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棉花原生质体培养和原生质体对称融合研究*

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根据增加棉花基因库多样性的迫切需要，棉花改良计划正在转向于多种分子育种技术的应用和资源的利用。丰富的棉花野生种(*Gossypium* spp.)是栽培棉遗传改良重要的种质资源和更新资源，并成为宝贵的遗传资源库，野生棉研究利用对栽培棉的遗传改良有着重大的现实和理论意义及潜在的应用价值。由于野生种和栽培种亲缘关系较远，杂交往往很难成功，或者 F_1 代没有育性，或者育性很低。寻求把野生棉的有益基因转入到栽培种中去的途径对于栽培棉种的遗传改良具有重要意义。利用生物技术进行棉花的种质资源创新是一条有效途径，笔者拟通过体细胞培养、原生质体培养和原生质体融合创造新的棉花种质。本研究内容主要涉及到野生棉的体细胞胚胎发生和植株再生；陆地棉和野生棉的原生质体培养及植株再生；栽培棉种和野生棉的原生质体对称融合及杂种植株再生。本研究取得的主要结果如下：

1. 系统研究了影响野生棉愈伤组织诱导、胚胎发生和植株再生的影响因素，首次从野生棉获得体细胞再生植株，并将建立的技术方法应用于其他野生种获得成功。从9个野生棉种中诱导出愈伤组织，其中从 *G. davidsonii*，*G. klotzschianum*，*G. raimondii* 和 *G. stocksii* 愈伤组织通过体细胞胚胎发生得到正常的再生植株。从 *G. aridum* 也可以得到形态不正常的再生植株。*G. anomalum*，*G. africanum*，*G. thurberi* 和 *G. bickii* 的愈伤组织仍处于非胚性状态。详细地研究了胚性愈伤组织的诱导和保存、促进体细胞胚成熟和植株再生与移栽的方法和途径，基本建立起一套适合野生棉体细胞培养操作程序。其中2,4-D/KT对所有供试棉种愈伤组织诱导都很有效；不同激素组合、糖源、悬浮培养、环境胁迫等能够不同程度地促进胚性愈伤组织形成、体细胞胚成熟、萌发和植株再生；野生棉的胚性愈伤组织在含有 IBA 0.984 μmol/L，KT 0.232 μmol/L 的 MSB 固体培养基上保存 4 a 以上，仍然具有分化能力，这样就可以提供大量的试验材料。

2. 详细分析了影响棉花原生质体培养及植株再生的因素，打破了仅能从陆地棉胚性悬浮系分离原生质体再生植株的局面；将建立的技术应用于野生棉原生质体培养，首次实现了从野生棉种原生质体再生植株。重点分析了原生质体分离的酶液组合，培养密度和激素组合等因素对原生质体持续分离、愈伤组织再生的影响，建立起比较成熟的原生质体培养体系。从2个陆地棉品系(Coker 201 和 YZ1)的多个不同外植体(胚性细胞悬浮系、胚性愈伤组织、体细胞幼胚、下胚轴、幼根和叶片)分离原生质体进行培养。其中 Coker 201 的6个外植体来源的原生质体培养都得到再生植株，但是不同来源的原生质体的植板率差异显著。胚性细胞悬浮系分离的原生质体的植板率为10%，胚性愈伤组织和体细胞幼胚分离的原生质体的植板率为6%，下胚轴、幼根和叶片分离的原生质体的植板率不到2%。从 YZ1 的胚性细胞悬浮系、胚性愈伤组织、体细胞幼胚分离的原生质体培养得到再生植株，植板率比 Coker 201 相同来源的低1%～2%。

从野生棉 *G. klotzschianum* 的体细胞幼胚和胚性细胞悬浮系分离的原生质体进行培养得到再生植株，植板率为6%～8%，RAPD 分子标记扩增结果分析表明，这些再生植株具有遗传同质性。

3. 首次通过细胞融合获得棉花种间体细胞杂种，为棉花新种质创造提供了全新的技术途径。本研究中开展了8个组合的原生质体在电场介导的融

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合。Coker 201＋*G. klotzschianum*，Coker 201＋*G. davidsonii*，Coker 201＋*G. bickii*，Coker 201＋*G. stockii* 这 4 个组合再生出形态上介于融合双亲之间、偏向于野生棉亲本的植株。细胞学和细胞流式仪检测表明，再生植株是六倍体或近六倍体的非整倍体，为体细胞杂种。RAPD 和其他分子标记分析表明，检测的大多数再生植株扩增带型含有双亲之和。Coker 201＋*G. klotzschianum*，Coker 201＋*G. davidsonii* 这 2 个组合的杂种植株可以在温室里开花，而在室外比较困难；Coker 201＋*G. bickii*，Coker 201＋*G. stockii* 在室外的秋季和冬季的温室里开花结铃。这些组合的育性比较高，可能与光周期反应有关。同时，得到 *G. arboreum*＋*G. stockii* 的杂种愈伤组织，这种愈伤组织分化困难。

4. 从野生棉的体细胞培养、胚胎发生，野生棉原生质体培养和野生棉和栽培种体细胞杂种再生 3 个方面研究了促进有关野生棉通过体细胞胚胎发生得到再生植株的多个影响因子，以提高野生棉再生频率，扩大野生棉种再生范围。

关键词　野生棉；陆地棉；中棉；体细胞胚胎发生；原生质体培养；原生质体融合；植株再生；体细胞杂种

Protoplast culture and protoplast symmetric fusion in cotton

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In light of the critical need to increase genetic diversity in the gene pool, cotton improvement programs are increasingly turning to the application of molecular approaches to breeding and germplasm utilizations. The abundant species of wild cotton (*Gossypium* spp.) are an important renewable resources and have been the valuable genetic germplasms for cotton genentic improvement, and which has been significant in the reality and theory, potential in the application. It is very difficult to widecross between cultivars and wild species for the distant relationship, or no fertility of F_1 hybrids, or very low fertility. Application of biotechnology is an effective way for developing new germplasm in cotton, and we aim to develop new sources of cotton germplasm *via* somatic cell culture, protoplast culture and protoplast fusion. Our studies involved somatic embryogenesis and plant regeneration in wild cotton species, protoplast culture in *Gossypium hirsutum* L. and wild species, protoplast fusion between cultivars and wild species. The main results of this research were as follows:

1. Calli were induced from 9 wild cotton species. Among them, the normally regenerated plants were obtained from *G. davidsonii*, *G. klotzschianum*, *G. raimondii* and *G. stocksii via* somatic embryogenesis, regenerated plants with abnormal morphology from *G. aridum*. Only non-embryogenic calli were obtained from *G. anomalum*, *G. africanum*, *G. thurberi* and *G. bickii*. We studied the methods and factors for embryogenic callus induction and conservation, improving somatic embryos maturation and germination, plant regeneration in detailed, and then a new and elementary protocol has been developed for somatic cell culture, mainly somatic embryogenesis and plant regeneration in wild cotton species. The combination of 2, 4-D/KT was very useful for callus induction in all tested wild species. Different combinations of PGR, sugar sources, suspension culture and environmental stress *etc* improved the formation of embryogenic callus, the maturation and germination of somatic embryos, and plant regeneration to some degree. Embryogenic calli of wild species subcultured and conserved on MSB semi-solid medium supplementing with IBA 0.984 μmol/L, KT 0.232 mol/L for 4 years still have the capability of differentiation

and provide a mass of materials. It is the first report of regeneration of plants *via* somatic embryogenesis in many wild cotton species.

2. Protoplasts were isolated from different explants of 2 species (Coker 201 and YZ1) in *Gossypium hirsutum* L. (embryogenic cell suspension culture, embryogenic callus, immature somatic embryos, hypocotyls, young roots and leaves). Plants regenerated from cultured protoplasts of 6 explants in Coker 201, but the plating frequencies of protoplasts from different explants varied significantly. The plating frequency of suspension culture-protoplast, embryogenic callus-and somatic embryo-protoplast, hypocotyl-young root-and leaf-protoplast was 10%, 6%, less than 2%. The plating frequencies of plants regenerated form protoplast cultures isolated from embryogenic suspension cultures, somatic embryos and embryogenic callus in YZ1 were lower (1%-2%) than that of plants regenerated from same explants in Coker 201.

Plants regenerated from protoplasts isolated from somatic embryos and embryogenic suspension cultures in wild cotton *G. klotzschianum* with the plating frequencies ranging 6% to 8%. RAPD analysis demonstrated that the regenerated plants were genetically homogeneous.

This study emphasized on enzyme combinations for protoplast isolation, the influences of culture density and PGR combinations *etc* for protoplasts sustained division, callus formation, and then a practical protocol for protoplast culture in cotton is established.

3. In this research, symmetric fusion including 8 combinations mediated by electricity was carried out. Plants regenerated from Coker 201+*G. klotzschianum*, Coker 201+*G. davidsonii*, Coker 201+*G. bickii*, Coker 201+*G. stockii*, which were morphologically intermediated fusion parents, apt to wild cotton parent. Cytological examinations and flow cytometric analysis showed that all of the tested plants were hexaploids with chromosomes (2n=2x=78), or aneuploids nearing 78 chromosomes, the sum of that of parents, and were somatic hybrids. Analysis of RAPD and other molecular markers demonstrated that the majority of regenerated plants had its parents' specific bands. Somatic hybrid plants of Coker 201+*G. klotzschianum*, Coker 201+*G. davidsonii* flowered and set bolls in green house, but difficultly flowered outside in the field. Somatic hybrid plants of Coker 201+*G. bickii*, Coker 201+*G. stockii* flowered and set bolls at fall in the field and at winter in the green house. The fertility of four somatic hybrids was comparatively high, perhaps related with photoperiod response. Somatic hybrid callus was obtained from *G. arboreum* + *G. stockii*, but this callus differentiated very difficultly. It is the first report on production of somatic hybrid plants between cultivars and wild species *via* protoplast fusion in cotton.

4. In this research, we studied various factors in efforts to improve somatic embryogenesis and plant regeneration in wild cottons through 3 aspects of somatic cell culture, protoplast culture in wild cotton species and regeneration of somatic hybrids between wild speciesand cultivars, in order to improve the efficiency of regeneration in wild cotton species and broarden the range of wild species in which plant regenerated *via* somatic embryogenesis.

Key words wild cotton; *Gossypium hirsutum* L.; *G. arboreum* L.; somatic embryogenesis; protoplast culture; plant regeneration; somatic hybrid

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