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Tilling and Eco Tilling

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Introduction

TILLING and Eco TILLING have been proven to be highly effective reverse genetic tools for functional genomic studies in plants and animals. Since the inception of these techniques, many researchers have gained indispensable insight on gene function and have identified natural and induced variants. These methods are now well established for many model plant and animal systems regardless of their mating system, genome size or ploidy level. Eco-TILLING is a high throughput, low cost technique for rapid discovery of polymorphisms in natural populations. Eco-TILLING is similar to Targeting Induced Local Lesions in Genomes or TILLING, but Eco-TILLING differs in that naturally occurring polymorphisms are detected as opposed to experimentally induced mutations. Single Nucleotide Polymorphisms (SNPs), small insertions and deletions and variations in microsatellite repeat number can be efficiently detected using the Eco-tilling technique. Furthermore, in highly heterozygous outcrossing species, Eco-TILLING can be used to determine heterozygosity levels within a gene fragment.

History and Methodology

TILLING first began in the late 1990's from the effort of a graduate student, Claire McCallum (and collaborators from Fred Hutchinson Cancer Research Center and Howard Hughes Medical Institute), who worked on characterizing the function of two chromomethylase genes in *Arabidopsis*. Claire McCallum utilized reverse genetic approaches such as T-DNA lines and antisense RNA, but was unable to successfully apply these approaches to characterize CMT2. The TILLING technique was first utilized in *Arabidopsis*, a workshop to develop TILLING as a service to the *Arabidopsis* community, known as the *Arabidopsis* TILLING Project (ATP), was initiated in 2001. In the first year of public operation, the ATP has detected, sequenced and delivered over 1,000 mutations in more than 100 genes ordered by *Arabidopsis* researchers The approach that was successful turned out to be what is now known as TILLING (Targeting Induced Local Lesions in Genomes). This was accomplished by pooling chemically induced mutagenized plants together, amplifying the region of interest, creating heteroduplexes among the pooled DNA and performing DHPLC (Denaturing High Performance Liquid chromatography) to detect the mutants by chromatographic alterations. Since the inception of this method, TILLING has been streamlined, automated and utilized in many plant and animal taxa. TILLING, which is a reverse genetic high throughput approach, aims to identify SNPs (Single Nucleotide Polymorphisms) and INDELS (Insertions / Deletions) in a gene / genes of interest from a mutagenized population. Therefore, the first step in TILLING is the creation of a mutagenized population, which is often accomplished by treatment with a chemical mutagen such as EMS. Many plant species are well suited for this strategy because they can be selffertilized and seeds can be stored for long periods of time. In plants, seeds are treated with EMS and grown out to produce M1 plants, which are subsequently self-

fertilized to produce the M2 generation. Leaf tissues from M2 plants are collected for DNA extraction and then used for mutational screening .To avoid sampling of the same mutation only one M2 individual from each M1 is chosen for DNA extraction. The M2 progeny can be self-fertilized and the resulting M3 seed can be preserved in long term storage. EMS has been widely used as a chemical mutagen in TILLING in both plant and animal studies to generate mutant populations, although other chemical mutagens can be effective. EMS typically produces transition mutations (G/C : A/T) because it alkylates G residues and the alkylated G residue pairs with T instead of the conventional base pairing with C. It is a beneficial strategy for users to try a range of concentrations of the chemical mutagen being applied to evaluate the toxicity and sterility on germinal tissue before preparing large mutant populations.

One of the main advantages of TILLING is the amount of time and money this method can potentially save by not requiring resequencing of all individuals in a population to mine for frequent or rare SNPs. As a general rule for a diploid organism, Furthermore, TILLING is sensitive enough to detect homozygous mutations as well as heterozygous mutations in an 8 fold pool, which represent 1 of the 16 genomes in pools from diploid species EcoTILLING can be a good technique to employ especially when working with a well established population with thoroughly characterized morphological data. These can be used for Mapping, Association Analysis, Mutational Profiling and Biodiversity.

Mutation Breeding In India

Besides IARI, mutation breeding work is being pursued at several universities and research institutes, notably, at Bhabha Atomic Research Center (BARC) Mumbai, Tamil Nadu Agricultural University (TNAU), Coimbatore, National Botanical Research Institute (NBRI), Lucknow. The mutant varieties released includes cereals, grain legumes, oil seeds fibre crops, vegetables and ornamental crops. By 2004, a total 313 mutant varieties were developed in India (Kharkwal *et al.*, 2004), out of which, 16 varieties were developed in vegetables. The success story of mutation breeding in ornamental and horticultural crops in India is particularly impressive. In chrysanthemum alone, 46 mutants are commercially released. Considering the fact that the mutations are generally deleterious, the number of mutant cultivars released in major crops is impressive. It is relevant to emphasize here that in all cases (except ornamentals) that mutant varieties have emerged as superior to other entries in all India coordinated trials being approved for commercial release.

Gamma Garden

Gamma garden is an area subjected to gamma irradiation. This area is enclosed by thick-high walls to protect the plants and animals outside the area from radiation damage. The purpose of a gamma garden is to irradiate whole plants during different stages of development and for varying durations. The source of radiation is located in the center of gamma garden, which is usually circular in outline. The gamma ray source consisted of 6g ^{60}Co in form of small pellets in an aluminium capsule.

The first gamma garden was built in Long Island near New York, U.S.A. The first gamma garden in India was installed in Calcutta at the Bose Research Institute in 1959. After in 1960, gamma garden was built in New Delhi at the Indian Agricultural Research Institute (IARI), and later at the Bhabha Atomic Research Center, Trombay. The IARI gamma garden had an area of 3 acres and was surrounded by a wall 12 feet high and 3 feet thick.

Applictions of Mutation Breeding

Mutation breeding has been used for improving both oligogenic as well as polygenic characters. Mutagenesis has been used to improve morphological and physiological characters including yielding ability. Various applications of mutation breeding are:

- Development of Improved Varieties.
- Induction of Male Sterility.
- Production of Haploids.
- Creation of genetic variability.
- Overcoming self-incompatibility.
- Improvement in Adaptation.

Advantages

- Mutation creates inexhaustible variation.
- When no improvement is possible this method has to be adopted.

Limiations

a. Frequency of desirable mutations is very low about 0.1 percent. To detect the desirable one in M2 considerable time, labour & other resources are to be employed.

b. To screen large population, efficient quick and inexpensive selection techniques are needed.

c. Desirable mutations may be associated with undesirable side effects due to other mutations thus extending the mutation breeding programme.

d. Detection of recessive mutations in polyploids and clones is difficult and larger doses of mutagen have to be applied and larger populations are to be grown.

e. Mutation produce *pleiotropic* effects.

f. There may be problems in the registration of mutant variety.

g. Most of the mutations are recessive.

Achievements

The world's first variety developed from a mutagenesis programme was X-rays induced cotton variety MA-9 released in 1948 in India, it had enhanced drought tolerance.

The total 2,252 mutant varieties, 1,585 were developed 'directly' after mutagenic treatment and selection in the subsequent generations. However, in many cases mutants or already released mutant varieties have been used as sources of desired characters in cross breeding programmes; in this way, 667 new varieties were developed. 1585 directly developed mutant varieties, a great majority (1411) were obtained with the use of radiation as mutagen.

Number of officially released mutant varieties in the top six countries (total 2252)

Country	Number of released mutant cultivars	Percentage of total
China	605	26.8
India	259	11.5
USSR + Russia	210	9.3
Netherland	176	7.8
USA	128	5.7
Japan	120	5.3

Number of officially released mutant cultivars developed with different types of radiation

Types of mutagen	Number of released mutant cultivars	Percent total
Radiation*	1411	100.00
Gamma rays*	910	64.49
X – rays*	311	22.04
Gamm chronic	61	4.32
Fast neutrons**	48	3.40
Thermal neutrons	22	1.56
Others	24	1.70

* including various treatments,
** including "neutrons"

Mutant used and trait improved in mutant cultivars released in India

Mutagen	Number of mutants	Main attribute	Number of occurrence
Gamma rays	169	High yield	86
X – rays	26	Early maturit	65
Neutrons	7	Disease resistance	57
Ethyl methane sulphonate	15	Quality characters	39
Dimethyl sulphate (DMS)	4	Grain quality	67
Ethylene imine (EI)	2	Abiotic stress resistance	65
Sodium azide (NaN3)	2	Improved plant type	181
Other mutagens	29	Other	9
Cross bred	47		
Natural mutants	12		

Mutant varieties of different crops released for cultivation in India

Crop	Number of varieties released	Specific crop and number of varieties
Cereals	69	Rice (39), Barley (13), Pearl millet (5), Finger millet (4), Foxtail millet (1), Wheat (4), Sorghum (3)
Pulses	53	Mungbean (14), Black gram (7), Chickpea (7), Cowpea (7), Mothbean (5), Pigeonpea (5), Lentil (3), Lablab bean (2), Cluster bean (1), Common bean(1), Pea (1)
Oilseeds	33	Groundnut (16), Mustard (6), Castor bean (4), Sesame (3), Soybean (4)
Fibre crops	14	American cotton (8), Tossa jute (3), White jute (2), Desi cotton (1)
Vegetables	12	Tomato (4), Turmeric (2), Bitter gourd (1), Brinjal (1), Green paper (1), Okra (1), Ridge gourd (1), Snake gourd (1)
Cash crops	10	Sugarcane (9), Tabacco (1)
Medicinal crops	16	Citronella (8), German chamomile (1), Indian henbane (2), Isabgol (1), Khasianum (1), Opium poppy (2), Spearmint (1)
Fruit trees	2	Mulberry (1), Papaya (1)
Forage crops	1	Egyptian clover (1)
Ornamentals	103	Chrysanthemum (46), Rose (16), Dahlia (11), Portulaca (11), Bougainvillea (10), Wild sage (3), Gladiolus (2), *Hibiscus* sp., (2), Tuberose (2)

Conclusion

Among various method of breeding in crop plant mutuation breeding i.e., induced mutation is one of the preeminent methods of creation of variation/genetic variation. Conventional method of breeding takes long time to improve a crop variety due to a very slow increase in genetic variation. To overcome this induced muttion play a crucial role which helps in creation of genetic variation in a short period. Over last several year's mutation breeding is getting popular and is adopted by several countries. It improves several qualitative and quantitative character of crop plant and is sucessfully applied in several cereal, grain legume, oil seed, vegetable, fruits, medicinal plant, ornamental plants and fodder crops.

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