Interspecific hybridization for transfer of cotton leaf curl disease resistance in Upland cotton

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Donors for CLCuD resistance

G. armourianum (DD)	Synthetic Allotetraploid	*G. arboreum* (AA)
		

Donor 1: *G. armourianum*

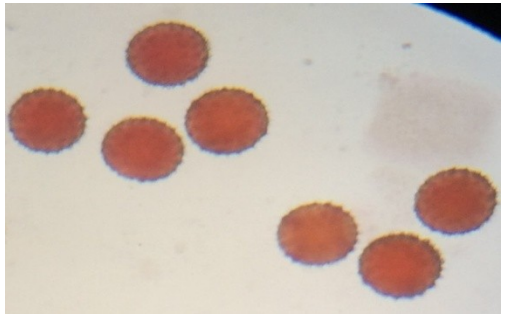
Development of interspecific F_1 hybrid

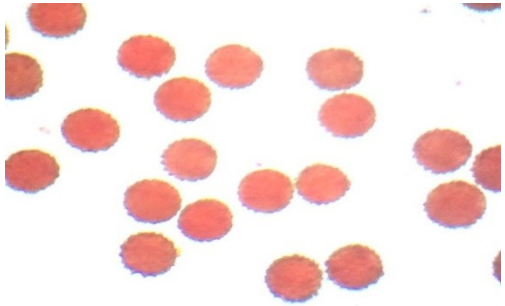
G. hirsutum x *G. armourianum*

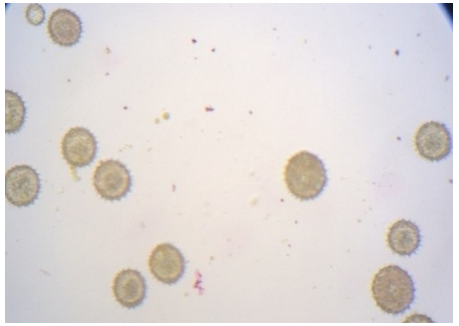
No crossability barrier

Pollen fertility

Genotypes	Total number of pollens observed	Number of unstained pollens	Number of stained pollens	Per cent fertility	Avg. pollen size (µm)
G. hirsutum	1220	70	1150	94.26	117
G. armourianum	1308	44	1264	96.63	103
Interspecific F_1 hybrid	1235	1208	27	2.18	70







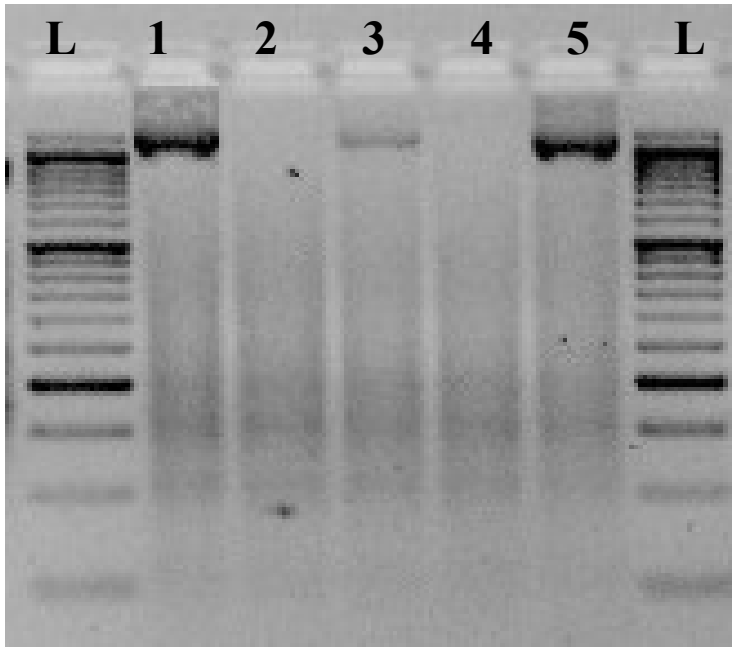
Hairiness

G. hirsutum
(Hairy)

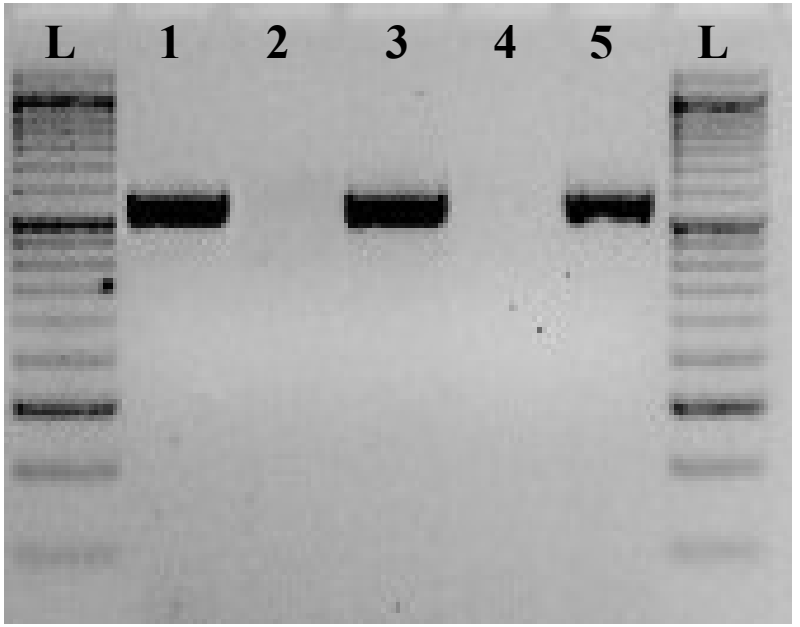
F_1 Hybrid
(Glabrous)

G. armourianum
(Glabrous)

Molecular confirmation of CLCuV



DNA Beta: Briddon *et al* (2002)



Av Ac primers: Wyat and Brown (1996)

1- positive control, 2- *G. armourianum*, 3- Triploid F_1 hybrid, 4- *Desi* cotton, 5- Synthetic allotetraploid

Development of BC_1F_1 through hybridization between F_1 hybrid (*G. hirsutum* x *G. armourianum*) and *G. hirsutum*

No. of pollinations	Seeds obtained	Seeds germinated
15898	5	4

4 BC_1F_1 plants

- 2 resistant to CLCuD
- 2 susceptible to CLCuD

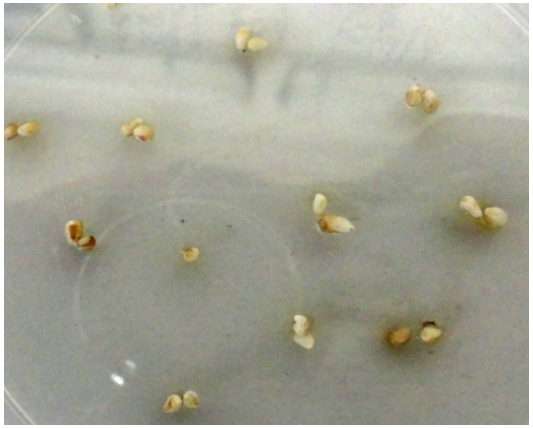
Ovule Culture – Media used

- **M1 media having MS+ IAA @1.0 mg/l + Kinetin @ 0.2 mg/l (Gill and Bajaj 1984)**
- **M2 media consisting of MS + Gamborg's B5 vitamins + 1.9 g/litre KNO_3 (Sacks 2008)**
- **And their various modifications were tried**

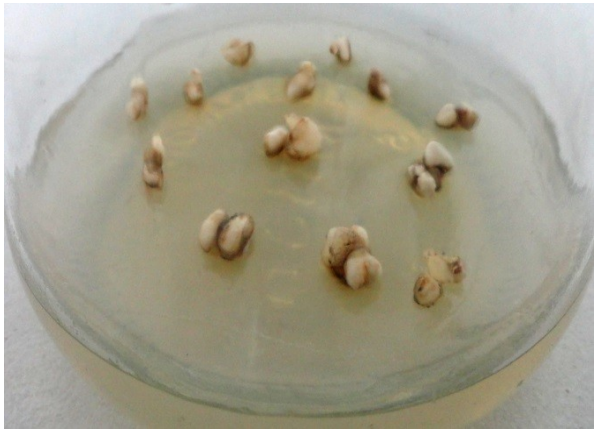
Modified Media used

Name	Components of medium
M3	MS
M4	MS + TDZ @ 0.1 mg/l
M5	MS + TDZ @ 0.2 mg/l
M6	MS + TDZ @ 0.2 mg/l + 1.9 g/l KNO_3
M7	MS + IAA @ 1.0 mg/l + Kinetin @ 0.2 mg/l + CH (250 mg/l)
M8	MS + malt extract @ 0.5 g/l + adenine @ 25 mg/l + sucrose @ 50 g/l
M9	MS + malt extract @ 0.5 g/l + sucrose @ 50 g/l
M10	MS + BAP @ 3 mg/l + malt extract @ 0.5 g/l + sucrose @ 50 g/l
M11	MS + Kinetin @ 1 mg/l + malt extract @ 0.5 g/l + sucrose @ 50 g/l
M12	MS micronutrients (except Fe), MS vitamins (nicotinic acid, pyridoxine-HCl, thiamine-HCl), KOH, $MgSO_4 \cdot 7H_2O$, Fructose 1,6-diphosphate monocalcium salt, Casein hydrolysate, NH_4NO_3, Malic acid, FeEDTA, $CaCl_2 \cdot 2H_2O$, Ammonium citrate dibasic, NH_4Cl, Myoinositol, Sucrose, Melibiose, Arabinose, Glucose, Proline, Serine, Alanine, Arginine, Glycine, Leucine, Asparagine, Methionine, Glutamic acid, Aspartic acid, Glutathione. (Fuller *et al* 2011)

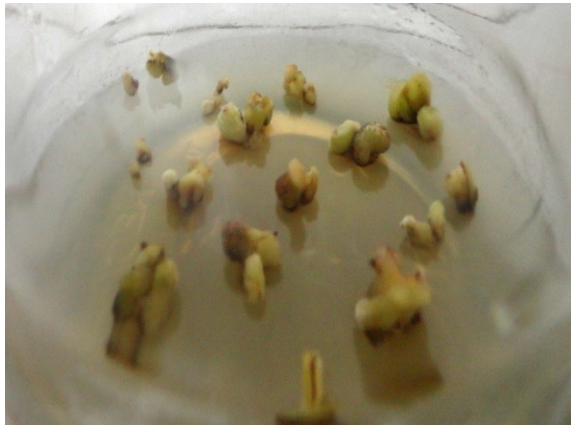
Cross	Number of pollinations made	Number of bolls picked for culturing 3 DAP	Number of ovules cultured
F_1 x F 1861	140	121	2420
F_1 x LH 2108	145	129	2580
F_1 x F 846	117	99	1980
F_1 x PIL 8	117	103	2060
F_1 x PIL 43	126	109	2180
F_1 x F 2164	109	94	1880
F_1 x LH 900	130	111	2220
F_1 x LH 1556	109	98	1960
F_1 x LH 2107	104	87	1740
F_1 x LH 2076	98	71	1420
Total	1195	1042	20440



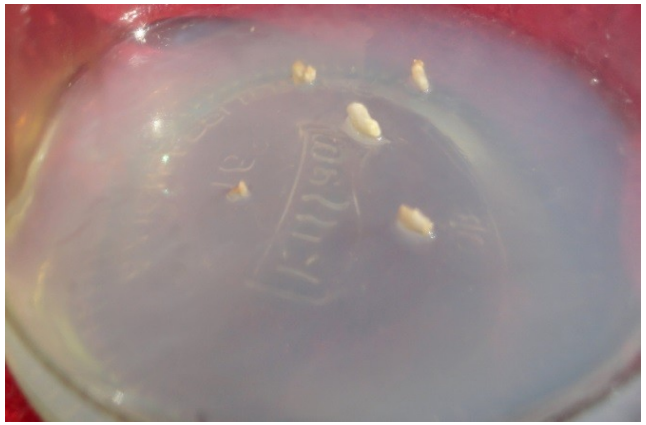
One week old ovules



One month old ovules



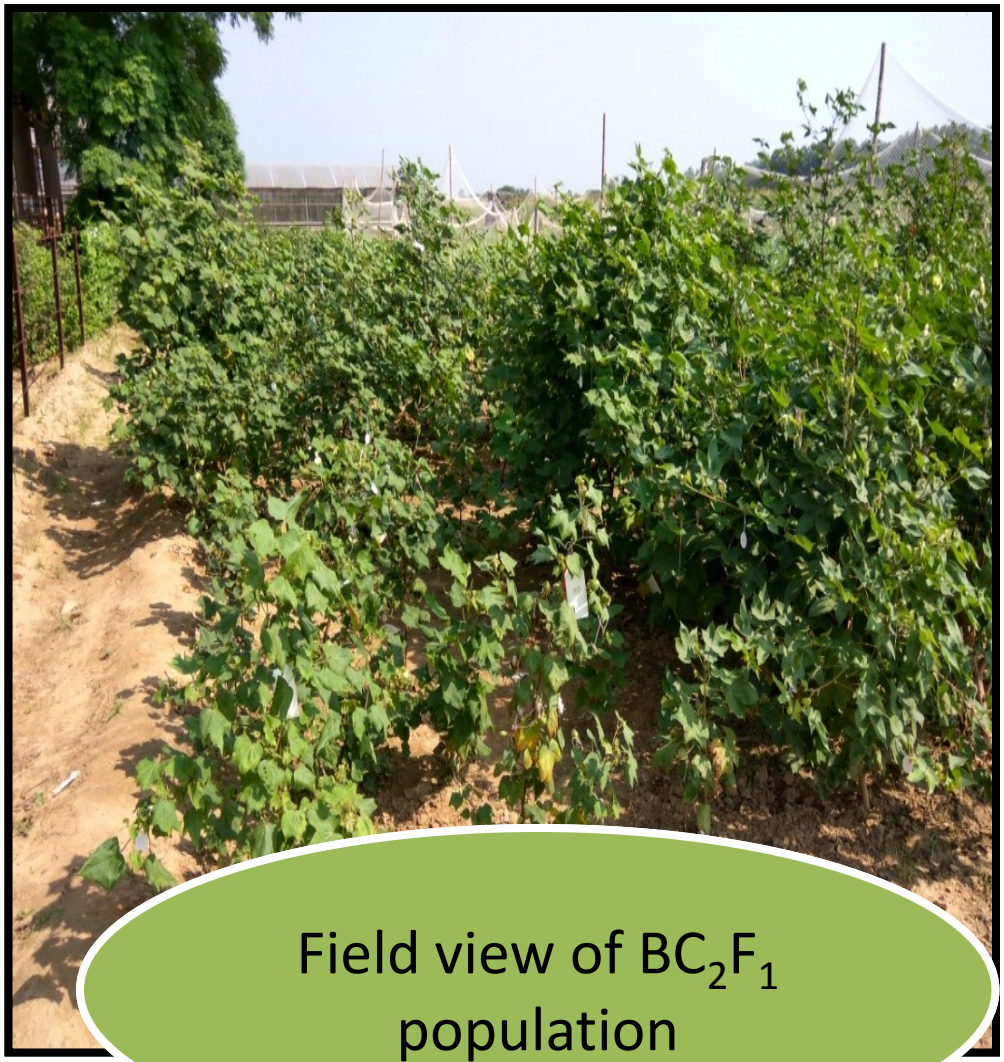
Three months old ovules



Dead and atrophied embryos excised from three months old ovules

Development of BC_2F_1 population

Plant No.	No. of flowers pollinated	Seeds obtained/ sown	Seeds germinated	Per cent germination
1 (S)	228	800	167	20.8
2 (R)	464	1551	129	8.7
3 (R)	306	50	0	0
4 (S)	79	60	0	0

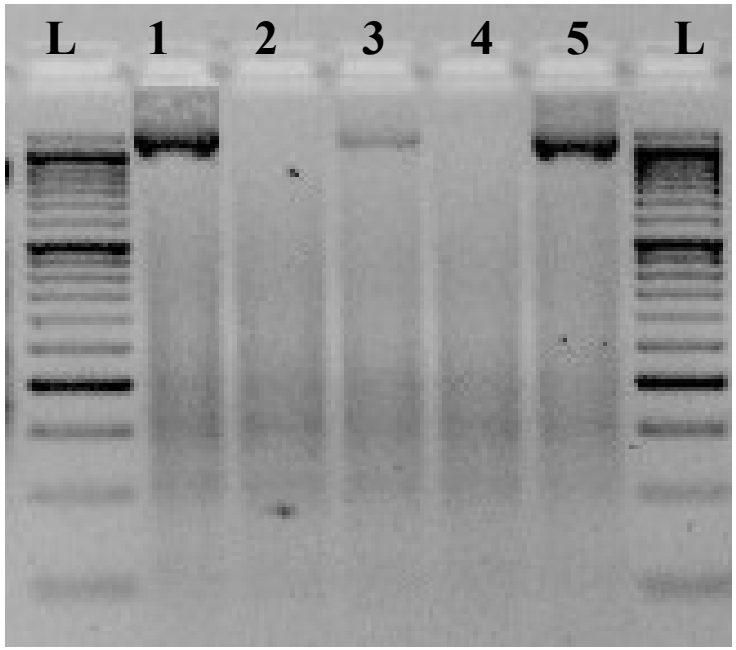


Field view of BC_2F_1 population

Plant resistant to CLCuD

Donor 2: Synthetic Allotetraploid

Molecular confirmation of CLCuV



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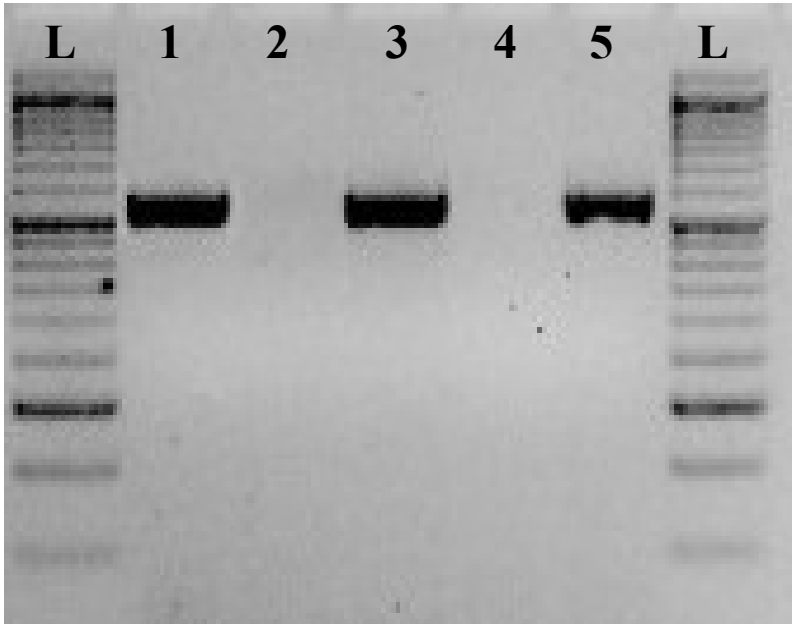

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Maximum boll size attained from Synthetic Allotetraploid x *G. arbor*

✓**Flowers pollinated: 3158**

✓ **Mature crossed bolls obtained: 28**

✓**Seeds obtained: 25**

Crossed bolls retained on the Synthetic amphidiploid

Opened bolls containing F_1 seeds

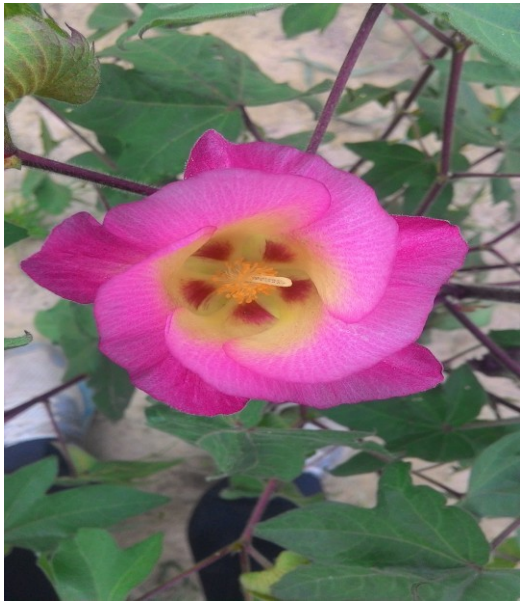
F_1 hybrids between *G. hirsutum* Acc. PIL 43 x Synthetic amphiploid

Back crosses : (a) F_1 (SA x PIL 43); (b) PIL 43 x SA

Backcross	Flowers pollinated	Bolls set	Per cent setting	Seeds obtained
				1868
F_1 x PIL 43	2173	15	0.69	
PIL 43 x F_1	5261	92	1.74	

Field view of BC_1F_1 population

Donor 3: *G. arboreum*



Many Thanks