

INTERNATIONAL COTTON RESEARCHERS ASSOCIATION

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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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Honorable ICRA Members!

As you know "Cotton Innovations" newsletter is published by ICRA on monthly basis and distributed to the registered members of ICRA. The newsletter covers all aspects related to cotton research starting from seed to lint fiber and beyond. The newsletter under the title of "Cotton Innovations" was started in March 2021 and since then nine issues have been published regularly. ICRA is thankful to the managing editors who took responsibility for compiling one or more issues to get these published in time.

To maintain continuity in 2022, ICRA is seeking your cooperation to act as "Managing Editor" of at least one issue of "Cotton Innovations". The main role of the managing editor is to collect the articles from the contributors, select the appropriate ones, do the editing and submit them to Dr. Negm (ICRA Chair) for its publication. You may select one slot for any month from February to December 2022.

Kindly reply to our mails if you are willing to extend your services for an issue of the ICRA newsletter.

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ALTERNATE INTERCROPPING OF COTTON AND PEANUT

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Since appropriate intercropping can make full use of agricultural resources such as sunlight, nutrients, water and land resources, it has been widely adopted as a traditional agronomic measure to improve crop yield and income all over the world. Intercropping is also considered an effective way to improve biodiversity, increase the populations of natural enemies and control pests and diseases. Legume/cereal, cotton/cereal. and legume/cotton intercropping are the most popular combinations, which not only allow crops utilize natural resources such as light, heat, fertilizer and water, but also reduce disease incidence (Chi et al., 2021).

China is one of the largest producers of peanut and cotton in the world. Since most major cotton growing areas are also the dominant production bases for peanut in the country, cotton/peanut intercropping has been increasingly adopted in recent years to achieve a simultaneous harvest of cotton and Although peanut peanut. cotton and intercropping can meet farmers' requirement of harvesting two crops in one year, there still exist continuous cropping constraints that yields decline during crop continuous planting under the traditional cotton/peanut intercropping (Ci et al., 2017). Crop rotation refers to growing a different crop on a given land area every growing/planting cycle and season. Rotation of cotton with legume crops usually increases yield not only due to the nitrogen fixation effect of legume crops but also to the reduction of disease incidence (Johnson et al., 2000). However, the traditional rotation of peanut with cotton is not attractive because most small-holder farmers

in the Yellow River valley of China prefer to harvest the two cash crops in the same year due to economic consideration. To address this concern, a strip intercropping system with 4 rows of cotton and 6 rows of peanuts has been proposed for mechanized field management and harvesting of the two crops (Meng et al., 2017). Intercropping can satisfy the need to harvest two crops a year, and rotation can reduce continuous cropping constraints. Therefore, a new intercropping system, alternate intercropping system, was established with a wide-strip intercropping combined with strip rotation (Chi et al., 2019). Alternate intercropping is a new cropping system which cotton and peanut are not only intercropped in a wide strip but also rotated inter-annually. while in the traditional intercropping system the two crops were not rotated (Fig. 1). In recent years, there have been studies on the advantages of alternate intercropping system of cotton in China, cotton-peanut including alternate intercropping (Chi et al., 2019), cotton-hot pepper alternate intercropping (Zhang, 2019), cotton-maize alternate intercropping (Meng et al., 2017). The net return from these alternate intercropping systems were much higher than that from traditional intercropping svstems because of the considerable increase in crop productivity without additional input.

In a cotton-peanut alternate intercropping study, the traditional intercropping of cotton/peanut (TIC) increased seed cotton yield by 16.9% and decreased peanut yield by 5.6%, while the cotton-peanut alternate intercropping (AIC) increased cotton yield by 21% without sacrificing peanut yield (Chi et al., 2019). Crop output value under AIC was 4.5% higher than that under TIC but the input value was the same; thus the net return under AIC exceeded that under TIC by 10%. The AIC increased the biological yield of peanut by 3.8% and harvest index by 2.4%; it increased the biological yield of cotton by 3.8% compared to TIC. AIC also increased uptake of N, P and K in peanut by 6.3, 11.5 and 7.3%, as well as net photosynthetic rate, chlorophyll content and maximum leaf area index of peanut by 7.2, 8.9 and 4.4%, respectively, relative to TIC (Chi et al., 2019). The soil fertility and microbial community structure in the rhizosphere of cotton and peanut under AIC were greatly improved compared with those under TIC. At the boll opening stage of cotton, the total amount of pathogens in cotton rhizosphere under AIC was significantly lower than that under TIC and monocropping. The total abundance of plant growth promoting rhizobacteria (PGPR) of cotton and peanut rhizosphere under AIC was also higher than those under other systems. Therefore, the yield advantage of cotton under AIC over monoculture cotton was mainly attributed to greater partitioning of assimilates to reproductive organs as indicated by increased harvest index, while the yield advantage of peanut under AIC relative to TIC was mainly due to improved nutrient uptake. The improved soil fertility and community structure microbial the in rhizosphere of cotton and peanut under AIC greatly contributed to the increased harvest index of cotton and improved nutrient uptake of peanut. The alternate intercropping of cotton can be a promising alternative cropping system in the areas with suitable ecology.

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Fig. 1 Model illustrations of alternate intercropping of cotton-peanut



Cotton is a perennial woody plant originated in the tropics. It is an important economic crop and the main raw material of the textile industry, which occupies an important position in the national economy. The world's cotton production regions are distributed between 38°-46° N and 35° S. The main cotton countries are China, India, America, Brazil, Pakistan, Uzbekistan and Turkey. These seven major cotton-producing countries accounts for about 84 % of the world's total cotton production in output. China and India both produced 6.314 10⁴t of cotton in 2020, ranking first in the world (Figure 1) (USDA, 2021).



Figure 1. Distribution of global cotton production in 2020

Cotton production was highly dependent on natural conditions, especially climatic conditions. Climate change will change the ecosystem elements such as light, temperature, water, soil and gas through the effect temperature combined of and precipitation changes, and then affecting crop planting system, control, pest

will lead to the increase of accumulated temperature during crop growth period, the extension of crop growth period, and great changes in agricultural production layout, planting system and crop varieties (Li et al., 2021).

Cotton is a C3 crop, which likes warm and light. It needs sufficient light, heat, and suitable humidity conditions during the growth period, and was sensitive to light and temperature factors. Temperature change plays an important role in cotton phenology, yield and fiber quality (Luo et al., 2014). Low temperature will lead to slow emergence in the early stage of cotton growth, susceptible to diseases, and even cause seed rot. The temperature was lower than 15 °C, and there was more rain, which was adverse to seedling growth. Some leaves suffers from freezing damage when the ground temperature drops to 3-6 °C, when it drops to 1-2°C, the plant partly or completely freezes to death, so, cotton must pay close attention to the harm of low temperature frost during seedling growth. The optimum temperature for cotton flowering and boll development was 25-30°C, too low and too high temperature during flowering and boll stage are not conducive to boll formation and boll development (Gao, 1986). The temperature was lower than 20 °C, pollen viability decreases or even loses. When the temperature was above 32°C, for every 1°C increase, the shedding rate of buds and bolls increases 0.66%-2.2% (Gao, 1986; Zafar and Mehmood et al., 2018). High temperature, especially above 35°C, not only reduces the viability of pollen, affects fertilization into bolls, increases abscission, but also affects

the developing young bolls, resulting in reduced boll weight, increased shriveled grains, and decreased fiber weight in single bolls (Figure 2) (Ton, 2011).

Water is an indispensable raw material for photosynthesis and plays an important role in the physiological and ecological processes of crop production.



Figure 2. Effect of higher temperature on agronomic besides physiological attributes of cotton at various developmental stages (Zafar et al., 2018).

Light is the only energy source for photosynthesis of plants. Sufficient sunshine was conducive to cotton flowering and boll setting; insufficient light increased the rate of shedding of young bolls. In the later stage of cotton growth, sufficient light was beneficial to photosynthesis, which could increase boll weight and improve cotton boll quality. It is the most afraid of continuous autumn rain and insufficient light, resulting in crazy growth of stems and leaves, bolls were susceptible to pests and diseases (Pettigrew, 2008).

The shortage of water resources restricts the sustainable development of cotton industry. Climate change will lead to changes in the temporal and spatial distribution of water resources, and water stress caused by unbalanced temporal and spatial distribution of precipitation will adversely affect cotton yield and quality (McMichael and Hesketh, 1982). Drought affected seedling emergence, while heavy rain made cotton field hardening, hypoxia, poor respiration, humid and obstructed seedling emergence; continuous rain could lead to poor root development of cotton and susceptible to seedling diseases such as anthrax and wilt. Excessive rainfall affects cotton yield by promoting vegetative growth and biomass accumulation, which ultimately leads to yield decline (Iqbal, 2011).

In addition, affected by extreme weather such as spring cold, hail, strong wind, continuous high temperature and rainfall, pests and diseases in cotton fields are becoming more and more serious, which has a great impact on cotton yield and quality. Thus, the changes of light, heat and water resources in the process of climate change have an important impact on cotton growth and yield formation. Rational utilization of climate resources plays a vital role in the development of cotton industry.

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PLANT DENSITY MEDIATES CHEMICAL TOPPING EFFECTS ON COTTON YIELD

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Cotton (Gossypium hirsutum L.) has indeterminate growth with strong apical dominance. Manual removal of the mainstem growth tip increases the partitioning of assimilates to reproductive organs and ultimately harvestable bolls and lint yield (Li et al., 2006), and thus has been widely used in China and other cotton-growing countries with abundant agricultural labor (Dai and Dong, 2014). However, manual topping is labor intensive and is becoming a great challenge as the rapid urbanization in China (Dai et al., 2017). Chemical topping that uses plant growth regulators to inhibit apical dominance may be a promising alternative in cotton, but the efficacy of chemical topping may be mediated by planting density or ecological condition.

In order to determine whether plant density or ecological condition mediated the efficacy of plant topping, a three-year field experiment with a split-plot design with plant density as the main plot treatment and planttopping mode as the subplot treatment was conducted in different ecological regions of China. At three sites including Hutubi 87°12'E), (36°61′N, (44°68'N, Linging 115°42'E) and Jinxiang (34°52'N, 116°7'E), the main plots were assigned low, moderate, or high plant density, and the subplots were assigned no topping, manual topping, or chemical topping. Chemical topping was conducted by foliar spray of a mixed liquor with 45 mL/ha mepiquat chloride and 75 flumetralin (Patent mL/ha No. CN105613008B) at peak flowering (Dai et al., 2019) when the number of fruiting branches reached 8 to 10 per plant in Hutubi site, 10 to 12 in Linqing site, and 9 to 11 in Jinxiang site, respectively.

Manual topping increased seed cotton yield regardless of plant density, but plant density significantly influenced the effect of chemical topping on seed cotton yield (Table 1). At low plant density, chemical topping reduced seed cotton yield by 4.0% to 6.0% at the three sites compared with the yield under no topping and by 5.5% to 10.8% compared with that under manual topping. However, at moderate and high plant densities, seed cotton yield with chemical topping was not different from that with manual topping, and compared with no topping, increased by 12.8% to 16.4% at Hutubi site, 8.6% to 16.1% at Linging site, and 11.9% to 13.8% at Jinxiang site. Thus, we concluded that chemical topping produced higher seed cotton yield than no topping only at moderate or high plant density.

Further analysis showed that plant topping, plant density, or their interaction significantly affected biological yield and harvest index (Table 1). Averaged across the three sites. chemical topping reduced biological yield by 12.7% at low plant density, but the biological yield was adequate at moderate and high densities. Moreover, similar to manual topping, chemical topping also promoted the allocation of total biomass to reproductive organs at each site. Although chemical topping increased the harvest index, it could not recover the loss in biological yield at low density, resulting in a significant decline in seed cotton yield. At moderate and high plant densities, greater

partitioning of assimilates to reproductive tissues and adequate accumulation of biological yield increased seed cotton yield. The differences in ecological sites did not affect the efficacy of chemical topping. In addition, because of comparable seed cotton yield and 22.6% to 24.2% lower labor input, the economic benefits of chemical topping under moderate and high plant density were 8.1% and 20.9% greater, respectively, than those of manual topping (Table 2).

Considering that chemical topping is less labor-intensive, time-consuming, and wideadaptability, chemical topping is a promising alternative to manual topping in cotton production at moderate and high plant densities. However, the formation of redundant buds and much more immature fruits should be noted, especially in rainy years. Thus, future studies to improve chemical topping should focus on developing highly efficient agents and matched spraying machinery.

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Table 1 Effects of plant density and topping mode on biological yield, seed cotton yield, and harvest index of field-grown cotton at Hutubi, Linging, and Jinxiang averaged across three years (2016 to 2018)

		Hutubi site			Linqing site			Jinxiang site		
Plant density	Topping mode*	Biological yield (kg/ha)	Seed cotton yield (kg/ha)	Harvest index	Biological yield (kg/ha)	Seed cotton yield (kg/ha)	Harvest index	Biological yield (kg/ha)	Seed cotton yield (kg/ha)	Harvest index
Low	NT	13925c**	4625e	0.332c	9452c	3573cd	0.378bc	6229c	2473f	0.397c
	MT	12924e	4980c	0.385ab	9221c	3707c	0.402a	6045c	2545f	0.421a
	СТ	11777f	4440f	0.377b	8613d	3404de	0.395a	5470d	2325g	0.425a
Moderate	NT	14511b	4580ef	0.316d	9986b	3515d	0.352d	8877b	3329d	0.375f
	MT	13485d	5250a	0.389a	9969b	3898b	0.391ab	9445a	3797a	0.402b
	СТ	13164d	5165ab	0.392a	9995b	3818b	0.382b	9359a	3725ab	0.398c
High	NT	14908a	4485f	0.301e	10486a	3471d	0.331e	8961b	3199e	0.357g
	MT	13521d	5200ab	0.384ab	10564a	4099a	0.388b	9460a	3680bc	0.389bc
	СТ	13541d	5220b	0.385ab	10630a	4029a	0.376bc	9531a	3641c	0.382d
Source of var	iance (p)									
Year	(Y)	0.0000	0.00245	0.0000	0.035	ns	0.0120	0.0021	0.000	0.0236
Densi	ty (D)	0.0033	ns	ns	0.0002	0.2591	ns	0.0001	0.0009	0.0001
Toppir	ng (T)	0.0041	0.0181	0.0018	0.0008	ns	0.0217	ns	ns	0.0002
Y×	D	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y×	т	ns	ns	ns	ns	ns	ns	ns	ns	ns
D>	кт	0.0001	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000	0.0000	0.0009
Y×E)×T	ns	ns	ns	ns	ns	ns	ns	ns	ns

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* Topping mode includes no topping (NT), manual topping (MT), and chemical topping (CT). The low, moderate and high plant

density is 9, 18 and 27 plants/m² in Hutubi site, 3, 6 and 9 plants/m² in Linging site, and 4.5, 9 and 13.5 plants/m² in Linging site, respectively.

** Means within a column followed by different letters are significantly different at p < 0.05.



FINE ROOT PHENOTYPES AND LIFESPANS OF COTTON UNDER DROUGHT STRESS

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Drought is one of the most important environmental factors affecting cotton growth and yield. Root systems, which play vital roles in both anchoring plants and water absorption, connect the above-ground shoots of plants with the soil environment and directly affect the morphogenesis of shoots. The early establishment of cotton roots is extremely important for the effective utilization of soil water, especially in areas with limited water.

Fine roots (less than 2 mm in diameter), also known as functional roots, are involved in water transport and account for most of the total length and total surface area of the root system (Lima et al., 2010). Fine roots are the first roots to detect the onset of drought, at which point they rapidly generate chemical signals, causing the pores to close to reduce water loss. Soil moisture has a strong influence on the distribution of fine roots with highly plastic phenotypes, which significantly affects the dynamics and lifespan of fine roots (Zhou and Shangguan, 2007). However, whether these factors are related to root water detection and adaptation to soil water stress remains unclear.

Root research methods and equipment are essential for assaying the phenotypes and lifespan of cotton fine roots.

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Hebei Agricultural University/State Key Laboratory of North China Crop Improvement and Regulation/Key Laboratory of Crop Growth Regulation of Hebei Province, Baoding 071000, China. A custom-made *in-situ* root observation device, RhizoPot, was employed in this study to monitor the dynamics of root phenotypes of cotton seedlings under drought stress (Fig. 1) (Zhou et al., 2021). The device enabled *insitu*, accurate, non-destructive, and continuous observation of the root system.



Fig. 1 Schematic representation of the integrated growth-imaging device (left) and an image obtained by the scanner (right).

'Guoxin No.9,' a local commercial cotton (*Gossypium hirsutum* L.) cultivar, was used for this study. Experiments were conducted with nine replicates and two levels of soil relative water content (SRWC) as follows: CK, well-watered (set as the control group), $75 \pm 5\%$ SRWC; DS, drought stress, $45 \pm 5\%$ SRWC. Drought treatment was initiated at the four-leaf stage of cotton seedlings. Results showed that drought stress inhibited the morphological development of cotton shoots and affected photosynthetic indexes while improving water use efficiency (WUE).

Drought stress promotes fine root elongation and thinning

Drought stress increased the number of fine roots and increased the root length

density (RLD) (Figure 2a). An analysis of the average root diameter explained these phenomena to some extent (Figure 2b). Drought stress was able to promote root elongation, but the diameter of these new fine roots was generally small. under normal irrigation, while drought increased and reduced their median lifespans to 33 and 36 d, respectively (Figure 3). Correlation analysis indicated that fine root lifespan and diameter were significantly positively correlated under CK and DS conditions. The lifespan of different diameter classes under DS conditions was generally



Figure 2 Trends in root length density (RLD) (a) and average diameter (b).

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When we counted the proportion of fine roots in terms of total root length, the fine roots tended to be thicker under normal watering conditions, while the proportion of very fine roots under drought conditions was very high. This suggests that very fine roots account for a significant proportion of the fine roots induced by soil drought stress.

Drought stress shortened the fine root lifespan. In this study, survival analysis was performed on cotton roots with a diameter between $300-700 \mu m$, revealing that the median lifespan increased continuously with the increase in root diameter.

The median lifespan of fine roots in the two root diameter ranges of 400–500 μ m and 600–700 μ m were 28 and 43 d, respectively,



Figure 3 Survival curves of the fine roots of two different average diameter (AD) classes, 400µm≤AD≤500µm (a) and 600µm≤AD≤700µm (b)

Our research has demonstrated the root length density of cotton increased significantly under drought stress and that the average fine root diameter showed the opposite trend; drought also shortened the lifespan of fine roots. Cotton developed more slender fine roots and longer root hairs under drought stress. thus promoting the development of new fine roots. Changes in the phenotype and longevity of fine roots enable cotton plants to absorb as much water as possible under drought stress. These results provide solid reference for the development cotton varieties of and cultivation measures against drought stress.

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APPLICATION OF CRISPR SYSTEMS IN PRECISE CREATION OF COTTON MUTANTS Lin Sun

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Cotton is an important cash crop and one of the four major genetically engineered crops worldwide. Breeding and Functional genomics research rely on a large number of mutants, however the low natural mutation rate can't meet the research needs. Generating plant mutants using traditional methods are mainly depends on physical and mutagenesis genetic chemical or engineering. Agrobacterium-mediated Т-DNA insertion is an important strategy to study plant functional genomics study. However, T-DNA insertion is randomly inserted in the genome and often in intergenic or non-coding regions of genome, resulting in a low rate of available mutant phenotypes. In addition, cotton is a polyploid plant. Due to the complexity of cotton genome, most genes have several homologous copies at different chromosome loci, therefore, one member of gene family mutated by T-DNA will not result in apparent phenotype. These limitations of traditional methods are very detrimental to cotton with complex genomes. Therefore, new strategies are needed to create a large mutant resource.

In recent years, a genome editing tool termed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system originally found in the adaptive immune system of bacteria and has become a powerful and universal tool for gene editing. CRISPR-Cas9 is more user-friendly tool consisting of two components, a Cas9 endonuclease and a guide RNA (gRNA) can target the DNA sequence of 5'-N20-NGG-3' (N indicates any base). Cas9 identifies the target sequence by base pairing between the target and 20-nt variable region at the 5' end of the gRNAs and NGG is the protospaceradjacent motif (PAM). Cas9 endonuclease can cause DNA double-stranded breaks (DSBs) in the target region and the DSBs sites can be repaired by error-prone nonhomologous end joining (NHEJ) pathway that leads to gene knockout. PAM appears frequently in the genome, making CRISPR-Cas9 capable to target almost all genetic elements.

CRISPR-Cas9 svstem has been applied successfully in cotton and demonstrated strong adaptability. The editing efficiency of PRGEB32-GHU6.7 vector system in cotton was up to 87%, and the offtarget effect was low(Wang et al.2018; Li et al.2019). So far, several research departments have used CRISPR-Cas9 genome editing system to create cotton mutants for molecular mechanism studies.

Even though, the CRISPR-Cas9 system works well in cotton genome, it still needs further optimization. For example, the selection of CRISPR-Cas9 target sites NGG PAM, which makes it requires impossible to screen suitable sgRNA under certain genome regions. The CRISPR-LbCpf1 system which can recognize the rich TT regions of cotton genome , which expands the scope of genomic editing in cotton and makes up for some deficiencies of CRISPR-Cas9 system(Li et al.2019). The researchers also found that the CRISPR/Cas12b system has potentially valuable for editing the

genomes of plant species, such as cotton, that are resistant to high temperatures(Wang et al.2020). In addition, genome editing efficiency is also an event that needs continuous attention in cotton research. Temperature can affect the efficiency of the genome editing system, and Cas9 and Cpf1 genome editing system showed temperaturesensitivity in plants(Li et al.2021). Since cotton is a thermophilic crop, researchers can increase the temperature in the incubation room for cotton tissue culture and plant regeneration, which will greatly improve the efficiency of genome editing.

Mutagenesis plays an important role in functional genomics and breeding. CRISPR-Cas9 system can rapidly and efficiently obtain gene knockout mutants in plants especially in ployploid species. Several Cas9 variants can also be further developed and utilized in cotton, such as xCas9 and Cas9 NG.

There is no exogenous insertion in the genome of mutant material obtained by using the genome editing system, so the CRISPR system is more secure. Moreover, genome editing technology can simultaneously improve multiple agronomic traits of crops, thus accelerating genetic improvement of crops. Genome editing technology shows great prospects in cotton breeding and functional genomics in the future.

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A NOVEL COTTON CULTURE TECHNIQUE: DRIP IRRIGATION UNDER PLASTIC MULCHING

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The northwest inland region, dominated by Xinjiang, has currently become the largest cotton-growing region in China in terms of yield, total production and planting acreage. Xinjiang has little rainfall but a large amount of evaporation, and the crop production is completely dependent on irrigation. Agricultural water consumption in Xinjiang accounts for about 97% of the total water consumption, of which 42.1% is used for cotton planting (Zhang, 2000). The shortage of water resources has become the bottleneck and obstacle restricting the economic and social development of this region.

Drip irrigation is one of the most advanced water-saving irrigation techniques in the world, while plastic mulching is a widely used cotton cultivation technique in China. In the 1990s, drip irrigation was introduced into Xinjiang and integrated with plastic mulching technology to form a new technique drip irrigation under plastic mulching (Dai et al., 2014). This technique has not only become a popular water-saving practice, but also an important practice to increase cotton yield in the region via further integration with high plant density, smaller singly plant size, and fertigation. In 2020, the yield per unit area was 1900 kg hm⁻², which is 21% higher than the national average in the same year, being largely attributed to the adoption of this technique.

1.Principles of drip irrigation under plastic mulching

This new technique is a combination of drip irrigation and plastic mulching. To realize this, a specific seeder was developed to achieve drip irrigation pipe laying, seeding and plastic mulching simultaneously during seeding. It not only increases temperature, preserve moisture and reduce surface evaporation due to the effects of film mulching, but also achieves a uniform, regular and quantitative water application to the root zone of cotton through the pipeline and emitter with a controlled pressure piping system (Fig.1). As a result, it significantly improves the water use efficiency and cotton yield. The principle of under-mulching drip irrigation is as follows:

a. Increasing ground temperature. Due to a large temperature difference between day and night in Xinjiang, it is hard to maintain the required temperature for cotton growth at sowing, and therefore causes a poor emergence. The plastic film mulching creates a more favorable environment for cotton growth by significantly increasing the ground temperature by 1°C-5°C daily, which not only promotes early sowing and emergence, but also seedling growth and development (Feng et al., 2020).

b. Reducing water consumption. Compared to conventional irrigation, under-

mulching drip irrigation not only improves ground temperature and reduces evaporation between plants, but also reduces deep leakages and balances water application, which promotes the emergence and growth of cotton seedlings and finally increases cotton yields.

c. Loosening the soil. Under-mulching drip irrigation directly applies water to the root zone, which not only ensures the water supply to the cotton plants, bus also helps loosen the soil to increase air permeability.

d. Increasing the use efficiency of water and fertilizer. With the technique of undermulching drip irrigation, soluble solid or liquid fertilizer can be dissolved in the water tank and applied to the cotton root zone with irrigation to maintain the appropriate moisture and soil fertility in the root zone, which is easy for cotton crops to absorb. Water consumption was only approximately 12% of that in traditional irrigation methods and 50% of that in sprinkler irrigation because this method reduces evaporation between plants and deep leakage (Mao et al., 2019). Additionally, fertilizer was reduced by 15%-20% by reducing fertilizer volatilization and loss and, in particular, by effectively avoiding the volatilization and loss of ammonium and urea nitrogen fertilizers (Mao et al., 2019).

e. Preventing diseases and pests. By directly applying water and fertilizer to the root zone of cotton, under-mulching drip irrigation effectively avoids the spread of diseases and pests caused by the flow of water. As a result, the costs of disease and pest control is greatly reduced.

2. The development of under-mulching drip irrigation

a. Under-mulching drip irrigation with winter and spring irrigation

The potential evapotranspiration is far greater than moisture input in Xinjiang and consequently salt gathers in surface soil, causing common secondary salinization. Presently, about 32% of cultivated land is saliferous in Xinjiang (Mao et al., 2019). To ensure good soil moisture for seedling stand emergence and establishment. irrigation before planting was recommended, which mainly included winter irrigation performed after cotton harvest and spring irrigation performed in the coming spring. The average irrigation guota is 3000 m³/hm² for winter and spring irrigation, accounting for 30-50% of annual irrigation quota. Currently, the drip irrigation under plastic mulching system is laid during seeding in the cotton field by a specific seeder in which drip irrigation pipe laying, seeding and plastic mulching can be completed simultaneously. Under-mulching drip irrigation bands are usually laid according to the standards of mechanical cotton harvest, that is, two or three drip bands are laid under each piece of plastic film mulch with a width of 200cm, which covers 6 rows of cotton plants, with the row spacing of (10+ 66+10+66+10) cm (Fig.1). The irrigation times for three or two drip bands are the same, but the irrigation rate of two drip bands is 70-80% that of the three drip bands. Before irrigating for the first time, the branch and auxiliary pipes are laid by hand on the surface and connected to the main pipe, and the capillary inlet is connected with the corresponding branch pipe and auxiliary equipment. Cotton is drip-irrigated through the system 8-12 times throughout the entire growth and development period,

and each irrigation application consumes $225-300 \text{ m}^3 \text{ hm}^{-2}$ water.

b. Dry-sowing and wet-emergence technique

Water consumption in Xinjiang has been increasing as a result of continued cotton acreage increases in the region. In recent years, winter and spring irrigation water is increasingly scarce. In response to this, an improved under-mulch drip irrigation technique of dry sowing and wet emergence has been widely implemented in the region. That is, neither winter irrigation nor spring irrigation is applied prior to sowing, but following soil preparation, plastic film and drip irrigation bands are directly laid and seeds are sowed. Once the optimal emergence temperature has been reached, a small amount of water is applied with drip irrigation so that the moisture content of soil under the mulch meets the requirements for emergence of seedlings (Feng et al., 2017). This technology requires a high standard of field preparation and timely sowing. This an obvious effect technique has on preventing wind damage and sand damage in spring, and can save water by 2550 m³/hm² which significantly alleviates the stress from water shortages in the region. For dry-sowing and wet-emergence drip irrigation technique, the irrigation regime during the growth stages of cotton is different from that under normal winter and spring irrigation conditions.

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Fig.1 The technique of drip irrigation under plastic mulching.



VARIATION OF INSECTICIDAL PROTEIN CONCENTRATION IN BT COTTON AND ITS AGRONOMIC REGULATION

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Bt transgenic cotton (Bt cotton) has been planted widely in the world, but its insect resistance is unstable. Reduced insecticidal ability was observed at flowering and bollsetting, the most important period for both vield formation and bollworm control. The reduction of insect resistance was associated with decreased Bt toxin content. Therefore, it is important to decipher the underlying mechanism and to find solutions to increase Bt protein concentration in cotton. In recent years, studies led by Yangzhou University in China and supported by NSFC of China, have been undertaken to better understand the effect of adverse environment and development process on Bt protein concentration in Bt transgenic cotton. Our research found that extreme temperature, drought, flooding, and growth stage was closely related to Bt protein concentration in both vegetative and reproductive organs of the cotton plants. The reduction of Bt toxin concentration was attributed to reduced protein synthesis and enhanced protein degradation, which guided us to find some agronomic practices to enhance Bt protein concentration.

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1. The change of Bt protein concentration in cotton under adverse environment and during development process.

The insecticidal efficacy reduced under the stressed high temperature, low temperature, drought, waterlogging, and soil salinity in leaves and reproductive organs of Bt transgenic cotton (Table1). The greatest

reduction of the Bt protein content was observed in bolls, followed by squares and flowers, and the minimum decrease was recorded in leaves. Low temperature seemed to have greater impact on the reduction of Bt protein content. The effect of drought was greater than that of waterlogging. Soil salinity exhibited less impact on Bt protein concentration of cotton plants among all the adverse environment mentioned above.

The Bt protein content decreased with growth advances (Table1). The highest Bt toxin content of cotton plants was detected at seedling and squaring stage, while the lowest Bt insecticidal efficacy was recorded at bollsetting stage. Among the target organs of cotton bollworm, the insecticidal efficacy of leaf is the highest, that of Boll is the lowest, and that of square and flower is between leaf and boll.

Factor		Leaf	Square	Flower	Boll
Stress	High temperature	+	++	+++	++++
chritent	Low temperature	++	+++	+++	+++++
	Drought	++	+++	+++	++++
	waterlogging	+	++	++	+++
	Salinity	+	++	++	+++
Growth stage	Seedling stage	+			
	Squaring period	+	++		
	flowering period	++	+++	++++	
	boll period	+++	++++	++++	+++++

Table1 The change of Bt protein concentration of cotton plants under adverse environment and during development process

+indicates decrease of Bt protein concentration, and the more + indicates the greater reduction of Bt protein concentration.

2. Agronomic measures to increase Bt toxin content

Sowing date, planting density, nitrogen and plant growth regulators (PGR) are major agronomic practices to regulate crop growth in Yangtze River region of China, and also the potential measures to increase Bt toxin content in cotton. Our research showed that earlier sowing date reduced boll Bt toxin concentration (Fig.1), which suggested that late sowing within suitable timing could increase Bt toxin content at boll-setting stage. Increased planting density could increase boll insecticidal protein content (Fig.2).

Increased nitrogen rates in soil could enhance Bt toxin content of cotton plants markedly, but rich nitrogen in soil caused overgrowth of vegetative organs and thus resulted in reduction of lint yield. Incontrast, foliar spraying of nitrogen (such as urea or amino acid) could increase both Bt protein concentration at boll-setting stage and lint yield (Fig.3). Some PGR could enhance boll Bt toxin content, with DPC (100×10^{-6}) and 6-A (10×10^{-6}) enhanced both boll Bt toxin content and boll weight effectively (Fig.4).

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Fig.1 Sown dates on Bt toxin concentration on bolls at 10 days after flowering and lint yield during 2015 and 2016 cotton growth seasons, vertical bar represents S.E. of the mean (n = 4).



Fig.2 Planting density on Bt toxin concentration on bolls at 10 days after flowering during 2013 and 2015 cotton growth seasons, vertical bar represents S.E. of the mean (n = 4).



Fig.3 Foliar spraying of the amino acid and urea on Bt toxin concentration on bolls at 10 days after flowering and lint yield during 2017 and 2018 cotton growth seasons, the concentration of amino acid was 20 mg/kg, urea was 2%, and were both applied at peak flowering period. Vertical bar represents S.E. of the mean (n = 4).



Fig.4 PGR application on Bt toxin concentration on bolls at 10 days after flowering during 2015 and 2016 cotton growth seasons, concentration of DPC (1,1-dimethyl piperidinium chloride (C7H16CIN) and 6-BA were 100 mg/kg and 5 mg/kg respectively, and were both applied at peak flowering period. Vertical bar represents S.E. of the mean (n = 4).



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As a result of climate change, flooding or waterlogging stress has been increasingly frequent and unpredictable worldwide in recent years. The damage caused by waterlogging was mainly due to oxygen deficiency, hypoxia, or anoxia. In addition, the imbalance between production and consumption of carbohydrates coupled with an accumulation of toxic metabolic products, is often fatal for most terrestrial plants.

Cotton possesses an indeterminate growth habit and is often impacted by waterlogging. Waterlogging stress leads to decreases in chlorophyll content of cotton leaves, leaf water potential, and rubisco activity, ultimately reducing photosynthetic capacity and accelerating leaf senescence and abscission. Waterlogging inhibits the uptake of most major nutrients. It also leads to an imbalance between C and N metabolism. The lack of energy supply hinders the growth and development of cotton plants, further affecting dry matter accumulation and yield formation of cotton.

However, cotton is able to make certain physiological and molecular adjustments to adapt to water over-saturation, because it has evolved adaptation and protection mechanisms that allow it to respond to waterlogging stress, including the escape mechanism, quiescence mechanism, and self-regulating compensation mechanism.

Plants trigger the escape strategy when subjected to short-term waterlogging stress by increasing adventitious root growth, producing aerenchyma, and accelerating

stem elongation. The quiescent adaptation strategy differs from escape adaptation. It minimizes energy consumption by retarding plant growth and development via a series of metabolic changes under waterlogging. Waterlogged will utilize cotton its indeterminate growth habit and compensatory ability to accelerate plant growth and development once the stress is relieved in order to compensate for losses due to waterlogging stress.

Given these three mechanisms, there are several measures that can improve the adaptation of cotton to waterlogging stress, such as the adoption of waterlogging-tolerant cotton varieties, foliar or soil fertilization, and the application of plant growth regulators and other agronomic management measures as shown in Table 1. Our recent study showed that earthing up (pile the top soil between rows to the base of the cotton plants until two centimeters above the cotyledon nodes) at squaring could efficiently reduce waterlogging damage occurred at flowering and boll-setting.

On one hand, waterlogging stress can alter the expression of numerous genes in cotton. The research on these aspects will assist with conventional and transgenic breeding approaches to enhance waterlogging tolerance of cotton. The use of transgenic cotton may elucidate the physiological role of waterlogging stressrelated pathways and their contribution to waterlogging tolerance. On the other hand, plant growth regulators, such as sodium nitroprusside (SNP). brassin. diethyl aminoethyl hexanoate (DA-6), aminoethoxyvinylglycine (AVG), and 1methylcyclopropene (1-MCP), can improve plant tolerance to waterlogging stress, but the efficacy of their single use is very limited. Optimizing or developing formulas or mixtures of various plant growth regulators might offer a solution. Therefore, selecting tolerant varieties, improving agronomic measures, and applying suitable fertilizers and plant growth regulators can enhance the adaptation of cotton plants and reduce damage caused by waterlogging stress. Given that significant improvements in waterlogging tolerance could be made by exploiting genotype x management x environment interactions, we suggest that bioengineering approaches in conjunction with appropriate crop management practices could be highly effective for improving the waterlogging tolerance of cotton.

In summary, the effects of waterlogging stress on plant growth and development, as well as the yield and fiber quality of cotton, is determined by the severity of waterlogging, cotton adaptability to waterlogging and corresponding agronomic measures. Timely adoption of agronomic measures like using tolerant cultivars, earthing up, and foliar spray of plant growth regulators can increase the adaptability of cotton to waterlogging stress, and thus reduces the damage and yield loss.

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Measures	Application	Effects	References	
Sodium	Foliar spray 0.5 mmol·L ⁻¹	Reduced membrane system	Zhang et al.	
nitroprusside	SNP	damage, and promoted cotton growth	(2021)	
(SNP)		after waterlogging.		
Ν	N fertilization (240 kg ha ⁻¹) Reduced waterlogging damage by			
		improving root growth, vigor, and	(1982), Rose et	
		photosynthesis.	al. (2002)	
K	Foliar or soil application of	Significant improvement in growth,		
	K(60kg⋅hm⁻²)	photosynthetic pigments, and capacity.		
FeSO ₄	A foliar spray of iron sul fate	Ameliorated the negative effects of iron	Li et al. (2013)	
	(FeSO ₄) prior to	chlorosis, returning cotton foliage to its	(, , , , , , , , , , , , , , , , , , ,	
	waterlogging	normal color.		
AVG/1-MCP	Application of AVG/1 -MCP	Improved boll number and vield	Liu et al. (2020)	
	1d prior to waterloaging			
AVG	Spraved of AVG 1d prior to	Improved leaf growth N acquisition	Naieeh et al	
	waterloaging	photosynthetic and fruit production	(2015)	
Growth	Urea (1%) + notassium	Improved metabolism of cotton plants	liana et al	
rogulators and	chlorido (0.5%) + brassin	contributing to the crops turning groop	(2012)	
fertilizero	(0.02 mg s) = -1)	contributing to the crops turning green	(2013)	
renunzers	$(0.02 \text{ mg L}^{-1}) + \text{dietry}$			
	mg L⁻')			
ABA	Foliar application	Increased tolerance to subsequent	Pandey et al.	
		waterlogging-induced injury in cotton	(2002)	
		through improving leaf photosynthesis.		

Table1: Main measures for alleviating waterlogging damage in cotton.