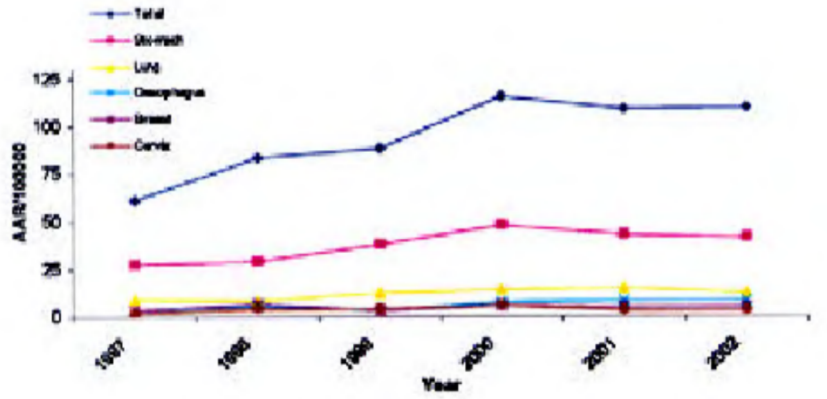

Figure 1. Age adjusted rate of the five most common cancers and total cancer cases seen at Aizawl Civil Hospital between 1997 and 2002.

be grouped under high-prevalence area for stomach cancer. The cases presented here required surgical resection or they were in such an advanced stage that only palliative intervention was feasible depending on the clinical condition of the patient.

From the above finding, Mizoram can be grouped under high-risk region for stomach cancer within the lower prevalence area for stomach cancer in the world, which calls for further in-depth study.

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Ovulo embryo cultured hybrid between amphidiploid (*Gossypium arboreum* × *Gossypium anomalum*) and *Gossypium hirsutum*

Cottons are widely distributed over five continents and Oceania[1]. In addition to being a leading fibre crop, it is also the second-best source of plant proteins and fifth-best oil-producing plant[2]. *Gossypium* contains about 50 species, including diploid ($2n = 2x = 26$; A through G and K) and tetraploid ($2n = 4x = 52$; AD)[2]. A considerable amount of genetic variability can be produced by crossing two or more species as they have mostly wild or important genes like those with resistance to drought, pests and diseases; even genes of some of the economic characters. Many of these species can be intercrossed by traditional hybridization. Earlier experiments on 27 species revealed that 147 out of 351 potential hybrids produced at least some viable seeds. In spite of this, some of the *Gossypium* species are sexually incompatible; hence new breeding material cannot be developed from them unless the crossability barrier is overcome[3].

Broadening of the existing genetic base of the world's major cultivated tetraploid is of major interest to cotton breeders[4]. Resistance to certain pathogens, insects, male sterility and certain morphological traits found in the old-world cottons (*G. herbaceum* A_2 *and G. arboreum* A_1) are useful in tetraploid (*G. hirsutum*; A_1D_1) and cotton production. Further, nearly complete homology between A chromosomes of *G. herbaceum* (A_2) and *G. hirsutum* (A_1D_1) enables ready meiotic gene exchange. However, such gene transfer has been achieved on a limited scale due to the difficulty in producing hybrids between these species[5], because of abortion of hybrid embryo and endosperm at various developmental stages[4]. In American × Asiatic crosses, embryo endosperm development was aborted soon after initiation[6]. The nature of species incompatibility such as the abortion of endosperm and embryo in $AD_1 \times A_2^4$ and $A_2 \times AD_1$ crosses[7] respectively, not only result in greater difficulty in producing hybrid plant but can also determine the relative success of *in vitro* techniques.

Ovulo embryo culture technique has been successfully utilized for *Gossypium* since the 1960s[1] to obtain various hybrids which were otherwise difficult or impossible to grow[5,7,8]. Frequent failures of hybrids to grow can be overcome by culture of excised immature embryos in satisfactory medium to raise hybrid seedling. Recently, ovulo embryo culture has created a growing interest in the use of *in vitro* fertilization to circumvent natural barriers of fertilization[9]. Attempts made to culture ovules and embryos for inter-

specific hybridization in *Gossypium* by earlier researchers are reviewed in Altman[1] and Mehetre & Aher[10]. Recent[10] and earlier[11] reviews encompass the reports of synthesized amphidiploid of *G. arboreum* × *G. anomalum*, but their utilization in transfer of desired characters into *G. hirsutum* and *G. barbadense* either by conventional or biotechnological approaches has not been cited.

Recently, we[12] reported the desirable characters of the wild African diploid *G. anomalum* ($2n = 2x = 26$, B_1B_1) like extremely fine fibre with high maturity, good strength, low fibre weight, resistance to insect pests, immunity to black-arm (angular leaf spot) and its narrow bracts which ensure clean picking[11]. Lint fineness is already transferred from *G. anomalum* to the cultivated *G. arboreum* ($2n = 2x = 26$, A_1A_1)[13,14]. Synthesized allopolyploids of *G. arboreum* × *G. anomalum* are fertile[15,16], thus they possess immense possibilities in transferring useful interspecific gene transfer to cultivated American (*G. hirsutum*) cottons[11]. We[12] synthesized amphidiploid (C_1) of *G. arboreum* × *G. anomalum* and confirmed $n = 2x = 26$ chromosome number from meiotic studies. It had very high (35.0 g/tex) fibre strength. Thus, it is considered as an excellent source for transferring high fibre strength to the cultivated tetraploid *G. hirsutum* or *G. barbadense* cottons. Few germinable selfed seeds were obtained from amphidiploid and a population of nine adult plants ($C_2$1 to 9) was raised. These plants were crossed with *G. hirsutum* varieties with a view to combine desirable characters of *G. anomalum*, *G. arboreum* and *G. hirsutum*.

During the present investigations we made several cross-pollinations of emasculated buds (one day before anthesis) with pollen from *G. hirsutum* varieties. All the nine plants (C_2 is a progeny of amphidiploid C_1) as seed parent with *G. hirsutum* variety JLH-168, LRA-5166 and Boll Worm Resistant (BWR) line. After pollination of 1198, 1057 and 968 emasculated buds with pollen from JLH-168, LRA-5166 and BWR pollen respectively, few bolls (Figure 1 *a* (top)) were set. The development of crossed bolls was found arrested after 15 days after pollination (DAP). Dehiscence of such bolls was after 28 DAP but they were found without any developed seed or lint (Figure 1 *b* (bottom)), which may be due to embryo abortion. Hence embryo rescue was attempted by *in vitro* ovule embryo culture (i) to overcome the difficulty of non-viability of seed due to embryo abortion often experienced during interspecific hybridization of *Gossypium* species and (ii) to obtain successful hybrid.

Developing crossed bolls were harvested after 3, 5, 7, 10 and 14 DAP. These bolls were surface sterilized and opened aseptically in a laminar flow cabinet, and excised ovules were planted on twelve different media supplemented with various concentrations of casein hydrolysate (CH), indoleacetic acid (IAA) and kinetin, viz. Murashige–Skoog[17] and Beasley–Ting[18] media M1 (MS + 1.5 mg/l IAA + 0.5 mg/l kinetin + casein hydrolysate 250 mg/l); M2 (MS + 2.0 mg/l IAA + 1.0 mg/l kinetin + casein hydrolysate 300 mg/l); M3 (MS + 2.5 mg/l IAA + 1.0 mg/l kinetin + casein hydrolysate 200mg/l) and M4 (BT + 1.5 mg/l IAA + 1.0 mg/l kinetin + 1.0 mg/l NAA + casein hydrolysate 275 mg/l) M5 (BT + 2.0 mg/l IAA + 2.0 mg/l kinetin + 1.0 mg/l NAA + casein hydrolysate 250 mg/l) respectively. These five media were selected on the basis of genotypically oriented response of ovules for callusing, regeneration and direct germination[19].

After incubation in dark for 21 days these cultures were exposed to light (3000 lux). Thereafter, some immature ovules showed callus formation or elongation and germination while others showed varied response (Table 1). Germinated ovules were transformed on fresh respective medium to allow further growth in light (3000 lux). Plantlets were transformed to pots

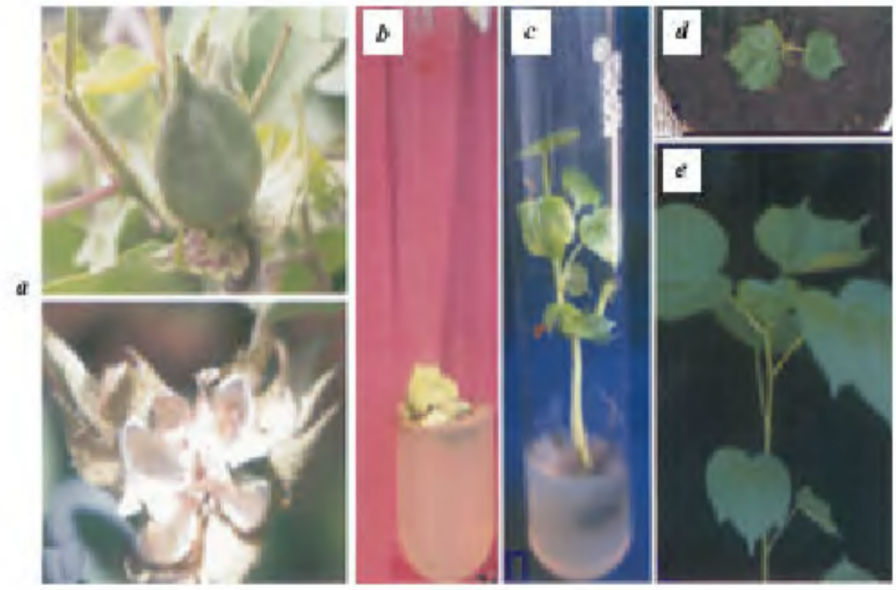

Figure 1 *a–e*. ***a***, (top) Developing boll of a cross A_2 × BWR harvested 14 DAP for excision of ovules for culture; (bottom) The opened boll after 28 DAP without any developed seed/lint. ***b***, Callus growth from 3 DAP ovules of a cross C_2–4 × JLH-168 formed on M1 media. ***c–e***, Seedlings formed 3 DAP ovules of a cross A_2–9 × BWR formed on M3 media; ***c***, Five-to-six leaf stage before hardening; ***d***, established after two weeks; ***e***, after six weeks before transplanted in field.

Table 1. Response of different culture media for *in vitro* growth of ovules *Gossypium hirsutum* varieties

	Age (in days after pollination DAP) of cross pollinated ovules of A_2-9 with different *Gossypium hirsutum* varieties															
	JLH-168					LRA-5166					BWR					
Media	3	5	7	10	14	3	5	7	10	14	3	5	7	10	14	Mean
M1	17.00 (4.18)	7.5 (2.82)	10.00 (3.24)	11.50 (3.46)	3.50 (1.96)	20.00 (4.52)	10.50 (3.30)	5.00 (2.34)	6.00 (2.55)	4.00 (2.11)	15.00 (3.94)	12.5 (3.61)	10.50 (3.32)	6.50 (2.64)	4.00 (2.11)	9.57 (3.07)
M2	15.00 (3.94)	8.00 (2.91)	12.00 (3.53)	8.50 (3.00)	7.50 (2.82)	14.50 (3.87)	10.00 (3.24)	13.50 (3.74)	7.00 (2.07)	8.00 (2.91)	17.50 (4.24)	7.00 (2.73)	11.00 (3.39)	6.50 (2.64)	3.50 (1.96)	9.97 (3.18)
M3	20.00 (4.52)	8.50 (2.99)	10.50 (3.31)	7.00 (2.74)	5.50 (2.45)	12.50 (3.61)	10.50 (3.31)	11.00 (3.39)	5.00 (2.35)	4.50 (2.23)	9.50 (3.16)	8.50 (3.00)	12.00 (3.53)	6.50 (2.64)	3.50 (2.00)	9.00 (3.02)
M4	15.00 (3.94)	7.50 (2.82)	10.50 (3.31)	9.00 (3.08)	10.00 (3.21)	13.50 (3.74)	6.00 (2.54)	2.50 (1.73)	6.50 (2.64)	7.50 (2.82)	15.00 (3.94)	16.00 (4.06)	7.00 (2.73)	5.50 (2.44)	7.50 (2.83)	9.27 (3.05)
M5	15.50 (4.00)	11.50 (3.46)	7.50 (2.83)	8.50 (3.00)	0.50 (0.97)	12.00 (3.53)	10.50 (3.31)	7.00 (2.73)	4.50 (2.23)	8.50 (2.99)	10.50 (3.31)	5.00 (2.34)	11.50 (3.46)	7.00 (2.73)	7.50 (2.82)	8.50 (2.91)
Varietal means for all the media and age (DAP) of ovules					9.90 (3.14)					8.82 (2.98)					9.06 (3.02)	

	Media	Crossed ovules	Age of ovules (DAP)	Interaction
S.E ±	0.0023	0.0014	0.0023	–
C.D. at 5%	0.13	0.1037	0.1339	0.2300

Figure 2. ***a***, Leaf shape; ***b***, Bract shape; ***c***, Flower colour and petal spot; ***d***, Boll shape, size and lint colour; ***e***, Meiotic metaphases. 1. *G. arboreum* var. Y–1, ($2n = 2x = 26$, A_1A_1); 2. F_1 hybrid, ($2n = 2x = 26$, A_1B_1); 3. *G. anomalum*, ($2n = 2x = 26$, B_1B_1); 4. Amphidiploid 2 (*G. arboreum* × *G. anomalum*; $2n = 4x = 52$; A_1A_1 B_1B_1); 5. Plant no. 6 from second generation of amphidiploid (*G. arboreum* × *G. anomalum*; $2n = 4x = 52$; A_1A_1 B_1B_1); 6. *G. hirsutum*, ($2n = 4x = 52$; $A_hA_hD_hD_h$); 7. Cultured F_1 hybrid (A_2–9 × *G. hirsutum*, $2n = 4x = 52$; $A_1A_hB_1D_h$).

containing mixture of soilrite after attaining a height of 5–6 cm at 5–6 leaf stage.

From the data (Table 1) it appeared that culture of immature ovules of age 3 and 6 DAP resulted in growth of calli (Figure 1 *b*). However some ovules of cross involving BWR pollen resulted in elongation and germination in M4 medium only. The germinated ovules were carefully sub-cultured on fresh medium for further development, which later on developed into seedlings (Figures 1 *c*, *d*). After hardening (Figure 1 *e*) the plants were transplanted in field. It was interesting to note that callus formation from ovules was highly specific to media and age of ovules. Ovules of cross $C_2 \times$ JLH-168 formed calluses on all media tested. It appeared that M1 and M3 media were found appropriate for growth of callus and germination of C_2 plant no $9 \times$ BWR ovules, respectively. These ovules germinated after 72–77 days of planting on media. During this period, the media were changed twice. A total of 15 ovules were geminated and out of these we were able to establish five adults in the field. Others died during the process of changing media, hardening and transplanting in field. The responses due to media, genotypes of ovules, and age including interaction effects, etc. were significant. Genotypic specific response for *in vitro* germination of ovules as reported earlier[20] is confirmed. The results obtained in present studies are similar to earlier workers[1,10,21]. Post-fertilization barrier, i.e. abortion of seed/embryos (Figure 1 *a* (bottom)) in cross A_2–9 (*G. arboreum* $\times$ *G. anomalum*) $\times$ *G. hirsutum* variety 'BWR' was successfully overcome by ovule embryo culture.

The hybrid nature of the plant was confirmed from both morphological and cytological studies. The shape and size of leaf (Figure 2 *a*, *b*) and bract boll lint colour (Figure 2 *d*) of cultured hybrids were different from its female parent and observed to be more tending towards the pollen parent. While colour intensity and size of petal spot and petal colour expressions (Figure 2 *c*) were intermediate in between C_2–9 and *G. hirsutum* variety 'BWR' (seed and pollen parents respectively). Meiotic studies *G. arboreum*, *G. anomalum*, F_1 (*G. arboreum* $\times$ *G. anomalum*) and amphidiploid, C_2–9 *G. hirsutum*, and cultured hybrid (C_2–9 $\times$ *G. hirsutum*) had $n = x = 13$ and $n = 2x = 26$ chromosomes respectively (Figure 2 *e*). Thus the hybridity of a cultured plant was confirmed cytomorphologically. The hybrid under report will be source material for combining desired characters like resistance to various stress and quality characters from *G. arboreum*, *G. hirsutum* and *G. anomalum*. It is being utilized further in current cotton breeding progamme on varietal improvement.

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Monoclonal antibody-based immunodot test for epizootic ulcerative syndrome pathogen, *Aphanomyces invadans*

Epizootic ulcerative syndrome (EUS) is one of the most destructive diseases affecting fresh- and brackishwater fish in Asia and the Indo-Pacific region. The definition of EUS is 'a seasonal epizootic condition of freshwater and estuarine warm-water fish of complex aetiology characterized by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to a granulomatous response'[1]. Furthermore, *Aphanomyces invadans* has also been demonstrated as a sole aetiologic agent in experimental infections[2]. The continuous spread of EUS from one country to another for over 30 years confirms that EUS is an international concern, particularly in the south and southeastern parts of the