



## IMPROVED BOTTLE SHOOT GRAFTING TECHNIQUE / METHOD FOR THE TRANSMISSION OF COTTON LEAF CURL VIRUS (CLCUV)

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An artificial inoculation technique for the screening of cotton germplasm against cotton leaf curl virus (CLCuV) is described in this article. Present technique is improved shape of bottle shoot grafting method and is equally successful in field under high temperature conditions. The technique is simple and faster. Success of grafting and disease transmission was 100% in our experiments.

**Key words :** Cotton, CLCuV, Improved grafting method, High temperature, Field conditions, *Gossypium hirsutum* L..

### 1. Introduction

Cotton (*Gossypium hirsutum* L.) is the major cash crop having vital importance in the economy of Pakistan. In 1991-92 Pakistan achieved a record production of 12.8 million bales which further declined to 7.9 million bales in 1994-1995 [1]. It was estimated that more than seven million bales have been lost during the last decade [2]. This down ward trend in production was mainly due to cotton leaf curl virus (CLCuV) [3-4]. Resistant varieties are the only permanent solution of the problem but for the successful breeding program reliable screening technique is a prerequisite [5]. Field observations indicated that most of the commercially grown cultivars were susceptible to CLCuV but there were different grades of tolerance in different varieties. Those, thought to have some tolerance, eventually seemed to lose it. Screening procedure was only relied upon to expose the cotton germplasm to high inoculum pressure by planting in natural hot spots. In the absence of a uniform technique no meaning full results could be drawn [6]. Grafting may succeed in transmission of a virus where other methods fail [7]. Ali *et al.* [8] described four methods *i. e.* side grafting, petiole & cleft grafting, budding and bottle leaf grafting method for the transmission of CLCuV. Most researchers followed these methods and found that the bottle leaf grafting method is quicker and reliable ( especially for old plants). But they

observed it bit laborious and found that it does not work in high temperature because of quick evaporation from the bottle / test tube. Due to this it can not be adopted for screening on large scale (under field conditions) [5-8]. Secondly the old method was not properly discussed in literature for its materials and method. Keeping in view the reliability of bottle leaf grafting technique developed by Mirza [9], present study was carried out to improve this technique for large scale screening of cotton varieties against CLCuV under high temperature conditions.

Materials used for this technique were cotton plants (healthy & CLCuV infected), scalpel (with blade), parafilm, polythene sheet, test tubes, fine string (to tie the test tube with grafted stem), distilled water and beaker.

### 2. Grafting Method

Six week old plants were selected for grafting and one sliced cut of 1 to 2 cm long and 0.1 to 0.2 cm deep was made on the stem near 2nd last internode of test plant. A CLCuV infected branch with growing tip, about 20 cm long was detached from diseased plant (maintained culture). A similar cut (as on test plant) was made on this branch and corresponding cut surfaces were brought together and tied with parafilm to avoid drying and to stop the entry of air (Figure 1). Care was taken to bring

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the corresponding cambium surfaces into contact. This stem was then placed in a test tube of 2 cm diameter and 16 cm length, filled with distilled water. Distilled water was changed daily at an interval of 12 to 13 hours for five days (according to the requirements). After five days these tubes were removed, plants were watched daily to

observe its success. An other experiment following same material and methods was also laid down. The only difference was the use of polythene sheet instead of parafilm to tie the grafted portions.

It was observed that the improved method is excellent under high temperature conditions.

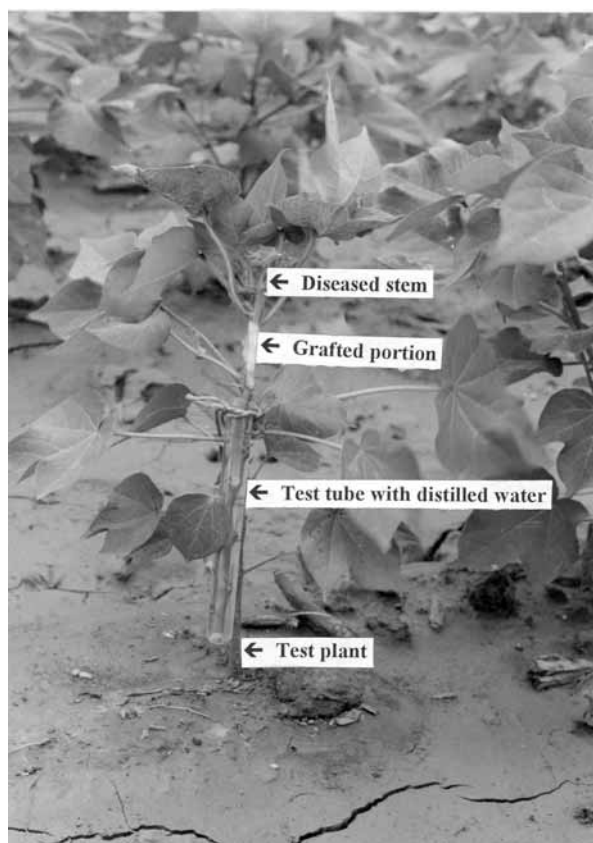


Figure 1. Improved bottle shoot grafting method.

Table 1. Response of improved grafting method for its success and disease transmission in field under high temperature conditions against cotton leaf curl virus (CLCuV).

Plant No.	Grafting date	Temp. on grafting day (°C) *	Grafting success**	Disease transmission	Time taken for disease appearance (Days)
1	16/7/1999	40.5	+	+	14
2	16/7/1999	40.5	+	+	14
3	16/7/1999	40.5	+	+	15
4	16/7/1999	40.5	+	+	16
5	16/7/1999	40.5	+	+	14
6	16/7/1999	40.5	+	+	15
7	16/7/1999	40.5	+	+	16
8	16/7/1999	40.5	+	+	15
9	16/7/1999	40.5	+	+	16
10	16/7/1999	40.5	+	+	15
Average		40.5	100%	100%	15

\* Average max. temperature during the 1st 15 days of grafting was 37.73°C.

\*\* Success of grafting was "+" when grafted stem (scion) survived for more than 10 days after grafting.

Note: Grafting was successful till the end of the crop.

Disease transmission and success of grafting was 100% (Table 1). Possible primary reason for the 100% survival rate under high temperature is the use of narrow necked test tube, which reduced the evaporation rate of water. Observations showed that during the 1st three days of grafting, scion needs more water and if no more water is added, the grafted stem will utilize the whole water present in the tube and as a result grafting may fail or took more time to recover. It is therefore, suggested that for an excellent results addition of distilled water at least for 1st three days is necessary. In case of second experiment success of grafting and disease transmission was same as in case of 1st experiment but the observed difference was that the grafted portion tied with polythene sheet becomes sunken that ultimately affects the growth of graft union. Graft union becomes depressed and may girdled in most of the cases. While in case of parafilm the growth of the graft union was normal. Reason was the expansion of parafilm with the growth of graft union, as a result parafilm expired after 10 to 15 days without injuring the grafted portion.

#### References

- [1] Economic survey, Government of Pakistan, Finance Division Islamabad, Pakistan, 1996-1997.
- [2] T. Mahmood, The Pak. Cottongrower. **3** (1999) 17.
- [3] S. S. Hameed, S. Khalid, Ehsan -ul- Haq and A. A. Hashmi, Plant Dis. **78** (1994) 528.
- [4] T. Hussain, M. Tahir and T. Mahmood, Pak. J. Phytopathol., **3** (1991) 57.
- [5] F. Muhamad, A. H. Tariq, J. Ihsan and A. Saleem, Pak. J. Phytopathol, **10** (1998) 18.
- [6] H. Shah, S. Khalid and S Hameed, Response of cotton germplasm to cotton leaf curl virus. Proceedings, ICAC- CCRI, Regional consultation insecticide resistance management in cotton. Multan, Pakistan, June 28 to July 1, (1999) 250 .
- [7] R. E. F. Mathews, Methods of transmission and infection in: Plant Virology, Academic Press, New York and London, (1970) p. 778.
- [8] M. Ali, Pak. J. Phytopathol. **9** (1997) 1.
- [9] M. S. Mirza, Virus problem in cotton and its control, Proceedings of National Seminar on Cotton Production at Ayub Agric. Res. Inst., Faisalabad on April 30, (1992).