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Review Article

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Characterization of Advanced Tilling and Its Application in Cotton

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Abstract

Among all the fibre crops, cotton is most important economic fibre crop. It is known as white gold because it contributes in the economic, industrial and agricultural sectors. In GDP, it contributes to about 1.6% and have share 7.8% in agricultural products. Pakistan is ranked 5th in world in term of consumption and has 4th in term of total cotton production in 2015-2016. The seed cotton yield and production are stagnant for more than decade in Pakistan, biotic and abiotic stresses are considered as major reasons of this stagnancy. Mutagenesis is an important tool in crop improvement. In breeding programs, mutation is an important tool for creating the variations. Powerful reverse genetic strategies allow the detection of induced point mutation. TILLING (Target Induced Local Lesions in Genomes) is genomic approach which is used for the screening of mutant and germplasm collection for the for the allelic variant in targeted gene. This kind of research explores an advanced TILLING population for various parameters. The main advantage of TILLING is that this technique can be used for any plant species, irrespective of its genome size, ploidy level and method of propagation.

Keywords: Biotic, Genomics, Mutagenesis, Propagation, TILLING.

Introduction

Cotton (Gossypium hirsutum L.) known as a white gold, is the leading fibre crop of the world that earns a considerable economic worldwide fibre lint production. The rise and fall in cotton production greatly affects GDP as well as affect the earnings and employment in Pakistan. Cotton seed is a source of oil, which is used for cooking purpose, dressing of salad and as a raw material in soap industry. Seed meal is used as animal feed. It is a source of many commercial products, like cellulose, which is consumed in cosmetics and in paper industry. Seed hull contained 100% cellulose, is frequently used in livestock feed and used as mulch to protect the loss of water [1].

Cotton is placed in the genus Gossypium of the family Malvaceae that consist of about 50 sprecies. These cotton species are extensively classified into 45 diploid and 5 tetraploid species. tetraploid species are classified as primary gene pool, diploid species as secondary and tertiary gene pool [2]. Cotton is cultivated in old world

approxmately 7000 yeara ago. Now most of the cotton is grown in USA, China, India and Pakistan.

Cotton act as a backbone of the pakistan economy. It contribute 7.3% in value added of agriculture and about 1.53% in GDP. In 2014-2015, cotton crop was cultivated on the area of 2.961 millions hactares in Pakistan. Increased in cotton growing area was seen in last few years. There is about 5.5% more area was cultivated under cotton crop than that of the previous records. In 2014-2015, the production was about 13.98 million bales againts 12.769 million bales previously 9.5% increased [3]. During 2015-16, there was a sharp decline in the production of cotton due to the bad climatic changes and insects attack.

Any crop with the narrow genetic base is at more risk to attack by natural diasters. Breeders have been utilizing the same parents that have few economic traits in their breeidng programs.

Due to their dependence on the available elite germplasm for cultivar development, genetic base of cultivated varieties has become narrow [4,5]. Molecular studies using RAPD marker had also confirmed the low level of genetic diversity in upland cotton germplasm [6]. The genetic similarities of cotton among the cotton varieties lead to their susceptability to cotton leaf curl virus CLCuV that is a major threat to cotton production [7].

The success of any breeding program is largely associated with the presence of high level of genetic variability in the gene pool. Genetic variability increases the heterogeneity that increase the resistance against biotic and abiotic stresses [8]. Genetic variation of desired traits is a pre-requisite for any crop improvement program.

The progress in either single or few economic traits and quailty characteristics can be achieved with the help of induced mutation by giving rise to many diverse mutant alleles with different degree of trait modification [9]. These induced variations may express in the phenotype or remain salient on DNA. Identification of these variations or mutations is needed for the breeder to know the function of the genes. Many traits that are important for the production would benefit from the ability to understand and modify the function of specfic gene [10]. High throughput reverse genetic approches link the sequence information of genes with the biological function and also have the capacity to recognize the novel variation in gene that would be a new source for crop improvement program [11].

TILLING a reverse genetic based approach is used for the identification of SNPs in a specfic gene [12]. This technique screened the mutated genotype to find the induced mutations in the sequence of desired genes. It used the CELL I endonuclease enzyme to cut the hetero duplexed DNA strands followed by the detection of cleavage products [13].

In the previous studies by using this technique novel genotype were developed that further linked with phenotype e.g. wheat with low amylose contents, sorghum with low lignin [14,15].

Exposure of a cotton plant to a potential pathogen activates the many defense related gene [16]. Research for the defense mechanisms used by the cotton plant has far been concentrated on the role of antibiotic flavonoids, terpenoids and structural defense e.g. vascular occlusion. Recent advances in molecular techniques have enabled the investigation of genes underlying the observed defense reponse in Gossypium spp [17]. It is considered that characterization of defense gene analogues (DGAs) in cotton can help to identify DNA markers that are closely linked to the defense genes and ultimatley lead to the mapping and cloning of cotton defense genes. The present study was aimed to assess the genetic diversity of the advanced TILLING population (Ms) of cotton and also to characterize the selected mutant genotypes at molecular level, to identify the mutation in defense related gene.

Review Literature

It decribes the genetic variabiloty in population and guides to achieve the specfic breeding objective [18,19]. Heterogeneity is to develop buffering capacity in crops againts the climatic stresses [20].

Genetic diversity is essential for sustainable crop production. Genetic diversity studies are important to know associations among varieties, to identy the diversity of germplasm and to develop effective strategies for preservation and management of germplasm [21]. Genetic diversity provides insight view of genetic structure of varieties that have different breeding origins; this data enables the breeders to select the suitable material that is used as a parent in breeding programs or directly released as a variety.

Genetic Diversity of Cotton

To access the genetic diversity different type of data sources are used by several researchers, including breeders. In the pre-genomic era source such as morphological, agronomic, pedigree, proximate and biochemical were used while in postgenomic era molecular marker data, especially single nucleotide polymorphism (SNPs) were used [22]. The first step in the description and classification of genetic resources in morphological characterization that is the strongest base of the agronomic and taxonomic value of the crop plant [23].

It is the performance of a plant in field that determine that either it is suitable for a breeding program or not. Phenotypic varience that is determined from environmental and genotypic interaction, has great connection with the genetic diversity at molecular level [24].

Morphological data is based on visually accessible and measurable trait such as plant height, seed weight, seed shape, flower color, growth habits and pigmentation; it does not require expensive technology like NMR spectroscopy, ELIZA reader to measure the data. These traits allow estimation of diversity in the presence of environmental variation that cannot be ignored from the genotype variation [25].

Data generated through biochemical and molecular markers also used in genetic studies. Biochemical markers are allelic variant of enzyme known as isozyme. These are co-dominant in nature, detected by electrophoresis and specific staining. By these enzymes diversity can be studied at functional level. It requires only small amount of molecular markers that are used for analysis of genetic and molecular variation. These markers can detect the variation that arises from deletion, duplication, insertion and inversion in chromosome [26].

Techniques for the Obervation of Genetic Diversity

Different statistical method are used to detect the genetic diversity by using morphological, biochemical and molecular data. The choice of any statistical method that has to use is depend on the objectives of the studies.

Metro Glyph Analysis

Andersion 1975 work suggested the use of metro glyph and index-score to check the morphological variation in data of given traits. This method was used to assess the morpholigical variation in green gram [27]. Use of metro glyph analyses and index score to access the genetic diversity of cotton genotypes under salinity condition was explored in 13 cotton genotypes for various physiological and biochemical traits. The genotypes were grouped into five cluters with maximum index score of 97 per cluster. Susceptible and tolerant genotypes under different salinity level were also identified [28].

Metro glyph analyses were used for assessing patterns of morphological variartion and selecting early maturing genotypes. Fifty-one lines of cotton subjected to metro glyph analyses. High significant genotypic difference were observed for different morphological characters. On the basis of early maturing while varieties with lowest index score as late maturing [29].

As the sample size of an experiment increased, approaches to determine the genetic variability are also changed. Multivariate statistical algorithms are used to classify germplasm, assembling variation and analyze the genetic relationships in breeding materials. Multivariate techniques are extensively used in assessing genetic diversity regardless of data either it is morphological,biochemical, or molecular markers. Among these cluters analysis, principal coordinate analysis (PCOA), Principal component analysis (PCA), Multidimensional scaling (MDS) and Canonical Correlation are widely used.

Custer Analyses

This analyses show the patterns of association among the genotype and exclusive grouping in such a way that mathematically similar descriptiors are grouped togather into the same cluter [22].

Each cluster showed high homogeneity within a cluster and high heterogeneity among clusters. Cluster analysis are classified into five methods un weighted group method, single linkage, complete linkage and median linkage [30].

Genetic diversity among the hundred accessions of cotton was assessed by using cluster and PCA analysis. Cluster analysis classified the hundred accession into four diverse clusters. Cluster 3 and 4 contain the genotype that were tolerat to CLCuV and have good fibre quality and higher yield potential. Dedogram explained the adequate diversity among the cotton genotype and some extent of association also demonstrated among different cluster [31].

In the last few years demand of naturally pigmented cotton is increased, but its cultivation is not enhanced due to its poor fibre characteristics and objectionable micronaire value. To develop elite colored cotton genotype that have good quality and high yield of fibre, genetically diverse parents are needed. Extent of genetic diversity among the colored cotton genotypes was assessed by cluster analyses [32].

Prinicpal Component Analysis

Principal component is used to develop a 2 or 3-dimensional scatter plot of individuals in which genetic distance among genotypes are represent by geometrical distance present among the individual genotype. To check the contribution of characters towards variation of genotypes, principal component analysis was used to analyst 100 cotton genotypes. Thses genotypes were evaluated for different morphological, disease as well as yield and yield related traits.the first five principal axes togather explained above 70% of the totaol variation among 10 characters that describe the hundred genotypes [31].

Fifty upland cotton genotype were analyzed by linkage cluster abd principal component analyses to identify the major characters which account for the variation in yield contributing traits. PC analysis showed that first 4 PCs had eigen values greater than 1 which, explained 64.8% of the total variation among the cotton accession [33].

Present Condition of Cotton Varieties

Genetic diversity present in specific species continuously decreased due to the selective breeding, environmental adaptation and species extinction. During crop evolution and domestication selective breeding decrease the genetic diversity. In this era, breeder only focused on the development of high yielding and genetically uniform cultivars for greater improvement regarding yield, adaptation and quality. With the passage of time elite cultivars replaced the land races and obsolete cultivars. The replacement of land races with a few genetically uniform varieties decreases the genetic diversity [34].

Genetic diversity is thought to be narrowing down gradually in modern cotton varieties. Information about the narrow genetic diversity in commerical varieties is less. Increased pressure for higher productivity has contantly stimulated the same gene pool that lead to a narrow genetic base which is becoming a big hinderance in breeding programs [35]. In an experiment, genetic variability in commerical as well as in popular transgenic varieties in cottonseed market was evaluted by using SSR markers. 177 SSR bands were amplified by using 88 primers. The average Jaccard's genetic similarity coefficient was 0.77. heighervalue of genetic similarities was found in varieties belonging to same company [36].

Colored and white lined cotton genotypes belonging to G. hirsutum and G. arboreum were used to assess the genetic diversity. RAPD markers were used to check the diversity. 45 random decamer primer were used , out of which 23 primers displayed polymorphism and they produced 205 fragments. One hundred and forty four showed polumorphism with a rate of about 70.24%. Data generated through primers were analyzed by cluster analysis. Genotypes belonged to different species group togather showing that these genotypes have narrow genetic base. All the genotypes belonging to G. hirsutum grouped togather except NIAB-801 and CIM-446. Genotypes belonging to G. arboreum clustered togather [37].

Pakistan cotton varieties released before and after year 1975 were used to test the level of diversity. Phenotypic data of 16 characters were analyzed by principal component analysis and cluster analysis. PCA exhibited 41% genetic diversity in pre-1975 group while 37% genetic variability was found was found in the psot-1975 group. Score plot analysis result showed that modern varieties were present very near to each other while varieties of pre-1975 group scattered. In cluster analysis based on similarity index all cluster, while showed that the genetic basis of upland cotton has been narrowed down due to the current breeding efforts [38].

Mutation Breeding and Genetic Variability

Crop improvement program cannot be started without the presence of genetically diverse germplasm. To meet current and future challenges, diverse genetic variation is needed that enable the plant breeder to make decision regarding proper use of genetic resources. These genetic variations may already exist in nature or may be created by man [39].

Breeders use many tools like the intoduction of exotic germplasm, mutation, hybridization and polyploidy to increase the variation. Parental crosses also acts as a source for creating genetic variation in subsequent progenies [40].

The induction of new genetic variation offers a unique way to identify novel traits in inbred elite cultivars as well as helps to retain the agricultural excellence of these line. Mutagenesis has generated enormous genetic variability and played an improtant role in plant breeding program throughout the world. The role of mutation breeding for increasing the genetic variation in desired traits of various crop plants proved to be beyond doubt [41].

By using physical and chemical mutagens wide range of genetic variability has been induced to utilize for crop improvement. Mutation breeding had been used to produce the genetic variation that were further successfully utilized to increase the yield and yield components and change the structure of plant in various crop like rice [42].

This utilization showed that this technique has a potential to improve crop production. This economic importance of mutant crop varieties, mainly based on manifestations like annual acreage; increased yield; enhanced market values; saved use of agricultural inputs; increased earning of the farmers; decrease in food importations. Mutant varieties database (MVD) is maintained by joint the division of international atomic energy agency and the food agriculture organization of the United Nations in Vienma, Austria. FAO/IAEA Dvision documentated more than 3200 mutant varieties with one or more useful traits. More than 1000 mutant varieties of staple crops, increase rural income, improve human mutrition and contribute to environmentally sustainable food security in world.

Utilization of Spontaneous Mutation

Spontaneous mutation naturally occur and remove certain undesired wild characteristics. These are unusual types of mutation for which no intervention has been made by man. For the development of agriculture these spontaneous mutation acted as a driving force in different agricultural areas e.g. in the Fertile Crescent of West Asia, Northern and Southern China, New Guinea, Africa's sahel, the Andes and several regions of America. these variation exploited by man to develop varieties with desired traits. The sami-dwarf varieties of cereal crops. E.g. wheat and rice also rexult due to spontaneous mutations. Spontaneous mutation occurred in the WG variety lead to develop DG (Di Jiao) form of rice that was utilized for the development of semi variety taichung Native 1.

In the bread wheat spontaneous mutations occurred in Japanese variety Daruma that reduced its height. These mutation were utilized for the development of semi dwarf variety Tohuko 34 also known as Norin 10. Two genes Rht1 and Rht2 were responsible for semi dwarfness of Norin 10 [43]. These short stature varieties were fertilizer responsive characters increased the grain yield in 1960s and became the reason of "green revolution". In many other crops spontaneous mutations were also reported that were utilized by the man for their improvement e.g. abolishment of bitterness toxicity in almond, seedless and parthenocarpic genotype in banana and grapes, compact spike unsuitable for shattering in wheat and barley, compact pod in pea and self-compatible, hermaphroditism in grapes [44].

Utilization of Induced Mutation

Spontaneous mutations have low frequency, often the desired variations are not present in nature. These problems of natural mutation can be resolved by aritifical induction of mutation known as induce mutation. In middle of the 20th ceentury, various techniques were emerged to induce the mutation and artifically increase variation [45].

Inducdion of mutations has become a sustainable way to create genetic variation in the crop variety. Mutation inducing agents are known as mutagens. These mutagens are classified into two physical and chemical mutagens.

The discoveries of X-rays, radioactivity and radioactive derived the ability of man to induce mutation in living organisms. Muller used the x-rays as a mutagenic agent for Drosophila population and presented that x-rays treatment increased the mutation rate by 15,000% [46].

After the successful use of X-rays in animals, it was practiced in plants. Stadler exposed the plant of barley and maize with X-rays and radium, he observed a strong phenotypic variations in barley seedlings and sterility in poisonous chemical. The gas contained nitrogen mustard that would be shown to cause mutations in living cells. After this radiations-based techniques were replaced by chemical mutagens that were less harmful, easily available and peaceful to apply.

Mutation in Pakistan

In Pakistan, the mutation program was started at the Nuclear Institute for Agriculture and Biology (NIAB) to improve the food and fibre crops. This institute is working to improve the crops such as rice, chickpea, mung bean, plant architecture, abiotic and biotic stress resistance. The result achieved by the mutation treatment have helped the scientist to evolve the varieties which are suitable for the country need. The first achievement of NIAB is 4 varieties of rice. These varieties were proved breakthrough for rice produc-

tion and increased the yield per hectare in Pakistan [47].

In Pakistan, Iratom 38 waas 1st mutant variety of rice released in 1970 developed by irradiation. Main improved attributes of the variety were early mutarity and higher protein content. Futher mutation were introduced in "Basmati-370" that resulted in Kashmir Basmati that was early maturing, had cold tolerance and retained some characteristics of its parents like aroma and cooking quality [48].

Three mutatn varieties of wheat Jauhar 78, Soghat 90 and Kiran 95 were released in Pakistan. The cultivar Jauhar 78 had high yield, wider adapatibility, resistance to shattering and amber grain color. Soghat 90 developed from Pavon, had high yield, high protein, amino acid contents e.g. lysine and tolerent to leaf rust. The cultivar Kiran 95 was also high yielding mutant variety [49].

In mung bean mutation breeding was used to develop the early maturing, short stature varieties. 11 mutant varieties of mung bean were released in Pakistan. NIAB mung-92 and NIAB mung-98 got more popularity. Both cultivar was high yielding and resistant to diseases especially yellow moasic virus and Cercospra leaf spot [50].

In 2006, NIAB 2006 was released by the hybridization using NIAB 92 as one parent had purple hypocotyl and stem, high number of pod and resistance to diseases [51].

In the cotton breeding mutation program was very furitful, yielding many mutants with high yield and early maturity. NIAB-78 was released in 1983, adopted widely and brought a revolution in cotton production of country. Main attributes of this variety were short stature, determinate growth habit, tolerance to abiotic and biotic stress such as heat and bollworm due to its early maturing nature. Due to its early maturing nature it considered as an ideal cultivar in cotton-wheat rotation. Within 5 years of release, it supported the textile industry of cotton by contributed highly in the growth of textile Punjab it covered about 70.8% of total cotton area. During heat stress conditon, when boll shedding started it produced a second flush of flowers [49].

Selection among the Mutants

Traditional forward based mutant screening were carried out to find out the genes involved in a specfic pathway of a traits development e.g. if a researcher had an interest to study flower development pathway, firstly he would collect mutants that could be generated by chemical treatment or by other means. Isolated mutants were then studied and crossponding genes involved in flower development pathway were eventually identified [52].

Separation of gene families was diffcult because they were obscured phenotypically due to the functional blurrines among closely related genes. To solve this problem, reverse genetics should be utilized that allows the identification of mutations in all association of a gene family. In the past few years, many reverse gentics based techniques were evolved to identify gene mutations. These techniques are insertion mutagenesis, virus induced gene silencing, agrobacterium mediated transformation, transposon tagging, RNA- mediated interference, TILLING, error-prone PCR, next generation sequencing [41,53].

Tilling For Mutant Screening

In the world of post-genomic squencing, the modern genomic technologies were applied for studying the gene function. The approaches which give the information about the targeted inactivation genes are reverse genetics approaches that are identified by the squenceing analysis including TILLING [54].

He enlisted the 3 steps of TILLING. The 1st step is treatment of seed with mutagen and lead to the formation of mutagenized population M1 and M2 followed by pooling of DNAs from M2. 2nd step is the amplification of targeted DNA fragment then formation and identification of heteroduplex for detection of mutant plants, 3rd step is analysis of mutant phenotypes [55].

A cheaper and faster amendment was made in TILLING protocol, which uses a celery nuclease enzyme and L1-COR gel analyzer system to detect the mutations [53].

In 2001, the standard protocol was made with practical software that makes the TILLING techniques a routine ethod to detect mutations to get satisfactory results [56].

The main adventage of TILLING is that it can be practiced for any plant species, irrespective of its genome size, ploidy level and method of propagation. For TILLING fully sequenced genome of the species do not require, the sequence of required gene can be retrieved from gene database like gene bank or by searching homology by basic local alignment search tool (BLAST). TILLING needed small amount of individuals to rearch saturation mutagenesis as compared to other reverse genetics based approches, TILLING needed 5000 M1 plants as compared to T-DNA mutagenesis that needed 36000 [53].

This method offers an allelic series of mutants; knockouts are also included in them. During all steps in TILLING strategy bioinformatics analyses are required. These analyses are needed to determine the sequence of gene that is more prone to mutantes and also to determine impact of allele on function of protein [41].

Defence Mechanism of Cotton Plant

Cotton plants are susceptible to a wide range of pathogens including bacteria, viruses, fungus and nematodes which are harmfull effects for the yield and for the product quality [57]. Many genes involved in plant-pathogen interactions have been identified. Genes providing resistance to plants classfied into two main groups; resistance and defence group. Resistance group composed of genes that involved in pathogen recognition and signals transduction [58]. While the defense group consist of genes that are concerned with defense mechanism and their products synthesis occurs de novo in response to pathogen recognition [59].

The defense responsive gene plays a role in limiting pathogen invasion of plant tissues, by limiting pathogen growth, development or propagation within the plant [60].

The cotton plant organizes a coordinate response that is specifically directed towards the rapid containment of the pathogen to stop the colonization in the vascular system. The success of the defense reponse to center on the ability of the cotton plant to rapidly negate

the systemic spread of pathogen with a combination of constitutive and induced defense mechanisms [61].

Research into the defense response mechanisms employed by the cotton plant has thus far been concentrated on the role of antibiotic flavonoids and terpenoids and structural defences such as vascular occlusion. Recent advances in molecular techniques have enabled the investigationa of genes involved in defense response in Gossypium spp. [62].

Conclusion

Cotton is an important fibre crop. It has a share of about 7.3% in value added of agriculture and about 1.53% in GDP. The economy of Pakistan is depent on the cotton because the industrial sectors depend on the cotton It is known as white gold because it contributes in the economic, industrial and agricultural sectors. Now a days, abiotic and biotic stress has a great influenced on the cotton yield. For maintaining its yield, we should use the effective techniques of plant breeding so that we develop those varieties which have the excellence resistance against different stresses just like cotton leaf curl virus etc. In plant breeding, mutation breeding is very effective for creating the genetic variability in the population. TILLING is an excellence technique in mutation breeding for targeting the inactivation gene and amplification of targeting gene. In this technique, we don't want sequence the whole genome which is a laborious. Powerful reverse genetic strategies allow the detection of induced point mutation. TILLING (Target Induced Local Lesions in Genomes) is genomic approach that make condition possible for screening the mutant and germplasm collections for allelic variants in target genes. The main advantage of TILLING is that this technique can be used for any plant species, irrespective of its genome size, ploidy level and method of propagation.

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