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Conditioning of Fasciation by Gibberellin and Genotype in Cotton (*Gossypium hirsutum* L.)

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Abstract. The round-leafed mutant cotton line L-501 developed fasciation of the upper stem when field grown in Central Asia. Fasciation co-segregated with the mutant gene for round leaves *In*.[1] Fasciation developed at the flowering stage, but removal of floral buds did not prevent fasciation. Fasciation in L-501 could be inhibited by the gibberellin (GA) biosynthesis inhibitor chlorocholine chloride or by fusicoccin. GA_3 application in the field induced fasciation in the mutant's parental line L-463, which has five-lobed leaves and does not normally develop fasciation. Fasciation did not develop in either line, even after GA_3 treatment, in UK glasshouse conditions.

Key Words. Fasciation—Gibberellin—*Gossypium*—Leaf-shape—Mutant

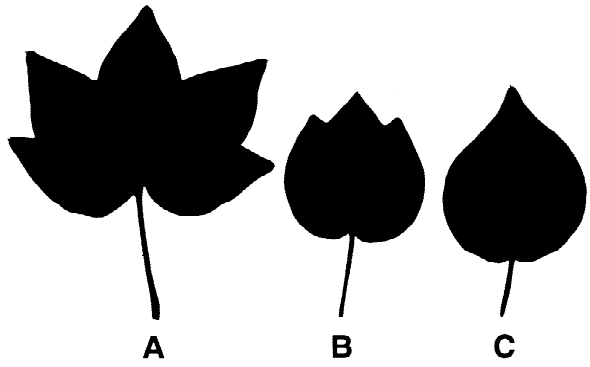

Fig. 1. *A*, normal five-lobed leaf of *G. hirsutum; B*, three-lobed leaf; *C*, round leaf.

The mechanisms responsible for the organization of the dicot shoot apex remain largely unknown. An example of disruption of apical organization which has been observed in a number of species and developmental contexts is fasciation (LaMotte et al. 1988). Stem fasciation is generally manifested in the later stages of growth and involves loss of normal branching pattern, clustering of flowers, and abnormal stem broadening. Fasciation could be of use in species such as cotton, in which mechanical harvesting might be facilitated by determinate shoot growth with clustering of fruits at the top of the stem.

The developmental mechanisms that trigger fasciation are not well understood. There is evidence for a hormonal basis in pathological fasciation caused by *Rhodococcus fascians*, which has a plasmid-borne virulence gene *fas-1* encoding an isopentenyl transferase that produces cytokinin (Crespi et al. 1994, Stange et al. 1996). Changes in hormone metabolism also correlated with fasciation in hybrid *Populus* transformed with the *Agrobacterium rhizogenes rolC* gene (Nilsson et al. 1996). We show here that GA_3 can promote fasciation in certain cotton genotypes.

Effects of genotype on fasciation are found in a wide variety of species, although legumes have been particularly well investigated (Beardsell et al. 1993, Gottschalk and Wolff 1983, Knights 1993, LaMotte et al. 1988, Mahna et al. 1993, Tang and Skorupska 1997). In *Arabidopsis*, several genetic loci that can cause fasciation associated with apical meristem enlargement and altered floral development have been examined in detail recently (Clark et al. 1993, 1995, Leyser and Furner 1992). Fasciated regenerants occur in micropropagation of strawberry (Jemmali et al. 1994). We report here a cotton gene causing fasciation in typical field conditions for this crop.

Abbreviations: CCC, chlorocholine chloride; FC, fusicoccin; GA, gibberellin; GA_3, gibberellic acid.

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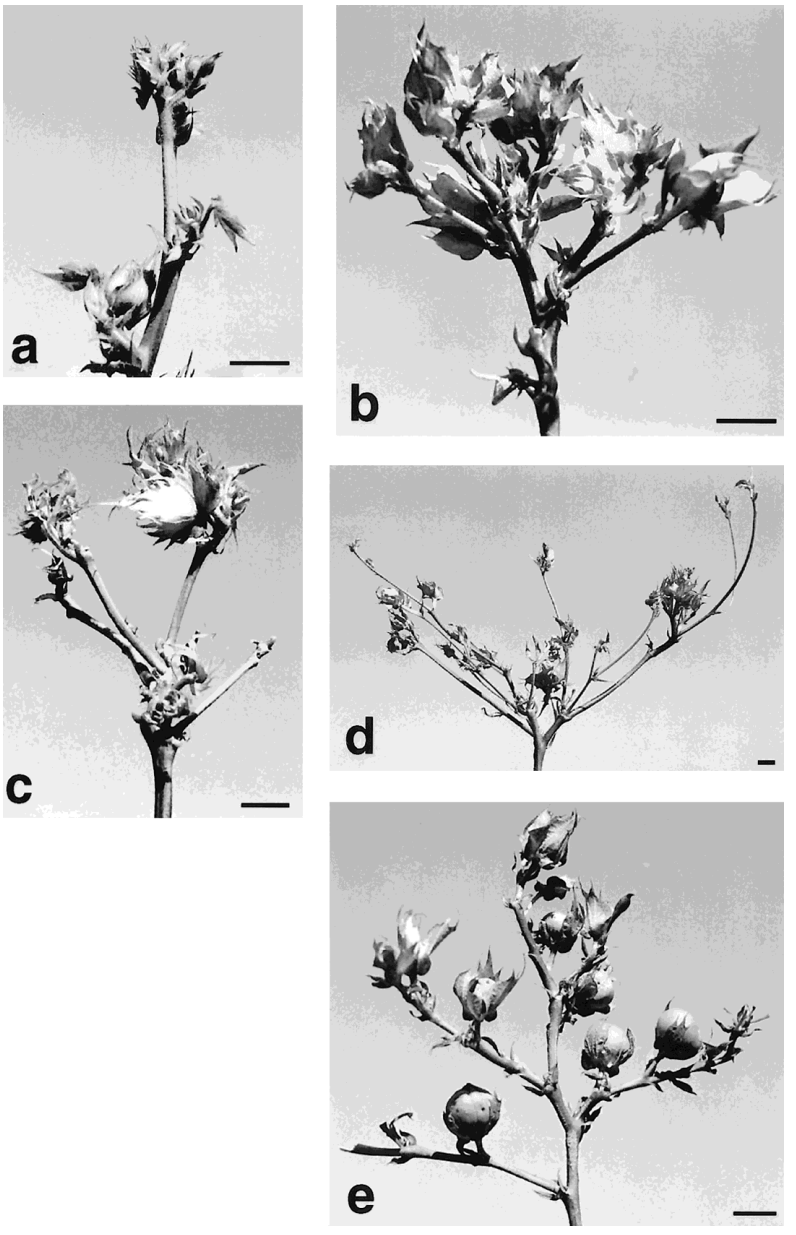

Fig. 2. Shoot apices of cotton plants (leaves were removed to reveal shoot structure): *a*, normal apex of L-463; *b*, fasciated apex of L-501; *c*, fasciation induced in L-463 by GA_3 treatment; *d*, elongated fasciation shoots of L-501 treated with GA_3; *e*, lack of fasciation in L-501 treated with FC. *Bars* indicate 10 mm.

Materials and Methods

Plants

Pure inbred lines of cotton (*Gossypium hirsutum* L.) from the collection of Tashkent State University (T.S.U.), Republic of Uzbekistan, were used. The T.S.U. line numbers are used in this paper: L-463 (an agricultural line, Akstafa-43) is the parental line of the mutant L-501; L-458 is the agricultural line 108-F (Absalov 1991).

Growth Conditions and Treatments

Field-grown plants were cultivated from late April to August in an irrigated plot at T.S.U. Growth regulators (Sigma Chemical Co., Poole, Dorset, UK unless stated) were sprayed onto the shoot apex and the three youngest expanding true leaves. All spray solutions, including controls, contained 1% (v/v) ethanol and 0.025% (v/v) Tween 80. GA_3 was applied at 0.9 mM and chlorocholine chloride (CCC) at 1.27 mM. Fusicoccin (FC, gift of Prof. G. S. Muromtsev, Institute of Agricultural Biotechnology, Moscow) was applied at 0.1 mM. For each growth regulator, two sets ($n = 5–8$) of plants were treated at weekly intervals, with the first application commencing after 6 weeks. Similar experiments were carried out on cotton plants in 10-cm diameter pots of compost with weekly nutrient applications, cultivated in a glasshouse in Aberystwyth, UK (minimum 18°C, with day length supplemented to 16 h with 1,000-W mercury vapor lights).

Results and Discussion

The cotton line L-501 carries a spontaneous mutation selected because its leaves are round rather than the normal five-lobed shape of *G. hirsutum* (Fig. 1). This leaf

Table 1. Cosegregation of fasciation with the mutant leaf shape gene *In*.[1] See Fig. 1 for the leaf shapes.

Genotype	Leaf shape	Fasciation[a]	*n*	Mean height (cm ± S.E.)	χ^2
463	5-lobed	−	25	90.4±1.6	
501	Round	+	25	57.8±0.9	
F_1 (463 × 501)	3-lobed	+	25	84.6±1.4	
F_2 (463 × 501)	5-lobed	−	20	103.3±2.9	0.79[b]
	3-lobed	+	50	84.6±2.3	
	Round	+	25	60.0±1.8	
F_B(463 × 501) × 501	3-lobed	+	20	77.5±2.0	0.21[c]
	Round	+	23	57.0±2.4	

[a] +, fasciation; −, no fasciation.
[b] not significantly different from Mendelian 1:2:1 ratio at 5%;
[c] not significantly different from Mendelian 1:1 ratio at 5%.

shape change is governed by a single semi dominant gene *In*[1] (Absalov 1991). When L-501 was grown under field conditions in Tashkent, Central Asia, fasciation of the upper stem occurred at the flowering stage, whereas the parental line L-463 showed normal stem development (Fig. 2, *a* and *b*). The fasciation involved cessation of vertical growth because of a loss of a vegetative apex; irregular production of an abnormal number of flower buds with loss of the normal branching pattern, so that a number of bolls clustered at the top of the shoot; and abnormal broadening of the stem. As a result, L-501 plants typically reached a final height of little over half that of L-463 plants at the end of the growing season (Table 1).

Fasciation in L-501 appears to be a pleiotropic effect of the *In*[1] mutation. The fasciated phenotype cosegregated with the leaf shape effect of *In*[1] through F1, F2, and back-cross generations (Table 1). The gene *In*[1] is semidominant with respect to both leaf shape and fasciation; heterozygotes exhibited an intermediate, three-lobed leaf shape (Fig. 1) together with an intermediate final stem height caused by fasciation occurring at a higher node (Table 1). The pleiotropic effects of *In*[1] could conceivably be the result of an action in the meristems of both the shoot apex and the developing leaves.

Fasciation in L-501 was first detectable at the stage of flower bud formation. Two hypotheses to explain this correlation might be advanced: either the flower buds produce a signal that directly induces fasciation, or fasciation is a secondary consequence of physiological changes in the shoot apex at the stage of floral induction. The latter hypothesis is favored because removal of all floral buds did not prevent fasciation. Indeed, the disbudded plants exhibited more pronounced stem broadening, perhaps as a consequence of increased resource allocation to the stem tissues.

The spontaneous fasciation of L-501 plants was prevented by spray application of the plant growth retardant CCC. Because CCC is believed to be a GA biosynthesis inhibitor (Grossmann 1992), this suggests an involvement of endogenous GAs in fasciation in this genotype. The CCC treatment also reduced stem growth by 49% ($n = 8$) and the number of flower buds in July from 15–18 to 4–6.

Table 2. Effects of various treatments on fasciation in field-grown cotton lines. All plants within a treatment group responded similarly ($n = 5$–8). For details, see "Materials and Methods." +, fasciation; −, no fasciation; nt, not tested.

Genotype	Untreated	Disbudded	GA_3	CCC	FC
L-501	+	+	+	−	−
L-463	−	nt	+	−	−
L-458	−	nt	−	nt	−

L-463, the parental line of L-501, has not been observed to develop fasciation under normal conditions. However, fasciation was induced in L-463 plants by spray treatment with GA_3 (Fig. 2*c*). Application of GA_3 to L-501 plants resulted in elongation of the fasciated organs (Fig. 2*d*). In contrast, another agricultural line, L-458, did not exhibit fasciation even when sprayed with GA_3. Additional genes that cause a propensity to fasciation in response to GA_3 therefore appear to exist.

Another treatment that prevented the spontaneous fasciation of L-501 was spraying with FC (Fig. 2*e*), which inhibited stem growth by 35% ($n = 10$). Very few studies have been made on the effects of FC on shoot development in intact plants (Lavee and Cleland 1993), and there is not yet sufficient information to interpret this novel observation that FC can influence shoot apex development. We have reported previously the general effects of FC on the growth and morphology of field-grown cotton plants (Nadjimov et al. 1996).

Conditions under which fasciation was observed in the various cotton lines are summarized in Table 2. This shows typical experiments with plants grown in the field in Tashkent, but the same responses have been obtained consistently in independent trials during several seasons. However, it should be noted that fasciation in these cotton lines is dependent on environmental conditions. In glasshouses in the UK, fasciation could not be induced, even with multiple GA_3 treatments, in plants of L-501 or L-463. It is known that differing light qualities of glasshouse vs field conditions can affect expression of some mutant phenotypes (e.g. Jones and Burgess 1977), but further investigation of environmental effects on cotton fasciation are required.

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