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Dessiccation Using Saturated Salt Solutions and Improvement Germination Rate of Walnut (*Juglans regia* L.) Somatic Embryos

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Abstract: Desiccation of somatic embryos under different air humidities conditioned by saturated salt solutions for different durations; relationships between desiccation and germination or root formation in the dark; and effects of desiccation and gibberellic acid on germinating of 'Su-2' walnut somatic embryonic embryo line were investigated. Survival of embryos was low (about 10%) or zero when loss of fresh weight was about 90% and dry matter was about 70%. The highest germinating percentages (13.3-23.3%) were obtained with about 40.4-77.3% of loss of fresh weight and 9.1-34.6% of dry matter of desiccated embryos in covered plates over saturated $MgCl_2.6H_2O$ for 4 days, $Mg(NO_3)_2.6H_2O$ for 5 days or NaCl for 5-6 days, and with uncovered plates over $ZnSO_4.7H_2O$ for 6-7 days or NH_3SO_4 for 6-8 days. The highest rooting percentage (80.9%) in the dark were obtained with 56.2% of loss of fresh weight and 16.8% of dry matter of desiccated embryos in covered plates over saturated $MgCl_2.6H_2O$ for 4 days. To improve germination, both desiccation and GA_3 treatments were more effective than only desiccation or GA_3 treatments on germination. Germinating was best (46.0%) with desiccation followed by incubation in the dark for 15 days prior to being transferred to a medium containing 9 mg/l GA_3 in the light.

Ceviz (*Juglans regia* L.) Somatik Embriyolarının Doymuş Tuz Solusyonları ile Kurutulması ve Embriyolarda Çimlenme Oranının Artırılması

Özet: Cevizin (*Juglans regia* L.) somatik embriyonik embriyo hattı 'Su-2'da, doymuş tuz solusyonları ile oluşturulan farklı hava nemi koşullarında değişik sürelerde somatik embriyonların kurutulması; kurutma ile karanlık koşullarda çimlenme ya da kök oluşumu arasındaki ilişkiler ve embriyoların çimlenmesi üzerine kurutma ve gibberellik asitin etkileri araştırılmıştır. Somatik embriyolarda yaş ağırlığın yaklaşık %90'ı kaybolduğunda ve kuru madde yaklaşık %70 olduğunda canlılık oranı düşük (yaklaşık %10) ya da sıfırdır. Embriyolarda en yüksek çimlenme oranı (%13.3-23.3) kapaklı petri kapları içerisine yerleştirilmiş embriyoların doymuş $MgCl_2.6H_2O$ üzerinde 4 gün, $Mg(NO_3)_2.6H_2O$'da 5 gün ya da NaCl'de 5-6 gün ve kapaksız petri kapları içerisinde doymuş $ZnSO_4.7H_2O$ üzerinde 6-7 gün ya da NH_3SO_4'de 6-8 gün süreyle kurutularak yaş ağırlığının yaklaşık %40.4-77.3'ü kaybolduğunda ve kuru madde oranının % 9.1-34.6 olduğunda elde edilmiştir. Karanlıktan en yüksek köklenme oranı (%80.9) kapaklı petri kapları içerisinde doymuş $MgCl_2.6H_2O$ üzerinde 4 gün kurutulmuş embriyoların yaş ağırlığının %56.2'si kaybolduğu ve kuru maddesi %16.8 olduğunda ortaya çıkmıştır. Çimlenmeyi artırmada kurutma ve GA_3 uygulamaları, sadece kurutma ya da GA_3 uygulamalarına göre çimlenme üzerine daha etkili olmuştur. Çimlenme, embriyoların kurutulmasından sonra, 9 mg/l GA_3 içeren ortama ve ışık altına transfer edilmeden önce 15 gün karanlıkta inkübasyona alınmasıyla en yüksek (%46.0) bulunmuştur.

Introduction

Somatic embryogenesis is a rapid propagation method and an important tool in making genetic improvements using molecular and cellular techniques (1). Somatic embryogenesis has been achieved for the first time in the family Juglandaceae, including *Juglans regia* L., *J. hindsii* L., and *Pterocarya* sp., from cultures of immature cotyledon tissue, by Tulecke and McGranahan (2). Cornu (3), Long et al. (4), and Neuman et al. (5) then tried this method for *J. nigra* L. and its hybrids. However, utilization of somatic embryogenesis for clonal propagation of a great number of species, such as *Juglans*, has been limited by the low frequency of embryo germination (6, 7, 8). Tulecke and McGranahan (2) reported that mature somatic embryos of the walnut were germinated after a cold treatment of 8-10 weeks at 2-4°C to overcome apical dormancy. Another method,is the embryo drying that occurs naturally in most seeds, and which has a role in the developmental transition between maturation and germination. Thus dessication led to enhanced germination of both zygotic and somatic embryos. Desiccation of whole somatic embryos is another alternative method of germplasm storage (9). According to Deng and Cornu (8), desiccation

pretreatment promotes germination and, as compared to a cold storage of 2 months, desiccation pretreatment (3-5 days) combined with the use of a liquid germination medium is more efficient in promoting germination (45%) of somatic embryos from the immature cotyledons of *J. nigra* x *J. regia* L. The desiccated embryos have also shown higher percentages of root elongation than non-desiccated ones (48%) (8). *In vitro* root culture can be useful for studing the biology of host-parasite interactions (10).

The aims of this study were to determine the desiccation of somatic embryos under different air humities conditioned by saturated salt solutions for different durations, to develop the germination of embryos with the desiccation and gibberellic acid treatments, and to improve root formation from somatic embryos for *in vitro* root cultures with desiccation and dark applications in the 'Su-2' walnut embryo line.

Materials and Methods

A repetitively embryonic embryo line, designated Su-2, was used in these studies. Su-2 was originally cultured from the immature cotyledon of an open pollinated walnut cv. Sunland (2). It has been in culture for over five years, and has been used in several previous studies (11, 12). For maintenance, it is grown in darkness at room temperature on basal DKW media (13) and transferred biweekly. Secondary embryos were cultured to increase the numbers available for experimentation.

Experiment I - *The Desiccation of Su-2 Walnut Somatic Embryos:* Young white embryos (4-5 mm in diameter) were evenly distributed in empty sterile petri plates (25/plate). These were individually placed, with or without covers, over 40 ml of saturated $MgCl_2.6H_2O$, $Mg(NO_3)2.6H_2O$, $ZnSO_4.7H_2O$, NH_3SO_4 and NaCl salt solutions or water in sterile Nalgene desiccators (1,500 cm^3) for 1-8 days in darkness. Embryos were weighed prior to desiccation and again on removal from the desiccators. For determination of the moisture content of desiccated embryos, a subset of 10 embryos per treatment was further dried at 65˚C for 24 hours and reweighed. For determine of survival and germination 10, desiccated embryos per treatment were transferred to a solid DKW basal medium and cultured under cool white fluorescent lights (uE) with a 16- hour photoperiod, for 30 days. If the embryos turned green they were scored as survivors; if survivors had both root and shoot present, they were scored as germinated. For determination of the effect of desiccation on rooting percentages of somatic embryos, embryos (25/plate) were desiccated in desiccators containing saturated $MgCl_2.6H_2O$, $Mg(NO_3)_2.6H_2O$, $ZnSO_4.7H_2O$ and NaCl salt solutions or water over 1-8 days. Cultures were incubated at 25˚C in darkness for 20 days. Rooted embryos (1 cm or longer) in cultures were counted at the end of this period. Each treatment consisted of 25 embryos. The experiments were repeated three times.

Experiment II - *The Germination of Su-2 Walnut Somatic Embryos by Desiccation and Gibberellic Acid Treatments:* One of the best desiccation treatments, saturated $MgCl_2.6H_2O$ for 4 days in covered plates, was selected from 'Experiment I' for germination treatments. In this experiment, the following treatments were investigated:

- Desiccated or non-desiccated embryos were placed on a solid DKW basal medium involving GA_3 at concentrations 0, 1, 3, 5, 7 and 9 mg/l and were cultured at 25˚C under cool white fluorescent lights with a 16-hour photoperiod.

-Desiccated embryos were placed on a solid DKW basal medium without GA_3 for 15 days at 25˚C in darkness and then were transfered to solid DKW basal medium involving 0, 1, 3, 5, 7 and 9 mg/l GA_3, were incubated at 25˚C under cool white fluorescent lights with a 16- hour photoperiod.

For each treatment, rate of germination were determined after 30 days. Each germination test consisted of 5 plates with 10 embryos per plate. The experiment was repeated two times.

In both experiment data were analyzed by a variance in SAS software in accordance with F-test (p=0.05) and means were compared by Duncan`s Multiple Range Test ($p \leq 0.05$). Transformed angle values were used for percentage data.

Results

The Desiccation of Su-2 Walnut Somatic Embryos

In the first experiment, the effects of various saturated salt solutions, desiccation durations and covered or uncovered petri plates on desiccation of Su-2 walnut somatic embryos were tested. Analysis of variance showed that there were significant differences among treatments (Table 1-5).

In the experiment, the drying of embryos in uncovered petri plates was much faster than in covered ones over all saturated salt solutions. In this condition,

the loss of fresh weight was the highest and the fastest with saturated $MgCl_2.6H_2O$ salt solution. This solution resulted in a 66.4% loss of fresh weight and 30.8% dry matter in embryos for one day. The results showed that survival of embryos was low or zero when loss of fresh weight was about 90% and dry matter was about 70% (Table 1, 2 and 3).

The highest germinating percentages (13.3-23.3%) were obtained with about 40.4-77.3% of loss of fresh weight and 9.1-34.6% of dry matter of desiccated embryos in covered plates over saturated $MgCl_2.6H_2O$ for 4 days, $Mg(NO_3)_2.6H_2O$ for 5 days and NaCl for 5-6 days and with uncovered plates over $ZnSO_4.7H_2O$ for 6-7 days and NH_3SO_4 for 6-8 days (Table 1, 2 and 4). As a germinating percentage, rooting percentage depended on the loss of fresh weight during desiccation. The highest rooting percentage (80.9 %) in the dark was obtained with 56.2% of loss of fresh weight and 16.8 of dry matter of desiccated embryos in covered plates over saturated $MgCl_2.6H_2O$ for 4 days (Tables 1, 2 and 5).

The Germination of Su-2 Walnut Somatic Embryos by Desiccation and Gibberellic Acid Treatments

In the second experiment, there was a significant difference among germinating treatments according to the analysis of variance. Only GA_3 treatments was found to have no effect on the germination of Su-2 walnut somatic embryos. The germinating percentages were higher in both desiccation and GA_3 treatments than in treatments with only desiccation or GA_3. For improvement of the germination percentage, after desiccation, incubation of embryos in the dark for 15 days was effective prior to GA_3 treatments. In the presence of GA_3 at 9 mg/l, a germinating percentage of

Table 1. Effect of desiccation of walnut somatic embryos in covered or uncovered plates with saturated salt solutions on loss of fresh weight (%)

Day	$MgCl_2.6H_2O$	$Mg(NO_3)_2.6H_2O$	$ZnSO_4.7H_2O$	NH_3SO_4	NaCl	Water
			With Cover			
1	14.3 x*-h_2	9.2 b_2-h_2	4.7 d_2-h_2	4.2 d_2-h_2	8.1 b_2-h_2	0.3 h_2
2	32.7 p-u	14.9 x-g_2	8.6 b_2-h_2	7.9 b_2-h_2	15.1 x-f_2	0.3 h_2
3	49.8 k-m	29.8 p-v	11.5 a_2-h_2	11.1 a_2-h_2	20.1 u-c_2	0.3 h_2
4	56.2 i-l	27.6 r-x	13.0 y-h_2	15.2 w-e_2	29.3 r-w	0.4 g_2-h_2
5	82.5 a-f	43.6 l-p	19.3 u-c_2	17.7 v-d_2	40.4 m-r	0.5 f_2-h_2
6	91.9 a-b	56.3 i-l	26.6 r-x	19.6 u-c_2	52.1 k-m	0.2 h_2
7	93.7 a	69.1 f-i	26.0 s-x	27.4 r-x	50.1 k-m	0.5 f_2-h_2
8	93.8 a	78.8 b-f	31.9 p-v	25.1 t-a_2	73.7 e-h	0.8 e_2-h_2
			Without Cover			
1	66.4 g-j	30.6 p-v	12.2 z-h_2	12.7 y-h_2	35.9 n-t	1.4 e_2-h_2
2	84.8 a-e	52.2 k-m	19.3 u-c_2	22.2 t-a_2	51.5 k-m	2.1 e_2-h_2
3	92.4 a-b	76.0 d-h	34.7 o-t	32.3 p-u	71.6 e-h	2.9 e_2-h_2
4	92.0 a-b	88.0 a-d	40.0 m-s	41.2 m-q	83.5 a-e	3.5 d_2-h_2
5	93.1 a-b	89.1 a-d	48.7 l-n	47.3 l-o	91.9 a-b	4.6 d_2-h_2
6	91.8 a-b	90.8 a-c	55.0 j-l	66.4 g-j	90.9 a-c	4.7 d_2-h_2
7	88.6 a-d	90.1 a-c	62.7 h-k	73.9 e-h	90.9 a-c	6.3 c_2-h_2
8	92.1 a-b	90.9 a-c	71.1 e-h	77.3 c-e	90.8 a-c	6.0 c_2-h_2

* Means with the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 2. Effect of desiccation of walnut somatic embryos in covered or uncovered plates with saturated salt solutions on dry matter (%)

Day	$MgCl_2.6H_2O$	$Mg(NO_3)_2.6H_2O$	$ZnSO_4.7H_2O$	NH_3SO_4	NaCl	Water
			With Cover			
1	6.7 p*-o	6.9 p-o	6.6 p-o	6.6 p-o	6.7 p-o	6.0 p
2	8.9 n-p	6.5 p	7.0 p-o	6.7 p-o	6.2 p	5.6 p
3	13.7 l-p	8.2 n-p	7.0 p-o	6.4 p	6.5 p	5.3 p
4	16.8 l-p	8.9 n-p	6.5 p	6.9 p-o	7.8 n-p	5.3 p
5	43.8 h-i	10.0 n-p	6.8 p-o	6.9 p-o	9.1 n-p	5.8 p
6	70.6 f	14.3 l-p	7.6 p-o	7.2 p-o	13.9 l-p	5.6 p
7	94.1 a	22.1 j-p	7.2 p-o	8.0 n-p	12.2 m-p	5.9 p
8	93.3 a-b	50.2 g-h	7.9 n-p	8.1 n-p	25.6 j-n	5.6 p
			Without Cover			
1	30.8 i-l	9.5 n-p	7.5 p-o	7.1 p-o	10.9 n-p	5.5 p
2	66.5 f	13.4 m-p	8.4 n-p	8.5 n-p	14.7 l-p	6.4 p
3	88.8 a-e	29.5 i-m	8.1 n-p	9.0 n-p	24.6 j-o	5.7 p
4	90.1 a-d	65.9 f	10.3 n-p	10.3 n-p	49.9 g-h	6.3 p
5	92.1 a-c	64.3 f-g	13.1 m-p	13.1 m-p	73.0 e-f	6.0 p
6	89.5 a-e	74.1 d-f	13.8 l-p	16.0 k-p	74.1 d-f	5.6 p
7	89.8 a-d	74.2 d-f	16.8 k-p	31.5 i-k	75.7 c-f	6.1 p
8	91.3 a-c	77.1 b-f	21.8 j-p	34.6 i-j	76.1 c-f	6.3 p

* Means with the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

46.0% in embryos was obtained with desiccation and then incubation in darkness for 15 days prior to transferred to medium containing 9 mg/lGA_3 (Table 6).

Discussion

In this study, desiccation of somatic embryos under different air-humidities conditioned by saturated salt solutions for different durations; relationships between desiccation and germination or root formation in the dark; and effects of desiccation and gibberellic acid on germinating of 'Su-2' walnut somatic embryos were presented.

Desiccation is an obvious process for regulating plantlet regeneration from somatic embryos (8, 9, 14, 15). Gray (16), demonstrated the efficacy of dehydration treatments for germinating and producing plants from grape somatic embryos. According to Gray (16), ,dehyration plays a role in breaking dormancy, and probably in the regulatory mechanisms that control post germination growth from mature embryos. However, Kermode and Bewley (17) reported that desiccation switches the pattern of gene expression from a maturation program to the program required for germination. Robert et al. (18) showed that the highest germination percentage (95%) was exhibited by somatic embryos of interior spruce that were incubated in humidities of 95%. It is possible that water loss triggers metabolic changes in the embryo and that these changes occur over the remainder of the treatment. Embryos under high relative humidity treatment only lose about 15% water content and may remain metabolically active in sitka spruce somatic embryos (18). Embryos reach specific water contents at specific relative humidities.

Table 3. Effect of desiccation of walnut somatic embryos in covered or uncovered plates with saturated salt solutions on survival (%

Day	$MgCl_2.6H_2O$	$Mg(NO_3)_2.6H_2O$	$ZnSO_4.7H_2O$	NH_3SO_4	NaCl	Water
			With Cover			
1	100.0 a*	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
2	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
3	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
4	100.0 a	100.0 a	96.7 a	100.0 a	100.0 a	100.0 a
5	73.3a-c	86.7 ab	100.0 a	100.0 a	100.0 a	100.0 a
6	33.3 d-f	90.0 a	100.0 a	100.0 a	100.0 a	100.0 a
7	0.0 f	70.0 a-c	100.0 a	100.0 a	100.0 a	100.0 a
8	0.0 f	26.7 d-f	100.0 a	100.0 a	53.3 b-d	100.0 a
			Without Cover			
1	66.7 a-c	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
2	33.3 d-f	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
3	0.0 f	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
4	0.0 f	40.0 c-e	100.0 a	100.0 a	53.3 b-d	100.0 a
5	0.0 f	33.3 d-f	100.0 a	100.0 a	6.7 ef	100.0 a
6	0.0 f	13.3 ef	100.0 a	100.0 a	6.7 ef	100.0 a
7	0.0 f	0.0 f	100.0 a	73.3 a-c	13.3 ef	100.0 a
8	0.0 f	6.7 ef	100.0 a	70.0 a-c	0.0 f	100.0 a

* Means with the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 4. Effect of desiccation of walnut somatic embryos in covered or uncovered plates with saturated salt solutions on germination (%)

Day	$MgCl_2.6H_2O$	$Mg(NO_3)_2.6H_2O$	$ZnSO_4.7H_2O$	NH_3SO_4	NaCl	Water
			With Cover			
1	0.0 e*	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
2	3.3 d-e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
3	6.7 c-e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
4	13.3 a-d	10.0 b-e	0.0 e	6.7 c-e	3.3 de	0.0 e
5	10.0 b-e	16.7 a-c	0.0 e	0.0 e	13.3 a-d	0.0 e
6	0.0 e	10.0 b-e	10.0 b-e	3.3 de	20.0 a-b	0.0 e
7	0.0 e	10.0 b-e	0.0 e	3.3 de	6.7 c-e	0.0 e
8	0.0 e	0.0 e	0.0 e	10.0 b-e	0.0 e	0.0 e
			Without Cover			
1	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
2	0.0 e	0.0 e	0.0 e	3.3 de	0.0 e	0.0 e
3	0.0 e	3.3 d-e	0.0 e	3.3 de	0.0 e	0.0 e
4	0.0 e	0.0 e	0.0 e	3.3 de	0.0 e	0.0 e
5	0.0 e	0.0 e	6.7 c-e	10.0 b-e	0.0 e	0.0 e
6	0.0 e	0.0 e	13.3 a-d	16.7 a-c	0.0 e	0.0 e
7	0.0 e	0.0 e	23.3 a	13.3 a-d	0.0 e	0.0 e
8	0.0 e	0.0 e	10.0 b-e	16.7 a-c	0.0 e	0.0 e

* Means with the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 5. Effect of desiccation of walnut somatic embryos in covered or uncovered plates with saturated salt solutions on rooting (%) in the dark

Day	$MgCl_2.6H_2O$	$Mg(NO_3)_2.6H_2O$	$ZnSO_4.7H_2O$	NH_3SO_4	NaCl	Water
			With Cover			
1	17.4 h*p	2.6 n-p	4.2 m-p	-	5.6 l-p	1.4 p-o
2	50.3 a-k	23.2 f-p	22.8 g-p	-	7.0 k-p	2.8 n-p
3	50.2 a-k	53.5 a-i	24.0 e-p	-	41.7 a-p	2.8 n-p
4	80.9 a	48.6 a-l	45.4 a-o	-	44.5 a-o	0.0 p
5	70.9 a-d	56.0 a-i	23.6 e-p	-	38.9 a-p	4.2 m-p
6	76.3 a-b	52.3 a-j	46.7 a-n	-	77.8 a-b	4.2 m-p
7	67.2 a-f	71.2 a-d	47.2 a-m	-	75.0 a-c	5.6 l-p
8	52.9 a-i	75.4 a-b	44.0 a-p	-	54.2 a-i	0.0 p
			Without Cover			
1	19.4 h-p	5.6 l-p	4.3 n-p	-	15.3 i-p	0.0 p
2	66.7 a-g	17.4 h-p	4.0 m-p	-	29.2 d-p	4.2 m-p
3	61.1 a-h	53.6 a-i	41.1 a-p	-	51.4 a-j	2.8 n-p
4	54.8 a-i	66.5 a-g	53.4 a-i	-	55.5 a-i	1.4 p-o
5	37.7 a-p	67.9 a-e	41.7 a-p	-	48.6 a-l	4.2 m-p
6	31.1 c-p	67.0 a-g	35.6 b-p	-	34.8 b-p	8.3 j-p
7	1.8 p-o	57.5 a-i	27.8 d-p	-	59.7 a-h	5.6 l-p
8	0.0 p	53.3 a-i	48.9 a-l	-	47.2 a-m	2.8 n-p

* Means with the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Table 6. Effects of desiccation, darkness and gibberellic acid on germination (%) of walnut somatic embryos

Treatments	GA_3 concentrations (mg/l)						
	0	1	3	5	7	9	Average
Non desiccation	0.0 e**	0.0 e	0.0 e	2.5 e	0.0 e	0.0 e	0.4
Desiccation*	14.0 d	20.5 cd	33.2 b	25.0 bcd	27.5 bc	35.0 ab	25.9
Desiccation* + dark	18.5 cd	28.0 bc	27.2 bc	36.2 ab	24.7 bcd	46.0 a	30.1
Average	10.8	16.2	20.1	21.2	17.4	27.0	

*Desiccation of somatic embryos in covered plates using saturated $MgCl_2.6H_2O$ for 4 days.

**Means with the same letter are not significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

Rate of water loss and time needed to reach a given water content are partially dependent on embryo size of species (16). Variation in desiccation tolerance among different species may reflect differences in the degree of embryo maturation (18). The results of our experiment showed that desiccation was most important for root formation and germination of 'Su-2' walnut somatic embryos. After desiccation, the highest germination and rooting percentages of embryos were obtained with 40-77% of fresh weight and 9-35% of dry matter content over saturated salt solutions for different durations (Table 1, 2, 4 and 5). Deng and Cornu (8) also reported that the

desiccated embryos which lost 50-70% of fresh weight had higher percentages of root elongation and of normal germination than non-desiccated ones, and that desiccation pretreatment (3-5 days) combined with the use of a liquid germination medium is more efficient in promoting germination (45%) of somatic embryos from the immature cotyledons of *J. nigra* x *J. regia* L. However, according to Komatsuda et al. (19), exogenous gibberellic acid was effective in promoting precocious germination (77% from semi-wild soybean and 60-64% from cultivated soybean) in premature soybean somatic embryos, but was not necessary for the germination of mature somatic embryos. In our results, the highest germinating percentage (46.0%) of Su-2 walnut somatic embryos was obtained with desiccation followed by incubation of embryos in darkness for 15 days prior to transfer to a medium containing 9 mg/l GA_3 (Table 6).

In conclusion, the best desiccation for germination and root formation of walnut somatic embryos is obtained with covered plates over saturated $MgCl_2.6H_2O$ for 4 days, $Mg(NO_3)_2.6H_2O$ for 5 days and NaCl for 5-6 days, and with uncovered plates over $ZnSO_4.7H_2O$ for 6-7 days and NH_3SO_4 for 6-8 days. Incubation of desiccated embryos on a solid DKW basal medium in the dark enhances root formation. After rooting, transferal solid DKW basal medium containing 9 mg/l GA_3 under light improves the germinating percentage of 'Su-2' somatic embryos.

References

1. Robacker, C., Somatic Embryogenesis and Plant Regeneration from Muscadine Grape Leaf Explants, HortScience 28(1), 53-55, 1993.
2. Tulecke, W., McGranahan, G., Somatic embryogenesis and plant regeneration from cotyledons of walnut, *Juglans regia* L., Plant Sci. 40, 57-63, 1985.
3. Cornu, D., Somatic Embryogenesis in Tissue Cultures of Walnut (*Juglans nigra, J. major*, and hybrids *J. nigra x J. regia*), In: Somatic Cell Genetics of Woody Plants, Ed: Ahuja, M.R., Kluwer Academic Pub., Dordrecht, The Netherlands, pp 45-49, 1988.
4. Long, L.M., Preece, J.H., Gaffney, G.R., Van Sambeek, J.W., Somatic embryogenesis and organogenesis of eastern black walnut (*J. nigra*), HortScience 27(6), 584, 1992.
5. Neuman, M.C., Preece, J.E., Van Sambeek, J.W., Gaffney, G.R., Somatic embryogenesis and callus production from cotyledon explants of Eastern black walnut, Plant Cell, Tissue and Organ Culture 32, 9-18, 1993.
6. Tulecke, W., Somatic Embryogenesis in Woody Perennials, In: Cell and Tissue Culture in Forestry, 2, Ed: Bonga, J.M., Durzan, D.J., Martinus Nijhoff Pub., Boston, 1987.
7. Webster, F.B., Roberts, D.R., Mcinnis, S.M., Sutton, B.C.S., Propagation of Interior Spruce by Somatic Embryogenesis, Canadian J. of Forest Research 20, 1759-1765, 1990.
8. Deng, M.D., Cornu, D., Maturation and germination of walnut somatic embryos, Plant Cell, Tissue and Organ Culture 28, 195-202, 1992.
9. Attree, S.M., Moore, D., Sawhney, V.K., Fowke, L.C., Enhanced Maturation and Desiccation Tolerance of White Spruce (*Picea glauca* (Moench) Voss) Somatic Embryos, Annals of Botany 68, 519-525, 1991.
10. Wolf, S.J., Timko, M.P., *In vitro* Root Culture: A Novel Approach to Study the Obligate Parasite *Striga asiatica* (L.) Kuntze, Plant Science 73, 233-242, 1991.
11. McGranahan, G.H., Leslie, C.A., Uratsu, S.L., Martin, L.A., Dandekar, A.M., *Agrobacterium*- Mediated Transformation of Walnut Somatic Embryos and Regeneration of Transgenic Plants, Bio/Technology 6, 800-804, 1988.
12. McGranahan, G.H., Leslie, C.A., Uratsu, S.L., Dandekar, A.M., Improved Efficiency of the Walnut Somatic Embryo Gene Transfer System, Plant Cell Rep. 8, 512-516, 1990.
13. Driver, J.A., Kuniyuki, A.H., *In vitro* Propagation of Paradox Walnut Rootstock, HortScience 19, 507-509, 1984.
14. Gray, D.J., Quiescence in Monocotyledonous and Dicotyledonous Somatic Embryos Induced by Dehydration, HortScience 22(5), 810-814, 1987.
15. Janick, J., Kim, Y.H., Kitto, S., Saranga, Y., Desiccation Synthetic Seed, In: Synseed, Ed: Redenbaugh, K., CRC Press, Inc., Boca Raton, pp. 11-33, 1993.
16. Gray, D.J., Effects of Dehydration and Exogenous Growth Regulators on Dormancy, Quiescence and Germination of Grape Somatic Embryos, *In vitro* Cell.Dev.Biol. 25, 1173-1178, 1989.
17. Kermode, A.R., Bewley, D.J., The Role of Maturation Drying in the Transition from Seed Development to Germination, J. Experimental Botany, 1916-1927, 1985.
18. Roberts, D.R., Sutton, B.C.S., Flinn, B.S., Synchronous and High Frequency Germination of Interior Spruce Somatic Embryos Following Partial Drying at High Relative Humidity, Can.J.Bot. 68, 1086-1090, 1990.
19. Komatsuda, T., Lee, W., Oka, S., Maturation and Germination of Somatic Embryos as Affected by Sucrose and Plant Growth Regulators in Soybeans *Glycine gracilis* Skvortz and *Glycine max* (L.) Merr., Plant Cell, Tissue and Organ Culture 28, 103-113, 1992.