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CONTENT

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Future of Mutation Breeding in Cotton: A Step Towards Smart Cotton

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Abstract

Cotton is a basic source of industrial fiber. Cotton genetic variability has been narrowed via using of excessive conventional breeding and eliminated beneficial alleles from germplasm. So, when there is no source of variations in cotton germplasm, then mutation breeding comes in action. It is a useful technique to create variation in germplasm and helps to broaden the genetic variability in cotton. Mutation induction is a useful approach for enhancing fiber quality as well as its yield. Unlike transgenic approach, it does not alter genetic background of cotton crop. Till now, total 3402 varieties via mutation breeding have been developed according to IAEA. Almost, forty-eight varieties of cotton via mutation breeding have been released for economical use. The mutation breeding has brought revolution in cotton breeding. In this review, we have summarized various mutation induction approaches in cotton. Likewise, modern mutagenesis techniques TILLING has also been summarized in cotton. Similarly, genome editing techniques are emerging and it also alternative approach for mutation creation and widely used in various crops and in cotton but still have limitations, but insecurity to accept genome edited crop is still a matter of concern. In this context, the wise use of mutagenesis holds a great potential to improve cotton crop productivity and sustainability. The advent of sequencing data of cotton genome has enabled the scientist to dig out specific mutation related to specific trait, to understand the gene function and use of this knowledge in developing smart cotton. This review paper commences with the introduction of conventional mutagenesis approaches (physical, chemical, and antisense) and advanced mutagenesis genome

editing techniques (MNs, ZFNs, TALENs and CRISPR/Cas9) and their applications in cotton crop as well as limitations. We also stated mutation mapping approaches including Mutmap, MutChromeSeq along with whole-genome sequencing-based. It will help to provide an overview of different mutagenesis approaches which can be used to speed up cotton breeding program.

Keywords: TILLING, Mutation Induction, Whole Genome Sequencing, Cotton, Mutation Breeding

Introduction

Cotton (*Gossypium spp.*) is well known as a white gold due to its quality fiber production, therefore it is a cash crops in many countries (S. Singh et al., 2022). Commercially it's a highly valuable crop, promising contribution in textile fiber, oil and protein products industries (Rojo-Gutiérrez, Buenrostro-Figueroa, López-Martínez, Sepúlveda, & Baeza-Jiménez, 2020). The major cotton growing countries are China, United States, India, and Pakistan which contribute more than 70% of the total cotton production throughout the globe (H. Wang & Memon, 2020). The genus Gossypium possess around 50 total species, among which only four species are cultivated while remaining are wild in nature. The cultivated species, two tetraploid (*G. hirsutum* and *G. barbadense*) and two diploid cotton species (*G. herbaceum* and *G. arboreum*) are grown globally (Hu et al., 2019). The upland cotton (*G. hirsutum*) promisingly contributing more than 90% of overall cotton production (Vejan, Khadiran, Abdullah, & Ahmad, 2021). Due to a polyploidization event (1–1.5 million years ago) among A-genome species *G. arboreum* and a D-genome species *G. raimondii* develop tetraploid cotton, *G. hirsutum*, cultivar which produces high lint yield, quality fiber. The cultivated cotton variety *G. hirsutum* is mostly susceptible to multiple abiotic and biotic stresses, therefore hereditary variations have a significant impact in copping the abovementioned disasters (Pan, Meng, & Wang, 2020).

Natural variations play a key role in developing genetic and phenetic diversity of different crop species (barley, maize, wheat, rice, cotton etc.) which assist in screening of desirable traits to achieve better crop yield (Hasan, Choudhary, Naaz, Sharma, & Laskar, 2021). The spontaneous naturally occurring genetic mutations is the major cause for greater phenotypic variations among different crop species through artificial, evolutionary and natural selection processes (Crisp et al., 2022). The studies of natural variations among different crop

species brought great advances to understand the process of domestication which occurs in thousands of years e.g., in cotton about 4000-5000 years ago, and it triggers the genetic modifications for several developmental and adaptive characters (Parris, 2022). Natural variations elucidate the genotypic basis that shows phenotypic variations among various crop species which were highly adaptive in nature after domestication process. This process also helpful in interpreting the evolutionary and phenotypic variations among multiple crop species (Orteu & Jiggins, 2020). Natural variations also valuable for seeking the morphological attributes of cultivated cotton lines under biotic and abiotic stresses (Shim, Bandillo, & Angeles-Shim, 2021). Among wild and cultivated cotton varieties the analyses of natural variation play a significant role in understanding and utilization of diverse crop resources for enhancing the information of genetic variations for the improvement of cultivated cotton genotypes (He et al., 2021).

The valuable and highly promising source of genetic diversity can only be achieved by developing a gene bank through which desirable alleles are selected by screening the germplasm (Sharma et al., 2021). A versatile gene bank provides desirable traits in cultivated cotton varieties through breeding strategies to enhance the crop yield and resistance against several biotic and abiotic sources (Adlak et al., 2019). During the process of domestication and modern breeding approaches, the genetic variability narrowed down due to bottlenecks effect, and it antagonistically affected the crop productivity, yield and adaptability (Yongfeng Zhou, Muyle, & Gaut, 2019). The greater genetic diversity can only be obtained by mutation breeding. Induced mutagenesis play a key role in identification of different regulatory genes and molecular attributes (Ahmar et al., 2020).

Mutation breeding technique play a significant role in developing elite crop varieties which have improved agronomic attributes, resistance against abiotic and biotic stresses (F. Zhang & Batley, 2020). Mutagenic approaches are highly promising in developing more genetic diversity and study of evolutionary relationship among different crops (Garland & Curry, 2022). In mutation breeding several techniques are used for site specific mutation e.g., EMS induced mutation, physical and chemical mutation, zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENS), cluster regulatory interspaced short palindromic repeats (CRISPR-Cas9) nucleases. All these types of mutation provide assistance in crop genetic

improvement (Mushtaq et al., 2019). In cotton several elite genotypes with desirable attributes have been developed through mutagenesis (Rahman, Zafar, Hussain, Abbas, & Till, 2021). The development of such type of superior cotton varieties are highly supportive for increasing country economy (Ali et al., 2019). Table 1 contain mutagenic cultivated cotton varieties that have been reported in last several decades.

Table 1: Overall cotton varieties developed via mutational breeding

Sr. No.	Variety Name	Scientific Name	Common Name	Country	Reported Year
1.	113	Gossypium sp.	Cotton	China	1985
2.	Agdash 3	Gossypium sp.	Cotton	Russian Federation	1983
3.	AN-20	Gossypium sp.	Cotton	Uzbekistan	1986
4.	AN-401	Gossypium sp.	Cotton	Uzbekistan	1971
5.	Badnawar-1	Gossypium sp.	Cotton	India	1961
6.	C-3585	Gossypium sp.	Cotton	Uzbekistan	1979
7.	C-7503	Gossypium sp.	Cotton	Uzbekistan	1980
8.	C-7510	Gossypium sp.	Cotton	Uzbekistan	1991
9.	Chandi 95	Gossypium sp.	Cotton	Pakistan	1995
10.	Chuanpei 1	Gossypium sp.	Cotton	China	1982
11.	DS-1	Gossypium sp.	Cotton	India	1985
12.	Emian 15	Gossypium sp.	Cotton	China	1991
13.	Helius	Gossypium sp.	Cotton	Bulgaria	2007
14.	Indore-2	Gossypium sp.	Cotton	India	1950
15.	Jimian 8	Gossypium sp.	Cotton	China	1984
16.	Karisma 1	Gossypium sp.	Cotton	Indonesia	2008
17.	Khandwa-2	Gossypium sp.	Cotton	India	1971
18.	Lumian 1	Gossypium sp.	Cotton	China	1976
19.	M.A.9	Gossypium sp.	Cotton	India	1948

Sr. No.	Variety Name	Scientific Name	Common Name	Country	Reported Year
20.	MCU 10	Gossypium sp.	Cotton	India	1982
21.	MCU 7	Gossypium sp.	Cotton	India	1972
22.	ML-11	Gossypium sp.	Cotton	Uzbekistan	1979
23.	Mutant 1	Gossypium sp.	Cotton	Uzbekistan	1966
24.	Mutant 5	Gossypium sp.	Cotton	Uzbekistan	1969
25.	NIAB 111	Gossypium sp.	Cotton	Pakistan	2004
26.	NIAB 777	Gossypium sp.	Cotton	Pakistan	2009
27.	NIAB 999	Gossypium sp.	Cotton	Pakistan	2003
28.	NIAB Karishma	Gossypium sp.	Cotton	Pakistan	1996
29.	NIAB-2008	Gossypium sp.	Cotton	Pakistan	2016
30.	NIAB-26N	Gossypium sp.	Cotton	Pakistan	1992
31.	NIAB-78	Gossypium sp.	Cotton	Pakistan	1983
32.	NIAB-846	Gossypium sp.	Cotton	Pakistan	2008
33.	NIAB-852	Gossypium sp.	Cotton	Pakistan	2012
34.	NIAB-86	Gossypium sp.	Cotton	Pakistan	1990
35.	Oktyabr	Gossypium sp.	Cotton	Russian Federation	1984
36.	Pusa Ageti	Gossypium sp.	Cotton	India	1978
37.	Rasmi	Gossypium sp.	Cotton	India	1976
38.	Sohni	Gossypium sp.	Cotton	Pakistan	2004
39.	Trakia	Gossypium sp.	Cotton	Bulgaria	2007
40.	VNIISSKh-101	Gossypium sp.	Cotton	Uzbekistan	1986
41.	Xinhai 2	Gossypium sp.	Cotton	China	1979
42.	Yanmian 48	Gossypium sp.	Cotton	China	1985
43.	Yunfu 885	Gossypium sp.	Cotton	China	1977
44.	Zhong 297-5	Gossypium sp.	Cotton	China	2005

Sr. No.	Variety Name	Scientific Name	Common Name	Country	Reported Year
45.	Zhong 297-5	Gossypium sp.	Cotton	China	2005
46.	Zhongmiansuo 42	Gossypium sp.	Cotton	China	2002
47.	Zhongmiansuo 50	Gossypium sp.	Cotton	China	2007
48.	Zhongmiansuo 69	Gossypium sp.	Cotton	China	2008

This review aims the overview of different mutagenesis techniques, their applicability in cotton and also discussed next generation approaches including Mutmap, MutChromeSeq and whole genome sequencing which will speed up cotton breeding program.

Induced Mutagenesis

Mutational breeding is a tool of plant breeders and exploits to broaden the genetic bases of plant. Mutagenesis involves beneficial use of mutagens to induce or create mutation in genome of plant to study the function of particular gene in vitro/in vivo (Engqvist & Rabe, 2019). All type of genetic variation is frequently depends on mutations even conventional breeding (works on principle of natural variation exists in wild/domesticated plants) has harnessed the power of variations to develop the high yield varieties. The advent of physical as well as chemical mutagens have brought revaluation in plant breeding. It has been effectively used to understand the gene function and to discover the important mutation that control beneficial traits in plants (Shelake, Pramanik, & Kim, 2019).

Physical and Chemical Mutagenesis

Conventional methods to induce mutation are as follows: physical and chemical mutagens. Physical mutagenesis comprises of ionizing radiations such as gamma rays, X-rays, high energy ions beams and neutrons (Bado et al., 2015). Plant breeders preferably use gamma rays as they are convenient, power of deep penetration into entire plant body (Suprasanna, Mirajkar, & Bhagwat, 2015). The physical mutagens such as radiation has been extensively exploited for the purpose of cotton breeding, for example, heat resistant as well as early maturity mutants were developed via irradiation of seed with gamma rays (Maluszynski, Ahloowalia, & Sigurbjörnsson, 1995). Another study on cotton was carried out for the development of mutant resistant to glyphosate via using ^{60}Co-γ-ray mutagen. Furthermore, gamma rays (^{60}Co) were used to develop the chicken foot leaves and gossypol free gland cotton mutants. They revealed that chicken foot leaves in cotton mutant were more advantageous in terms of light assimilation, resistance against insect, and contributed to fiber strength and yield (Zhao et al., 2022). Currently, ion beam technology has also been employed to produce novel germplasm for improvement of quality in cotton. For instance, scientists revealed that use of laser technology may promote growth, enhance quality of fiber as well as yield in cotton (Zhao et al., 2022).

Currently, scientists are also using space-based mutagenesis as space environment is very harsh. Song et al. developed a leaf-yellowing cotton mutant in space (Song et al., 2012). Wang and his colleagues used physical mutagen named 'atomic energy' to develop the cotton dwarf mutant (Zhao et al., 2022).

Chemical mutagens are used to create point mutation within genome at greater rate. Chemical mutagens are believed to be milder as compared to physical mutagens. Various types of chemical mutagen have been stated, however, some chemical mutagens have been employed in plant breeding (Oladosu et al., 2016). Chemical mutagens are as follows: hydroxylamine, alkylating agents, nitrous acid, base analogs and acridine dyes. Use of chemical mutagens is advantageous as compared to physical mutagen because it has easy application, causes less destruction in genome and increased mutation rate. Plant breeders prefer ethyl methanesul-fonate (EMS) as it is easy to handle in terms of detoxification (Pathirana, 2011). Cotton breeder also use EMS to create mutation (Brown et al., 2015; J. D. Patel et al., 2014). EMS has been employed in cotton for improvement of fiber quality as well as its yield (Brown et al., 2015), improved oil content, alterations in seed composition (Muthusamy & Jayabalan, 2011), enhance resistance against biotic stresses (X. Zhang, Yao, Shi, & Dong, 1998). In summary, physical and chemical have been used in conventional mutational breeding and will play a vital rule in the future. But conventional mutagenesis techniques have some limitations, for example, traditional mutagenesis may create mutation randomly, perilous to mankind and environment and dose rate of every mutagens is different for every crop and needs proper check and balance (Bado et al., 2015).

Insertional Mutagenesis

It is another effective tool to create a mutant population via employing exogenous DNA which acts as a mutagen. This technique has been used effectively to separate genes directly via identification of mutant phenotypes. Insertional mutagenesis is more effective because of its dual role. Insertional mutagenesis is used to modify the function of gene and also delivers knowledge of sequence which can help to recover the sequence of flanking region of genome directly via employing various PCR-based techniques. Therefore, genes impacted via mutagenesis might be discover rapidly, characterize, so there is no need of positional cloning as well as gene mapping. In plants, two types of insertional mutagenesis have been widely applied effectively; transposable elements and T-DNA of Agrobacterium (Auld, Light, Fokar, Bechere, & Allen, 2009). The T-DNA technology involves Ti-plasmid of agrobacterium, when inserts into gene, it can alter normal gene function in plants. T-DNA performs two functions; first is gene disruption to make a mutant, then providing a novel tag sequence which will lead to the identification of physical insertion in genome (Krysan, Young, & Sussman, 1999). Subsequently, T-DNA mutant genotyping is simple as compared to point mutations created by EMS/fast neutrons. Xing Qi from the Texas Tech University used T-DNA and inserted into gene rich regions within genome (Barakat et al., 2000). It is widely employed in Arabidopsis as well in other plants in which transformation is easy. Various T-DNA knockout lines have

been generated for different genes. However, this technique could not disrupt function of some genes in Arabidopsis. Moreover, new technologies are emerging and evaluated to attain above mentioned limitation of T-DNA (Woody, Austin-Phillips, Amasino, & Krysan, 2007). So, the solution of above-mentioned limitation of T-DNA technology is the use of transposon mutagenesis or tagging to insert mutation in crops. Transposons also known as jumping genes have been firstly reported in maize and revealed that these jumping gene are involved in suppression of gene function (McClintock, 1956). These transposon elements have an ability to jump within genome and during jumping they insert themselves within genomes. When these transposons inserted within gene or promoter region, it can alter gene function. For example, the installation/deletion of various transposon elements within promoter region of two transcription factor genes in both At and Dt subgenomes of cotton caused alteration of gene expression (K. Wang, Huang, & Zhu, 2016).

Mutagenesis by Antisense Approach

Antisense technology is used by breeders for the development of varieties. This technology suppresses the function of desired gene and helps to understand the possible role of that gene (Chaudhary et al., 2019). Antisense technology is a broad term: gene knock down is performed via various techniques such as antisense RNA (asRNA), long non-coding RNA (lncRNA), RNA interference (RNAi), and various enzymes as well as molecules. In this technology, antisense sequence which is complementary sense RNA, is inserted and that sense RNA becomes unavailable to translation, thus stops the gene expression (M. Patel, 2019). Additionally, many review papers about mechanism of antisense technology have been published (Basso et al., 2019; Brant & Budak, 2018; Budak, Kaya, & Cagirici, 2020; Rajam, 2020; Summanwar, Basu, Rahman, & Kav, 2020; Tilahun, Bezie, Kerisew, & Taye, 2021). RNAi technology has been used to develop resistance against cotton boll worm via silencing *CYP6AE14* gene in cotton plant (Younis, Siddique, Kim, & Lim, 2014). In another study, drought tolerance was attained via silencing two genes such as *GhSnRK2* and *GbMYB5* (Bello et al., 2014; Chen et al., 2015). Even though, this technique has much potential but still encounters numerous limitations such as off-target effects, difficulty in delivering adequate amount of small RNA in desired tissue, unknown degradation time, and various ethical issues (R. K. Singh, Krishnamachari, & Sharaff, 2020).

Mutagenesis by Genome Editing Approaches

An advance technique to produce precise mutation within genome is genome editing. The genome editing techniques have ability to precisely edit the gene of interest and extensively employed in various crops (Venezia & Creasey Krainer, 2021). Currently, four type site specific nucleases (SSNs) have been employed to enhance the yield as well as quality of crops. These SSNs includes meganucleases (MNs), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) and have been engineered for editing of genes (Zaman, Li, Cheng, & Hu, 2019).

Meganucleases (MNs)

Meganucleases also known as homing endonucleases (HEs) are first site specific nucleases. They are called meganuclease because they have large recognition site from 20-30 base pairs (Iqbal, Iqbal, Ahmad, Memon, & Ansari, 2020). MNs known to be first reported SSNs which created double strand breaks (DSBs) at targeted location within genome. DSBs generated via MNs could be repaired with nonhomologous end joining (NHEJ) or homology-directed repair (HDR) mechanism (Silva et al., 2011). This technology has been employed successfully in bacteria, mosquitoes, mice, flies and plants. MNs has also been used as an editing tool in cotton crop, for example, resistance against herbicide tolerant and insect were developed in cotton (D'Halluin et al., 2013). Additionally, this technology is not widely employed because of off-target sequences. However, to engineer MNs is difficult, as single protein domain has to perform dual function such as recognition as well as cleavage (Arnould et al., 2011; Smith et al., 2006).

ZFNs and TALENs

ZFNs are artificial endonucleases that is produced via fusing of three components; DNA binding domain fused with DNA recognition module and DNA cleavage domain. Cys2–His2 zinc fingers makes DNA binding domain, both identify 3-6 base pairs of DNA while DNA cleavage domain also known as Zinc finger proteins is fused with the catalytic domain of the *Fok* I restriction enzyme. *Fok* I domain is very important for ZFNs because it plays an important role in targeted cleavage within genome. The successful use of ZFNs has been reported in Arabidopsis, tobacco and maize. Similarly, ZFNs has also been employed to small or large segments in chromosomes. But the major limitation in use of ZFNs is laborious, low efficiency as well as lower reproduce-bility in germ cells as compared to somatic cells (Zaman et al., 2019).

The plant bacterial pathogen *Xanthomonas* infects many crop species including rice, citrus, tomato, and soybean (Boch & Bonas, 2010). To create TALEs, catalytic domain of *Fok* I endonuclease was fused with TALE DNA binding domain. TALENs is advantageous as it enables to target one DNA locus at every 10 base pairs as compared to ZFNs. The binding domains of TALENs are capable to recognize particularly any DNA sequence (Zaman et al., 2019). Precise gene stacking for significant traits could be attained via using TALENs (Khan, Khan, Mubarik, Sadia, & Ahmad, 2017). Still, to design ZFNs and TALENs for a specific target is lengthy procedure and do have limitations (Uniyal, Yadav, & Kumar, 2019).

CRISPR/Cas System

Even though, significant improvements have been made for transgenic cotton, however, it still requires advanced molecular tools. Currently, CRISPR/Cas9 technology holds a potential to modify the significant traits in cotton and open a new horizon for precision breeding. CRISPR/Cas9 is a natural defense mechanism of bacteria against viruses. Scientists adopted bacterial natural CRISPR/Cas system to edit the gene in plants. In contrast to transgenic technique, CRISPR/Cas9 has diverse applications such as gene can be knock out/knock in, moreover it is also able to do epigenetic modification of a single gene even at transcriptional level too. Both industrial as well as academic are

extensively using CRISPR/Cas9 for precise targeted gene editing to improve cotton production (Peng, Jones, Liu, & Zhang, 2021). The fiber initiation and development are both important phenomenon in cotton plant, therefore, they are of great concern in cotton fiber quality as well as yield. Li and his team used CRISPR/Cas9 to knock out *MYB-25like* transcription factor gene and attained fibreless mutation with great editing efficiency without off target sequence. This mutation brought no change in other phenotypic trait of cotton plant (C. Li, Unver, & Zhang, 2017). The brassinosteroid signaling pathway, *14-3-3* is a master player for the early production of fiber and its differentiation in cotton, therefore, *14-3-3l* gene was overexpressed via used of CRISPR technology resulting longer fiber, however, inhibition the expression of this gene negatively influenced initiation and elongation (Ying Zhou et al., 2015). Gossypol content in cotton oil is considered as toxic, thus, knockout of genes involved in its biosynthesis might be produced cotton without gossypol which will help to enhance the value of cotton (Peng et al., 2021).

Climate change is a serious issue and aggravating both abiotic and biotic stress. So, CRISPR/Cas9 could be used to develop resistance against both abiotic/biotic stresses in cotton. For example, *14-3-3d* gene was knocked out to achieve resistance against *Verticillium* dahlia which is a pathogen in cotton plant (Z. Zhang et al., 2018). Nutrients very important for crop development, so, scientists used CRISPR/Cas9 technology to knockout the *arg* gene which increased the total number of lateral roots as well as total root surface area under both normal and nitrogen deficiency conditions (Y. Wang et al., 2017). It might be helpful for plant to absorb water and nutrient and improved resistance against abiotic stresses.

Mutation Mapping Approaches

MutMap Approach

MutMap is a newly developed cost-effective and efficient forward genetic technique that relies on next-generation high-throughput sequencing techniques. This technique is based on crossing an interesting (desirable) mutant plant with the parental line which was used for mutagenesis, following F1 individual's selfing to develop F2 progenies (X. Wang et al., 2022). If the phenotypic changes are due to the single recessive mutation, then it is expected that F2 population will segregate 3:1 for wild/type and mutant progeny. From F2 population at least 20 individuals confirming mutations through phenotypic screening are used for DNA extraction. The high-quality extracted DNA is pooled with an equal ratio and subjected to a newly developed sequencing technology such as PacBio for whole genome sequencing with more than 10x coverage (Sugihara et al., 2022). After sequencing, the short/small reads are aligned to the reference genome to find out the SNPs (Figure 1).

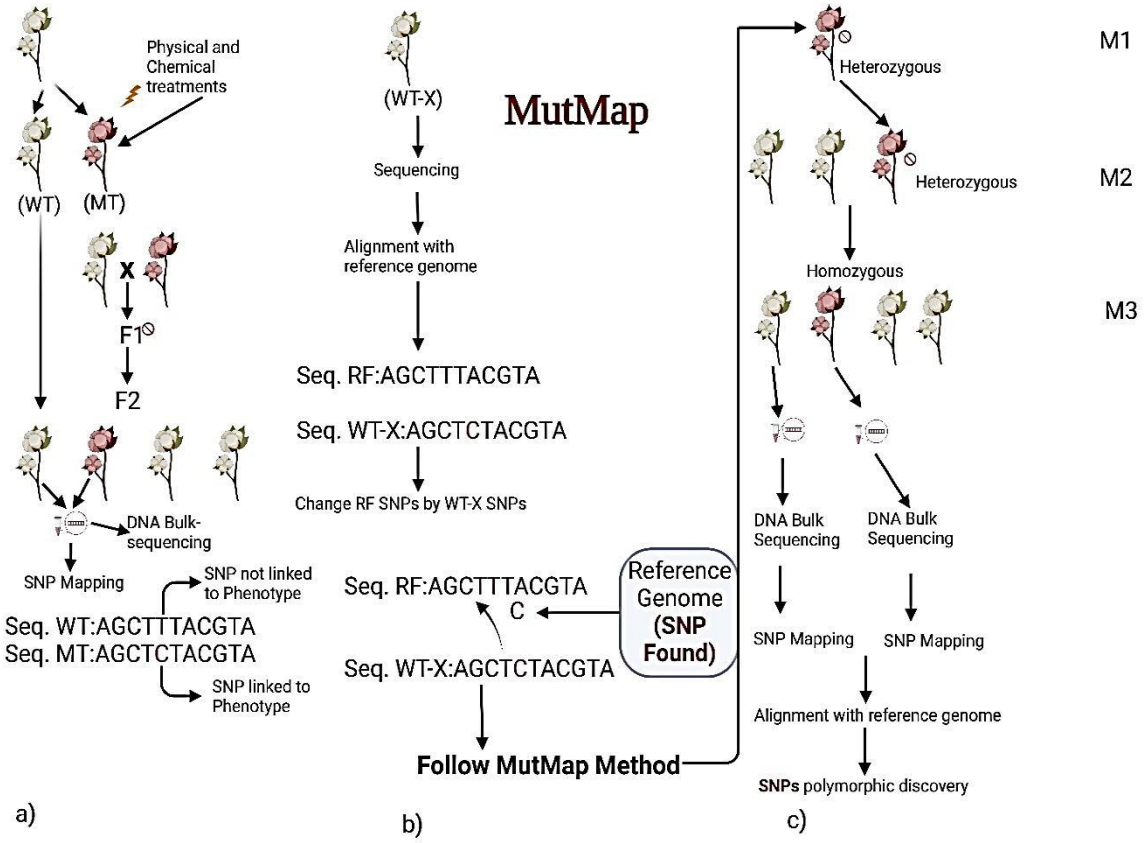

Figure 1: Pictorial illustration of MutMap approach, a) mutation induction via chemical/physical mutagens, b) exploitation of NGS approach for identification of mutant, c) identification of SNPs associated with mutant phenotype.

Since the mutant lines from F_2 population carry SNP due to the mutation, the re-sequenced short reads carrying that SNP must carry the nucleotide that differs from the reference genome to confirm the SNP associated with the phenotypic change (Qiao, Yan, Wang, & Huang, 2020). On the other hand, the SNPs that are not associated with the phenotypic changes must segregate in 1:1 ratio in F2 population. In most cases, about 50% of the short reads covering such positions carry the nucleotides/SNPs that differ from the reference genome. The find out the total number of changed nucleotides/SNPs in the short reads as compared to the reference genome, a recently developed concept called SNP-index is used. If only half of the short reads cover the position that carries SNP then the SNP-index will be 0.5 and if it involves the entire short reads for that SNP then the SNP-index will be 1 (X. Wang & Wang, 2022). SPN-index is calculated for the SNPs induced by mutagenesis and the relationship among genomic position and the SNP-index is graphically plotted.

In MutMap technique, the desirable mutants are selected from M3 to M5 generations and then crossing these mutants with parental lines, followed by evaluating through phenotypic changes in F2 segregating

progenies. Mutants with unwanted changes; for example, mutants with sterility or lethal mutations are not acceptable for MutMap applications. However, to address this issue a newly developed MutMap+ (an updated version of MutMap) is used that is based on selfing of those heterozygous lines which display phenotype just like wild/type and identified in segregated progenies of M2 population for wild/type and mutant with a desirable phenotype that is recessive homozygous. The resulted M3 population will segregate for desirable mutations and then selected mutants will be directly used for whole genome sequencing. The isolation of mutations in genes that prevent artificial crossover can be accomplished with MutMap^{+} since it does not require backcrossing to the wild-type parent (Fekih et al., 2013). Therefore, MutMap+ broadens its applications for improving the genetics of cotton and other agricultural plants.

MutChromSeq Approach

Identification of a randomly induced mutation in a genome is like finding a needle in a haystack. Anyhow, the advancements in the next generation sequencing technologies have made the identification of mutations in a genome much easier than before. However, identifying mutations with high confidence in a whole genome using sequencing technologies is easier for plants with small genome and become difficult for plants with bigger genome. A newly introduced method for rapid detection of mutations called MutChromSeq has been widely applied in different crop species to identify novel genes in plants. MutChromSeq deals with the identification of DNA sequences and genes that controlling the expression of a particular gene in plants with polyploid and complex genomes. In this approach, chromosome flow sorting and classical mutagenesis are combined to filter the region of interest where we can identify our gene of interest. In MutChroSeq the chromosomes having desire gene are filtered on the basis of fluorescent marker tags, followed by the sequencing of both non-mutant and mutant chromosomes to identify the targeted/desirable gene (Chaudhary et al., 2019). This approach can be utilized in cotton and other crop species with complex or large chromosomes which devoid of recombination and thus, plummeting the need for recombination based genetic mapping. This method is used on plants that are amenable to mutagenesis, the targeted mutation produces a noticeably altering phenotype, and prior knowledge of the chromosome where the gene is situated is accessible. Therefore, it may be a reliable and affordable method of cloning known troublesome genes (Kulus, 2022).

Whole Genome Sequencing based Mapping

The availability of a high quality cotton reference genome has made the re-sequencing of mutant cotton genotypes much easier and cost effective to detect the genomic alterations at nucleotides level across whole genome. Till now, different genotypes of cotton including varieties, cultivars and advanced lines have been re-sequenced and aligned to the reference genome to identify the variations (SNPs) occurs due to mutation throughout the genome associated with phenotypic changes. The recently developed techniques like GWAS are widely used across all plant species including cotton to study the mutations and identify the beneficial SNPs associated with some desirable phenotypic

changes and then mapped these changes across the genes/chromosomes. Till now millions of SNPs have been identified in cotton using whole genome sequencing (WGS) and forwarded for generating high-quality WGS map. WGS map has high resolution and reduces the chances for the occurrence of errors during constructing WGS map. However, constructing WGS map using GWAS still has some limitations for example the complete genetic variations (SNPs) associated with phenotypes cannot identified even after alignment with the reference genome (Su et al., 2020). This is due to the hidden variations or missing variations even in the reference genome. To address this issue, scientists have introduced a novel technique called Pan-genomics. This technique allows the alignments across the different plant species like cotton, maize and any-other related specie rather than a reference genome to detect the whole variations. This technique allows the detection of more variations within a crop that cannot be detected through GWAS and increase the resolution of the WGS map (W. Li et al., 2022).

TILLING

The discovery of various mutation breeding techniques have been employed by breeders to develop new variation in crops(Ahmar et al., 2020). TILLING was brought a revolution in mutation breeding as it is comparatively easy technique to identify lesions in a desired sequence (M. K. R. Khan et al., 2022). It is reverse genetic technique; combination of both conventional mutagenesis and advanced screening to recognize the point mutation/nucleotide polymorphisms in a sequence of interest. This approach works best when genetic basis of a species is narrowed. EMS is preferred in TILLING as it has ability to create the single nucleotide polymorphism (SNP). Likewise, a single change in the coding region of targeted gene will brought the change in gene expression leading to alternation in both transcriptional as well as translational levels (Wilde, Chen, Jiang, & Bhattacharya, 2012). There are plenty of excellent review paper published on TILLING mechanism (Henikoff & Comai, 2003; Irshad, Guo, Zhang, & Liu, 2020; Shaheen et al., 2012; Sikora, Chawade, Larsson, Olsson, & Olsson, 2011). TILLING has been widely applied in various plant such as Arabidopsis, barley, rice, wheat, maize, soybean, pea, and cotton (Rahman et al., 2021; Ulukapi & Nasircilar, 2018). TILLING can be used to strengthen the genetic bases of cotton and thus studies have been carried out to improve cotton production.

Herring et al. (2004) used cotton variety namely Paymaster HS 200 and developed three mutant generations such as M1, M2, and M3 for the improvement of lint as well as fiber quality (Herring et al., 2004). Lowery et al. (2007) employed 3% EMS, 0.05% Na azide to mutagenize three cotton varieties which are as follows; *G. hirsutum* var. (FiberMax 958, ACALA 1517-99, and TAM 94L-25) *G. barbadense* var. (Pima S7) *G. arboreum* var. (GA-TAMU, A2-60T, and A2-120W) to enhance the lint percent, lint yields as well fiber quality (Lowery et al., 2007; Rahman et al., 2021). Another study was carried out by Aslam et al. (2013) and they used 0.2% and 0.3% EMS to mutate *G. hirsutum* var. PB-899, pb-900 for elucidating gene function in cotton (Aslam, Ali Khan, Naseer Cheema, Imtiaz, & Malik, 2013).

Fiber quality is an important trait in cotton, as cotton is grown basically for its fiber, so, Bechere et al. (2012) conducted an experiment and subjected the *G. hirsutum* var. SC 9023 to 2.45% EMS for improvement of agronomic and fiber traits (Bechere, Turley, Auld, & Zeng, 2012). Bechere et al. (2018) used 3.2 % EMS to mutate the *G. hirsutum* lines and developed fiber length, uniformity, and strength (Bechere, Zeng, & Auld, 2018). Witt et al. (2018) used EMS in *G. hirsutum* Raider 276 Tamcot Sphinx and TTU 774 and enhanced agronomic as well as fiber quality traits in cotton (Rahman et al., 2021; Witt, Ulloa, Pelletier, Mendu, & Ritchie, 2018). Fiber development is an important stage in cotton, and brassinosteroids receptor gene is involved in fiber development, thus, TILLING technique was employed to identify this gene in cotton, thus indicating that TILLING is highly applicable in cotton (Shaheen et al., 2012). Y. Wang et al. 2019 and Fang et al. (2020) also used EMS to create short fiber in *G. hirsutum* while thompson et al. (2019) utilized EMS to reduce the palmitic acid content in cotton plant (Khan, Ali, & Khan, 2022).

Furthermore, Aslam et al, 2016 used EMS to mutagenize the two cotton cultivars named "PB-899 and PB-900'' and tilled five gene classes successfully and they tilled five important gene classes involved in various traits (Aslam et al., 2016). Scientists also employed TILLING technique in *G. hirsutum* var. NIAB-777 to introduce high yield, early maturity, resistance to diseases in cotton. EMS have been extensively employed in cotton to create mutants which have high yield as well enhanced fiber quality, enhanced/decreased oil contents, seed composition alteration (Zhao et al., 2022).

The amalgamation of bioinformatics tools has been made TILLING easy and applicable. Various softwares are used to detect and help to observe variation. For instance, conservation-based SIFT (sorting intolerant from tolerant) is a tool used to detect a difference in codon of amino acids. Any type of change in gene sequence can be identify via PARSESNP (for Project Aligned Related Sequences and Evaluate SNPs, http: wwwproweborgparsesnp) (Yadav et al., 2022).

The updated TILLING techniques integrated with the bioinformatic tools can be used to create mutants and released as a variety and may help to broaden the genetic base of cotton germplasm. It is advanced as compared to conventional phenotyping techniques because this technique may be used to identify recessive mutations within homozygous individuals (Wilde et al., 2012).This technique can also be used to address many difficulties for genetics in diploids, so, that each homologue of a multigene family can be targeted independently as well combined into a single line using genetic crosses (Rahman et al., 2021). However, TILLING still face some challenges such as production of false positive/negative result, expensive and requires skillful labour.

Limitations and Challenges for Mutagenesis in Cotton

Cotton is an important cash crop known also white gold and basic sources of textile fiber. However, the cotton genetic base has been narrowed because of repetitive selection and eliminated the beneficial alleles. A

successful cotton breeding program aims to improve cotton yield as well as its quality under climate change scenario. Cotton breeders are using aggressive mutagens for developing variations/broaden genetic base in cotton genome. However, mutation breeding itself is a challenge, random, and uncontrolled. For example, created mutation can be lethal and interact with ploidy level of cotton genome or may enhance/reduce the ploidy level. Even though, mutagen may interfere with positive beneficial genes related to fiber quality and yield as well. It may cause pleiotropic effect. Moreover, the desirable mutant frequency is very low. If mutation occurs in recessive alleles, it cannot be detected. It is difficult to identify a desirable mutant from large number of mutated populations.

It is laborious, time-consuming, expensive and needs sophisticated protocol. However, the notable achievement of mutation breeding is that 48 cotton varieties have been released worldwide according to IAEA. The mutation breeding in the era of omics techniques may be used to study the function of genes in cotton crop. There are still twenty first century challenges like cotton production is in serious decline due to rapid and uncontrollable climate change. TILLING seems to be powerful technique to create point mutation and can be helpful to study gene function. CRISPR/Cas9 technology has also become popular for gene editing, however, gene editing in polyploid cotton is very challenging, for example if a single gene is targeted in cotton, it may silence that gene in one genome, but the genome buffering interferes in editing of that gene and still expression of that gene occurs. So, there is a need to use the amalgam of conventional mutagenesis as well as modern breeding technologies to develop smart cotton variety, as limitation of one technique can be overcome via benefits of other techniques. As, scientist should decide which technique is better at specific time period.

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CHEMO-ENZYMATIC PROCESSES FOR PREPARATION OF ABSORBENT COTTON

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Abstract

Cotton is the raw material for preparation of absorbent cotton. Raw cotton has to be subjected to scouring and bleaching processes for making it absorbent by removing the naturally present wax, protein and minerals in the fibre. The scouring is done at 115°C using alkali followed by bleaching at boiling condition using alkaline hydrogen peroxide solution. The effluent coming out of such processes contains high COD and BOD values. Due to the stringent environmental regulation and great awareness among the public about environment, worldwide attempts have been made to develop green and sustainable chemical processing of materials. Based on the above, in the present study efforts have been made to develop an eco-friendly preparatory process for the production of absorbent cotton using chemo-enzymatic formulation and single bath enzymatic scouring and bleaching Process . The result indicated that absorbent cotton produced using the developed processes fulfilled the required performance properties as per pharmacopeia with less energy, water and chemical requirement and also with minimum damages to the cotton compared to the absorbent cotton produced with the conventional process.

Keywords: Absorbent cotton, Bleaching, Chemo-enzyme, Enzyme pretreatment, Eco-friendly, Enzyme, Scouring.

Introduction

Since the ancient time, fibrous materials have been used in the field of medicine especially for absorbing body fluids during surgery, wound healing treatment etc. Cotton and other cellulosic fibres have been widely used for the above purposes. Absorbent cotton is one such medical product developed during 18th century. This is used in the hospitals for absorbing and wiping body fluids. Since, absorbent cotton is a medical product, the required performance

properties are defined by pharmacopeia. Apart from medical use, absorbent cotton is a basic raw material for the production of ear buds and cosmetic wipes. Cotton has to be subjected to scouring and bleaching processes for making it absorbent. Scouring is the process to remove natural and added impurities present in the raw cotton fibre. The scouring process removes pectin, wax, nitrogenous matter and minerals. Conventionally, this process is carried out by treating the raw cotton with sodium hydroxide at elevated temperature of 110-115°C under pressure using a machine called kier. The scouring process is followed by alkaline hydrogen peroxide based bleaching processes for removing residual colour present in the fibre.

Textile processing industries generally combine both scouring and bleaching processes since both the processes are done under alkaline conditions at boiling temperature. However, in the case of absorbent cotton, the adoption of such one bath process is limited due to the requirement higher absorbency which requires maximum removal of natural impurities present in the fibre. Due to the stringent environmental regulation and great awareness among the public about environmental problems, worldwide attempts have been made to develop green and sustainable chemical processing of materials. Enzyme based processes are considered as eco-friendly in nature. Enzymes are bio-catalyst which can increase the rate of reaction in moderate temperature. The literature survey reveals that several enzymatic processes are attempted for scouring and bleaching of cotton. Tzanov et al. (2001) attempted to develop enzymatic scouring process for cotton using acid and alkaline pectinase enzyme. Chung et al (2004) developed a FT-IR spectrometric based method to characterize the effects of enzymatic scouring process. Karapinar & Sariisik (2004) compared the conventional alkaline scouring and enzymatic processes and found that the combination pectinase, protease and cellulose were suitable for scouring. Špička et al (2015) studied the pectinase based scouring of cotton and low temperature bleaching process. Based on the above, in the present study efforts have been made to develop an eco-friendly preparatory process for the production of absorbent cotton using chemo-enzymatic formulation and enzymatic pretreatment process.

Material and Methods

Virgin cotton was used for this study. The fibre quality of raw cotton used for this study had 25.3 mm staple length, 4.9 mic fineness and 20.1 g/tex strength. An scouring enzyme (Xymo Nat Scour) supplied by United Alarcity Pvt Ltd, Chennai was used for the scouring and bleaching purposes. This enzyme formulation also contains activators. A non-ionic wetting agent supplied by Dupont India Ltd was used for the processing. Analytical grade hydrogen peroxide, sodium hydroxide, acetic acid and sodium silicate were used.

Conventional Process

Scouring was done in an autoclave under 15 lbs/sq in (psi) pressure and at 115°C using 3% sodium hydroxide and 0.5% of nonionic wetting agent on the weight of fibre for 1 hr. The material to liquor ratio was kept at 1:10. After the scouring process, the fibre was rinsed with water. The scoured fibre was then subjected to bleaching treatment using 3 g/L hydrogen peroxide , 1.5 g/L sodium

silicate 1 g/L sodium hydroxide keeping material to liquor ratio 1:10 at 98°C for 1 hour. Finally, the fibre was washed and dried

Enzymatic Process

The raw cotton was taken in a beaker and treated with 0.5% of nonionic wetting agent on the weight of fibre and optimized concentration of enzyme using shaking water bath at 98°C . A preliminary optimization trial was conducted to optimize concentration of enzyme required for getting required performance properties by keeping non-ionic wetting agent concentration as constant. For this purpose, different enzyme concentrations of 2%, 2.5%, 3%, 3.5%, 4% and 4.5% were used.

Chemo-enzymatic Process

In this process, one bath scouring and bleaching process were carried using 0.5% of nonionic wetting agent, optimized concentration of enzyme formulation along with 1g/L sodium hydroxide and 1.5 g/L hydrogen peroxide in order to get required properties as per the pharmacopeia standards. Other conditions of the experiment were similar to enzymatic process mentioned in the previous section.

Single Bath Enzymatic Scouring and Bleaching Process

In this process, the enzyme scouring was done using the enzyme mixture with optimized concentration of pectinase and cellulase enzymes in a beaker dyeing machine keeping material- to- liquor ratio at 1:20.The pH of the bath was adjusted at 6.8-6.9. The scouring process was done for 30min at 60-65°C. After that the bath was allowed to cool to 40- 45°C temperature. Then the bleaching chemicals were added in the same bath. The concentrations of hydrogen peroxide, sodium hydroxide and sodium silicate were kept at 3% (owm), 1g/L and 1.5g/L respectively. The temperature of the bath was then raised to 95°C and the bleaching was done for 45 min. Finally, the fibre was washed thoroughly and dried.

Testing of performance Properties

As per the Indian pharmacopeia, absorbent cotton should fulfill more than twenty parameters. The important requirements of absorbent cotton such as absorbency, water holding capacity and sulphated ash were measured as per the Indian Pharmacopeia. As per the pharmacopeia, the absorbency/sinking time should be less than 10 sec, water holding capacity should be more than 23 g/g of fibre and shulphated ash should be less than 0.5%. Another important parameter requirement of absorbent cotton is that it should be pure white. Though pharmacopeia standards do not specify any value or methods, in the present study whiteness of the fibre was measured using visible spectrophotometer. The CIE 2000 whiteness value was taken as the measure of whiteness. Ideally, absorbent cotton should exhibit the whiteness index value value more than 70 units.

Results and discussion

The result of preliminary study to find out optimum enzyme concentration showed that 2.5% enzyme treated fibre fulfilled the absorbency requirement of cotton i.e. below 10 sec. Further increasing the concentration of enzyme didn’t reduce the sinking time significantly. The water holding capacity values were decreased when the concentration of enzyme increased. The sulphated ash percentage was increased with the increase in the concentration of enzyme

from 2.5% to 4%. The whiteness of the fibre was also increased with the increase in concentration of enzyme from 2% to 3.5%. However, further increase in the concentration did not show any significant improvement. Based on the above result, it was concluded that 2.5% enzyme concentration was the optimum one to get absorbency.

The performance testing results of absorbent cotton produced using conventional and enzymatic process is given in Table 1. The result indicated that the conventional process made absorbent cotton fulfilled the requirement as prescribed by the pharmacopeia. The enzymatically produced absorbent cotton fulfilled the absorbency requirement of below 10 sec and water holding capacity of 24 g of water per gram of fibre. However, it failed to fulfil the other two more important requirements of sulphated ash and whiteness. The sulphated ash content of enzymatically produced cotton was above 0.5% and whiteness value was below 70. The enzyme taken for the study was intended for scouring and bleaching of textile material where the requirements are different than absorbent cotton.

In order to improve the whiteness and decrease the sulphated ash content, a modified enzymatic process termed as chemo-enzymatic process was attempted in which the chemicals such as 1% sodium hydroxide and 1.5 g/L hydrogen peroxide was added in the enzymatic bath. The performance properties of the absorbent cotton prepared by chemo-enzymatic process are also given in Table 1. The result indicated that that absorbent cotton produced using chemo-enzymatic process fulfilled the requirement of sulphated ash percentage and whiteness value. The sulphated ash percentage of such produced absorbent cotton was below 0.5% and the whiteness index value was above 70 units. The addition of sodium hydroxide in the enzymatic bath supplemented the removal of wax and mineral content from the cotton fibre. Similarly addition of hydrogen peroxide leads to the increased whiteness of the fibre. The performance property values obtained in the fibre was comparable to conventionally produced absorbent cotton. The chemo-enzymatic process developed in this study is one bath process and required less chemical addition as well as lower temperature.

The performance properties of the absorbent cotton prepared by Single Bath Enzymatic Scouring and Bleaching Process are also given in Table 1.The result indicated that the absorbent cotton produced by this process is fulfilling the pharmacopeia requirement just like conventional process. However, due the use of enzyme, the energy, water and time requirement is reduced.

Table 1: Performance properties of absorbent cotton produced from different methods

S.No	Parameters	Conventional process	Enzymatic Process	Chemo-Enzymatic Process	Single Bath Enzymatic Scouring and Bleaching
1	Ash content (%)	0.40	0.73	0.41	0.37
2	Absorbency/Sinking time(sec)	1.6	3	2.5	1.8
3	Water holding capacity(g of water/g of fibre	25.3	26.5	26.0	24.2
4	Whiteness Index	75	58	72	78

Physico-chemical Changes in Treated Cotton Fibre

Purification treatments can cause physical and chemical damages to the cotton fibre. Extent of this damage is assessed by studying carboxyl content and FTIR spectra based crystalinity index of conventionally and enzymatically (single bath) scoured and bleached cotton fibres.

Carboxyl Content

The carboxyl content in the conventional and enzymatic scoured and bleached cotton fibres is given in Fig 1. The result indicates that the cotton fibre treated with conventional process has higher carboxyl content than enzymatic process. The carboxyl content in the cotton increases with the formation of oxy cellulose. During alkaline scouring at high temperature, higher amount of hydroxyl groups (-CHOH) in the cellulose is oxidized into aldehyde (-CHO) and further into carboxyl groups (–COOH) which implies more chemical damage to cotton fibre as compared to enzymatic process. The damage to the cotton is lesser when subjected to enzymatic process due to the specific nature of action of the enzymes.

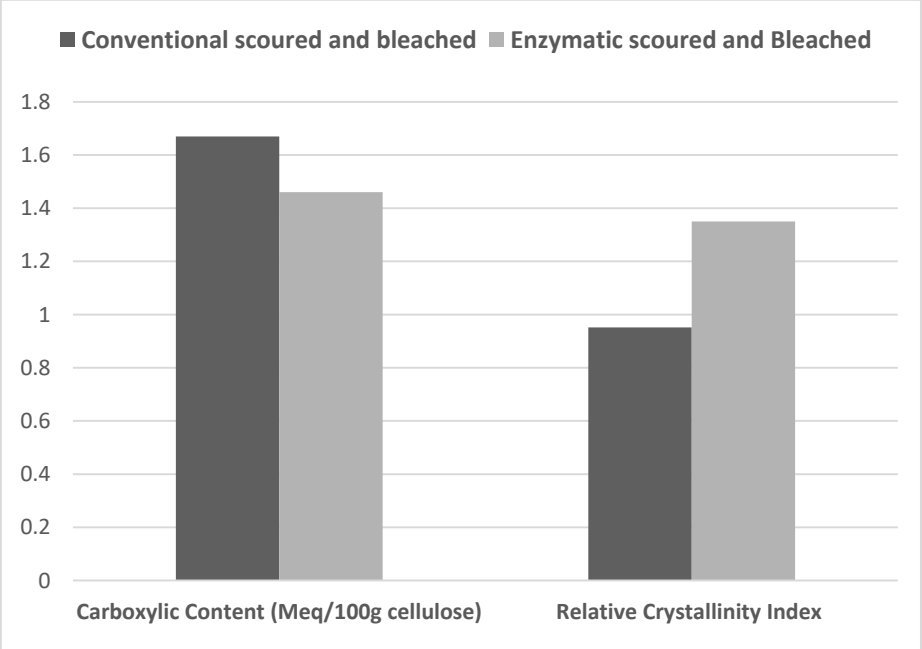

Fig. 1 Carboxylic content and relative crystallinity index of cotton fibres treated with conventional and enzymatic processes.

Relative Crystallinity Index

The FTIR spectra of conventional and enzyme treated cotton are used to calculate the relative crystallinity index by measuring ratio of absorbance at 1428 cm^{-1} and at 900 cm^{-1} (A_{1428}/ A_{900}). The index measures the relative changes in crystalline and amorphous region of the cotton fibre. The results are depicted in Fig.1. From the result, it is found that conventional scoured cotton showed less crystallinity index as compared to enzymatic processed cotton. The reduction in the index may be due to the severity of alkali on cellulose chain during scouring and bleaching. The result is in good correlation with the carboxylic content test reported in section *3.5.1* of this paper.

Based on the above results, it is clear that the enzymatic process is gentle on cotton fibre with less chemical damage as compared to conventional scouring and bleaching process. At the same time, it produces required performance on cotton fibre as prescribed by pharmacopeia.

Conclusion

The developed single bath process is eco-friendly as compared to conventional process with savings in energy, water and time. Apart from being eco-friendly, the enzymatic process causes less chemical damage to cotton fibre as compared to conventional process.

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Cotton harvesting machine suitable for small farms

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Introduction

Near 40% of the cost of cotton production is manual harvesting. Harvesters can reduce a large percentage of this cost (Saeedi Rad et al., 2017). Difficulty of manual harvesting and lack of labor in recent years, reveal the use of cotton harvesters. Cotton harvesters reduce costs and speeds up harvesting and finally, it will eventually increase income and increase farmers' desire to cotton cultivation (Nowrouzieh et al., 2003). Today, in developed countries, cotton farming is done by machine. This has led to an increase in the area under cotton cultivation and a reduction in production costs. Unfortunately, in Iran, the degree of cotton mechanization in planting operations is about 70%, in growing and protection near 10% and in harvesting is less than 1% (Saeedi Rad et al., 2017).

Although during the golden age of cotton production in Iran, some efforts carried out to use cotton harvesters, but these efforts were unsuccessful, and now, except in special cases, the cotton harvest by labors. Limited harvest time and lack of seasonal labor increase the cost of manual harvesting and decrease economic justification to produce cotton in Iran. Also there are no suitable cotton harvester in Iran and general cotton harvester are very expensive. The use of these harvesters requires large farms, which of course is small farms in Iran (majorities of cotton farmes are 3 hectares) (Rezaei Asl et al., 2013).

Cotton is harvested in October, November and December. The cotton harvest season coincides with the grain harvest season as well as the harvest of many crops such as saffron and orange. Therefore, lack of labor and workload in this crop season creates problems such as delays in cotton harvesting (Saeedirad et al., 2017). If the cotton harvest is delayed, this product will face a decline in quality and a reduction in the purchase price. The use of cotton harvester can play an effective role in reducing harvesting costs and on the other hand, with timely harvesting, the damage caused by cold and early autumn rainfall can also be reduced (Rahimi et al., 2017). These problems have reduced the area under cotton cultivation in Iran. In this case, there is no choice to use a cotton harvester.

Nazarzadeh Oghaz et al. (2014) conducted a study to evaluate the portable cotton picker machine with the aim of evaluating the

portable cotton picking and comparing it with the labor harvesting method. The results of this study showed that the uniformity of fibers and purity of cotton of portable cotton picker were 84.1% and 97.88%, respectively, which is more than 80.9% and 96.15% reported for manual method. Due to the cotton farms are mostly small; the use of large cotton harvesters in these farms is not economic justified. The purpose of this study is to design a suitable harvester without the limitations of conventional cotton pickers such as incapability of row distance with head distance of cotton picker, the need to use defoliator, the high price of cotton harvester. This machine harvests with maximum efficiency and performance.

Material and methods

The new harvester that designed and made in this project, was a semi-mechanized self-propeller machine with three-wheels that runs on the cotton farm. It has four picking units. The picking unit is moved by labor between the cotton plants and picks up the open bolls. The picking unit consists of a number of fingers that are rotated by an electric motor and pull out seedcotton from the open bolls and transfer them to the basket. For harvesting with this machine do not need to defoliator. Also, row or non-row cultivation does not hinder the work of the harvester. Due to the type of harvest, its field losses are almost zero and it is easy to service and maintenance. The seedcotton is transferred to the basket by pull and push air mechanism. Then seedcotton is collected in large bags and put out at the end of the field. The price of this machine is quite economic justified for farmers who have about 3 hectares of cotton cultivation. This harvester works easily in small farms and transport easily from farm to farm.

This cotton harvester has three parts: picker unites, transportation and electrical. The initial design of the mechanical part of the cotton harvester machine carried out by SolidWorks 2015 software.

This harvester machine was evaluated on two cotton cultivars in Hashemabad cotton research station and the working speed and quality of harvested seedcotton were compared by manual harvesting method. The evaluation was performed in a randomized complete block design with two factors in three replications in the field. The main factor was cultivar (Khorshid and Golestan) and the secondary factor was harvest method (manual and small cotton harvester (SCH)). 4 rows of each cultivar were considered, two rows were harvested by SCH and two rows were harvested manually, and the harvest time and amount in both methods were recorded separately and then analyzed.

Results and discussion

The small cotton harvester was built after the initial design and modifications is shown in Figure 1. This harvester can work with 4 picker units. However, due to the limitations, the prototype was evaluated with two picker units.

Fig 1- 3D model of small cotton harvester created by Solidworks softwear

Field comparison of SCH with manual harvesting showed that cultivar only affects significantly on the performance (hour per hectare) of SCH, which is due to cotton plant form and different number of bolls in the studied cultivars.

Fig 2- field test of small cotton harvester

The interaction of cultivar and harvest method was not significant on any of the parameters. The average field capacity of both methods in Figure 3 shows that the average material capacity of SCH is about 163.45 (g/min) and the manual harvesting is about 137.72 (g/min).

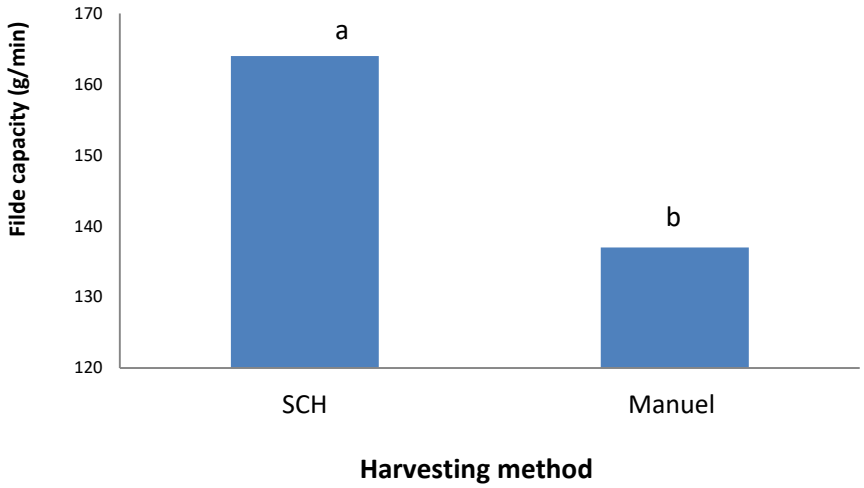

Fig 3- Field capacity comparison of SCH and manual harvesting

In the other words, the capacity of SCH was 18.7% more than the capacity of manual harvesting. Although Rahimi et al. (2014) reported that air suction machines were not able to work and do not harvest clear, this study shows, if the air suction machine was equipped with a picker unit, it increases the field capacity of the air suction machines.

In manual harvesting with two labors, it will take about 117 hours to harvest one hectare of cotton farm, but one hectare of cotton field took about 90 hours with using SCH with two picker units (Fig 4). That's mean 23% of the harvest time was reduced.

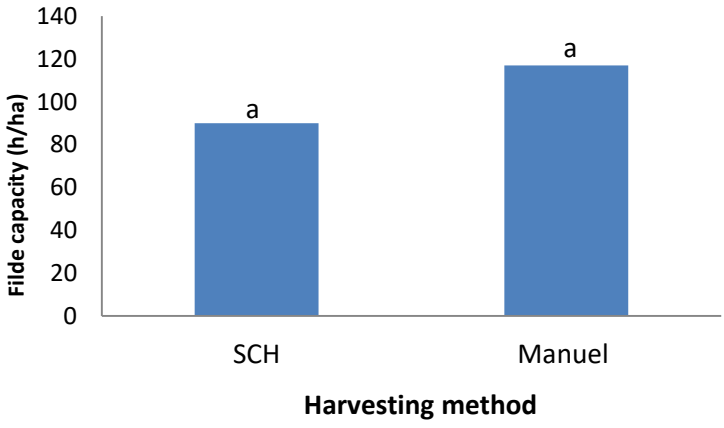

Fig 4- Speed harvesting comparison in SCH and manual harvesting

Seedcotton remaining on the plant in SCH was calculated to be 6.7% and manual method to be 6.2%. The harvester not only reduces the purity percentage, but also increases the purity by about 3%, due to the fingers of picker unite that extracted only the seedcotton. Also the dust and fine particles in cotton harvested by SCH went out by the pull- push air system in basket. The length of fibers (UHML) that measured by HVI shown in SCH it was 28.2 (mm) and in manual harvesting method was 27.5 (mm).

Due to the simplicity and cheapness of SCH, all cotton farmers can use this machine in cotton fields. Also SCH will surely satisfy the farmers because the cotton losses are very low. No need for defoliator, non-row harvesting capability, maintaining fiber quality and low price of SCH are the unique features of this harvesting machine.

SCH has been evaluated in different cotton cultivars and has shown that cotton cultivar has no effect on its efficiency.

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