

Pre-breeding efforts for low gossypol seed and high gossypol plant in *G. herbaceum* L. cotton utilizing *G. australe* Mueller

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Among the 49 species of *Gossypium*, the Australian species belonging to *Hibiscoidia* and *Sturtia* possess unique features of glandless seed and glanded plant. In an effort to transfer this trait to cultivated species of *Gossypium*, distant hybridization was effected between *G. herbaceum* race *wightianum* ($2n = 2x = 26$) and an Australian species *G. australe*. This species has its origin in arid zones of Australia, and behaved as facultative cleistogamous in transitional tracts of India. Upon two years of crossing only 0.9% success was achieved to obtain the crossed seeds. The F_1 seeds of the hybrid possessed dormancy. The F_1 plant was sterile due to abortive ovary, and 8–11% of pollen fertility was noticed. The plants have also been found to be of the best genetic stocks for nectar character, bract shape, calyx morphology and other inflorescence traits as both the parents exhibited diversity for many traits. The cross is expected to carry some genetic load, as the species involved are genetically and geographically distant.

COTTON is a leading fibre and food crop of the world^{1–3} belonging to genus *Gossypium*. This genus consists of 49 species, which have an aggregate geographic range that encompasses most tropical and subtropical regions of the world⁴. Among the 49 species, 44 are diploid ($2n = 2x = 26$) and fall into A, B, C, D, E, F, G and K genomes^{5–7} and the remaining are allotetraploids ($2n = 4x = 52$ AADD) with progenitors of *G. herbaceum* var. *africanum* (AA) and *G. raimondii* (D₅)⁷. There are four cultivated species, the new world allopolyploids *G. hirsutum* and *G. barbadense* ($2n = 4x = 52$ AADD) and the old world diploids *G. herbaceum* L. and *G. arboreum* L. ($2n = 2x = 26$ AA). These cultivated species embody considerable genetic diversity, but the diploid cultivated cottons are in restricted cultivation only in the Indian subcontinent and are facing a threat of genetic erosion⁸.

Though cotton is primarily grown for textile fibres, cotton-seed is an inexpensive but valuable source of edible oil, cake, linters and hulls. In fact, cotton is the second best source of plant proteins (16–24%) and the best oil-producing plant (13–24%)⁹. However, the presence of gossypol glands (which is a characteristic feature of the genus *Gossypium*) in seeds reduces the nutritive value of cotton-seed oil and protein. This is

because gossypol in excessive quantity is toxic in nature and it has been found highly reactive with other cotton-seed constituents during storage^{2,10}. Whereas presence of gossypol on aerial plant parts confers tolerance to insect pests¹².

Hence, a combination of low or no gossypol seed and high gossypol plant character in the same variety appears to be ideal. The trait 'glandless seed and glanded plant' exists in *Gossypium* sections, *Sturtia* and *Hibiscoidia*^{3,4}. The sections consist of Australian species belonging to C and G genomes and are phylogenetically remote from upland cotton and from diploid desi cottons^{3,4}. Nevertheless, these offer a great scope for breeders. Earlier efforts were made for transferring glandless seed and glanded plant trait using *G. sturtianum* Willis (CC)₁ by Dilday¹³, Altman *et al.*¹⁴ and Rooney *et al.*¹⁵, through direct crossing strategies, but were not successful. However, the trispecific method tried at Gembloux, Belgium using *G. thurberi* Todaro (DD)₁ and *G. raimondii* Ulbrich (DD)₅ as bridge species to transfer trait from *G. sturtianum* into cultivated *G. hirsutum*, has been found to be viable^{16,17}. Zhu and Li¹⁸ also tried to use *G. arboreum* as a bridge species to transfer the trait from *G. bickii* (GG)₁ to *G. hirsutum*.

In our study, efforts were made to produce a hybrid between *G. herbaceum* ($2n = 2x = 26$, AA) and *G. australe* ($2n = 2x = 26$ GG) through distant hybridization. The study has been initiated to transfer glandless seed and glanded plant trait from *G. australe* to *G. herbaceum* and to use *G. herbaceum* as the bridge species in improvement of *G. hirsutum*. The present investigation aims at assessing polymorphism for different plant and seed traits between parents and hybrid.

G. herbaceum race *wightianum* ($2n = 2x = 26$ A₁A₁) cultivar Jayadhar was used as female parent. This is indeterminate, possesses gossypol glands on seed and plant, and usually flowers during September to February (post-rain). *G. australe* ($2n = 2x = 26$ GG), used as a pollinator, is native of Trans Australian North Arid zone and belongs to tertiary gene pool of cotton⁶.

In the crossing technique, hand emasculation and pollination with some modifications involving brushing out of anther column was used for crossing¹⁹. To overcome premature fall of the crossed boll due to formation of abscission layer, a cotton swab dipped in a solution containing 50 mg/l NAA and 100 mg/l GA was placed at the pedicel base.

A comparative assessment of parents and F_1 s was made. The observations listed in Table 1 were recorded on both parents and whenever possible on F_1 plants that were being maintained as perennials. Leaf-related observations were recorded as specified in Figure 1. The total number of gossypol glands on bracts and calyx was counted by selecting the bud to be opened the next day. The number of glands per square centimetre was observed on the leaf using 10X lens. A cross-section of

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Table 1. Performance of parents and hybrid plants for leaf, flower traits and differences in boll, fibre and seed features in parents

Character	<i>G. herbaceum</i>	<i>G. australe</i>	F ₁	Percentage heterosis over	
				Female	Male
<i>Leaf trait</i>					
Leaf area (cm ²)	25	25	42	68.0	68.0
Petiole length (cm)	4.20	1.30	2.70	−35.7	107.7
Lobes	5.00	0	3.00	−40.0	
Lobe length (cm)					
L1	5.12	—	7.30	42.6	
L2	4.65	—	6.16	32.5	
L3	3.16	—	—		
Lobe width (cm)					
W1	2.13	—	3.26	53.1	
W2	1.62	—	2.50	54.3	
W3	1.13	—	—		
Lobe angle (degrees)					
A2	51.00	—	36.00	−29.4	
A3	39.00	—	—		
Nectary colour	Colourless	Crimson red	Yellowish pink		
Nectary length (mm)	1.0	5.0	3.0	200.0	−40.0
<i>Flower trait</i>					
Pedicel length (mm)	17.0	4.0	7.0	−58.8	75.0
Calyx area (mm ²)	11	7	10	−9.1	42.9
Calyx teeth	0	5.0	5.0		0.0
Calyx type	Truncate	Lobed	Lobed		
Bract area (mm ²)	340.0	2.8	43.6	−87.2	1479.7
Bract teeth	6.0	—	3.0	−50.0	
Bract type	Normal	Highly lanceolate	Frego (intermediate)		
Corolla colour	Yellow	Pinkish red	Pink		
Corolla spot	Present	Present	Present		
Corolla length (cm)	4.1	2.7	3.4	−17.0	20.5
Corolla width (cm)	2.6	1.2	2.5	−3.8	108.3
Ovary length (mm)	7.0	3.0	6.0	−14.3	100.0
Ovary width (mm)	5.0	2.0	5.0	0.0	150.0
Staminal column length (mm)	12.0	7.0	11.0	−8.3	57.1
Style length (cm)	17.0	7.2	17.0	0.0	136.1
Nectary colour	Colourless	Crimson red	Pink		
Nectary place	Within involucll	Outer involucellar	Outer involucellar		
Nectary number	3.0	3.0	3.0	0.0	0.0
Anther number	68.0	42.0	74.0	8.8	76.2
<i>Boll, fibre and seed trait</i>					
No. of locules	3.0	3.0	3.0	0.0	0.0
Boll weight (g)	2.1	0.2	—		
Thickness of boll rind (mm)	2.0	1.0	—		
Seed index (g)	5.8	1.1	—		
Seed percentage	92.8	91.0	—		
Fuzz percentage	7.2	9.0	—		
Oil content (%)	17.2	13.2	—		
Fibre length (mm)	21.2	6.0	—		
Fibre colour	White	Brown	—		
Fibre stiffness	Wrinkled	Straight	—		
Nature of uniformity	Less	Very high	—		
Nature of fibre attachment to the seed coat	Loose	Very tight	—		
<i>Gossypol gland</i>					
On leaf (per cm ²)	129	116	118	−8.5	1.7
On calyx (total)	167 (1.38)*	67 (1.36)*	221 (2.21)*	32.3	229.9
On bracts (total)	99 (0.29)*	3 (1.0)*	20 (0.45)*		
On seeds (cross-section)	47	0	—		

*Number of glands per mm.

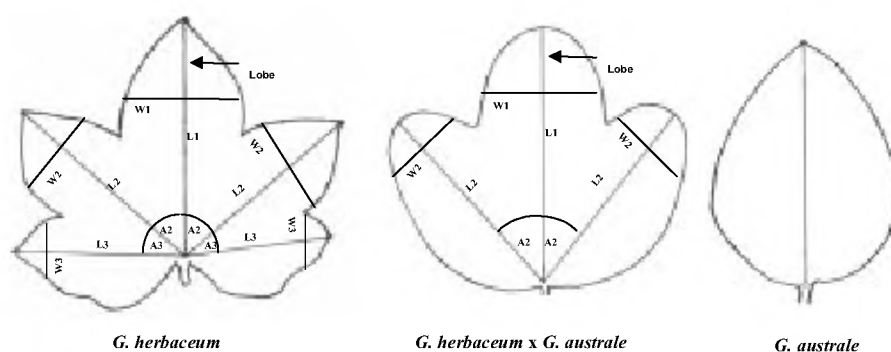


Figure 1. Cotton leaves showing different measurements.

seed was taken and the gossypol glands were counted on it. The observations were recorded on five samples each. Oil content was estimated by the petroleum ether principle, using Soxhlet apparatus. Pollen fertility was assessed by standard 2% acetocarmine staining method. The pollens that had taken stain were considered as fertile and those that had not absorbed the stain and were irregular in shape, were considered as sterile. The observations were made on 500 pollens.

G. australe has its origin in the arid zones of Australia. Its flowering behaviour was quite peculiar at the Dharwad Centre, which falls under a transitional zone of Karnataka. The plant produced around 53 flowers (mean of 2 years) between June and March (Table 2). About 27.3% of flowers did not open and were found to be cleistogamous, indicating facultative cleistogamous nature of *G. australe*. The cleistogamous nature of flowering was observed specially in cloudy and humid weather conditions. Dharwad experiences bimodal rainfall with two phases of cloudy and humid weather during June–July and September–October. The corolla morphogenesis was not observed during June–July, as small-sized buds gave rise to fruits. However, during September–October petals grew to a visible level in the bud but did not open, eventually setting into fruits. However during bright sunshine days, flowers were chasmogamous, where opening and anther dehiscence was complete and visible.

The cross between *G. herbaceum* and *G. australe* was successful only up to 0.90% in terms of boll set (Table 3). Among the large number of flowers emasculated and pollinated (330), only 55 bolls developed and after twenty-one days, 52 of them dropped before attaining maturity. Maximum fruit-dropping was observed between 19 and 24 days. Finally only three bolls were developed completely and eight seeds were harvested during two years. Some of the probable reasons for boll dropping are (1) genome (AA vs GG) incompatibility, (2) structural differences of chromosomes and cryptic structural changes, and (3) delicate flower structure of *G. herbaceum*.

Table 2. Data on flower opening in *G. australe*

Year	Cleistogamous	Chasmogamous	Total
1999–2000	16 (26.2)	45 (73.8)	61
2000–2001	13 (28.8)	32 (71.2)	45
Total/average	29 (27.3)	77 (72.7)	53.0

Figures in parenthesis indicate percentages.

Among the five seeds obtained during 1999–2000, three were planted after two weeks of harvest. But, none of them germinated even after three weeks of planting. When one of the remaining seeds was broken, proper seed filling and completely developed embryo were observed. The failure of germination was attributed to dormancy. During 2000–2001, after 12 weeks of incubation, all the three seeds along with the remaining one of the previous year were planted in earthen pots and F_1 plants were obtained. The study hence indicated requirement of substantial period of incubation after harvesting of crossed seeds. Ndungo *et al.*²⁰ and Mergeai *et al.*²¹ have also reported dormancy in distantly hybridized seeds.

The leaf of *G. herbaceum* consists of five lobes with moderate lobe depth. On the contrary, *G. australe* possessed palmate and entire leaf devoid of lobes (Figure 1). The F_1 plant was found to be trilobed, deviating from both the parents. The observations hence confirm the incomplete dominance of lobing pattern. The leaf area of F_1 hybrid was 68% higher than either of the parents. The increased size of the leaf was attributed to the increased lobe length and increased lobe width when compared to the female. Lobe sizes as measured in terms of length and width were higher in F_1 in comparison with female. The reduction of angle between the lobes was also noticed in F_1 when compared to female. The leaf of F_1 exhibited a marked phenotypic deviation from both the parents. In both female and F_1 , length of the main lobe was higher than the other lobes and similar observations were made for width of the lobe. The

Table 3. Details of crossing programme between *G. herbaceum* and *G. australe*

Year	No. of flowers emasculated	Boll dropped between 19 and 24 DAP*	Boll developed	No. of locules with completely developed seed	No. of seeds obtained
1999–2000	172	25	1	2	5
2000–2001	158	27	2	2	3
Total	330	52	3	4	8

*Days after pollination.

lobes in F_1 were not pointed like *G. herbaceum* and the margins were smooth. F_1 also possessed intermediate petiole length and was sparsely pubescent when compared to parents.

In general, foliar nectary of *G. herbaceum* exudates a colourless fluid and was a dot-like structure of one mm size located little away from the leaf sinus (Figure 2a). In *G. australe*, secretal tissue of the foliar nectaries was bright crimson red and prominent, with a larger size of 5 mm length, situated near the sinus of the leaf. In the F_1 , foliar nectary was intermediate in size (3 mm) between two parents and placed away from the leaf sinus. Further, the colour of the foliar nectary is yellowish pink. In the cultivated species of *Gossypium*, polymorphism for the foliar nectary is very limited. Studies in this regard assume importance in developing wide genetic diversity for size and colour of nectar gland trait that could be used as a phenotypic marker.

Flower of *G. australe* is very attractive with pink–red colour corolla and a prominent red spot at the claw of the petal (Figure 2e). However, *G. herbaceum* flowers possess yellow petals, with a spot at the claw of the corolla. In F_1 also, corolla spot was observed with equal prominence in comparison with either of the parents, but the colour of the petals was pink. This petal colour in F_1 indicated dominance of pink petal colour of *G. australe*. Most of the genetic work on petal colour has been reported only on yellow and white petal colours^{5,22}. Flower size of F_1 hybrid as measured by corolla length, corolla width, ovary length, ovary width, staminal column length and style length were smaller than the female parent (Figure 2d). Similar visual observations were made with respect to flower buds (Figure 2b). However, there was 50–150% increase in various flower parts when compared to size of the male flower, indicating increased flower size over the male parent. The calyx of *G. herbaceum* is truncate with no teeth. However, *G. australe* possesses pentamerous calyx with deeply-lobed five teeth. In the F_1 also, deeply-lobed pentamerous calyx was observed. In cultivated species, calyx has no morphological variation, and all the species possess truncate calyces with no distinguishable teeth. Hence, genetics of calyx morphology has not been studied in detail^{5,22}. In *G. herbaceum* species, the

bracts of the involucler are highly developed into broad (340 mm²) foliaceous trimerous and usually cordate structures with less deeply-lobed teeth (normally 6 in number; Figure 2c). Further, bracts are found flared when compared to closed structures of other cultivated species. However, in *G. australe* bracts are completely lancinate and very small in size (2.8 mm²). The F_1 was found to possess fregobract.

In domesticated cotton, an additional set of nectary occurs in association with the involucler. The trimerous nectaries that occur immediately below each of the three bracts that subtend the flower, usually known as ‘outer involucellar nectaries’, were present in *G. australe*. Further, these nectaries were crimson bright-red in colour, and obtriangular in form (Figure 2b and c). But in *G. herbaceum*, trionectaries were found to be inserted at the base of the calyx, with colourless secrete. This type of dotted ‘inner involucellar nectaries’ is not generally known in other species of *Gossypium*, except in *G. arboreum*²³. In F_1 , outer involucellar, pink-coloured obtriangular nectary was observed. These observations indicate the dominance of outer location nectaries over inner location nectaries in the involucler and bright-red colour was incompletely dominant over colourless nectary (Figure 2b and c).

Anther number in *G. herbaceum* and *G. australe* was less than in F_1 hybrid. But only 8–11% pollen fertility was observed in the F_1 plant. The flowers were found to drop after pollination. The reason for non-setting of fruits on F_1 plants was the abortive ovary along with inadequate pollen fertility. Ndungo²⁰ and Stewart⁶ have also reported abortive ovary as a common feature for F_1 sterility, when tertiary germplasm was involved in the cross.

The distribution of gossypol glands on aerial plant parts of both the parents and hybrid was fairly good. The density of glands on bracts and leaves was less than the calyx, in both parents and hybrid. The seeds of *G. australe* were found to be devoid of glands as against high-density gland on the seeds of *G. herbaceum* (Figure 2g). The glands on the plant parts have been found to confer resistance to insect pests and diseases^{11,12}. However, gossypol-free seed and high-gossypol plant are found to be ideal. With this primary view, these F_1

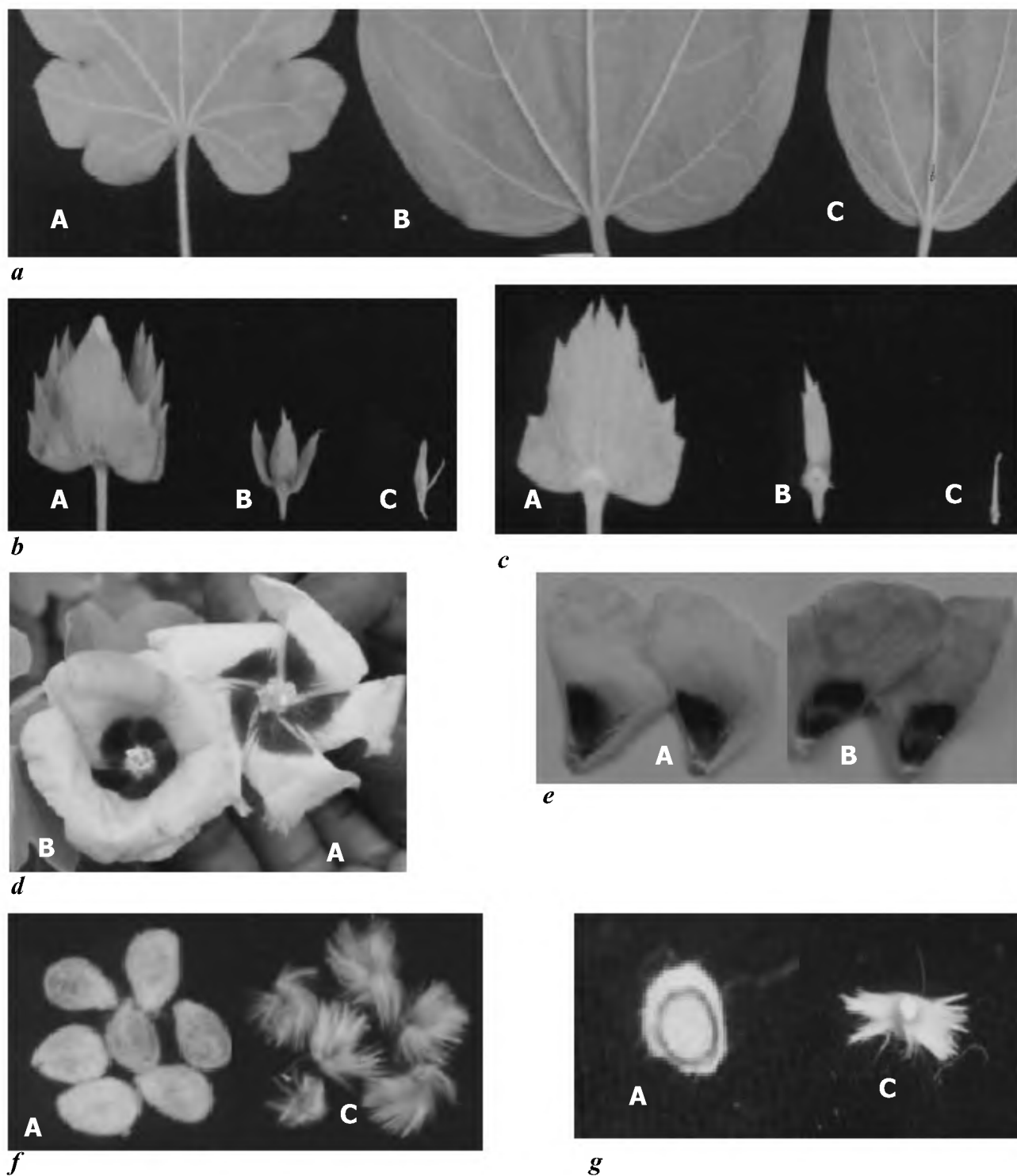


Figure 2. Morphological variations among parents and interspecific hybrid for leaf (*a*), flower bud; (*b*), bract; (*c*), flower; (*d*), petals; (*e*), seeds; (*f*) and gossypol glands on seed (*g*). a, *G. herbaceum*; b, *G. herbaceum* × *G. australe* (F₁); c, *G. australe*.

hybrid plants were generated between *G. herbaceum* and *G. australe*, to transfer the character of glandless seed and glanded plant trait. The pollen fertility in F₁ is now being utilized in generating back crosses and the plants are also being treated with colchicine to double

the chromosome number. Such a colchiploid is expected to hybridize with tetraploid cultivated cotton⁶.

This cross also bears considerable amount of genetic load because boll, fibre and seed features of *G. australe* have been found to be undesirable. The boll weight as

well as boll-rind thickness of *G. australe* was less when compared to domesticated *G. herbaceum* race *wightianum*. Further, seed index (Figure 2f), oil content and fibre length also fall in same line with boll observations, as considerable decrease in values of *G. australe* were noticed. In *G. herbaceum*, lint or fibre of nearly 21 mm length can be removed easily through ginning. On the contrary, in *G. australe* fibres of 6 mm length which are attached very tightly to the seed have been found to be unginnable (Figure 2f). The seed hairs are perfectly straight and radiate in all directions from the seed. Upon maturation, the capsules of *G. australe* flare widely, releasing the seeds to wind or gravity and enabling them to tumble quickly away with the first breeze. Hence, this species is most widely distributed in Australia. The colour of the fibre in *G. australe* was found to be brown as against white in *G. herbaceum*. Further, unmatched uniformity exists in the fibres of this Australian species, indicating a source for fibre colour and uniformity.

The observations made by Stewart⁶ indicated possibility of getting recombinants through back-cross for various traits discussed here. However, substantial efforts need to be made in terms of generating abundant back-cross populations and isolating glandless seed and glanded genotype without deleterious genes.

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Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102

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The field soil at Taegu, South Korea was screened for phosphate solubilizing fungal strains. Such strains were identified to be *Fomitopsis* sp. Phosphate solubilizing ability of *Fomitopsis* sp. PS 102 was studied on four different insoluble phosphates, viz. tricalcium phosphate, rock phosphate, aluminium phosphate and hydroxyapatite. Tricalcium phosphate was found to be solubilized maximally, while hydroxyapatite could not be solubilized by the isolated fungal strain. Further, the effect of salinity under *in vitro* conditions on the solubilization activity of rock phosphate was also observed. Presence of 1% NaCl enhanced the solubilization of rock phosphate by *Fomitopsis* sp. PS 102.

PHOSPHORUS is one of the major plant nutrients, second only to nitrogen in requirement. However, a greater part of soil phosphorus, approximately 95–99% is present in

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