Chromosomal location of *Fov* disease response in *G. hirsutum* X *G. sturtianum* chromosome addition lines

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Summary

Fusarium oxysporum f. sp. vasinfectum (Fov) is considered the most destructive pathogen of cotton in Australia. In this study, BC_3 progenies of chromosome addition lines between G. sturtianum (C genome), an Australian wild Gossypium species shown to be resistant to fusarium wilt, and G. hirsutum were genetically characterized (using G. sturtianum chromosome-specific AFLPs) to determine the number and identity of the G. sturtianum chromosomes in 47 G. hirsutum X G. sturtianum chromosome addition families. The 47 families were challenged with Fov (VCG 11) in glasshouse trials using root-dipping inoculations to determine their levels of fusarium wilt resistance. Overall, 20 of the BC_3 families showed enhanced fusarium wilt resistance relative to their G. hirsutum parent. Logistic regression nominated five G. sturtianum linkage groups as having a significant effect on fusarium wilt resistance in a G. hirsutum background. Two linkage groups were associated with improved resistance, while three linkage groups were associated with increased susceptibility.

Introduction

fusarium wilt of cotton in Australia is caused by Fusarium oxysporum f. sp. vasinfectum (Fov). fusarium wilt was first recorded in 1993 in the Cecil Plains/Brookstead region of Queensland (Kochman, 1995). Since then, the incidence of fusarium wilt in Australian cotton crops has increased drastically, spreading rapidly to most cotton growing regions in New South Wales and Queensland (Reid et al., 2002). Despite, robust improvements in the fusarium wilt resistance of Australian cotton cultivars, cotton breeders continue to look for new sources of resistance. Genetic variability for resistance to fusarium wilt seems to be limited in the cultivated cotton germplasm, and consequently cotton breeders are looking beyond the cultivated gene pool In addition, remarkably little is known about the genetics of resistance to fusarium wilt in cotton, particularly in Australia. Reports on the mode of inheritance of resistance to Fov have been inconsistent with respect to the number and effect of genes involved (Fahmy, 1927; Smith and Dick 1960; Mohamed, 1963). Understanding the genetics of fusarium wilt resistance in cotton will allow breeders to develop future cultivars more efficiently and quickly.

The Australian cotton relatives may be a valuable pool of germplasm that can be used as a source of genetic resistance to fusarium wilt (Brubaker and Beasley per. comm.), and because they have a simpler genome, they could also serve as useful genetic models for elucidating the genetics of fusarium wilt resistance. One Australian cotton relative, G. sturtianum has been shown to improve levels of fusarium wilt resistance when hybridized with cultivated cotton (G. hirsutum) (McFadden et al., in press). In a preliminary attempt to elucidate the genetics of fusarium wilt resistance in G. sturtianum, 47 G. hirsutum X G. sturtianum chromosome lines were assessed for the levels of fusarium wilt resistance to determine if differential fusarium wilt resistant responses could be attributed to specific G. sturtianum chromosomes.

Methods

Two-week old seedlings of 47 G. hirsutum X G. sturtianum chromosome addition families and the common G. hirsutum parent (CPI138969) were inoculated by dipping the roots into a VCG 11 ($2x10^6$ conidia per ml) conidial suspension. Plant responses were assayed six weeks after inoculation using a categorical scoring system, the vascular browning score (VBS): [0 = no vascular discoloration, 1 = discoloration restricted to base of stem only, 2 = discoloration of the hypocotyl, 3 = discoloration of the epicotyl, 4 = complete vascular discoloration of stem and 5 = plant dead]. After the trials total genomic DNA was extracted from leaves following Brubaker and Brown (2003) and a suite of 181 G. sturtianum chromosome-specific AFLP molecular markers was used to identify the G. sturtianum chromosomes present in the BC_3 individuals following Becerra Lopez-Lavalle et al., (2002).

All disease assays were conducted in randomized complete blocks in three consecutive experiments; the G. hirsutum parental line (CPI138969) was included in every experiment as a control. The VBS values were analyzed by univariate analysis of variance under a generalized linear model (GLM) (□=0.01). The means were compared for significant differences using least significant differences (LSD). The association between fusarium wilt resistance and the presence of G. sturtianum chromosomes in the G. hirsutum background was evaluated using binomial logistic regression. The binomial logistic regression was used for modeling the relationship between the fusarium wilt response variable and a set of explanatory variables (e.g., the G. sturtianum chromosomes present in each individual).

Results

The 47 G. hirsutum X G. sturtianum chromosome addition lines (1600 BC_3 individuals) were assayed for fusarium wilt resistance in three consecutive trials. The G. hirsutum parental (CPI 138969), whose Fov disease response is comparable to the Fov susceptible standard cultivar Siokra 1-4 (McFadden et

al., in press), was included in all three trials. The disease assay showed that fusarium wilt symptoms were variably expressed both in the parental control and in the chromosome addition lines, ranging from very resistant (0) to very susceptible (5) (Fig. 1).

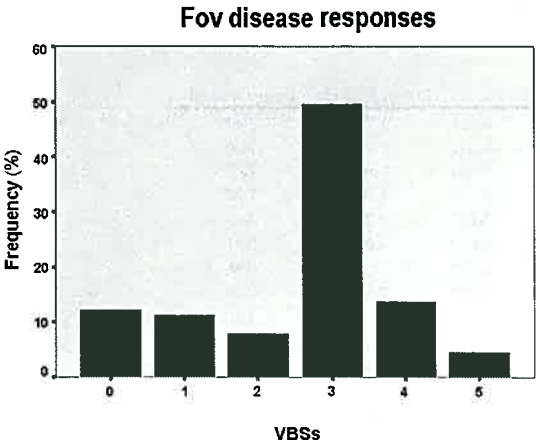

Figure 1: Pooled frequency distribution of VBS scores of *G. hirsutum* (CPI138969) and 47 *G. sturtianum* X *G. hirsutum* chromosome addition lines.

Twenty of the 47 chromosome addition lines had significantly higher resistance levels than the G. hirsutum parental control (P□ 0.01; Table 1). To determine if this enhanced resistance could be attributed to specific G. sturtianum chromosomes, a suite of 181 G. sturtianum chromosome-specific AFLP molecular markers were selected to identify the G. sturtianum chromosomes in 625 BC_3 individuals with extreme fusarium wilt responses [370 resistant (VBS of 0&1s) and 255 susceptible (VBS of 4 & 5s) individuals). Binomial logistic regression analyses identified five putative G. sturtianum linkage groups that were associated with significant differences in fusarium wilt response relative to the G. hirsutum parent (Table 2). Linkage groups "C", "H", and "O" were significantly associated with Fov susceptibility, while linkage groups "N" and "Q" were associated with Fov disease resistance.

Table 1: Mean comparisons of means vascular browning scores using a least significant differences (LSD) test between *G. hirsutum* (A; CPI138969) and 47 *G. sturtianum* chromosome addition lines (B).

LSD test (mean vascular discolouration index)

G. hirsutum (A)	C genome addition lines (B)	Mean Difference (A-B)		Std. Error	Sig.	99% Confidence Interval	
						Lower Bound	Upper Bound
CPI138969	Hyb645-2	0.793	*	0.243	0.001	0.17	1.42
	Hyb686-7	0.780	*	0.239	0.001	0.16	1.40
	Hyb687-15	1.180	*	0.239	0.000	0.56	1.80
	Hyb687-17	0.705	*	0.239	0.003	0.09	1.32
	Hyb687-8	0.330		0.239	0.167	-0.29	0.95
	Hyb710-18	-0.662		0.377	0.079	-1.63	0.31
	Hyb710-21	0.303		0.256	0.238	-0.36	0.96
	Hyb710-24	-0.113		0.273	0.679	-0.82	0.59
	Hyb710-25	0.155		0.259	0.550	-0.51	0.82
	Hyb710-26	0.005		0.239	0.983	-0.61	0.62
	Hyb710-28	0.286		0.241	0.235	-0.34	0.91
	Hyb710-30	0.330		0.239	0.167	-0.29	0.95
	Hyb710-35	0.086		0.248	0.730	-0.55	0.73
	Hyb710-37	0.030		0.307	0.922	-0.76	0.82
	Hyb710-38	0.430		0.266	0.106	-0.26	1.12
	Hyb710-40	-0.100		0.295	0.734	-0.86	0.66
	Hyb710-41	0.095		0.263	0.719	-0.58	0.77
	Hyb710-42	0.105		0.239	0.660	-0.51	0.72
	Hyb710-43	0.155		0.259	0.550	-0.51	0.82
	Hyb710-44	-0.021		0.241	0.930	-0.64	0.60
	Hyb710-45	-0.160		0.307	0.601	-0.95	0.63
	Hyb710-46	0.105		0.239	0.660	-0.51	0.72
	Hyb710-47	0.271		0.269	0.314	-0.42	0.97
	Hyb710-48	0.267		0.243	0.273	-0.36	0.89
	Hyb710-51	0.669	*	0.248	0.007	0.03	1.31
	Hyb710-52	-0.013		0.295	0.964	-0.78	0.75
	Hyb710-56	0.155		0.239	0.517	-0.46	0.77
	Hyb711-124	0.095		0.263	0.719	-0.58	0.77
	Hyb711-78	0.724	*	0.248	0.004	0.08	1.36
	Hyb712-82	0.953	*	0.281	0.001	0.23	1.68
	Hyb712-87	0.824	*	0.254	0.001	0.17	1.48
	Hyb712-89	0.355		0.239	0.138	-0.26	0.97
	Hyb712-91	0.744	*	0.251	0.003	0.10	1.39
	Hyb712-92	1.259	*	0.251	0.000	0.61	1.91
	Hyb734-100	1.271	*	0.269	0.000	0.58	1.97
	Hyb734-102	0.373		0.251	0.137	-0.27	1.02
	Hyb734-104	0.598		0.246	0.015	-0.04	1.23
	Hyb734-93	1.272	*	0.256	0.000	0.61	1.93
	Hyb734-94	0.973	*	0.251	0.000	0.33	1.62
	Hyb734-95	0.824	*	0.254	0.001	0.17	1.48
	Hyb735-107	0.859	*	0.251	0.001	0.21	1.51
	Hyb735-108	0.755	*	0.239	0.002	0.14	1.37
	Hyb735-110	1.001	*	0.251	0.000	0.35	1.65
	Hyb735-125	0.805	*	0.239	0.001	0.19	1.42
	Hyb735-126	1.267	*	0.243	0.000	0.64	1.89
	Hyb736-115	0.942	*	0.254	0.000	0.29	1.60

* The mean difference is significant at the .01 level.

Table 2: Logistic regression analysis on 47 C-genome chromosome addition lines

C genome Linkage Groups	Fov response	B	S.E.	Wald	df	Sig[a].	Exp(B)
CHRC	S	1.13	0.63	3.25	1	0.07	3.1
CHRH	S	0.60	0.28	4.53	1	0.03	1.8
CHRN	R	-0.41	0.23	3.26	1	0.07	0.7
CHRO	S	0.98	0.46	4.62	1	0.03	2.7
CHRQ	R	-0.92	0.45	4.09	1	0.04	0.4
Constant		-0.39	0.10	14.20	1	0.00	0.7

a 95% confidence level
R Resistance
S Susceptibility

Discussion

Introgression of agronomically important traits, such as fungal disease resistance, from novel germplasm into cultivated cotton to improve current commercial cultivars represents one of the major aims of cotton breeding today (Brubaker and Brown 2003). Species-specific molecular markers can facilitate such breeding approaches considerably. AFLP analysis (Vos et al., 1995) has proven to be an efficient method for tracking the inheritance of G. sturtianum chromosomes in G. hirsutum X G. sturtianum chromosome addition lines as they are recurrently backcrossed to G. hirsutum.

The significant improvement in fusarium wilt resistance observed in G. hirsutum x G. sturtianum hybrids suggests that G. sturtianum has the potential to contribute useful genes to cotton breeding programs (McFadden et al., in press). The fusarium wilt response disease resistant response observed in 20 of the 47 G. hirsutum X G. sturtianum chromosome addition lines also indicates that G. sturtianum can confer good levels of resistance to the Fov pathogen. In this preliminary analysis the G. sturtianum contribution could be traced to five specific linkage groups. Two of these linkage groups, "N" and "Q", were associated with improved levels of fusarium wilt resistance while, three linkage groups were associated with increased susceptibility. The molecular markers used to identify the chromosomes can also be used to select individuals carrying the "N" and "Q" linkage groups while simultaneously eliminating the individuals carrying the "C", "H", and "O" linkage groups. These results also highlight the need to broaden our genetic understanding of fusarium wilt resistance, because this, in turn, will greatly facilitate our ability to effectively transfer genes from novel germplasm to cultivated cottons.

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References

Becerra Lopez-Lavalle, L.A., McFadden, H.G. and Brubaker, C.L. 2002. Genetic characterization of chromosome inheritance in G. hirsutum x C genome alien chromosome addition lines: fusarium wilt resistance in wild Australian Gossypium. In: Field to Fashion, Proceedings 11th Australian Cotton Conference. ACGRA. (Brisbane, 13-15 August, 2002) 7 pp.

Brubaker CL and AHD Brown. 2003. The use of multiple alien chromosome addition aneuploids facilitates genetic linkage mapping of the Gossypium G genome. Genome 46 (5): 774-791.

Famhy T. 1927. The fusarium wilt disease of cotton and its control. Phytopathology. 17: 749-767.

Kochman JK. 1995. fusarium wilt in cotton - a new record in Australia. Australasian Plant Pathology 24: 74.

McFadden, H. D. Beasley, and C.L. Brubaker. In press. Gossypium sturtianum is a potential source of fusarium wilt resistance in cotton breeding. Euphytica

Mohamed HA. 1963. Inheritance of resistance to fusarium wilt in some Egyptian cottons. Empire Cotton Growing Review. 40: 292-295.

Reid P, G Constable, W Stiller, S Allen and G McNamara. 2002. The CSIRO Fusarium Breeding Project. In: Field to Fashion, Proceedings of the 11th Australian Cotton Conference. ACGRA. (Brisbane, 13-15 August, 2002), pp 653-656.

Smith AL and JB Dick. 1960. Inheritance of resistance to fusarium wilt in Upland and Sea Island cotton as complicated by nematodes under field conditions. Phytopathology. 50: 44-48.