甘藷種原試管中保存技術之研究

Ⅰ.碳素源對培殖體生長之影響[1]

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摘要：甘藷無病毒苗之莖段培養於含 MS 基本無機鹽類，0.4 mg/l thiamine-HCl，100mg /l myo-inositol，1 mg/l IAA，6% sucrose 及0.8% Difco agar 之改良式MS固體培養基，可使培殖體生長旺盛，且移植後生長勢強，對加速種原繁殖極具功效。爲延長種原保存的期限，於上述改良式 MS 培養基中，以3% glucose 取代6% sucrose，則可抑制培殖體生長，延長繼代培養 (subculture) 週期達6個月，其培殖體若再移植培養於3% sucrose之改良式MS培養基中，即可迅速恢復其生長勢。

關鍵詞：甘藷、無病毒苗、培殖體、繼代培養。

植物種原保存爲近十數年來作物研究學者所注意，保存種原的方法，除傳統的種子及無性繁殖作物繁殖器官的低溫保存外，植物組織培養保存法，亦廣受重視，且已發展出冷凍保存法[4,15]、試管內維持緩慢生長 (minimal-growth maintenance in vitro) [11,13,24]及 DNA 或 cDNA[22]貯存等方法，其中以試管內維持緩慢生長法，較被廣泛應用[5,6]。有些學者指出培養基內加入 ABA (abscisic acid)、CCC (chloromequat)、mannitol、sorbitol 及高濃度蔗糖等物質，可抑制試管內甘藷苗的生長[8,12]，據 Jarret & Gawel (1991) 報導培養基中添加10 mg/l ABA 則甘藷莖段的腋芽生長，受抑制達一年之久，可達到種原短中期保存之目的[14]。Eapen & George 在不同碳源對於芸香科體胚的發育與再生的研究結果顯示，3% glucose 及 sucrose 之植株再生率最高，lactose、galactose 及 arabinose 則使植株再生率完全被抑制[9]。

本試驗的主要目的爲尋求最適當的甘藷種原試管中培養的條件，以求短期間內，由試管苗移植至溫室後，其生長勢最爲旺盛，藉此加速健康苗的繁殖，同時研究甘藷種原長期保存於試管內之技術，以減低培殖體因頻繁的繼代培養所引起的突變，並可避免田間種原保存時，因天然災害及人爲疏忽所引起的種原遺失，且可節省人力、物力的支出。

材料與方法

本試驗所選用的材料，爲甘藷臺農57號 (TNG 57) 及臺農66號 (TNG 66) 無病毒苗，培養基配方爲 Murashige and Skoog (1962) 基本無機鹽類 (MS) [19]添加0.4 mg/l thiamine-HCl，100 mg/lmyo-inositol，1 mg/l IAA，3% sucrose 及0.8% Difco agar 之改良式 MS 培養基，培殖體置於25±1°C恒溫，光照1,500 lux，光期16小時之環境下培養，經30天取下長約1.5 cm (2個莖節) 之莖段，進行下述各項試驗：

1.臺灣省試驗所　研究報告第 1657 號。本研究承行政院農業委員會經費補助 (80農建—7.1—糧—121 (22))，文稿承本所農藝系劉大江博士及蔡新聲博士斧正，謹致謝意。
2.本所嘉義農業試驗分所助理及副研究員。臺灣省　嘉義市。

一、不同碳素源對培殖體生長之影響；本試驗採用改良式 MS 培養基，以 lactose, maltose, D(−) fructose, D(+)mannose, D(+)galactose,α-D(+)glucose,α(+)arabinose 及sucrose 等八種醣類爲主要碳素源，使用濃度均爲3%，每處理20重複。

二、蔗糖濃度對培殖體生長的影響：採用改良式 MS 培養基，配合0，3，6，9，12及15%等六種不同蔗糖濃度，每處理40重複。

三、葡萄糖濃度對培殖體生長的影響：採用改良式 MS 培養基，並以葡萄糖取代試驗二所列不同濃度之蔗糖，每處理40重複。

各試驗培養1～6個月，分別定期調查鮮重、株高、莖徑、葉片數、乾重、試管內成活率、移植後成活率等項目，以鄧肯氏多變域測驗法（Duncan's multiple range test）進行處理平均值間之差異顯著性測驗。

結　果

一、不同碳素源對培殖體生長之影響：

表1之結果顯示，在芽體培養兩個月後，以 lactose、mannose 及 glucose 爲碳源之培殖體，較以 sucrose 爲碳源之培殖體有顯著抑制生長的作用，其中 lactose 對於不同品種間芽體生長力的影響較不一致，甚至使臺農66號的生長完全停滯，而 mannose 及 glucose 能有效地抑制品種間芽體的生長，且仍保有生命，而 maltose 及 fructose 對培殖體生長抑制效果較小，若以galactose 及 arabinose 爲碳素源，對甘藷臺農57號及臺農66號皆造成芽體生長停滯，且有褐化死亡之現象。

表1. 不同碳源對甘藷莖生長及發育之影響

Table 1. Effect of sugar source on the growth and development of sweet potato shoot segments cultured for two months on modified MS medium.

Sugar[1]	Fresh wt. (mg/shoot)		Plant height (cm)		Number of expanded leaves		Dry weight (mg/shoot)	
	TNG 57[2]	TNG 66[2]	TNG 57	TNG 66	TNG 57	TNG 66	TNG 57	TNG 66
Lactose	101de[3]	84ef	2.2d	1.5d	2.6d	0.3e	8.2de	8.1e
Maltose	354c	637b	5.1c	10.7b	7.2c	6.6c	25.1c	45.6b
Fructose	596b	527c	7.3b	9.1c	12.1b	7.6b	42.0b	43.1b
Mannose	84de	201d	1.9d	2.4d	2.6d	2.2d	10.0d	24.0c
Galactose	0e[4]	0f	0.0d	0.0d	0.0e	0.0e	0.0e	0.0f
Glucose	202d	158de	2.6d	2.2d	4.5d	2.1d	16.9cd	16.5d
Arabinose	0e[4]	0f	0.0d	0.0d	0.0e	0.0e	0.0e	0.0f
Sucrose	877a	1,024a	9.4a	13.8a	14.8a	8.8a	67.7a	77.5a

1 Concentration of all sugars was 3%.
2 TNG 57 : Tainung 57 ; TNG 66 : Tainung 66.
3 Means with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 20 replications.
4 Explants did not survive under the experimental conditions.

二、不同濃度之蔗糖對培殖體生長之影響：

表2顯示，培養基分別添加3～9% sucrose 使培殖體的成活率達100%，而含0及15% sucrose 者，則不利於培殖體生長，含12% sucrose 者，因品種不同，對培殖體鮮重、莖徑與成活率有相異的表現；6% sucrose 對培殖體的鮮重及莖伸長速度有顯著增加的效果，有利於健康種苗大量繁殖，而9% sucrose 雖可促進莖徑加粗，但老化葉片隨之增加，致使展開綠葉減少。

表2. 不同濃度之蔗糖對甘藷莖生長及成活率之影響

Table 2. Effect of sucrose concentration on the growth, development and in vitro survival rate of sweet potato shoot segments cultured for one month on modified MS medium.

Sucrose conc. (%)	Fresh wt. (mg/shoot)		Stem diameter (mm)		Plant height (cm)		Number of expanded leaves		In Vitro survival rate (%)	
	TNG 57[1]	TNG 66[1]	TNG 57	TNG 66	TNG 57	TNG 66	TNG 57	TNG 66	TNG 57	TNG 66
0	13e[2]	97e	0.1e	0.7c	1.6c	1.7d	0.1e	0.3d	12.5	56.7
3	538c	766c	1.4c	1.8b	6.6b	5.4b	6.1b	4.1a	100.0	100.0
6	1,110a	1,177ab	1.6b	2.3a	9.2a	6.6a	7.0a	3.4b	100.0	100.0
9	956b	1,309a	1.8a	2.6a	6.3b	5.4b	5.0c	2.5c	100.0	100.0
12	252d	1,148b	0.8d	2.4a	2.1c	3.8c	1.1d	2.4c	50.0	100.0
15	38e	255d	0.2e	1.0c	1.6c	1.8d	0.1e	0.6d	22.5	56.7

1 TNG 57 : Tainung 57 ; TNG 66 : Tainung 66.
2 Meas with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 40 replications.

三、不同濃度之蔗糖對培殖體移植後生長之影響：

由表3，4可知，培養基中添加3～15% sucrose 之培殖體，移植後之結果顯示，兩個受測品種之移植成活率皆達90%以上，而培養基中不含 sucrose 者呈現低成活率，含6%sucrose 者，對植株鮮重、株高、展開葉片數及乾重均呈顯著增加，其次是含3%及9% sucrose者，兩者之間對於促進植株生長的效果差異不大，且顯示移植後莖的厚度，並未隨著蔗糖濃度的增加而增加，若培養基 sucrose 濃度超過12%以上時，則有抑制植株生長之負面效果。

表3. 不同濃度之蔗糖對臺農57號移植後莖生長及成活率之影響

Table 3. Agronomic characters measured at 45 days after transplanting of pot-cultured Tainung 57 sweet potato plants originated from shoot segments cultured for one month on modified MS medium with different sucrose concentrations.

Sucrose conc. (%)	Fresh weight (g/plant)	Stem diameter (mm)	Plant height (cm)	No. of expanded leaved	Number of branches	Dry weight (mg/plant)	Survival rate after trans-planting(%)
0	0.03d[1]	0.1d	1.6d	0.1e	0.1d	2.7d	20.0
3	2.64b	2.4a	12.6b	5.1b	1.1b	506.5b	100.0
6	3.60a	2.4a	15.5a	7.6a	1.7a	659.2a	100.0
9	2.99b	2.4a	14.6a	5.5b	1.3b	483.0b	100.0
12	1.27c	1.2b	6.4c	2.2c	0.5c	219.5c	100.0
15	0.34d	0.5c	2.5d	1.0d	0.2d	43.8d	100.0

1 Means with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 40 replications.

表4. 不同濃度之蔗糖對臺農66號移植後莖生長及成活率之影響

Table 4. Agronomic characters measured at 45 days after transplanting of pot-cultured Tainung 66 sweet potato plants originated from shoot segments cultured for one month on modified MS medium with different sucrose concentrations.

Sucrose conc. (%)	Fresh weight (g/plant)	Stem diameter (mm)	Plant height (cm)	No. of expanded leaved	Number of branches	Dry weight (mg/plant)	Survival rate after trans-planting(%)
0	0.15e[1]	0.3c	2.4e	0.7d	0.2c	13.0d	41.2
3	3.19c	2.6a	11.9b	3.8b	1.1a	473.7b	100.0
6	4.10a	2.6a	14.8a	4.5a	1.1a	584.7a	100.0
9	3.96ab	2.7a	12.6b	4.3ab	1.1a	544.7ab	100.0
12	3.47bc	2.6a	9.6c	3.8b	1.0a	489.3b	100.0
15	1.14d	1.3b	3.6d	1.6c	0.6b	166.3c	94.1

1 Means with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 40 replications.

四、不同濃度之葡萄糖對培殖體生長的影響：

由表5知，培養基中含3～6% glucose 者其成活率可達100%，而含0%及9%glucose 者其成活率低於40%，含12%及15% glucose 不利於培殖體生長，甚至有褐化死亡的現象；培養基中含3% glucose 比含6% glucose，使培殖體生長更緩慢且根發生率達100%。有一特殊現象即培養基所含 glucose 濃度漸增加，其莖基部會形成較多的 callus，故含6%glucose 者，其培殖體的總鮮重呈顯著增加，且以臺農66號培養於6% glucose 較3% glucose者鮮重增加約一倍。

表5. 不同濃度之葡萄糖對控制甘藷莖生長之影響

Table 5. The inhibition effect of glucose concentration on the growth of sweet potato shoot segments cultured for one month on modified MS medium.

Glucose conc. (%)	Fresh wt. (mg/shoot)		Plant height (cm)		Number of expanded leaves		Rooting rate (%)		In Vitro survival rate (%)	
	TNG 57[1]	TNG 66[1]	TNG 57	TNG 66	TNG 57	TNG 66	TNG 57	TNG 66	TNG 57	TNG 66
0	24cd[2]	48d	1.6b	1.6bc	0.2c	0.3b	15.0c	32.5b	11.3	35.0
3	224b	368b	1.9a	2.1a	2.5a	1.9a	100.0a	100.0a	100.0	100.0
6	291a	620a	1.9a	2.2a	1.9b	1.9a	82.5b	97.5a	100.0	100.0
9	57c	200c	1.5b	1.7b	0.0c	0.5b	20.0c	35.0b	13.8	37.5
12	0d[3]	0d[3]	0.0b	0.0c	0.0c	0.0c	0.0d	0.0c	0.0	0.0
15	0d[3]	0d[3]	0.0b	0.0c	0.0c	0.0c	0.0d	0.0c	0.0	0.0

1 TNG 57：Tainung 57；TNG 66：Tainung 66.
2 Means with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 40 replications.
3 Explants did not survive under the experimental conditions.

表6，7顯示，經培養6個月後，含0% glucose 者其成活率顯著下降，臺農57號成活率由11.3%降至7.5%，臺農66號成活率由35%降至0%，且有褐化死亡的現象，含3～6% glucose 者其培殖體成活率仍達100%，而含3% glucose 者使植株生長緩慢，且展開葉片數較含6% glucose 有顯著的增加，將培養於含3% glucose 培養基達6個月之久的培殖體，再移植至含3% sucrose 之原改良式培養基，可恢復其原有的生長活力。

表6. 不同濃度之葡萄糖對臺農57號連續培養六個月之影響

Table 6. Influence of glucose concentration on the growth of Tainung 57 shoot segments cultured continuously for six months on modified MS medium.

Glucose conc. (%)	Fresh weight (g/plant)	Stem diameter (mm)	Plant height (cm)	No. of expanded leaved	Rooting rate (%)	Dry weight (mg/plant)	In Vitro survival rate (%)
0	0.01d[1]	0.1d	1.6c	0.2c	5.0c	0.8d	7.5
3	1.12b	1.9b	8.6b	9.2a	100.0a	115.8b	100.0
6	1.92a	2.4a	9.7a	4.3b	100.0a	208.0a	100.0
9	0.55c	0.6c	2.0c	0.9c	40.0b	67.0c	40.0
12	0.00d[2]	0.0d	0.0c	0.0c	0.0c	0.0d	0.0
15	0.00d[2]	0.0d	0.0c	0.0c	0.0c	0.0d	0.0

1 Means with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 40 replications.
2 Explants did not survive under the experiimental conditions.

表7. 不同濃度之葡萄糖對臺農66號連續培養六個月之影響

Table 7. Influence of glucose concentration on the growth of Tainung 66 shoot segments cultured continuously for six months on modified MS medium.

Glucose conc. (%)	Fresh weight (g/plant)	Stem diameter (mm)	Plant height (cm)	No. of expanded leaved	Rooting rate (%)	Dry weight (mg/plant)	In Vitro survival rate (%)
0	0.00d[1]	0.0d[2]	0.0c	0.0c	0.0c	0.0c	0.0
3	1.19b	1.8b	10.4a	2.4a	100.0a	102.8b	100.0
6	1.59a	2.2a	9.5a	0.9b	100.0a	261.0a	100.0
9	0.51c	1.0c	2.8b	0.2c	45.0b	108.3b	45.0
12	0.00d[2]	0.0d	0.0c	0.0c	0.0c	0.0c	0.0
15	0.00d[2]	0.0d	0.0c	0.0c	0.0c	0.0c	0.0

1 Means with the same letter of a column are not significantly different at 5% level by Duncan's Multiple Range Test. Each data represents the mean of 40 replications.
2 Explants did not survive under the experiimental conditions.

討 論

利用廖、鐘[1]所報導甘藷無病毒苗之培育及病毒檢定方法，可大量獲得無病毒苗，然無病毒苗之保存及大量繁殖種苗，常須投入大量人力、物力，爲了達到短中期保存的目的，Murashige *et al.*提出難以用種子貯藏法保存之作物如甘藷、馬鈴薯，採用莖頂培養保存法和莖項液態氮（−196°C）保存法有助於避免遺傳基因的遺失[11,16,18,20]。然經冰凍過的莖項，再生爲植株的比率仍偏低，尙不十分實用，故本試驗嘗試採用抑制試管苗生長之組織培養保存法，結果顯示在25°C培養條件下，以 sucrose 爲主要碳源，使培殖體生長活力旺盛（表1）；而 Ko 等人報導，利用香蕉莖頂培養於17°C低溫下培養16個月，調查顯示 sucrose 的保存效果僅次於ribose[15]，結果與表1不同，可能因培養溫度的不同所造成，表1同時顯示 galactose 及arabinose 則完全抑制培殖體生長甚至死亡，Eapen & George 亦報告 galactose 及 arabinose 可完全抑制芸香科體胚發育及植株再生，可能原因爲培殖組織無法適當吸收、轉運及利用 galactose及 arabinose 所造成[9]，本試驗結果發現3% glucose 及 mannose 均可有效地抑制培殖體生長，但 mannose 商品價格昂貴，不符合經濟成本；至於 ribose 及低溫處理的效果，值得進一步探討。

蔗糖主要的功用是提供生長所需的碳源及調節滲透壓，隨著蔗糖濃度的增加可使植株生長旺盛，以6%蔗糖爲主要碳源的效果最好，蔗糖（sucrose）濃度太低或太高均造成培殖體生長勢減弱，莖伸長受阻及乾物率降低，同時隨著蔗糖濃度增加，培殖體展開葉卻逐漸減少（表2，3，4）。蔡等[2,3]亦曾指出6%蔗糖濃度最適於甘藷花藥 callus 的誘導，過高濃度的蔗糖對胚後期的生長發育有相反的效果；Michel[17]及 Purves[23]發現增加蔗糖濃度，可使 IAA 作用降低造成莖伸長受阻；蔡、林[2]指出不含蔗糖的培養基，甘藷花藥的 callus 無法形成，隨著蔗糖濃度的提高可增加callus 的鮮重；有關葡萄糖抑制培殖體生長的效果，國內外均少有研究；本研究顯示以3～6%glucose 爲碳源，其培殖體的成活率可達100%，且對抑制培殖體生長高度，二受測品種間均顯示相同趨勢，又含3% glucose 者根形成率可達100%（表5），另一特殊現象即 glucose 濃度愈高，培殖體基部所形成的 callus 亦愈多，或許可利用 glucose 誘導 callus 的產生，以替代2，4-D 或 NAA，值得探討；高濃度12～15% glucose 使培殖體褐化且死亡，不利於培殖體生長；以3～6% glucose 較適宜培殖體緩慢生長的條件，經連續培養6個月不行繼代培養條件下，培養在6% glucose 培殖體之黃化葉明顯增加，其原因有待探討，若以3% glucose 作爲抑制培殖體生長的主要碳源，可延長繼代培養週期達6個月之久（表6，7），若再配合低溫之培養環境，是否能再延長保存的期間，則需更進一步探討證實。

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Studies on the In Vitro Maintenance Techniques of Sweet Potato (Ipomoea batata L.) I. Influence of Carbon Source[1]

S. F. Lo and C. H. Liao[2]

Summary

Experiments were conducted to study the influence of sugar sources on the in vitro growth and maintenance of sweet potato cuttings for the purpose of efficient germplasm preservation. Stem segments of virus-free sweet potato plants were successfully cultured on a medium containing Murashige and Skoog (MS) inorganic salts supplemented with 0.4 mg /l thiamine-HCl , 100mg/l myo-inositol , 1mg/l IAA , 6% sucrose and 0.8% Difco agar. In addition to active growth of the explants in the test tube, the survival rate and growth vigor of the cuttings after transplanting to pots were also high as compared to other treatments. In oreder to prolong the time interval between subcultures of the in vitro preserved sweet potato stem segments, the addition of 3% glucose as the sole carbon source to the medium was effective to lower the growth rate of the explants. The interval between successive subcultures could be extended to as long as 6 months. The growth vigor could be resumed after transfer the explants to culture medium containing 3% sucrose.

Key words : Sweet potato, Virus-free plant, Explant, Subculture.

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