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延龄草属花粉形态的研究*

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摘要 对延龄草属(*Trillium* L.)23 种进行了光镜和电镜的观察研究。其中 14 种作了透射电镜的研究。研究结果表明，本属花粉的形状和大小在种间变化不大，但在外壁纹饰上却有较大的差异，大致可分为 4 个纹饰类型，即颗粒状纹饰（多数种类），疣状纹饰、星状纹饰及刺状纹饰。外壁结构上，大多数种类结构层次模糊，无明显的复盖层和柱状层分化。但 *T. grandiflorum* 和 *T. catesbaei* 2 个种则有较明显的复盖层和柱状层的分化。*T. lancifolia* 和 *T. lanceolatum* 具有截然不同的纹饰特征，前者为颗粒状纹饰，后者为星状纹饰。从孢粉学考虑这 2 个种不宜合并，应独立为 2 个种。本文还对产于亚洲和北美的种类进行了比较和讨论。

关键词 延龄草属，花粉形态

POLLEN MORPHOLOGY OF TRILLIUM

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Abstract The pollen of 23 species of *Trillium* was examined by LM, SEM and TEM. The result showed that the pollen shape, the size and the aperture type are similar basically, characterized by spherical or subspherical pollen grains, inaperturate, size ranging from 20−38 um in diameter. The exine sculpture, however, shows different and four types are recognized, i.e., granulate−, verrucate−, starred−and spinulate types. The wall exine stratification is faint in most species except *T. grandiflorum* and *T. catesbaei* which have obvious tectum and collumellae in wall structure. *T. lancifolia* and *T. lanceolatum* have different sculpture, the former has granulate sculpture,the latter starred sculpture .Palynology suggests that the two species be treated as separate species.

Key words Pollen morphology, *Trilium*

The genus *Trillium* L., together with *Paris* L., *Medeola* L. and *Scoliopus* Torr. composes the family Trilliaceae according to most taxonomists, though some authors retained it in Liliaceae. In the family, *Trillium* is the largest genus with 50 or so species distributed disjumctly between North America and eastern Asia, other two genera *Medeola* and *Scoliopus* are small and restricted to North America, especially in eastern N. America [1−2]. Freeman J.D. divided the genus *Trillium* into two subgenera, subgerus *Trillium* amd subgerus *Phyllanterum*, the former comprises the pedicellate−flowered species and the latter

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includes all other sessile-flowered species except *T.pusillum* which has pedicellate and sessile forms.Freeman believes that the pedicellate-flowered species probably preceded the sessile-flowered ones phylogenetically by reason of the presence pedicels in more primitive related genus *Paris*. In general, the external morphology of the two genera is similar except for the leaf number and flowermery,i.e.,*Trillium* with a whorl of 3 leafy bracts and trimerous flower, and *Paris* a whorl of 4 leafy bracts and tetramerous flower. *Paris* distributes in north timperate zone from Asia to Europe, and *Trillium* in both Asia and North America.

In *Trillium*, the remarkable uniformity in external morphology and internal structure makes the delimitation of interspecies difficult,some species which were separated by some taxonomists are incorporated into one species by other authors,for example *T. lancifolia* and *T.lanceolatum*. Palynology data sometime possess the potential in determing the delimitation of species and in assessing the taxonomic relationships.

Pollen morphology of *Trillium* has beem studied by Ikuse (1965), Nair& Sharma (1965) and Takahashi M.[2,3]. Takahashi examined 17 species from North America in 1982 and five species and two interspecies hybrids from eastern Asia in 1983 ,and his study being the most detailed and systematic so far. However, because of the large number of species in the genus, his study has not involved half of the total species of the genus, it is necessary to examine the other more than half species for summarizing the pollen morphology character of the whole genus. In this paper, 23 species have been examined by SEM and TEM (14 species). From the comparison four types of exine sculptures are divided. We hope that this study would supply a deficiency in pollen morphology of the genus and provide some additional data for the classification and systematic study of *Trillium*..

Materials and Methods

All pollen grains were removed from herbarium material deposited at the University of Southwestern Louisiana (LAF) and the collections and locations are listed in Appendix.

Pollen was treated by 5% KOH first and then acetolyzed after the method of Erdtman (1960). Pollen sizes are based on the measurement of 15—20 acetolyzed pollen grains mounded on slides with a polystyrene mounting medium. For SEM, acetolyzed pollen was dehydrated in an alcohol series and mounted on stubs with the high vacuum Wax Apiezon W-100, coated with gold palladium by Balzer CDS 040, viewed and photographed with a Hitachi S-450 scanning electron microscope. However, due to the very thin pollen wall, the acetolyzed pollen of most species collapsed and the shape of the pollen grain changed very much, unacetolyzed pollen was mounted directly on stubs and photographed again as the same manner stated above. For TEM, acetolyzed pollen was put into 1% heated agar, after centrifugation and cool, cut the bottom part of agar containing pollen and dehydrated in an alcohol series from 30% to 100% and at last in 100% acetone. After 2—3 changes of acetone, the agar containing pollen sample was embedded in Spurr's low viscosity resin, sectioned and stained with Patassium Permanganate ($KMnO_4$), and viewed and photographed with a Hitachi H-600 transmission electron microscope.

Result

All species of *Trillium* examined in this study have pollen grains characterized by spherical or subspherical (rarely) shape, inaperture, size ranging from 20 to 38 μm in diameter. Four types of sculpture are recognized, that is granulate-, verrucate-, starred- and spinulate types. The pollen wall has very thin exine which is usually collapsed and make the shape change form time to time. The pollen wall stratification is faint except *T. grandiflorum* and *T. catesbaei* which have stratified wall structure composed of tectum, collmellae and footlayer.

Detailed Description

T. stamineum (plate Ⅰ: 1—4; Ⅳ-A)

pollen grain spherical to subspherical, inaperturate, size ranging from 22 to 36 μm in diameter, exine sculpture is composed of verrucae, and two types of verrucae can be recognized, one is more than 1 μm, the other 0.3—1 μm. the surface of verrucae is striato-reticulate, the pollen wall is unstratified, composed only of verrucae and the underlying very thin footlayer.

T. decumbens (plate Ⅰ: 7—8; Ⅳ-B)

Pollen grain spherical, inaperturate, size 20— 38 μm in diameter, exine sculpture is comprised of granula which are multangular and evenly distributed over the wall surface of pollen. The pollen wall is unstratified, composed only of granula and underlying, discontinuous, very thin footlayer.

T. discolor (plate Ⅰ: 5—6; Ⅳ-C)

Pollen grain spherical or subspherical, inaperturate, size ranging from 18 to 28 μm in diameter. Exine sculpture is composed of granula which are multangular. The pollen wall is unstratified, composed only of granula and the underlying, discontinuously very thin foolayer.

T. gleasoni (plate Ⅰ: 9—10; Ⅳ-D)

Pollen grain spherical, inaperturate, size 20—32 μm in diameter. Exine sculpture is composed of small and compact granula which are multangular. Pollen wall is unstratified and only composed of granula and underlying thin footlayer.

T. grandiflorum (plate Ⅰ: 11—12; Ⅳ-E)

Pollen grain spherical, inaperturate, size ranging from 24 to 36 μm in diameter. Exine surface is covered by extremely few and scattered (sparse) Spinulose, but the surface between the spinulose is rugulate. The pollen wall is stratified and three layers are recognized, that is relatively continuous tectum, poorly developed columellae and footlayer.

T. lanceolatum (plate Ⅰ: 13—15; Ⅳ-F)

Pollen grain spherical, inaperturate, size 26— 34 μm in diameter. Exine sculpture is composed of sparsely starred units which are above 1.5 μm in diameter and easily separate from wall surface (plate Ⅰ :15) The surface between starred units is scabrate——rugulate The pollen wall is unstratified and only composed of starred units and the underlying footlayer.

T. recurvatum (plate Ⅰ: 16—17; Ⅳ-G)

Pollen grain spherical, inaperturate, size ranging form 22 to 32 μm in diameter. Exine surface is covered by starred units which are smaller than that in T. lanceolatum, ca. 1 μm in diameter but densely distribution. The pollen wall is unstratified, composed only of starred units and the underlying footlayer.

T. catesbaei (Plate Ⅱ: 1—4; Ⅳ-H)

Pollen grain spherical, inaperturate, size ranging from 20 to 30 μm in diameter. Exine sculpture is composed of granula which are round and smooth ca. 0.5 to 1 μm in diameter. Pollen wall is stratified and can be divided into discontinuous tectum, distinctive and high columellae and relatively thick footlayer.

Under the footlayer, a thin lamellate layer (endexine ?) can be seen (plate Ⅳ-H)

T. flexipes (plate Ⅱ:5—6)

Pollen grain spherical, inaperturate, size ranging from 23 to 28 μm in diameter. Exine sculpture is composed of granula

T. reliquum (plate Ⅱ: 7—8)

Pollen grain spherical, inaperturate, size ranging from 24 to 30 μm in diameter. Exine sculpture is composed of granula and two kinds of which are recognized, one is large but irregular in shape, the other smaller.

T. viride (plate Ⅱ: 9—10)

Pollen grain spherical, inaperturate, size ranging from 22 to 28 μm in diameter. Exine sculpture is composed of different size of granula which are smooth.

T. sessile (plate Ⅱ: 11—12; Ⅳ-1、J, K)

Pollen grain spherical, inaperturate, size ranging from 22 to 30 μm in diametor. Exine sculpture is composed of different sized granula. Pollen wall is unstratified, composed only of granules and underlying footlayer. The granules stand side by side on the footlayer (plate Ⅳ-J, K).

T. lancifolium (plate Ⅱ: 13—14)

Pollen grain spherical, inaperturate, size ranging from 20 to 30 μm in diameter. Exine sculpture is composed of different sized granules which are smooth.

T. maculatm (plate Ⅱ: 15—16)

Pollen grain spherical, inaperturate, size ranging from 24 to 32 μm in diameter. Exine sculpture is composed of various sizes and shapes of granules.

T. gracile (plate Ⅱ: 17—18)

Pollen grain spherical, inaperturate, size ranging from 25 to 33 μm in diameter. Exine sculpture is composed of different sized granules which are round and smooth.

T. erectum (plate Ⅲ: 1—2; Ⅳ-L, M、N)

Pollen grain spherical, inaperturate, size ranging from 22 to 30 μm in diameter. Exine sculpture is composed of various sized granules which are round and smooth. Pollen wall is unstrarified, only composed of numerous granula and underlying footlayer.

T. vaseyi (plate Ⅲ: 3—6; Ⅳ-O)

Pollen grain spherical, inaperturate, size ranging from 20 to 30 μm in diameter. Exine sculpture is composed of various sizes of granules which are round and smooth. Pollen wall is unstratified, composed only of granules and underlying footlayer which is basically continuous.

T. texanum (plate Ⅲ: 7—8; Ⅳ-P)

Pollen grain spherical, inaperturate, size ranging from 24 to 28 μm in diameter. Exine sculpture is composed of small granules which are compactly distributed over the surface of pollen grain. Pollen wall is unstratified, composed only of granula and underlying, thin and discontinuous footlayer.

T. foetidissimum (plate Ⅲ: 9—10; Ⅳ-Q)

Pollen grain spherical, inaperturate, size ranging from 24 to 28 μm in diameter. Exine sculpture is composed of various sized and shaped granules. Pollen wall is unstratifiod, only composed of granula and thin, discontinuous footlayer.

T. ludovicianum (plate Ⅲ: 13—14)

Pollen grain spherical, inaperturate, size ranging from 25 to 30 μm in diameter. Exine sculpture is composed of different sized granules which are round and smooth.

T. rugelii (plate Ⅲ: 11—12; Ⅳ-R)

Pollen grain spherical, inaperturate, size ranging from 24 to 32 μm in diameter. Exine sculptue is composed of different sized granules which are smooth. Pollen wall is unstratified, composed only of granula and thin, discontinuous foolayer.

T. cuneata (plate Ⅲ: 15—16)

Pollen grain spherical, inaperturate, size ranging from 24 to 28 μm in diameter. Exine sculpture is composed of granules which are round and smooth but unevenly distributed over the surface of the pollen grain.

T. underwoodii (plate Ⅲ: 17—18)

Pollen grain spherical, inaperturate, size ranging from 24 to 32 μm in diameter. Exine sculpture is composed of different sized granules which are unevenly distributed over the surface of the pollen grain.

Discussion

From above description of pollen morphology of *Trillium*, we can see there are no remarkable differernce in pollen shape, size and aperture among different species. The greatest variation was observed in exine sculpture, of which four types are recognized:

a) granular sculpture which can be subdivided into two kinds, one kind of granules is round and smooth, for example *T. catesbaei*, *T. gracile*, *T. erectum*, *T. vaseyi* etc; the other kind of granules is multangular, such as *T. decumbens*, *T. discolor* and *T. gleasoni*.

b) verrucate sculpture, only one species, *T. stamineum*.

c) starred sculpture, i. e., *T. lanceolatum* and *T. recurvatum*.

d) spinulate sculpture, only one species, *T. grandiflorum*.

As far as the wall structure is concerned, most species in the genus have only faint stratification and the wall exine is composed only of granules, verrucae, spinules or other structure units and the underlying, thin footlayer. *T. grandiflorum* and *T. catesbaei*, however, have stratified exine structure composed of tectum, poorly developed columellae and footlayer, especially in *T. catesbaei*, the exine structure at nonapertural area consists of perforate and thick tectum, well-developed columellae and thick footlayer, the columellae stand side by side on the footlayer, and two columellae support a tectum unit (plate Ⅳ-H), the footlayer is underlain by a thin lamella (endexine ?). The exine structure of T. catesbaei is very similar to that of *Paris* [4].

T. lanceolatum and *T. lancifolium* have been treated as one species by some authors, such as Gates (1917), Freeman (1975) and Takahashi (1982). The pollen morphology, especially the exine sculpture, however, shows that the two species are remarkably different, *T. lancifolium* has exine of granulate sculpture but *T. lanceolatum* has multangular-starred sculpture exine. Palynology supports that tbe two species are treated as separate species.

T. lanceolatum and *T. recurvatum* have very similar exine sculpture, i. e. starred sculpture, showing that the two species are closely related. The result gives support to Takahashi's view [2].

T. grandiflorum is the only one example with spinulate sculpture in our study. According to Takahasi, *T. ovatum* and *T. nivale* also have spinulate exine sculpture. The similarity of exine sculpture shows that they are closely related to each other.

Relationship of asiatic species and North American species of *Trillium*. According to Takahashi, most species except *T. govanianum* have exine sculpture composed of granules, such as *T. smallii*, *T. apetalon*, *T. kamtschaticum* and *T. tschonoskii*. In our study, of 24 species examined, 19 species also have granulate sculpture. This suggests that they are closely related to each other. With regard to *T. govanianum*, Nair and Sharma (1965) reported that the pollen was monocolpate with "crystallate" exine surface and Takahashi described as "angular and striately sculptured". Haga and Watanabe (1966) reported that *T. govanianum* was an allotetraploid composed of two different genomes, neither of which is found in Japanese or American species of *Trillium*. They treated this species as *Trillidium govanianum*, a distinct genus, and so did Takahashi in 1983, he said "The isolated features in pollen morphology of *Trillium govanianum* support the exclusion of the species from *Trillium*". Indeed, when we compared the SEM micrographs and description of North American species *T. undulatum* (Takahashi 1982, Figs 25—28) with those of *T. govanianum* (Takahashi 1983, Figs 16—17), we found that the two species have the same exine sculpture. In the pollen of *T. govanianum*, he said "exine ornamentation is clavate, characterized by projections which are sculptured striately and angular"[3] and in *T. underlatum* he said "exine ornamentation verrucate, characterized by verrucae which are sculptured striately and are angulous". The descriptions show that the two species have the same exine sculpture. The resemblance in exine sculpture would suggest that *T. govanianum* and *T. underlatum* are closely related each other, and the exclusion of *T. govanianum* from *Trillium* is worthy of further consideration.

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Appendix Species names and voucher specimens

Trillium stamineum Harbison LAF (Herbarium of The University of Southwestern Louisiana) 51581.

T. decumbes Harbison LAF 43601.

T. discolor Wray ex Hooker LAF 30931, S. Carolina.

T. gleasoni Fern. LAF 65856

T. grandiflorum (Michx.) Salisb. LAF 71783.

T. lanceolatum Boykin LAF 30927, S. Carolina.

T. recurvatum Beck LAF 26853.

T. catesbaei Elliott LAF 66016.

T. flexipes Raf. LAF 55039.

T. reliqum Freeman LAF 01, Georgia, Richmond.

T. viride Beck LAF 7019, Missouri.

T. sessile L. LAF 69904.

T. lancifolium Raf. LAF 16346.

T. maculatum Raf. LAF 65938.

T. gracile Freeman LAF 46423.

T. erectum L. LAF 46302, N. Carolina.

T. vaseyi Harbison LAF 31385.

T. texanum Buckl. LAF 44932.

T. foetidi ssimum Freeman LAF 46439, W. Feliciana.

T. ludovicianum Harbison LAF 72803.

T. rugelii Rendle. LAF 16341.

T. cuneatum Raf. LAF 43575.

T. underwoodii Small LAF 43599.

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Explanation of plates

Plate Ⅰ figs 1—4 *Trillium stamineum*, figs 2 & 4 showing partial areas of the frames in figs 1 & 3 respectively and magnifying the frames 5 diameters; figs 5–6 *T. discolor*, fig 6 showing a partial area of the frame in fig 5 and magnifying the frame 5 diameters; fig 7—8 *T. decumbens*, fig 8 showing a partial area of the frame in fig 7 and magnifying the frame 5 diameters; fig 9—10 *T. gleasoni*, fig 10 showing a partial area of the frame in fig 9 and magnifying the frame 5 diameters; fig 11—12 *T. grandiflorum*, fig 12 showing a partial area of the frame in fig 11 and magnifying the frame 5 diameters; fig 13—15 *T. lanceolatum*, fig 14 showing a partial area of the frame in fig 13 and magnifying the frame 5 diameters, fig 15 showing a partial wall surface separated from pollen grain, the magnification is the same as in fig 14; fig 16—17 *T. recurvatum*, fig 17 showing a partial area of the frame in fig 16 and magnifying the frame 5 diameters.

Plate Ⅱ figs 1—4 *T. catesbaei*, figs 2 & 4 showing partial areas of the frames in figs 1 & 3 and magnifying the frames 5 diameters; fig 5—6 *T. flexipes*, fig 6 showing a partial area of the frame in fig 5 and magnifying the frame 5 diameters; fig 7—8 *T. reliquum*, fig 8 showing a partial area of the frame in fig 7 and magnifying the frame 5 diameters; figs 9—10 *T. viride*, fig 10 showing a partial area of the frame in fig 9 and magnifying the frame 5 diameters; figs 11—12 *T. sesslie*, fig 12 showing a partial area of the frame in fig 11 and magnifying the frame 5 diameters; figs 13—14 *T. lancifolium*, fig 14 showing a partial area of the frame in fig 13 and magnifying the frame 5 diameters; figs 15—16 *T. maculatum*, fig 16 showing a partial area of the frame in fig 15 and magnifying the frmae 5 diameters; figs 17—18 *T. gracile*, fig 18 showing a partial area of the frame in fig 17 and magnifying the frame 5 diameters.

Plate Ⅲ fig 1—2 *T. erectum*, fig 2 showing a partial area of the frame in fig 1 and magnifying the frame 5 diameters; figs 3—6 *T. vaseyi*, figs 4 and 6 showing partial areas of the frames in figs 3 and 5 and magnifying the frame 5 diameters; figs 7—8 *T. texanum*, fig 8 showing a partial area of the frame in fig 7 and magnifying the frame 5 diameters; figs 9—10 T. foectidissimum, fig 10 showing a partial area of the frame in fig 9 and magnifying the frame 5 diameters; figs 11—12 *T. rugelii*, fig 12 showing a partial area of the frame in fig 11 and magnifying the frame 5 diameters; figs 13—14 *T. ludovicianum*, fig 14 showing a partial area of the frame in fig 13 and magnifying the frame 5 diameters; figs 15—16 *T. cuneatum*, fig 16 showing a partial area of the frame in fig 15 and magnifying the frame 5 diameters; figs 17—18 *T. underwoodii*, fig 18 showing a partial area of the frame in fig 17 and magnifying the frame 5 diameters.

Plate Ⅳ (A) *T. stamineum*, × 10 000; (B) *T. decumbens*, × 15 000; (C) *T. discolor*, × 17 000; (D) *T. gleasoni*, 17 000; (E) *T. grandiform*, × 8 000; (F) *T. lanceolatum*, × 12 000; (G) *T. recurvatum*, × 20 000; (H) *T. catesbaei*, × 10 000; (I–K) *T. sessile*, I × 2 500, J & K × 10 000; (L–N) *T. erectum*, L × 3 000, M & N × 8 000; (O) *T. vaseyi*, × 10 000; (P) *T. texanum*, × 8 000; (Q) *T. foetidissimum*, × 15 000; (R) *T. rugelii*, × 12 000.

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粉叶小檗愈伤组织的培养及小檗碱的含量*

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摘要 由粉叶小檗（*Berberis pruinosa*）的茎、腋芽、叶、实生苗的子叶及胚轴均可诱导出愈伤组织。B_5+2, 4-D 0.5 mg／L+KT 0.2 mg／L 培养基对诱导较好，而 B_5+2, 4-D 0.5 mg／L+KT 0.5 mg／L 对愈伤组织生长较适宜。接种量在 0.4—0.8g（20 mL 培养基）之间较为适宜。经薄层层析-分光光度法、薄层扫描法鉴定，证明愈伤组织具有合成小檗碱的能力，含量高达 1.8148%，接近原植物体含量（茎，1.58%）。

关键词 粉叶小檗，小檗碱，愈伤组织 组织培养

CALLUS CULTURE OF BERBERIS PRUINOSA AND BERBERINE CONTENT IN CALLUS

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Abstract Form the excised stem, axillary bud, leaf, cotyledon, plumulax axis of *Berberis pruinosa* Franch. the callus could been induced on Gamborg(B_5) medium or Murashige-Skoog(MS) medium, but B_5 was better than MS. The combination of 2,4-D 0.5 mg／L and KT 0.2 mg／L was found most effective for induction, while the combination of 2,4-D 0.5 mg／L and KT 0.5 mg／L was better to growth and alkaloids synthesis. The opitimum inoculum quantities for callus growth was about 0.4—0.8g／20 mL media.

Identification by thin-layer chromatography, ultraviolet and visible absorption spectrum of alcohol extracts of callus and stem, leaves of *B. pruinosa* Franch. proved that the callus had the capability to synthesize berberine. The contents of berberine in the callus was 1.8148%, it is slightly higher than the contents of berberine in the stems of infact plants (it was 1.58%).
The growth curves of the callus are sigmoid. When the callus had cultured for about 27 days, the growth rate and contents of berberine were highe than other times.

Key words *Berberis pruinosa*, Berberine, Callus

粉叶小檗（*Berberis pruinosa* Franch.）又称大黄连刺、三颗针等，多年生灌木，分布于广西北部，云南中部至西北部，西藏南部及东南部[1]。它的化学成分中有小檗碱、掌叶防已碱及药根碱，是西南几省提取黄连素的主要原料之一。经昆明市第一人民医院临床试验，该药对菌痢、肠炎、结膜炎、中耳炎、感冒、滴虫性阴道炎等病确有疗效，对浸润性肺结核、渗出性胸膜炎、流行性腮腺炎等方面亦有一

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定疗效[2]。但由于对植株需要量大，植物生长又缓慢，经历年大量采挖，野生资源已渐枯竭。我们试应用细胞培养技术，从粉叶小檗培养物中提取小檗碱，对细胞生长和产物积累等种种因素进行研究。国内曾有小檗属植物的有关报道[3]，但目前未见粉叶小檗组织培养研究的报道。

材料和方法

1. 愈伤组织的诱导

用粉叶小檗野生植株的茎、腋芽、叶片及种子萌发苗的子叶和胚轴作外植体材料，用自来水冲洗 15 min 后，在无酶洗衣粉液中振荡 10 min，再用自来水冲洗 15 min，无菌条件下于 75%酒精中浸泡 10 s 后，用 0.1%升汞作表面灭菌 8 min，无菌水冲洗 4—5 次后，将茎和胚轴切成 0.5 cm 长的小段，叶片切成 0.5 cm^2 的小块，腋芽剥除外层老叶，子叶切下，接种于 MS[4] 和 B_5[5] 培养基上，激素为 2，4-D 0.5+KT0.2，或 2，4-D1.0+KT0.2（激素浓度单位为 mg／L，以下同）。在温度 20±1℃的恒温箱中暗培养。子叶及胚轴在培养 1 周左右出现浅黄色愈伤组织，腋芽及茎的愈伤组织在 2 周左右产生，叶片愈伤组织在 2 周后陆续形成。

2. 愈伤组织的培养

来自子叶的愈伤组织在 B_5+2,4-D 0.5+KT0.5 培养基上继代培养选择（3—4 周继代一次），经 7 代培养后选择出生长快、颜色黄、质地疏松的愈伤组织无性系，转移到 13 种不同的培养基上避光培养，培养用 50 mL 三角烧瓶，内盛 20 mL 培养基，每接种愈伤组织 2—3 块（约 0.5—1.5 gFW），生长速度以每天每升培养基上平均增加的鲜重或干重的克数表示（$g.d^{-1}.L^{-1}$）。

3. 小檗碱的鉴定和含量测定

小檗碱的提取：愈伤组织干粉（65℃下烘干）100 mg，用 50 mL 甲醇在索氏提取器中提取至甲醇无色，提取液浓缩后，用甲醇定容为 5 mL 或 10 mL，作点样用[6, 7]。

小檗碱的鉴定及含量测定：小檗碱含量测定用薄层层析-分光光度法[8]，点样量 50—100 μm，相当于样品 0.5—1 mg。色斑刮取后用 0.05 mol／L 硫酸乙醇浸提 30min，4000 r／min 离心 15 min，提取上清液在 UV-120 型分光光度计（日本岛津）上 420nm 处，以同等面积的硅胶硫酸乙醇提取液（离心上清液）作对照测量吸光度，并在 200—500nm 波长范围内测定吸收光谱，另用 CS-910 型双波长薄层扫描仪（日本岛津）在 200—500nm 波长对样品的与标准品（硫酸小檗碱）的层析斑点进行扫描（狭缝 1mm×1mm，扫描宽度 1×5mm，锯齿扫描，反射吸收）。

以上实验均重复 3—6 次。小檗碱含量以干重的百分比（%）表示。

结果和讨论

1. 不同培养基对愈伤组织诱导和生长的影响

愈伤组织的诱导结果如表 1，B_5 培养基较 MS 培养基好，两种培养基中，KT 一定浓度时，2，4-D 浓度稍低更有利愈伤组织诱导，单加 2，4-D 也能诱导出愈伤组织，但没有 2，4-D 和 KT 复合使用效果好。

对愈伤组织的生长，B_5 培养基仍优于 MS，其生长速度较 MS 高一倍左右，当 KT 浓度一定时，2，4-D 过高会抑制生长。根据这一结果，以后继代培养主要采用 B_5 培养基。

接种量多少对愈伤组织生长亦会影响，结果如表 2，接种量偏低或偏高都不利于愈伤组织的生长。最适宜的接种量是 0.4—0.8 gFW（20 mL 培养基），此范围内接种、鲜重和干重增长较高。

表 1 不同培养基对愈伤组织诱导和生长的影响

Table 1 Effect of different medium on growth and induction of callus

Basic medium	2,4-D (mg／L)	KT (mg／L)	Percent of callus formation (%)	Increment of FW ($g.d^{-1}.L^{-1}$)	Increment of DW ($g.d^{-1}L$)
MS	1.0	0.2	17.72		
	0.5	0.2	25.11		
	0.5	0	15.00		
	1.0	0.5		2.7874 ± 0.9817	0.1635 ± 0.0595
	0.5	0.5		3.2627 ± 1.0485	0.1762 ± 0.0434
B_5	1.0	0	15 00		
	1.0	0.2	55.77		
	0.5	0.2	58 95		
	1 0	0.5		4.1999 ± 1.0485	0.2400 ± 0.0654
	0.5	0.5		4.3868 ± 0.8085	0.2655 ± 0.0661

• Percent of callus formation calcuated at 19 days after inoculating explant (cotyledon). Callus grew for 23 days

表 2 接种量对愈伤组织生长的影响

Table 2 Effect of inoculum quantity on callus growth

Inoculative quantity (g FW／20 mL media)	Increment of FW ($g.d^{-1}.L^{-1}$)	Incement of DW ($g.d^{-1}L^{-1}$)
0.3602	3.7385	0.2044
0.4567	4.0059	0.2191
0.5707	4.6511	0.2544
0.6518	4.3412	0 2374
0.7476	5.2399	0.2866
0.8477	5.1769	0 2832
1.3207	0.3985	0.0217

• Medium: B_5+2,4-D 0.5 mg／L+KT 0 5 mg／L. Callus grew for 23 days

2. 粉叶小檗愈伤组织中小檗碱的含量

为了解愈伤组织是否含有合成原植物所含的有效成分小檗碱的能力，进行了分析鉴定。薄层层析的结果表明，愈伤组织中含有和原植物相同的有效成分小檗碱，其 Rf 值和标准品（硫酸小檗碱）的 Rf 一致，为 0.43。样品和标准品的层析斑点扫描结果（图 1）表明，扫描峰在同一位置，且两者的分别峰面积之和与二者叠点样得到的峰面积近相等。据文献[2]记载，粉叶小檗地上部分含有掌叶防已碱，但我们做的另一试验（展层剂为苯-氯仿-异丙醇＝8∶8∶2, v／v），愈伤组织中无掌叶防已碱，Rf0.32 的斑点和文献报道的药根碱位置相近[2]。愈伤组织（甲醇提取液）层析层，小檗碱斑点洗脱液的紫外光和可见光吸收光谱，除 228nm 处吸收峰有所前移外，其余与标准品吸收光谱是一致的（图 2）；小檗碱斑点薄层扫描结果，显示两者的吸收光谱基本相同，证明从愈伤组织中分离出的是小檗碱。

表 3 愈伤组织、植物茎、叶小檗碱含量比较

Table 3 The comparision of berberine contents between callus and stems, leaves of infact plant

Material	Callus 3	Callus 4	Stem**	Stem*	leave*
berberine cont. (%DW)	1.5210	1.8148	0.8941	1.5800	0.2130

3,4: Grown medium of callus is B_5+2,4-D 0.5mg／L+KT 0.5mg／L, B_5+0.5mg／L+KT 0.5mg／L respectively.

• *Result of this test.* ** *The information in literature. Callus grew for 20—23 days.*