CSIRO Publishing

Australian Journal of Plant Physiology

An International Journal of Plant Function

Volume 28, 2001

All enquiries and manuscripts should be directed to:

Australian Journal of Plant Physiology
CSIRO Publishing
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7625
Fax: +61 3 9662 7611
Email: ajpp@publish.csiro.au

Published by CSIRO Publishing
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/ajpp

Aust. J. Plant Physiol., 2001, **28**, 1243–1247

Enhanced shoot regeneration in nine Australian wheat cultivars by spermidine and water stress treatments

Harjeet K. Khanna[A] *and Grant E. Daggard*

Department of Biological and Physical Sciences, University of Southern Queensland, Qld 4350, Australia.
[A]Corresponding author; email: khanna@usq.edu.au

Abstract. The regeneration potential of ageing calli initiated from isolated scutella of immature embryos was increased in nine elite Australian cultivars (QT7208, QT9685, QT7709, Kennedy, Lang, Sunvale, Giles, Petrie and Veery) of wheat (*Triticum aestivum* L.). Firstly, the effects of 4–32 h of dehydration stress on regeneration of 4- to 20-week old calli were evaluated. Cultivars such as Veery, Kennedy and Sunvale showed significant improvement in regeneration from calli up to 12-weeks old that had undergone 16 h of dehydration stress. Secondly, 4- to 20-week old callus cultures were treated with 0.05–5 mM spermidine to evaluate its effect on regeneration. While spermidine had a negative effect on regeneration from 4-week old calli at all tested concentrations (as compared with untreated controls), there was a 3–50% improvement in the regeneration ability of older calli (16- to 20-week old) of all cultivars. Finally, exogenous application of 1 mM spermidine to 16-week old cultures, in combination with 16 h dehydration stress, improved plant regeneration by 10–65% in all nine cultivars.

Keywords: regeneration, spermidine, tissue culture, water stress, wheat.

Introduction

Wheat is Australia's most important grain crop. Australia contributes between 8 and 15% of world trade, making it the fourth largest exporter after the United States, Canada and the European Union. Australian wheat is recognized throughout the world for its consistently high quality. Because of the importance of wheat as one of the worlds major food crops, numerous laboratories have developed wheat transformation protocols using biolistics (Weeks *et al.* 1993; Becker *et al.* 1994; Nehra *et al.* 1994; Altpeter *et al.* 1996; Chibbar *et al.* 1998; Bliffeld *et al.* 1999), electroporation (He and Lazzeri 1998), and more recently an *Agrobacterium tumefaciens*-mediated approach (Cheng *et al.* 1997). However, recovery of transformants has remained at around 1% of the number of scutella bombarded, and 4% using *Agrobacterium*.

Variation of tissue culture media, culture protocols, and selection systems has failed to enhance the transformation efficiency. This may not necessarily be because of the limitation of plasmid vectors or the efficiency of transformation systems, but because regeneration from calli more than 4-weeks old remains low. Selection following transformation demands that cultures be maintained in callus phase for at least 6–8 weeks, but the loss of morphogenic potential with increasing age of calli derived from almost all sources, prevents high frequency recovery of transformants. Immature embryos, and calli from immature embryos, remain the best explant source for transformation of cereals, but loss of regeneration potential following long periods of dedifferentiation has been one of the major factors limiting transformation frequencies in most wheat cultivars (H. K. Khanna and G. E. Daggard, unpublished data). Therefore, efficient regeneration from secondary and tertiary level calli is a critical step toward obtaining an optimal transformation system for wheat.

With this objective in mind, we have been developing plant regeneration protocols for some elite Australian wheat cultivars, namely QT7208, QT9685, QT7709, Kennedy, Lang, Sunvale, Giles, Petrie and Veery. We have been developing alternative approaches for enhancement of plant regeneration and subsequently, improvement of transformation efficiencies in these cultivars.

Several stress conditions that have been implicated in the improvement of plant regeneration in rice, may be suitable for manipulation of callus in wheat. For example, water stress has been reported to stimulate regeneration from rice cultures (Jain *et al.* 1996). Exogenous application of spermidine has also been implicated in somatic embryogenesis of celery and carrot (Robie *et al.* 1989; Danin *et al.* 1993). Bajaj and Rajam (1995; 1996) have reported the effect of polyamines like spermidine and putrescine on promotion of plant regeneration via somatic embryogenesis in long-term callus cultures of rice. The importance of cellular polyamines, and the ratio of putrescine to spermidine, to regeneration ability in several commercially grown rice varieties has also been reported recently (Shoeb *et al.* 2001).

In this report we describe a comparison of regeneration experiments, carried out over a period of 8 months, using

 10.1071/PP01115 0310-7841/01/121243

scutella isolated from immature embryos. The objective of this investigation is to examine the effects of dehydration stress, as well as exogenous application of spermidine, both in isolation and in combination with dehydration stress, on regeneration from 4- to 20-week old scutellar calli induced from immature embryos.

Materials and methods

Plant material

Seeds of *Triticum aestivum* L. cvv. QT7208, QT9685, QT7709, Kennedy, Lang, Sunvale, Giles, and Petrie were obtained from Dr Phil Banks at Leslie Wheat Research Institute, Toowoomba, Qld, Australia. Seeds of cultivar Veery were kindly provided by Dr Keith Gatford from the University of Adelaide Waite Campus, Glen Osmond, SA, Australia. Seeds were sown in peat and sand (1:1) potting compost in 8-inch pots in the glasshouse. Pots were fertilized with Osmocote® (Scotts PBG, Marysville, OH, USA) at 2-week intervals. Temperature in the glasshouse varied between 12–26°C, with a 10–14 h photoperiod.

Isolation and culture of explants

Developing caryopses were harvested 14–18 d post-anthesis. Grains were surface-sterilized with 10% Domestos® [Lever Rexona, Sydney, Australia (active ingredients: 5.25% w/v sodium hypochlorite, 1.25% w/v sodium hydroxide, 0.5 g L^{-1} 'alkaline salts')] for 20 min and washed five times with sterile distilled water. Immature embryos were excised from the grain under the dissecting microscope and embryonic axes were carefully removed (Nehra *et al.* 1994). Isolated scutella were placed scutellum up in 90-mm petri dishes containing 25 mL solidified agar medium. Details of media are given in Table 1. Petri dishes were sealed with Parafilm® (Structure Probe Inc., West Chester, PA, USA) and incubated at 24–26°C in the dark for 4 weeks.

Culture treatments

Normal culture

Control treatment 'a–4' for each cultivar consisted of culturing the scutella on media A (see Table 1 for details) for 4 weeks before transfer to media B for regeneration. Control treatments 'a–8' to 'a–20' consisted of subculturing the 4-week old scutella once every 4 weeks, and transferring them to media B after one to four subcultures. So, 'a–8', 'a–12', 'a–16' and 'a–20' cultures were 8-, 12-, 16-, and 20-weeks old, respectively, at the time of transfer to regeneration media (and had been subjected to one, two, three or four cycles of subculture on media A at 4-week intervals, respectively). Thirty-five calli were cultured per dish. Three dishes were used per treatment, and each treatment was replicated three times.

Table 1. Culture media

2,4D, 2,4-dichlorophenoxyacetic acid; BAP, benzylaminopurine; K, kinetin; NAA, napthaleneacetic acid; MS, Murashige and Skoog (1962)

Media	Composition (L^{-1})
A	2 × MS macro salts, 1 × MS micro salts, 1 × MS vitamins, 40 mg thiamine, 150 mg asparagines, 60 g sucrose, 2.5 mg 2,4D, 9 g agar, pH 5.8
B	1 × MS macro salts, 1 × MS micro salts, 1 × MS vitamins, 40 mg thiamine, 150 mg asparagines, 60 g sucrose, 2 mg BAP, 1 mg K, 1 mg NAA, 9 g agar, pH 5.8

Dehydration treatments

Treatments 'DT–1' to 'DT–5' consisted of subjecting 'a-4' to 'a–20' treated scutella to various durations of water stress (dehydration on 90-mm diameter sterile Whatman Paper # 121) before transfer to media B for regeneration. Results of the experiment are given in Table 2. Thirty-five calli were cultured per dish. Three dishes were used per treatment, and each treatment was replicated three times.

Spermidine treatment

Treatments 'SP–1' to 'SP–5' consisted of transferring 'a-4' to 'a–20' treated scutella to media B supplemented with different concentrations of filter-sterilized polyamine spermidine for regeneration. Results of the treatments are shown in Fig. 2. Thirty-five calli were cultured per dish. Three dishes were used per treatment, and each treatment was replicated three times. When transferred to media B for regeneration, all calli were kept in dim light for 2 weeks, and then exposed to 16 h photoperiod at 24–26°C for 6 weeks.

Data analysis

Total number of calli showing regeneration were counted, and percent regeneration frequency was calculated (number of calli showing regeneration/total number of calli × 100) and compared across treatments. Means of three replicates were compared using Fischer's LSD test, and are presented in Table 2.

Results and discussion

Plant regeneration from routine in vitro *culture*

Four-week old primary callus of all cultivars showed the best regeneration, with the response greatest for Giles (67%) and lowest for QT9685 (17%) (Fig. 1). At the 4-week stage, the calli were white to off-white in colour, compact and morphogenic, with well-formed somatic embryos that differentiated into green shoots. With age, the calli became yellowish and softer, and the organized structures dedifferentiated into a loose mass. By the time most calli reached 20-weeks of age, they had turned yellowish-brown and become non-morphogenic, with some even beginning to root. Regeneration frequency decreased significantly with increasing age in all nine cultivars tested. In 20-week old calli subcultured four times, the regeneration response dropped from 68 to 11% in Giles, and from 17 to 0% in

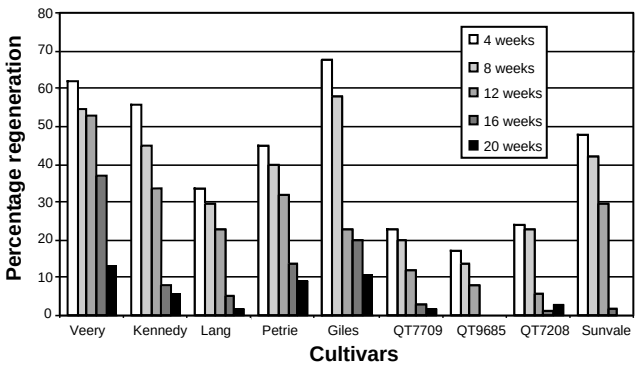

Fig. 1. Effect of the age of immature embryo-derived scutellar calli of nine wheat cultivars on plant regeneration (n = 105).

Table 2. Regeneration from immature embryo-derived scutellar calli of different ages, from nine wheat cultivars, subjected to dehydration stress for different periods

Callus age is measured at time of stress treatment. Each value is the mean ± SE based on three replicates. Values followed by different letters within rows and columns of a block representing a particular cultivar, are significantly different at $P<0.05$ using Fischer's LSD test ($n=105$)

Treatment code	Callus age (weeks)	Length of time callus was exposed to stress (h) Veery 4	Veery 8	Veery 16	Veery 32	Kennedy 4	Kennedy 8	Kennedy 16	Kennedy 32	Lang 4	Lang 8	Lang 16	Lang 32
DT-1	4	60 ± 3.6 c	59 ± 8.3 bc	78 ± 0.6 ab	64 ± 6.2 bc	40 ± 4.7 b	55 ± 5.1 a	52 ± 6.9 a	39 ± 2.2 b	36 ± 3.7 c	41 ± 4.7 b	57 ± 5.6 a	28 ± 1.5 de
DT-2	8	52 ± 5.2 de	84 ± 4.9 a	72 ± 2.1 b	68 ± 5.3 b	28 ± 3.2 c	38 ± 3.9 b	52 ± 4.2 a	19 ± 1.3 ef	34 ± 2.5 c	38 ± 4.9 bc	40 ± 3.6 b	42 ± 2.9 b
DT-3	12	51 ± 3.6 de	79 ± 5.7 a	70 ± 3.2 bc	59 ± 6.2 cd	20 ± 2.9 ef	22 ± 2.3 de	38 ± 2.1 b	25 ± 1.7 cd	28 ± 4.1 de	31 ± 6.2 cd	30 ± 2.3 d	22 ± 1.7 e
DT-4	16	43 ± 6.9 e	39 ± 7.2 e	43 ± 3.2 e	23 ± 4.9 f	5 ± 0.9 h	17 ± 1.1 f	23 ± 1.4 d	19 ± 0.7 ef	7 ± 3.9 gh	9 ± 1.3 gf	13 ± 1.3 fg	7 ± 0.4 gh
DT-5	20	12 ± 2.1 e	19 ± 2.1 g	20 ± 1.7 fg	7 ± 1.2 e	8 ± 0.6 h	11 ± 1.2 g	12 ± 0.8 g	6 ± 0.1 h	8 ± 1.9 g	3 ± 0.2 h	8 ± 0.4 g	5 ± 0.1 h

		Length of time callus was exposed to stress (h) Petrie 4	Petrie 8	Petrie 16	Petrie 32	Giles 4	Giles 8	Giles 16	Giles 32	QT7709 4	QT7709 8	QT7709 16	QT7709 32
DT-1	4	52 ± 1.2 b	50 ± 5.2 bc	68 ± 5.8 a	35 ± 4.2 e	66 ± 5.0 b	62 ± 7.6 cde	74 ± 5.4 a	37 ± 1.3 g	37 ± 5.8 b	39 ± 4.7 ab	46 ± 3.9 a	32 ± 4.2 bc
DT-2	8	43 ± 4.9 d	39 ± 5.0 de	43 ± 8.7 d	54 ± 4.2 b	58 ± 4.0 ef	59 ± 5.8 de	70 ± 2.2 ab	43 ± 4.3 g	25 ± 4.2 cd	28 ± 5.0 c	25 ± 4.6 d	12 ± 1.2 h
DT-3	12	31 ± 6.9 g	34 ± 4.2 ef	30 ± 6.0 g	48 ± 3.9 c	37 ± 2.8 g	62 ± 4.2 cde	53 ± 4.6 f	7 ± 1.9 k	23 ± 2.9 de	20 ± 5.8 fg	16 ± 2.1 h	9 ± 1.9 hi
DT-4	16	22 ± 1.7 h	32 ± 3.1 gf	20 ± 1.9 h	9 ± 1.9 ij	32 ± 1.5 h	16 ± 3.5 j	22 ± 5.0 i	2 ± 0.6 l	22 ± 4.1 def	18 ± 4.1fg	8 ± 0.2 i	4 ± 0.6 j
DT-5	20	13 ± 1.2 i	7 ± 1.2 j	11 ± 2.1 ij	3 ± 0.6 j	23 ± 6.2 i	8 ± 1.9 jk	12 ± 3.3 j	3 ± 0.2 l	7 ± 1.2 ij	9 ± 0.6 hi	7 ± 1.2 ij	2 ± 0.2 j

		Length of time callus was exposed to stress (h) QT9685 4	QT9685 8	QT9685 16	QT9685 32	QT7208 4	QT7208 8	QT7208 16	QT7208 32	Sunvale 4	Sunvale 8	Sunvale 16	Sunvale 32
DT-1	4	34 ± 4.2 b	42 ± 3.3 ab	51 ± 3.1 a	12 ± 1.2 de	34 ± 2.9 bc	32 ± 2.2 c	38 ± 2.1 ab	24 ± 5.0 d	56 ± 6.3 ab	51 ± 4.2 b	64 ± 5.8 a	26 ± 2.6 de
DT-2	8	23 ± 1.9 c	21 ± 1.8 c	18 ± 1.4 cd	7 ± 0.1 ef	43 ± 3.7 a	37 ± 2.7 abc	25 ± 1.7 d	11 ± 2.8	52 ± 4.2 b	47 ± 3.2 c	50 ± 4.2 b	24 ± 1.3 e
DT-3	12	18 ± 1.4 cd	17 ± 1.3 d	12 ± 1.1 de	0.0 f	12 ± 1.3 e	8 ± 0.1 efg	10 ± 0.2 e	2 ± 1.5 gh	49 ± 4.4 b	39 ± 2.6 cd	58 ± 7.6 a	15 ± 3.2 f
DT-4	16	2 ± 0.1 f	1 ± 0.1 f	0.0 f	0.0 f	9 ± 0.1 ef	3 ± 0.1 gh	4 ± 0.1 gh	0.0 h	0.0 g	5 ± 0.5 g	0.0 g	3 ± 1.2 g
ST-5	20	0.0 f	0.0 f	0.0 f	0.0 f	0.0 h	0.0 h	3 ± 1.0 gh	0.0 h	0.0 g	2 ± 1.0 g	0.0 g	0.0 g

QT9685. Other cultivars showed a similar trend in regeneration (Fig. 1). In fact, QT9685 showed no regeneration after 12 weeks.

Plant regeneration from dehydrated callus

Four-week old cultures exhibited significant enhancement of the regeneration response after up to 16-h water stress in all cultivars. Calli subjected to dehydration showed enhanced maturation of pre-existing somatic embryos, rather than callus proliferation. With the exception of Veery, Kennedy and Giles, no cultivar showed any significant regeneration enhancement following dehydration stress, once the calli had passed 4 weeks of age. In fact, dehydration stress caused a drop in regeneration in older calli of most cultivars. The older calli lacked any organized structures that could be induced to start morphogenesis. Only calli of the Kennedy cultivar continued to respond positively to 16 h of dehydration stress until the age of 16 weeks (Table 2). In almost all 4-week old cultures, shoot proliferation started at least 4 d earlier following 4–16 h of stress treatment, as compared with non-stressed cultures.

Continuing dehydration beyond 16 h had a negative effect on all calli of all cultivars. Shoot regeneration was highly reduced in cultures of all ages and cultivars stressed for 32 h (Table 2). Jain *et al* (1996) made similar observations for suspension cultures of rice in which partial desiccation of calli was found to increase shoot regeneration 3-fold.

Plant regeneration after spermidine treatment

The effect of exogenous spermidine on plant regeneration from 4- to 20-week old cultures of all cultivars is summarized in Figs 2*a–e*. When treated with spermidine at concentrations greater than 0.05 mM, younger calli (4 weeks of age) tended to turn soft and watery, with the somatic embryos slowly dedifferentiating into a disorganized mass. At a concentration of 0.05 mM, spermidine had no significant effect on regeneration from 4-week old cultures (Fig. 2*a*), but above this concentration, there was significant reduction of regeneration in all cultivars. With increased culture age, the effect of spermidine on regeneration was positive in all cultivars, up to a concentration of 1 mM — ranked in order of most positive to least positive regeneration response as follows: Veery, Petrie, Giles, Kennedy, Lang, QT7709, QT7208, QT9685, and Sunvale. At a spermidine concentration of 1 mM, 20-week old cultures of all cultivars exhibited higher regeneration compared with cultures not treated with spermidine (Fig. 2*e*). When treated with 1 mM spermidine, older calli tended to turn whiter in colour, become compact and organized, and show signs of improved embryogenesis.

A clear difference in shoot regeneration was also observed when examining the percent increase in regeneration for 12- and 16-week old cultures treated with spermidine. Eight-week old cultures appeared to show an improvement in shoot regeneration in all cases (except

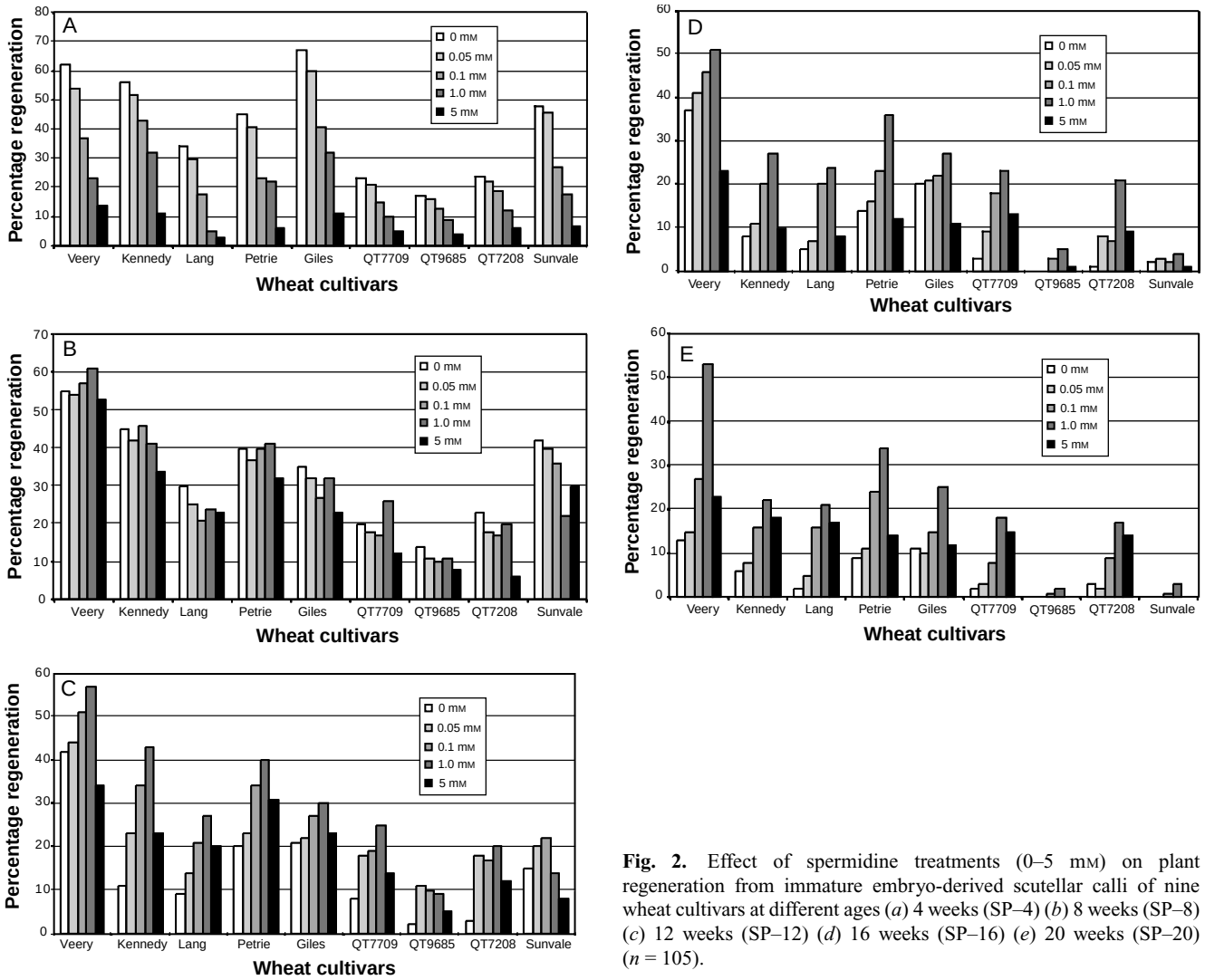

Fig. 2. Effect of spermidine treatments (0–5 mM) on plant regeneration from immature embryo-derived scutellar calli of nine wheat cultivars at different ages (*a*) 4 weeks (SP–4) (*b*) 8 weeks (SP–8) (*c*) 12 weeks (SP–12) (*d*) 16 weeks (SP–16) (*e*) 20 weeks (SP–20) (n = 105).

Sunvale), but this improvement was not statistically significant (P>0.05). Early initiation of shoot bud formation was observed to occur, as compared with untreated controls (data not shown).

When 16-week old cultures were subjected to dehydration for 16 h, before treatment with 1 mM spermidine, a very significant improvement (P<0.05) in regeneration was observed across all cultivars tested, including Sunvale and QT9685 (Fig. 3), as compared with 16-week old calli treated with 1 mM spermidine alone (Figs 2*c*, 3).

Bajaj and Rajam (1995; 1996) have reported similar observations, made using long-term rice callus cultures. Three-week old rice cultures showed a drop in regeneration from 76 to 28% as they aged to 12 months. These authors found a reduction in regeneration frequency with fresh cultures, even at a spermidine concentration of 0.1 mM, whereas 3- to 6-month old callus cultures exhibited the same level of regeneration as fresh 3-week old cultures without any spermidine treatment. The results indicated that this was caused by increased putrescine to spermidine ratios, as the calli advanced in age. Analysis of polyamine levels in freshly-induced and older calli has shown that older calli accumulate more putrescine than spermidine, leading to an increased putrescine : spermidine ratio. Exogenous addition of spermidine, that brought the ratio closer to that found in fresh cultures, improved the regeneration potential of older calli. In a recent study, Shoeb *et.al*. (2001) have shown that rice cultivars with a putrescine : spermidine ratio of 2.3 respond much better in culture than cultivars with a ratio of 3.8 or higher. They have also shown that favourable modification of cellular polyamine titers and their putrescine : spermidine ratio [either by addition of exogenous polyamines, or the biosynthetic inhibitor of putrescine, difluoromethylarginine (DFMA)] led to induction and/or promotion of plant regeneration in poorly-responding genotypes. DFMA at a concentration of

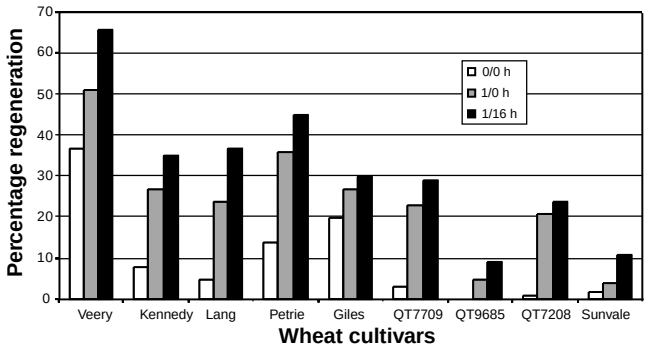

Fig. 3. Effect of 1 mM spermidine treatment and 16 h dehydration stress on plant regeneration from 16-week old immature embryo-derived scutellar calli of nine wheat cultivars (n = 105). Key: value before slash indicates duration of spermidine treatment; value following slash indicates duration of dehydration treatment.

0.5 mM caused a considerable decrease in the putrescine content of all rice genotypes, but did not affect conversion of putrescine to spermidine, thus resulting in favourable polyamine ratios that improved regeneration.

This could explain why fresh wheat callus is negatively responsive to spermidine treatment as compared with older callus. Fresh callus that has the correct ratio of polyamines regenerates to its maximum potential. Addition of spermidine to these cultures disturbs the balance and regeneration drops. But in long-term cultures, as the putrescine level increases, an exogenous supply of spermidine might help restore the balance. However, increasing spermidine concentration beyond a certain limit had an adverse effect, as it may have decreased the putrescine : spermidine ratio below an optimal level, thereby reducing regeneration. In our studies using older calli, the ratios of putrescine to spermidine might have been restored by exogenously supplied spermidine, resulting in enhanced shoot regeneration. Bagni and Mengoli (1985) reported that addition of putrescine inhibitors could cause an increase in the rate of conversion of somatic embryos into plantlets in carrot. Altering the putrescine : spermidine ratio by addition of spermidine was also found to enhance plantlet formation from somatic cultures of celery (Danin *et al.* 1993).

In conclusion, regeneration from older wheat calli could be significantly improved by subjecting the cultures to either water stress before transfer to regeneration medium, or by supplementing the regeneration medium with optimal levels of spermidine. The optimal level of spermidine may vary between cultivars, but seems to be in the range of 0.1–1 mM. A detailed analysis of the cellular polyamine titers of the cultivars, and measurement of ratios of spermidine to putrescine in the calli at different ages, would lead to a better understanding of why some wheat genotypes respond poorly in tissue culture. It would also help determine the optimal polyamine ratios needed for greater morphogenesis in wheat.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Grain Research Development Corporation of Australia, and the facilities provided by University of Southern Queensland.

References

Altpeter F, Vasil V, Srivastava V, Stöger E, Vasil IK (1996) Accelerated production of transgenic wheat (*Triticum aestivum* L.) plants. *Plant Cell Reports* **16**, 12–17.

Bagni N, Mengoli M (1985) Characterization of a carrot callus line resistant to high concentrations of putrescine. *Plant Growth Regulation* **3**, 371–380.

Bajaj S, Rajam MV (1995) Efficient plant regeneration from long-term callus cultures of rice by spermidine. *Plant Cell Reports* **14**, 717–720.

Bajaj S, Rajam MV (1996) Polyamine accumulation and near loss of morphogenesis in long-term callus cultures of rice: restoration of plant regeneration by manipulation of cellular polyamine levels. *Plant Physiology* **112**, 1343–1348.

Becker D, Brettschneider R, Lörz H (1994) Fertile transgenic wheat from microprojectile bombardment of scutellar tissue. *The Plant Journal* **5**, 299–307.

Bliffeld M, Mundy J, Potrykus I, Fütterer J (1999) Genetic engineering of wheat for increased resistance to powdery mildew disease. *Theoretical and Applied Genetics* **98**, 1079–1086.

Cheng M, Fry JE, Pang S, Zhou H, Hironaka CM, Duncan DR, Corner TW, Wan YC (1997) Genetic transformation of wheat mediated by *Agrobacterium tumefaciens*. *Plant Physiology* **115**, 971–980.

Chibbar RN, Baga M, Caswell K, Repellin A, Leung N, Abdel Aal E, Hucl P (1998) Genetic transformation strategies to alter starch structure in wheat. In 'Proceedings of the 9th international wheat genetics symposium'. Saskatoon. pp. 167–170. (University of Saskatchewan, Saskatoon, Canada)

Danin M, Upfold SJ, Levin N, Nadel BL, Altman A, Van Staden J (1993) Polyamines and cytokinins in celery embryogenic cell cultures. *Plant Growth Regulation* **12**, 245–254.

He GY, Lazzeri PA (1998) Analysis and optimisation of DNA delivery into wheat scutellum and Tritordeum inflorescence explants by tissue electroporation. *Plant Cell Reports* **18**, 64–70.

Jain RK, Jain S, Wu R (1996) Stimulatory effect of water stress on plant regeneration in aromatic Indica rice varieties. *Plant Cell Reports* **15**, 449–454.

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

Nehra NS, Chibbar RN, Leung N, Caswell K, Mallard C, Steinhauer L, Baga M, Kartha KK (1994) Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. *The Plant Journal* **5**, 285–297.

Robie CA, Minocha SC (1989) Polyamines and somatic embryogenesis in carrot I. Effect of difluoromethylornithine and difluoromethylarginine. *Plant Science* **65**, 45–54.

Shoeb F, Yadav JS, Bajaj S, Rajam MV (2001) Polyamines as biomarkers for plant regeneration capacity: improvement of regeneration by modulation of polyamine metabolism in different genotypes of indica rice. *Plant Science* **160**, 1229–1235.

Weeks JT, Anderson OD, Blechl AE (1993) Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). *Plant Physiology* **102**, 1077–1084.

Manuscript received 27 April 2001, accepted 12 July 2001.