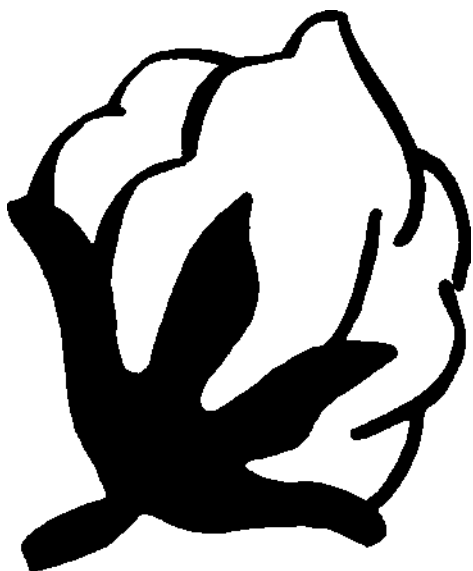




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International
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Technical
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- **Update on cotton production research**
- **Nouvelles recherches cotonnières**
- **Actualidad en la investigación de la producción algodонера**

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Introduction

China (Mainland) is the largest cotton producing country in the world. Since 1992, cotton production has been badly hit by wide spread losses in the Yellow River Valley due to severe cotton bollworm attacks. The cotton bollworm built up slowly in China over four decades and is now the most dangerous pest in this country, having developed resistance to a group of insecticides and becoming difficult to control at economical costs. High insecticide costs in the Yellow River Valley have resulted in the abandonment of cotton area in Shandong, Hebei and Henan and in the expansion of the Northwest Region area, particularly in Xinjiang. Currently, pesticide use is minimal in the Northwest Region and yields are higher compared to the Yellow and Yangtze River valleys. Since the bollworm disturbed the economics of cotton cultivation in the Yellow River Valley, cotton area in the Northwest Region has more than doubled. But, recently, the cotton bollworm has also spread to the Northwest Region, becoming a problem. In the first article, the cotton bollworm situation with particular reference to the reasons for the spread and its effects on the production system in China are discussed.

DNA markers provide an invaluable tool to breeders and geneticists by allowing selection by markers rather than complex quantitatively controlled characters. Utilization of molecular markers requires DNA extraction, development of a methodology for scoring large numbers of polymorphic molecular markers and creation of a mapping population. Molecular markers are a comparatively new area of research. A lot of work is being done to identify and tag various markers for the most important characters and tracking their transfer to offspring. Four different methods used in various labs include Restriction Fragment Length Polymorphisms, Randomly Amplified Polymor-

phic DNAs, Simple Sequence Repeats and Amplified Fragment Length Polymorphisms. Dr. Saha and his colleagues in the United States Department of Agriculture in Stoneville, Mississippi, have compared different molecular marker techniques in the second article, in addition to reporting on the Polymerase Chain Reaction based DNA markers labeled with fluorescent.

Bt cotton is spreading. It is estimated that 2.63 million hectares of transgenic cotton were planted in the world during 1998/99. In the USA, 45% of total cotton area was planted to genetically engineered varieties. Argentina, Australia, China (Mainland), Mexico and South Africa are other countries where transgenic cotton was grown on a commercial scale during 1998/99. Development of transgenic varieties involves heavy investments, and private companies who commercialize this technology need to be paid back. Farmers in countries where Bt cotton has been adopted have agreed to pay the technology fee to private companies who own the technology. But there is fear that the technology may spread uncontrolled and that not only won't biotech companies get paid for their research and development efforts, but that the technology could be misused and may result in losses instead of benefits. A new system called Technology Protection System is being researched to prevent the free spread of transgenic technology: seed companies can convert transgenic seed to produce infertile seed at any stage they want. Pros and cons of the Technology Protection System are discussed briefly in the third article.

Global Working Group: Transgenic Organisms in Integrated Pest Management and Biological Control

Transgenic cotton varieties not only provide resistance to insects or herbicides, but they could have a significant effect on

the pest complex, as some minor pests may become major pests. Resistance to the Bt toxin is another major concern for the industry. There is a need to monitor the effects of transgenics on the production system as a whole. A working group called Global Working Group: Transgenic Organisms in Integrated Pest Management (IPM) and Biological Control has been established with a new initiative designed to engage and bring together scientists from public sector institutions in developed and developing countries around the world. It will encourage scientific studies and multidisciplinary cooperation in areas outlined below. While the working group is intended for scientists from public sector institutions, it will establish mechanisms to regularly exchange information and ideas with private industry and non-governmental organizations.

The scope and activities of the working group are to

- Provide a network for information exchange among scientists.
- Identify research needs to understand the efficacy and environmental impact of transgenic plants, and develop methods and protocols for their evaluation.
- Develop methods and protocols for resistance management.
- Develop methods and protocols for monitoring.
- Provide a scientific basis for making decisions about the value of deploying or developing transgenic organisms for particular IPM systems.
- Advocate the role of scientific information as a basis for policy making.

Methods will be developed to measure the non-target effects of transgenic organisms that need to be improved and related to the impact on the field and landscape level.

Successful IPM involves the participation and cooperation of farmers, extension workers, researchers, regulatory authorities and policy makers in designing and implementing appropriate local IPM systems. All of these stakeholders need to be empowered and involved in the process of deciding whether and how to use transgenic organisms as components of IPM.

The working group is committed to rapid and extensive communication of scientific information and progress to the public, and to the proper use of scientific information in developing policy on the use of transgenic organisms in IPM and biological control. Its outputs, in the form of publications and protocols, will be made available to all. The working group will be producing its first newsletter in early 1999. For more information or participation in the group activities, please contact the chair of the group or Prof. Waage of CABI, at the following addresses:

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Meetings

- Over 40% of the world cotton production during 1997/98 was produced in three countries: China (Mainland), India and Pakistan. Currently, all three countries are faced with a common problem of insect resistance to a variety of insecticide groups. With other smaller cotton producing countries like Thailand and Vietnam also affected by the same problem, resistance to insecticides has emerged as a regional problem in Asia.

ICAC is sponsoring a Regional Consultation on Insecticide Resistance Management in Cotton in Asia. The Consultation will be held at the Central Cotton Research Institute, Multan, Pakistan, from June 28 to July 1, 1999. The objectives of the Consultation are to review the current status of insecticide resistance in the region, research programs on insecticide resistance, management strategies being developed in different countries, exchange experiences in the implementation of resistance management practices, and consider possible collaboration among different countries to tackle the resistance problem. Experts from outside the region are also welcome. More information on the Consultation can be obtained from the Technical Information Section of the ICAC or Dr. Zahoor Ahmad, coordinator of the Consultation.

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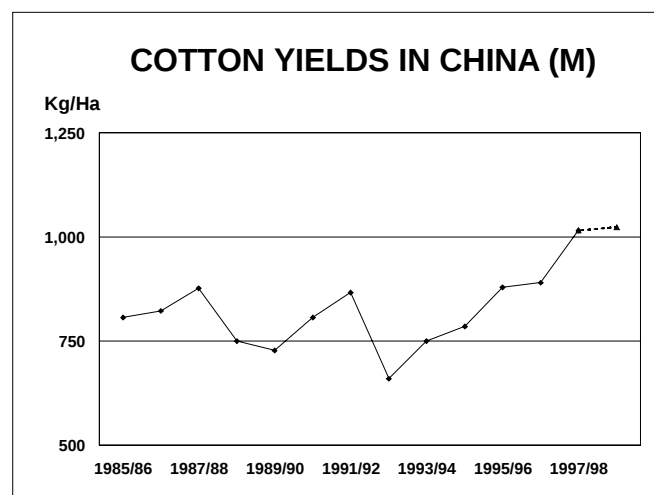
- The 58th Plenary Meeting of the ICAC will be held in Charleston, South Carolina, USA, from October 25-29, 1999. A one-day technical seminar on Fiber Quality Needs of the Modern Spinning Industry, including papers on ginning research, will take place during the plenary meeting. Visits to cotton fields, a ginning factory and a USDA classing office are also planned. More information on the meeting will be available soon from the ICAC Secretariat.

The Cotton Bollworm in China (Mainland)

China (Mainland) has been the largest cotton producing country in the world since 1982/83. In 1991/92, China produced 5.672 million tons of lint from a total area of 6.54 million hectares. The following year, area increased by 4.5% to 6.837 million hectares but production dropped by 20% to 4.51 million tons. Over 200 kg/ha of the drop in the average lint yield in 1992/93 was attributed to insect damage, mainly the cotton bollworm *Helicoverpa armigera*. Cotton production in China (Mainland) has been continuously affected by wide-spread attacks of the cotton bollworm since 1992/93, the first year China suffered heavy losses in cotton production due to the bollworm. There are two possible explanations: first, the bollworm population moved from other cotton areas to China's northeast; second, the bollworm changed status from a minor to a major pest and developed resistance. The second explanation, which seems reasonable and is considered responsible for the outbreak of the bollworm in China, is discussed here.

Production Regions

On average, China (Mainland) grows cotton on 4 to 5 million hectares divided into seven different regions: Northeast, North (Yellow River Valley), Northwest, East, Central, South and Southwest. The East Region, also called Mouth of Yangtze River Valley, and the Southwest Region, comprised of the Guizhou, Sichuan and Yunan provinces, can be grouped with the Central Region to form the Yangtze River Valley. The Northeast (Liaoning province) and South (Guangxi province) Regions together produce less than 1% of the total. Thus, the Yellow River Valley, the Yangtze River Valley and the Northwest Region which includes the province of Xinjiang, are the three main cotton-producing regions in China (Mainland). Production characteristics among the regions are quite different. The federal government has only one central research institute on cotton located in the province of Henan, which develops varieties for all regions. It is estimated that about 50% of the total cotton



area in China is grown under varieties developed by this institute. Most varieties have a high ginning outturn ranging from 38% to 43%.

Population Build-up

The five main cotton pests that affect cotton production in China are cotton aphids *Aphis gossypii*, cotton bollworm *Helicoverpa armigera*, pink bollworm *Pectinophora gossypiella*, cutworm *Agrotis* spp., and spider mites *Tetranychus* spp. The spotted bollworm *Earias* spp., plant bug *Adelphocoris* spp. and jassid *Amrasca* spp. may also show up but are rarely in the first five most important pests. With the exception of the pink bollworm, which is not yet a common pest in the Northwest Region, the other four pests are found in all cotton production areas. However, since 1992, the cotton bollworm has emerged as the most destructive pest throughout the country. The Yellow River Valley has been affected the worst and now the bollworm has also moved to the Northwest Region.

Cotton Production Regions in China (Mainland)

Region	Provinces in the Region	Main Characteristics
Yellow River Valley	Beijing, Hebei, Henan, Shandong, Shanxi, Tianjin	Planting under plastic is common; some transplanting is done; intercropping is also done; verticillium and seedling diseases are common; cotton bollworm resistance to insecticides is a problem; pheromones are used.
Yangtze River Valley	Anhui, Guizhou, Hubei, Hunan, Jianghai, Jiangxi, Shanghai, Sichuan, Yunan, Zhejiang	Transplanting is common; some planting under plastic; wheat intercropped with cotton; verticillium and boll rot are common; pink bollworm is a major pest.
Northwest	Gansu, Shaanxi, Xinjiang	Short growing season; high density plant stand; planting is done under plastic; verticillium wilt is common; aphids are a major problem; high yields; lately, the common bollworm has become a problem.

The cotton bollworm emerged as the most important pest in China only during the 1990s. During the 1950s, aphids, spider mites and the pink bollworm were the major insects. The cotton bollworm did exist on cotton, but there was hardly a need for spraying against it. During the 1960s and 1970s, the second generation caused economic losses and the third and fourth generations appeared on cotton. During the 1980s, the second, third and fourth generations caused serious losses in yield. During the early 1990s, the fifth generation also appeared at the end of the crop season, and insect pressure on the plant further increased. In addition, overlapping of generations started occurring, resulting in heavy losses in production. Similarly, egg counts per 100 plants increased from 200-300 in the 1950s to 925 in the 1960s, 1351 in the 1970s, and 2000 in the 1980s. Consequently, the pest complex changed, which had a big impact on cotton production in China.

Reasons for Flare-up

The outbreak of the cotton bollworm has increased steadily. In the northern areas forming the Yellow River Valley, the bollworm population started increasing during the 1950s, while to the South it started multiplying from the 1960s. According to the data collected by local authorities (Sheng et al, 1994), it is estimated that in parts of Hebei Province, the area infested with the cotton bollworm was only 26% in 1953. The bollworm was recorded affecting 57% of the area in 1957 and, in 1970, it had spread to 100% of the cotton area. The cotton bollworm has certain inherent characteristics that have helped to establish it on cotton in China. They include euryphagy, high reproductive rate, quick development of resistance to pesticides and survival under a variety of climatic conditions. Though the bollworm outbreak built up slowly, 1992 had special significance in boosting the bollworm population to extraordinary levels. The following factors played a role in building the cotton bollworm population:

- Chinese agriculture is characterized by smallholdings with an average cultivated area of less than one-tenth of a hectare per family. Cotton production is highly intensive and double cropping is commonly practiced, particularly in the Yangtze and Yellow River Valleys. In the Yangtze River Valley, double and even multiple cropping systems have become popular since the 1960s. In the Yellow River Valley, changes in the cropping system started a little later. However, it is estimated that in 1976, 0.7 million hectares were double-cropped in the Yellow River Valley, increasing to 1.236 million in 1988, 1.296 million in 1989, 1.638 million in 1990, and 1.955 million hectares in 1991. In Shandong, the worst affected province, the cotton-wheat double-cropped area increased from 6% in 1981 to 54% of the total cotton area in 1991. Double cropping and multiple cropping systems delayed planting and extended the harvesting period. Thus, cotton plants were left in the field for a longer period of time.
- According to Sheng et al (1994), another change in addition

to intensive farming, particularly from the beginning of the 1980s, has been a general decrease in the plowing of cotton fields during the off season. In the cotton growing areas, where double cropping was not followed, cotton and corn fields were not tilled, thus, there was less disturbance of the pupae hibernating in the soil, and a favorable chain for the bollworm to survive and multiply at a higher rate was established. Delayed maturity increased the number of bollworms going into hibernation thus increasing the over-wintering population. Wheat served as a host for the survival of the first generation.

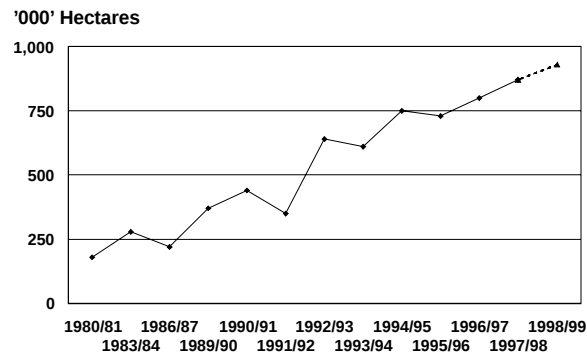
- Cultivation of mulch crops and their incorporation into the soil dramatically increased. According to the Chinese Ministry of Agriculture, the green manured area in the seven cotton growing provinces of Anhui, Hebei, Henan, Jiangsu, Shanxi, Shaanxi and Shandong increased by 81% in four years. In 1986, crops were mulched on 0.741 million hectares, increasing to 1.341 million hectares in 1990. Not only did the mulched crops provide a new source of food for the over-wintering population, the data showed that mulched cotton fields had about three-times higher egg density of second-generation bollworms compared to non-mulched fields.
- The bollworm's host range during winter was further extended through the cultivation of more vegetables and flowers in the cotton areas. Tomatoes, beans and eggplants were very popular vegetables and strong hosts. It is estimated that in the seven provinces mentioned above, vegetables and flowers were cultivated on 1.921 million hectares in 1991 against 1.094 million hectares in 1979. Vegetables and flowers received fewer insecticide applications compared to cotton.
- All over the world the development of short stature varieties enhanced the ability of the cotton plant to tolerate higher doses of nitrogenous fertilizers. According to data from the Chinese government, in Anhui, Hebei, Henan, Jiangsu, Shanxi, Shaanxi and Shandong the quantity of fertilizers used in 1991 increased by 83% over 1980. In the same period, irrigation facilities were extended to 10% more area. Higher fertilizer doses, coupled with irrigation, gave rise to healthier crops, which were preferred by insects also.
- Up until 1982, the cotton bollworm had been controlled with DDT and carbaryl, products to which the pest had already developed resistance. According to Xia (1993), in the provinces of Hebei and Henan resistance was as high as 116 to 136-fold in 1975. The resistant population was exposed to extensive use of pyrethroids at least since 1983. In the next seven years, particularly from 1986 to 1990, the bollworm developed high resistance to fenvalerate and monocrotophos. However, the first field failure associated with pyrethroid resistance in the third and fourth generations was noticed in 1990 in Hebei, Henan and Shandong. Extensive use of pyrethroids continued and high resistance was wide spread in the north in 1992. Chemical control became almost ineffective and resulted in a higher use of insecticides.

- In an effort to control the bollworm chemically, the natural control of the pest was disturbed.
- Over-wintering conditions are very important for the survival of the pest through the next generations. It is claimed that extremely cold temperatures in Uzbekistan are one of the reasons for a lower pest population there. Similarly, favorable off-season temperatures result in low mortality during the winter. In the Yellow River Valley, the bollworm population was already at the verge of exploding, and average temperatures were 5°C higher than usual from December 1991 to February 1992, favoring a high bollworm survival rate for the 1992/93 crop.
- Cotton bollworm populations were large in 1970-72 and 1982, and it is believed that 1992 marked a ten-year outbreak cycle for the bollworm.
- Wheat intercropping served as an additional host for aphids. According to Xia (1994), there were two types of cotton-wheat intercropping. In the first type, cotton was planted in April and overlapped with wheat for two months, while in the second type cotton was planted in May and overlapped with wheat for one month. The aphid population multiplied on wheat and moved to cotton. At the very beginning of the season, cotton started being attacked by a large aphid population and, consequently, the need to control aphids on cotton at an early stage increased significantly. Insecticides used to target the aphid population were automatically received by the cotton bollworm, which in the meantime adapted to low insecticide doses.
- One of the main reasons for the development of resistance in most countries is the misuse of insecticides. There was no control on insecticides in the Yellow River Valley and farmers applied whatever they wanted, particularly against the cotton bollworm. The number of sprays increased significantly, to 15-20 per season in some areas, with the ultimate result being the development of resistance.

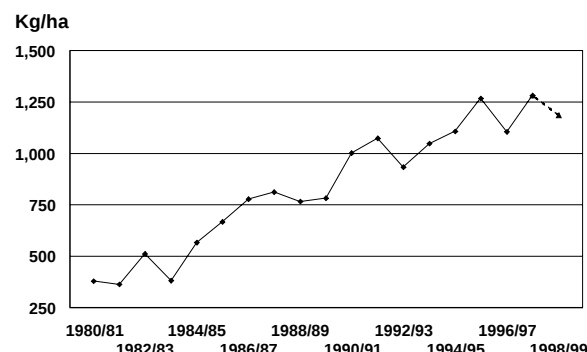
Shift in the Production Area

One of the most significant effects of the inability to control the bollworm through insecticides in the Yellow River Valley was the abandonment of the highly affected areas. Insecticide use increased to the extent that it was not economical to produce cotton in many parts of the Hebei, Henan and Shandong provinces. For the last fifteen years, China has consumed over 4 million tons of cotton locally. In order to meet the domestic needs, China must produce at least 4 million tons of cotton or become a permanent importer of cotton. Abandonment of some production areas in the North resulted in the expansion of cotton in the Northwest Region, particularly in Xinjiang. Since 1991, the cotton area in the Northwest Region has increased by almost 300%, and it is estimated that during 1998/99, about 93% of the total cotton crop in the region was produced in Xinjiang.

COTTON AREA IN NORTHWEST REGION OF CHINA (M)



COTTON YIELDS IN NORTHWEST REGION OF CHINA (M)



In the late 1980s, cotton yields in the three regions were comparable. But now, yields in the Northwest Region are over 300 kg/ha higher than in the other two regions. Higher yields and a lower need for insecticide use has encouraged expansion in area. Plans are to grow 1.5 million hectares in the Northwest Region in 2000.

The Cotton Bollworm in Xinjiang

It is understood that many cotton growers from the Northeast moved to the Northwest Region, and while they took rich cotton-growing experiences with them, they also carried some production practices that encouraged the multiplication of pests. According to Sheng (1999), during 1980, less than 50,000 hectares were affected by the cotton bollworm in Xinjiang, but since 1991 the bollworm has increased significantly and it is estimated that over 70,000 hectares were affected in 1993. In 1996, the affected area increased to 360,000 hectares, or 45% of the total cotton area in the Xinjiang, of which 52,000 hectares had high bollworm infestation. In some pockets, the attack was so

severe that about 300 hectares were devastated and had to be abandoned. This was a clear indication that the path of the Yellow River Valley was being followed.

Mild weather conditions at the end of 1996 and early 1997 significantly reduced the mortality rate of the over-wintering population, particularly in the east and south of Xinjiang. It was the first year that emergence of the bollworm started in early April and reached its peak in early May, two weeks earlier than usual. In some places, moth catches were greater than in Shandong. The majority of first generation moths emerged in middle-to-late June, and the density was a new record for the area.

Resistance Management Program

Currently, China has two situations: management of resistance to insecticides in the Yellow River Valley and management of the pest in the northwest so that multiplication is controlled before it develops resistance. A program, Strategies and Tactics to Reduce the Cotton Bollworm, was launched in the Yellow River Valley in 1993. The resistance management program has been discussed in detail in the September 1997 issue of the *ICAC RECORDER*. The program seems to be working effectively as cotton yields in the region, which had dipped to below 500 kg/ha, have come back to normal. In 1998/99, 1.6 million hectares of cotton were planted in the Yellow River Valley, 2.2 million hectares less than in 1991/92 and 1992/93. Elimination of the worst affected area might have had a positive effect on yields and decreased losses. If the program is sustained for years, the resistant population may be eliminated or at least successfully reduced. The approach followed in Xinjiang in the last two seasons is discussed below.

Learning from the experience in the Yellow River Valley, the expert advice of federal researchers to the local government has been to adopt a community-wide approach. The proposal, applied by provincial authorities, included measures on three different fronts at the same time. They are

- Kill over-wintering pupae
- Moth trapping during the season
- Effective control in the field

One of the most successful approaches to control any insect is to minimize its multiplication. To successfully control the boll weevil in the USA, a minimum population is allowed to go into hibernation and the maximum possible emerging population is killed to minimize multiplication. On the same principle, a three-step approach was started in China before the bollworm developed resistance to insecticides. A large scale campaign was started in the fall of 1997 to plow as much area as possible. It is estimated that almost a million hectares of wheat and corn area

was plowed to expose the pupae. A large number of pupae was physically collected and destroyed in the early spring of 1997 and 1998.

Efforts were made to collect the first two generations of moths with pheromone traps. Poplar tree branches and corn were used as trap crops to attract the moths. According to Sheng (1999), the data showed that in areas where moth catching was done, a lower population emerged in subsequent generations. In areas where no moth catching was done, the population kept increasing.

The third approach was equally important and was also strictly followed. At the beginning, a non-insecticide control was adopted through the application of Bt and nuclear polyhedrosis viruses (NPV). Soft control followed applications of commonly used conventional insecticides. The bollworm population decreased and, accordingly, threshold levels were revised to depress the population to further lower levels. One of the important recommendations and revisions has been to base threshold levels on fertilized egg counts. It was recommended that cotton be sprayed only when fertilized egg (yellow, brown or dark colored) counts reach 50 per 100 plants.

Data for the last two seasons have shown that pest pressure as well as boll damage have significantly decreased. The approach can successfully avert the resistance problem for a longer period of time, and if the program is continued, the situation in the Yellow River Valley will not be repeated in the Northwest.

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Gel Based DNA Marker Technologies in Cotton

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Introduction

Genetic mapping with DNA markers has become an essential tool for revealing the genetic basis of both qualitative and quantitative traits in crop plants. DNA markers have special advantages over morphological traits in genetic analysis because they are normally polymorphic, have no pleiotropic or epistatic effects, employ non-destructive methods requiring very small amounts of plant materials and there is no effect of environment on the phenotype. Many of the economically important traits of crop plants are controlled by complex quantitative traits (QTL). An accurate and detailed genetic map of DNA markers linked to QTLs provides an invaluable tool to the breeders to use molecular markers in marker-assisted selection by allowing selection for the markers rather than the complex QTL phenotype. Recently several laboratories in USA and abroad have launched special initiatives on molecular genome mapping programs in cotton to exploit the available gene pools and enhance the improved germplasm resources (Cantrell et al., 1999; Yu and Kohel 1999; Rungis et al. 1999; Cantrell et al., 1998, 1999; He et al. 1999; Wright et al. 1998; Luo et al. 1999; Akbari and Lyon, 1999).

Scientists have had to face three primary challenges in molecular mapping the cotton genome: 1) developing an extraction method to produce high quality DNA suitable for molecular analysis; 2) developing an appropriate method that can quickly score large numbers of polymorphic molecular markers and 3) creating a suitable mapping population that can be used for identifying molecular markers linked to the traits of interest. However, the method for creating the appropriate mapping population varies based on the research interest. The overall objectives of this paper are to address these concerns and also provide a summarized report on the current status of molecular mapping in cotton.

DNA Extraction

High quality DNA is an essential requirement for developing DNA markers in cotton. Cotton tissue is notoriously recalcitrant to many common DNA extraction methods due to high levels of polyphenolic compounds (e.g. gossypol). When cells are disrupted during sample grinding, phenolic compounds interact with proteins and nucleic acids causing problems in molecular analysis. Therefore, special precautions must be taken during DNA extraction from cotton tissues. Several methods

have recently been developed for DNA extraction in cotton (Callahan and Mehta 1991; Paterson et al., 1993). However, these methods require either fresh tissue or tissue stored at very low temperatures (-50° to -70°C). Saha et al. (1997) reported a DNA extraction method using freeze-dried cotton tissue. The freeze-dried tissue can be ground as a dry powder and stored in a freezer (-20°C) for an indefinite period. Recently, the method has been modified to be more efficient and less time consuming for large scale DNA analyses using a slightly modified method of Dneasy Plant Mini Kit (Qiagen, Santa Clarita, CA). Freeze-dried crushed cotton leaf tissue powder (35 mg) was used instead of liquid N₂. The remainder of the method was followed as per the manufacturer's protocol. This method yielded high molecular weight DNA that was readily digestible with restriction enzymes and served as a template for PCR amplification.

Molecular Genome Methods

Four different types of molecular methods were used for developing DNA markers in cotton namely Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLPs).

Restriction Fragment Length Polymorphisms

RFLP techniques involve restriction enzyme digestion of DNA into small fragments which are electrophoretically separated on an agarose gels and transferred to a solid support or filter (nitrocellulose membrane) and detected as discrete bands following hybridization to a radioisotope or non-radioisotope labeled probes. Alleles are recognized by differences, due to mutation at the restriction sites, in the size of the restriction fragments to which probes have hybridized. The advantages of the RFLP method are that it is normally polymorphic and markers are co-dominant distinguishing heterozygote from the homozygote (Figure 1A). However, the conventional detection of RFLPs by Southern blot analysis is laborious, time consuming, costly and it always requires sufficient quantity of good quality DNA and a probe.

Reinisch et al. (1994) published the first report on a RFLP map using an interspecific tetraploid cultivated *Gossypium* spp. They reported 705 RFLP loci assembled into 41 linkage groups cov-

ering 4675 cM. They also located several RFLP markers to different chromosomes using aneuploid chromosome substitution lines. Shappley et al. (1996) reported 16 RFLP loci arranged in 6 linkage groups in an intraspecific F_2 population of upland cotton. Shappley et al. (1998) presented another report of 120 loci arranged into 31 linkage groups covering 865 cM in upland cotton. Shappley et al. (1998) also reported the first report of RFLP-QTL linkage map using an intraspecific population of upland cotton. They determined the location of 100 QTLs in 24 linkage groups. They also observed that several QTLs are located on the same position in some of the linkage groups. This might be due to several reasons including a master QTL gene controlling several traits, the same genetic effect (QTL) measured in different ways or the QTL loci are strongly linked. Jiang et al. (1998) reported 261 RFLP markers in 27 linkage groups in an RFLP-QTL linkage map using an inter-specific population. They observed that 14 QTLs affecting fiber-related traits were located within the D subgenome group. They mentioned that fiber-related genes had already been fixed in the A sub genome and that the discovery of genes from a non-cultivated genome (D) is a reflection of a genetic revolution during polyploidization in tetraploid cotton. We reported the chromosomal association of several RFLP markers linked to important QTLs in upland cotton (Saha et al., 1998). However, our report indicated more influence of the A sub genome in fiber-related QTLs in the upland cotton demonstrating the importance of further investigation and introgression of diploid A-genome species in improving fiber traits in upland cotton. Our results also showed the portability and strength of RFLP markers that could be used between two different intra and inter-specific cotton populations. The high density genetic map of RFLP markers and QTLs of defined chromosome regions will be very helpful in transferring useful orthologous QTL loci among the tetraploid cultivated species of cotton. Wright et al. (1998) identified RFLP markers linked to the resistance allele for the bacterial blight pathogen (*Xanthomonas campestris* pv. *malvacearum* (Xcm).

To complement conventional RFLP markers, molecular markers based upon Polymerase Chain Reaction technology (PCR) have recently been widely used in plants. PCR is done following an enzymatic amplification of genomic DNA that is flanked by two synthetic oligonucleotide primers (3' and 5' end of DNA) that hybridize to complementary strands of the target sequence in template DNA. Repeated cycles of the heat denaturation of the template DNA, annealing of the primers and primer extension by *Taq* polymerase in the PCR reaction result in amplification of the specific segment of the template genomic DNA defined by the synthetic 5' and 3' primers. Normally the banding patterns are scored directly under UV light after electrophoresing the amplified products of PCR, stained with ethidium bromide, in an agarose gel. Polymorphism in the genome is detected due to 1) nucleotide changes that prevent amplification due to mismatch of bases at any one of the priming sites; 2) deletion of a priming site; 3) insertion or deletion in the tem-

plate DNA that changes the size of the amplified products; and 4) insertion that causes the priming site to be too distant to support amplification. The PCR method has become one of the most popular tools because of its ease, speed, sensitivity and versatility to study large populations. In recent years 3 different types of PCR-based DNA markers have been used in cotton namely: RAPD, SSR and AFLP.

Random Amplified Polymorphic DNAs

Most commonly used PCR-based DNA techniques that fall in this category in which about 10 to 15 base-size oligonucleotide arbitrary primers are used for amplification of DNA segments to develop molecular markers (Figure 1B). The advantage of this method is that it does not require any prior DNA sequence information. Our results using inter and intra-specific tetraploid cotton showed that normally about 8 to 12 DNA markers ranging from 120 to 1000 bp in size per primer combination, are generated using RAPD method (Figure 1B). We observed about 2% to 10% polymorphic markers/primer combination in upland cotton depending on accessions. The disadvantages of using RAPD markers are that they are dominant markers and sometimes produce non-specific DNA markers. Yu et al. (1998) identified several RFLP, RAPD and SSR markers linked to fiber length and other properties in an inter-specific population. Wajahatullah and Stewart (1997) evaluated genomic affinity among the selected *Gossypium* species using RAPD method.

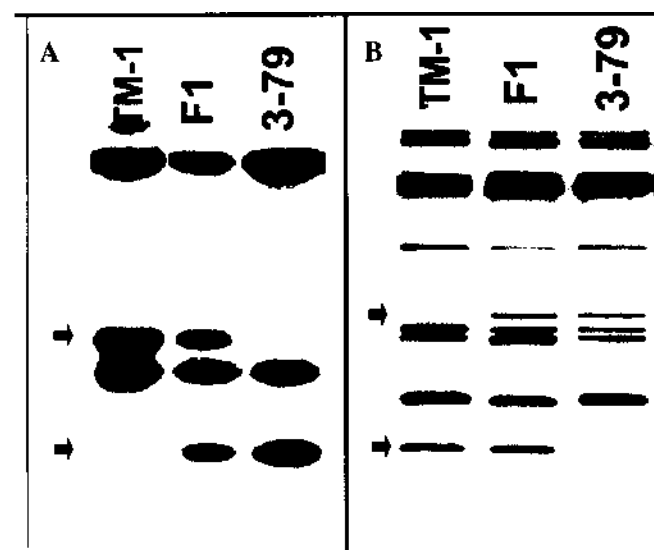


Figure 1A. Arrows indicated the presence of co-dominant RFLP markers in samples of TM-1, 3-79 and a F1. TM1 is an inbred line of *G. hirsutum*, 3-79 is a double haploid line of *G. barbadense* and F1 is a hybrid between TM-1 and 3-79.

Figure 1B. Agarose gel picture of RAPD analysis in TM-1, 3-79 and a F1. Note arrows indicated the presence of polymorphic dominant bands.

Simple Sequence Repeats

SSRs are tandemly repeated di-, tri- or tetra nucleotide loci in a genome. These repeats vary in number and occur in many sites within the genome. The analysis of short tandem repeat (SSR) loci by PCR methods has proven to be very useful because of the high degree of repeat number polymorphisms at many loci within the genome. The development of SSR markers is time consuming and requires sequencing of the genome to identify flanking primers for SSR markers. Dr. Ben Burr's lab at Brookhaven National Laboratory, New York, has developed several hundred SSR primer pairs from the cotton genome which are currently available commercially through Research Genetics, Huntsville, Alabama. SSR primers were also developed from a cotton genomic library enriched in (GA)_n repeats (Reddy et al., 1998). The primary limitations in the use of SSR markers are their cost of development and the presence of non-specific stutter or shadow bands due to the slippage of the *Taq* polymerase during PCR.

Agrawal et al. (1999) reported that cross-species SSR primers could be used to detect polymorphism in tetraploid cotton. We observed that SSR markers can be dominant, similar to RAPD (unpublished data) or co-dominant (Figures 2). Cantrell et al. (1998) identified the chromosomal location of over 50 SSR markers in cotton. They also used this method to develop linkage map of QTL and SSR markers in tetraploid cotton.

Amplified Fragment Length Polymorphisms

AFLP is a PCR-based method of DNA fingerprinting. This method is based on the selective amplification of restriction enzyme digested fragments from genomic DNA using different primer combinations. In comparison to other methods, the AFLP method generates virtually unlimited numbers of DNA fragments from nanogram quantities of genomic DNA. AFLP techniques use normally very stringent PCR conditions, which provide a better reproducibility of the results. In this method, genomic DNAs are digested by two restriction enzymes; subsequently the oligonucleotide adapters, specific to the restriction site, are ligated to the digested DNA fragments to generate template DNA and these template DNAs are amplified using primers complementary to the adapter sequence. Our results indicated that AFLP method can be used to develop both dominant or co-dominant markers in cotton depending on the appropriate primer combination (unpublished data). Typically, detection of AFLP requires radioactive-labeling (Feng et al., 1997) or fluorescence labeling of the primers or silver staining of the gel (Feng et al., 1997). However, in silver staining both strands of the PCR products are detected, resulting in the formation of multiple double-band patterns that may complicate the interpretation of AFLP results. Moreover, we observed that low sensitivity of silver staining sometime limits the power of detected AFLP, especially with DNA fragments in the lower molecular weight range.

Khan et al. (1998) used AFLP and RAPD methods to identify DNA markers linked to three morphological traits. They constructed a linkage map comprising 51 linkage groups covering 6663 cM with 332 AFLPs, 91 RAPDs and 3 morphological traits. Feng et al. (1997) identified the chromosomal location of several AFLP markers. Currently, we are using AFLP markers to identify root-knot nematode resistance genes in cotton (unpublished data).

Next Generation of PCR-based DNA Markers Labeled with Fluorophores

Currently we are using a Capillary Electrophoretic system (CE) to separate fluorescently-labeled amplified DNA markers that are visualized as peaks on electropherograms using the automated PE-Applied Biosystems ABI PRISM 310 Genetic Analyzer equipped with Genotyping and GeneScan analysis software (PE Applied Biosystem, Foster City, CA; Figure 2A, 2B and 2C). The multiple fluorescent detection system in CE permitted correction of lane to lane variation by co-electrophoresis of an internal size standard DNA labeled with a dye different from that of the sample. Automatic sample loading, digitized output of peak position, automated data collection and analysis and no need for gel preparation makes CE attractive to process large numbers of samples within a very short time for analysis. This method is sensitive enough to detect one/two base pair DNA fragment size differences between alleles of a DNA marker. This feature makes this method more attractive to distinguish a large number of polymorphic markers that can not be detected in a regular agarose gel.

The SSR markers were received from Research Genetics, Huntsville, AL and the PCR method was used as per manufacturer's protocol in the presence of fluorescently labeled base or primer. The AFLP analysis was done using AFLP Selective Amplification Module for large plant genome from PE Applied Biosystem (Foster City, CA 94404). The overall AFLP method was performed according to the manufacturer's protocol.

It was observed that SSR markers in CE can produce about 3-4 times more DNA markers of cotton in comparison to detection on agarose gel. This non-radioactive method requires very small, nanogram amounts of amplified product, very short time for sample analysis and accordingly very fast and cost-effective. This method also provides the capacity to visualize different amplified products of the same sample in one sample from multiplexing different primers labeled with multicolor fluorescence labeling techniques. DNA marker system in cotton using CE is an effective tool replacing gel electrophoresis. It provides high speed, sensitivity and reproducibility because it requires less human intervention and the automation of the system. The advantage of automated computer data storage and analysis of the sample for DNA marker makes it easy to analyze a large number of samples. Currently we are using CE routinely to screen a large number of germplasms for SSR and AFLP analyses (Figure 2). However, this CE system is only

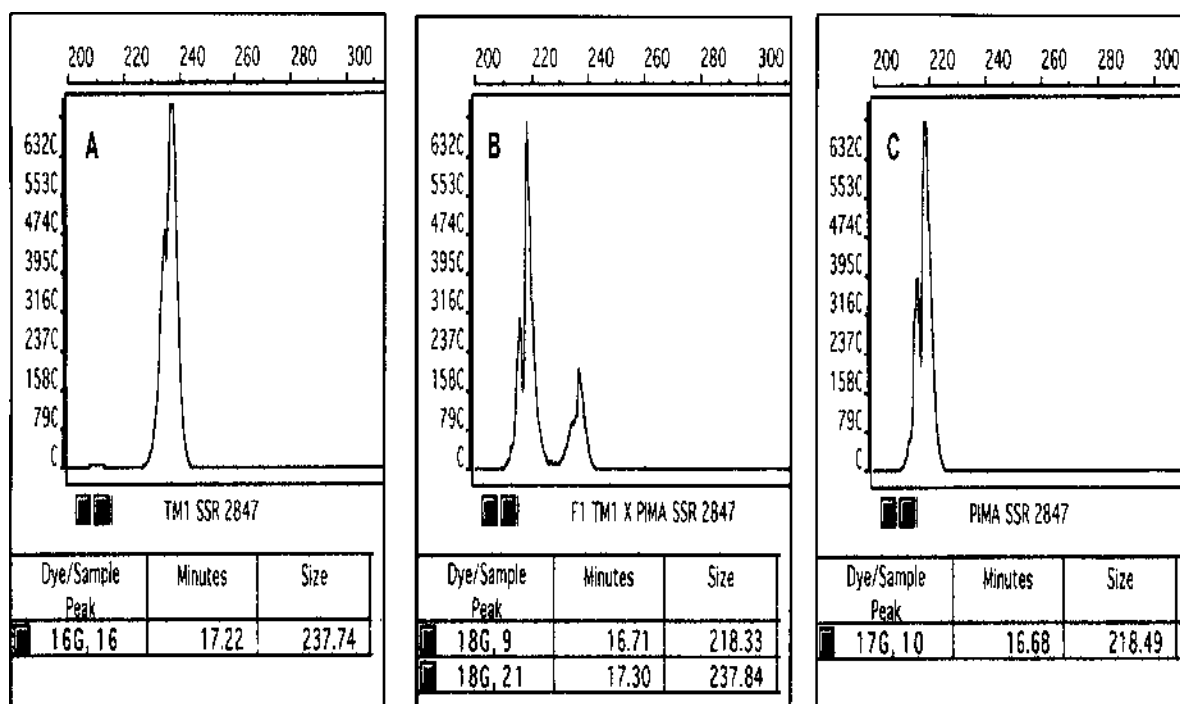


Figure 2. Automated Capillary Electrophoretic System (CE) showing polymorphic fluorescently-labeled amplified SSR markers which are visualized as peaks on electropherograms. X axis represented the size of the DNA fragment and the y-axis showed the amount of the amplified products. Note the presence of co-dominant DNA markers in F1 (B) showing the presence of same size allelic DNA fragments of TM-1 (A) and Pima (C), the two parents of the F1. The automated system provides also the table showing the size of different DNA fragments.

efficient at detecting DNA fragments ranging from 40 to 500 bp in size.

Preliminary results from CE detected about 25 polymorphic markers/AFLP primer combination at the inter-specific level of the tetraploid cotton (Table 1). Seven polymorphic SSR markers at the inter-specific level in comparison to 5 polymorphic SSR markers at the intra-specific level of the tetraploid cotton (Table 1) were also observed.

In conclusion, results demonstrated that AFLP method detected more polymorphic markers in comparison to the SSR technique

(Table 1). Accordingly, it is suggested that the AFLP method can be used as a very useful tool for germplasm screening in cotton. Automated fluorescently-labeled amplified DNA markers using PE Applied Biosystem ABI 310 may be an alternative replacement for gel based DNA marker for screening large number of samples in cotton.

Acknowledgements

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Table 1. Polymorphic DNA Markers in Cotton

Marker type	Number of primer combinations		Number of DNA markers		Number of polymorphic DNA markers		Number of DNA markers /primer combination		Polymorphic DNA markers /primer combination		% Polymorphism	
	inter-specific	intra-specific	inter-specific	intra-specific	inter-specific	intra-specific	inter-specific	intra-specific	inter-specific	intra-specific	inter-specific	intra-specific
AFLP	10	10	607	631	256	166	60.7	63.1	25.6	16.6	42.17	26.30
SSR	10	10	150	130	79	53	15	13	7.9	5.3	52.66	40.77

This preliminary survey was conducted with the lines of TM-1, HS-46, and MARCABUCAG8US-1-88 (*G. hirsutum*) and 3-79 (*G. barbadense*).

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Technology Protection System

Commercial production of Bt and herbicide tolerant varieties has been conditional on a fee to be paid to the owner of the genes. In the case of transgenic varieties resistant to lepidopteran insects, U.S. farmers have to pay a technology fee of US\$80/ha. It was assumed that the Bt gene would save more than US\$80/ha in insecticide costs. The ability of the cotton plant to tolerate herbicide applications over the top of the plant and the ability to produce a specific toxin injurious to a variety of bollworms are heritable characteristics. Once these resistant genes are inducted into the cotton plant, they are automatically transmitted to the next generation, and farmers can use the transgenic seeds year after year. However, farmers sign agreements with biotech companies to prevent them from storing and planting the same transgenic seed the next year. Also, farmers are not allowed to transfer seed to other growers.

Biotech companies maintain a list of their transgenic cotton growers. Although the technology fee varies between Australia and the USA, similar practices are followed in both countries. This is also true in Argentina, Mexico and South Africa, which have already gone into commercial production of Bt cotton. China (Mainland) also planted about 52,000 hectares of Bt cotton in 1998/99, but the arrangements were slightly different from other countries. Agreements with biotech companies may or may not have been abused, and the illegal transfer of seed may or may not have taken place. Nevertheless, theft is a potential threat that could affect the recuperation of research and development costs by biotech companies. Chances are that the technology could be leaked free to other farmers and countries by growers receiving the advantages of Bt traits.

In 1993, research was started in the USA on a system to limit the use of planting seed to only one year. After almost five years of collaborative research, the USDA and Delta and Pine Land Company were awarded a patent in 1998 on technology called Technology Protection System (TPS). TPS is a clever three-gene system that forces plants to produce a toxin that is fatal to their own seeds, compelling farmers to buy new seeds every year. TPS is a transgenic system comprised of a complex array of gene promoters which, in a normal state, are inactive. This means that a transgenic variety with TPS will produce viable seed like a normal variety. But, if the same seed carrying TPS is given a treatment prior to sale, the treated seed will germinate as normal, but will not produce a viable seed. The treatment will trigger an irreversible series of events rendering the seed non-viable for replanting. The toxin is harmless to people and is produced late enough in the season so that the commercial value of the seed is not affected. The seed matures like a normal seed and is perfectly fit for feeding or oil extraction.

TPS varieties are not yet available, but farmers will soon have a choice of TPS or non-TPS varieties. However, there may be

an additional fee for TPS. Economically speaking, the technology should have a discount to encourage farmers to grow TPS varieties, which would allow biotech companies to reap the benefits of the technology fee for a longer period of time. According to TPS-technology owners, the long-term benefits of specific technologies will bring more money to research and ultimately benefit cotton growers.

One of the additional advantages of TPS is biosafety of the transgenic species. The currently available non-cotton genes, transferred to the cotton genome, have been tested for many years and do not have any deleterious effects. But, as more and more genes are being explored, there are chances that transgenic genotypes could spoil the germplasm particularly in areas having high out-crossing. TPS can help to preserve wild species and other varieties, as the cross-pollinated seed will not germinate.

Commercial production is the second stage. First, seed companies need a viable seed for multiplication for at least three generations: nucleus, basic and certified. The stages of multiplication may vary in different countries but normally seed production involves at least three stages before the seed reaches farmers for commercial production. Thus, multiple seed generations should be able to produce mature fertile seed to sell to growers. To do so, researchers have manipulated the plant's DNA to control yet another genetic mechanism, which suppresses the effect of the suicide genes indefinitely. In the suppressed stage, transgenic plants having TPS always produce fertile seeds.

The TPS system can be activated according to the desire of the companies to produce infertile seeds at any stage. The fertile seeds will be sprayed with a chemical (in one version, it is said to be tetracycline antibiotic in nature) called "inducer," which awakens the dormant self-infertile genes to overcome the suppression effect. An inducer application revives the ability of the plant to produce seed toxins such as Ribosomal Inhibitory Protein that induces seed infertility. Such a seed, if planted, gives normal germination and produces a normal crop but lacks the ability to form mature seeds.

The commercial availability of the TPS system is still some years away. The technology's simplicity and the companies' confidence in its performance on cotton implies that the technology will be available for commercial use in less than five years, or even two to three years.

The currently available Bt genes or herbicide tolerant varieties have limited applicability because of the farming systems used in many countries. There are only two ways by which countries can get the transgenic technology:

1. To develop their own transgenic genotype systems. Some statistics show that on average over US\$200 million are spent to identify, induct and commercialize a single gene. Some

countries do not have the resources for expensive research facilities and for making heavy investments in fundamental research.

2. To buy the technology through joint ventures with multinationals. This option is easy, but has a cost, and multinationals have limitations to enter into such agreements. Some of these limitations could be that the seed production systems are not well developed yet or fear that farmers could keep seeds for the next season. Smallholdings present another hurdle as agreements would have to be made with millions of growers in one country.

Farmers' associations or governments in some countries using the second option have a choice to buy the technology by paying a one-time fee and give it free to farmers or devise a system to collect the fee at the gin level. But, such a procedure would require a sound seed production system and also expertise for monitoring and management of problems like resistance in Bt cotton, quantity of toxin produced, and effectiveness of the toxin.

Commercial utilization of the TPS technology will encourage the expansion of transgenic technology to many more countries. Farmers will have to come back to seed companies every year, and seed companies will be guaranteed to recoup their

costs for as many years as they want. Owners of transgenic technologies could go into agreements with governments to use TPS for a limited number of years and then have the seed available free.

Technically, cotton is a cross-pollinated crop, but in most countries it behaves like a self-pollinated crop. It is safe to use TPS technology under self-pollinated conditions, but, if there are conditions where natural cross-pollination in cotton is enough, TPS technology could create a disaster in the germplasm through cross-pollination. Pollen from terminator plants grown on one farm could fertilize nearby native or commercial crops and make them sterile. Such an outcrossing could trigger an epidemic of crop sterility.

While TPS technology could be a solution to expand biotechnological developments to developing countries more safely and quickly, TPS could be considered an additional financial burden on billions of poor farmers around the world. Subsistence farmers in many countries are in the habit of keeping seed for next year. Not only will they be forced to buy seed every year, but they will have to pay for the TPS technology, in addition to the technology fee for the novel gene.
