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Content

The Cotton Innovations Newsletter is published twelve times annually. Contributed articles, pictures, cartoons, and feedback are welcome at any time. Please send contributions to the General Editors (see below). The editors reserve the right to edit. The deadline for contributions is one month before the publication date.

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News

The ICAC is delighted to announce 'Prof. Tabashnik' and Prof. Wendel' as joint winners of the ICAC RESEARCHER OF THE YEAR AWARD 2021

Prof. Bruce Tabashnik
Head of the Department of Entomology,
Regents Professor, University of Arizona, USA

Professor Tabashnik is one of the most influential scientists of our times in entomology and biological sciences. He led the Department of Entomology at the University of Arizona for 24 years. Tabashnik's research has provided fundamental knowledge about insects for enhancing agricultural sustainability and reducing the use of harmful insecticides. His research team studies the evolution and management of insect resistance to insecticides and transgenic plants. Current work focuses on the evolution of resistance to insecticidal proteins from the *Bacillus thuringiensis* (*Bt*). Web of Science/Clarivate Analytics recognized his work as highly cited in 2018 and 2019. Tabashnik is Fellow of the Royal Society of Entomology, UK. He is the recipient of several Awards including the Nan-Yao Su Award for Innovation and Creativity in Entomology, Entomological Society of America; Koffler Prize in Research/Scholarship/Creative Activity, University of Arizona. He recently won the Plant-Insect Ecosystems Lifetime Achievement Award in Entomology from the Entomological Society of America.

Jonathan F. Wendel
Distinguished Professor
Department of Ecology, Evolution, and Organismal Biology.
Iowa State University. USA

Professor Wendel's research focuses on the mechanisms underlying plant genomic and phenotypic diversify, focusing on the phenomenon of whole genome doubling, or polyploidy. Most of his ~300 publications focus on the cotton genus (Gossypium), in which two diploid and two polyploid species were each independently domesticated thousands of years ago. This natural evolutionary diversification, followed by parallel strong directional selection under domestication, provides a model framework for exploring the comparative basis of domestication, the origin of form and diversity in nature, and the evolutionary consequences of genome doubling. His research has helped shape our understanding of the myriad genomic consequences of allopolyploid, where two diverged diploid genomes become reunited in a common nucleus. His laboratory has illuminated the evolutionary processes of intergenomic gene conversion, homeolog expression bias, duplicate gene coregulation and expression dominance, biased fractionation, and the evolutionary trajectories of duplicated networks. Moreover, his contributions have been recognized in all three major domains of professorial life - Master Teacher, 2005, for his role as graduate mentor and educator, Distinguished Professor, 2012, for national research prominence, and Outstanding Achievement in Departmental Leadership, 2009, for leadership excellence during his 15 years (2002-2017) as department chair, His work has garnered national recognition, as evidenced by his election as a AAAS Fellow (2010), Distinguished Fellow of the Botanical Society.

World Cotton Research Conference Has Been Delayed until October 2022

When the seventh World Cotton Research Conference (WCRC-7) was cancelled last year after it became clear it would not be possible to hold an in-person event during a pandemic, it was a great disappointment to the research community. Unfortunately, lingering uncertainty regarding COVID has forced the organizers to delay once again the event, which is held every four years.

'We waited as long as we could before making this decision, especially after the disappointment over last year's postponement', said Dr Keshav Kranthi, Chief Scientist of the International Cotton Advisory Committee (ICAC). 'But with uncertainty about vaccines and possible new variants, we came to the conclusion that delaying the event again in 2021 was our best choice'.

As a result, the WCRC has been rescheduled for 2022 at the same place and time as the 2021 conference which was supposed to be held: 3-7 October in Sharm el-Sheikh, Egypt. The organizers announced that they would reconvene to evaluate the situation in March or April of next year. It was agreed that it was important to keep the event as an in-person event as this was where the maximum benefit could be gained from cotton researchers interacting with their peers.

Because the 2021 WCRC Proceedings — a massive tome packed with a variety of in-depth research papers — has already been completed, it will be released in June 2021 as Volume 1 and a new set of Proceedings – Volume 2 — will be created for the 2022 WCRC. An announcement will be made when Volume 1 of the Proceedings are available.

Source: www.icac.org

Status Of Genetically Modified Cotton In Pakistan

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INTRODUCTION

Cotton is a multipurpose crop in Pakistan. Mostly grown for its fiber, it is also the biggest oilseed crop, contributing 70% to local edible oil production (Iqbal *et al.*, 2003). From an economic perspective, it stands at the 2nd position after wheat and represents 0.8% of the country's GDP (Abbas *et al.*, 2015). Cotton is also termed as a problem crop. As all the possible production and plant protection problems can be faced by cotton growers, whether they are germination issues or biotic and abiotic stresses. Toxic chemicals are sprayed to control insect pests and diseases. Throughout the world, pesticides are being used to limit insect pest attacks. In the last thirteen years, a number of pesticides have been used to save the cotton plant from insect pests. Off all the insects found in cotton fields in Pakistan, lepidopteran insects are the major ones causing substantial economic losses, which account for 30% of production losses. Several approaches have been used to develop the resistance/tolerance against the lepidopteran insects, including American Bollworm (*Helicoverpa armigera*), spotted bollworm (*Earias vittella* Fabr.), armyworm (*Spodoptera frugiperda*), and pink bollworm (*Helicoverpa Armigera*). The development of *Bt* cotton, has proven to be the only successful approach (PARC, 2007). The development of *Bt* cotton was possible by using biotechnological tools, as it carries a foreign gene from the *Bacillus thurengenesis* bacteria. Identification, isolation, and transformation protocols are needed to develop a transgenic plant, and biotechnological applications made it possible (Gandhi and Namboodiri, 2006). Monsanto, together with Delta and pineland cotton seed company produced *Bt* cotton for the USA and was sanctioned for general cultivation in 1996 (Pray *et al.*, 2002). Transgenic were approved for general cultivation in 1996 (Pray *et al.*, 2002).The transgenic event was called MON-531, and the varieties and hybrids containing the cry1Ac gene were sold in the markets under the trademark Bollgard®. Bollgard® was found to be effective in protecting the cotton plant against the attack of most of the bollworms, including pink bollworm (*Pecthinofora gossypiella*), American bollworm (*Helicoverpa armigera*), tobacco budworm (*Heliothis virescens*), and spiny bollworm (*Earias* spp.). The effectiveness of Bollgard® was lower against fall armyworm (Spodoptera frugiperda) and cotton leafworm (*Spodoptra litura*). Insect pests have developed resistance for Bollgard® in many areas of the world, and to cope with this issue Monsanto has issued an advanced form of Bollgard®, which was Bollgard II ® that contains Cry1Ac and Cry2Ab genes (Arshad *et al.*, 2018). This advanced form was highly effective against American and pink bollworm as well as fall armyworm and cotton leafworm (Showalter *et al.*, 2009). Resistance against lepidopterans in cotton is not dependent on the introduction of Cry genes only. Several other factors are also involved in resistance like, genetic background, mode of gene transformation, breeding technique used to multiply the seeds, and the environment of the locality in which cotton plants are cultivated. Gene expression is also affected by technical restraints such as gene segregation in hybrids (F_1 generations), inappropriate backcrossing, growing conditions, and ultimately resistance against lepidopteran (Guo *et al.*, 2001, Lanqin *et al.*, 2005, Showalter *et al*.,, 2009).

MODE OF ACTION in Bt COTTON

Cry 1 Ac gene, introduced in Bt cotton, produces δ- endotoxins that protect the cotton plant against pink bollworm (Arshad et al., 2018;). Cry1Ac protein attacks especially lepidopteran insects and is harmless to other beneficial insects (McClintock *et al.*, 1995). Proteins start their action after binding to the receptors of midgut epithelium (Van Rie *et al.*, 1990), and these types of receptors are not present in mammalian cells (HOFMANN *et al.*, 1988). Therefore, it is specific to the target organisms only (Sims and Martin, 1997). Ingestion of endotoxins by chewing mouthparts of lepidopterans causes crystal solubilization in the mid-gut. Activation of these crystal proteins is largely dependent on the splitting of toxins (Perlak *et al.*, 2001). Feeding of larvae is inhibited, as up taking of these toxins causes preliminary paralysis of the midgut. Moreover, the epithelium starts dissolving and the permeability of the midgut is also affected. It opens the way for insect's starvation, resulting in insect death (Gill *et al.*, 1992).

Bt COTTON ADOPTION IN PAKISTAN

Bt cotton was the first GM (genetically modified) crop to be used on a commercial scale (Qaim *et al.*, 2003). In Pakistan, exotic *Bt* cotton, integrated the cultivation system through an informal channel. Seeds of transgenic cotton were mainly coming from India and other countries through informal network and in 2008, before the official approval of *Bt* cotton, these varieties were planted on ¾ of total cotton land (Sharma, 2009). Later, local plant breeders developed *Bt* cotton with extensive backcrossing of local cultivars with actual *Bt* cotton. Like in the rest of the world, adoption of *Bt* in Pakistan was unusual, and in 2010 0.6 Million farmers had cultivated *Bt* cotton. In Pakistan, the Punjab seed council (PSC), in 2010 for the first time, approved two Bt cotton events, including GFM event containing both Cry1Ac and Cry1Ab and MON-531. This approval included 8 varieties containing Mon531 event and one hybrid expressing a fusion of both genes (GFM event) (Punjab seed Council, 2010, NBC,2010). The following year, PSC renewed the approval of previously approved four transgenics (*Bt*) varieties namely, Neelam-121, Ali Akbar-802, IR-1524, and FH-113 (Pakistan Today, 2011). *Bt* cotton was being planted by more than 60% of farmers locally before official approval in Pakistan (Pakistan Central Cotton Committee, PCCC, 2008).

Cotton production one year (2011-12) after the approval of *Bt* cotton, had increased to a record production of 14.8 million bales which was a 25% increase from the previous year (2010-11). Before that the highest production was recorded in 2004-05, which was 14.3 million bales (James, 2011). In the mid-2000s the first conflict of unapproved Bt usage in Pakistan arises, when local progressive farmers and officials of local seed industries decided to use and multiply MON-531 event. Monsanto did not register any patent for their event in Pakistan. Therefore, breeders easily introgressed this event (informally arrived to Pakistan) into local germplasm (Spielman *et al.*, 2015). By the start of the last decade, it looked like the issue of bollworms was solved forever.

PINK BOLLWORM ISSUE IN PAKISTAN

Pink bollworms, army bollworms, and spotted bollworms are responsible for 30-40% production losses in Pakistan (Abro *et al.*, 2004), as these bollworms attack boll, squares, and flowers (Ashfaq *et al.*, 2006). Pink bollworm is frequently present in cotton fields (2001), and due to its mode of attack, this bollworm's control is very difficult compared to others. Cry1Ac gene can efficiently protect the plant against it (Naranjo, 2002). Despite of GM cotton, the issue of Pink bollworm is yet unresolved in Pakistan (Karar *et al.*, 2015). It appeared in epidemic form in 2015 and is seen intermittently every year since. Cotton production was reduced to 8.95m bales from 2014 to 2016, and pink bollworm control has been one of the limiting factors (Shuli *et al.*, 2018). Despite the adoption of Bollguard1, small farmers in Pakistan, sow self stored and contaminated seeds, which gave rise to the incidence of lepidoptron pests especially pink bollworm. Pink bollworm affected 9 to 11% of the cotton crop in Sindh province. The minimum damage to the crop was recorded to be 9.18% in the Khairpur district (Khuhro *et al.*, 2015). Pink bollworm has developed resistance against *Bt* cotton grown by up to 70 to 80% local farmers (Khuhro, 2014). It is probably due to lower expression of the Bt gene in floral buds which is the target tissue for pink bollworm and the tendency of farmers not to spray insecticides against bollworms. A survey was conducted to assess the pink bollworm population in 17 different *Bt* cotton varieties in different cotton growing regions. All these varieties/lines showed susceptibility by exhibiting infestation ranging from 24.49% to 100%. Average intensity of alive larvae on each boll was recorded at 109.6% since they had collected 1449 bolls for the study and 1698 larvae were present in the infested bolls (Abbas *et al.*, 2016).

WHY WE ADOPT GM COTTON?

Cotton bollworm complex causes huge production losses. The use of *Bt* cotton carrying endotoxins has changed pests control (Alvi *et al.*, 2012). Along with lower use of pesticides, it has brought other benefits, such as the safety of beneficial insects and low input cost with high production (Kouser and Qaim, 2015). In the past, several studies (Ali and Abdulai, 2010, Kouser and Qaim, 2013, 2015) were conducted to assess the impact of *Bt* cotton agricultural productivity in Pakistan and reported positive impact of *Bt* cotton by using cross-sectional data. Adoption of new technology in agriculture is always dependent on its economic benefits, and *Bt* contains several economic benefits such as low production cost with better yields, safety of micro-climate and farming community against toxic chemicals. Along with the above-mentioned points, the distribution of transgenic cotton with insect pest resistance could enhance per hectare income up to 280 USD (Pakistan Textile Journal, 2010, Kakakhel, 2010).

Major contributions to the Cotton improvement of Biotechnology sectors of Pakistan

Both the private and public sector has been contributing to research and development of GM cotton. In the public sector, NIBGE (National Institute for Biotechnology and Genetic Engineering) Faisalabad, and CEMB (Centre for Excellence in Molecular Biology), Lahore have taken the lead.

Single-gene technology

As discussed previously Mon-531 entered Pakistan informally, it was later bred into local cotton cultivars and resulted in the control of major cotton bollworms. This had been a single gene technology that has spent its natural life now. This technology was developed for the first time in USA and was allowed for general cultivation in 1995 (James and Krattiger, 1996). Still, in Pakistan, its introduction happened somewhere in early 2000s. Now 96 % of the total acreage is planted by Bt cotton, the only commercialized GMC (Genetically modified Crop) in Pakistan. It provides a more economical and environmentally friendly alternative to pesticides (Bennett *et al.*, 2013). In the case of Bt cotton, single-gene technology was mainly utilized for its resistance against *lipidoptera* by the Cry1 series gene (Bernhard *et al.*, 1997), Because of its protein high solubility at the higher PH solubility in insect gut contrary to other organisms causing it to become toxic to that specific insect group (Bennett *et al.*, 2013). But because of many factors single gene has been compromised (Arshad *et al.*, 2016) and paved the path for double/triple gene technology. Single gene transformations like herbicide tolerance, stress tolerance, and quality characters were also attempted and proved successful. Research on cotton improvement through improving seed viability with improved stress resistance against heat and drought utilizing AtNHX1 gene from

Arabidopsis thaliana (Arshad *et al*., 2016), Vip3A against *Lepidopteran herbivores* resistance (Khan *et al.*, 2020), and Hvt spider toxin against American Bollworm (*Helicoverpa armigera)* (Shah *et al.*, 2011) is ongoing in many research institutes in the country.

Double-Gene Technology

Double gene transformation started in cotton during the early 2000s (Li *et al.*, 2000.) But the age of double gene transformation in Pakistan started from the Cry1 Ac and Cry1 AB gene pyramiding for chewing insects by using Agrobacterium assisted transformation systems (Rashid *et al.*, 2008). One of the most recent advances in Pakistan is the development of Bt cotton to combat the weakened single gene resistance by introducing Cry 1Ac-Cry1 Ab in single cassette resulting in improved resistance to Pink Bollworm *(Pectinophora gossypiella)* with a relative efficiency of 93% (Siddiqui *et al.*, 2019). The unique event is validated for its authenticity (Asif *et al.*, 2021) and it is hoped that soon it will go through regulatory stages to come into the field.

Triple-Gene Technology

Then a series of contributions started the stacking of Cry1Ac+Cry2A and GT genes (Cp4-EPSPS) for insect resistance and herbicide tolerance using agrobacterium assisted transformation (Ali *et al.*, 2016, Awan *et al.*, 2015, Butt and Awan, 2016, Latif *et al.*, 2015, Puspito *et al.*, 2015). Moreover, the idea of using a fusion protein (Tp2) with the Cry1Ac+Cry2A enhances its localized expression in the chloroplast for that a single cassette having Cry1Ac-Cry2A and Tp2 was transformed using agrobacterium with an efficiency of 0.7% and resulted in 100% mortality in the targeted insect type (Muzaffar *et al.*, 2015). Then another major landmark is the transient expression of Cry1Ac-Cry2Ab-EPSPS in chloroplast using a single casette transformation to limit the toxicity to the green parts of the plant and is validated in the Tabbaco model plant (*Nicotiana benthamiana*) (Naqvi *et al.*, 2017). Bt cotton entered Pakistan informally and benefitted the farming community for more than a decade, unfortunately the farmer has been kept away from the advanced technologies like Bollguard-II and Bollguard-III. This is because these technologies could not enter Pakistan informally and the government could not develop a consensus to import these technologies from their developers. At the same time, locally developed technologies are yet to benefit the cotton growers and the efficacy of Mon-531 is now under question. Farmers are at the losing end, the need of the hour is to either import the advanced technologies or develop alternatives locally and bring them to farmers' field.

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Current Scenario of Cotton in Pakistan

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Cotton, earlier considered a gold crop, has lost some of its advantages over the past few years. Being considered as the backbone of the economy, the cotton crop adds its share ~0.8 % to GDP and 4.1% in total value added in agriculture. In Pakistan, >1200 km area, along the Indus River, is designated as a cotton belt. Mainly upland cotton is cultivated in the province of Punjab and Sindh. In the Sindh province, the weather is very hot and dry, and the temperature ranges from 45 to 50 °C. Although this year the overall cotton area increased by 6.5% over last year's area (2,373 hectares), several biotic and abiotic stresses led cotton farmers not to prioritize cotton to earn a profit. A decline of 6.9% was observed in cotton production last year (9.178 million bales) compared to the previous year *i.e.*, 9.861 million bales (Anonymous, 2019-20). Moreover, Pakistan is lagging far behind in terms of per acre yield (679 kg/h), as compared to major cotton producing countries like Turkey (1826 kg/h), China (1719 kg/h), Australia (1816 kg/h), and USA (985 kg/h) (ICAC, 2018). Unfavorable weather due to climate change and low water availability during critical stages of plant development along with pest attacks hampered cotton production and yield seriously. The situation led the textile sector (yarn manufacturers) to import cotton worth around USD 2.0 billion this year. Abiotic stresses such as high temperature, drought, salinity, metal toxicity, and biotic stresses, pests, and pathogens are major production limiting factors (Mahajan and Tuteja, 2005). These factors not only affect plant growth and yield but also affect harvest quality. The normal functioning of the defense system in plants is disturbed by abiotic stresses which ultimately lead to more production and accumulation of reactive oxygen species (ROS) inside the cells. This high level of ROS causes oxidative stress, which is harmful to plants and changes all the metabolic processes responsible for the normal functioning of plants (Mullineaux and Baker, 2010; Qamer *et al*., 2021). In recent times, a rise in temperature has emerged as a major threat to cotton productivity. Global temperature is estimated to increase by 0.4~0.8 °C/year (PMD 2016). The cotton plant has an indeterminate growth habit and possesses sensitivity to high temperature, which sometimes rise up to 45°C, which is also a major constraint in cotton production.

The versatile impacts of high temperature stress are reported on biochemical and morphophysiological attributes of plant growth and development. High temperature (air and soil) severely affects the essential processes, including carbon assimilation and leaf chlorophyll content, ultimately reducing photosynthesis. The plant shows different responses to high temperatures depending on species and developmental stages (Azhar *et al*., 2020). High temperature (> 35 °C) causes not only deleterious effects at the time of flowering (pollen sterility and abortion) and boll setting and formation (boll shedding) but also disturbs the photosynthetic activity, elongation of sympodial branches, and gossypol content (Ekinci *et al*., 2017). Several osmoprotectants, including proline, salicylic acid (SA), and ascorbic Acid (AA) can be applied exogenously for increasing thermal stability (Qamer *et al*., 2021). Due to climate change, global warming, and unpredictable rainfall, growth of crop plants is negatively affected. Rainfall occurrence is significantly less as compared to previous years, resulting in drought (Mittler and Blumwald, 2010). Drought refers to less water availability for an extended period, from the optimum level required for healthy growth and productivity (Abdelraheem *et al*., 2019). However, growth enhancers, foliar application of supplements, and minerals can also be used to increase resistance against drought stress on a short-term basis.

Due to poor quarantine measures and climate change, many insects emerge as viruliferous pests and cause serious damage to the cotton crop which was already considered as a perfect and favorite host. Insects of cotton in Pakistan show great diversity. More than 1250 species of insects are reported to attack the cotton crop, and approximately 93 insects and mites are damaging the cotton crop in Pakistan. Whitefly (*Bemisia tabaci*) is considered as the most notorious pest for upland cotton because of its ability to transmit cotton leaf curl virus. Dry weather with high temperature conditions always favors the spread of whitefly. Due to consistent efforts of cotton breeders and researchers for several years, high yielding and virus resistant varieties were developed (Mansoor *et al*., 2003), but due to the emergence of a recombinant strain (Burewala strain) during 2001, all the resistant cotton varieties collapsed. The recombinants dependents upon a balanced nucleotide substitution rate besides the combination of different genetic backgrounds and adaptation to vector (whitefly) (Saleem *et al*., 2016), and unfortunately in case of cotton nature played its role and favored the pathogen. Likewise, jassid, thrips and bollworms are also threatening pests of cotton crop. Most of the farmers do not know how to identify a thrips infestation. Bollworms cannot attack the Bt cotton but few bollworms can hibernate in cotton seeds, debris in ginning factories, and cotton sticks. Therefore, farmers should refrain from dumping cotton sticks in open fields. There are many biological, chemical and cultural control methods to regulate these pests, but chemical control is expensive and increasing the number of sprays results in lower profit.

Since, the onset and utilization of tools of genetic engineering and biotechnology for the improvement of cotton, besides traditional breeding, an overall boost in the economy of Pakistan through agriculture was observed. Several traits such as fiber quality, drought resistance, pest resistance, and herbicide resistance were successfully improved by incorporating foreign genes. The utilization of germplasm resources by exploiting molecular markers in breeding programs also played a major role in the development of several improved varieties of cotton in terms of quality and yield (Saleem *et al*., 2021). The rise in popularity and cultivation of genetically modified (GM) cotton resulted in increased acreage, ultimately enhancing the productivity of many cotton growing countries globally, including Pakistan. Many improved varieties have been approved for general cultivation in the last few decades. Several new genes which confer drought tolerance are identified by using transgenic approaches and QTL techniques. Central Cotton Research Institute, Multan (Pakistan) has maintained 6030 accessions of cotton and worked hard to develop new stresses tolerant varieties with high quality fiber traits. Due to ever increasing demand for cotton, improvements are still needed to enhance production in areas where less irrigation water and low rainfall. In this situation, varieties that require less water and give better yield are desirable. Keeping in mind global warming and an alarming climate change situation, it is time to develop climate smart varieties that could withstand harsh climatic conditions and associated pest and pathogen attacks.

The farmer's preference shift towards corn, rice and sugarcane has tremendously decreased the area for cotton in Punjab. Meanwhile due to less biodiversity of insect pests in Baluchistan, farmers are more willing to grow cotton over there, which is an encouraging situation. This area switch to cotton and the decrease in cotton production in Punjab affects production cost of the textile industry, and costlier cotton has been imported to meet the requirement of the international textile market. In Balochistan, a lot of efforts are required to establish cotton as a commercial profit giving crop especially for farmers with small land holdings. It is highly recommended and suggested that government should take appropriate action to help farmers to get more profit from cotton by lowering the price of pesticide, fertilizers, provision of pure seed and subsidy but also increase the budget for applied research to face the challenges of ongoing and upcoming climate change situation.

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Known and Unknown Effects of Heat Stress on Cotton Productivity in Pakistan

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1. Introduction

Pakistan is the world's fifth largest producer of cotton, which is grown primarily along the hotter regions of the Indus River in Punjab and Sindh. In Punjab, cotton is regularly cultivated in various districts; Multan, D.G. Khan, Rahim Yar Khan, Khanewal, Bahawalnagar, Rajanpur *etc*, whereas in Sindh, cotton is mostly grown in the areas of Sanghar, Ghotki, Nawabshah, Nosheroferoz *etc*. However, recently, cotton cultivation tends to increase in various regions of Balochistan province of Pakistan. Cotton is considered the backbone of the Pakistani economy because it provides the raw material for the country's largest manufacturing sector, which accounts for more than 60% of total export earnings. Pakistan is one of the largest producers and leading exporter of cotton yarn. Therefore, millions of farmers, factory workers, and traders depend on a single crop for their livelihood (Abbas, 2020). Though production of cotton has been increased manifold, it has also seen enormous yield uncertainties during the last decade. During 2019-20, the total area under cotton cultivation was 24.5 million ha, which decreased to 22 million ha in 2020-21. Pakistan's total cotton production in 2020-21 was at an all-time low of 4.7 million bales for the marketing year (MY) (August/July) (USDA, 2020). Cotton is facing competition from other crops, especially sugarcane, as well as unfavourable prices and rising sugar demand and price. Further, global warming impedes crop production capacity while also posing a danger to agricultural productivity.

2. Effect of heat stress on cotton growth and development

Since climate change has a major impact on crop development, Pakistan is more vulnerable to climate change as a result of its geographical position (Hussain *et al.*, 2020). In comparison to previous decades, the decade of the 2000s was the warmest in Pakistan, with the years 2014 to 2016 being the hottest. The highest temperatures during the cotton season in Sindh province, where cotton is widely grown, range from 45°C to 50°C. Similarly, a warming trend has been observed in Punjab province over the last three decades, with a focus on the years 2000–2016 (Ahmad *et al.*, 2016). According to future climate model forecasts, noticeable differences in the frequency and intensity of the mean temperature would increase more in Pakistan than the projected global average of 1.4-3.7°C (Ahmad *et al.*, 2019).

Cotton has a vertical tap root system and is more tolerant to elevated temperature and droughts than other crops, but temperatures above 32°C can trigger cotton growth and development to be impaired (Ton, 2011). The increased temperature would cause buds and fruits to shed at an early stage of growth. High temperatures can also affect boll growth and size, as well as the maturity period. During the cotton season in Pakistan, the average temperature is 40 to 45°C, with temperatures sometimes exceeding 50°C. The average temperature change during the cotton season since 1980 is increasing (Fig. 1). As a result, heat stress in Pakistan is seen as a major stumbling block to increasing overall cotton production.

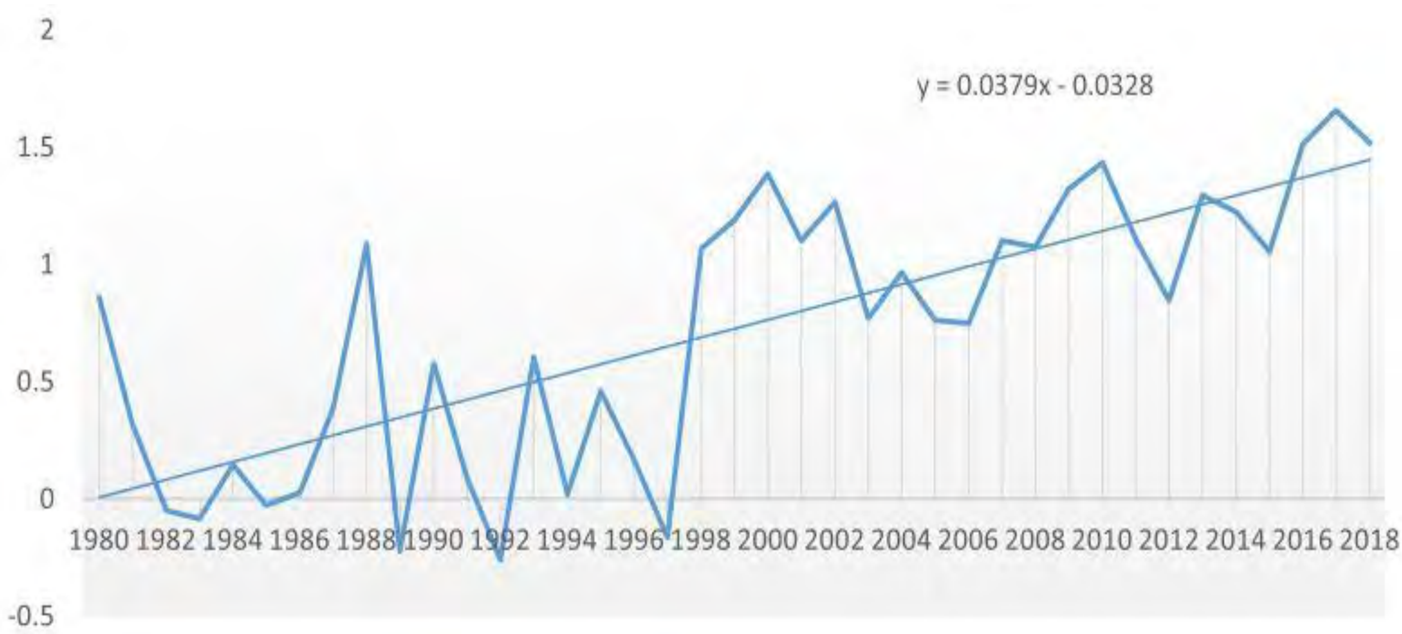

Figure 1. Mean temperature change in Pakistan (April to October) from 1980 to 2018, Meteorological

Source: Abbas, S. (2020). Climate change and cotton production: an empirical investigation of Pakistan. Environmental Science and Pollution Research, 27, 29580-29588.

In recent years, extreme temperatures during plant flowering and boll growth stages in Punjab have resulted in significant abortion of cotton bolls (Iqbal *et al.*, 2017). Cotton plants are also much more susceptible to insect-pest attacks and viral diseases due to high temperature. In addition, the natural response of self-defense was also hampered, resulting in the loss of vegetative and fruiting parts. Cotton produced in Pakistan is famous worldwide for its good quality and long fiber that made it popular for textile products (Zulfiqar *et al.*, 2017).

Cotton fruit quality is highly influenced by earlier exposure to local weather conditions during the development stages. Moreover, the fiber quality forestalls the relationship between the plant's fruit positioning architecture and air temperature. Correspondingly, high temperatures have also been linked to poor fiber quality (Ton, 2011) (Fig. 2). Additionally, the most heat-sensitive stages of the reproductive growth process in cotton are pollen production, pollen tube growth, and reproductive stages (Zinn *et al.*, 2010).

3. Mitigation strategies:

3.1 Agronomic practices:

Various crop production practices can minimize the effects of heat stress on cotton crop. One possible way is to enhance the number of irrigations which can provide surface cooling through evaporation and reduce the intensity of high temperature (Lobell *et al.*, 2008). This strategy doesn't seem to be suitable as the International Monetary Fund (IMF) has already declared Pakistan as third among the countries facing water shortage (Nabi *et al.*, 2019). Under such circumstances, the crop irrigation paradigm must be shifted from flood irrigation to high efficiency irrigation systems. In the Punjab province, the cotton crop is sown after the 10th of April for the management of pink bollworm. Crop sown in mid April is approaching the reproductive or flowering stages during June which is the hottest month of the year for this region. Thus, flowering stage (the most vulnerable to heat stress) is coincide with maximum temperature of the region. While the late sowing can disturb the wheat-cotton system of the region, and early sowing increase the pink bollworm infestation. The adoption of determinate types of cultivars with early maturing habits is a suitable strategy. To-date, the cultivars of cotton grown in the country are indeterminate in habit and usually 3-4 pickings are practiced.

Antioxidants and plant hormones can also improve the tolerance to high temperature by modulating metabolic activities, neutralizing reactive oxygen species, and improve gaseous exchange. Experimental results showed that foliar application of these chemicals significantly reduces the impact of oxidative stress on the crop (Abdelhamid *et al.*, 2013; Hanif *et al.*, 2021). The availability of these chemicals on commercial scale and their utilization in farmer's field is still very limited. The frequent use of these chemicals can add more to the cost of cotton production as farmers are already paying a huge amount to control biotic stresses in terms of both *i.e.*, purchase of Bt-cotton seeds and pesticides. If Pakistan successfully achieves the pink-free cotton zones, such inputs can be added to the production cost of cotton.

One of the best managements for heat tolerance can be achieved through genetic modification of crop and introducing cultivars with a natural ability to withstand temperature extremes without compromising fibre yield and quality. As a result of continuous breeding efforts from various public and private sector researchers, the cotton cultivars of Pakistan can tolerate the 44 ºC temperature without much compromising yield. But, the maximum temperature in cotton growing regions now exceeds 47 ºC. It is, therefore, needed to accelerate the breeding efforts. Cultivars released in the past were the results of conventional breeding through screening, hybridization, and selection processes or selection following random mutations. These breeding approaches are laborious and time consuming. Selection was mostly limited to morphological and fibre quality parameters. Moreover, the genetic base of cotton germplasm narrowed due to intensive selection from particular genotypes, limited cross-pollination and wide hybridization (Rahmat *et al.*, 2014).

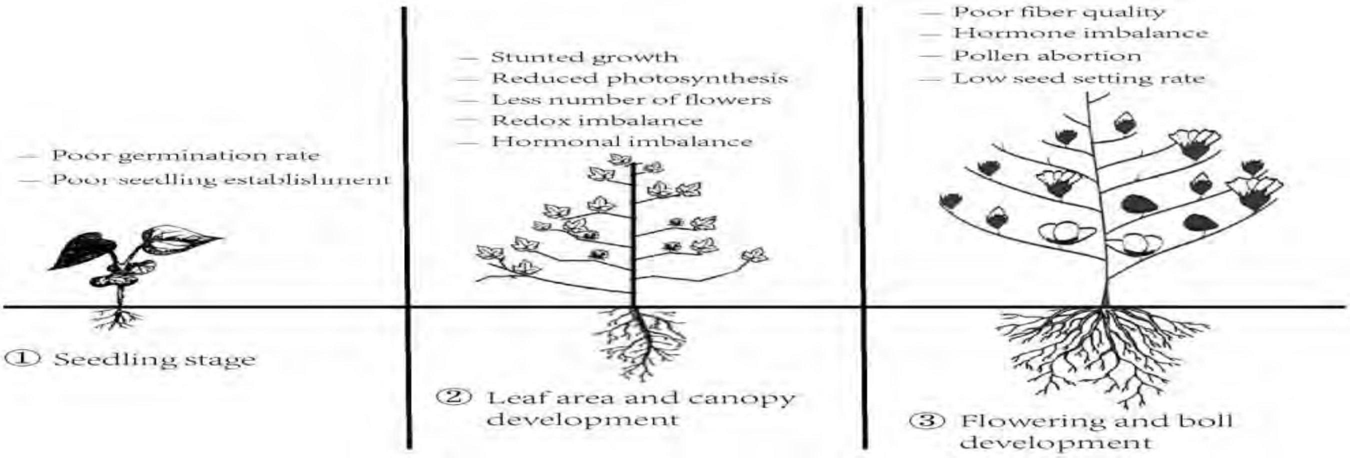

Figure 2. The impact of high temperatures on cotton's agronomic and physiological characteristics at different stages of growth.

***Source**: Zafar, S. A., Noor, M. A., Waqas, M. A., Wang, X., Shaheen, T., Raza, M., & Rahman, M. U. 2018. Temperature extremes in cotton production and mitigation strategies. Past, present and future trends in cotton breeding. 23:65-91.*

related to heat tolerance in cotton. These parameters include viability and germination of pollen grains, the integrity of cell membrane structure, antioxidant activities, and ROS production (Majeed *et al.*, 2018). These parameters must be taken into account while breeding cultivars under changing climatic scenarios. Moreover, the exchange of germplasm must be appreciated and promoted by fully implementing the "plant breeding rights" for broadening the genetic basis of germplasm. Non-involvement of multinational seed companies directly in cotton research programs is also a limiting factor to adapt modern technologies. Implementation of plant breeding rights will encourage those companies to invest directly in cotton seed sector of Pakistan. The use of biotechnological tools in cotton research and cultivar development is also limited in Pakistan. To-date, no literature reports on the use of next generation sequencing technology to develop genomic markers in cotton breeding programs in the country, and the use of recent genome editing tools is also limited. Most of the molecular breeding of cotton is conducted by public research institutes, while a few private companies are involved in molecular breeding of cotton. The focus of most of the labs is towards development of insects and herbicide tolerance genotypes. In a nutshell, revamping cotton breeding programs by integrating classical breeding approaches, functional genomics, and genome editing technologies would open new avenues for selection, characterization, and incorporation of heat-tolerant traits in cotton cultivars (Mubarik *et al.*, 2020).

3.3 Tree plantation drive

The overall effect of global warming can be minimized or reversed through various approaches to minimize the greenhouse gases. The one, most important and highly needed for Pakistan is the intensive plantation of trees and reforestation. This approach could reduce the region's overall temperature and make it more suitable for cotton cultivation. For this purpose, various projects have been accomplished or are still on-going. One remarkable achievement is completing the "Billion Tree Tsunami" project in 2017 by the government of Khyber Pakhtunkhwa (KPK), Pakistan. Currently, the "10 Billion Tree Tsunami" program is being run by the Ministry of Climate Change, Government of Pakistan. There is a dire need for such projects on all levels and all over the country to mitigate the effects of high temperature in the country.

Conclusion

Cotton cultivation in Pakistan is dropping each year, and the country is now ranked fifth among the top cotton-producing countries, down from third only a few years ago. The decrease of cotton production in the country involves various factors while high temperature stress is one of the major factors among them. Pakistan is badly hit by climate change, and the country's annual temperature is rising every year. Sustainable cotton production under such temperature extremes can be achieved through the combination of various agronomic practices. The adoption of recent biotechnological and genomic tools in cotton breeding programs has also become indispensable. The implementation of "plant breeding rights" will also open a new horizon in cotton research by involving multiple seed companies. Tree plantation drives in Pakistan can also sustain the agriculture and cotton production in the country by mitigating the adverse effects of high temperature.

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Role of Breeders in Cotton Productivity enhancement of Pakistan

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Introduction

Cotton is a leading fiber crop in the World and known as white gold of Pakistan. The economic viability of Pakistan depends on this crop as it is a major source of foreign exchange earnings (60%) and provides livelihood to 1.5 million people in Pakistan. It is also catering the needs of 400 textile mills and 1200 ginning factories. In Pakistan, cotton is cultivated on about 2.5 million hectares annually with average lint production of 660 kg ha-^{1}. Cotton is mainly planted in two provinces. Punjab province produces 70% whereas rest of 30% production is received from Sindh province with a little contribution of Baluchistan. Historically cotton cultivation in Pakistan dated back to 6000 BC with *Gossypium arboreum* L. as identified in the ancient remains of Monjadharo (Sindh). Farming community of Pakistan has been cultivating G. *arboreum* L. till 1920s which was gradually replaced by the introduction of high yielding upland cotton varieties. Cotton is now mainly cultivated in South Punjab and upper and mid Sindh parts of Pakistan. Cotton mostly fits in cotton-wheat-cotton cropping system in Pakistan.

Cotton breeding in Pakistan started with the establishment of the Agriculture Department in 1902. The first step towards cotton research was with the establishment of Agriculture college in Faisalabad and later on the establishment of different Research centers. The main research work on cotton began in 1962 when Ayub Agricultural Research Institute, Faisalabad (AARI, FSD) was established, which is the foremost research organization working on various crops in Pakistan. Under the umbrella of AARI, FSD, there has been continuous expansion in cotton research system in Punjab to cater to the needs of farmers, ginners, oil mills and textile industry. During 1935, one cotton research Sub-Station was established in Multan for testing the performance of varieties in relatively hotter Southern zone. This Research Sub-Station, Multan was upgraded in 1958, and in addition to testing varieties developed at Lyallpur (Faisalabad), the task of developing varieties was also entrusted to this center. Later on, Directorate of cotton was shifted from Faisalabad to Multan in 2017. It is the main Cotton Research Center in Pakistan which has developed 66 varieties since 1914 (Table 1) with economic contribution of 13.22 billion dollar since 1980. There are 2 eras of cotton breeding in Pakistan.

Pre-transgenic cotton era (1947-2009)

Cotton was grown on an area of 1.2 million ha with the production of 1.1 million bales in 1947. Plant breeders continued to work on development of new and improved cultivars of cotton. A number of high yielding cultivars were developed which helped farmers increase their per acre yield, expansion of cotton area and overall cotton production. Most popular varieties during these years were MNH-93, S-12, NIAB-78 and CIM-496. MNH-93 is the first break-through in 1980 when ginning out turn/lint %age improved from 32.0 to 37.5%, whereas NIAB-78 was early in maturity. The second break-through came in 1991 when S-12 was introduced to farmers and Pakistan achieved the highest production of 12.8 million bales with average yield of 769 kg/ha this year. S-12 variety of CRI Multan was cultivated on about 50% of total area of cotton in Pakistan. Later on, in 1992 cotton

MNH-93 S-12 NIAB-78 CIM-496

crop was not successful due to the Cotton Leaf Curl Virus (CLCuV) disease and all the available cotton cultivars were susceptible. From here onward, the main research focus shifted towards the development of new CLCuV varieties, and a large number of germplasms was screened for resistance to CLCuV. Later on, a large number of CLCuV resistant cotton varieties were developed by various institutes including the Cotton Research Institute, Multan. A new strain of cotton leaf curl virus, *i.e.*, Burewala strain emerged in 2002 and all germplasm in the country became susceptible to the CLCuV disease. However, all cotton research institutes in Pakistan continued breeding efforts for the development of leaf curl resistant varieties, which culminated in the development of CIM-496 and MNH-786 and other varieties which proved highly tolerant to leaf curl virus disease. In 2004, cotton production jumped to 14.2 million bales. It could not be sustained due to cotton leaf curl virus disease and bollworms attacks.

Post- transgenic cotton era (2010-2020)

This decade can be termed as an era of Bt. cotton. Breeding for Bt cotton actually started in 2005 but it was first commercialized in 2010. The first Bt. cotton variety introduced in Pakistan was N-121 by a public sector organization/Neelum Seeds Pvt Ltd. Cotton Research Institute Multan also started work on development of Bt cotton varieties and the first Bt variety of this Institute was commercialized for general cultivation in 2012. Later on, several other Bt. varieties were introduced but FH-142 of this research centre was the most prominent one commercialized in 2013. A jump in cotton production was witnessed in 2011 when we achieved a mile stone by producing 14.8 million bales and Punjab production also stood at ever highest figure of 12.2 million bales. Pakistan harvested good crops till 2014 and was among the top three players worldwide. Cotton varieties which dominated during these years were MNH-886 and FH-142. MNH-886 covering up to 90% area in Punjab from 2011 to 2015. Later on, climatic change, pests attack, and diseases adversely hit cotton productivity in Pakistan, particularly in Punjab and cotton production dropped to 10 million bales. All these factors collectively made the cotton crop uneconomical, and a sharp decline in area and production was observed in 2020. Cotton Research Institute actually started working on interspecific hybridization in 2009 to tackle the issue of CLCuV by transferring CLCUV resistant genes from G. arboreum to G. hirsutum. Germplasm was collected from all over the world and screened against CLCuV.

Resultantly some highly tolerant lines were isolated, and these were used in breeding programs for development of CLCuD resistant varieties.

As a result, CLCuD losses in 1993 were 70% which have been narrowed down to only 16% in 2019. Moreover, owing to continuous efforts of the breeding team at CRI Multan, six varieties i.e. MNH-1016, MNH-1020, MNH-1026, MNH-1035, FH- Super cotton and FH-490 have been approved in 2021. Among these varieties MNH-1020 and MNH-1026 are highly heat tolerant and early maturing (150 days) whereas, other varieties are suitable for marginal lands with shortage of water and low fertility.

We have been working with USDA for the development of cotton leaf curl virus resistance / highly tolerant cotton varieties and so far, we have identified one most promising line i.e. MAC-07 which is being utilized for the development of CLCuV varieties. One variety NIBGE-11 possessing genes of MAC-07 has been approved in 2021. Two strains (MNH-1050 and MNH-1090) have been developed at CRI Multan through hybridization of desi and American cotton possessing high degree of CLCV tolerance, high yield potential and harmonious combination of fiber traits, which is a unique work in the world.

MNH-1050 is at the approval stage as it has completed all national trials whereas, another variety MNH-1090 has been included in the National Trials in 2021. Another strain MNH-872 has been developed through hybridization of G. anomalum (a wild spp.) and American cotton possessing high degree of CLCV tolerance, high yield potential and harmonious combination of fiber traits (GOT = 39%, staple length = 32.0, Mike = 4.6 µg/inch, Fiber strength = 35g/tex). Keeping in view the challenges of insect pests and weeds, biotechnologists and plant breeders are working for the improvement of Bt. cotton. MNH-1045 possessing Bt. + glyphosate resistant genes, is in advance stages of its development. Significant improvement has been made in Gossypium arboreum- native to this country and cultivated on about 5% of the total area of cotton. Staple length has been improved from 19 mm to 27mm, and its fiber fineness is better than the commercial desi variety mollisoni (standard). This institute is also working on insecticide resistance and integrated pest management to reduce the use of chemical insecticides and bring down the overall cost of production.

MNH-1050	
Boll weight (g)	4.5
GOT (%)	40.5
Staple Length (mm)	28.46
Mic (µg/inch)	4.58
strength (g/tex)	31.0

MNH-1090	
Boll weight (g)	4.5
GOT (%)	42.2
Staple Length (mm)	30.2
Mic (µg/inch)	4.2
strength (g/tex)	34.2

MNH-1093	
Boll weight (g)	3.5
GOT (%)	38.5
Staple Length (mm)	28.0
Mic (µg/inch)	4.2
strength (g/tex)	29.7

MNH-1092	
Boll weight (g)	4.5
GOT (%)	35.0
Staple Length (mm)	28.0
Mic (µg/inch)	5.5
strength (g/tex)	32.1

Overall genetic improvement in cotton in Punjab/Pakistan

There has been remarkable genetic improvement in various agronomic and quality traits of cotton after the initiation of breeding programs. Modern varieties are early maturing (41%) which makes them a best fit in our wheat cotton cropping system. Fiber traits have been improved with an increase of 34% in ginning outturn, 35% in staple length and 15% in fiber fineness.

Bottlenecks in cotton production in Punjab/Pakistan.

Since 2014, climate change emerged as a key factor for cotton productivity in Pakistan and particularly in Punjab.

Along with this, whitefly, pink bollworm, cotton leaf curl virus disease, dusky cotton bug, and shortage of water are other major threats to cotton productivity in Pakistan particularly in Punjab. Keeping all these factors in consideration, breeders have been focusing on disease resistance, insect pest resistance, drought tolerance, heat tolerance, and better fiber quality. The material is being developed by employing various conventional and non-conventional techniques.

Future thrust

- Development of climate resistant varieties
- Improvement of fiber quality
- Implementation of IPM
- International collaboration

GENETIC IMROVEMENT OF COTTON TRAITS

Traits/ Prod.	4-F 1914	AC134 1959	MNH-93 1980	S-12 1988	MNH-886 2012	FH-142 2013	MNH-1020 2021	% Gain
Lint (%)	32	34.5	37.7	41.3	41	40	43.0	34
S.L (mm)	20.8	26.4	28.3	28.2	28.2	28.5	28.1	35
Fineness (µg/inch)	5.8	4.5	4.3	4.2	4.9	4.7	4.9	15
Maturity (Days)	310	300	290	270	210	200	180	41
Yield Potential (Kg/ha)	66.7	118	600	800	1000	950	800	1099

LIST OF THE APPROVED VARIETIES DEVELOPED AT CRI, MULTAN

#	Varieties	Year of Release	G.O.T %	SL (mm)	Mike (µ/inch)	Strength (tppsi)	#	Varieties	Year of Release	G.O.T %	SL (mm)	Mike (µ/inch)	Strength (tppsi)
1	4-F	1914	32	21.5	5	85	34	MNH552	2000	40	27.5	5.2	96.3
2	LSS	1933	32	23.0	5	85	35	FH-1000	2003	38.8	27.5	4.9	96.9
3	289 F/43	1934	30	24.0	4.5	93.5	36	BH-160	2004	35.3	29.5	4.6	101.9
4	124-F	1945	33	25.0	4.7	95	37	MNH-786	2006	39.5	27.5	4.7	101
5	199-F	1946	35	25.0	4.5	90	38	CRSM-38	2009	39.5	29.0	4.5	95
6	AC-134	1959	34.5	26.5	4.5	92.5	39	FH-113	2010	40	28.0	4.7	98
7	L-11	1959	33.7	27.7	4	90	40	FH-114	2012	39.6	28.1	4.85	95.5
8	BS-1	1962	34.5	25.0	4.5	91.5	41	MNH-886	2012	41	28.2	4.9	99.5
9	MS-39	1970	33.6	31.5	3.8	87.5	42	FH-942	2012	38	29.6	4.2	95.1
10	MS-40	1970	32.7	31.3	3.8	88	43	B H-167	2012	41.2	29.1	4.8	92.7
11	149-F	1971	34.5	28.0	4.1	94.3	44	SLH-317	2012	38	29.8	4.4	96.7
12	B-557	1975	34.5	27.5	4.5	92.5	45	FH-118	2013	39.2	28.0	4.2	100.8
13	MNH-93	1980	37.5	28.4	4.5	94	46	FH-142	2013	40	29.0	4.7	99.6
14	MS-84	1983	34.5	33.0	3.9	91.4	47	BH-178	2013	41.5	29.5	4.9	98.6
15	MNS-79	1983	36.5	27.5	4.4	99.3	48	VH-259	2013	39.5	27.5	4.9	93
16	MNH129	1985	38.6	28.7	4.4	95.4	49	FH-Lalazar	2016	42.04	28.9	4.8	98.6
17	SLH-41	1985	36.5	27.7	4.4	89	50	MNH-988	2016	42	28.5	4.8	96.1
18	S-12	1988	41.2	28.2	4.4	93	51	VH-305	2016	39.8	28.1	4.8	96
19	FH-87	1988	33.8	28.5	4.3	95	52	BH-184	2016	40	28.7	4.9	95.5
20	Gohar-87	1990	34.5	27.2	4.4	92.6	53	RH-647	2016	40.2	28.3	4.2	99.2
21	RH-1	1990	31.5	29.0	3.9	103.7	54	VH-327	2016	37.7	30.2	3.9	101
22	MNH147	1992	42.5	27.3	4	96.6	55	FH-326	2017	38.8	29.2	4.3	95.3
23	BH-36	1992	35	28.3	4	87	56	FH-152	2018	40.3	28.9	4.2	115.5
24	S-14	1995	43.9	29.5	4.2	93.6	57	RH-662	2018	39.9	29.1	4.3	105.3
25	SLS-1	1995	36.8	27.4	4.6	95.3	58	RH-668	2018	39.4	28.8	4.5	103.2
26	MNH-329	1996	41.8	28.5	4.2	96.6	59	SLH-8	2018	41	30.1	4.3	96.25
27	FH-634	1996	36.3	28.5	4.1	95.1	60	FH-444	2019	40.4	28.5	4.6	98.5
28	RH-112	1996	34.3	27.6	4.6	95.1	61	MNH-1016	2021	43	29.0	4.5	29
29	FVH-53	1998	35.8	27.5	4.8	101.2	62	MNH-1020	2021	43	28.1	4.9	32
30	BH-118	1999	37.5	28.5	4.7	96.9	63	MNH-1026	2021	42	28.0	4.7	31
31	FH900	2000	37.5	28.5	4.5	94	64	MNH-1035	2021	39	28.0	4	33
32	FH901	2000	38.2	26.7	5.1	92	65	FH SUPR C	2021	42	30.0	4.7	30
33	MNH554	2000	43	28.5	4.3	98.9	66	FH-490	2021	43	28.0	4	31