Plant Cell Rep (2007) 26:285–290
DOI 10.1007/s00299-006-0252-0

CELL BIOLOGY AND MORPHOGENESIS

Ethylene influences green plant regeneration from barley callus

Ajay K. Jha · Lynn S. Dahleen · Jeffrey C. Suttle

Received: 25 July 2006 / Revised: 12 September 2006 / Accepted: 17 September 2006 / Published online: 17 October 2006

Abstract The plant hormone ethylene is involved in numerous plant processes including in vitro growth and regeneration. Manipulating ethylene in vitro may be useful for increasing plant regeneration from cultured cells. As part of ongoing efforts to improve plant regeneration from barley (*Hordeum vulgare* L.), we investigated ethylene emanation using our improved system and investigated methods of manipulating ethylene to increase regeneration. In vitro assays of regeneration from six cultivars, involving 10 weeks of callus initiation and proliferation followed by 8 weeks of plant regeneration, showed a correlation between regeneration and ethylene production: ethylene production was highest from 'Golden Promise', the best regenerator, and lowest from 'Morex' and 'DH-20', the poorest regenerators. Increasing ethylene production by addition of 1-aminocyclopropane 1-carboxylic acid (ACC) during weeks 8–10 increased regeneration from Morex. In contrast, adding ACC to Golden Promise cultures during any of the tissue culture steps reduced regeneration, suggesting that Golden Promise may produce more ethylene than needed for maximum regeneration rates. Blocking ethylene action with silver nitrate during weeks 5–10 almost doubled the regeneration from Morex and increased the Golden Promise regeneration 1.5-fold. Silver nitrate treatment of Golden Promise cultures during weeks 8–14 more than doubled the green plant regeneration. These results indicate that differential ethylene production is related to regeneration in the improved barley tissue culture system. Specific manipulations of ethylene were identified that can be used to increase the green plant regeneration from barley cultivars. The timing of ethylene action appears to be critical for maximum regeneration.

Keywords ACC · *Hordeum vulgare* · Silver nitrate

Communicated by K. K. Kamo

A. K. Jha
Department of Plant Sciences,
North Dakota State University,
Fargo, ND, USA

L. S. Dahleen (✉) · J. C. Suttle
USDA-ARS, Northern Crop Science Laboratory,
Fargo, ND 58105, USA
e-mail: dahleenl@fargo.ars.usda.gov

Present address:
A. K. Jha
Department of Botany,
Oklahoma State University,
Stillwater, OK, USA

Introduction

Ethylene is a gaseous plant hormone that regulates numerous cellular and developmental processes including germination, flowering, senescence, fruit ripening, and response to different stresses (Abeles et al. 1992). Ethylene has positive and negative effects on callus growth, shoot and root production, and embryogenesis, depending on the plant species (Biddington 1992). Almost all plant tissues have the capability to produce ethylene. Ethylene biosynthesis is highly regulated, and ethylene affects plants at a concentration $\geq 0.01\ \mu l\ l^{-1}$ (Reid 1995).

Ethylene production and action can be manipulated by certain chemicals to study the effects of ethylene in vitro. These include silver nitrate ($AgNO_3$), which blocks the activity of ethylene by reducing receptor capacity to bind ethylene (Beyer 1976), and 1-aminocyclopropane 1-carboxylic acid (ACC), an ethylene precursor that promotes ethylene production (Bleecker and Kende 2000). $AgNO_3$ has been used to increase plant regeneration from cultured tissues of several species (Purnhauser et al. 1987; Vain et al. 1989;

Songstad et al. 1988). $AgNO_3$ promoted shoot regeneration in tissue cultures of two wheat cultivars and a number of *Nicotiana plumbaginofolia* mutants. Exogenous ethylene reduced shoot regeneration in these genotypes (Purnhauser et al. 1987). $AgNO_3$ also enhanced ACC synthase activity and ACC accumulation. The addition of 17.6 μM $AgNO_3$ to culture media enhanced shoot elongation in lemon (*Citrus limonum* L.) (Kotsias and Roussos 2001). Cho and Kasha (1989) found that differences in callus and embryo production from anthers of barley (*Hordeum vulgare* L.) cultivars were due to differing capacities to produce ethylene and respond to ethylene.

Genetic transformation is an important tool for crop improvement and genetic studies. A prerequisite of transformation for crop improvement is a high rate of regeneration from tissue culture (Goldstein and Kronstad 1986). Efficient plant regeneration methods would facilitate genetic transformation of commercial barley cultivars (Bregitzer et al. 1998; Dahleen and Bregitzer 2002). In vitro performance measures such as growth rate and number of green plants regenerated are influenced by genotype, culture media, and genotype by culture media interactions (Bregitzer 1992). Despite the relatively large number of in vitro studies of ethylene effects (Biddington 1992), systematic use of ethylene manipulation as part of the in vitro systems used for barley transformation is not widespread. Stage- and genotype-specific effects have been reported, and therefore no standard system has been established. Nevertheless, the literature indicates a great potential to increase regeneration, justifying further efforts to investigate this issue and formulate specific protocols for genotypes of interest.

The objectives of the present research were to (1) determine if a relationship exists between ethylene production and green plant regeneration in specific barley genotypes that have been investigated in our laboratories by comparing the ethylene evolution from the tissue cultures of six genotypes that differ in regeneration capacity, (2) confirm any relationship found by manipulating ethylene synthesis or action in vitro and measuring the effects on regeneration, and (3) establish critical timing of ethylene action (inhibition/promotion) for maximum green plant regeneration.

Materials and methods

Donor plant growth

Single plant-derived lines of the barley cultivar Conlon selected from breeder's seed and Golden Promise provided by Dr. P. Bregitzer (USDA-ARS, Aberdeen, ID), and the Steptoe and Morex parents and two doubled haploid lines from the Steptoe/Morex mapping population (DH-12, DH-20; Kleinhofs et al. 1993) were used as explant sources. The seeds were sown in an environmentally controlled greenhouse at 25–28°C. Supplemental lighting was provided by mercury halide lamps to maintain a day length of 16 h. Two plants were grown per clay pot (15 cm in diameter by 18 cm deep), using approximately 1.2 L mixture of peat and vermiculite supplemented with a slow release blend of macro- and micronutrients.

Tissue culture

The barley genotypes were sown at different times to synchronize seed production. The immature seeds were collected approximately 14–21 days after pollination. The seeds were dehusked and surface sterilized with 70% (v/v) ethanol for 1 min, 2.6% (w/v) sodium hypochlorite for 5 min, and rinsed three times in sterile distilled water for 5 min each. Embryos were aseptically removed from the immature seed, placed on initiation medium, and incubated in the dark at 18.5°C to produce callus. After 12 days, shoots derived from the zygotic apical meristem were removed from the developing calli. After 4 weeks, roughly equal amounts of friable calli from each Petri plate were removed from the initiation medium and subcultured on maintenance medium for 3 weeks (weeks 5–7), subcultured again, and allowed to proliferate for an additional 3 weeks (weeks 8–10), and then subcultured onto regeneration medium and placed under lights (3.1–5.5 μmol m^{-2} s^{-1}, warm and cool white fluorescent lamps) at 21–25°C. Following 4 weeks on regeneration medium (weeks 11–14), developing green plants with a complete root and shoot were counted and removed; green callus sectors and unrooted shoots were transferred to rooting medium and kept under the same light conditions. Final green plant counts were taken after 4 weeks.

Culture media were prepared as described in Dahleen and Bregitzer (2002). These media contain decreased levels of iron and increased levels of copper and boron relative to those found in standard MS medium (Murashige and Skoog, 1962), and are supplemented with various growth regulators (Table 1). Four embryos were placed on 15 mL of media in each 60 × 20 mm Petri plate.

Ethylene measurement and manipulation

For investigations of ethylene production and green plant regeneration, 12 Petri plates cultured from each of the six genotypes were used for ethylene analysis. Ethylene was measured twice per week by headspace analysis (described later). Plates 1–6 were used for the first measurement each week and plates 7–12 for the second measurement to reduce the time each plate was in a sealed container for headspace sampling. The experiment was replicated twice.

Plant regeneration in response to manipulation of ethylene levels was investigated using the ethylene precursor ACC

Table 1 Temperatures, light conditions, and growth regulators used during barley tissue culture

Tissue culture stage	Stage length (weeks)	Temperature and light conditions	Growth regulators[a] and concentration
Initiation	4 (0–4)	18–20°C, dark	2, 4-D; 3 mg/L
Maintenance-1	3 (5–7)	18–20°C, dark	2, 4-D; 3 mg/L and BAP; 0.2 mg/L
Maintenance-2	3 (8–10)	18–20°C, dark	2, 4-D; 3 mg/L and BAP; 0.2 mg/L
Regeneration	4 (11–14)	20–25°C, light	BAP; 0.1 mg/L
Rooting	4	20–25°C, light	None

[a]2,4-D, 2,4 dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine.

(100 μM) to increase ethylene production, or the ethylene perception inhibitor $AgNO_3$ (25 μM) to reduce plant responses to ethylene. Golden Promise and Morex were used for these experiments, as they were the highest and lowest ethylene producers and regenerators from the previous experiment. Seven treatments of $AgNO_3$ or ACC were compared: the first 3 weeks of maintenance (weeks 5–7), the second 3 weeks of maintenance (weeks 8–10), the 4 weeks of regeneration (weeks 11–14), M1–M2 (weeks 5–10), M2–G (weeks 8–14), M1–G (weeks 5–14), and the control with no $AgNO_3$ or ACC added. Four Petri plates were compared for each treatment for each cultivar. All Petri plates were kept under the same environmental conditions described earlier and were randomly placed on shelves in an 18.5°C incubator. Two Petri plates in one gas-tight container (described later) were considered as an experimental unit and plate pairs were the replicates. The experiment was conducted using a completely randomized design (CRD) and means were compared using Tukey's Studentized Range Test at the 95% probability level. Analysis of variance (ANOVA), regression, and correlation analyses were conducted using SAS (SAS Institute 1999).

Ethylene quantification

Ethylene diffuses from the calli and accumulates in the headspace of the Petri plates. On the days of ethylene measurement, two Petri plates were sealed in each air-tight glass jar (230 mL), which was incubated for 6 h in the same conditions as other calli. After incubation, 1 mL of gas was withdrawn with a syringe through a rubber septum in the container lid and injected into a gas chromatograph. A standard peak was also generated by using a known concentration of ethylene. The height and known ethylene concentration of the standard peak were used to calculate the ethylene concentration of experimental samples. The instrument conditions were as follows: oven temperature 80°C; injector temperature 150°C; detector temperature 250°C, 45 cm activated alumina column, nitrogen carrier gas (Suttle and Kende 1978). In initial experiments, Petri plates containing only media were included for ethylene measurements. No ethylene was detected from any of the media used, so these controls were not included in the later experiments.

Results

Cultivars exhibited significant variation for regeneration rates, from a high of 12.8 plants per Petri plate for Golden Promise to a low of 0.3 plants per Petri plate for Morex (Table 2). Cumulative ethylene production in vitro, measured twice per week, also showed significant differences among cultivars (Fig. 1, Table 2). Ethylene production was correlated with green plant regeneration rates. Measurements during the first maintenance stage among different genotypes showed the strongest relationship with the number of green plants regenerated (Pearson correlation coefficient, $r = 0.91$). The correlation between green plant regeneration and ethylene production also was significant at the regeneration stage but not at culture initiation and the second 3 weeks of maintenance. The six genotypes showed a linear regression coefficient (r^2) of 0.77 between cumulative ethylene production and regeneration; the highest green plant regeneration occurred in the highest ethylene producer and the lowest green plant regeneration occurred in a low ethylene producer. There were no significant genotype by stage interactions for either ethylene production or green plant regeneration.

Manipulating ethylene action by the use of the ethylene antagonist $AgNO_3$ resulted in significant differences in green plant regeneration (Table 3). Regeneration from Morex almost doubled when $AgNO_3$ was added during the 6 weeks on maintenance media (weeks 5–10). The lowest regener-

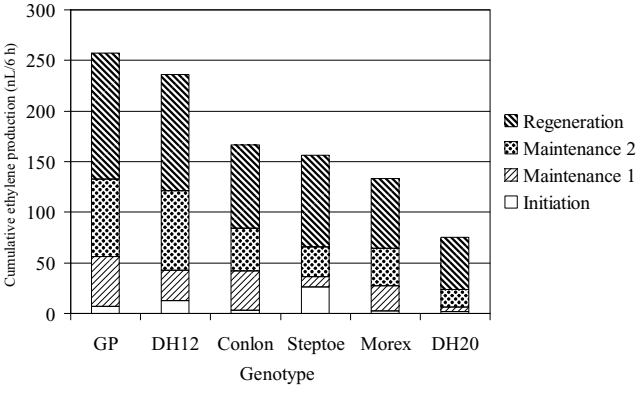

Fig. 1 Genotypic differences in the amount of ethylene produced (nL/6 h) during barley tissue culture. Stacked column shows cumulative amounts of ethylene produced at different tissue culture stages. DH-12 and DH-20 are doubled haploid lines derived from a cross between Steptoe and Morex. GP, Golden Promise

Table 2 Comparison of cumulative ethylene production at each tissue culture stage and average number of green plants regenerated/Petri plate from tissue cultures of six barley genotypes

Genotype	Ethylene (nL)				Regeneration (plants/Petri plate)
	I[b]	M1	M2	G	
GP	6.81 ab[a]	49.48 a	76.46 a	124.48 a	12.8 a
Conlon	3.39 ab	38.82 a	41.97 b	82.54 bc	7.2 b
Steptoe	26.13 a	10.16 b	29.15 bc	90.84 b	4.2 bc
DH-12	13.07 ab	29.55 ab	78.45 a	115.10 a	3.5 cd
DH-20	1.64 b	4.93 b	17.06 c	51.95 d	0.8 d
Morex	2.87 ab	24.28 ab	37.41 bc	69.08 cd	0.3 d
Correlation	0.64ns	0.91*	0.56ns	0.84*	

Ethylene was measured by headspace analysis twice per week in nL/6 h. Pearson correlation coefficients were calculated between mean ethylene production at each stage and green plant regeneration.

[a]Means of two replicates in a column followed by the same letter are not significantly different at $p < 0.05$. Correlations were non-significant (ns) or significant at $p < 0.05$ (*).

[b]I, initiation medium; M1, first 3 weeks of maintenance medium; M2, second 3 weeks of maintenance medium; G, regeneration medium.

ation occurred when $AgNO_3$ was applied during regeneration (weeks 11–14). In experiments with Golden Promise, $AgNO_3$ added throughout the second maintenance and regeneration stages (weeks 8–14) more than doubled the regeneration compared to the control (Table 3). Inhibition of ethylene during regeneration (weeks 11–14) and during all 10 weeks of maintenance and regeneration (weeks 5–14) regenerated the fewest plants in Golden Promise.

The addition of ACC significantly increased the ethylene production and had non-significant affects on regeneration in Morex (Table 4). Regeneration was highest when ACC was added during the second maintenance stage (weeks 8–10), with reduced regeneration when ACC was applied at either earlier or later stages. Increased ethylene synthesis during the first 3 weeks of maintenance (weeks 5–7) reduced regeneration. Golden Promise did not show improved regeneration when ACC was added at any time, and regeneration was highest for the control. Increased ethylene production during the maintenance stages (weeks 5–10) resulted in regeneration rates similar to the untreated calli, but treatment at any other stage significantly reduced regeneration (Table 4).

Table 3 Mean green plant regeneration per Petri plate (three replicates, eight plates/replicate) from barley tissue cultures treated with 25 μM $AgNO_3$ at different tissue culture stages

Treatment (weeks)	Morex	Golden Promise
Control	9.2 b[a]	19.7 bc
M1[b] (5–7)	10.3 ab	13.5 c
M2 (8–10)	10.5 ab	16.3 bc
G (11–14)	6.5 b	8.5 c
M1–M2 (5–10)	16.3 a	30.8 ab
M2–G (8–14)	10.3 ab	42.8 a
M1–G (5–14)	9.7 ab	3.3 c

[a]Means in a column followed by the same letter are not significantly different ($p < 0.05$).

[b]I, initiation medium; M1, first 3 weeks of maintenance medium; M2, second 3 weeks of maintenance medium; G, regeneration medium.

Discussion

An important aspect in these studies was minimizing the variability in experimental material. All plant materials used were originally derived from a single seed, removing the variability inherent in cultivar seed lots. The seeds were planted to obtain embryos for culturing from different genotypes, at the same time to reduce environmental differences in ethylene production during tissue culture. Seasonal variation in the amount of ethylene produced and green plants regenerated was observed when comparing the different experiments and replicates. For example, the genotype DH-12 regenerated a comparable number of green plants to Golden Promise and Conlon in one replicate, but regenerated much fewer plants in the other replicate, resulting in average regeneration less than predicted (Table 2) from DH-12's ethylene production (Fig. 1). DH-12 may be more sensitive to environmental conditions than the other genotypes. Comparison of Golden Promise and Morex data in Table 2 to the control data in Table 4 also shows these seasonal effects. Experiments in Table 2 were conducted using embryos from plants grown in the summer and early autumn that were exposed to high temperatures during heading, especially in the second replicate when temperatures were higher than 30°C for more than 12 days of growth. Higher temperatures increased the ethylene production and reduced the regeneration compared to the embryos from donor plants in experiments in Table 4. These plants were grown under more optimal conditions in mid-autumn and mid-spring, when greenhouse temperatures were rarely more than 30°C and natural light levels were still high. These environmental effects can make it difficult to interpret the effects of ethylene on green plant regeneration. The large variation in ethylene production and the green plant regeneration between replicates also reduced the statistical power to differentiate between treatments. Similar seasonal effects of temperature and light intensity on regeneration have been

Table 4 Cumulative ethylene production (three replicates, eight Petri plates/replicate) during each barley tissue culture stage (M1, M2, and G) and mean green plant regeneration per plate after treatment with 100 μM 1-aminocyclopropane 1-carboxylic acid (ACC)

Period of ACC treatment (weeks)	Golden Promise ethylene production (nL)[a]				Morex ethylene production (nL)			
	M1[c]	M2	G	Regeneration	M1	M2	G	Regeneration
Control	36.9 c[b]	186.7 bc	119.7 c	19.7 a	32.2 bc	155.3 a	76.8 b	9.2 ab
M1 (5–7)	336.2 a	239.5 bc	149.1 c	5.3 b	79.3 ab	213.8 a	91.2 b	3.0 b
M2 (8–10)	55.4 bc	725.5 a	150.0 c	8.5 b	14.3 c	488.0 a	206.3 ab	14.0 a
G (11–14)	35.9 c	137.5 c	546.2 ab	6.7 b	21.6 bc	93.2 a	490.7 ab	5.5 b
M1–M2 (5–10)	293.7 a	756.9 a	284.2 bc	17.2 a	105.1 a	630.9a	105.4 b	8.8 ab
M2–G (8–14)	36.5 c	622.6 ab	627.1 ab	8.2 b	33.5 bc	627.8 a	541.2 ab	7.7 ab
M1–G (5–14)	285.4 ab	757.9 a	692.3 a	6.0 b	94.9 a	645.1 a	726.9 a	11.8 a

[a]Ethylene was measured after a 6-h incubation, twice weekly, by headspace analysis.

[b]Means in a column followed by the same letter are not significantly different ($p < 0.05$) using Tukey's Studentized Range Test.

[c]I, initiation medium; M1, first 3 weeks of maintenance medium; M2, second 3 weeks of maintenance medium; G, regeneration medium.

reported in other barley tissue culture experiments (Dahleen 1999; Sharma et al. 2005). Growth of the donor plants in controlled conditions of a growth chamber might yield more clear-cut results; unfortunately, such space is often limited for experiments requiring many donor plants.

Different barley genotypes (Golden Promise, Morex, Steptoe, DH-12, DH-20, and Conlon) produce varying amounts of ethylene during in vitro culture (Fig. 1). Cho and Kasha (1989) determined that differences in callus and embryo production from anthers of barley cultivars were due to differing capacities to produce ethylene and sensitivity to higher levels of ethylene. The present results showed Golden Promise was the highest ethylene producer and the best green plant regenerator, while Morex and DH-20 had the lowest ethylene production and minimal green plant regeneration. Bregitzer (1992) tested 15 barley genotypes and observed that Golden Promise gave the highest plant regeneration, while Morex was recalcitrant and regenerated few plants. A high correlation ($r = 0.91$) was observed between ethylene production during the first 3 weeks of maintenance and green plant regeneration ($r = 0.77$ between cumulative ethylene production and green plant regeneration), so in vitro manipulation of ethylene action can improve the green plant regeneration in barley. The development and use of ethylene-optimized media for improved regeneration from specific genotypes will facilitate genetic manipulation of additional recalcitrant cultivars. Other factors that have improved green plant regeneration from elite barley lines include the use of smaller immature embryos (Chang et al. 2003) and optimized media culturing protocols (Dahleen and Bregitzer 2002). Dahleen (1995) showed that optimal concentrations of copper and 2,4-D were genotype specific and suggested the development and use of genotype-specific protocols for improved regeneration.

Manipulation of ethylene synthesis and action is possible in vitro by the application of ACC, an ethylene precursor, and $AgNO_3$, an ethylene antagonist. ACC is the immediate precursor of ethylene biosynthesis in higher plants, often acting as the rate-limiting factor (Bleecker and Kende 2000). Silver compounds (Ag^+) non-competitively block ethylene perception by binding to ethylene receptors and altering their conformation, so ethylene cannot bind to the receptor, and initiate an ethylene response (Beyer 1976). $AgNO_3$ promotes shoot regeneration in tissue cultures of two wheat cultivars and a number of *Nicotiana plumbaginofolia* mutants (Purnhauser et al. 1987). Exogenous ethylene reduces shoot regeneration in these genotypes. The addition of 17.6 μM $AgNO_3$ to culture media enhances shoot elongation in lemon (Kotsias and Roussos 2001), but inhibits both germination and seedling growth in barley (Locke et al. 2000) at 75 μM. In *Coffee canephora* L., $AgNO_3$ (30–60 μM) increases regeneration, while higher doses (≥ 60 μM) reduce regeneration (Fuentes et al. 2000). Turhan (2004) found a genotypic-dependent response to $AgNO_3$ on in vitro shoot development in potato (*Solanum tuberosum* L.).

Cho and Kasha's (1989) experiments on barley anther culture showed that embryogenesis from pollen grains was stimulated by ethylene promoters (ACC or ethrel) or an ethylene production inhibitor (putrescine) depending upon genotype. Cultivars with low endogenous ACC produced ethylene slowly and had low rates of embryogenesis. These cultivars showed increased embryogenesis when ACC was incorporated into the media. High ethylene-producing cultivars showed improved embryogenesis when ethylene production was inhibited by putrescine. We saw similar effects with Golden Promise, a high ethylene producer, which showed significantly increased regeneration when $AgNO_3$ was added during weeks 8–14. Morex, which had lower ethylene production, showed a more complex response and had significantly increased regeneration with decreased ethylene during weeks 5–10.

The developmental context in which hormones act has a large influence on their synthesis (Chow and McCourt 2003). Ethylene appears to coordinate cell growth and development during tissue culture in barley in a highly regulated manner. In this study, ethylene greatly affected the green plant re-

generation at certain critical stages of tissue culture. When $AgNO_3$ was applied to Golden Promise cultures throughout maintenance and regeneration (Table 3) only 3.3 plants were regenerated on average, one-sixth the level of the control, although not significantly different. Regeneration from Morex also was low with this treatment. Thus, the inhibition of ethylene effects throughout tissue culture can be detrimental to callus growth and green plant regeneration. In maize, ACC and $AgNO_3$ were used throughout the culturing process (Songstad et al. 1988). Evans and Batty (1994) added ACC and $AgNO_3$ during induction and regeneration media for barley anther culture. In experiments with shoot regeneration of wheat and *Nicotiana plumbaginofolia*, $AgNO_3$ also was added throughout the culturing process (Purnhauser et al. 1987). These long-term exposures may mask the effects of ethylene in specific tissue culture steps.

This study also indicates that ethylene levels are critical during maintenance and growth stages in callus tissue. Inhibition of ethylene action during all 6 weeks of maintenance (weeks 5–10) or the last 3 weeks of maintenance through regeneration (weeks 8–14) for Golden Promise increased the green plant regeneration (Table 3), whereas increased ethylene production during tissue culture reduced the regeneration (Table 4). This indicates that Golden Promise is likely to produce supra-optimal amounts of ethylene, and further addition of ethylene reduces regeneration. The mixed responses of Morex to ACC and $AgNO_3$ indicates there may be a critical amount of ethylene required at certain stages for improved regeneration. Organ development begins during the second maintenance stage and continues on regeneration medium as the roots and shoots starts to appear, so ethylene may be more important in callus tissue development and maintenance. At this point, it is not known whether blocking action or increasing ethylene production throughout the critical stage(s) or only a brief exposure is needed for optimum growth. Further research should be carried out to determine the optimal amount and exposure time of ethylene during crucial tissue culture stages.

References

Abeles FB, Morgan PW, Saltveit ME Jr. (1992) Ethylene in plant biology, 2nd edn. Academic, San Diego, CA

Beyer EM (1976) A potent inhibitor of ethylene action in plants. Plant Physiol 58:268–271

Biddington NL (1992) The influence of ethylene in plant tissue culture. Plant Growth Regulation 11:173–187

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–18

Bregitzer P (1992) Plant regeneration and callus type in barley: effects of genotype and culture medium. Crop Sci 32:1108–1112

Bregitzer P, Dahleen LS, Campbell RD (1998) Enhancement of plant regeneration from embryogenic callus of commercial barley cultivars. Plant Cell Rep 17:941–945

Chang Y, Zitzewitz V, Hayes PM, Chen THH (2003) High frequency plant regeneration from immature embryos of an elite barley cultivar. Plant Cell Rep 21:733–738

Cho UH, Kasha KJ (1989) Ethylene production and embryogenesis from anther cultures of barley. Plant Cell Rep 8:415–417

Chow B, McCourt P (2003) Hormone signaling from a developmental context. J Exp Bot 55:247–251

Dahleen LS (1995) Improved plant regeneration from barley callus cultures by increased copper levels. Plant Cell Tiss Org Cult 43:267–269

Dahleen LS (1999) Donor-plant environment effects on regeneration from barley embryo-derived callus. Crop Sci 39:682–685

Dahleen LS, Bregitzer P (2002) An improved media system for high regeneration rates from barley immature embryo derived callus cultures of commercial cultivars. Crop Sci 42:934–938

Evans JM, Batty NP (1994) Ethylene precursors and antagonists increase embryogenesis of barley anther culture. Plant Cell Rep 13:676–678

Fuentes SRL, Calheiros BP, Manetti-Filho J, Vieira GE (2000) The effects of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffee canephora*. Plant Cell Tiss Org Cult 60:5–13

Goldstein CS, Kronstad WE (1986) Tissue culture and plant regeneration from immature embryo explants of barley, *Hordeum vulgare*. Theor Appl Genet 71:631–636

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kotsias D, Roussos PA (2001) An investigation on the effect of different plant growth regulating compounds in in vitro shoot tip and node culture of lemon seedlings. Sci Horticult 89:115–128

Locke JM, Bryce JH, Morris PC (2000) Contrasting effects of ethylene perception and biosynthesis inhibitors on germination and seedling growth of barley. J Exp Bot 51:1843–1849

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 15:473–497

Purnhauser L, Medgyesy P, Czako M, Dix PH, Marton L (1987) Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginofolia* tissue cultures using the ethylene inhibitor $AgNO_3$. Plant Cell Rep 6:1–4

Reid MS (1995) Ethylene in plant growth, development, and senescence. In: Davis PJ (ed) Plant hormones, 2nd edn. Kluwer, Dordrecht, The Netherlands, pp 486–508

SAS Institute Inc. (1999) SAS/STAT User's Guide, version 7–1. SAS Institute Inc., Cary, North Carolina

Sharma VK, Hansch R, Mendel RR, Schulze J (2005) Seasonal effect on tissue culture responses and plant regeneration frequency from non-bombarded and bombarded immature scutella of barley (*Hordeum vulgare*) harvested from controlled environment. Plant Cell Tiss Organ Cult 81:19–26

Songstad DD, Duncan DR, Widholm JM (1988) Effect of 1-aminocyclopropane-1-carboxylic acid, silver nitrate, and norbornadiene on plant regeneration from maize callus cultures. Plant Cell Rep 7:262–265

Suttle JC, Kende H (1978) Ethylene and senescence in petals of tradescantia. Plant Physiol 62:267–271

Turhan H (2004) The effect of silver nitrate on in vitro shoot development in potato. Biotechnology 3:72–74

Vain P, Yean H, Flament P (1989) Enhancement and regeneration of embryogenic type II callus in *Zea mays* L. by AgNO3. Plant Cell Tiss Org Cult 18:143–151