This is usually an assumption, because no data are usually available to confirm or deny this property.
- ✓ **Inexpensive, Easy,** fast and cheap in detecting across numerous individuals. If possible, the equipment should be of multipurpose use in the experiment.

Molecular markers:

Strauss, *et al.*[17], distinguished between two classes of molecular marker viz. molecular genetic markers (those derived from direct analysis of polymorphism in DNA sequences), and biochemical markers (those derived from study of the chemical products of gene expression).

- ➢ Molecular markers consist of specific molecules, which show easily detectable differences among different strains of a species or among different species.
- ➢ Molecular markers analyze genes directly.
- ➢ Molecular markers reveal neutral sites of variation at the DNA sequence level. These variations do not necessarily show themselves in the phenotypes.
- ➢ Use of linked molecular markers would allow indirect selection for desirable traits in early segregating generations at the seedling stage and independent of environmental influences.
- ➢ This, in turns will save time and other resources that are needed.

- They have the big advantage that they are much more numerous than morphological markers.

Classification of molecular markers based on the basic strategy

- **Non PCR based approaches**

– RFLP (Restriction fragment length polymorphism)

- **PCR-based approaches**

– RAPD (Random Amplified Polymorphic DNA)
– AFLP (Amplified fragment length polymorphism)
– SSR (Simple Sequence Repeat)
– ISSR (Inter-simple sequence repeats)
– SCAR (Sequence characterized amplified regions)
– CAPS (Cleaved amplified polymorphic sequence)

RFLP (Restriction fragment length polymorphism) (Non PCR)

- Restriction fragment length polymorphism (RFLP) analysis was one of the first techniques to be widely used for detecting variation at the DNA sequence level. The principle behind the technology rests on the possibility of comparing band profiles generated after restriction enzyme digestion in DNA molecules of different individuals. Diverse mutations that might have occurred affect DNA molecules in different ways, producing fragments of variable lengths. These differences in fragment lengths can be seen after gel electrophoresis, hybridization[16] and visualization.

Procedure

- Genomic DNA digested with Restriction Enzymes.
- DNA fragments separated via electrophoresis and transfer to nitrocellulose membrane.
- Membranes exposed to probes labelled with P^{32} via southern hybridization.

Film exposed to X-Ray.

Random Amplified Polymorphic DNA (RAPD) technique

First developed independently by Welsh & McClelland[21] and Williams *et al.*, and considered the most widely used molecular marker type in molecular studies (White *et al* 07). It is a **first PCR-based marker.** The random amplified polymorphic DNA (RAPD) technique is a PCR-based method that uses a short primer (usually 10 bases) to amplify anonymous stretches of DNA. With this technique, there is no specific target DNA, so each particular primer will adhere to the template DNA randomly. As a result, the nature of the obtained products will be unknown. The DNA fragments generated are then separated and detected by gel electrophoresis. RAPDs can be detected by running PCR products through electrophoresis on an agarose or acrylamide gel.

In both cases, the gel is stained with ethidium bromide. The difference obtained by running RAPD products in acrylamide versus agarose lies only in the degree of resolution of bands. In most cases, agarose gel electrophoresis gives sufficient resolution.

Main features:

Specific regions of the DNA molecule are amplified for analysis of variation by the PCR, using primers (usually 10 bases),

- Random stretches of the genome will be sampled and amplified and used as the basis for variation analysis
- The no. of amplified fragments generated by PCR will depend on the length and sequence of the primer and the genome size
- In most plant s a 10 nucleotide primer will generate on an average 2-10 amplification products[20].

Many different fragments (corresponding to multiple loci dispersed throughout the genome) are normally amplified, using each single primer. The technique is therefore rapid in detecting polymorphisms. Although most commercially produced primers result in several fragments, some primers may fail to give amplification fragments from some material. The technique is simple. RAPD analysis does not require expertise to handle hybridization of DNA or other highly technical activities.

Amplified fragment length polymorphism (AFLP)

This is a highly sensitive method for detecting polymorphism throughout the genome and it is becoming increasingly popular. It is essentially a combination of RFLP and RAPD methods, and it is applicable universally and is highly reproducible. It is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of few nucleotide bases[19]. it is a novel DNA fingerprinting technique. DNA fingerprinting involves the display of a set of DNA sample. Fingerprints are produced without prior sequence knowledge, using a limited set of genetic primers. AFLP technique uses stringent reaction conditions for primer annealing and combines the reliability of RFLP technique with the power of PCR technique.

Interpreting AFLP bands:

- The AFLP technique detects polymorphisms arising from changes (presence or size) in the restriction sites or adjacent to those.
- Different restriction enzymes can be used, and different combinations of pre- and selective nucleotides will increase the probability of finding useful polymorphisms.
- The more selective bases, the less polymorphism will be detected.
- Bands are usually scored as either present or absent.
- Heterozygous versus homozygous bands may be detected, based on the thickness of the signal, although this can be tricky.

Procedure

DNA is digested with two different restriction enzymes. Oligonucleotide adapters are ligated to the ends of the DNA fragments. Specific subsets of DNA digestion products are amplified, using combinations of selective primers. Polymorphism detection is possible with radioisotopes.

Microsatellites (SSRs, STMS or SSRPs)

Microsatellites are also called **simple sequence repeats (SSRs**) and, occasionally, sequence-tagged microsatellite sites (STMS) or simple sequence repeat polymorphisms (SSRPs). The term "Microsatellites" was coined by Litt and Lutty. SSRs are short tandem repeats, their length being 1 to 10 bp, most typically, 2-3 bp. SSRs are highly variable and evenly distributed throughout the genome. This type of repeated DNA is common in eukaryotes, their number of repeated units varying widely among organisms to as high as 50 copies of the repeated unit. These polymorphisms are identified by constructing PCR primers for the DNA flanking the microsatellite region. The flanking regions tend to be conserved within the species, although sometimes they may also be conserved in higher taxonomic levels.

PCR product size variation is caused by differences in the number of microsatellite repeat units. SSR polymorphisms can be visualised by agarose or polyacrylamide gel electrophoresis. Microsatellite alleles can be detected, using various methods: ethidium bromide, silver staining, radioisotopes or fluorescence.

If fluorescence-labelled primers are used, and the products are different enough in size and not overlapping, then multiplexing that is, loading more than one sample per lane of reaction products can greatly increase the already high efficiency of these markers.

Inter-simple sequence repeats (ISSRS)

- Reported by Zietkiewicz *et al*[27].
- They are regions found between microsatellite repeats.
- The technique is based on PCR amplification of inter-microsatellite sequences.
- Because of the known abundance of repeat sequences spread all over the genome, it targets multiple loci.

Application of molecular marker in floriculture:

- Genetic diversity analysis
- Disease diagnostics
- Gene mapping
- Marker assisted selection
- Marker assisted backcrossing
- Identification of qtls / marker assisted pyramiding

Institutes working on molecular markers in floricultural crop

IIHR	Genetic diversity analysis in Bougainvillea and Carnation
	Molecular markers for Bacterial blight resistance in Anthurium andreanum
NBRI	Molecular characterization of plant viruses affecting ornamental plants.
	Varietal identification and assessment of genetic relationships in bulbous plants
IARI (NBPGR)	Genetic diversity analysis in Gladiolus cultivars
IHBT (Palampur)	Diversity analysis and characterization of viruses affecting ornamentals

Use of Molecular Markers in Bulbous plants

Crop	Marker used	References
Gladiolus	Morphological and RAPD markers (Genetic diversity)	Pragya *et al*, 2010
Lilium	RAPD	Benedetti *et al*, 2000
Gladiolus	RAPD (Fusarium Wilt)	Nasir *et al*, 2012
Alstroemeria	AFLP (Genetic diversity)	Han *et al*, 2000
Tulip	SNP Markers (Genetic mapping of resistance to *Fusarium oxysporum)*	Tang *et al*, 2015
Tuberose	Morphological and ISSR markers (Genetic diversity)	Kameswari *et al*, 2014

Marker assisted selection (MAS):-

It is the selection of trees with desirable traits based on their molecular genotype. It can be used alone or in combination with the classical methods of selection.

Benefits:

- ✓ Early selection that can potentially decrease the breeding cycle time.
- ✓ Decrease cost by reducing expensive progeny test establishment, maintenance and measurement.Increasing selection intensity because more individuals can possibly be evaluated.
- ✓ Increasing the relative efficiency of selection on low heritability traits

Molecular analyses of Gladiolus lines with improved resistance against *Fusarium* wilt[8].

Procedure:-

DNA from leaves of *In-vitro* selected, Gamma irradiated cell lines and control of Gladiolus cultivar **Friendship (CV-1)** was extracted using the **CTAB method** 29 RAPD primers were used for screening.The amplified products varied from 200bp to 1800bp. 5 primers were found to be polymorphic and produced different percentages of polymorphism. The average number of fragments per primer was 6 from which 62% were found to be polymorphic fragments

Molecular assisted breeding for disease resistance in lily[7].

The variation in resistance to Fusarium was determined in four different green house tests in four different years on the same 100 descendants of a backcross population. Asiatic hybrid lilies **'Connecticut King'** and **'Pirate'** were crossed. One F1 hybrid, the cultivar 'Orlito', was used as father in a backcross with 'Connecticut King'.'Connecticut King' is partially resistant to Fusarium wilt.15 AFLP

primers were used for analysis in 527 segregating population.Of the 527 segregating markers in the 'Connecticut King' x 'Orlito' cross 176 were originating from 'Connecticut King'201 from '**Orlito** and remaining 150 markers were present in both parents and segregated in the offspring population.

BEST DNA MARKERS FOR VARIOUS APPLICATIONS:

Applications that require large number of loci:

- ✓ Measuring genetic diversity and differentiation.
- ✓ Estimating rates of gene flow or migration (between population).
- ✓ Genetic linkage mapping or quantitative trait loci localization.

Markers of Choice- AFLP's OR RFLP's

Applications that require high discrimination power:

- ✓ Characterizing mating systems.
- ✓ Analyzing paternity or parentage.
- ✓ Characterizing patterns of gene flow or migrating within populations.
- ✓ Assessing seed orchard efficiency.
- ✓ Quality control in breeding, DNA fingerprinting or cross verification.

Markers of Choice- Microsatellites

Applications that require DNA sequence information:

- ✓ Phylogeny and taxonomy

Markers of Choice- PCR and sequencing

FUTURE PERSPECTIVES:

- ➢ DNA fingerprinting, through its high precision in identifying plant genotypes, holds considerable promise as a reliable tool of intellectual property protection of crop varieties and germplasm. In order to fully harness the potential of this technology, it is necessary to establish a network of DNA fingerprinting laboratories using uniform set of standardized protocols for each crop.
- ➢ protection of crop varieties or for verifying varietal identity. It has included electrophoresis of seed proteins in barley and wheat and of isozymes in maize, soybean and sunflower as additional characters for establishing distinctness of varieties.
- ➢ In India also similar sort of coordinated efforts are required to test the suitability and application of these marker techniques for molecular profiling of varieties and parent lines of hybrids.
- ➢ Molecular marker technologies can be used to attack trade secrets by rapid identification of female parent inbred line contaminants in bags of hybrid seed. These inbred lines might then be used directly as parents of hybrids or as parents for further breeding.
- ➢ Molecular marker technology can be used to identify segregating molecular characteristics in an otherwise uniform variety and thus to select a distinct "new" variety from the segregating source without any breeding effort being expended.
- ➢ Microsatellite markers have been used successfully to determine the degree of relatedness among individuals or groups of accessions, to clarify the genetic structure, or partitioning of variation among individuals, accessions, population and species of rice and similar trends can be applied in the field of forestry for the registration of hybrids.

CONCLUSIONS

- ➢ No genetic barriers
- ➢ Novelty through genetic engineering
- ➢ Speed of improvement
- ➢ Altered plant byproduct, form and colour
- ➢ Creation of genetic variation
- ➢ Easy acceptability than GM food crops
- ➢ Molecular markers provide valuable information about genetic diverstity and used to broaden the gene pool of ornamental crops.

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