ATTACK OF LEAF CURL VIRUS ON COTTON CROP IN PAKISTAN GENETIC ENGINEERING APPROACHES TO DEVELOP TRANSGENIC COTTON RESISTANT TO LEAF CURL VIRUS

Nasir Ahmad Saeed, Yusuf Zafar
Kauser A. Malik
NIBGE, Faisalabad, Pakistan
Jane Dever, Linda Koonce, Norma Trolinder
Biotex, Lubbock, TX

Abstract

Cotton Leaf Curl Virus (CLCuV) is a white fly (*Bemisia tabaci*) transmitted Gemini virus which caused significant losses to the cotton crop during the last three years in Pakistan. Production was reduced to 8.5 million bales in years1992-93 TO 1996-97 from 12.8 million bales in 1991-92. At present no virus resistant variety is available for commercial cultivation. Genetic Engineering approaches are being employed to develop transgenic CLCuV resistant plants. The CLCuV has been characterized and antisence viral constructs were made. Efforts are under way to develop transformation protocols for Pakistani cotton varieties. The present work is an attempt to develop these protocols which would be used for producing CLCuV resistant cotton plants in Pakistan.

Introduction

In Pakistan, Cotton is grown on an area of 2.835 million hectares with a total production of 12.8 million bales (Anonymous 1991-92). But the production was reduced to 8.5 million bales during the last three years due to attack of Leaf Curl Virus (CLCuV). Cotton is the main cash crop and foreign exchange earning (> 60 %) product and in fact is the back bone of the economy of the country.

The rate of infection varies with different varieties/ germplasm lines (Table -1) but no completely resistant source is available which could be used for crossing to other commercial varieties. Alternative approaches are being adopted to develop transgenic virus resistant cotton plants.

Cotton Leaf Curl Virus (CLCuV) is a whitefly (Bemisia tabaci) transmitted Gemini virus (Mansoor et el 1992; Hameed et al 1994). Studies were made at NIBGE, Faisalabad, Pakistan to characterize and sequence (Kauser et al 1995) the virus and antisence viral constructs were cloned into pBIN19 vectors for use in plant transformations. For development of regeneration protocols 19 Pakistani/exotic varieties of cotton were tested (Nasir et el 1994).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1508-1509 (1997)
National Cotton Council, Memphis TN

In order to develop cotton regeneration and transformation protocols, work is being done in collaboration with BIOTEX (Trolinder and Goodin 1987). The present work is the development made so for in Agrobacterium-mediated transformation work. Further work is in progress for development of Leaf Curl Virus Resistant cotton in Pakistan.

Materials And Methods

Agrobacterium-mediated Transformation of Cotton.

Varieties: S-12, Siokra 1-3 and Coker 312 (as control)
Plant material: Hypocotyl sections of 7-10 days old seedlings.
Bacterial strains: a) AGL1 (pBIN19GUSINT)
b) LBA4404 (pBI121) c) LBA4404 (without any Ti-plasmid)
Media Compositions:
Seed germination: MS + 30 g/l glucose + 7.5 g/l agar
Bacterial Inoculation: MS + 30 g/l glucose + 2.5 g/l phytagel + 0.75 g/l $MgCl_2$.
Selection media: MS + B_5-Vit. + 0.1 mg/l 2,4-D + 0.5 mg/l Kin. + 30 g/l glucose + 2.5 g/l phytagel + 0.75 g/l $MgCl_2$ + 50-100 mg/l Kanamycin + 250 mg/l Cefotaximine.
Suspension culture: MS + 1.9 g/l KNO_3 + 30 g/l glucose
Plant Development: Vermiculite saturated with SH media + 0.1 mg/l of each of the IAA, Kin and GA_3.
Incubation Conditions: Hypocotyl sections dipped in bacterium culture (108 cells/ml), gently shaked, blotted dry, placed on filter paper on callus induction media and incubated at 28 ^{0}C for three days and then transferred to selection medium under 16/8 hours photoperiod.
Plant development: Subcultures were made after 4-6 weeks. Embryonic calli were transferred to liquid media and shaken for 5-6 weeks. Suspension cells were transferred to embryo maturation media and mature embryos were transferred to vermiculite for plant development.

Results and Discussion

Histochemical GUS staining after 48 hours of cocultivation with LBA4404 (pBI121) showed 70 % of the explants (Var. Coker-312) with blue expression on the edges of the cut surfaces. AGL1GUSINT also gave 85 % transient expression on Coker-312 and Siokra 1-3 varieties. This expression is lower with S-12 variety. Forty eight hours co-cultivation time is optimum for good expression. Extended cocultivation favored the overgrowth of bacteria which was difficult to control especially in the later stages of callus growth. Hypocotyl sections taken from 8-10 days old seedlings gave good expression as compared to older tissues. Pre-culturing of tissues before agro-inoculations did not significantly increase the expression. After 10-15 days of co-cultivation, tissues started producing callus at the edges; while no callus grew on control tissues (on kanamycin) or from tissues inoculated with LBA4404 with out any plasmid. Number of independent transformation events varied from 0-5 per explant. Over all 25 %

transformation efficiency was recorded in Coker-312 variety. Hypocotyl tissues cultured on normal callus induction media without any antibiotics produced callus on all wound sites. In the initial stages, callus formation started at the same rate from all the varieties but later on after 45 days embryogenic callus was seen only on Coker-312 and Siokra 1-3 varieties. S-12 did not produce any somatic embryos. Coker-312 and Siokra 1-3 varieties produced GUS positive callus. Suspension cultures gave somatic embryos which in the later stages formed complete plants.

Regeneration of cotton is a highly genotype dependent phenomenon (Trolinder and Goodin; 1987) which limits the application of *Agrobacterium*-mediated transformation. Efforts are under way to get regeneration from recalcitrant cotton varieties. Alternatively regenerable cotton can be transformed and then crossed with commercial varieties for transfer of useful traits. The other possible way is to try novel genotype independent transformation systems (Oral presentation by Dr. Linda Koonce).

Acknowledgements

This research was supported by Ministry of Food, Agriculture and Co-operatives, Food and Agriculture Division, slamabad, Pakistan.

References

Anonymous. 1991-92. Agricultural Statistics of Pakistan. Ministry of Food, Agriculture and Co-operatives. Food and Agriculture Division (Economic Wing), Islamabad, Pakistan.

Hameed, S., Khalid, S and Harrison B. D. 1994. State of CottonLeaf Curl Disease in Pakistan. In: World Cotton Research Conference-1. Brisbane, Australia, Feb. 14-17, p 95.

Kauser A. Malik, Mansoor ,S., Nasir A. Saeed, Asad, S., Zafar, Y., John Stanely and Peter Markham. 1995. Development of Leaf Curl Virus resistant Cotton varieties through Genetic Engineering. Paper presented at National Seminar on Cotton: Strategies for increasing cotton production, April 26-27. Published by Research Information Section, AARI, Faisalabad, Pakistan.

Mansoor, S., Bedford, I. D., Pinner, M. S., Stanley, J., and Markham, P. G. 1993. A whitefly transmitted geminivirus associated with cotton leaf curl disease in Pakistan. Pakistan J. Of Botany 25: 105-107.

Nasir A saeed, Zafar Y and Kauser A. Malik. 1993. Development of in-vitro techniques for transformation of cotton. In: Proceedings of International Symposium on Biotechnology for Sustainable Development. Dec 15-20, held at National Institute for Biotechnology & Genetic Engineering, P.O.Box 577, Faisalabad, Pakistan.

Trolinder N. L. And Goodin, J. R. 1987. Somatic embryogenesis and plant regeneration in cotton (G. Hirsutum L.). Plant Cell Reports 6: 231-234.

Table 1. Infection of Leaf Curl Virus (CLCuV) on different Pakistani cotton varieties/lines at NIBGE campus, Faisalabad, Pakistan during year 1995 and 1996.

	Name of var./line	% Infection	
		Year 1995	Year 1996
1.	NIAB -78	40	45
2.	S -12	80	90
3.	SLS -1	10	20
4.	CIM -109	20	15
5.	NIAB -26	20	43
6.	B -557	50	33
7.	Karishma	30	80
8.	BH -36	70	50
9.	RH-1	80	40
10.	SLH - 41	41	35
11.	FH -87	80	70
12.	S -14	90	80
13.	NIAB -86	30	50
14.	MNH-93	70	64
15.	CIM -240	60	40
16.	V3NCVT	00	20
17.	AEM -185	80	65
18.	AEM -52	60	60
19.	AENS 18/87	50	55
20.	RAVI (Desi)	00	10