`貝利 A´及`巨峰´葡萄胚乳癒傷組織之誘導

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關鍵字：胚乳培養、三倍體、培養基

摘要：本研究測試`貝利 A´及`巨峰´葡萄胚乳培養之最適合誘導癒傷組織之胚乳發育階段及培養基條件。結果顯示，以 MSMO 為基礎配方添加 5 μM BA 及 1 μM NAA(或 2,4-D)，`貝利 A´及`巨峰´花後 2 週胚乳留胚培養 2 個月後誘導癒傷組織發生較高，均可達 90%以上。而胚乳去胚培養則需添加酪蛋白水解物以獲得較高之癒傷組織發生率。而以 MS 為基礎配方，雖可誘導癒傷組織發生，但誘導率顯著低於以 MSMO 基礎配方之處理。胚乳留胚及去胚比較，`貝利 A´及`巨峰´之胚乳留胚培養之誘導癒傷組織發生較高。綜合上述結果，誘導`貝利 A´及`巨峰´胚乳癒傷組織發生以取花後 2 週內胚乳培養之癒傷組織發生率較高；胚乳留胚培養高於去胚；MSMO 為基礎配方高於 MS；培養基有添加酪蛋白水解物者高於未添加者。

前 言

大部分的被子植物胚乳的形成來自於雙重受精時三個單倍體核融合，其中兩個來自於胚囊的極核(polar nuclei)，一個來自花粉管內之精核。超過 80%開花植物在發育的種子裡有能提供養份給胚生長發育之胚乳(Williams and De Latour, 1980)。三倍體植株因染色體不對稱而形成高不稔性及無子性狀，若是能將無子性狀加以利用，可提升果實品質，將會有更大的經濟價值，且三倍體一般比二倍體植株生長勢強(Thomas and Chaturvedi, 2008)。因此，利用胚乳培養可直接獲得三倍體無子植株，不需要經過傳統以二倍體與四倍體相互雜交之手續。

胚乳培養需要選擇適當的種子發育階段才能成功誘導植株再生。不同植物胚乳培養之適當時間有所不同，可分為未成熟胚乳與成熟胚乳兩種培養方式。未成熟胚乳培養，如核桃(*Juglans regia*)授粉後 8 週為最理想取樣時間點(Tulecke *et al*., 1988)、桑椹(*Morus alba*)

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則為開花後 17-20 天(Thomas *et al*., 2000)，百香果為授粉後 36-49 天(李等，1998)。成熟胚乳培養，如油柑(*Emblica officinale*)、番荔枝(*Annona squamosa*)、柑桔類(*Citrus* spp.)及獼猴桃(*Actinidia deliciosa*)，均需取成熟胚乳才能誘導形成胚乳癒傷組織或不定芽(Sehgal and Khurana, 1985; Nair *et al*., 1986; Gmitter *et al*., 1990; Goralski *et al*., 2005)，但接近成熟的葡萄胚乳則幾乎不能誘導癒傷組織產生(母等，1977)。

胚乳培養時，是否去胚也是關鍵因子之一，一般胚萌芽需要成熟胚乳刺激，如番茄(Kagan-Zur *et al*., 1990)。成熟胚乳需要與初期胚結合以誘導癒傷組織，如楊桃(吳等，2002)，但未成熟胚乳增殖則通常不依賴胚，如獼猴桃(Goralski *et al*., 2005)。然而印度棟需要胚來誘導未成熟胚乳形成癒傷組織(Chaturvedi *et al*., 2003)，另外桑椹未成熟胚乳有留胚或是去胚皆可以誘導形成癒傷組織，但有留胚者誘導率較高(Thomas *et al*., 2000)。

植物生長調節劑方面，需要選擇適合之基本培養基及添加適合之植物生長調節劑，大部份植物可使用 MS (Murashige and Skoog, 1962)基本培養基誘導胚乳癒傷組織形成，此外有些植物則適用 WM (White, 1963)、NN (Nair et al., 1986)或 MT (Murashige and Tucker, 1969)等，而如能找到最合適的培養基時，甚至可以直接誘導不定芽形成，如百香果未成熟胚乳培養於 MS 添加 2.27 μM TDZ (thidiazuron)之培養基，可直接誘導生成不定芽(李等，1998)。另外也可以添加不同的有機物質來提高胚乳癒傷組織誘導率，如酪蛋白水解物(casein hydrolysate, CH)、番茄汁、酵母萃取液(yeast extract)、椰奶、玉米萃取液及牛奶等。本研究的主要目的為尋找適合`貝利 A´與`巨峰´葡萄胚乳癒傷組織誘導之胚乳發育階段及合適的培養基配方。

材 料 與 方 法

一、植物材料

本試驗材料為位於台中縣霧峰鄉之國立中興大學園藝試驗場葡萄中心所栽種的`貝利 A´ (`Muscat Bailey A´)與`巨峰´ (`Kyoho´)葡萄自花授粉著果後，於花後不同發育階段採取果粒進行胚乳培養試驗。

二、試驗方法

1.方法一(2008 年 4 月 28 日開始進行)

(1) 胚乳處理方式：

取花後 1、2、3 及 4 週之果粒，在果粒表面用 70%酒精擦過，接著用 1 %次氯酸鈉(sodium hypochlorite)含 1-2 滴 Tween 20 消毒果粒表面 10 分鐘，然後在無菌操作台裡用無菌水沖洗 3 次，使用滅菌過之鑷子與解剖刀將果粒剖開取出胚珠，在解剖顯微鏡下去除胚珠種皮即剩胚乳並留胚，胚乳留胚培植體移至誘導癒傷組織培養基。花後 1 週，每處理 10 個，共 2 重複，花後 2-4 週，每處理 10 個，共 3 重複。胚乳培養放置暗處，溫度維持在 26 ± 1℃，

每 6 週繼代 1 次。

(2) 初代癒傷組織培養基配製：

培養基以 MSMO (Murashige and Skoog basal salt with minimal organics, sigma)為基礎配方，培養基中含有全量 MSMO、3% sucrose 及添加植物生長調節劑(BA、NAA 及 2,4-D)，培養基的 pH 值調整至 5.8，之後加入 0.8 % agar 加熱至沸騰，將培養基放入滅菌釜，經高溫高壓(121℃及 1.2 kg cm^{-2})滅菌 20 分鐘後，等培養基降溫至 50℃以下，取約 20 ml 分裝至 90 mm × 15 mm 無菌培養皿(petri dishes)，等培養基凝固及培養皿風乾後備用。培養基濃度分為 2 種：(1) 5 μM BA + 1 μM NAA 及(2) 5 μM BA + 1 μM 2,4-D。記錄培養 2 個月癒傷組織發生率。

2.方法二(2008 年 9 月 17 日開始進行)

(1) 胚乳處理方式：

取花後 1、2、3 及 4 週之果粒，經消毒滅菌後取出胚珠，在解剖顯微鏡下去除頂部約 1/4 之位置(去除胚的部位)及去種皮，胚乳去胚培植體移至誘導癒傷組織培養基。每處理 10 個，共 3 重複。胚乳培養放置暗處，溫度維持在 26 ± 1℃，每 6 週繼代 1 次。

(2) 初代癒傷組織培養基配製：

培養基以 MSMO 為基礎配方，培養基中含有全量 MSMO、3% sucrose 及添加植物生長調節劑(BA、NAA 及 2,4-D)，培養基的 pH 值調整至 5.8，之後加入 0.8% agar 加熱至沸騰，將培養基放入滅菌釜，經高溫高壓滅菌 20 分鐘後，等培養基降溫至 50℃以下，用 0.22 μm 過濾膜(Millex, minipore)將酪蛋白水解物(casein hydrolysate，CH)過濾至培養基裡，取約 20ml 分裝至 90 mm × 15 mm 無菌培養皿，等培養基凝固及培養皿風乾後備用。培養基濃度分為 4 種：(1) 5 μM BA + 1 μM NAA；(2) 5 μM BA + 1 μM 2,4-D；(3) 5 μM BA + 1 μM NAA + 500 ppm CH 及(4) 5 μM BA + 1 μM 2,4-D + 500 ppm CH。記錄培養 2 個月癒傷組織發生率。

3.方法三(2009 年 4 月 15 日開始進行)

(1) 胚乳處理方式：

取花後 1、2、3 及 4 週之果粒，分為 2 種處理方式，一處理在解剖顯微鏡下去除胚珠種皮即剩胚乳並留胚，另一處理在解剖顯微鏡下去除頂部約 1/4 之位置(去除胚的部位)及去種皮，將胚乳留胚及去胚培植體移至誘導癒傷組織培養基。每處理 10 個，共 3 重複。胚乳培養放置暗處，溫度維持在 26 ± 1℃，每 6 週繼代 1 次。

(2) 初代癒傷組織培養基配製：

培養基同方法二，但培養基以 MS (Murashige and Skoog basal salt, sigma)為基礎配方。記錄培養 1、2、4 週及 2 個月癒傷組織發生率。

結　果

`貝利 A´葡萄花後 1、2、3 及 4 週胚乳留胚分別培養於 5 μM BA + 1 μM NAA 及 + 5 μM BA + 1 μM 2,4-D (以 MSMO 為基礎配方)之 2 種癒傷組織誘導培養基 2 個月後，花後 1 週胚乳培養於 5 μM BA + 1 μM NAA 者無法誘導癒傷組織發生，而培養於 5 μM BA + 1 μM 2,4-D 者誘導癒傷組織發生率為 50%。花後 2 週胚乳癒傷組織發生率顯著較高，分別為 90% 及 96.67%，在花後 3 週胚乳則誘導癒傷組織發生較低，而花後 4 週胚乳則無法誘導癒傷組織產生。培養基比較方面，花後 1 週胚乳培養於 5 μM BA + 1 μM 2,4-D 較佳，而花後 2-4 週胚乳培養於上述 2 種培養基則無顯著差異(Table 1)。

表 1. `貝利 A´葡萄不同胚乳齡之胚乳留胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 1. Callus induction rate from `Muscat Bailey A´ endosperms with embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Callus induction rate (%) [z]	
	5 μM BA + 1 μM NAA	5 μM BA + 1 μM 2,4-D
1 week after bloom	0 b[y]B[x]	50.00 bA
2 weeks after bloom	90 aA	96.67 aA
3 weeks after bloom	10 bA	3.33 cA
4 weeks after bloom	0 bA	0.00 cA

[z] Endosperms were cultured with embryo and without seedcoat for 2 months. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 2 replicates at treatment of 1 week after bloom and 3 replicates at treatments of 2, 3 and 4 weeks after bloom; 10 explants per replicate. The basal medium was MSMO.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

`貝利 A´葡萄花後 1、2、3 及 4 週胚乳去胚分別培養於 5 μM BA + 1 μM NAA、5 μM BA + 1 μM 2,4-D、5 μM BA + 1 μM NAA + 500ppm CH 及 5 μM BA + 1 μM 2,4-D + 500 ppm CH (以 MSMO 為基礎配方)之 4 種癒傷組織誘導培養基 2 個月後，花後 2 週胚乳誘導癒傷組織發生除了培養於 5 μM BA + 1 μM NAA 者無顯著差異外，其它 3 種培養基均較高於花

後 1、3 及 4 週者，誘導癒傷組織發生率分別為 6.67%、10%、90%及 80%，而花後 3 及 4 週胚乳則無法誘導癒傷組織發生。培養基方面，花後 1、3 及 4 週胚乳培養於上述 4 種培養基均無顯著差異，花後 2 週胚乳以培養於 5 μM BA + 1 μM NAA + 500 ppm CH (酪蛋白水解物)及 5 μM BA + 1 μM 2,4-D + 500 ppm CH 者皆顯著高於未添加酪蛋白水解物之培養基者(Table 2)。

表 2. `貝利 A´葡萄不同胚乳齡之胚乳去胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 2. Callus induction rate from `Muscat Bailey A´ endosperms without embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Callus induction rate (%)[z]			
	BA+NAA	BA+2,4-D	BA+NAA+CH	BA+2,4-D+CH
1 week after bloom	3.33 a[y]A[x]	0 bA	0 bA	6.67 bA
2 weeks after bloom	6.67 aB	10 aB	90 aA	80.00 aA
3 weeks after bloom	0 aA	0 bA	0 bA	0 bA
4 weeks after bloom	0 aA	0 bA	0 bA	0 bA

[z] Endosperms were cultured without embryo and seedcoat for 2 months. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 3 replicates per treatment; 10 explants per replicate. The basal medium was MSMO. The concentrations of growth regulators: BA 5 μM, NAA 1 μM, 2,4-D 1 μM and CH 500ppm.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

`巨峰´葡萄花後 1、2、3 及 4 週胚乳留胚分別培養於 5 μM BA + 1 μM NAA 及 5 μM BA + 1 μM 2,4-D (以 MSMO 為基礎配方)之 2 種癒傷組織誘導培養基 2 個月後，花後 1 及 4 週胚乳則無法誘導癒傷組織產生，花後 2 週胚乳癒傷組織發生率顯著較高，分別為 93.33%與 90%，花後 3 週胚乳培養於 5 μM BA + 1 μM NAA 誘導率為 33.3%，而培養於 5 μM BA + 1 μM 2,4-D 者則誘導率較低，只有 6.67%。培養基方面，花後 1-4 週胚乳培養於上述 2 種培養基皆無顯著差異(Table 3)。

表 3.`巨峰´葡萄不同胚乳齡之胚乳留胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 3. Callus induction rate from `Kyoho´ endosperms with embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Callus induction rate (%)[z]	
	5 μM BA + 1 μM NAA	5 μM BA + 1 μM 2,4-D
1 week after bloom	0 c[y]A[x]	0 bA
2 weeks after bloom	93.33 aA	90.00 aA
3 weeks after bloom	33.33 bA	6.67 bA
4 weeks after bloom	0 cA	0 bA

[z] Endosperms were cultured with embryo and without seedcoat for 2 months. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 2 replicates at treatment of 1 week after bloom and 3 replicates at treatments of 2, 3 and 4 weeks after bloom; 10 explants per replicate. The basal medium was MSMO.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

`巨峰´葡萄花後 1、2、3 及 4 週胚乳去胚分別培養於 5 μM BA + 1 μM NAA、5 μM BA + 1 μM 2,4-D、5 μM BA + 1 μM NAA + 500 ppm CH 及 5 μM BA + 1 μM 2,4-D + 500 ppm CH (以 MSMO 為基礎配方)之 4 種癒傷組織誘導培養基 2 個月後，花後 2 週胚乳誘導癒傷組織發生除了培養於 5 μM BA + 1 μM 2,4-D 培養基與花後 1 週無顯著差異外，其它 3 種培養基皆顯著高於花後 1 週者，分別為 33.33%、26.67%、63.33%及 43.33%，而花後 3 週胚乳無法誘導癒傷組織產生，花後 4 週胚乳則只有培養於 5 μM BA + 1 μM NAA + 500 ppm CH 者有 3.33%之癒傷組織發生率，但與其它 3 種培養基均無顯著差異。培養基方面，花後 1、3 及 4 週胚乳培養於上述 4 種培養基均無顯著差異，花後 2 週胚乳培養於 5 μM BA + 1 μM NAA + 500 ppm CH 者顯著高於其它 3 種培養基(Table 4)。

`貝利 A´葡萄花後 1、2、3 及 4 週胚乳留胚及胚乳去胚分別培養於 5 μM BA + 1 μM NAA、5 μM BA + 1 μM 2,4-D、5 μM BA + 1 μM NAA + 500 ppm CH 及 5 μM BA + 1 μM 2,4-D + 500 ppm CH (以 MS 為基礎配方)之 4 種癒傷組織誘導培養基 2 個月後，胚乳留胚培養方面，花後 1、3 及 4 週之胚乳均無法誘導癒傷組織發生，只有花後 2 週胚乳能誘導癒傷組織發生，分別為 10%、13.3%、6.67%及 16.67%。培養基方面，花後 2 週胚乳留胚

培養於上述4種培養基均無顯著差異，並且培養時間在2週內即可誘導癒傷組織發生(Table 5)。胚乳去胚培養方面，花後1及4週之胚乳無法誘導癒傷組織發生，花後2週胚乳培養於5 μM BA + 1 μM NAA + 500 ppm CH之癒傷組織發生率為23.33 %，顯著較高於花後1、3及4週胚乳。培養基方面，花後1、2、3及4週胚乳去胚培養於上述4種培養基均無顯著差異，此外，花後2週胚乳培養於5 μM BA + 1 μM NAA + 500 ppm CH之培養時間在1週內即可誘導癒傷組織發生，其它3種培養基則是培養2週內可誘導癒傷組織發生，花後3週胚乳培養於5 μM BA + 1 μM NAA也是培養2週內可誘導癒傷組織發生(Table 6)。

表 4. `巨峰´葡萄不同胚乳齡之胚乳去胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 4. Callus induction rate from `Kyoho´ endosperms without embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm[z]	Callus induction rate (%)[z]			
	BA+NAA	BA+2,4-D	BA+NAA+CH	BA+2,4-D+CH
1 week after bloom	3.33 b[y]A[x]	10.00 abA	6.66 bA	0 bA
2 weeks after bloom	33.33 aB	26.67 aB	63.33 aA	43.33 aB
3 weeks after bloom	0 bA	0 bA	0 bA	0 bA
4 weeks after bloom	0 bA	0 bA	3.33 bA	0 bA

[z] Endosperms were cultured without embryo and seedcoat for 2 months. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 3 replicates per treatment; 10 explants per replicate. The basal medium was MSMO. The concentrations of growth regulators: BA 5 μM, NAA 1 μM, 2,4-D 1 μM and CH 500 ppm.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

表 5. `貝利 A´葡萄不同胚乳齡之胚乳留胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 5. Callus induction rate from `Muscat Bailey A´ endosperms with embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Culture period	Callus induction rate (%) [z]			
		BA+NAA	BA+2,4-D	BA+NAA+ CH	BA+2,4-D+ CH
1 week after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 b [y]A [x]	0 bA	0 bA	0 bA
2 weeks after bloom	1 week	0	0	0	0
	2 weeks	10	13.33	6.67	16.67
	4 weeks	10	13.33	6.67	16.67
	2 months	10 aA	13.33 aA	6.67 aA	16.67 aA
3 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 bA	0 bA	0 bA	0 bA
4 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 bA	0 bA	0 bA	0 bA

[z] Endosperms were cultured with embryo and without seedcoat. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) ×100%. 3 replicates per treatment; 10 explants per replicate. The basal medium was MS. The concentrations of growth regulators: BA 5μM, NAA 1μM, 2,4-D 1μM and CH 500 ppm.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

表 6. `貝利 A´葡萄不同胚乳齡之胚乳去胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 6. Callus induction rate from `Muscat Bailey A´ endosperms without embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Culture period	Callus induction rate (%) [z]			
		BA+NAA	BA+2,4-D	BA+NAA+ CH	BA+2,4-D+ CH
1 week after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 a[y]A[x]	0 aA	0 bA	0 aA
2 weeks after bloom	1 week	0	0	3.33	0
	2 weeks	3.33	3.33	23.33	10
	4 weeks	3.33	3.33	23.33	10
	2 months	3.33 aA	3.33 aA	23.33 aA	10 aA
3 weeks after bloom	1 week	0	0	0	0
	2 weeks	3.33	0	0	0
	4 weeks	3.33	0	0	0
	2 months	3.33 aA	0 aA	0 bA	0 aA
4 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 aA	0 aA	0 bA	0 aA

[z] Endosperms were cultured without embryo and seedcoat. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 3 replicates per treatment; 10 explants per replicate. The basal medium was MS. The concentrations of growth regulators: BA 5 μM, NAA 1 μM, 2,4-D 1 μM and CH 500 ppm.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

表 7. `巨峰´葡萄不同胚乳齡之胚乳留胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 7. Callus induction rate from `Kyoho´ endosperms with embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Culture period	Callus induction rate (%) [z]			
		BA+NAA	BA+2,4-D	BA+NAA+ CH	BA+2,4-D+ CH
1 week after bloom	1 week	3.33	6.67	10	13.33
	2 weeks	3.33	10.00	10	13.33
	4 weeks	6.67	10.00	10	13.33
	2 months	6.67 b [y]A [x]	10.00 abA	10 abA	16.67 abA
2 weeks after bloom	1 week	13.33	3.33	30	20.00
	2 weeks	26.67	13.33	40	36.67
	4 weeks	26.67	26.67	40	36.67
	2 months	26.67 aA	30.00 aA	40 aA	43.33 aA
3 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 bA	0 bA	0 bA	0 bA
4 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 bA	0 bA	0 bA	0 bA

[z] Endosperms were cultured with embryo and without seedcoat. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 3 replicates per treatment; 10 explants per replicate. The basal medium was MS. The basal medium was MS. The concentrations of growth regulators: BA 5 μM, NAA 1 μM, 2,4-D 1 μM and CH 500 ppm.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

表 8. `巨峰´葡萄不同胚乳齡之胚乳去胚培養於不同植物生長調節劑培養基之癒傷組織發生率

Table 8. Callus induction rate from `Kyoho´ endosperms without embryo harvested at different stages of development and incubated on different growth regulator mediums.

Developmental stage of endosperm	Culture period	Callus induction rate (%) [z]			
		BA+NAA	BA+2,4-D	BA+NAA+ CH	BA+2,4-D+ CH
1 week after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 a[y]A[x]	0 aA	0 bA	0 aA
2 weeks after bloom	1 week	0	0	6.67	0
	2 weeks	10.00	6.67	10	6.67
	4 weeks	13.33	10.00	13.33	6.67
	2 months	16.67 aA	10.00 aA	13.33 aA	6.67 aA
3 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 aA	0 aA	0 bA	0 aA
4 weeks after bloom	1 week	0	0	0	0
	2 weeks	0	0	0	0
	4 weeks	0	0	0	0
	2 months	0 aA	0 aA	0 bA	0 aA

[z] Endosperms were cultured without embryo and seedcoat. The rate of callus induction = (the number of cultured endosperms with callus initiation / the total number of endosperms cultured) × 100%. 3 replicates per treatment; 10 explants per replicate. The basal medium was MS. The concentrations of growth regulators: BA 5 μM, NAA 1 μM, 2,4-D 1 μM and CH 500 ppm.

[y] Means separation within columns by LSD test at $p \leqq 0.05$.

[x] Means separation within rows by LSD test at $p \leqq 0.05$.

`巨峰´葡萄花後 1、2、3 及 4 週胚乳留胚及胚乳去胚分別培養於 5 μM BA + 1 μM NAA、5 μM BA + 1 μM 2,4-D、5 μM BA + 1 μM NAA + 500 ppm CH 及 5 μM BA + 1 μM 2,4-D + 500 ppm CH (以 MS 為基礎配方)之 4 種癒傷組織誘導培養基 2 個月後，胚乳留胚培養方面，花後 1 及 2 週之胚乳可誘導癒傷組織發生，花後 2 週胚乳培養於 5 μM BA + 1 μM NAA 者之癒傷組織發生率為 26.67%，顯著高於花後 1 週，其它癒傷組織發生率分別為 30 %、40 %及 43.33 %，與花後 1 週無顯著差異，但與花後 3 及 4 週有顯著差異，花後 3 及 4 週胚乳皆無法誘導癒傷組織產生。培養基方面，花後 1 及 2 週胚乳培養於上述 4 種培養基均無顯著差異，而花後 1 及 2 週胚乳留胚之培養時間皆在 1 週內即可誘導癒傷組織發生(Table 7)。胚乳去胚培養方面，花後 1、3 及 4 週之胚乳皆無法誘導癒傷組織發生，只有花後 2 週胚乳可以誘導癒傷組織發生，分別為 16.67%、10%、13.33%及 3.33%，其中以培養於 5 μM BA + 1 μM NAA + 500 ppm CH 者顯著高於花後 1、3 及 4 週者。培養基方面，花後 1-4 週胚乳培養於上述 4 種培養基均無顯著差異，但花後 2 週胚乳培養於 5 μM BA + 1 μM NAA + 500 ppm CH 者之培養時間在 1 週內即可誘導癒傷組織發生，其它 3 種培養基則培養 2 週內可誘導癒傷組織發生(Table 8)。

討　論

本研究發現以未成熟的葡萄胚乳為材料，可成功誘導癒傷組織發生，但胚乳齡太早則未必能誘導出癒傷組織；如`貝利 A´與`巨峰´葡萄在花後 1 週之未成熟胚乳是呈現半透明膠狀，此時胚乳癒傷組織誘導率很低。在花後 2 週胚乳則呈現半透明乳白色，此時誘導癒傷組織率高。在花後 3 週種皮已開始硬化，`貝利 A´之部分胚乳開始有白色硬化現象，但`巨峰´胚乳則還是呈現半透明乳白色，但此時誘導癒傷組織率也是很低。在花後 4 週時`貝利 A´種皮堅硬且胚乳呈現乳白色硬狀，而`巨峰´胚乳則是部分開始有白色硬化現象，此階段胚乳誘導癒傷組織率更低，此結果與母等人(1977)報導之結果相似。而 Thomas 等人(2000)以開花後 17 至 20 天的未成熟桑椹胚乳誘導癒傷組織率較高。本研究之`貝利 A´與`巨峰´胚乳在培養 7-14 天內便可以誘導癒傷組織產生，而吳等(2002)及劉等(2004)取成熟紅楊桃胚乳培養於 9.05 μM 2,4-D + 0.89 μM BA 培養基裡，培養第 5-8 天便可誘導出癒傷組織並且癒傷組織率可以高達 93.5-94.7 %。因此，不同植物胚乳之最佳培養年齡及誘導癒傷組織之速度並不相同。胚因素方面，隨品種可能有所不同，`巨峰´胚乳在有留胚的情形下誘導癒傷組織率都較去胚高，代表胚可以幫助`巨峰´胚乳癒傷組織產生，與桑椹的情形相似(Thomas *et al*., 2000)，但`貝利 A´則是以胚乳去胚誘導癒傷組織率較高，且兩品種胚乳有留胚或是去胚皆可以誘導癒傷組織產生。

基本培養基及植物生長調節劑對於胚乳是否能誘導癒傷組織極為重要。基本培養基方面，本研究發現`貝利 A´與`巨峰´葡萄胚乳留胚及去胚使用之基本培養基 MSMO (MS 添加

有機質)誘導癒傷組織之效果較 MS 佳。植物生長調節劑方面，`貝利 A´及`巨峰´胚乳的培養基裡添加較高濃度細胞分裂素 BA 與較低濃度生長素 NAA 或 2,4-D 及另外添加酪蛋白水解物(CH)有利於癒傷組織之誘導。其他的植物生長調節劑如細胞分裂素 TDZ、Kinitin、Zeatin，生長素 IAA、IBA 及激勃素 GA_3 或者是椰子乳等隨作物之不同都可能影響癒傷組織是否能誘導成功(母等，1977；吳等，2002；劉等，2004；Sehgal and Khurana, 1985; Nair *et al*., 1986; Tulecke *et al*.,1988; Gmitter *et al*.,1990; Thomas *et al*., 2000; Goralski *et al*., 2005)。

本研究也嘗試以胚乳癒傷組織來誘導體胚或不定芽，但並未成功，其適當之培養條件有待更進一步探討。

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Callus Induction from Endosperms of `Muscat Bailey A´ and `Kyoho´ Grapes

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Key words: Endosperm culture, Triploid, medium

Summary

This study examined the effect of developmental stage of endosperm and culture mediums for callus initiation from endosperms of `Muscat Bailey A´ and `Kyoho´ grapes. The results indicated that using MSMO as basal medium supplemented with 5 μM BA and 1 μM NAA (or 2,4-D), over 90% of callus induction rates were obtained from endosperms of `Muscat Bailey A´ and `Kyoho´ grapes harvested at 2 weeks after bloom and cultured with embryo for two months. When endosperms were cultured without embryo, casein hydrolysate (CH) had to be added into the culture medium to obtain higher callus induction rate. Although callus induction was obtained when MS was used as basal medium, yet the induction rates were significant lower than those in using MSMO as basal medium. Higher callus induction rates were obtained from endosperms cultured with embryo from both `Muscat Bailey A´ and `Kyoho´ grapes than those cultured without embryo. In summary, to have higher callus induction rate, endosperms of `Muscat Bailey A´ and `Kyoho´ grapes, should be harvested at 2 weeks after bloom and cultured with embryo on MSMO basal medium supplemented with CH.

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