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Short Communication

Application of Molecular Markers in characterization of Vegetable Crops

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India is the second largest producer of vegetables in the world and the per capita consumption of vegetables is about 174 g/day against 300 g/day. India accounts for one-third of all the vegetable pesticide poisoning cases in the world and 50-60% of vegetables are contaminated with insecticide residues. So, there is poor availability of good varieties and lack of improved production technologies for vegetables. Hence, there is a need for germplasm conservation and evaluation, genetic enhancement, varietal development, selection of indigenous lines, seed production, safe and sustainable vegetable production systems, post-harvest management, market opportunities and nutritional security. The vegetable breeders at government institutions and seed companies have to face some challenges in order to develop newer crop varieties possessing useful traits. The techniques employed for testing and screening will aid the possibility of maintaining a desired trait during the process of breeding.

Removal of undesirable traits and selection of desired traits in the preexisting varieties or lines lead to the development of cultivars. The traits controlled by genes that can be visualized or measured are selected. Genes provide all the instructions to determine the characteristic features of an organism. These are the hereditary units and are composed of the genetic material or DNA (deoxyribonucleic acid). This knowledge is applied for the development of potential DNA-based molecular markers to assist plant breeders in sorting large groups of plants more efficiently and in lesser time. Molecular markers are 'tags' that can be used to identify and locate specific genes. A plant breeder is capable to develop "fingerprint" or bar code like pattern with the use of molecular markers for easy identification of desirable or undesirable traits in a selection and/or breeding program. DNA-based molecular markers have been very significant in the cloning and identification of disease resistance genes from numerous plants.

DNA based markers are being used in the seed industries to develop "fingerprint" patterns for grouping or clustering complex beneficial traits of vegetable crops. The "cluster analysis" will help the plant breeders to assess local performance or environmental adaptation based on molecular marker patterns. For example, processing tomatoes that perform best in Ohio environment cluster differently from the tomato varieties adapted for California conditions. Therefore, the outcome and efficiency of selection strategies can be significantly improved by optimization of breeding crosses to a desired cluster of DNA markers. Identification of seed lot purity of asparagus at UC Riverside is another utility application of molecular markers. It is reported with the help of molecular markers that crown production in some of the seed lots consisted of more than 70% poor yielding types along with the ones with low postharvest quality. Such information can be of great use in relation to a perennial crop that is believed to be productive for a long span of time. Undoubtedly, classical breeding and selection techniques can't be replaced by the DNA-fingerprinting techniques. Infact, DNA-fingerprinting has become a potential accelerating tool so as to enhance the development and durability of plant-based traits that are disease resistant. Specific molecular monitoring in case of complex traits has provided the confidence in selection of traits and overall cost-effectiveness of the breeding programs. The expense of using the molecular markers is 100 to 1000 times higher as compared to the conventional phenotypic screening according to the latest data. Use of Marker Assisted Selection technique is not favored if we look at the cost: benefit ratio but has a immense use in management of complex traits like sensory quality and yield. Hence, the plant breeders apply the knowledge of biotechnology in daily routine so as to have more production and monitoring of postharvest traits.

Recent research in the field of plant biotechnology have resulted in the development of various advanced breeding technologies that meet these goals. Some of the examples of vegetable disease resistant traits identified with molecular markers are Downy Mildew resistance genes in lettuce, Anthracnose resistance gene in common bean, Powdery Mildew resistance gene in peas, Bacterial Spot resistance gene in pepper, Cyst Nematode resistance gene in potato and Tobacco Mosaic Virus resistance gene in tomato. The vegetable crops targeted by Monsanto Company for improvement by the help of molecular markers are mainly tomato, rootstock, pepper, eggplant (Solanaceous), Squash, cucumber, melon, watermelon, pumpkin (Cucurbits), Onion, carrot, leek (Root and Bulb), Sweet corn, garden bean (Large Seed), Broccoli, cauliflower, cabbage, radish, Chinese cabbage (Brassica) and Lettuce, spinach, fennel (Leafy).

- A sweet tasting lettuce, very low in bitterness and crunchier compared to traditional Romaine Would Be Delivered with Frescada™. It is going to provide a better eating experience as it would be greener in color and having more flavour. It has been preferred by many consumers in blind taste tests. It is currently trialing as specialty products and the second generation varieties to expand market opportunity are near launch.
- The Geminivirus resistance trait would protect the tomatoes against their most significant disease threat and would provide an excellent opportunity to improve marketable yield and fruit quality under disease pressure. The multiple resistance sources would have to be combined into elite germplasm to provide best protection package.
- Marker Assisted Breeding has to be broadly applied to improve resistance of Pepper to *Phytophthora*, one of the most destructive Pepper diseases globally. Significant improvements in *Phytophthora* resistance have been demonstrated by field evaluations. *Phytophthora* resistance has been incorporated into a broad range of pepper types through advanced breeding techniques.
- Molecular breeding would help in improving Downy Mildew Resistance in multiple varieties of slicing Cucumber and would yield an opportunity to reduce costly fungicide chemical applications for disease control.
- Seminis® EasyHarvest™ Broccoli could contribute in improving both harvest efficiency and consumer appeal as compared to the conventional varieties. Several key features to help make harvest easier are raised head even with leaf canopy, fewer leaves on stem to hand-strip, uniform maturity could reduce passes through field and consistently greener florets improves consumer appeal.

Uses of molecular marker in vegetable crops

Development of saturated genetic maps

Molecular markers are large in number and are not influenced by environment and development stage. For gene tagging, marker assisted selection and map based gene cloning, saturated linkage maps are the prerequisite. Based on SNPs, SSRs and RAPDs the first genetic linkages in male fertile garlic accessions were identified by Yayeh in 2005 [1]. Nine linkage groups were formed by 37 markers covering 415 centrimorgans (cM) with average distance of 15 cM between loci. A male fertility locus was located on the map. By using RAPD and SCAR markers a linkage map for watermelon was constructed by Zhang., *et al* [2]. Zhang., *et al.* [2] used recombinant inbred lines (RILs) developed by a cross between the high quality inbred line 97103 and the fusarium wilt resistant plant. This map is useful for identification of genes conferring resistance to fusarium wilt and for further development of quantilative trait loci (QTLs) affecting fruit quality.

Assessment of genetic diversity

For the assessment of genetic diversity in a wide range of plant species molecular markers have been proved as an excellent tool. This information is of direct utility to plant breeders as it indicates performance, adaptation or other agronomic qualities of the germplasm. Molecular markers provide important information about the overall genetic range of crop germplasm. This information is vital for the breeders for proper utilization of germplasm, particularly to search rare and unique genes. Germplasm of narrow genetic base is not likely to harbor novel genes e.g. genes conferring biotic and abiotic stresses resistance. Analysis of pepper breeding lines by RAPD markers, revealed narrow genetic base with more than 50% of DNA bands being common among all the lines [3]. Assessment of the world collections of tomato by Villand., *et al*. [4] revealed that the South American accessions of tomato have greater diversity than old world accessions. Shim and Jorgensen [5] carried out AFLP analysis in wild and cultivated carrots and observed that the old varieties released between 1974-76 are more heterogeneous than the newly developed F1 hybrids varieties. RAPD marker analysis of the old introductions and locally developed varieties of 1970s of tomato revealed significantly larger number of variation than the ones released in 1990s [6].

Gene tagging

Molecular markers have one of the most important application in facilitating the method of "conventional" gene transfer. Gene tagging refers to mapping of genes of economic significance close to known markers. Molecular markers that are closely linked to gene act as tag and these tags can be used for indirect selection of genes in breeding programs. By the construction of molecular map, several genes of economic significance such as disease resistance, insect resistance, stress tolerance, yield attributing traits have been tagged. For marker assisted selection and map based gene cloning, gene tagging is a pre-requisite. In case of tomato TMV resistance *Tm-2* locus, powdery mildew resistance gene, *Mi gene*, nematode resistance, *Fusarium oxysporum* resistance gene, has been tagged. In tomato the powdery mildew resistance gene ol-1 on chromosome 6 was tagged by using RAPD and SCAR markers [7].

DNA fingerprinting for varietal identification

DNA fingerprinting serves as important tool for varietal identification and for ascertaining variability in the germplasm. PCR based markers like RAPDs, RFLPs and microsatellite, are preferred as prior information on nucleotides is not essential. The fingerprinting information is useful for characterization of accessions in plant germplasm collections, to assess the genetic diversity, and for protection of germplasm especially the cms lines. For DNA fingerprinting of cultivars and breeding lines of a number of vegetable crops like beans [8], tomato [9], pepper [10], molecular marker has been used extensively. DNA technology has huge potential for enhancing purity valuation in hybrids. To distinguish the closely related lines as well as high yielding varieties, the available molecular tools from the simplest ISSRs, RAPDs, DNA amplification fingerprinting (DAF) to more elaborate, precise but robust microsatellites, AFLPs, and RFLP based variable number of tandem repeats (VNTR) have been used.

Breeding lines and accession identification

In a breeding programme, identification of accessions and breeding lines is very important. In breeding experiments large number of lines are handled and thus they can get contaminated due to mixing of seed samples and cross contamination in field. This may lead to mislabeling of the plants/seeds. Such accessions are difficult to distinguish as they differ in few morphological traits. But the molecular markers can easily distinguish such closely related genotypes. By using RAPD markers, Waycott and Fort [11] successfully differentiated nearly identical germplasm lines of bitterhead lettuce. Microsatellite probes were used to fingerprint various accessions of tomato and reported the efficacy of microsatellite probes to assess the purity of breeding lines and F1 progeny testing [9]. Tivang., *et al*. [12] used RAPD markers to identify variation among and within artichoke breeding populations. RAPD and RFLP markers were used in asparagus to distinguish F1 from F2 seeds and to evaluate the purity of seeds [13]. Microsatellite markers were used by Fisher and Bachmann [14] to distinguish 83 accessions of onion.

Sex identification

Considerable efficiency in breeding programmes of dioecious species can be brought by early identification of male and female plants. SCAR markers in the asparagus were developed by Jiang and Sink [15]. These were linked to the sex locus at a distance of 1.6 cM. To facilitate the differentiation of XY from YY males in asparagus, STS markers were developed by Reamon Buttner and Jung [16].

Identification of cultivar

Microsatellites were developed to allow vastly reliable identification of cultivars in crops like potato, tomato, cucurbits, pepper, lettuce and spinach. In tetraploid potato, the comparative assessment of different DNA fingerprinting techniques showed that the AFLP have the maximum discrimination power followed in decreasing order by multilocus SSR, RAPD, ASSR and single locus SSR. In pepper, ISSR markers were observed to be the most effective in detecting polymorphism by Gaikwad., *et al*. [17]. However, the marker index of AFLP markers was prominently higher than that of ISSR and RAPD due to very high number of markers generated per assay by AFLP. The intellectual property rights are granted to the developers of new crop varieties in the form of plant breeders' rights under The Indian Plant Variety Protection and Farmers' Rights (PVPFR) Act 2001. A candidate variety must meet the criteria of distinctness, uniformity and staibility (DUS) to be qualified for registration and protection under this act. The basis for DUS testing is provided by the morphological data. To determine the distinctness of a variety, the variety is compared with the extant varieties for a number of characters. Under the PPVFR there is a provision for the Essentially Derived Varieties (EDV). In this provision the protection benefits are to be shared with breeders of the variety from which the EDV has been derived. At present, as a proof of unique identity of a plant the DNA profiles of the variety alone is not adequate. However, molecular profiles may strengthen the claim of the plant breeders for protection of new varieties to establish the distinctness of their varieties. In cases of biotechnologically developed varieties, molecular profiles may have high significance where only slight phenotypic differences exist between new variety and an extinct one.

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