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生長素與通氣處理對仙草組織培養苗增殖與生育之影響[1]

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摘　要

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本研究探討不同生長激素 [NAA (α-naphthaleneacetic acid)、IAA (indole-3-acetic acid) 及 IBA (indole-3-butyric acid)] 及透氣處理對仙草組培苗育成之影響。莖頂培植體接種於 0.5 mg/L NAA 之 1/2 MS 固體培養基，暗培養 4 週所誘導的團粒狀癒合組織，再經光照 4 週培養後可形成叢生芽，以供仙草種苗大量繁殖利用；將叢生芽截切為單節培植體，移植至含 0.5 mg/L 之 NAA、IBA 或 IAA 之 1/2 MS 基礎鹽類培養基，於 25℃、光照 30–35 μmol m^{-2} s^{-1}、培養 6 週以比較植株生育情形；於另一個試驗探討不同瓶口覆蓋物及透氣時間處理對培養於含 0.5 mg/L IBA 之 1/2 MS 基礎鹽類培養基之植株苗玻璃質化的影響，結果顯示 3 個生長素處理，以 IBA 可得生長較佳的瓶苗，根重為 0.031 克/株，而 IAA 處理者只有 0.011 克/株；透氣試驗以 4 層藥包紙覆蓋瓶口，再覆以石蠟膜以減少透氣，經培養 5 週後再除去石蠟膜以透氣者，可得移植存活率高之優質組培苗，移植存活率為 98.7%，較鋁箔處理者 92.1%為高。藉由生長素 IBA 的添加及透氣處理可育成健康、生長良好之仙草組培苗，此技術可應用於增殖種苗，並免除非季節性佔用種植土地之情形。

關鍵詞：仙草、組織培養、生長素、透氣處理。

前　言

仙草 (*Mesona procumbens* Hemsl.) 屬唇形科高經濟價值之特用作物，依據古代文獻中藥大辭典記載，仙草味澀、甘、寒，具有清熱、解渴、涼血及降血壓之功效，可治臟腑熱毒 (Hung 2001)，說明仙草是具生理機能性之中草藥。仙草的許多療效目前均已得到科學證實；Capellades (1990) 報告指出，仙草水萃取物具有良好的清除自由基及抗氧化能力，內含熊果酸 (ursolic acid)、齊墩果酸 (oleanolic acid) 等三萜類 (triterpenes) 成分，這些成分在文獻上均指出具減緩急慢性肝炎、抗發炎、抗高血脂及抗腫瘤之生理功效 (Liu 1995)。仙草栽培之種苗來源慣以扦插育苗以防種質變異，在非種植季節仍需佔地種植母株，若以育成組織培養瓶苗的技術增殖種苗，則可省去種植母株土地空間，並可避免天候病蟲害等不確定之傷害種

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苗因素。因此本研究擬利用農試所栽種之農試 1 號仙草品種，進行組織培養苗之繁殖試驗，探討苗體繁殖之合適條件，以供相關產業參考利用。

材料與方法

供試材料

本試驗使用之材料，係採自農試所內栽種的農試 1 號仙草。

癒合組織與不定芽誘導之觀察

切取仙草莖頂組織長約 3 mm，以自來水沖洗乾淨後，於 70%酒精中浸泡 30 秒，再以 0.5% 次氯酸鈉 (每 100 mL 含 2–3 滴 Tween 20) 震盪消毒 10 分鐘，最後以無菌去離子水清洗 3–4 次，於無菌接種箱中切去白化的組織後，接種於含 0.5 mg/L NAA、3%蔗糖、0.9%洋菜 (Difco agar) 及 pH = 5.7 ± 0.1 之 1/2 MS (Murashige & Skoog 1962) 斜面固體培養基 (Cassells *et al*. 1994)，培養容器為 45-mL 試管，培養環境為 (25 ± 1)℃，進行暗培養以誘導癒合組織。莖頂組織經 4 週暗培養後形成團粒結構之癒合組織，移至每日照光 16 小時、光強度約 30–35 μmol m^{-2} s^{-1} 之環境，約 1 個月後可誘導為不定芽。

不同生長素對植株生長與移植存活試驗

前項試驗誘導形成之不定芽切成含一分枝之節段作為培植體，分別接種於含 0.5 mg/L 之 NAA、IBA 或 IAA 之 3 種不同生長素 (auxin) 的 1/2 MS 培養基 (成分如前述)，培養容器為 125-mL 三角瓶，每種培養基各 10 瓶，瓶內各 5 個節段培植體，於 6 週後調查其育成瓶苗之農藝性狀，調查項目為株高、葉數、芽體數、根重、植株鮮重。將上述不同培養基之組培瓶苗，分別移出栽種於經高溫高壓滅菌之泥炭土、蛭石 (3 號)、珍珠石 (4 號) 1 : 1 : 1 (v/v) 之混合介質，不同處理均各種植 3 盤，於 1 個月後調查其存活率。

不同透氣處理對組培苗生長與移植存活試驗

將與前項試驗相同之節段培植體接種於含 0.5 mg/L IBA 之 1/2 MS 固體培養基，瓶內各 5 個節段培植體，瓶口覆蓋 4 層滅過菌藥包紙，最後再以石蠟膜 (Parafilm M®) 包覆於藥包紙外，分別於培養 3、4、5、6 週後去除外部石蠟膜以續行透氣培養，達培養週期共 8 週 (代碼分別為 P3 + DP5、P4 + DP4、P5 + DP3 及 P6 + DP2)，取出調查組培苗之農藝性狀；對照組為以二層鋁箔封住瓶口，試驗全程期間皆不除去石蠟膜 (代碼 AF8)，每種透氣處理各 10 瓶 (Chen *et al*. 2006a; Tsay *et al*. 2006)。將上述不同透氣處理之組培瓶苗，分別移出栽種於經高溫高壓滅菌之泥炭土、蛭石 (3 號)、珍珠石 (4 號) 1 : 1 : 1 (v/v) 混合介質，不同透氣處理處理均各種植 3 盤，於 1 個月後調查其存活率。

統計分析

上述試驗資料均經 SAS 統計分析套裝軟體進行變方分析 (analysis of variance, ANOVA) 後，以最小顯著差異性測驗 (least significant difference test, LSD test)，在 5%顯著水準下比較各處理平均值間之差異，若為百分率之數據，則先經平方根轉換。

結　果

不同生長素對植株生長與移植存活率之影響

仙草莖頂接種於 1/2 MS 含 0.5 mg/L NAA 之培養基，基部會形成團粒結構之癒合組織，將此癒合組織移至照光環境下再經 4 週培養後，可誘導大量不定芽產生，平均每個莖頂約可誘導 10–20 個不定芽 (圖 1)，將此不定芽截切為多個含一個節之培植體，接種於含有不同生長素之培養基，結果顯示 3 種生長素對植株葉數、根重及鮮重等性狀，以 0.5 mg/L IBA 處理，效果顯著優於含 0.5 mg/L NAA 或 IAA 之

圖 1. 仙草癒合組織接種於 0.5 mg/L NAA 之 1/2 MS 固體培養基移至照光環境 4 週後，誘導出之不定芽生長情形。

Fig. 1. Development of *Mesona procumbens* plantlets induced by calluses placed under light for 4 weeks cultivated in half strength MS basal solid medium containing 0.5 mg/L NAA.

處理；移植出瓶後植株之存活率也以 0.5 mg/L IBA 為佳，達到 92.3%，由結果顯示，誘導仙草節段發根及生長之最佳培養基為 0.5 mg/L 之 IBA (表 1)。瓶苗植株外觀上，玻璃質化之情形極為普遍。

不同透氣處理對組培苗生長之影響

為培育無玻璃質化之優質瓶苗，以上述試驗 0.5 mg/L IBA 處理之培養條件，進行透氣無菌培養，即捨棄鋁箔改以滅菌過的四層藥包紙封住瓶口外面再封以石臘膜，並分別在培養第 3、4、5、6 週後去除石蠟膜，以加大瓶內氣體和外界之通透率，經 8 週週期後，取出瓶苗作農藝性狀調查同時進行移植存活試驗，結果顯示以全程以鋁箔紙封瓶口之對照組，瓶苗有玻璃質化之現象，而藥包紙處理則只有封口 6 週者出現玻璃質化，且不論莖長、葉寬、根重及植株鮮重皆以在第 5 週去除石蠟膜者為佳，且較其它組有顯著性差異，取出之瓶苗進行移植試驗時，亦以第 5 週者存活率最高，較其它組有顯著性差異 (表 2)；其次以第 4 週處理根重大於第 6 週，其存活率也較優於第 6 週 (表 2)。本試驗結果亦顯示，太晚進行通氣處理不利於仙草瓶苗之生長，且仍出現玻璃質化，但太早通氣 (第 3 週) 則易使培養基失水龜裂，也不利於仙草瓶苗之生長 (圖 2)。

表 1. 仙草不定芽以不同生長調節劑處理六週後之組培苗農藝性狀調查

Table 1. Agronomic characteristics of regenerated plantlets of *Mesona procumbens* grown on 1/2 MS medium amended with different auxins for 6 weeks [z]

Auxins treatment	plantlet height (cm)	No. of leaves/ plantlet	No. of shoots/ plantlet	Root weight/ plantlet (g)	Fresh weight/ plantlet (g)	Survival rate (%)
IAA	3.5 ± 0.3 b [y]	17.4 ± 2.1 b	2.4 ± 0.5 a	0.011 ± 0.001 c	0.42 ± 0.01 b	87.5 ± 1.1 b
IBA	4.3 ± 0.3 ab	25.5 ± 2.9 a	2.1 ± 0.3 ab	0.031 ± 0.002 a	0.62 ± 0.01 a	92.3 ± 0.6 a
NAA	4.9 ± 0.2 a	17.5 ± 1.6 b	1.1 ± 0.4 b	0.021 ± 0.001 b	0.29 ± 0.01 c	91.3 ± 0.6 ab

[z] Single nodes explants were cultured on half strength MS basal solid medium containing 0.5 mg/L of IAA, IBA or NAA.
[y] Values are mean ± SE (n = 10). Means with different letter(s) in the same column are significantly different ($P < 0.05$) by LSD test. Percentage data of survival rate were square-root transformed prior to analysis.

表 2. 仙草苗培養行不同時間之透氣處理後之農藝性狀調查

Table 2. Agronomic characteristics of regenerated plantlets of *Mesona procumbens* grown under different gas exchange treatments

Ventilation closures [z]	Stem length (cm)	Leaf width (cm)	Root weight/plantlet (g)	Fresh weight/Plantlet (g)	Survival rate (%)
AF8	1.36 ± 0.09 b [y]	1.04 ± 0.07 b	0.021 ± 0.001 d	0.500 ± 0.010 c	92.1 ± 0.33 d
P3 + DP5	1.32 ± 0.06 b	1.05 ± 0.04 b	0.030 ± 0.001 c	0.504 ± 0.009 c	92.9 ± 0.18 d
P4 + DP4	1.44 ± 0.05 ab	1.08 ± 0.04 ab	0.041 ± 0.013 b	0.602 ± 0.019 b	97.2 ± 0.28 b
P5 + DP3	1.59 ± 0.08 a	1.21 ± 0.06 a	0.051 ± 0.001 a	0.718 ± 0.023 a	98.7 ± 0.29 a
P6 + DP2	1.35 ± 0.08 b	1.06 ± 0.05 ab	0.031 ± 0.001 c	0.610 ± 0.021 b	96.0 ± 0.24 c

[z] AF8: The opening of each container was covered with two layers of aluminum foil (AF) during 8 weeks of incubation; P3 + DP5, P4 + DP4, P5 + DP3, and P6 + DP2: Two additional layers of Parafilm (P) were removed after 3, 4, 5, and 6 weeks of incubation, followed by four layers of dispense paper for the next 5, 4, 3, and 2 weeks of incubation.

[y] Values are mean ± SE (n = 10). Means with different letter(s) in the same column are significantly different ($P < 0.05$) by LSD test. Percentage data of survival rate were square-root transformed prior to analysis.

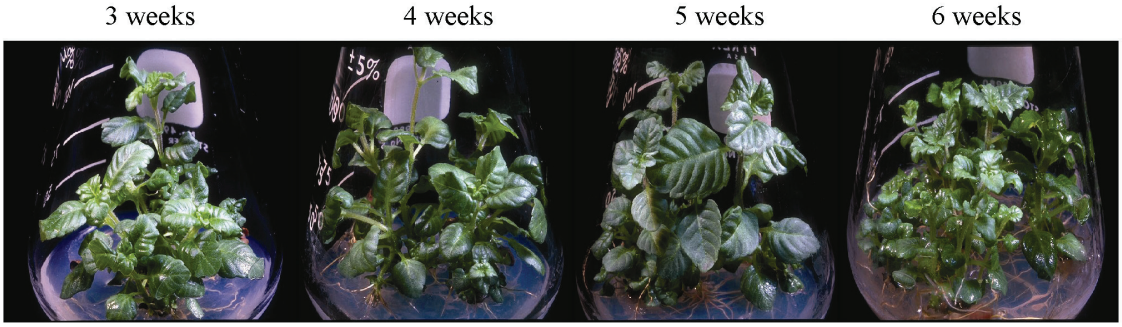

圖 2. 仙草苗在 0.5 mg/L IBA 培養下行不同時間之透氣處理後之生長情形。

Fig. 2. Effect of duration of ventilation treatment on growth of *Mesona procumbens* seedlings cultivated on half strength MS basal solid medium containing 0.5 mg/L IBA. Note vigorous growth of seedlings in the treatments of 3-, 4- or 5-weeks old seedlings but poor growth in the treatment of 6-weeks old seedlings.

討　論

利用組織培養大量繁殖種苗是商業種苗生產之重要技術，本試驗進行仙草瓶苗生長與發根試驗時，以 1/2 MS 基礎培養基分別添加 0.5 mg/L IAA、NAA、IBA 三種處理，皆可育成仙草瓶苗，存活率也都有 80%以上，觀察其仙草株型，在培養至第 3 週時，分枝大致已成型。

量產組織培養種苗常利用 IAA、NAA、IBA 為促進組培苗發根之生長調節劑，其中又以 NAA、IBA 效果為佳 (Gaspar *et al*. 1996; Moncousin 1991)。由本研究可見以 IBA 處理者，根重大於 NAA 處理。以 IBA 處理者植株較重、株形發展均衡且根系形成較佳；亦有研究指出若 IBA 或 NAA 濃度太高，易造成基部癒合組織化現象 (basal callusing) (Chang *et al*. 2000)，會不利於後續的移植馴化成苗，在本研究中並未發生此現象，可見 0.5 g/mL IBA 為培育仙草瓶苗之適當生長調節劑及濃度；由於此試驗皆以鋁箔封住瓶口，不論何種處理皆出現植體玻璃質化 (hyperhydricity) 之情形，以鋁箔封住瓶口是為防止污染及培養基水分散失，但由於其材質透氣性低，導致培養瓶內濕度高、有害氣體 (如二氧化碳及乙烯) 及致毒形態氧

(toxic oxygen forms) 的累積，致使植體葉面氣孔及表皮臘質層的發育異常或喪失功能，如此造成苗體外觀似玻璃，透明易碎，移植馴化存活率不高 (Chen *et al*. 2006b)。

為改善仙草組培苗玻璃質化現象，本試驗續探討通氣處理對瓶苗之育成影響，以上述生長較佳之 IBA 培養基條件接續以下試驗，摒除上述之鋁箔改用透氣性較高之 4 層藥包紙封口，藥包紙外部再加封 2–3 層石蠟膜，並分別在培養 3、4、5、6 週後，去除封在藥包紙上之石蠟膜，使氣體能通透，在第 8 週時取出瓶苗調查苗體之農藝性狀，顯現於第 3 週就去除石蠟膜者，由於過早透氣處理，易使培養基的水分散失，因而苗體在瓶內培植生長不良，莖短葉形小，移植存活率較差；第 5 週去除石蠟膜之瓶苗生長最健康，葉片較大，根系及植株發育佳，移植存活率亦提高；第 6 週才去除石蠟膜者，可見瓶苗則已出現玻璃質化之情形，本試驗結果與 Chen (1998) 康乃馨組培試驗有相同之情形；植體雖出現玻璃質化，但只要即時將氣體通透至瓶中，亦可以使植株回復為正常苗株，此玻璃質化苗移植於混合蛭石、泥炭土、珍珠石亦可回復為正常苗，存活率亦有 90%以上，顯示仙草生命力極為強健。

影響玻璃質化的因素很多 (Chen *et al*. 1998)，玻璃質化之組培苗直接移植至溫室環境下存活率會下降，瓶內馴化 (acclimatized *in vitro*) 主要為降低瓶內相對溼度及增加氣體的交換率，如此可增加葉面臘質之形成及氣孔之功能維持，有助培養出健康苗體，於移植出瓶時易存活，因此多數研究報告利用通氣性佳的覆蓋材質，皆能有效控制植體玻璃質化情形發生及增加瓶苗移出馴化的存活率 (Capellades *et al*. 1990; Cassells & Wash 1994; Lai *et al*. 2005; Ritchie *et al*. 1991; Smith *et al*. 1990; Wardle *et al*. 1983; Yue & Gosselins 1993; Ziv 1986; Ziv 1991)。依本研究結果顯示，1/2 MS 固體培養基、添加 0.5 mg/L IBA 並配合藥包紙封口之透氣處理，可有效防止仙草瓶苗玻璃質化之情形，並育成高存活率之優質仙草植株。

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Effects of Auxins and Ventilation on Growth and Multiplication of Plantlets of *Mesona procumbens* Hemsl.[1]

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Abstract

Lo, S. C. and H. S. Tsay. 2011. Effects of Auxins and Ventilation on Growth and Multiplication of Plantlets of *Mesona procumbens* Hemsl. J. Taiwan Agric. Res. 60:293–299.

This study was conducted to determine effects of auxins [NAA (α-naphthaleneacetic acid), IAA (indole-3-acetic acid) and IBA (indole-3-butyric acid)] and ventilation on *in vitro* micropropagation of *Mesona procumbens* Hemsl. Shoots of *M. procumbens* were grown in dark on 1/2 MS media supplemented with 0.5 mg/L of NAA for 4 weeks for formation of granule-like calluses and then placed under light for another 4 weeks for the development of plantlets. The plantlets were cut into segments, transferred on 1/2 MS media supplemented with 0.5 mg/L of NAA, IAA or IBA, and incubated at 25℃ under light 30–35 μmol m^{-2} s^{-1} for 6 weeks to compare effects of auxins on plantlet growth and development of plantlets. In another experiment, plantlets were transferred on 1/2 MS media supplemented with 0.5 mg/L of IBA to study effect of different container sealing materials and ventilation closures on hyperhydricity of plantlets. Results showed that among the three auxins tested, the medium containing 0.5 mg/L of IBA was most suitable for growth of plantlets of *M. procumbens* with a root weight of 0.031g/plant, compared to 0.011g/plant for the treatment of IAA. Ventilation affected growth of plantlets. When the tissue culture containers were sealed with dispense paper and Parafilm for 5 weeks and then removed the Parafilm for ventilation, the survival rate of *ex vitro* acclimation of plantlets increased to 98.7%, compared to 92.1% in the treatment of plantlets in aluminum foil**.** Thus, it is possible to produce healthy plantlets of *M. procumbent* in tissue culture with proper amount of IBA in the growth medium and proper control of ventilation. The protocol established in this study would be useful for *in vitro* propagation of *M. procumbent* and it could be a potential alternative to the conventional propagation of this crop in the field.

Key words: *Mesona procumbens*, Tissue culture, Auxins, Ventilation.

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