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Introgression of pink bollworm resistance from wild *Gossypium thurberi* Tod. to cultivated *Gossypium arboreum* L. cotton: pre-breeding efforts

S. S. Mehetre*, J. M. Patil and S. B. Kharbade

Directorate of Research, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722, India

Pink bollworm (PBW, *Pectinophora gossypiella* Saund.) is a pest of economic importance in cotton producing countries. Earlier results of host-plant resistance to the PBW indicated races of *Gossypium hirsutum* L. and some wild species including *Gossypium thurberi* Tod. which are resistant to PBW. Hence prebreeding efforts were made to transfer PBW resistance from *G. thurberi* to *G. arborum*. F_1, F_2 and BC_1 populations along with parents were studied for morphological, boll anatomical characters and infestation by PBW larvae. The F_1, F_2 and BC_1 generations were distinct from both the parents for all morphological characters. The highest boll toughness was found in *G. thurberi*, followed by F_1, whereas it was lowest in *G. arboreum*. Significant negative correlation was found between toughness with locule damage by PBW larvae. Promising prebreeding material is identified which will be excellent source of developing lines combined with PBW resistance, fibre strength and drought tolerance.

Keywords: *Gossypium hirsutum* L., *Gossypium thurberi* Tod., *Pectinophora gossypiella*, pink bollworm, wild species.

COTTON fibre is an important raw material for the textile industry. Of the several pests attacking cotton pink bollworm (PBW), *Pectinophora gossypiella* Saunders is a serious pest of substantial economic importance in cotton producing countries[1].

Several species of *Gossypium*[2,3] including its taxonomic races are resistant to this pest. The presence of unidentified antibiotic factors in certain strains of upland cotton[4] provides significant resistance to PBW[3].

PBW infestation in the bolls of *G. thurberi* (*Thurberia thespesioides*) was lower than 4% as against 100% in cultivated cotton grown nearby[5]. *G. thurberi* has been reported to be either free from the attack of PBW or its complete absence in Trinidad, Brazil and in the Anglo-Egyptian Sudan, although the neighbouring commercial cotton had 97% attack[6]. Immunity of *G. thurberi* and its hybrid *G. arboreum* × *G. thurberi* is due to some repellant scent present especially in the petals, that prevents the oviposition by the moth on the plant[7] in addition to other characters, viz. smallness of bolls, smoothness of boll surface, repellant and unpalatable principle in the seed[7,8]. Bolls of *G. thurberi* were essentially ignored by the PBW when grown near a commercial cultivar of *G. hirsutum*[5] probably because of the extremely small bolls of *G. thurberi*, that increased mortality and extremely slower growth rates that were due to lack of food. Further, the resistance in *G. thurberi* is due to tight-fitting calyx with triangular lobes and flared bracts causing either escape or non-preference. In addition to *G. thurberi*, *G. stocksii* showed similar resistance but had very small bolls[5].

Despite several attempts made by the cotton breeders to incorporate PBW resistance of *G. thurberi* in the new world cottons, limited success has been reported[9]. In an attempt to transfer bollworm immunity from *G. thurberi* to upland cottons[10], a synthetic tetraploid F_1 *thurboreum*,

*For correspondence. (e-mail: subhashmehetre@rediffmail.com)

i.e. doubled (*G. thurberi* × *G. arboreum*) was crossed to *G. hirsutum*[10]. An enormous amount of variability was noticed in the F_2 for morphological, physiological and economical characters[9].

Even though wild cottons with smaller bolls than *G. thurberi* were found attacked by PBW, both *thurborsutum* (*G. thurberi* × *G. hirsutum*) and *thurborense* (*G. thurberi* × *G. arboreum* × *G. barbadense*) F_1s were highly susceptible similar to those of cultivated cottons[11]. The F_1 hybrids *thurbadense* (*G. thurberi* × *G. barbadense*) and *armadense* (*G. armourianum* × *G. barbadense*) obtained by crossing[12–14] wild species of *G. thurberi* and *G. armourianum* respectively, to commercial cotton variety Sakel of *G. barbadense* were found to be resistant[12–14].

Efforts were made to establish host plant resistance[2] that included cultivars of old-world Asiatic cultivated species *G. arboreum* and *G. herbaceum*, and races of *G. hirsutum*[15]. Interspecific hybrids *G. hirsutum* × *G. tomentosum*[16] and *G. arboreum* × *G. thurberi* × *G. hirsutum*[5], intraspecific hybrid *G. hirsutum marie-galante* and Demetor-15 (ref. 16); wild species, viz. *G. thurberi*[2,3,16–18] and *G. trilobum*[2], *G. armourianum*[2] and *G. somalense*[2] showed appreciable resistance. Apart from wild species, races, viz. *palmeri* and *marie-galante* of *G. hirsutum*[16] are also resistant to PBW.

Antibiosis[19] type of resistance found in *G. hirsutum* race *marie-galante* to PBW is genetically controlled and is of partial dominance in nature[16]. In this, cotton proliferation of boll tissue after injury by boll worm larvae was found responsible for larval mortality. Exudation of the proliferated tissue from the larval hole and deposition masses of such tissue on external and internal surface of the boll was observed[16].

Several morphological characters[20–24] have been evaluated extensively since 1977. These include nectarilessness[18–25] and okra leaf[21]. Reduced insect numbers and amount of seed damage was reported in glabrous and nectariless as compared to the normal nectaried pubescent variety[25]. In contrast, high plant hair density is also reported to be a promising mechanism for resistance to the PBW in cotton. Nectarilessness, i.e. the absence of nectaries on leaves, bracts and flowers imparts a low but consistent level of resistance to the PBW[26,27]. Even though the glabrous or smooth plant character is less preferred for oviposition, these cottons are not resistant to the PBW[28].

The genetic variability for PBW resistance is rather limited among the available commercial cultivars of upland cotton as no resistance to PBW was noticed[29]. Whatever, the stocks of cotton resistant to PBW is usually inferior to commercial cultivars in agronomic and fibre properties[30]. 'Okra leaf' cotton imparts resistance to PBW because of changed behaviour of the PBW because altered microclimate around the bolls. However, when this character was evaluated on different genetic backgrounds consistent results were not obtained[30].

An unidentified mechanism of resistance[26] was found in upland cotton, viz. Cocker, Foster 300 (American); Laxmi (Indian); genotypes, viz. T-86 mut × Delta pine G24-2 (a race stock × cultivar hybrid); AET-Br-2, AET-Br-2-8 and 7203-14-104. In addition to these, one breeding line AET-5 (derivative of triple hybrid material (*G. arboreum* × *G. thurberi*) × *G. hirsutum*) has shown consistent antibiosis[19] type of resistance[15,20,31] and *G. arboreum* and *G. thurberi* showed resistant reaction to PBW[5,32].

Even though considerable work on the chemical and morphological aspects of resistance has been reported earlier by several workers, very little attention has been paid towards improving the mechanical and physical aspects of resistance such as tissue toughness/stiffness of various plant parts and boll rind thickness that prevents insect feeding and oviposition[32]. Strength required for penetrance determines the extent of damage caused by insect pests during its feeding and hence the pests consider boll rind toughness and its thickness as a barrier or permissive factor in selection and preference of their food[32].

Variability for rind toughness of 20-day-old green bolls of the upland cotton genotypes is reported[33]. Bolls of the resistant varieties showed improved mechanical properties during early stages of growth[34–37]. Lesser damage by PBW was noticed due to increased boll rind thickness after spraying the growth regulators[38].

As reported earlier, cotton boll rind thickness imparts higher degree of resistance to *Earias fabia*[39,40] as it detracts the bollworm larvae in penetrating the cotton fruiting bodies[41–44] and hence it is considered as one of the important traits responsible for resistance to PBW. Genotype 'JK 97-LPS' had the thickest boll rind at three stages of development[44] while strains 'JK-276-4' and 'JK-260' also possessed bolls with thick rind as compared to susceptible cultivars like 'Laxmi' and 'Sharada' varieties. Negative and positive correlations between thicknesses of rind with the incidence of bollworms and yield respectively were established[45].

The pericarp of capsule/boll has both the sclerenchyma and the parenchyma tissues in variable proportions[46–48]. However, collenchyma walls become modified at successive growth stages and are differentiated into lignified secondary walls, thus changed into sclerenchyma cell[49–51]. Boll rind of tolerant genotypes, viz. JK-345-3-3, JK-119 and JK-276-4 had higher area and amount of mechanical tissues[52]. In contrary, the susceptible genotypes showed comparatively more area of parenchyma cells and intercellular spaces.

These results indicated the advantage of boll rind toughness and thickness as one of the criteria in breeding bollworm-resistant genotypes. These characters provide mechanical and physical resistance and are governed by the anatomical structures[53–56].

The experimental material consisted of *G. arboreum* var. MPKV GMS (Regn. No. INRG 03071 and Identity

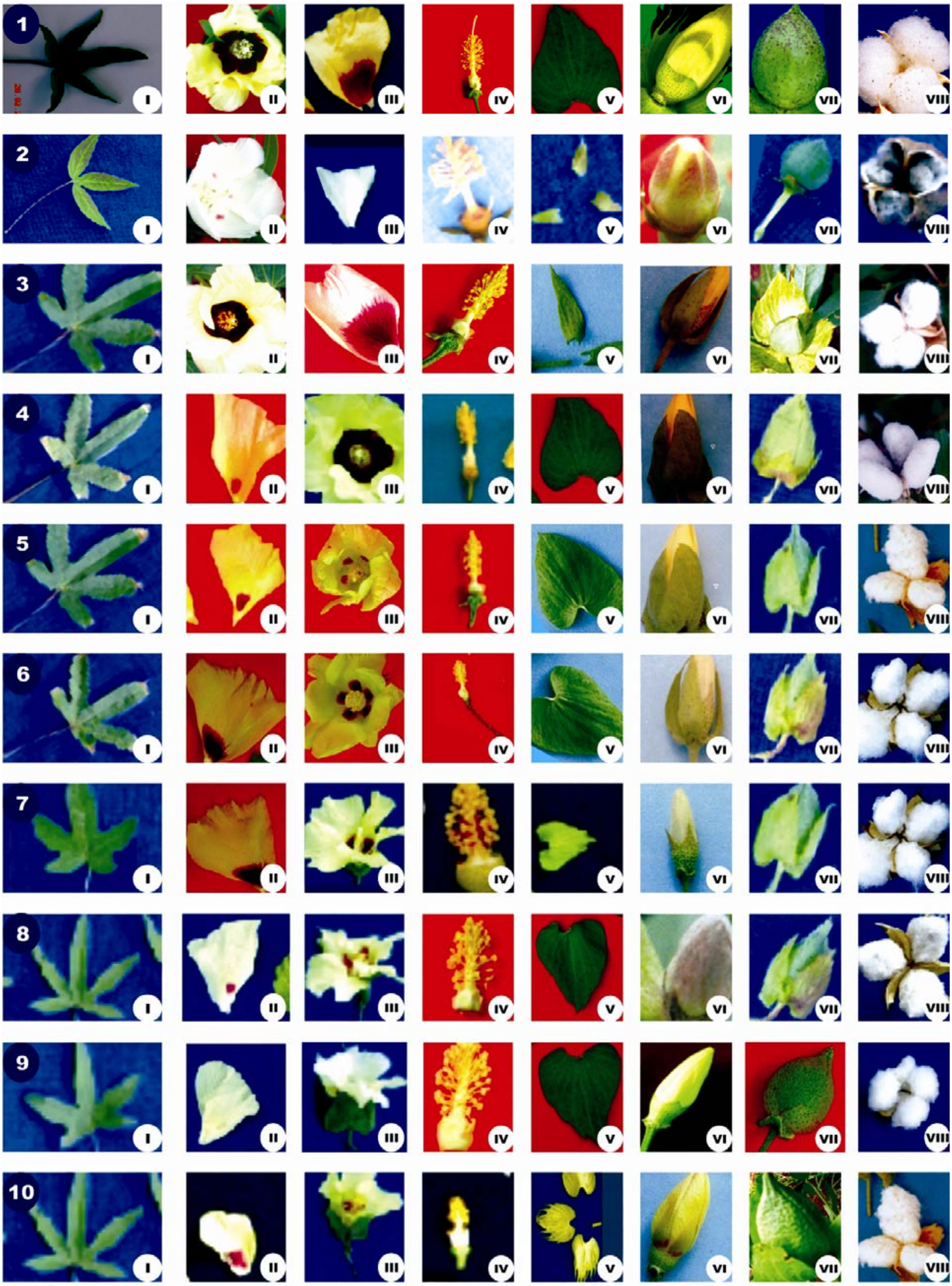

Figure 1. Morphological characters of leaf (I), floral parts (II and III), Androecium (IV), epicalyx (V), flower bud – a day before flower opening (VI), boll morphology (VII) and boll opening (VIII) in P_1, P_2, F_1, F_2 and BC_1. 1, *Gossypium arboreum* var. MPKV GMS; 2, *Gossypium thurberi*; 3, F_1 (*G. arboreum* var. MPKV GMS × *G. thurberi*); 4–7, F_2 (*G. arboreum* var. MPKV GMS × *G. thurberi*); 8–10, Backcross plants (F_1 (*G. arboreum* var. MPKV GMS × *G. thurberi*) × *G. arboreum* var. MPKV GMS).

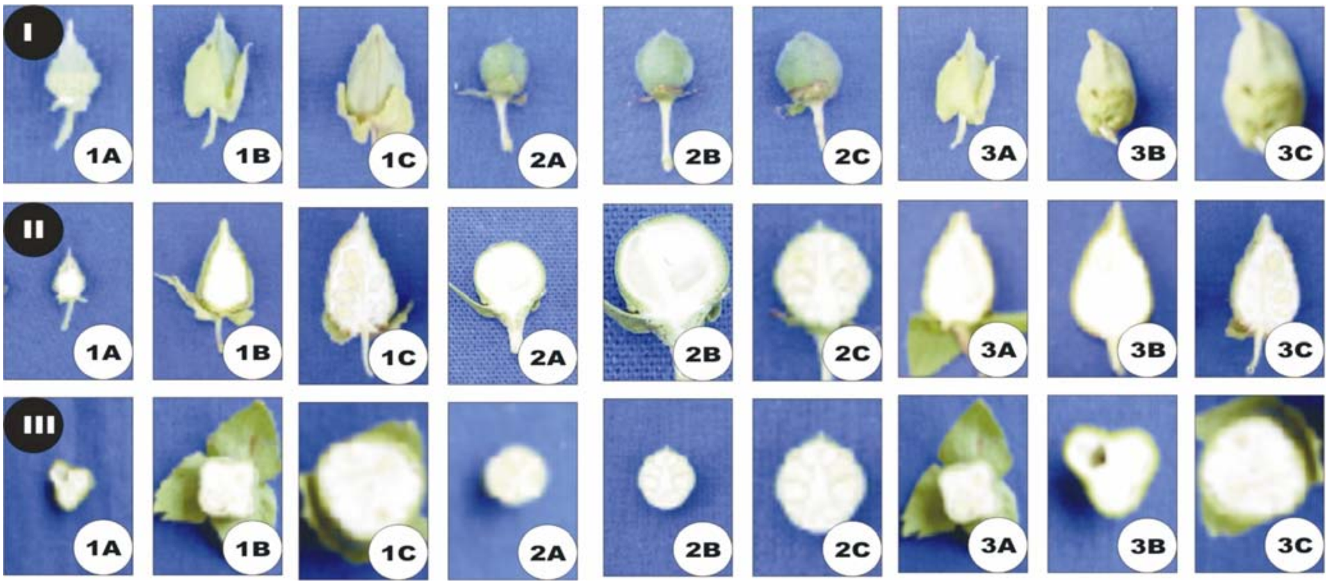

Figure 2 A–C. Observation recorded at **A:** 15, **B:** 30 and **C:** 45-day-old bolls after flower opening. **1–3**, Bolls of **1**: *Gossypium arboreum* var. × MPKV GMS (P_1); **2**: *Gossypium thurberi* (P_2); **3**: F_1 (*Gossypium arboreum* × *Gossypium thurberi*). **I–III**, I, Bolls of different ages; II, Longitudinal, and III, Cross section of the 15, 30 and 45-day-old bolls after flower opening.

No. IC 29676) (P_1); *G. thurberi* (P_2); F_1 hybrid (*G. arboreum* var. MPKV GMS × *G. thurberi*)[57]; F_2 and BC_1 populations of 40 plants and 15 plants[58] respectively.

The growth of boll development was critically monitored every day. The date of the appearance of the first flower bud was recorded for each generation. The period from the appearance of floral bud to anthesis was recorded critically. At the stage of 50% flowering, 100 flowers for non-segregating generation and 400 flowers for segregating generations were tagged. From the date of tagging 15 bolls for P_1 and P_2, 10 bolls for F_1 and BC_1 and 20 bolls for F_2 generation were sampled at 15, 30 and 45 days interval. The observation on boll toughness was recorded with the help of hardness tester at three locations of the boll, viz. upper, middle and lower portion measured in shore. The boll rind thickness was recorded by cutting boll vertically with the help of blade and the green portion of boll rind thickness was recorded with the help of scale in milimetre.

Procedure for ensuring a heavy attack of PBW in experimental material was adopted[59]. Green bolls were collected periodically and held in emergence containers for 2–3 weeks. Laboratory dissection and examination were used to determine the degree of damage to boll contents, and insect mortality and seed damage[3,60].

The parents *G. arboreum* (P_1), *G. thurberi* (P_2), F_1, BC_1 and F_2 generations were morphologically distinct (Figure 1). The lowest toughness of boll rind (6.80, 16.40, 33.67 shore) at all the three stages, i.e. 15, 30 and 45 days was observed in *G. arboreum* (P_1) followed by BC_1 generation (8.00, 18.17, 38.73 shore), F_1 (9.29, 19.53, 36.30 shore) and F_2 (12.40, 23.58, 36.56 shore). The highest toughness was recorded in P_2 (*G. thurberi*) (22.87, 30.40, 46.00 shore) with more cell compactness (Figures 2–5).

The PBW moth lays eggs in flower immediately after fertilization. First instar larvae emerges after 4–5 days, which is the most important stage for entry of larvae inside the boll. For this mechanical resistance of boll, rind toughness plays an important role. If the boll rind toughness is high, there is more resistance for penetration for PBW larvae. The highest boll rind toughness was recorded in *G. thurberi* at all three stages of boll development. The percentage of locule damage and the number of PBW larvae observed in 20 green bolls in *G. thurberi* were nil, which reveal its resistance to PBW larvae. This is confirmed on the basis of significant negative correlation found between toughness and locule damage[56]. The toughness of boll rind of *G. thurberi* is highest among the different generations at all three stages. The importance of boll rind thickness for penetration by larvae and boll rind toughness in resistance breeding is already emphasized. Similar results regarding mechanical and physical resistance based on anatomical structure have been reported[46,51–56].

It is revealed that the toughness of boll rind was lowest in *G. arboreum* amongst all the generations studied at 15 day boll age (6.80 shore) which was further increased at 30 day boll age (16.40 shore) and 45 day boll age (33.67 shore). However, the highest bollworm infestation was recorded in *G. arboreum* as compared to *G. thurberi*. It might be due to lesser boll rind toughness during the early stage of boll development when PBW larvae entered into young bolls. Though the increased toughness of boll at 30 and 45 DAP was observed, they did not play any role in suppression of bollworm as the larvae had already entered the bolls. Hence, correlations between toughness of boll at 30 and 45 DAP and locule damage were negative[56].

Table 1. Boll rind toughness, thickness, locule damage and infestation of bollworm larvae observed in bolls of parents, F_1, F_2 and BC_1 generation of interspecific cross between *G. arboreum* and *G. thurberi*

	Boll rind toughness (shore)			Boll rind thickness (mm)				
	Days after flowering			Days after flowering				
Sl. no.	15	30	45	15	30	45	Locule damage (%)	Pink bollworm larvae (no.) 10 bolls
P_1	6.80	16.40	33.67	1.14	1.95	2.00	9.42	1.87
P_2	22.87	30.40	46.00	0.89	1.30	1.77	0.00	0.00
F_1	9.29	19.53	36.30	0.90	1.51	1.76	7.59	1.66
BC_1	8.00	18.17	38.73	0.98	1.64	1.80	9.98	2.33
F_2	12.40	23.58	36.56	1.77	2.29	2.81	8.87	2.05

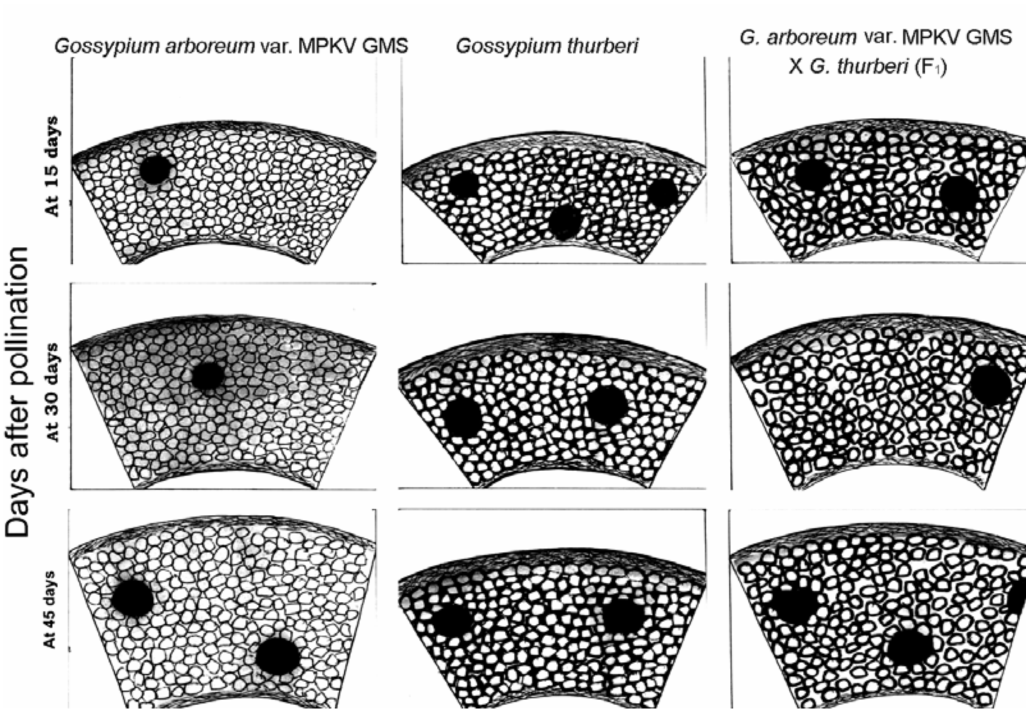

Figure 3. Drawing of cross sections sketches of boll rind *Gossypium arboreum* var. MPKV GMS, *Gossypium thurberi* and their hybrid at 15, 30 and 45-day-old bolls after flower opening.

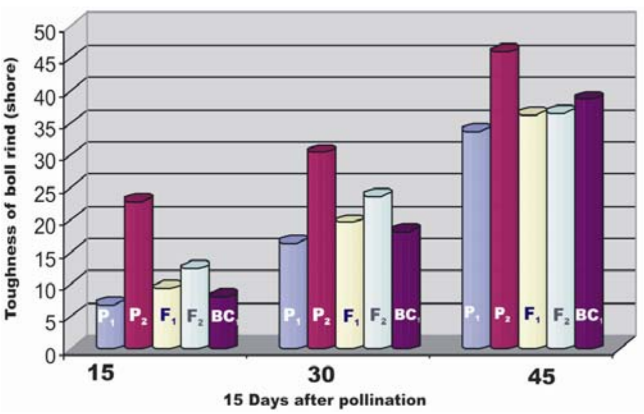

Figure 4. Toughness (shore) of boll rinds measured at different ages in parents (P_1 and P_2), F_1, F_2 and BC_1 generations of interspecific (*G. arboreum* × *G. thurberi*) cross of cotton.

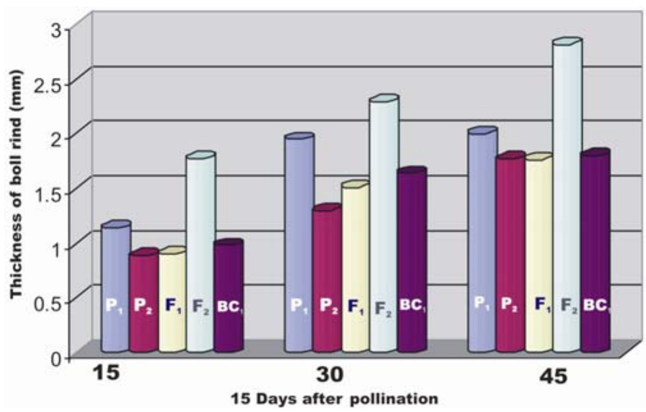

Figure 5. Thickness of boll rind measured at different ages in parents (P_1 and P_2), F_1, F_2 and BC_1 generations of interspecific (*G. arboreum* × *G. thurberi*) cross of cotton.

A similar trend was observed in F_2 generation (Table 1). Plants numbered 2, 9, 27 showed more toughness than *G. thurberi*. As the anatomical characters are under genetic control[61], boll rind toughness of *G. thurberi* holds promise to introgress it in both *G. arboreum* and *G. hirsutum*[10].

Positive correlation (*r*) at 15 days after flowering (DAS) between boll rind thickness (0.985), boll toughness (0.924) at 30 DAS (0.885), and negative correlations boll rind thickness at 15 DAS (–0.545), 30 days (–0.428) and 45 DAS (–0.426) observed agree with the earlier reports[45].

The range of fibre properties, viz. 2.5% SL (span length) (21.9–23.6); uniformity ratio (50–52%); micronaire (5.2–5.5); strength (19.6–21.4 g/tex) and fibre extensionability (5.0–5.2 %) were observed in F_2 as compared to *G. arboreum*, 23.3, 52.0, 5.1, 17.0, 5.0 respectively. This pre-breeding material will be excellent source of developing lines combined with PBW resistance[5,7,8], fibre strength and drought tolerance. After doubling of chromosomes[62], it will be possible to transfer these traits to *G. hirsutum*[10]. Such material will also be useful for incorporation of *Bt* genes[63] for developing tolerance to both bollworms, viz. *Helicoverpa* and *Pectinophora gossypiella*.

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Plant macro-remains from Neolithic Jhusi in Ganga Plain: evidence for grain-based agriculture

Anil K. Pokharia[1,]*, J. N. Pal[2] and Alka Srivastava[3]

[1]Birbal Sahni Institute of Palaeobotany, 53, University Road, Lucknow 226 007, India
[2]Department of Ancient History, Archaeology and Culture, Allahabad University, Allahabad 221 002, India
[3]Department of Botany, DG (PG) College, Civil Lines, Kanpur 208 001, India

The results of the study of archaeobotanical samples from Neolithic site, Jhusi, at the confluence of Yamuna and Ganga rivers in Allahabad, UP, are presented here and discussed in the light of information on prehistoric plants of subsistence in Ganga Plain during 7th millennium BC–2nd century BC. The study indicates that the likely staples were cereals (*Oryza sativa*, *Hordeum vulgare*, *Triticum aestivum* and *Triticum sphaerococcum*), pulses (*Lens culinaris*, *Pisum arvense*, *Vigna radiata*, *Lathyrus sativus* and *Macrotyloma uniflorum*) and two oil-yielding (*Linum usitatissimum* and *Sesamum indicum*) crops. In addition, there is evidence for viticulture or horticulture (*Vitis vinifera*).

Keywords: Archaeobotany, Ganga Plain, Jhusi, macro-remains, neolithic.

THE aim of this communication is to present the results of the charred/carbonized plant remains recovered through archaeological excavations at Jhusi, and compare with the information on agriculture remains from other sites in the Middle Ganga Plain.

*For correspondence. (e-mail: apokharia@yahoo.com)