The Inheritance of An Ultra-dwarf Mutant in Upland Cotton and Its Gene Location

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Abstract：Dwarf plant resources are few in upland cotton. A ultra-dwarf mutant from upland cotton was found by X.S. Chen in 2004. The inheritance of the mutant was studied, showing that the traits controlled by a pair of recessive qualitative genes. its gene signal was denominated *du*. Up to now, no similar mutation has be found in upland cotton. The mutation could not normally flower and produce bolls under natural condition, and reached last height only 10.5cm. If treated with exterior GA3, it could flower and set bolls.

Meanwhile, linkage inheritance of gene *du* based on SSR markers were conducted. Seventy pairs of polymorphic primers were picked out from 1350 primers，covering all the identified chromosomes by screened in parents and near-isogenic lines. Then mapped with F_2 population，from intraspecific crosses of “Ultra-dwarf No.1” and “Xinluzao 16” by bulked segregation analysis. Linkage analysis and molecular linkage map of ultra-dwarf mutant gene *du* was constructed. Thirty-six primers were linked to eight linked groups，and *du* was linked to LG01. Seven co-dominant markers linked to *du* were NAU2679、NAU2749、NAU905、NAU2838、NAU5373、NAU2238 and NAU4946. Based on the known genetic map of tetraploid cotton, markers NAU4946、NAU2238、NAU905、NAU5373 and NAU2679 were on the chromosome 6，and the target gene *du* was located between NAU2238 and NAU4946，their genetic distance were 3.5cM and 1.5cM, respectively. Hence，the *du* gene was located on the chromosome 6.

Keywords：Upland cotton(*G. hirsutum L.*)；qualitative inheritance; Ultra-dwarf mutant；Gene location；SSR

Introduction

A ultra-dwarf mutant was found by X.S. Chen in 2004 from Upland Cotton C119-2 groups, then it was purified and named “Chaoai No.1”. Its cotyledon and leaf is curling and extremely dwarf. If there is no treatment with exogenous GA3, the mutant cannot initiate the process of flowering and boll，but if treated with GA3, the mutant can normally flower and set boll. So far no similar mutant has been reported in Upland Cotton.

Objective

In this paper, through the analysis of the quality genetic law of the ultra-dwarf mutant and the location of its gene using SSR molecular marker technique, in order to lay a foundation for the subsequent fine mapping and cloning the ultra-dwarf mutant gene.

Material and Method

Experimental material is “Chaoai No.1” with dwarf plant height and wild type “Xinluzao 16” with normal one.

Crossing "Xinluzao 16" with “Chaoai No.1” got the combination of F_1. Then F_1 crossed with Xinluzao 16 gained backcross population BC1[(Xinluzao 16×“Chaoai No.1”)× Xinluzao 16] and

crossed with “Chaoai No.1” got backcross population BC_2[(Xinluzao 16×“Chaoai No.1”) × “Chaoai No.1”]. Inbred F_1 produced F_2. The separation ratio of BC_1\$BC_2$\$F_2$ was investigated according to the theory of Mendel genetics, theoretic segregation ratio was tested to use X^2.

The ultra-dwarf gene mapping population F_2 from intraspecific crosses of “Chaoai No.1” and “Xinluzao 16” by bulked segregation analysis. Used two near-isogenic pool and two parents of DNA samples to screen polymorphic primers. Then, the polymorphic primers for detection of 184 individual genotypes in group F_2, filtrated the SSR marker associate to ultra-dwarf mutant gene.

The rule of statistical analysis on polymorphism type was that the individual genotypes equal to " Xinluzao No. 16" recorded as "1", and equal to individual genotypes “Ultra-dwarf No.1” as "2", co-dominant genotypes as "3", missing band as " 0 ". Then, X^2 tested whether the separation ratio was consistent with Mendel's genetic law. The linkage analysis calculated genetic distance(cM) between SSR markers and mutant gene.

Results and conclusions

1. Observation of ultra-dwarf mutant phenotype

Ultra-dwarf mutant is a kind of new dwarf mutant in upland cotton. Phenotypically, ultra dwarf mutant can obviously be distinguished with normal seedling in cotton seedlings (Figure 1). The cotyledons and leaves of normal cotton seedling grew normally, but the mutant’s were crinkle and dwarf. Both of them in plant height have obvious differences. The final plant height of “Chaoai No.1” was only 10.5 cm; but the normal variety "Xinluzao 16" reached 89.2 cm (Figure 2).

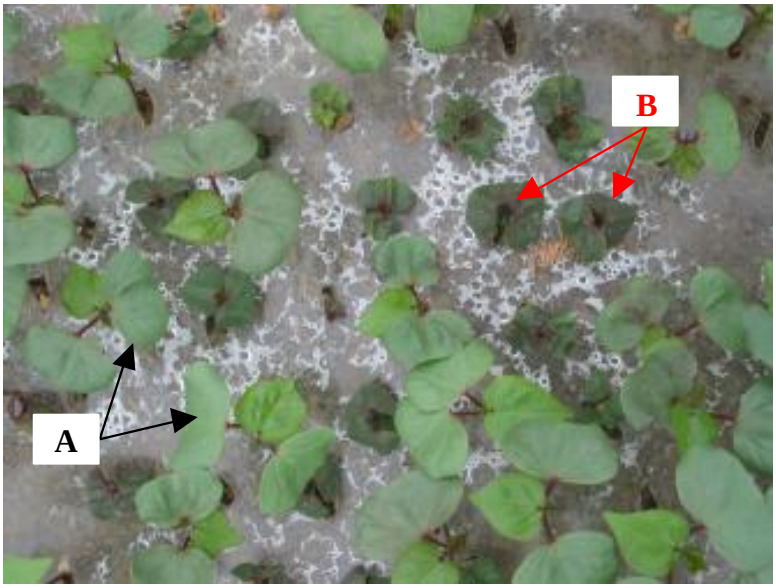

Fig.1 (A) Phenotypes of wild type with normal cotyledon & leaf
(B)Ultra-dwarf mutant with crinkled dwarf cotyledon and leaf in seedling bed

A. B.

Fig.2 A. Phenotype of normal plant variety "Xinluzao 16" grew in natural condition；
B. Phenotype of the ultra-dwarf plant of “Chaoai No.1” grew in natural condition

2. The mutant in response to GA_3

The mutant grows in extremely dwarf state; but spraying with 50 ppm of exogenous GA_3, it can restore normal growth, then it can bud, blossom and set less cotton boll. Compared to the wild type, the mutant after processed by GA_3 became upper leaves pale, bud less and blossom later (Figure 3).

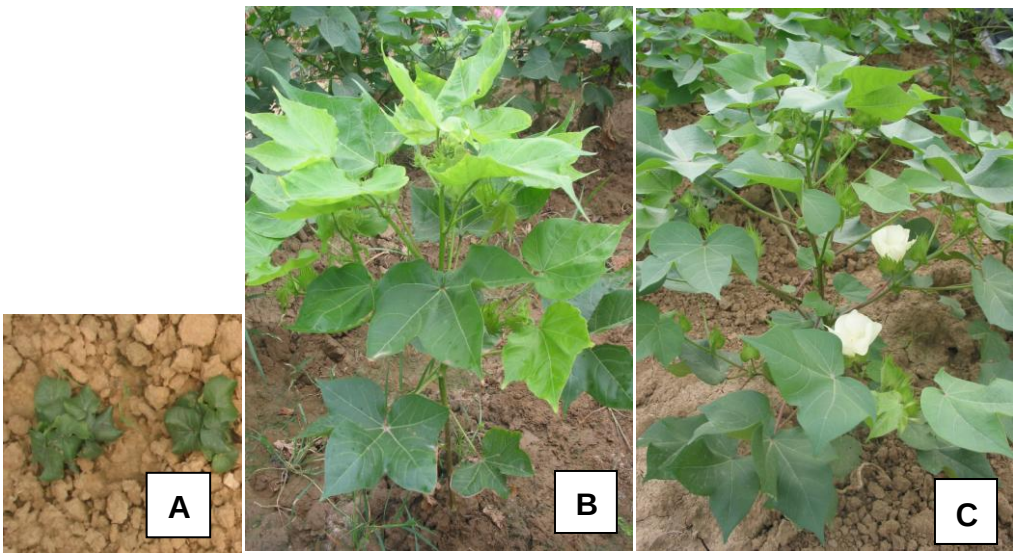

Fig 3. The phenotypic change of ultra-dwarf mutant after spraying GA_3
A: ultra-dwarf mutants; B: the mutant plant after spraying GA_3; C: the wild type plant

3. The inheritance of the ultra-dwarf mutant

The phenotype of ultra-dwarf mutant can express obviously in seedlings, and sustain dwarf state throughout the period of growth and development. Observation of six generation population in the outdoor seedling bed , their separation conditions were shown in table 1. We can see from the table 1 that the height of F_1 cotton seedling grow normally, indicated that normal height plant was dominant to ultra-dwarf one. The seedlings were all normal in backcross BC_1, the separation ratio of backcross BC_2 with normal seedlings : ultra-dwarf one = 1: 1; F_2 separation ratio with normal seedlings: ultra-dwarf one = 3 : 1. The above result showed that the ultra-dwarf is controlled by a pair of recessive qualitative gene, its gene signal was denominated *du*.

Table1. Phenotypic segregation of hybrid progeny between the cross Xinluzao16 with Chaoai No.1

gener-ation	parents/Cross	wild type	mutant type	X^2 value of expected ratio	probability
				BC_2→1∶1 F_2 →3∶1	
P_1	Xinluzao16	52	0	All nomal plants	
P_2	Chaoai No.1	0	42	A11 Ultra-dwarf	
F_1	Xinluzao16×Chaoai No.1	59	0	All nomal plants	/
BC_1	(Xinluzao16×Chaoai No.1) ×Xinluzao16	243	0	All nomal plants	/
BC_2	(Xinluzao16×Chaoai No.1) ×Chaoai No.1	107	85	2.5543	0.10-0.25
F_2	Xinluzao16×Chaoai No.1	258	83	0.07918	0.75-0.90

4. Location of gene *du* by SSR markers

Gene location groups F_2 had total 184 plants. Two plant phenotypes of normal plant and ultra-dwarf one appeared in F_2. Normal plant 136 to ultra-dwarf plant 48, fit to 3: 1 theoretical separation ratio ($X^2 = 0.1159$).

Use the parents as well as the near-isogenic lines to screen in 1350 pairs of SSR primers, and obtained 70 pairs of polymorphic primers. Then detecting each individual band type in F_2 separated groups, among 70 pairs of markers were found 59 pairs of markers in the F_2 group to have polymorphism. Linkage analysis found 36 pairs of markers can be linked and distributed in 8 linkage groups.

Seven SSR markers linked to the target gene *du*, namely NAU2679, NAU2749, NAU905, NAU2838, NAU5373, NAU2238 and NAU4946, they are all co-dominant markers. Five of them such as NAU4946, NAU2238, NAU905, NAU5373 and NAU2679 are located on chromosome sixth. Close on both sides of the target gene *du* are the molecular marker NAU4946 and NAU2238. The genetic distance between *du* and the marker NAU4946 is 1.4 cM, the genetic distance between the markers NAU2238 and *du* 3.3 cM (Figure 4). The marker NAU4946 and NAU2238 on sixth chromosome were known, so target gene *du* was located on sixth chromosome.

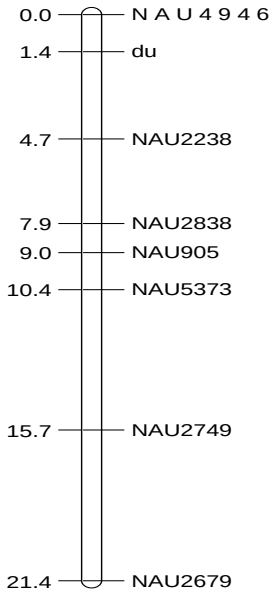

Fig 4. Linkage map of ultra-dwarf gene *du*