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Evaluation of Border Cell Number and Cry Protein Expression from Root Tips of *Gossypium hirsutum*

Oliver G.G. Knox[1,3], and Gupta V.S.R. Vadakattu[2,3*]

[1]CSIRO Land and Water, Locked Bag 59, Narrabri, NSW, Australia, 2390.
[2]CSIRO Land and Water, Gate 5, Waite Road, Urrbrae, SA, Australia, 5064.
[3] CSIRO Entomology, Gate 5, Waite Road, Urrbrae, SA, Australia, 5064.

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We investigated border cell numbers and levels of Cry proteins expressed in root tips, border cells and the mucilage of cotton seedlings. Border cell counts averaged 5 x 10^3 per radicle terminal, for the fourteen cultivars assayed, well below the previously reported 10^4 border cells for cotton root tips. Border cell counts for transgenic cultivars did not significantly differ from their transgenic donor or elite parents, with the exception of elite parent Sicot 189. Quantifiable ELISA detected expression of both Cry1Ac and Cry2Ab proteins from border cells, mucilage and root tips of all tested transgenic lines indicating that Bt cotton varieties could exude Cry proteins into the soil environment.

In transgenic cotton (*Gossypium hirsutum*), expression of the Bt genes Cry1Ac and Cry2Ab from the soil-borne bacterium *Bacillus thuringiensis* provides effective control against lepidopteran pests. However, the development of transgenic cotton cultivars is not a simple case of just inserting the insecticidal genes. The process involves several back-crossings of a transgenic donor with an elite non-transgenic cultivar, with continuous screening for desirable traits, such as yield, insecticidal protein expression and disease resistance. We investigated border cell production as a property of root architecture that might have been altered during development of transgenics. Border cells are terminally differentiated individual or small groups of cells that detach from the root cap (2). The significance of border cells in the environment is their production of several cell-specific proteins and signal molecules, which influence the direction of root growth, soil chemistry and plant-microbe interactions in the rhizosphere, and may contribute the majority of carbon-rich exudates released from roots into the soil (7).

By providing the desired insecticidal control (3), transgenic cotton can decrease the use of chemical insecticides considerably, thereby helping to develop more sustainable farming systems with reduced non-target environmental impacts (1). However, the majority of research on the expression of Cry proteins in cotton crops has concentrated on expression in above-ground plant tissue, and there is limited experimental information available on below-ground expression (4). Additionally, the below-ground implications of the presence of the transgenic proteins are largely unknown due to the complexity of soil ecosystems (8) and limited information on their accumulation and persistence in soil (10). We undertook an examination of Cry protein expression from roots on a morphological basis, using ELISA to detect and quantify Cry1Ac and Cry2Ab in root fractions. Acid delinted cotton seed was surface sterilised with an ethanol (50% v/v) and bleach (0.4 % m/v available chlorine) 3 min wash procedure, the seed was germinated and mucilage, border cells and homogenising root terminals recovered from twenty 72 h old radicles.

To establish the number of border cells produced by cotton cultivars we germinated surface sterilised seedlings, placed individual emerging radicles in 1 mL of water, allowed it to imbibe for 5 min before applying gentle agitation with ten repeated draws and returns of a 200 μl pipette, the 1 mL of solution of liberated border cells was transferred to a Sedgewick Rafter (Phyco-Tech) and counted under the compound microscope (200 times magnification).

Results demonstrated that root tips, mucilage and border cells produced detectable levels of Cry1Ac and Cry2Ab (Table1). The release of Cry1Ac protein by roots of two week old seedlings of transgenic cotton cultivars Sicot 289 Ingard® and 289 Ingard® Roundup Ready® was previously observed in solution culture experiments (5), although it has also been reported that the roots of cotton do not exude Cry proteins (9). Our

* Corresponding author. Mailing author : CSIRO Entomology ,PMB 2, Glen Osmond, SA, Australia, 5064. Tel: 61 8 8303 8579. Fax: 61 8 8303 8550. Email : gupta.vadakattu@csiro.au

results indicate that there is potential for commercial cotton cultivars to release Cry proteins from their roots and, due to differences in expression levels between varieties, they may serve to highlight the need to assess Cry protein exudation on a case by case scenario.

Border cell counts demonstrated differences between cultivars but, with the exception of elite parent Sicot 189, none of the transgenic derived cultivars differed significantly from either parent (Figure 1). This suggested that insertion of the transgenic material did not appear to have a significant impact upon border cell production. However, our results produced an average of 5000 border cells per root tip, considerably lower than the 10000 previously reported for cotton (6). The significance of difference in cotton border cell numbers is currently unknown.

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TABLE 1. Mean and standard deviation of quantified levels of Cry1Ac and Cry2Ab expression in ppb from five transgenic cultivars as detected by ELISA on root fractions from 20 seedlings.

		Mucilage		Border cell		Root	
		Mean	SD	Mean	SD	Mean	SD
Cry1Ac	**289B**	2	*1*	20	*7*	563	*366*
	289BR	17	*9*	18	*10*	481	*325*
	DP50BGII	11	*7*	11	*6*	1256	*808*
	41BR	50	*25*	15	*11*	1879	*754*
	71BR	170	*55*	156	*94*	2228	*852*
Cry2Ab	**289B**	7	*2*	19	*8*	453	*50*
	289BR	1	*1*	11	*2*	580	*158*
	DP50BGII	7	*2*	0	*0*	29	*9*
	41BR	3	*2*	17	*13*	857	*127*
	71BR	37	*3*	65	*8*	824	*43*

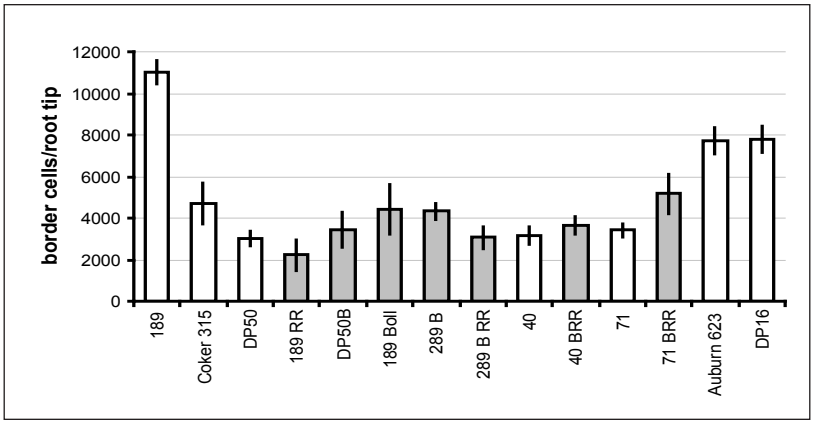

FIG. 1. Mean border cells counts from 72 h old cotton radicles for 14 cotton cultivars. Error bars represent the standard error of the mean. Shaded bars indicate transgenic cultivars.