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ORIGINAL PAPER

Microspore-derived cell suspension cultures of oilseed rape as a system for studying gene expression

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Abstract Abiotic stress, such as extreme temperature, drought, or excessive salinity, is one of the leading causes of crop loss worldwide. Microspore-derived (MD) cell suspension cultures of *Brassica napus* L. cv. Jet Neuf have been shown to be a useful system for studying the biochemistry of developing oilseeds. In the present study, we describe the application of MD cell suspension cultures of *B. napus* as a system for studying gene expression in response to abiotic stress, and demonstrate emybryogenic competence in cultures that have been continuously subcultured for more than 20 years. MD cell suspension cultures of *B. napus* L. cv Jet Neuf were exposed to low temperature or osmotic stress and the expression profile of known stress responsive genes was evaluated. The gene expression profile of *BN115*, a known cold-responsive gene in *B. napus*, was similar to that described for intact cold-acclimated plants. Likewise, two late embryogenesis abundant (*Lea*) genes were shown to be up-regulated in response to low temperature or osmotic stress. The results demonstrate that *B. napus* MD cell suspension cultures are a useful system for the investigation of changes in gene expression in plants brought about by abiotic stress.

Keywords Somatic embryogenesis · Regeneration · Long-term culture · Low-temperature · Osmotic stress · *Brassia* · Quantitative real time RT-PCR

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Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
BA	6-Benzylaminopurine
CRBC	Chicken red blood cells
CTR	Control
LT	Low temperature
MD	Microspore-derived

NAA α-Naphthaleneacetic acid
PEG Polyethylene glycol
PI Propidium iodide

Introduction

Oilseed rape (*Brassica napus* and *B. rapa*) has become Canada's second most valuable field crop and the third most important annual crop for vegetable oil production in the world (Canola Council of Canada 2006). *B. napus* (AACC, 2n = 38) is an amphidiploid species resulting from hybridization between *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18) (Morinaga 1934; UN 1935). Plant biotechnology has played a key role in the genetic modification of this crop, and microspore-derived (MD) cultures have proven to be a useful tool not only for breeding applications but also for fundamental studies in plant physiology, biochemistry and molecular biology (Weselake and Taylor 1999; Weselake 2005). Cell suspension cultures are convenient sources for selection of somaclonal variants with desirable characteristics (Gonzales 1994). The major concern when using cell culture systems for crop genetic modification is the tendency of cultures to lose embryogenic and organogenic competence over long-term culture (Bajaj and Rajam 1996; Ozeki et al. 1997). Morphologically uniform cells do, however, represent an experimental system for the study of mechanisms of responses to various abiotic stresses in plants (Pedras and Biesenthal 2000; Egierszdorff and Kacperska 2001; Gu et al. 2004).

MD cell suspension cultures of *B. napus* L. cv. Jet Neuf have been used in studies involving freezing tolerance (Orr et al. 1986; Johnson-Flanagan and Singh 1987; Johnson-Flanagan et al. 1991), mutant selection (Saxena et al. 1990), nutritional physiology (Moraes and Plaxton 2000) and storage lipid synthesis (Furukawa-Stoffer et al. 1998; Byers et al. 1999; Weselake and Taylor 1999; Furukawa-Stoffer et al. 2003). The cells were originally generated in 1983 during an attempt to induce androgenesis in microspores of *B. napus* (Simmonds et al. 1991). The cells have been sub-cultured in our laboratory on a bi-weekly basis since 1990. In this study, we report on the successful production of embryos from the MD cell suspensions cultures, which to our knowledge is the first report on embryogenesis from cells maintained for over 20 years through sub-culture. We further demonstrate that these extensively sub-cultured cells exhibit gene expression responses to abiotic stress that are characteristic of whole plants.

Materials and methods

Plant materials and treatments

MD cell suspensions of oilseed rape (*B. napus* L. cv Jet Neuf) were maintained on a rotary shaker (160 rpm) at 20°C under constant fluorescent light with an intensity of 40 μmol m^{-2} s^{-1}. NLN media (pH 6.0, containing 6.5% sucrose, 30 mg l^{-1} glutathione, 800 mg l^{-1} glutamine, 100 mg l^{-1} L-serine, 0.5 mg l^{-1} α-naphthaleneacetic acid (NAA), 0.05 mg l^{-1} 6-benzylaminopurine (BA) and 0.5 mg l^{-1} 2,4-D) was prepared according to Orr et al. (Orr et al. 1986). At 2-week intervals, one third of the mass of cells grown in 125 ml flasks was transferred to 50 ml of fresh NLN medium. On the tenth day of growth, cells were exposed to either cold or osmotic stress. For cold treatment, cells were placed in a growth cabinet set at 5°C. For application of osmotic stress, polyethylene glycol (PEG) 4000 (prepared in NLN medium) was added to the cell suspensions to a final concentration of 20% (w/v). An equal volume of fresh NLN media was added to control cells to account for any media replenishment effect. For both experiments, challenged cells and corresponding control cells were harvested by filtration over a nylon mesh at 0, 6, 12, 24, 48 and 72 h post treatment, flash frozen in liquid nitrogen and stored at −80°C until further use. Each experiment was independently repeated three times.

RNA extraction and reverse transcription

Approximately 100 mg of frozen cells collected from the abiotic stress experiments were used for RNA extraction. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, CA, US), according to the manufacturer's instructions. Genomic DNA contamination was removed by treatment with RNase-free DNase (Qiagen), and the RNA was quantified with a NanoDrop ND-1000™ spectrophotometer (NanoDrop Technologies, Inc. DE, USA). To

check the structural integrity of the RNA preparations, Agilent 2100 Bioanalyzer and RNA 6000 Nano kit (Agilent Technologies, Waldbronn, Germany) were used, following the manufacturer's protocols.

First strand cDNA synthesis from total RNA was carried out using the SuperScript III First Strand Synthesis System for RT-PCR (Invitrogen, CA, USA). Approximately 1.5 μg RNA was reverse-transcribed into cDNA in a total volume of 20 μl. The DNA-free RNAs were denatured at 65°C for 5 min in the presence of oligo-d(T) primer and dNTP mix, and cooled on ice before adding SuperScript III Reverse Transcriptase. The reaction was conducted at 50°C for 50 min and terminated at 85°C for 5 min. RNA in the mix was then removed by adding 1 μl of RNase H and incubating for 20 min at 37°C. The cDNA were then stored at −20°C and used as template for the quantification of target gene expression levels.

Quantitative real-time RT-PCR (qRT-PCR)

Real time RT-PCR assay was performed with gene-specific primers and TaqMan™ probes designed according to the deposited sequences of genes to be analyzed from Genbank using Primer Express™ Software 3.0 (Applied Biosystems, California, USA) as described in the user's manual (Table 1). Primers and probes labeled with VIC or FAM were synthesized by Applied Biosystems. The constitutively expressed actin gene of *B. napus*, used as the internal reference, was amplified in parallel with the target genes. PCR amplification was carried out on an Applied Biosystems 7900HT Fast Real-Time PCR System with TaqMan™ MGB chemistry. PCR conditions comprised an initial cycle of 50°C for 2 min, a hot-start activation step of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. No-template controls were also included to ensure no carryover contamination occurred in the experiment.

Statistical analysis was conducted using the Sequence Detection Systems Software version 2.2.2 (Applied Biosystems). The threshold cycle (C_t) values of the duplicate reactions were averaged and used for quantification of gene expression levels. Relative quantification of transcripts was achieved using $\Delta\Delta C_t$ method (Ali-Benali et al. 2005). The induction ratio of target gene against the reference gene (actin) was determined with $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ is:

$$(Ct_{Target} - Ct_{Actin})_{Treatment} - (Ct_{Target} - Ct_{Actin})_{Control}.$$

Estimation of ploidy level

Ploidy levels of the cells were estimated by flow cytometry following the method of Arumuganathan and Earle (Arumuganathan and Earle 1991), with minor modifications. Four days after sub-culture, cells were collected by centrifugation (10 min at 3,000 rpm) and re-suspended in 500 μl chopping buffer (45 mM $MgCl_2$, 30 mM sodium citrate, 20 mM MOPS, 0.1% (w/v) Triton X-100; pH 7.0). The cells were chopped for 10 min with a sharp razor blade to release the nuclei. The homogenate was then filtered through a 40 μm nylon mesh to remove debris, followed by centrifugation at 17,000×*g* for 20 s. The resulting pellet was re-suspended in 400 μl chopping buffer. The nuclei were stained by adding propidium iodide (PI; 100 μg ml^{-1} in H_2O) to a final concentration of 10 μg ml^{-1}. Samples were analyzed using a FACScan flow cytometer (Becton Dickinson, Sunnyvale CA) with a laser-emission wavelength of 488 nm and a minimum of 5,000 events were captured. Fresh CRBC were used as an internal standard (2.33 pg/nuclei). The nuclear content was calculated based on the fluorescence intensities of sample cells and CRBC, using the following formula: sample nuclear DNA content (pg) = (sample fluorescence intensity/CRBC fluorescence intensity) × 2.33.

Somatic embryogenesis

Cultured suspension cells were washed in MS liquid basal medium (Murashige and Skoog 1962) on a rotary shaker for 3–5 h. The cells were transferred into petri dishes containing B5 medium (Gamborg et al. 1968), supplemented with various concentrations of BA and NAA, 2.0% sucrose, 0.3 M mannitol and solidified with 0.8% agar. After 20 days of culture, the resulting calli were placed on solidified B5 medium with 2.0% sucrose. The resulting embryos were transferred onto MS or B5 media with

Table 1 Gene-specific primers and TaqMan™ probes used for qRT-PCR analyses

Gene	Accession number	Primers and probes (5′-3′)		Labelling	Product length (bp)
BN115	U14665	Forward	ATGTCACTCTCAGGATCAGC	FAM	62
		Reverse	GCTATGCCGCTGGAGAAA		
		Probe	TCATTGGGATTGGTTC		
LEA76	X15348	Forward	CCAACAAAGCTACAAAGCTGGTG	FAM	64
		Reverse	CATTGCTTGTCCTGTCTTCTCCT		
		Probe	AACCAGAGGCAAGACT		
ME-LeaN4	AB083361	Forward	CAAGCGCAAGACTCAGGAGAA	FAM	63
		Reverse	TCCTCAGCCTTGTCCCTCAT		
		Probe	AGGACAAGCAATGGG		
Actin	AF111812	Forward	TGGGTTTGCTGGTGACGAT	VIC	63
		Reverse	TGCCTAGGACGACCAACAATACT		
		Probe	CTCCCAGGGCTGTGTT		

Accession numbers refer to sequences in the NCBI Genbank database

or without plant growth regulators for further development and germination. All media were adjusted to pH 6.0 prior to autoclaving. Cultures were maintained at 20°C with a light intensity of 60 μmol m^{-2} s^{-1} under a 16 h photoperiod.

Results

Regulation of *BN115* gene expression by low temperature

To investigate the responses of cell suspension cultures under low temperature conditions, *BN115*, a well-characterized gene known to be specifically involved in cold acclimation, was used as a marker. Cells subjected to 5°C were collected at 0, 6, 12, 24, 48 and 72 h post-cold challenge. To minimize the effects of culture duration on gene expression in response to cold treatment, transcript levels of non-stressed control cells were also examined at each time point. RT-PCR with *BN115* specific primers revealed the up-regulation of gene expression by cold treatment (data not shown). This was further confirmed by qRT-PCR (Fig. 1). The relative transcript level of *BN115* began to increase after 6 h at 5°C, but after 24 h of cold exposure, the gene expression level was 13-fold higher than that of the control at the same time point. By 48 h, *BN115* expression was up to 40-fold higher than the control. The transcript level decreased thereafter, but still

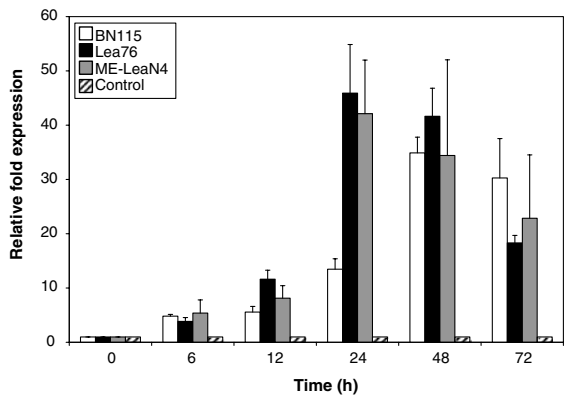

Fig. 1 qRT-PCR analysis of gene expression in *B. napus* MD cell suspension cultures exposed to low temperature. Transcript levels in cell suspensions derived from microspore cultures exposed to 5°C for 0, 6, 12, 24, 48, 72 h plotted as a fold-difference relative to expression in the corresponding control. *B. napus* actin was used as an internal constitutively expressed reference gene for qRT-PCR. Error bars indicate standard error from three independent treatments

remained at a level of 30-fold over control after 72 h of cold treatment.

To test if the cold-inducible gene *BN115* responded to osmotic stress, we also examined the transcript level changes in the RNA isolated from cell suspensions challenged with 20% polyethylene glycol (PEG) at the ambient temperature of 21°C. No significant differences in gene expression were observed at any of the treatment times when compared with corresponding controls (data not shown).

Regulation of *Lea76* and *ME-LeaN4* gene expression by osmotic stress

Since cDNAs of *Lea76* and *ME-LeaN4* were isolated from cDNA libraries of mature dry seeds or desiccated embryos, and their expression was detected only in embryos undergoing desiccation (Harada et al. 1989; Wakui and Takahata 2002), we examined the expression of representative genes in MD cell suspension cultures challenged with 20% PEG for 0, 6, 12, 24, 48 and 72 h. Quantitative real-time RT-PCR analyses using primers and TaqMan™ probes specific to *Lea76* and *ME-LeaN4* genes indicated that the expression profiles of *Lea76* and *ME-LeaN4* were almost identical (Fig. 2). The transcripts of both genes were present 6 h after the application of osmotic stress and steadily increased. At 48 and 72 h of PEG treatment, the transcript accumulations increased dramatically, going up to 20 and 60-fold higher than the corresponding controls, respectively. Since transcripts increased continuously during the treatment and the challenge did not extend beyond 72 h, it is unclear whether these accumulations represent the maximum possible level of induction.

Many Lea proteins have been shown to be induced not only by water stress but by low temperature stress as well (Ali-Benali et al. 2005). Therefore, we also examined the response of *Lea76* and *ME-LeaN4* in cells exposed to low temperature, and found that both genes were up-regulated (Fig. 1). The transcript accumulations of *Lea76* and *ME-LeaN4* gene occurred after 6 h of exposure to 5°C. The maximum induction ratios were measured at 24 h post treatment, and then decreases in transcript levels were observed thereafter. By 72 h, the transcripts were 17- and 11-fold of the corresponding control cells, higher than those at 12 h.

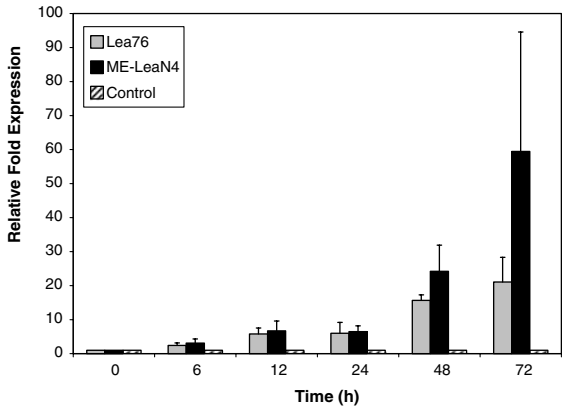

Fig. 2 qRT-PCR analysis of gene expression in *B. napus* MD cell suspension cultures exposed PEG-induced osmotic stress. Transcript levels in cell suspensions derived from microspore cultures exposed to 20% PEG for 0, 6, 12, 24, 48, 72 h plotted as a fold-difference relative to expression in the corresponding control. *B. napus* actin was used as an internal constitutively expressed reference gene for qRT-PCR. Error bars indicate standard error from three independent treatments

Ploidy level of MD cell suspensions after long-term culture

The MD cell suspension culture used in this study has been maintained for 23 years through continuous bi-weekly sub-culture, without apparent change in growth rate. Ploidy levels of the cells at the initiation stage were not reported. Plants regenerated from this cell line after seven years of maintenance were all aneuploid with chromosome numbers of 33 or 34, as identified with cytological analysis (Simmonds et al. 1991). To examine the current ploidy levels of the cells, flow cytometric analysis was performed. DNA flow cytometry measures the fluorescence of a large number of nuclei stained with propidium iodide and nuclear DNA content per 2C (C = DNA content of a haploid cell) is calculated based on the fluorescence. MD cell suspension cultures displayed two broad-base fluorescent peaks compared with chicken red blood cells (CRBC), indicating variability in the ploidy levels in the cell suspensions (data not shown). The amount of DNA of MD cell suspensions was estimated to be between 3.2 and 3.9 pg per nucleus. Nuclear DNA content of typical diploid *B. napus* ranged from 2.3 to 2.8 pg, according to previous estimations (Bennett and Leitch 1997).

Somatic embryogenesis and plant regeneration

When transferred onto agar-solidified B5 medium supplemented with 0.5–1.0 mg l^{-1} BA, 0.3 M mannitol, 2.0% sucrose, the cells gradually turned green and then grew vigorously (Fig. 3a). After one to two sub-cultures on the same medium, compact dark green globular embryo-like structures emerged on the surface of calli (Fig. 3b). Mature embryos were occasionally observed on the medium. The calli with

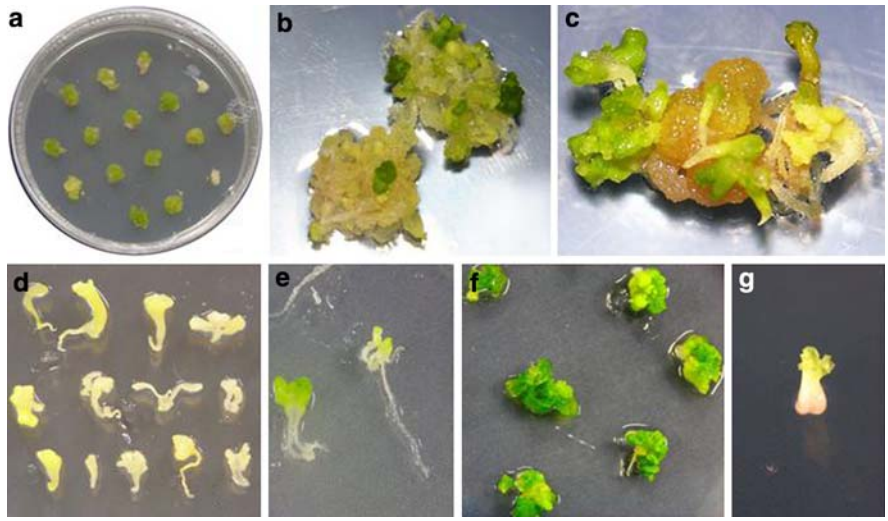

Fig. 3 Somatic embryogenesis of long-term MD cells of *B napus*. Panel A: Cells grown on agar-solidified B5 medium, supplemented with BA and NAA; Panel B: Globular embryo-like structures emerged on the surface of calli; Panel C, D: Embryos developed on B5 basal medium; Panel E: Roots produced from the embryos; Panel F: Secondary embryogenesis; Panel G: Abnormal plantlet regenerated from the embryos

globular structures were placed onto B5 basal medium without supplementation of plant growth regulators and developed into mature embryos, characterized by the appearance of cotyledons and hypocotyls (Fig. 3c, d). The embryos further produced roots (Fig. 3e) and consistently underwent secondary embryogenesis on the B5 basal medium (Fig. 3f). The majority of calli produced embryos, each producing 1–8 embryos. Although a large number of embryos were obtained, very few normal embryos were observed. Most exhibited developmental abnormalities. The cotyledons were usually fused and hypocotyls either thick with rough surface or slender but coiled, which were similar to those from rapid-cycling *B. napus* (Koh and Loh 2000). Attempts to germinate the embryos into plants, including adjusting the media compositions, supplementing with different combinations of plant growth regulators and activated charcoal, cold (4°C), PEG and ABA treatments, were carried out. A few embryos germinated (Fig. 3g). Most plantlets were vitrified with large leaves, thick and short stems, and failed to elongate. No plants were observed on the media that was previously proven effective (Simmonds et al. 1991).

Discussion

MD cell suspension cultures of *B. napus* L. cv Jet Neuf have proven to be useful system for studying various aspects of plant biology (Orr et al. 1986; Johnson-Flanagan and Singh 1987; Johnson-Flanagan et al. 1991; Moraes and Plaxton 2000). Previously, we have used these cell suspension cultures in the study of storage lipid biosynthesis in developing oilseeds (Weselake and Taylor 1999), and have characterized several lipid biosynthetic enzymes from this culture (Furukawa-Stoffer et al. 1998; Byers et al. 1999; Nykiforuk et al. 2002; Furukawa-Stoffer et al. 2003). In the present study, we extended this model to the examination of gene expression changes in response to abiotic stress and evaluated the ploidy levels and embryogenic potential of these cultures, which have been maintained via continuous bi-weekly sub-culture for 23 years.

The expression of the cold-responsive gene *BN115* in cell cultures exposed to low temperature closely reflected that previously described in intact leaves of *B. napus* L. cv. Jet Neuf (Weretilnyk et al. 1993). Northern blot analysis indicated that *BN115* transcripts were detectable in leaves from intact plants within 1 day of low temperature exposure and reached a maximum level after 3 days of treatment. After 10 weeks in the cold, the transcript was still present but at a lower level. Our results showed that the accumulation of *BN115* gene transcripts in MD cell suspensions changed in a similar manner as in the leaves from plants with respect to the duration of low temperature treatment (Weretilnyk et al. 1993). Further, the cell suspensions responded to the cold challenge (6 h exposure) faster than intact plants (1 day). The data implied that in vitro tissues or cells might be more sensitive than intact plants in response

to low temperature stress. Indeed, excised leaf discs of *B. napus* treated for 18 h at a low temperature condition gave a hybridization signal as strong as leaves from intact plants acclimated for 1 week, as shown with Northern blot analysis (Weretilnyk et al. 1993). Since the transcripts were also present in other cruciferous cold-acclimated plants, we might reasonably expect that the cell suspensions may be capable of developing a low-temperature tolerance after cold treatment. Indeed, cell suspensions of *Medicago falcata* (Wolfraim et al. 1993), *Prunus persica* (Arora and Wisniewski 1995), *Bromus inermis* (Ishikawa et al. 1990) and *B. napus* var *oleifera* (Egierszdorff and Kacperska 2001) showed significant increases in low temperature tolerance after exposure to cold challenge. *BN115* did not respond to PEG-induced osmotic stress, which is in agreement with earlier observations with intact plants, where drought stressed *B. napus* plants at room temperature only produced a very weak signal hybridizing to *BN115* in Northern blot analysis (Weretilnyk et al. 1993).

To investigate the response of the MD cell suspension to osmotic stress, we applied 20% polyethylene glycol, which has been used in previous studies to simulate drought in liquid culture (Rhodes et al. 1986; Leustek and Kirby 1988; Leone et al. 1994), and followed expression of two previously characterized desiccation-induced, late embryogenesis abundant (*Lea*) genes. *Lea76* and *ME-LeaN4* were shown to respond to both the PEG treatment and low temperature, consistent with observations in in vivo desiccating embryos, where mRNA of *Lea76* accumulated at late embryogenesis and remained at a high level in dry seeds (Harada et al. 1989). It should be noted, however, that since not all sequences of *B. napus* encoding Lea proteins have been identified to date, the transcript accumulations of these two *Lea* genes might represent the closely related genes in the family, although nucleotide BLAST indicated that the primers or TaqMan^{TM} probes might only allow the detection of genes of interest.

Plant cell cultures are prone to chromosomal, genetic and molecular changes, induced by physiological and biochemical factors, such as plant growth regulators and mutagens (Jayasankar 2005). A decrease in plant regeneration potential under long-term maintenance of callus and suspension cultures has been reported in numerous plant species (Zaghmout and Torello 1992; Komatsu et al. 1996). In some cases, the change of ploidy level of cells was thought to be associated with the decline of regeneration capacity (Murashige and Nakano 1967; Moyne et al. 1993). Several investigators, however, believe there is no strict correlation between the ploidy level and the ability to produce somatic embryos and plants (Winklemann et al. 1998; Nakano et al. 2000). Protein phosphorylation and enzymatic changes were detected in long-term cultured rice cells, and proposed to be related to the decrease of plant regeneration potential (Abe and Futsuhara 1989; Komatsu et al. 1996). In this study, the suspension cells have been sub-cultured more than 600 times in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D) and other plant growth regulators, which might induce somaclonal variation, such as chromosome aberration or point mutations. The nuclear DNA content of the MD cell suspension cultures, as estimated by flow cytometry, was increased relative to previously determined values for diploid *B. napus*, suggesting the cells have likely undergone some chromosomal changes during long-term culture, and are probably aneuploid. This agrees with the earlier finding that plants regenerated from this culture after seven years were aneuploid (Simmonds et al. 1991). Variation in nuclear DNA content in cell suspensions has been observed in *Euphorbia pulcherrima* (Geier et al. 1992) and *Rosa hybrida* (Moyne et al. 1993).

Despite these potential chromosomal abnormalities, the MD cell suspension cultures were able to undergo embryogenesis after long-term culture in the presence of plant growth regulators. The resulting embryos, however, did not regenerate under the conditions previously reported to be effective (Simmonds et al. 1991). One might expect that long-term exposure to exogenous hormones may have resulted in alterations to the endogenous hormone biosynthetic pathways and responses, which regulate cell differentiation and plant regeneration processes. To unveil the actual mechanism responsible for the failure of these embryos to regenerate requires further investigation. If plants could be regenerated, the MD cell suspensions might be useful in studies of mutagenesis, plant breeding and genetics. *B. napus* cell lines possessing a resistance to chlorsulfuron, a widely used herbicide, have been isolated from the MD cell suspension cultures through chemical mutagenesis followed by colony rescue (Saxena et al. 1990).

In the present study, we examined the effect of low temperature and osmotic stress on gene expression in MD cell suspension cultures of *B. napus* in order to validate its use as a system for studying the biochemistry and molecular biology of abiotic stress in oilseeds. Expression profiles for several stress-inducible genes from MD cell suspension cultures exposed to low temperature or osmotic stress were similar to those observed in intact plants, which suggests this culture can be used as a convenient, fast growing, scalable system for more in-depth studies of abiotic stress responses in oilseeds. The increased sensitivity with respect to *BN115* expression brought about by low temperature treatment of the cultures when compared to expression in leaves suggests the culture may be a particularly useful system for identifying low abundance transcripts. Despite an apparent increase in nuclear DNA levels compared to diploid *B. napus*, the cells retained their embryogenic competence, but not the ability to regenerate into plants, after more than 20 years of continuous sub-culture. The formation of rudimentary plant-like structures remains promising however, and suggests that regeneration may still be achievable upon further optimization of the experimental conditions. Currently, we are developing a transformation method for the MD cell suspension cultures to facilitate the investigation of the molecular biology and enzymology of fatty acid desaturation, which is known to increase in plants during low temperature exposure. These studies may be useful for identifying molecular or biochemical targets for future biotechnological approaches for decreasing saturated fatty acid content in edible seed oils.

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